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Haryana Veterinarian (June, 2026) **65(1),** 1-6 ISSN 0033-4359 (P) **Research Article**

SENSORY EVALUATION OF *SHRIKHAND* PREPARED FROM BUFFALO AND CAMEL MILK ENRICHED WITH GINGER AND BLACK PEPPER

VIMALA CHOUDHARY*, BASANT BAIS, PHOOL KANWAR, PRIYANKA MASAR, ANITA TANWAR and KAPIL GODARA
Department of Livestock Products Technology, CVAS, RAJUVAS, Bikaner-334001, Rajasthan

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ABSTRACT

Present investigation was carried out to assess the sensory evaluation of herbs enriched *shrikhand*. *Shrikhand* is a popular Indian dessert prepared by fermentation of milk. In present study the *shrikhand* is obtained by buffalo and camel milk (70:30) by incorporating spices. *Shrikhand* was prepared from dahi (70% buffalo + 30% camel milk) with a constant level of sugar (40%) and supplementing with ginger powder (0.5%, 1%, 1.5% - T_1G_1, T_1G_2, T_1G_3), black pepper powder (0.5%, 1%, 1.5%-T_2B_1, T_2B_2, T_2B_3) and ginger + black pepper powder (0.25-0.25%, 0.5+0.5%, 0.75-0.75% - $T_3(GB)_1$,$T_3(GB)_2$,$T_3(GB)_3$). T_0 served as control with no supplementation. The sensory evaluation of the developed *shrikhand* was carried out using an 8-point hedonic scale by semi-trained panellists to assess appearance and colour, flavour, body and texture and overall acceptability. Three treatment groups were prepared based on the spice incorporated (ginger, black pepper and ginger + black pepper) and each group consisted of three concentration levels (0.5%, 1.0%, and 1.5%). The comparison of sensory scores was therefore made within their respective treatment groups. It was observed that the 1% ginger, 1% black pepper and 0.5% + 0.5% ginger + black pepper treatments obtained significantly ($P < 0.01$) higher overall acceptability, scoring 7.44 ± 0.039, 7.70 ± 0.041 and 7.69 ± 0.031, respectively. After identifying the best compositions within each group, a subsequent comparison among the three most acceptable treatments-1% ginger (T_1), 1% black pepper (T_2) and 0.5% + 0.5% ginger + black pepper (T_3) was conducted. The pooled data were then used to determine overall acceptability. Sensory analysis showed a significant difference ($P < 0.01$) in various sensory attributes of the T_1 sample compared with the other treatments. *Shrikhand* prepared with 1% ginger powder (T_1) recorded the highest overall acceptability, scoring 7.69 ± 0.032.

Keywords: Black Pepper, Buffalo Milk, Camel Milk, Ginger, Hedonic Scale

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Shrikhand is one of the leading indigenous fermented milk products in the Indian diet, particularly enjoyed in the states of Maharashtra and Gujarat (Aneja *et al.*, 2002). Fermented milk products become more nutritious due to the increased vitamin content produced by selective starter cultures. Fermented milk products have better keeping quality and are easily digestible because of breaking down of proteins into peptides and free amino acids as a result of microbial action. *Shrikhand* is a popular Indian dessert, it's a creamy, yogurt-based sweet dish that's often flavoured with cardamom, saffron and sometimes fruit like mango and herbs like ginger, black pepper etc. (Aneja *et al.*, 2002). To make it, thick yogurt (usually hung curd or Greek yogurt) is sweetened with sugar and mixed with these flavors. *Shrikhand* is derived from the Sanskrit term '*Shikharani*', a curd produced with additional sugar, flavouring ingredients (saffron), fruits, nuts and almonds (Mane *et al.*, 2017). This chakka is then finely combined with sugar and flavourings to achieve a semisoft texture and sour-sweet flavour. *Shrikhand* is well-known for its excellent nutritional value, distinctive flavour, appetizing nature, and potential therapeutic value. Lactic acid bacteria reduce blood cholesterol levels while raising vitamin B levels in the product (Bhandage *et al.*, 2020).

*Corresponding author: dvimala111@gmail.com

Camel milk is an excellent source of health-beneficial substances in the human diet. Camel milk has an average of 3.1% protein, 3.5% fat, 4.4% lactose, 0.79% ash and 11.9% total solids. Water content is the most significant component in camel milk (Kaskous, 2016). Camel milk has less lactose than cow's milk, making it potentially more tolerable for people with mild lactose intolerance. Fermented camel milk, which contains more antioxidant peptides due to its higher β -casein level has been proven to be a medicinal substance (Izadi *et al.*, 2019). Buffalo milk has a significant impact on human nutrition.

Buffalo milk contains higher concentrations of fat (6.80%), ash, total solids (17.0 ± 0.1%), protein (3.91%), SNF (9.61%), lactose (4.83±0.01%), calcium and vitamins A and C. Because of its vitamins, minerals and bioactive components, buffalo milk exhibits notable antioxidant properties. The greater antioxidant capacity of buffalo milk, as compared with cow milk, has been attributed to its higher monounsaturated fatty acid (MUFA) content (Khan *et al.*, 2017).

Because of the potential toxicity of chemical preservatives in humans, there is a growing market that prefers meals devoid of them. Herbs, spices and seasonings have been familiar to humans for centuries.

Herb's bioactive components include antioxidant and antibacterial characteristics, preventing degradation and increasing the shelf life of dairy products. Moreover, their ingestion has been linked to several health advantages. (Barak *et al.*, 2023). Black pepper (Piper nigrum) is an excellent source of minerals, including manganese, phosphorus, iron, potassium and magnesium. Calcium, dietary fibre, copper, and vitamins B_6, C and K. Along with piperine, it also contains flavonoids, other alkaloids and monoterpenes (camphene, pinene and limonene) that have antibacterial properties against a variety of microbes. (Khawsud *et al.*, 2020). According to Ayurvedic medicine, ginger (Zingiber officinale) is a carminative stimulant that provides stimulating treatments. All the elements needed for healthy nutrition are present in ginger. Iron, calcium, vitamins C, E and B_6 and dietary fibre are also present. It contains no cholesterol. Therefore, the aim of this study was to evaluate the sensory parameters of *shrikhand* manufactured with buffalo and camel milk supplemented with different levels of ginger and black pepper powder.

MATERIALS AND METHODS

The experiment research work was carried out to prepare shrikhand with the addition of black pepper (Piper nigrum) and ginger powder (Zingiber officinale) which was conducted in the laboratory of the Department of Livestock Products Technology, College of Veterinary and Animal Science, (RAJUVAS) Bikaner during the year 2023-24. The materials used and the methodologies adapted are as under.

MATERIALS

Good quality Fresh buffalo milk was collected from local buffalo dairy farm of Bikaner. And fresh camel milk was collected from National Research Centre on Camel, Bikaner was standardized to 6% fat and filtered to remove dirt and other extraneous matter, before use. All samples were collected manually in sterile bottles. Good quality sugar, black pepper and ginger powder was procured from Bikaner local market.

Preparation of black pepper, ginger and ginger +black pepper added *Shrikhand*

Shrikhand was prepared as per procedure described by (Aneja *et al.*, 2002) with slight modification.

Fresh camel and buffalo (30:70) Milk
↓
Filtration
↓
Standardization
↓
Heating of Milk @ 85º C for 30 min
↓
Cooled to 30º ± 2º C
↓
Inoculation with starter culture @ (1-2% by V/V of Milk)
↓
Incubation at 35-37º C for 8-10 hrs
↓
Dahi
↓
Drainage of whey (16-18 hrs.) through muslin cloth
↓
Chakka
↓
Addition of sugar 40%
↓

Ginger enriched *shrikhand*	Black pepper enriched *shrikhand*	Ginger + black pepper enriched
(0% - T_0)	(0% - T_0)	(0% - T_0)
(0.5% - T_1G_1)	(0.5% - T_2B_1)	(0.25+0.25% - $T_3(GB)_1$)
(1% - T_1G_2)	(1% - T_2B_2)	(0.5+0.5% - $T_3(GB)_2$)
(1.5% - T_1G_3)	(1.5% - T_2B_3)	(0.75+0.75% - $T_3(GB)_3$)

↓
Sieving and kneading
↓
Filling of control and all treatments separately in pre-sterilized glass jars
↓
Refrigeration Storage (4 ± 1º C)

Sensory evaluation of the herbs-enriched *shrikhand* prepared as per the experimental formulations was conducted using an 8-point hedonic scale by a panel of eight semi-trained members drawn from academic staff, technical staff and students of the Department of Livestock Products Technology, RAJUVAS, Bikaner for various sensory attributes viz., appearance and colour, flavour, body and texture and overall acceptability. In the hedonic scale, a score of '8' denotes "excellent," whereas '1' denotes "extremely poor."

Each treatment was prepared in three independent replications, generating a total of n = 21 sensory observations per attribute. *Shrikhand* samples were presented on plates, coded with a three-digit digital code and the order of presentation was randomized for each panellist. To avoid flavour carryover, panellists rinsed their mouths with potable water between evaluations. The sensory data were statistically analysed by adopting appropriate analysis of variance (ANOVA) procedures as described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Sensory evaluation of various levels of ginger in *shrikhand*

Sensory evaluation of the various levels of ginger in

shrikhand showed that sample T_1G_2 with 1% ginger powder had significantly (P<0.01) higher acceptability in all the attributes analysed as presented in Table 1. This is in agreement with the report of Sahu & Pathak (2021), Kumari *et al.* (2021), Waghmare *et al.* (2021) and Yedatkar *et al.* (2022). where the control *shrikhand* samples were preferred by the panelists. Scores for Appearance and Colour, Flavour, Body and Texture, Overall Acceptability ranged from $7.58^{d} \pm 0.031$ - $5.89^{a} \pm 0.041$, $7.18^{d} \pm 0.072$ - $5.33^{a} \pm 0.046$, $7.49^{c} \pm 0.032$ - $6^{a} \pm 0.047$ and $7.44^{d} \pm 0.039$ - $5.72^{a} \pm 0.043$, respectively with significant difference (P≤0.01) between samples. Control had significantly lower scores compared to other samples. Colour and flavour is a very important parameter in judging properly prepared *shrikhand* products that not only reflect in the suitability of raw materials used for the preparation but also provides information about the quality of the product. A significant difference (p<0.01) was observed between treatment of ginger enriched *shrikhand* for appearance and colour, flavour, body and texture and overall acceptability of sensory evaluation. Thus, it may be interpreted that the different levels of ginger powder significantly affect the sensory quality of *shrikhand* but 1% ginger powder give best result in *shrikhand* preparation.

Sensory evaluation of various levels of black pepper in *shrikhand*

The mean score for appearance and colour ranged between 5.84±0.052 to 7.50±0.102. The highest mean score 7.50±0.102 was obtained in 1% ($T_2 B_2$) black pepper enriched *shrikhand* as compared to the control, $T_2 B_1$ and $T_2 B_3$. The addition of 1.5% black pepper to *shrikhand* resulted in a significant decline in the score. The acceptability of any dairy product is influenced by its flavour. The alterations in flavour score of developed *shrikhand* found that there was a significant difference in flavour score when black pepper was introduced at different levels. It was found that 1 per cent level of black pepper got maximum flavour score that was 7.87±0.031 which varied from control, 0.5% and 1.5% level of black pepper enriched *shrikhand* preparation. The average score for body and textural qualities of black pepper enriched *shrikhand* treatments were from 6.25±0.02 to 7.48±0.051. The maximum score 7.48±0.051 obtained of 1 per cent level of black pepper enriched *shrikhand* as shown in table 2. The addition of 1.5% black pepper resulted in a drop in the overall acceptability score. Out of all the treatments, the 1% black pepper added *shrikhand* sample had the highest overall acceptance score of 7.70±0.041. A significant difference (P<0.01) was reported between treatment of black pepper enriched *shrikhand* for appearance and Colour, flavour, body and texture and

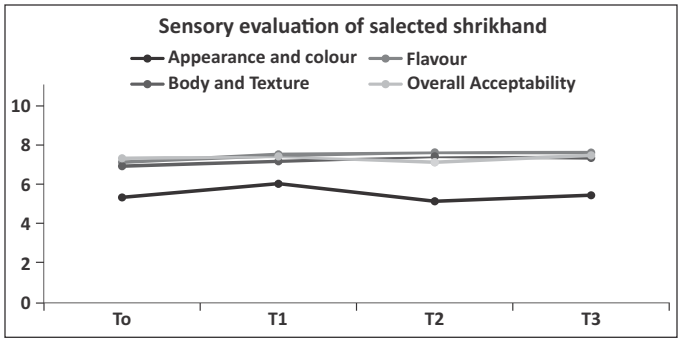

Fig. 1. Sensory evaluation of selected *shrikhand* T_0- Control *shrikhand*, T_1-1% ginger enriched *shrikhand*, T_2- 1% black pepper enriched *shrikhand* and T_3-0.5% ginger+0.5% black pepper enriched *shrikhand*.

overall acceptability. Similar findings have been reported by earlier researchers, where the incorporation of spices or herbal additives in fermented milk products such as *shrikhand*, yoghurt and flavoured dairy desserts showed that moderate levels enhanced sensory attributes, while higher levels led to reduced acceptability. Prajapat (2019) and Meena *et al.* (2020) documented similar trends in spice-enriched *shrikhand* and Sarkar & Ghosh (2020), Kumari *et al.* (2021), Sarnaik *et al.* (2021), Shori (2022) and Kumari *et al.* (2022) all confirmed that excessive addition of spices or botanicals tends to impart sharp or overpowering flavour, thereby reducing consumer preference.

Sensory evaluation of ginger + black pepper enriched *shrikhand*

Ginger + black pepper had a significant (P<0.01) influence on Appearance and Colour, Flavour, Body and Texture, Overall Acceptability of the *shrikhand*. The average score for appearance and colour varied from 5.84±0.041 to 7.38±0.043. The highest score was 7.38±0.043 at the 1(0.5+05) percent level. The score declined significantly when 1.5(0.75+0.75) percent ginger and black pepper were added to *shrikhand*. From the Table 3. it is observed that the flavour score increased on addition of ginger + black pepper up to 1 (0.5 + 0.5) per cent. In comparison to control (T_0), $T_3(GB)_1$ and $T_3(GB)_3$, the flavour of the ginger + black pepper enriched shrikhand sample [$T_3(GB)_2$] was the greatest, i.e. 7.70±0.035. However, subsequent addition of ginger + black pepper at the rate of 1.5(0.75+0.75) per cent resulted into reduction in score up to 7.30±0.082. The body and texture of $T_3(GB)_2$ sample of ginger + black pepper enriched shrikhand was highest i.e. 7.54±0.016 compared to T_0, $T_3(GB)_1$ and $T_3(GB)_3$. The results of overall acceptability have been provided in Table 3 found that the sample $T_3(GB)_2$ (0.5+0.5 per cent) level presents better outcomes score 7.69±0.031 than sample T_0, $T_3(GB)_1$ and $T_3(GB)_3$. There was a significant difference (P<0.01) in the body and texture,

Table 1. Sensory evaluation of various levels of ginger enriched shrikhand (Mean± SE)

Parameters/ Treatments	T_0	T_1G_1	T_1G_2	T_1G_3
Appearance and Colour	$5.89^{a} \pm 0.041$	$6.83^{b} \pm 0.053$	$7.58^{d} \pm 0.031$	$7.10^{c} \pm 0.054$
Flavour	$5.33^{a} \pm 0.046$	$6.62^{b} \pm 0.043$	$7.18^{d} \pm 0.072$	$6.83^{c} \pm 0.041$
Body and Texture	$6.00^{a} \pm 0.047$	$7.13^{b} \pm 0.046$	$7.49^{c} \pm 0.032$	$7.22^{b} \pm 0.051$
Overall Acceptability	$5.72^{a} \pm 0.043$	$6.80^{b} \pm 0.041$	$7.44^{d} \pm 0.039$	$7.06^{c} \pm 0.037$

Note: Means bearing different superscript in a row differ significantly. T_0- Control *Shrikhand*, T_1G_1- 0.5% ginger *Shrikhand*, T_1G_2 - 1% ginger *Shrikhand* and T_1G_3 - 1.5% ginger *Shrikhand*

Table 2. Sensory evaluation of black pepper enriched *shrikhand* (Mean± SE)

Parameters/ Treatments	T_0	T_2B_1	T_2B_2	T_2B_3
Appearance and Colour	$5.84^{a} \pm 0.052$	$6.22^{b} \pm 0.031$	$7.50^{d} \pm 0.102$	$6.80^{c} \pm 0.035$
Flavour	$5.34^{a} \pm 0.039$	$7.34^{b} \pm 0.041$	$7.87^{d} \pm 0.031$	$7.46^{c} \pm 0.031$
Body and Texture	$6.25^{a} \pm 0.020$	$6.83^{b} \pm 0.031$	$7.48^{d} \pm 0.051$	$7.11^{c} \pm 0.032$
Overall Acceptability	$5.76^{a} \pm 0.039$	$6.88^{b} \pm 0.041$	$7.70^{d} \pm 0.041$	$7.17^{c} \pm 0.06$

Means ± SE bearing different superscript in a row differ significantly. T_0 - Control shrikhand, T_2B_1 - 0.5% black pepper enriched *Shrikhand*, T_2B_2 - 1% black pepper enriched *shrikhand* and T_2B_3 - 1.5% black pepper enriched *shrikhand*

Table 3. Sensory evaluation of ginger + black Pepper enriched *shrikhand* (Mean± SE)

Parameters/ Treatments	T_0	$T_3(GB)_1$	$T_3(GB)_2$	$T_3(GB)_3$
Appearance and Colour	$5.84^{a} \pm 0.041$	$6.63^{b} \pm 0.031$	$7.38^{d} \pm 0.043$	$7.00^{c} \pm 0.102$
Flavour	$5.37^{a} \pm 0.046$	$7.15^{b} \pm 0.061$	$7.70^{c} \pm 0.035$	$7.30^{b} \pm 0.082$
Body and Textures	$6.12^{a} \pm 0.040$	$7.00^{b} \pm 0.102$	$7.54^{c} \pm 0.016$	$7.17^{b} \pm 0.047$
Overall Acceptability	$5.74^{a} \pm 0.096$	$6.90^{b} \pm 0.041$	$7.69^{c} \pm 0.031$	$7.02^{b} \pm 0.063$

Means±SE bearing different superscript in a row differ significantly. T_0 - Control *shrikhand*, $T_3(GB)_1$ - 0.5% ginger + 0.5% black pepper enriched *shrikhand*, $T_3(GB)_2$ - 1% ginger + 1% black pepper enriched *Shrikhand* and $T_3(GB)_3$ - 1.5% ginger + 1.5% black pepper enriched *shrikhand*.

Table 4. Sensory evaluation of selected *shrikhand* (Mean± SE)

Parameters/ Treatments	T_0	T_1	T_2	T_3
Appearance and Colour	$5.40^{a} \pm 0.054$	$7.20^{bc} \pm 0.108$	$7.00^{b} \pm 0.147$	$7.40^{c} \pm 0.061$
Flavour	$6.10^{a} \pm 0.082$	$7.60^{c} \pm 0.074$	$7.25^{b} \pm 0.104$	$7.50^{bc} \pm 0.041$
Body and Texture	$5.20^{a} \pm 0.108$	$7.70^{c} \pm 0.082$	$7.50^{c} \pm 0.054$	$7.20^{b} \pm 0.102$
Overall Acceptability	$5.51^{a} \pm 0.032$	$7.69^{d} \pm 0.032$	$7.42^{b} \pm 0.041$	$7.55^{c} \pm 0.054$

Means±SE bearing different superscript in a row differ significantly. T_0 - Control *shrikhand*, T_1 - 1% ginger enriched *shrikhand*, T_2 - 1% black pepper enriched *shrikhand* and T_3 - 0.5% ginger + 0.5% black pepper enriched *shrikhand*.

appearance and colour, flavour and overall acceptability of sensory evaluation between the ginger and black pepper enriched *shrikhand* treatments. From the sensory assessment of *shrikhand* prepared in preliminary trial, it was indicated that the addition of 1 per cent level of natural preservative in single or in combination provided best acceptable quality of *shrikhand*. Similar results have been reported by several researchers working on spice- or herb-incorporated fermented milk products, particularly *shrikhand* and yoghurt. Felfoul *et al.* (2017) observed that addition of plant extracts to yoghurt enhanced flavour and texture only up to an optimum concentration. Badola *et al.* (2018) found that spice-enriched *shrikhand* showed better acceptability at moderate levels, whereas excessive incorporation reduced panel scores. Prajapat (2019) and Meena *et al.* (2020) also demonstrated that *shrikhand* fortified with natural spices such as ginger, black pepper and cardamom achieved maximum scores at intermediate levels of addition. Similarly, Kumari *et al.* (2021), Waghmare *et al.* (2021) and Khandagale *et al.* (2022) reported improved sensory characteristics in spice-based dairy desserts but noted a decline at higher dosages due to overpowering flavour. Wakde *et al.* (2023) further confirmed that optimal incorporation of herbal/spice mixtures in fermented dairy products enhances appearance, flavour, body and texture, while excessive addition negatively

affects overall acceptability.

Sensory evaluation of selected *shrikhand*

Based on a sensory evaluation of *shrikhand* enhanced with different amounts of herbs, it was found that the maximum degree of acceptability for the *shrikhand* was achieved when 1% of ginger and 1% of black pepper were added, either separately or in combination. The mean values for all the attributes like flavour, body and texture, appearance and colour and overall acceptability for various *shrikhand* range from 5.20 ± 0.108 to 7.70 ± 0.082. The mean scores for the appearance and colour of the control *shrikhand* (T_0) were determined to be 5.40±0.054, while the scores for herbs enriched shrikhand i.e. for T_1, T_2 and T_3 were 7.20±0.108, 7.00±0.147 and 7.40±0.061, respectively. As a result, the panellists awarded T_3 (0.5+0.5% ginger + black pepper enriched buffalo and camel milk *shrikhand*) the maximum possible score of 7.40±0.061 and minimal score achieved by the control group (T_0) for appearance and colour was 5.40±0.054. The average score for flavour of control *shrikhand* (T_0) was found to be 6.10±0.082 and for herbs enriched *shrikhand* i.e. for T_1, T_2 and T_3 it was found to be 7.60±0.074, 7.25±0.104 and 7.50±0.041, respectively. Thus, it may be concluded that T_1 (1% ginger enriched *shrikhand*) had highest flavour score 7.60±0.074 for flavour by the panellist. The average body and texture score for control *shrikhand* (T_0) was 5.20±0.108, while T_1, T_2 and T_3 had scores of 7.70±0.082, 7.50±0.054 and 7.20±0.102, respectively. Therefore, it can be concluded that T_1 (1% ginger enhanced *shrikhand*) received a maximum score of 7.70±0.082 for body and texture from the panellists, while T_0 (control *shrikhand*) received a minimum score of 5.20±0.108 for body and texture. On the basis of results obtained in the present study as shown in Table 4 the ginger enriched *shrikhand* (T_1) had gained maximum overall acceptance 7.69±0.032. Whereas control and other treatments i.e. (T_0), (T_2) and (T_3) it was determined to be 5.51±0.032, 7.42±0.041 and 7.55±0.054, respectively that is shown in figure 1.

The present study demonstrated that ginger, black pepper and their combinations significantly improved the sensory attributes of *shrikhand* up to an optimum level, beyond which acceptability declined. These findings are in close agreement with earlier research on herb- and spice-enriched fermented dairy products. Rathod *et al.* (2019) reported improved sensory quality in shrikhand fortified with ginger, tulsi and cinnamon extracts, showing that moderate incorporation enhanced flavour and texture, similar to the effect of 1% ginger in the present study. Maji *et al.* (2020) evaluated yoghurt enriched with mint and tulsi extracts and found that optimum levels improved body and texture, consistent with the enhancement observed in our formulations containing 1% black pepper and 1% ginger. Meena *et al.* (2020) worked with *shrikhand* supplemented with cardamom, clove and cinnamon, reporting maximum acceptability at moderate spice levels, which supports our observation that higher concentrations (1.5%) reduce sensory scores. Mishra *et al.* (2021) studied fermented dairy desserts fortified with fenugreek, ginger and turmeric extracts and similarly found that botanical additives improved sensory perception, in line with our results for ginger and ginger–black pepper blends. Prajapat *et al.* (2021) used ginger, black pepper and cardamom in *shrikhand* and reported enhanced acceptability at middle-range levels, closely matching the outcomes of our study. Waghmare *et al.* (2021) observed that medicinal spice blends including ginger, cinnamon and pepper improved appearance, flavour and texture, which parallels our findings across all sensory attributes. Kumari (2022) documented improved sensory characteristics in *shrikhand* fortified with cardamom, clove and nutmeg, again reporting decline at higher inclusion levels, similar to the 1.5% treatments in our work. Khandagale *et al.* (2022) demonstrated that dairy products enriched with black pepper, cinnamon and ginger achieved maximum scores at moderate concentrations, comparable to the behaviour of 1% black pepper and combined ginger–pepper formulations in our study. Savaliya *et al.* (2023) found that yoghurt enriched with mint, ginger and tulsi exhibited improved sensory acceptance only up to an optimum level, supporting our conclusion that moderate incorporation (1% or 0.5+0.5%) provides the best results.

CONCLUSION

Sensory evaluation of herb-enriched *shrikhand* prepared from a standardized blend of buffalo and camel milk (70:30; 5.68% fat and 9.26% SNF), supplemented with 40% sugar and 1–2% starter culture, demonstrated that all formulations possessed desirable sensory attributes. Among the different incorporation levels of ginger, black pepper, and their combination, the formulation containing 1% ginger consistently achieved superior scores for colour, flavour, body and texture and overall acceptability. The findings clearly indicate that the buffalo–camel milk blend, when optimally fortified with ginger, can significantly enhance the sensory appeal of *shrikhand*, offering a promising approach for developing value-added fermented dairy products.

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IMPORTANT INFORMATION

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Research Article

IMPACT OF LUMPY SKIN DISEASE ON SERUM BIOCHEMICAL PARAMETERS IN CATTLE

ANKITA*, D.S. MEENA, ANITA SEWAG, RASHMI SINGH, DEVENDER YADAV[1], PRERNA YADAV and WARSHA CHAUDHARY
Department of Veterinary Medicine, Post Graduate Institute of Veterinary Education and Research (PGIVER), Jaipur-302031, Rajasthan, India
[1]HPVK, Mahendragarh, LUVAS, Hisar-125004, Haryana, India

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ABSTRACT

Lumpy skin disease (LSD) has emerged as a worldwide infectious disease in cattle, leading to significant devastation in the livestock industry. It is characterized by high fever and widespread nodules on the mucous membranes or skin, severely impacting the growth of the cattle industry and international trade. A total of 214 cattle affected by LSD were studied during year 2023-2024, with clinical progression, age and breed of the affected animals recorded. Out of 214 samples, 92 serum samples were found positive for LSD virus (74 in ELISA test and 8 in PCR). Among the total cattle affected by LSD, 31% were Holstein Friesian (HF), 28% were nondescript breeds, 27% were Rathi, 7% were Gir, 4% were Jersey and 3% were Tharparkar. Of the total cattle affected by LSD, 49% were over 5 years old, 39% were between 2 and 5 years, and 12% were under 2 years of age. Serum biochemical analysis showed a significant rise in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, along with hypoalbuminemia in cattle affected by LSD. This study contributes to a better understanding of the disease's pathogenesis, aiding in the early management and treatment of affected animals.

Keywords: Biochemical, Cattle, Jaipur, Lumpy skin disease

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Lumpy Skin Disease is characterised by fever, enlargement of lymph nodes, anorexia, depression, dysgalactia, emaciation and development of skin nodules, eventually resulting in abortion in pregnant cattle, and sterility in bulls (Tuppurainen *et al.*, 2017). LSD is a infectious viral disease of cattle caused by Lumpy skin disease virus (Murphy *et al.*, 2008). Lumpy skin disease virus is a double-stranded DNA containing virus of around 150 kilobase pairs (kbp) with relatively large sizes (230-260 nm), enclosed in a lipid envelope, belongs to Genus *Capripox*, which is genetically related to the sheep pox (SPPV) and goat pox (GTPV) viruses (Buller *et al.*, 2005; Givens, 2018). Lumpy skin disease virus (LSDV) is classified under Genus *Capripox*, Family *Poxviridae* and subfamily *Chordopoxvirinae* (Tuppurainen and Oura, 2012).

Depending on the virulence of the strains and the sensitivity of the cow breed, the severity of the clinical indications of LSD ranges from asymptomatic to lethal. Several clinical symptoms, including skin nodules, swollen lymph nodes, fever, nasal discharge, lacrymation, edema of mucous membrane and internal organ might be seen in infected animals (Abutarbush *et al.*, 2015). Viremia and fever are the first symptoms of the generalized type, later localization in the skin and production of inflammatory nodules can be noticed (Constable *et al.*, 2017). The severity of the early clinical manifestations of LSD varies, depending on the herd management, irrespective of the sex or age of the animal. The animal develops several solid, bounded nodules in epidermis (Beard *et al.*, 2016). Nodules may emerge within 1-2 days, which may remain as localized to a few lesions or may be extensive, can be seen at different locations including the head, neck, perineum, genitalia, udders and limbs (Al-Salihi *et al.*, 2014).

Increased levels of aspartate aminotransferase, alkaline phosphatase, and creatinine concentrations in serum biochemical analysis of infected animals, suggest the liver and kidney failures occur during LSDV infection (Murat *et al.*, 2016).

MATERIAL AND METHODS

Animal source: For this study, a total of 214 blood samples from LSD suspected cases of cattle were collected during year 2023-2024, from various parts of Rajasthan, for detection of LSD Virus. Out of 214 samples, 92 samples were found positive for LSD virus (74 in ELISA test and 18 in PCR, kept under Group-I. Twenty visibly healthy cattle, kept under optimal management conditions, were selected as the healthy control group (Group-II) to assess normal biochemical parameters. Cattle suspected of having Lumpy Skin Disease (LSD), based on symptoms such as skin nodules, fever, palpable superficial lymph nodes, brisket edema, lameness, excessive salivation and reduced feed and water intake, underwent a thorough physical examination. Clinical evaluation included measuring rectal temperature, assessing the conjunctival mucous membrane, palpating superficial lymph nodes, and recording other LSD-related symptoms, including limb swelling, brisket edema, nasal discharge, excessive tearing, and lumps on different body parts. Serum samples

*Corresponding author: drankita8408@gmail.com

from each group were obtained for biochemical assessment.

Blood collection: Whole blood of 5 ml was collected aseptically, from the jugular vein of the animal with a 10 ml syringe, in clot activator tube taking all precautions to avoid haemolysis.

Separation of serum: Serum was separated, by keeping the vial in slanting position for 1 hour at room temperature and centrifugation at 3000 rpm for 20 minutes.

ELISA assay: LSD suspected cases of cattle, during year 2023-2024 were subjected to rapid detection by ELISA assay, using Bovine Lumpy Skin disease (LSDV) ELISA Kit (SunLong Biotech Co., LTD).

Genomic DNA isolation: The viral DNA extraction was done by using HiPura Multi- Sample DNA purification kit (Himedia).

Molecular detection of LSD virus: Conventional polymerase chain reaction (PCR) was performed for the amplification of the VP32 gene of lumpy skin disease virus (LSDV). The forward primer sequence used was 5′-TTTCCTGATTTTTCTTACTAT-3′, while the reverse primer sequence was 5′-AAATTATATACGTAAATAAC-3′, yielding an expected amplicon size of 192 bp.

PCR amplification was carried out in a 10 µL reaction volume comprising 1 µL of 10X PCR buffer, 1 µL of 2 mM dNTP mix, 0.5 µL each of forward and reverse primers, 0.4 µL of Taq DNA polymerase (5 U/µL), 2 µL of template DNA, and 4.6 µL of nuclease-free water to make up the final volume. The PCR cycling conditions consisted of an initial denaturation at 94° C for 7 minutes, followed by 42 cycles of denaturation at 94° C for 1 minute, annealing at 52° C for 30 seconds, and extension at 72° C for 1 minute, with a final extension at 72° C for 10 minutes. The amplified products were subsequently held at 4° C until further processing.

The PCR products were resolved by agarose gel electrophoresis and visualized using a gel documentation system. Known LSDV-positive DNA procured from the National Centre for Veterinary Type Culture (NCVTC), ICAR-NRCE, Hisar, was used as the positive control, while nuclease-free water served as the negative control.

Biochemical estimations: Biochemical parameters included the estimation of serum total protein, albumin, globulin, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and alkaline phosphatase (ALP), estimated by using TURBOCHEM 100 Biochemistry Analyzer (CPC DIAGNOSTICS PVT. LTD).

RESULTS AND DISCUSSION

A total of 214 blood samples from LSD suspected cases of cattle were collected during year 2023-2024. Out of 214 samples, 92 samples were found positive for LSD virus (74 in ELISA test and18 in PCR). Fig. 2 displays that among the total cattle affected by LSD, 31% were Holstein Friesian (HF), 28% were nondescript breeds, 27% were Rathi, 7% were Gir, 4% were Jersey and 3% were Tharparkar. Fig. 3 displays that out of the total cattle affected by LSD, 49% were over 5 years old, 39% were between 2 and 5 years, and 12% were under 2 years of age. In Fig. 1 Graph is showing level of Serum biochemical parameters in the control group and Lumpy skin disease affected cases of various places. The serum biochemical analysis revealed an increase in serum AST and ALT levels, accompanied by a decrease in albumin levels across all age groups of affected cattle. Among them, older animals were the most susceptible to LSD, followed by younger ones, with very young animals being the least affected. However, no significant changes were observed in the levels of albumin, globulin, creatinine, and alkaline phosphatase (ALP), which remained within the normal range in LSD-affected cattle. Table 1 displays the Mean ± SE values of serum biochemical parameters for both the control group and LSD-affected cattle from different locations. Among the LSD-affected animals, region-wise variation, with exceptionally high AST levels in Udaipur followed by, Shriganganagar, Jaipur and Bikaner. ALT activity was elevated predominantly in animals from Jaipur, followed by Shriganganagar, Bikaner and Udaipur regions.

Levels of AST and ALT were significantly ($p<0.05$) elevated in LSD affected cases compared to healthy control group (Group-II) highlighted the influence of disease severity, management practices and environmental stressors on biochemical responses. The lowest mean albumin levels were recorded in Udaipur, followed by Shriganganagar, Jaipur and Bikaner suggested impaired hepatic synthetic function, increased vascular permeability and enhanced protein loss due to systemic inflammation. Alkaline phosphatase activity showed moderate elevation without significant regional differences, indicating possible involvement of hepatobiliary tissues and bone metabolism, though its diagnostic sensitivity appeared limited compared to transaminases. Mild to moderate elevation in serum creatinine, especially in older and Holstein Friesian cattle, pointed towards renal involvement or dehydration secondary to prolonged illness. Overall, the findings of the present study confirm that Lumpy Skin Disease induces characteristic biochemical alterations involving hepatic, muscular, renal and immune systems.

The present study elucidates significant serum biochemical alterations in cattle naturally infected with lumpy skin disease virus (LSDV), reflecting the multisystemic nature of the disease. Marked elevation of hepatic and muscle-associated enzymes, along with

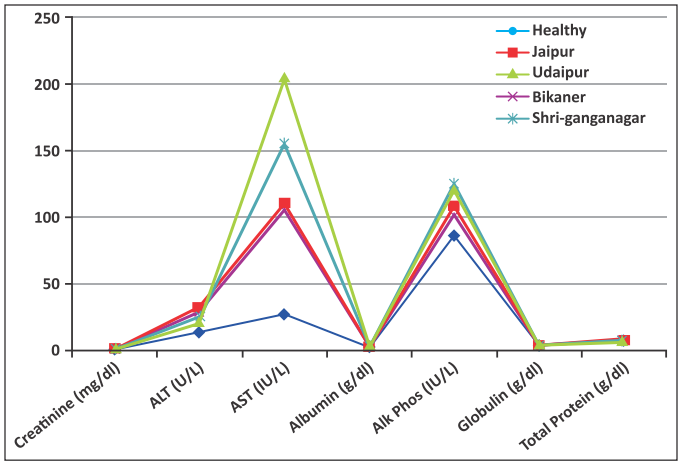

Fig. 1. Graph showing level of Serum biochemical parameters in the control group and Lumpy skin disease affected cases of various places.

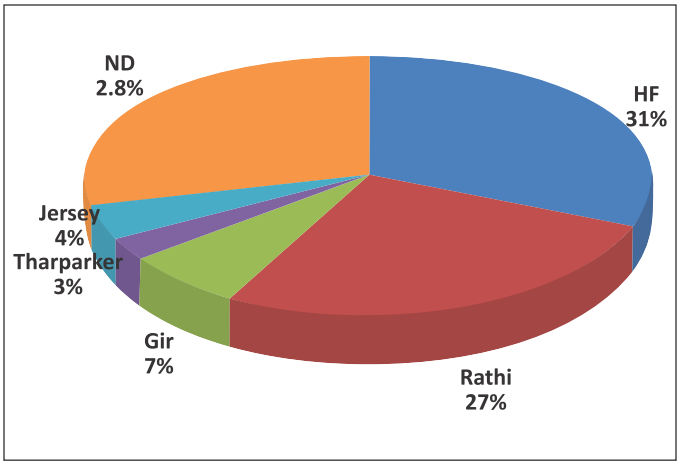

Fig. 2. Breed-wise distribution of infection of LSDV in Cattle during Year 2023-24.

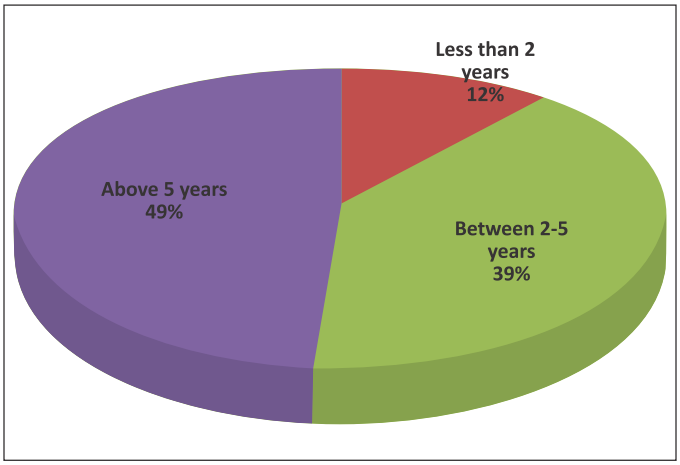

Fig. 3. Age-wise distribution of infection of LSDV in Cattle during Year 2023-24.

alterations in protein fractions and mild renal stress, highlights the extensive metabolic and inflammatory impact of LSDV infection. Significant elevation of alanine aminotransferase (ALT) in LSD-affected cattle, particularly in animals from Jaipur and in Holstein Friesian cattle, indicates hepatocellular injury associated with viral replication, hypoxia, and inflammatory mediator release. The progressive increase in ALT activity with advancing age further suggests diminished hepatic regenerative capacity and increased susceptibility to liver injury in older animals. Similar ALT elevations in LSD have been reported earlier and are attributed to hepatocellular degeneration and leakage of intracellular enzymes into circulation during active infection (Kaneko *et al.*, 2008; OIE, 2019; Elhaig *et al.*, 2019).

Aspartate aminotransferase (AST) exhibited the most pronounced elevation among all biochemical parameters, with significantly higher values observed in severely affected animals, particularly from Udaipur. Elevated AST likely reflects extensive muscle involvement due to nodular lesions, necrotizing dermatitis and myositis, in addition to hepatic damage. Higher AST levels in older cattle and Holstein Friesian breed further support the role of disease severity, muscle damage, and systemic inflammation in enzyme elevation. These findings are consistent with earlier reports describing AST as a sensitive indicator of muscular and hepatic involvement in LSD (Tuppurainen *et al.*, 2017; Zeedan *et al.*, 2019).

Alkaline phosphatase (ALP) activity showed mild to moderate elevation without consistent statistical significance, suggesting limited involvement of biliary tissues in LSD pathogenesis. The relatively stable ALP levels observed across age groups and breeds align with previous studies indicating that ALP alterations in LSD are usually mild and non-specific, reflecting secondary hepatic congestion or steroid-induced metabolic changes rather than primary cholestasis (Ali *et al.*, 2022; El-Kenawy *et al.*, 2018).

Hypoalbuminemia was a consistent biochemical abnormality observed across regions, breeds and age groups, particularly in older cattle. Reduced albumin levels likely result from impaired hepatic synthesis, negative acute-phase response, decreased feed intake, and increased vascular permeability during systemic inflammation. These findings corroborate earlier observations from LSD outbreaks in India and Africa, where hypoalbuminemia was associated with disease severity and prolonged clinical course (Abutarbush, 2015; Gamal *et al.*, 2020).

Conversely, globulin concentrations were markedly elevated in many LSD-affected cattle, particularly in adult animals, indicating strong humoral immune activation and prolonged antigenic stimulation. The concurrent increase in total protein levels and reversal of the albumin-to-globulin ratio further supports the presence of chronic inflammatory processes. Similar hyperglobulinemia and elevated total protein levels have been reported in LSD-positive cattle and are attributed to increased immuno-globulin synthesis

Table 1. Mean±SE of Serum biochemical values in the control group and Lumpy skin disease affected cattle of various places.

	HEALTHY (N= 20)	Jaipur region (N= 18)	Udaipur region (N= 22)	Bikaner region (N= 25)	Shriganganagar region (N=27)	Significance
Creatinine (mg/dl)	1.236 ± 0.082	1.653 ± 0.163	1.377 ± 0.116	1.623 ± 0.114	1.544 ± 0.131	NS
ALT (IU/L)	13.877^{a} ± 2.244	32.519^{c} ± 3.397	20.686^{ab} ± 3.032	29.348^{bc} ± 2.461	25.478^{bc} ± 3.043	**
AST (IU/L)	26.832^{a} ± 3.895	110.863^{b} ± 25.01	204.632^{c} ± 44.468	105.985^{ab} ± 15.107	155.467^{bc} ± 25.329	**
Albumin (g/dl)	2.909^{bc} ± 0.15	3.074^{c} ± 0.129	2.425^{a} ± 0.092	3.096^{c} ± 0.087	2.683^{ab} ± 0.112	**
Alk Phos (IU/L)	87.041 ± 10.578	108.419 ± 15.645	120.941 ± 12.399	102.056 ± 11.151	124.922 ± 12.004	NS
Globulin(g/dl)	3.672 ± 0.632	4.668 ± 0.82	4.402 ± 0.37	5.456 ± 0.639	4.728 ± 0.398	NS
Total Protein(g/dl)	6.64 ± 0.639	7.741 ± 0.846	6.826 ± 0.37	8.552 ± 0.655	7.411 ± 0.396	NS

Note: Means bearing different superscripts in same row differ significantly ($p \leq 0.05$). N : Number of samples.

during sustained viral infection (El-Nahas *et al.*, 2017). Serum creatinine concentrations showed mild, non-significant elevation in affected animals, particularly in Holstein Friesian and older cattle, suggesting transient renal stress rather than overt renal dysfunction. These changes are likely secondary to dehydration, fever, and systemic inflammatory response rather than direct viral nephropathy. Comparable observations have been reported in previous studies, indicating that renal function is generally preserved in LSD, except in severe or complicated cases (Sevik & Dogan, 2017; Sudhakar *et al.*, 2020).

CONCLUSION

The present study highlights the biochemical alterations in cattle affected by Lumpy Skin Disease (LSD), revealing significant increases in serum AST and ALT levels and a notable reduction in albumin levels. These changes indicate hepatic involvement and possible muscle damage in LSD-affected animals. Among the affected cattle, older animals were the most susceptible. Additionally, crossbred cattle exhibited a higher susceptibility to LSD. This knowledge can contribute to improved disease management, early diagnosis and the development of effective control strategies to mitigate the economic losses associated with LSD.

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Research Article

HISTOCHEMICAL STUDIES OF THYMUS GLAND OF SHEEP IN PRE AND POSTNATAL AGE GROUPS

BHAGYALAKSHMI, J.*, BALASUNDARAM, K.[1], SRAVANTHI, M.[2], SUJANA, K.M.[3] and CHARITHA, V.G.[4]
Department of Veterinary Anatomy, NTR College of Veterinary Science, Gannavaram
[1]Department of Veterinary Anatomy, Veterinary College and Research Institute, Namakkal
[2]State Level Diagnostic Laboratory, [3]Department of Veterinary Anatomy, Sri Venkateswara Veterinary University, Tirupati
[4]Department of Veterinary Parasitology, College of Veterinary Science, Proddatur

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ABSTRACT

The present study investigates the distribution of key histochemical components in the thymus gland of sheep across various prenatal and postnatal age groups. In prenatal thymus, strong PAS positive reactions were observed in Hassall's corpuscles and blood vessels, while the capsule and trabeculae showed moderate PAS positive reaction, indicating presence of glycogen. The Hassall's corpuscle exhibited mild reaction for neutral mucopolysaccharides and strong reaction for acid mucopolysaccharides with combined Alcian blue-PAS technique in all prenatal age groups. The capsule, septa and parenchyma showed moderate to strong reaction for the presence of protein tyrosine in all prenatal age groups. The septa showed positive reaction for the presence of lipid deposits in all prenatal age groups. A strong activity in parenchyma of cortex, medulla and Hassall's corpuscle was noticed for the presence of enzyme alkaline and acid phosphatases in all prenatal age groups. In postnatal ages with combined Alcian blue-PAS staining a moderate neutral and acid mucopolysaccharide positive reaction was noticed in the Hassall's corpuscle of all postnatal age groups. The capsule and septa showed moderate PAS activity for the presence of glycogen. Capsule and septa showed weak reaction whereas Hassall's corpuscles showed moderate reaction for the presence of tyrosine in all postnatal age groups. Parenchyma of thymus showed mild reaction for lipids whereas strong lipid activity was noticed in the interlobular septa in all postnatal age groups. Both acid phosphatase and alkaline phosphatase showed mild reaction in the stroma however, a strong reaction was noticed in the parenchymal region in all postnatal age groups.

Keywords: Histochemistry, Postnatal, Prenatal, Sheep, Thymus

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Sheep serve as a vital livestock species in global agriculture, contributing substantially to the rural economy through the production of meat, milk and wool (Bhagyalakshmi *et al.*, 2024). Owing to their well-characterized gestational timeline, immunological relevance and physiological similarity to other ruminants, sheep are extensively utilized as a model organism in developmental biology and comparative immunopathology studies (Bhagyalakshmi *et al.*, 2025). The Greek word "warty excrescence" originates from the word thymus, which resembles the flowers of the thyme plant. It is located in the superior mediastinum and anterior part of the inferior mediastinum. The thymus, being a retrosternal and well-protected organ, can be utilised for foetal age determination, highlighting its importance in accurately estimating foetal age. The thymus develops as a bilobed structure from the endoderm of the third pharyngeal pouch during the 6th week of embryonic life (Tamilselvan *et al.*, 2017). This endoderm interacts with neural crest cells, which become encapsulated within a capsule. Therefore, the gross features and histogenesis of the thymus can be used to estimate the gestational age of foetuses in cases of grossly mutilated foetal deaths (Sandhya *et al.*, 2023).

Size of the thymus grows rapidly during the embryonic life and pre-pubertal life, reaches its maximum absolute size about the time of puberty and then degenerates (Prabavathy, 2021). The lymphatic system performs a vital role in both normal and pathological processes. It contributes in the maintenance of normal tissue fluid balance, the immune functions such as cellular and antigen trafficking, the absorption of fatty acids and lipid-soluble vitamins in the gut (Elizabeth and Fredric, 2011). The thymus is the one of the primary lymphoid organs, which appear during embryonic development. The main function of the thymus is the production of 'T' lymphocytes, differentiate and assist in the clonal expansion of lymphocytes, which are necessary for the cell-mediated immunity thus recognized as a 'pacemaker' of lymphopoiesis. Thymus performs a vital part in the development of immune response in the foetal period. The distribution of the epithelial cells gives rise to the thymic epithelial reticulum, which becomes occupied by blood vessels from the contiguous mesenchyma. Lymphoblasts derived from the bone marrow stem cells invade the interstices, filling the spaces between epithelial cells. Therefore, the thymus also referred to as a lympho-epithelial organ. Thymus is essential for the growth of the peripheral lymphoid tissue and their associated adaptive immune function. The thymus determines the number of lymphocytes in the lymphatic organs and regulates the immunologic competence of animals. It is also important in the production of the

*Corresponding author: grace.vety@gmail.com

hormones (Prakash, 1988). The present study was undertaken to study the relative changes in histochemistry of thymus in different prenatal age groups of sheep foetuses.

MATERIALS AND METHODS

The present study was conducted on thirty-six sheep (prenatal and postnatal) from 0 to 150 days of prenatal sheep and 1month to 2 years and above of postnatal age groups. These animals were divided into six groups and each group containing six animals; Group-I (0 to 50 days), Group-II (51 to 100 days), Group-III (101 to 150 days), Group-IV Birth to 6 months (Pre-pubertal) and Group-V 6-8 months (Pubertal) and Group-VI 2 years and above (Adult). The lymphoid organ thymus of prenatal and postnatal age groups of sheep collected separately in thermos containing ice cubes from slaughterhouses in Andhra Pradesh and Tamil Nadu.

The approximate age of the prenatal age groups was estimated by obtaining the crown rump length (CRL) and substituting in the formula given below (Noakes, 2009).

$$A = 2.1(B+17)$$

Where 'A' is the developmental age of foetus in days and 'B' is crown rump length in centimetres.

In case of foetuses up to 3 cm CRL standard values given by Bryden *et al.* (1972a) were used for estimation of age in days. The postnatal age of sheep was determined based on the eruption of teeth as per Dyce *et al.* (1996). The foetuses of non-descript breed and irrespective of sex were collected from the pregnant sheep uteri immediately after slaughter. The body weight and CRL of foetuses were recorded. The foetuses were dissected by making a mid-ventral skin incision starting from the body of the mandible up to the inguinal region. The lymphoid organ, thymus was located and collected. The collected thymuses were rinsed in normal saline. The gross morphology such as colour, shape, location and topography was recorded and fixed in the various fixatives such as 10% neutral buffered formalin, chilled 10% neutral buffered formalin and Bouin's fluid.

For histochemical study, fresh tissue samples of the thymus were fixed in chilled 10% neutral buffered formalin. After fixation, thymic tissue samples of 1-2 mm thickness size were cut at 9 µm thickness of frozen sections were prepared at -20º C and stained with oil red O for fats, Gomori's cobalt and Napthol AS-BI method for Alkaline phosphatase, Gomori's lead nitrate method for Acid phosphatase. These cryostat frozen sections were then processed separately with Gomori's Cobalt and Napthol AS-BI method for demonstration of alkaline phosphatase, Gomori's modified lead nitrate method for acid phosphatase (Carleton and Drury, 1980) and Oil Red O method for lipids. These cryostat sections for histochemical studies of the samples were carry out at the State Level Diagnostic Laboratory, Sri Venkateswara Veterinary University, Tirupati.

Paraffin wax-embedded sections were prepared and stained with periodic acid Schiff's reagent (PAS) for glycogen, Million's reaction for protein tyrosine and Combined Alcian blue- PAS technique for acid and neutral mucopolysaccharides. The slides were observed under a compound microscope at magnifications of 40, 100, 400 and 1000 times for interpretation. Hence this study aims in determining the histochemical components of thymus gland at different gestational ages in foetuses and adult sheep.

RESULTS AND DISCUSSION

Prenatal thymus:

(a) Carbohydrate analysis:

In prenatal age groups the thymus of sheep, strong PAS positive reaction was observed in Hassall's corpuscles (Fig. 1a) and blood vessels whereas capsule and trabeculae showed moderate PAS positive reaction indicating the presence of glycogen. These results are in agreement with the reports by Yogesh (2007) in goat foetuses. The Hassall's corpuscle (Fig. 1b) showed mild reaction for neutral mucopolysaccharides and strong reaction for acid mucopolysaccharides with combined Alcian blue-PAS technique in all prenatal age groups (Table 1), which was in agreement with the reports of Yogesh (2007) in goat foetuses.

b) Protein dynamics:

The capsule, septa and parenchyma (Fig. 1c) showed moderate to strong reaction for the presence of tyrosine in all prenatal age groups (Table 1). The protein component was not noticed in the prenatal age group in any of the literature reviewed.

c) Lipid storage:

The septa (Fig. 1d) showed positive reaction for the presence of lipid deposits in all prenatal age groups (Table 1). Whereas Ramayya and Singh (2012) in buffalo foetuses and Geetha *et al.* (2016) in mice, rat and guinea pig reported that lipid content was less in prenatal age groups.

d) Enzymatic activities:

i) ***Alkaline phosphatase activity:***

A strong activity in parenchyma of cortex, medulla and Hassall's corpuscle was noticed for the presence of alkaline phosphatase (Fig. 2a) in all prenatal age groups whereas Ramayya

Table 1. Histochemical observations of thymus in prenatal age groups of sheep

Thymus	Prenatal age groups					
	PAS	Combined Alcian blue -PAS	Tyrosine	Lipid	Alkaline Phosphatase	Acid Phosphatase
Capsule	-	-	++ to +++	-	-	-
Septa	-	-	++ to +++	+	-	-
Cortex	-	-	++ to +++	-	+++	+++
Medulla	-	-	++ to +++	-	+++	+++
Hassall's Corpuscle	+++	+ to +++	++ to +++	-	+++	+++
Blood vessel	+++	-	++ to +++	-	-	-

+++ Strong; ++ Moderate; + Weak; - No reaction

Table 2. Histochemical observations of thymus in postnatal age groups of sheep

Thymus	Prenatal age groups					
	PAS	Combined Alcian blue -PAS	Tyrosine	Lipid	Alkaline Phosphatase	Acid Phosphatase
Capsule	++	-	+	-	+	+
Septa	++	-	+	-	+	+
Cortex	-	++	-	+	+++	+++
Medulla	-	++	-	+	+++	+++
Hassall's Corpuscle	-	++	++	+++	+++	+++

+++ Strong; ++ Moderate; + Weak; - No reaction

(2007) noticed weak activity in capsule and stroma in all prenatal age groups of buffalo foetuses. In contrast to the present results, Yogesh (2007) and Geetha *et al.* (2016) stated that there was no alkaline phosphatase activity in all prenatal age groups of goat and mice, rat and guinea pig, respectively.

ii) ***Acid phosphatase activity:***

The cortex, medulla and Hassall's corpuscle showed strong activity for the presence of acid phosphatases (Fig. 2b) in all prenatal age groups whereas Yogesh (2007) stated that the acid phosphatase activity was moderate to intense in the capsule, septa, Hassall's corpuscles and blood vessels. Whereas, moderate activity was found in cortex and medulla in all prenatal age groups of sheep. In contrast to the present results, Ramayya (2007) reported no acid phosphatase activity during prenatal period in buffalo foetuses. Acid phosphatase activity noticed in the present study was indication of lysosomal activity in the developing phase of thymus because of continuous tissue variation with age advanced. The moderate to strong acid phosphatase activity in thymus could be described to the release of lysosomal hydrolases, which might be associated with the cellular modifications occurring during histogenesis of thymus with the advancement of age (Yogesh, 2007).

Postnatal thymus:

a) Carbohydrate analysis:

In postnatal age groups, a moderate neutral and acid mucopolysaccharide reaction was noticed in the Hassall's corpuscle and parenchyma of all postnatal age groups (Fig. 3a). In accordance with the present observations, Ramayya and Singh (2012) reported strong to moderate PAS positive activity in Hassall's corpuscles during pre-pubertal age groups of buffalo calves (Table 2).

b) Protein dynamics:

The capsule and septa showed weak reaction whereas Hassall's corpuscles (Fig. 3b) showed moderate reaction for the presence of tyrosine in all postnatal age groups (Table 2). The protein content was not noticed in the postnatal age groups in any of the literature reviewed.

c) Lipid storage:

The parenchyma of thymus showed mild reaction for lipids whereas strong lipid activity was noticed in the interlobular septa (Fig. 3c) in all postnatal age groups (Table 2). In contrast to the present results, Ramayya and Singh (2012) observed no activity for

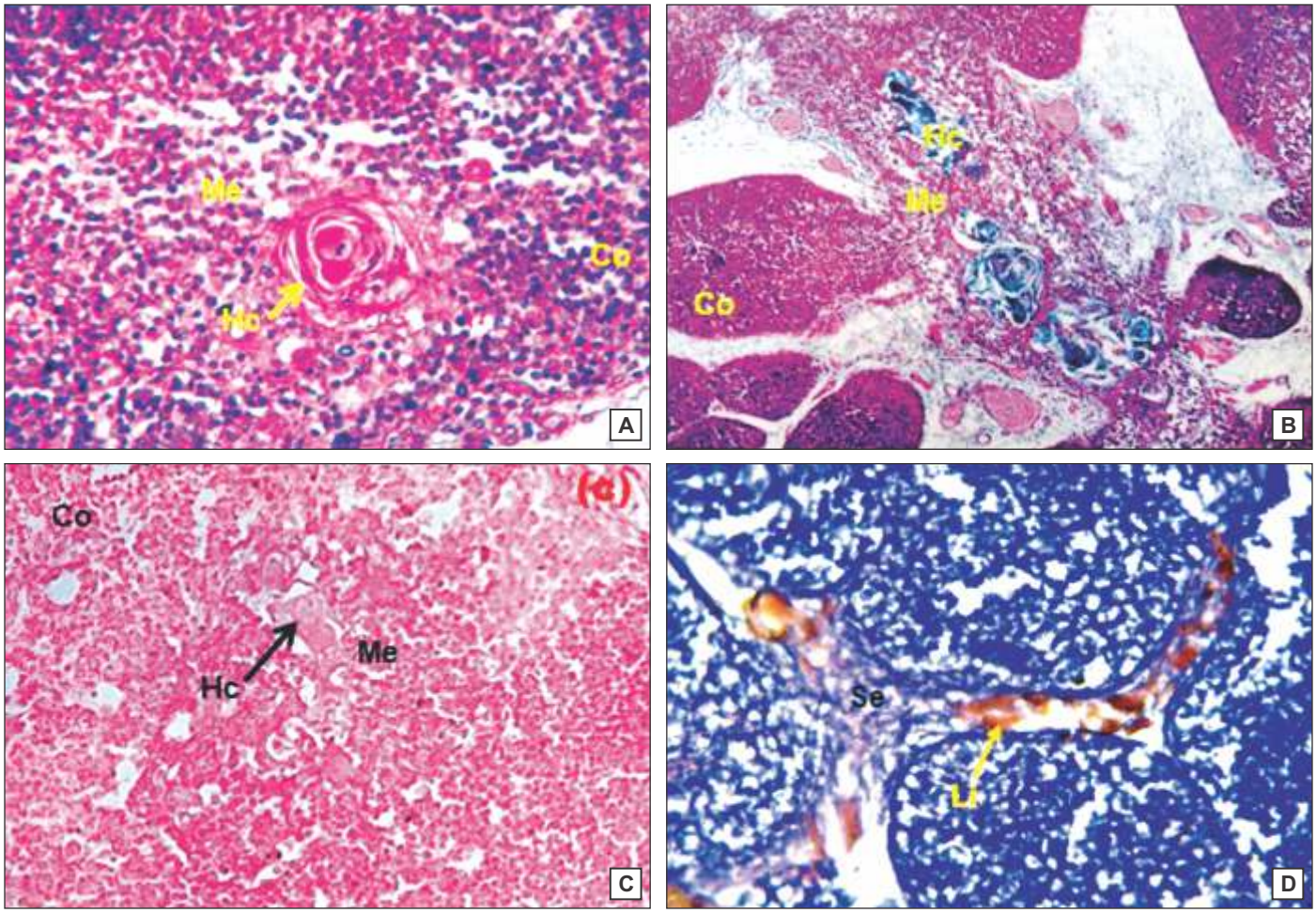

Fig. 1. **(a)** Photomicrograph showing PAS positive reaction in thymus of sheep foetus at 103 days of gestation (PAS×400). **(b)** Photomicrograph showing AB-PAS positive reaction in thymus of sheep foetus at 84 days of gestation (Combined Alcian blue-PAS×40). **(c)** Photomicrograph showing localization of tyrosine in thymus of sheep foetus at 126 days of gestation (Millon's reaction×400). **(d)** Photomicrograph showing lipid deposits in thymus of sheep foetus at 110 days of gestation (Oil Red O×400). [Hc- Hassall's corpuscle; Me- Medulla; Co- Cortex; Se- Septa; Li- Lipids].

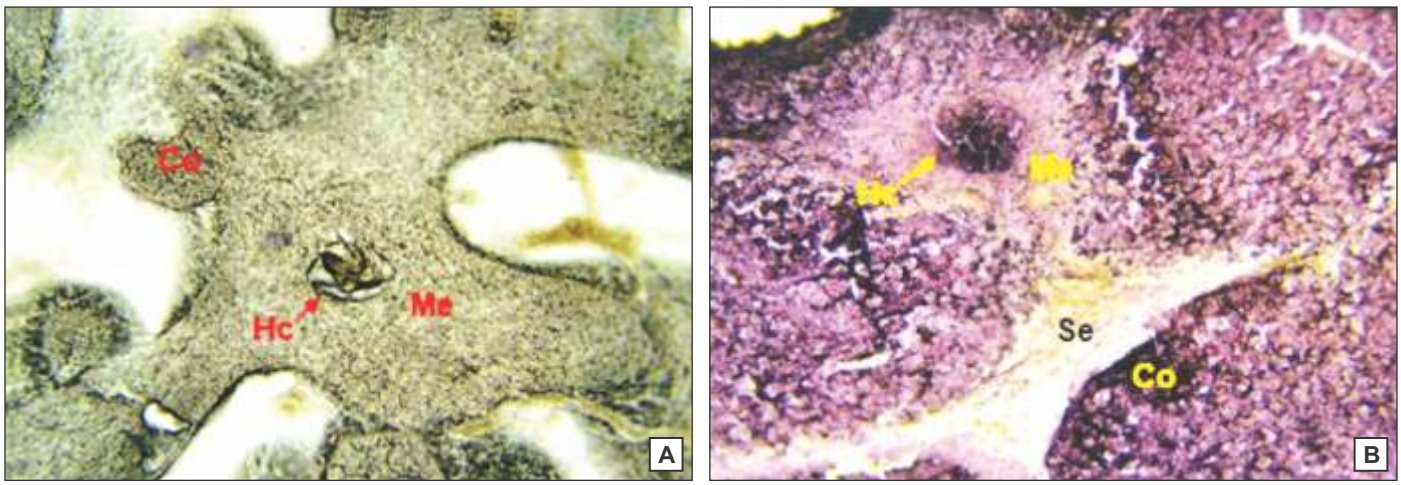

Fig. 2. **(a)** Photomicrograph showing alkaline phosphatase reaction in thymus of sheep foetus at 110 days of gestation (Gomori's cobalt ×100). **(b)** Photomicrograph showing acid phosphatase reaction in thymus of sheep foetus at 110 days of gestation (Gomori's lead×100). [Hc- Hassall's corpuscle; Me- Medulla; Co- Cortex; Se- Septa].

lipids in capsule of buffalo, but they have noticed lipids in reticular epithelial cells. According to Geetha *et al.* (2016), the accumulation of fats was more in the post-pubertal age groups when compared to prenatal and pre-pubertal age groups, which indicated the age-related involuntary changes

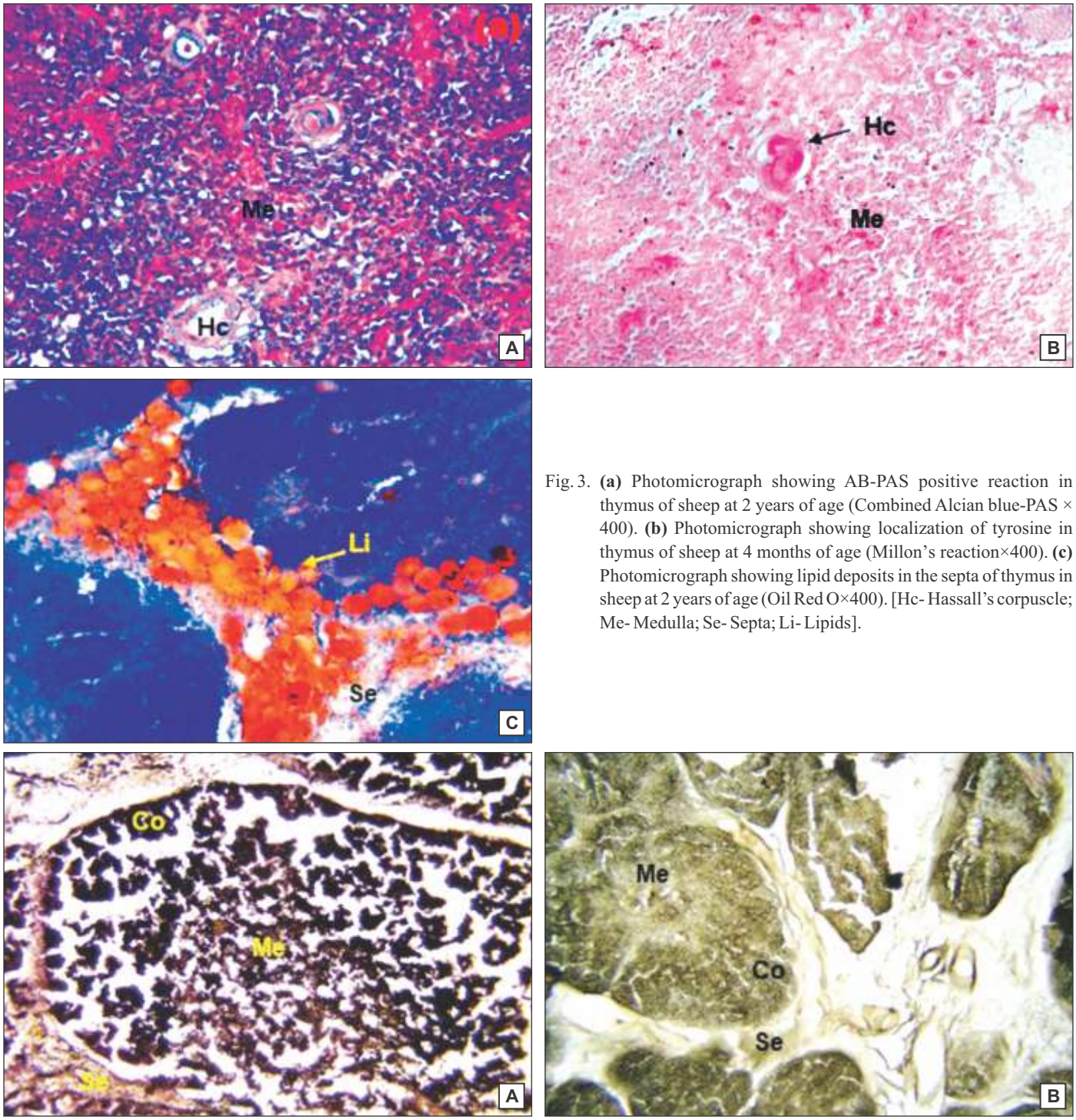

Fig. 3. **(a)** Photomicrograph showing AB-PAS positive reaction in thymus of sheep at 2 years of age (Combined Alcian blue-PAS × 400). **(b)** Photomicrograph showing localization of tyrosine in thymus of sheep at 4 months of age (Millon's reaction×400). **(c)** Photomicrograph showing lipid deposits in the septa of thymus in sheep at 2 years of age (Oil Red O×400). [Hc- Hassall's corpuscle; Me- Medulla; Se- Septa; Li- Lipids].

Fig. 4. **(a)** Photomicrograph showing acid phosphatase reaction in thymus of sheep at 7 months of age (Gomori's lead×100). **(b)** Photomicrograph showing alkaline phosphatase reaction in thymus of sheep at 7 months of age (Gomori's cobalt×100).

of thymus indicated by invasion of adipose tissue, loss of lymphocyte population and formation of small pits.

d) Enzymatic activities:

i) ***Alkaline phosphatase activity:***

The alkaline phosphatase showed mild reaction (Fig. 4a) in the stroma. However, a strong reaction was noticed in the parenchymal region in all postnatal age groups whereas Ramayya (2007) reported that the alkaline phosphatase activity was strong in cortex and medulla of buffalo calves during 1 week to 3 months and moderate in 6 to12 months of age.

ii) ***Acid phosphatase activity:***

The acid phosphatase showed mild reaction (Fig. 4b) in the stroma. However, a strong reaction

was noticed in the parenchymal region in all postnatal age groups (Table 2). In contrast to present results, Ramayya (2007) reported that there was no acid phosphatase activity in capsule, stroma and blood vessels during postnatal period.

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Research Article

MODELLING AND FORECASTING OF BREEDING EFFICIENCY IN HARIANA CATTLE

RAKSHIT*, VIJAY KUMAR, S.P. SINGH, JITENDRA KUMAR AGRAWAL and AMIT SINGH
Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry,
DUVASU, Mathura-281001, Uttar Pradesh, INDIA

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ABSTRACT

The present investigation was undertaken to predict the breeding efficiency by Wilcox method (BEW) using early life reproduction traits. Under this study, multiple linear regression (MLR) approach was used. Breeding data were obtained from history sheet registers and herd inventory records of Hariana cattle maintained at the Livestock Farm Complex, Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, DUVASU, Mathura. Information was collected on productive animals born between 1962 and 2024 with total 756 records. The overall least-squares mean for breeding efficiency (Wilcox method) estimated was 69.5±0.69 percent. Period of birth, AFC and parity had significant effect on breeding efficiency in the present study. The heritability estimates for BEW was 0.533. Breeding efficiency was predicted by multiple linear regression (MLR) analysis. In MLR study, it was observed that model 3 having only one independent variable (First Service Period) fulfilled most criteria such as higher R^2 and lower MSE, RMSE, MAE, MAPE and U values. The MLR explained 55.4% of accuracy of prediction of BEW in Hariana cattle. MLR method could be used for the prediction of BEW, which is a simple and easy method.

Keywords: AIC, BIC, Breeding efficiency, Hariana cattle, MLR

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Cattle hold immense significance in India's agricultural economy, culture, and everyday life. They play a crucial role in food production, rural livelihoods, and sustainable farming. As the world's largest producer of milk, India relies heavily on cattle for dairy products, with indigenous breeds such as Gir, Sahiwal, and Hariana supporting millions of dairy farmers. Small and marginal farmers depend on cattle for income, employment, and nutrition. In many rural areas, bullocks continue to be used for plowing, transportation and various farm activities, particularly where mechanization remains limited. Cattle dung serves as natural manure, enhancing soil fertility and reducing reliance on chemical fertilizers. It is also used for biogas production, offering an eco-friendly energy source. Cattle contribute to sustainable farming by improving soil health through natural fertilization. Additionally, in Hinduism, cows are considered sacred and hold a vital place in religious rituals. Milk and dairy products are an integral part of the Indian diet, providing essential nutrients like protein and calcium. Beyond milk production and farm labor, cattle are deeply embedded in India's rural economy, traditional agricultural practices and ecological balance. Cattle breeds in India are categorized based on their milk yield, with high-yielding breeds distinguished from low or medium-yielding ones. The Hariana breed, native to Haryana, is primarily found in the districts of Rohtak, Hisar, Jind and Gurgaon. These cattle have a white or light grey coat, a strong and muscular build, long legs and a small hump. While primarily used as draught animals in agriculture, Hariana cows produce a moderate amount of milk, approximately 5-8 liters per day under proper management. They are known for their resilience, disease resistance and adaptability to hot climates, making them highly valuable to farmers in northern India. Breeders have always aimed to evaluate the production potential of dairy animals. Since the economic viability of the dairy industry depends on the productive and reproductive performance of cattle, identifying effective selection criteria is essential. According to Bakir and Cilek (2009), cattle reproduction is the foundation of economic returns. Breeding efficiency directly impacts profitability. Negussie *et al.* (1998) emphasized that developing suitable breeding strategies requires an accurate assessment of the reproductive efficiency of native cattle under different production conditions. Since breeding efficiency is a lifetime trait, selecting cattle based on this factor alone is often delayed due to the age of the animal at the time of evaluation. Predicting breeding efficiency early, using reproductive traits, helps in identifying high-performing animals sooner. This approach also aids in the timely culling of less productive animals, reducing maintenance costs. According to Shetkar *et al.* (2021), lifetime productivity is a key factor in maximizing financial returns from an individual animal. Based on this understanding, research was conducted on Hariana cattle to develop a predictive model for breeding efficiency using multiple regression analysis.

MATERIALS AND METHODS

In the present study breeding data were obtained from history sheet registers and herd inventory records of

*Corresponding author: rakshitvashisht99@gmail.com

Hariana cattle maintained at the Livestock Farm Complex, Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, DUVASU, Mathura (UP). Information was collected on productive animals born between 1962 and 2024, totaling 756 records. To assess breeding efficiency, outliers were removed and only data within the mean ± 3SD range were considered to ensure a normal distribution.

Breeding efficiency was estimated by formula given by Wilcox *et al.* (1957).

$$BE = \frac{365 \times (N-1) \times 100}{D}$$

Where,

B.E. = Breeding efficiency in percentage

N = number of calving

D = Number of days from first to last calving

Means, standard deviations, standard errors and coefficients of variations of the BE were calculated according to the statistical method given by Snedecor and Cochran (1994).

The non-genetic factor effects such as period of birth, season of birth, AFC age group (lower and higher AFC age group based in individual value lower or higher than population average) and parity (class 1 with 2-3 parity; class 2 with 4-6 parity; and class 3 with 7 or more parity) of various traits were studied by least-squares analysis using the technique suggested by Harvey (1987). The models used were as follows:

$$Y_{ijklm} = \mu + P_i + S_j + A_k + L_l + e_{ijklm}$$

Where,

Y_{ijklm} - m^{th} observation in l^{th} parity, k^{th} age group, j^{th} season and i^{th} period of calving

μ - Overall mean

P_i - Effect of i^{th} period of birth (i = 1 to 6)

S_j - Effect of j^{th} season of birth (j = 1 to 4)

A_k - Effect of k^{th} AFC age group (k = 1 to 2)

L_l - Effect of l^{th} parity (l = 1 to 3)

e_{ijklm} - Random error ~NID $(0, \sigma^2_e)$

Heritability was estimated by paternal half-sib (PHS) correlation method as suggested by Becker (1975). Correlation of breeding efficiency with first service period and first dry period was estimated as suggested by Becker (1975).

The Variance Inflation Factor (VIF) tool is used to measure and quantify the extent of variance inflation. When predictors are correlated, the coefficient of determination becomes biased, leading to an increase in the standard error of predictor coefficients. As a result, the variance of predictor coefficients is inflated (Kumar *et al.*, 2019). VIF can be determined using the following formula:

$$VIF = \frac{1}{1-R^2_i}$$

Where R^2_i is the R^2– value obtained by regressing the ith predictor on the remaining predictors. A variance inflation factor exists for each of the i predictors in a multiple regression model.

The multiple regression analysis was done as suggested by Draper and Smith (1987) with the help of following model:

$$Y_i = a + b_1 X_1 + b_2 X_2 + \ldots + b_n X_n + e_i$$

Where,

Y_i - Variable to be predicted

a, b_1, b_2, …, b_n - Unknown parameters to be estimated.

$X_1, X_2, \ldots, X_n$ - Traits whose values are known.

e_i - Random error, NID $(0, \sigma^2_e)$

The coefficient of determination (R^2) represents the percentage of total variability in the prediction that is explained by a set of independent variables. It is calculated through analysis of variance and varies across different models.

Various statistical metrics, including Akaike information criterion (AIC), Bayesian Information Criterion (BIC), Mallow's Conceptual Predictive Value (MCp), coefficients of determination (R^2), mean absolute error (MAE), mean absolute percentage error (MAPE), Theil's U-statistic (U) and root mean square error (RMSE), were used to identify the optimal model(s) for predicting BEW in Hariana cattle. The best models were those with higher coefficients of determination (R^2) and lower values of RMSE, MAE, MAPE, BIC, and RMSE. Additionally, models developed using artificial neural networks (ANN) were compared with the best multiple regression analysis (MLR) model. According to Kumar *et al.* (2019), the primary goal of model development is to predict the response variable using predictor variables. Since model development is research-intensive and time-consuming, evaluating a model's predictive capability is crucial. Therefore, an ideal model should be both simple and highly accurate in its predictions.

RESULTS AND DISCUSSION

Information was collected on productive animals born between 1962 and 2024, totaling 756 records. After

Table 1. Least-squares means and standard error of breeding efficiency in Hariana cattle

Effects	BEW (%)
Overall mean (μ)	69.5 ± 0.69 (738)
Season of Birth	
Winter	69.2 ± 0.83 (365)
Summer	68.4 ± 1.20 (159)
Rainy	71.5 ± 1.59 (87)
Autumn	68.9 ± 1.33 (127)
Period of Birth	
1962-1970	69.0 ± 1.37^{a} (139)**
1971-1980	71.3 ± 1.20^{b} (155)
1981-1990	68.4 ± 1.21^{b} (171)
1991-2000	55.7 ± 1.21^{b} (167)
2001-2010	70.7 ± 1.98^{b} (57)
2011-2018	81.9 ± 2.13^{c} (49)
AFC Class	
Lower AFC Class	71.1 ± 0.88^{a} (404)**
Higher AFC Class	67.9 ± 0.95^{b} (334)
Parity Groups	
2-3	64.1 ± 0.96^{a} (270)**
4-6	67.9 ± 0.94^{b} (314)
>7	76.5 ± 1.26^{c} (154)

Winter: December to march, Summer: April to June, Rainy: July to August, Autumn: September to November. Similar/dissimilar superscript indicates the non-significant/significant differences between subclasses of means. Figures in parentheses are the number of observations. **Level of significance (P<0.01)

Table 2. VIF value for Breeding Efficiency by Tomar

Trait	VIF value
Age at first calving	1.005
First service period	2.262
First dry period	2.254

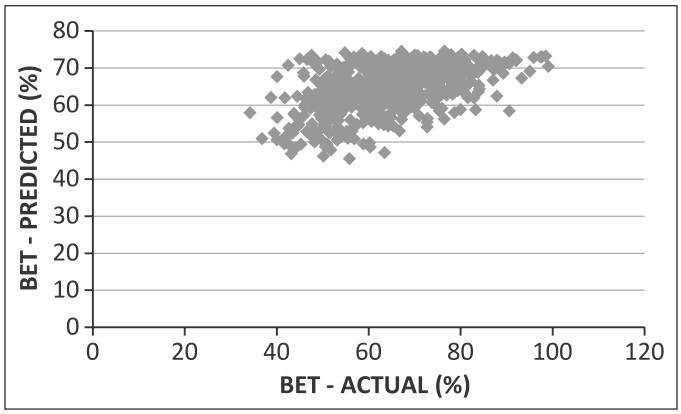

Fig. 1. Regression of predicted on the actual BEW using MRA model 3 in Hariana cattle

normalization, outliers were removed to estimate overall mean for breeding efficiency (Wilcox method). BEW was 66.3% with standard error and standard deviation of 0.61 and 16.9, respectively. The coefficient of variation estimated for breeding efficiency (Wilcox method) was 25.49. The higher average of breeding efficiency (Wilcox method) were reported by Dhangar and Patel (1991) in Jersey×Kankrej (JK) F1 cross, Shelke *et al.* (1992) in Jersey ×Red Kandhari half bred and Red Kandhari, respectively. Panwer *et al.* (1996) in Rathi, Habib *et al.* (2013) in Red Chittagong Cattle, Singh *et al.* (2016) in different Crossbred cattle and Barros *et al.* (2020) in Red Sindhi cattle also reported the higher mean of breeding efficiency (Wilcox method). Ebrahim (2018) in Friesian cattle estimated lower mean of breeding efficiency (Wilcox method) in Friesian cattle.

Table 3. Prediction equation for breeding efficiency (Wilcox)

S. No.	Dependent variable	Independent variables	R^2 (%)	Linear regression equation's
1.	Breeding	AFC	0.3	BE (W) = 70.609 -0.0022AFC
2.	efficiency	FDP	32.4	BE (W) = 81.998 – 0.0494FDP
3.	(Wilcox)	FSP	55.4	BE (W) = 88.134 – 0.0654FSP
4.		AFC, FDP	32.5	BE (W) = 85.511 - 0.00152AFC – 0.04933FDP
5.		AFC, FSP	55.4	BE (W) = 88.460 – 0.0002AFC – 0.0654FSP
6.		FDP, FSP	55.4	BE (W) = 88.305 – 0.0633FDP - 0.002FSP
7.		AFC, FDP, FSP	55.4	BE (W) = 88.673 – 0.0002AFC – 0.063FSP – 0.002FDP

Table 4. Estimation of intercept and regression coefficient(s) for breeding efficiency (Wilcox) in Hariana cattle

Model	Variable	A	AFC	FSP	FDP	RMSE	R^2	MAE	MAPE	U
1.	AFC	70.609	-0.002	-	-	16.51	0.3	13.67	22.84	0.239
2.	FDP	81.998	-	-	-0.049	13.59	32.4	10.92	17.68	0.197
3.	FSP	88.134	-	-0.065	-	11.04	55.4	8.90	14.47	0.160
4.	AFC, FDP	85.511	-0.001	-	-0.049	13.58	32.5	10.89	17.61	0.197
5.	AFC, FSP	88.460	-0.0002	-0.065	-	11.04	55.4	8.92	14.46	0.160
6.	FDP, FSP	88.305	-	-0.063	-0.002	11.04	55.4	8.92	14.45	0.160
7.	AFC, FDP, FSP	88.673	-0.0002	-0.063	-0.002	11.05	55.4	9.00	14.44	0.160

Least-Squares Analysis

The overall least-squares mean for breeding efficiency (Wilcox method) estimated was 69.5±0.69 percent (Table 1). In the present study it was observed that the season of birth had non-significant effect on breeding efficiency of the animal with least-squares mean of breeding efficiency 69.2 ± 0.83 percent in winters, 68.4 ± 1.20 percent in summer, 71.5 ± 1.59 percent in rainy season and 68.9 ± 1.33 percent in autumn season. Period of birth had highly significant effect on breeding efficiency in the present study, the animals born during 2011-2018 period had highest breeding efficiency with least-squares mean of 81.9 ± 2.13 percent and those born during the period of 1962-1970, 1971-1980, 1981-1990, 1991-2000 and 2001-2010 with observed least-squares means of 69.0±1.37, 71.3 ± 1.20, 68.4 ± 1.21, 55.7±1.21 and 70.7±1.98 percent, respectively. In this study age at first calving had highly-significant effect on breeding efficiency in Hariana cattle. Animals with Lower AFC were observed higher breeding efficiency of 71.1±0.88 percent as compared to higher AFC animals with least-squares mean of breeding efficiency 67.9 ± 0.95 percent. In the present study parity of animals had highly significant effect on breeding efficiency of animals. Cows with more than 7 parity had higher breeding efficiency of 76.5±1.26 percent as compared to the animals with 2 to 3 parity and 4 to 6 parity with least-squares mean of breeding efficiency 64.1 ± 0.96 and 67.9 ± 0.94 percent, respectively.

Singh *et al.* (2016) in crossbred, Dash *et al.* (2017) in Karan Fries, Ambhore *et al.* (2017) in Phule Triveni, Tomar *et al.* (2022) in Hariana reported higher estimates for least-squares mean of breeding efficiency (Wilcox method). Significant effect of period on breeding efficiency was reported by Singh *et al.* (2016), Dash *et al.* (2017), Ambhore *et al.* (2017) in Crossbred cattle, Karan Fries cattle and Phule Triveni cattle, respectively. Season of birth had significant effect on breeding efficiency in the studies reported by Singh *et al.* (2016) in Crossbred cattle.

Heritability and correlation

Precise approximations of the heritability of different reproductive traits are crucial for evaluating the improvement of different traits and designing the future breeding and selection plans. The heritability estimates for BEW was high 0.533. The breeding efficiency had genetic correlation of 0.134±0.198 with age at first calving. The breeding efficiency had negative genetic correlations with first service period, first dry period with estimates as -0.666±0.561, -0.946 ± 0.702, respectively. The breeding efficiency (Wilcox) had positive phenotypic correlations with age at first calving with estimate as 0.047. Breeding efficiency (Wilcox) had negative phenotypic correlations with first service period and first dry period with estimated as -0.684 and -0.451. The breeding efficiency (Wilcox) had positive environmental correlations with age at first calving with estimate as 0.248. Breeding efficiency (Wilcox) had negative environmental correlations with first service period and first dry period with estimate as -0.747 and -0.275.

Shetkar (2021) estimated similar heritability for breeding efficiency in Hariana cattle. Lower estimates for heritability of breeding efficiency was observed Tekerli and Kocak (2009) in HF cattle. Tomar (2022) in Hariana cattle reported the similar positive genetic correlation with AFC and negative genetic correlation with first service period and first dry period in Hariana cattle. Tomar (2022) in Hariana cattle reported the similar positive phenotypic correlation between AFC and BEW. Tomar (2022) in Hariana cattle also reported negative phenotypic correlation between BEW with FSP and FDP.

Prediction model

To determine the correlations between the independent traits in the study of prediction, the variance inflation factor (VIF) was employed. The lower VIF values for AFC, FSP and FDP were observed which means that these traits were not correlated (Table 2). Multiple regression analysis was carried out for the prediction of BEW. First lactation production and reproduction traits were taken as independent variables viz. AFC, FSP and FDP. Prediction equations for prediction of BEW from early reproductive traits are presented in the Table 3. The best model for the lifetime BEW were selected based on their higher co-efficient of determination (R^2) value and lower RMSE, MAE, MAPE and Theil's U-statistic values. AIC, BIC and MCp values were also estimated for various model for interpretation of results and get more accurate prediction.

Development of MLR model for breeding efficiency

To predict the breeding efficiency (Wilcox) reproduction traits like AFC, FDP and FSP were used as independent variable for multiple regression analysis method. The model for the prediction of breeding efficiency (Wilcox) using reproduction traits are represented in Table 3.

The developed models were in different combinations ofAFC, FDP and FSP. Out of 7 models developed model 3 having independent variable first service period was best for prediction of BEW. Although few models had similar coefficient of determination but model 3 using only one independent variable so will be more useful. Hence model 3 with coefficient of determination (R^2) 55.4% and lower

RMSE, MAE, MAPE, U-statistic values has fulfilled the criterions. AIC (Akaike information criterion), BIC (Bayesian Information Criterion) and MCp (Mallow's Conceptual Predictive Value) values for model 3 were -1973.905, -496.27 and 2.001, respectively. BIC value for Model 3 is lowest as compared to other models. The linear regression equation developed is given below.

$$BE(W) = 88.134 - 0.0654FSP$$

The R^2 value of prediction was relatively high suggesting that the relationship between the predictors and the response variable is linear. The regression of predicted on actual breeding efficiency (Wilcox) using MRA model 3 is represented in Fig. 1.

Step wise regression procedure was used to predict the breeding efficiency (BEW) from first lactation traits viz. AFC, FDP and FSP under the present study. On analysis the co-efficient of determinations of 0.3%, 32.4%, 55.4%, 32.5%, 55.4%, 55.4% and 55.4% for BEW were observed when AFC, FDP, FSP, AFC-FDP, AFC-FSP, FDP-FSP and AFC-FDP-FSP were used as independent variables, respectively. The model with FSP (R^2 = 55.4%) as independent trait has been selected as optimum model to predict the breeding efficiency (Wilcox method). The model using AFC as independent variable had very low co-efficient of determination (R^2 value) of 0.3%. Model using FDP for predicting BEW the R^2 was observed as 32.4 percent only. Results clearly depicted that the AFC and FDP didn't influence BEW as that of FSP alone. In this study the FSP had a more influential effect on animals BEW as compared to different combinations of independent variables. Tomar *et al.* (2023) predicted BEW with lower co-efficient of determination of 36.3%. Gahlot (2003) predicted life time milk production in Tharparkar cattle with higher co-efficient of determination of 74.48%. Shetkar *et al.* (2023) predicted the lifetime II milk yield with higher co-efficient of determination of 75.46%. Second lactation milk yield was predicted by Das *et al.* (2022) with higher co-efficient of determination of 83.7 percent in Deoni cattle. Dangar and Vataliya (2022) predicted life time milk yield with higher coefficient of determination of 98% in Gir cattle.

CONCLUSIONS

The present study found a significant difference in breeding efficiency based on periods of birth, AFC class, and parity. This suggests that the breeding efficiency of Hariana cattle can be enhanced through optimal management practices. Multiple regression analysis (MRA) was used to predict BEW, with the best models selected based on higher coefficients of determination (R^2) and lower values of AIC, BIC, RMSE, MAE, MAPE, and Theil's U-statistic. The analysis considered various first lactation reproductive traits as independent variables, while breeding efficiency served as the dependent variable. The optimal model for BEW included the first service period as an independent variable and was expressed as BE(W)= 88.134-0.0654FSP, with an R^2 of 55.4%. The MLR method proved to be a simple and effective approach for predicting BEW.

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Research Article

DETECTION AND PATHOTYPE ANALYSIS OF SHIGA TOXIN-PRODUCING *E. COLI* (STEC) SEROGROUPS: O26, O111 AND O157

BHUVA AKASH, VIBHA SINGH, MANOJ S., BHAVIN KATIRA, SANJUMON E.S., Z.B. DUBAL[1], VINODH KUMAR O.R. and M. SUMAN KUMAR*
ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, Uttar Pradesh,
[1]ICAR-National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru-560064, Karnataka

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ABSTRACT

Escherichia coli is a ubiquitous inhabitant of the gut microbiome in humans, animals and birds. The majority of *E. coli* strains maintain a harmless presence, however, certain high-risk serogroups such as O26, O111 and O157 acquire specialized virulence factors that enable them to cause a spectrum of clinical syndromes. In this study, we employed a PCR-based serogrouping approach to detect and characterize three major key O-serogroups (O26, O111 and O157) among *E. coli* isolates recovered from 341 diverse samples collected from cow and buffalo milk, rectal swabs of cows, buffaloes and goats as well as poultry cloacal swabs from Sambhal district of Uttar Pradesh, India. Initial biochemical screening was followed by molecular confirmation using *uid*A and *cyd*A gene targets to identify *Escherichia coli*. The isolation rate of *E. coli* was 73.02% (256/351). Subsequent PCR analysis revealed five O26, two O111 and one O157 isolates, corresponding to prevalence rates of 2.0%, 0.8%, and 0.4%, respectively. Notably, all the serogroup-positive isolates were Shiga toxin-producing *E. coli* (STEC), with various combinations of *stx*1, *stx*2 and *hly*A gene presence. Distinct antimicrobial resistance profiles were observed among the isolates, with universal susceptibility to amikacin and chloramphenicol, higher resistance to Ampicillin, Ceftazidime and Ceftriaxone and resistance to Imipenem amongst O157 isolates, highlighting that resistance traits are not solely determined by serotype or pathotype. The data provides critical insights into the occurrence of highly virulent STEC serogroups in livestock and poultry, emphasizing their potential impact on food safety and public health.

Keywords: *Escherichia coli*, Molecular serogrouping, O26, O111, O157, Pathotypes

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Escherichia coli, a key member of the *Enterobacteriaceae* family, is a common inhabitant of the gastrointestinal tracts of humans, animals and birds, as well as in environmental reservoirs. Although most *E. coli* strains exist harmlessly as commensals, certain clones have acquired unique virulence factors that enable them to cause a wide range of diseases. Pathogenic strains of *E. coli* are implicated in diverse clinical syndromes, including gastrointestinal infections that cause diarrhea, urinary tract infections, and severe systemic illnesses such as sepsis and meningitis. Shiga toxin-producing *E. coli* (STEC) are especially concerning on a global scale, being major foodborne pathogens responsible for outbreaks ranging from watery diarrhea and hemorrhagic colitis to life-threatening hemolytic-uremic syndrome (HUS). Historically, *E. coli* O157:H7 has been identified as the most important STEC serotype, accounting for a significant proportion of infections and fatalities (Scallan *et al.*, 2011). However, non-O157 serogroups such as O26, O45, O103, O111, O121 and O145 are emerging as major contributors to illness. Ruminants are the primary reservoirs, with additional evidence pointing to wild animals as carriers. India is a country of immense geographical diversity, data on *E. coli* serogroups and their pathogenic profiles remain limited. This study aims to assess the prevalence of O26, O111, and O157 serogroups in *E. coli* isolates from diverse sources, pathotyping and antimicrobial resistance profiling to inform effective public health interventions.

MATERIALS AND METHODS

A total of 341 samples comprising 65 cow milk samples, 64 buffalo milk samples, 66 cow rectal swabs, 64 buffalo rectal swabs, 33 goat rectal swabs and 49 poultry cloacal swabs were randomly collected from Sambhal district of Uttar Pradesh, India.

Milk samples were centrifuged at 3500 rpm for 10 minutes and the pellet was inoculated into MacConkey broth at 37° C for 18-24 hours, rectal and cloacal swabs were also inoculated into MacConkey broth under similar conditions. After incubation, broth was streaked onto MacConkey Lactose Agar plates from which the selected colonies were streaked onto eosin methylene blue (EMB) agar and incubated for 24 hours. After incubation plates were examined, typical *E. coli*-like colonies were selected and screened for the IMViC tests. The cultures giving typical (+, +, −, −) reaction were confirmed as *E. coli*. Further molecular conformation was done by targeting *uid*A and *cyd*A gene. A total of 249 isolates were confirmed as *E. coli* through phenotypic and molecular methods. The confirmed isolates were screened for the presence of the O26, O111 and O157 serogroups using PCR. Following molecular confirmation of these O serogroups, all positive isolates were further screened by PCR for the presence of

*Corresponding author: sumanvph@gmail.com

pathotypes (STEC, EPEC, ETEC and EAEC) to identify the serogroups-pathotype relationship. The primers, their sequences and the annealing temperatures are listed in Table 1.

Each PCR reaction was performed in a 200 µL Eppendorf tube with a total reaction volume of 25 µL. The reaction mixture consisted of 12.5 µL of 2X master mixture (containing dNTPs, Taq DNA polymerase, along with reaction buffer 1.5 mM $MgCl_2$) and 5 to 10 pmol of both forward and reverse primers. Additionally, 4 µL of DNA template was added and the volume was adjusted to 25 µL using nuclease-free water. The PCR cycling conditions for gene amplification were: denaturation at 94° C for 5 minutes, followed by 30 cycles of denaturation at 94° C for 30 seconds, annealing at the temperature specified for each primer (as listed in Table 1) and extension at 72° C for 45 seconds. A final extension was carried out at 72° C for 5 minutes. To prevent contamination, a non-template control (NTC) was included in each PCR run. PCR products (8 µL) were separated by electrophoresis on 1.5% agarose gels in 1X Tris-acetate-EDTA (TAE) buffer. The gels were stained with ethidium bromide (1 mg/mL) and visualized under UV light.

In addition, all verified O26, O111 and O157 strains were subjected to antimicrobial susceptibility testing via the disc diffusion method, following the protocols established by the Clinical and Laboratory Standards Institute (CLSI). For anti-microbial susceptibility pattern following antibiotics including ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), ceftriaxone (30 µg), cefpodoxime (10 µg), aztreonam (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftazidime (30 µg, amikacin (30 µg), chloramphenicol (30 µg), enrofloxacin (5 µg), imipenem (10 µg), nalidixic acid (30 µg), sulphamethoxazole-trimethoprim (1.25-23.75 µg) and tetracycline (30 µg) were used.

RESULTS AND DISCUSSION

A total of 249 *E. coli* isolates were recovered from 341 samples by targeting *uid*A and *cyd*A gene using PCR assay, resulting in an isolation rate of 73.02%. The sample sources included 50.76% cow milk samples, 54.68% buffalo milk samples, 86.36% cow rectal swabs, 85.93% buffalo rectal swabs, 81.81% goat rectal swabs and 85.71% poultry cloacal swabs. Conventional serotyping, the standard method for detecting O-antigens in *E. coli* isolates, involves labor-intensive agglutination reactions with specific antisera. This study used a PCR-based O-typing method utilizing 3 primer pairs, referred to as *E. coli* PCR-based serogrouping, which is a more efficient approach. The selected primers represent the most commonly employed molecular techniques for the identification of pathogenic bacteria, facilitating rapid and reliable screening of O types in *E. coli* isolates. In this study, we targeted the three most virulent O-serogroups of *E. coli* using a PCR assay. The serogroups included O26, O111 and O157. These O serogroups were detected by using PCR assay by targeting various genes such as *wzx* and *rfb*E. We found five O26, two O111 and one O157 serogroups with a positivity of 2.00%, 0.80% and 0.4%, respectively, among the 249 *E. coli* isolates screened. The O157 strain was isolated from buffalo fecal sample. Supporting our findings, Kumar *et al.* (2022) also reported that O157 was less common and associated with STEC. Abike *et al.* (2015) identified nine serogroups in dairy products, with O111 being the most prevalent. Pathotyping revealed that O111, O26 and O157 consistently harbored STEC-associated genes (*stx*1/*stx*2/*hly*A). Details of positive O serogroups isolates and its relationship with pathotype are mentioned in table 2.

All positive samples from PCR-based O antigen serogrouping were screened for the presence of various pathotypes, including enteropathogenic (EPEC), Shiga toxic *E. coli* (STEC), enterotoxigenic (ETEC), and enteroaggregative (EAEC) *E. coli* by detecting specific genes such as *stx*1, *stx*2, *hly*A, *bfp*, *lt*, *st*II and *agg*R, respectively. Pathotyping results revealed that all the O26, O111 and O157 isolates carried STEC virulence genes and were identified as STC pathotype. The O157 and O111 confirmed isolates revealed the presence of all the three (*stx*1, *stx*2, *hly*A) STEC genes. Among the five O26 isolates, four were having two STEC genes (*stx*1/*stx*2/*hly*A) whereas one isolate derived from buffalo rectal sample possessed only one (*stx*1) STEC gene. Amongst these STEC genes, *sxt*1 gene was present in all the O26, O111 and O157 serogroups whereas *hly*A gene was present in the only four isolates. Similar to our findings, Renter *et al.* (2005) confirmed that all O26, O111 and O157 serogroups possessed STEC genes. Merwad *et al.* (2014) identified STEC serogroups O26 and O111 in milk and fecal samples. In contrast to our findings, Malvi *et al.* (2015) detected the O26 serogroup within enteropathogenic *E. coli* (EPEC) strains, while Cardonha *et al.* (2004) identified the O111 serogroup in enteropathogenic *E. coli* from sewage and nearby coastal areas in Brazil. Tamaki *et al.* (2005) analyzed 42 isolates of the O111 serogroup, classifying 11 as EPEC, 6 as STEC and 14 as EAEC. Similarly, among 49 O26 isolates, three were identified as EPEC, nine as STEC, one as enterotoxigenic *E. coli* ETEC and one as EAEC.

The Shiga toxin-producing *E. coli* (STEC) is a significant foodborne pathogen that can cause gastroenteritis, hemorrhagic colitis and hemolytic-uremic syndrome (HUS). The O157 serogroup is particularly associated with HUS, while other non-O157 important pathogenic serogroups include O26, O103, O111, O121 and O145. Emerging serogroups, such as O104:H4, have also been implicated in outbreaks. The most prevalent pathogenic non-O157 STEC serogroups in the United

Table 1. Details regarding the primers, including their specific annealing temperature and product size

S.No.	Type	Gene	Primer	Annealing temp. (°C)	Product size (bp)	Reference
1.	*E. coli*	*cyd*A	F-CGCCGATGCAGATATTCG R-GCTGTGACGCACAGTTCATAG	60	398	Chopra *et al.*, 2021
2.		*uid*A	F- CGCCGATGCAGATATTCG R- GCTGTGACGCACAGTTCATAG	60	603	Chopra *et al.*, 2021
3.	O26	*wzx*	F-GGGGGTGGGTACTATATTGG R-AGCGCCTATTTCAGCAAAGA	67	241	Paddock *et al.* (2012)
4.	O111	*wzx*	F-CAAGAGTGCTCTGGGCTTCT R-AACGCAAGACAAGGCAAAAC	67	451	Paddock *et al.* (2012)
5.	O157	*rfb*E	F-CAGGTGAAGGTGGAATGGTTGTC R-TTAGAATTGAGACCATCCAATAAG	64	296	Bertrand and Roig (2007)
6.	STEC	*stx*1	F-CAGTTAATGTGGTGGCGAAGG R-CACCAGACAATGTAACCGCTG	62	348	Cebula *et al.* (1995)
7.		*stx*2	F-ATCCTATTCCCGGGAGTTTACG R-GCGTCATCGTATACACAGGAGC	62	584	Cebula *et al.* (1995)
8.		*hly*A	F-AGCTGCAAGTGCGGGTCTG R-TACGGGTTATGCCTGCAAGTTCAC	62	569	Wang *et al.* (2002)
9.	ETEC	*st*II	F-AAAGGAGAGCTTCGTCACATTTT R-AATGTCCGTCTTGCGTTAGGAC	62	129	Vidal *et al.* (2004)
10.		*lt*	F-GCACACGGAGCTCCTCAGTC R-TCCTTCATCCTTTCAATGGCTTT	62	218	Vidal *et al.* (2004)
11.	EPEC	*bfp*	F- GGAAGTCAAATTCATGGGGGTAT R- GGAATCAGACGCAGACTGGTAGT	62	254	Vidal *et al.* (2004)
12.	EAEC	*agg*R	F-GTATACACAAAAGAAGGAAGC R-ACAGAATCGTCAGCATCAGC	52	254	Ratchtrachenchai *et al.* (1997)

Table 2. Details of positive O serogroups and its relationship with pathotype

Isolate No.	Sample type	O serogroups	Pathtyping gene	Pathotypes
E736	Poultry cloacal	O26	*stx*1 and *hly*A	STEC
E797	Buffalo rectal	O26	*stx*1	STEC
E1083	Cow milk	O26	*stx*1 and *stx*2	STEC
E1090	Buffalo rectal	O157	*stx*1, *stx*2 & *hly*A	STEC
E1139	Poultry cloacal	O26	*stx*1 and *stx*2	STEC
E1158	Cow rectal	O26	*stx*1 and *stx*2	STEC
E1168	Buffalo rectal	O111	*stx*1, *stx*2 & *hly*A	STEC
E1160	Cow rectal	O111	*stx*1, *stx*2 & *hly*A	STEC

States are O26, O45, O103, O111, O121 and O145, causing over 70 to 83% of the total non-O157 STEC illnesses (Bettelheim, 2007).

The antimicrobial resistance patterns of O26, O111 and O157-confirmed isolates exhibited distinct variations. All three serotypes were found to be susceptible to Amikacin and Chloramphenicol. The highest resistance rates were recorded against Ampicillin (50%) followed by Ceftazidime and Ceftriaxone (37.5%) across all positive serotypes. Resistance against cephalosporin antibiotics has been reported previously by Bairwa *et al.* (2023). However, resistance to Imipenem was observed exclusively in O157-positive isolates. A detailed antimicrobial resistance pattern is presented in Table 3. There was no observed correlation between antimicrobial resistance patterns and serogroups or pathotypes, as these characteristics exhibited significant variation. Similar to our research Mora *et al.* (2005) also found that the resistance profiles of both O157:H7 and non-O157 STEC (which include serogroups such as O111 and O26) varied significantly among isolates, with no clear correlation between the resistance patterns and the serogroup or pathotype. This indicates that, despite classification into different serogroups (e.g. O111, O157, O26), the antimicrobial resistance traits are not strictly linked to these groups but rather show high variability.

CONCLUSION

This study revealed a low prevalence of highly virulent serogroups (O26, O111 and O157) among diverse samples collected from Sambhal district, Uttar Pradesh. All these O-types possessing STEC genes. The data further revealed that while O157 and O111 isolates consistently possessed all three virulence determinants, O26 strains displayed variable gene carriage, with some isolates harboring only *stx*1. This variability in virulence profiles, even at low prevalence, highlights the complex epidemiological landscape of STEC and underscores their significant public health potential. Overall, these findings advocate for the continued implementation of targeted molecular surveillance and enhanced food safety protocols to mitigate the risks associated with emerging STEC strains in livestock, poultry, and related food products. The study revealed that while all isolates were susceptible to amikacin and chloramphenicol, high resistance was observed to Ampicillin,

Table 3. Details of Anti-microbial susceptibility pattern of conformed O-types isolates

Isolate ID(O-type)	E736 (O26)	E797 (O26)	E1083 (O26)	E1090 (O157)	E1139 (O26)	E1158 (O26)	E1168 (O111)	E1160 (O111)
Ampicillin	I	R	S	R	S	R	S	R
Amoxicillin-clavulanic acid	S	S	I	R	S	S	I	S
Amikacin	S	S	S	S	S	S	S	S
Aztreonam	S	S	S	S	R	S	S	S
Chloramphenicol	S	S	S	S	S	S	S	S
Ceftazidime	R	S	S	S	R	I	S	R
Cefotaxime	S	S	S	S	R	S	I	S
Ceftriaxone	S	I	S	R	R	S	S	R
Cefoxitin	S	S	S	R	R	I	S	S
Cefpodoxime	S	S	S	S	R	S	S	S
Enrofloxacin	S	R	S	S	S	S	S	S
Imipenum	S	S	S	R	S	S	S	S
Nalidixic acid	I	S	S	S	S	R	I	R
Tetracycline	S	S	S	S	S	R	S	S
Trimethoprim-Sulfamethoxazole	S	S	S	S	S	R	S	S

* S- Sensitive *I- Intermediate *R-Resistance

Ceftazidime and Ceftriaxone with Imipenem resistance found exclusively in O157 isolates. These findings underscore that antimicrobial resistance traits vary significantly among serogroups and are not strictly linked to serotype or pathotype classifications.

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Research Article

ASSESSMENT OF TRAINING NEEDS OF PASTORALISTS OF PUNJAB

VEENU SHARMA, RAJESH KASRIJA*, JASWINDER SINGH, UDEYBIR SINGH CHAHAL, DALJEET KAUR and NALLAPATI SAI ANJANA
Department of Veterinary and Animal Husbandry Extension Education College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana

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ABSTRACT

Pastoralism, one of the world's oldest and most sustainable livestock management systems, remains integral to the livelihoods of several nomadic communities in India. In Punjab, pastoralist groups, especially the Gujjars, migrate seasonally from Jammu & Kashmir and Himachal Pradesh in search of better grazing lands. The present study aimed to assess the training needs of pastoralists in Punjab by examining their knowledge levels across six key areas of dairy farming: breeding, feeding, housing and management, healthcare, marketing, and government services. Data were gathered from 90 pastoralists across Majha (Group-I), Malwa (Group-II) and Doaba (Group-III) regions through structured personal interviews. The findings revealed significant regional differences in knowledge indices (at $P<0.01$), with Group-II (Malwa) consistently outperforming other regions. Notably, government services emerged as the area with the lowest awareness, highlighting a pressing need for institutional outreach. The training needs order of pastoralists of Punjab was reported to be at I, II, III, IV, V and VI for government services, feeding, marketing, housing & management, healthcare and breeding, respectively. Feeding practices and marketing skills also ranked high in training priority, whereas breeding was identified as the least urgent. The study emphasizes the necessity of culturally sensitive, community-based training interventions, particularly in government schemes, feed management, and market linkages, to enhance the livelihoods and well-being of pastoralist communities in Punjab.

Keywords: Knowledge Index, Pastoralists, Punjab, Training Needs

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There are several indigenous groups in India known as tribes that have not yet adopted the ways of the modern world. India has the second-largest tribal population in the world, numbering about 84.4 million. Pastoralism is one of the most ancient, robust, and sustainable systems of raising livestock; it originated in Northeast Africa as early as 9000 B.P. (Dong, 2016). Pastoral groups in India are classified as nomadic tribes. Pastoralism is the branch of agriculture where pastoralists raise cattle for the purpose of making money off of their products (Djordjevic Milošević & Milovanović, 2020). This could be nomadic, which means relocating frequently in pursuit of water and grazing places in the form of new pasture, or stationary, which involves maintaining cattle in a semi-permanent region, typically close to farms and villages (Turner & Schlecht, 2019). About 200 million transhumant and nomadic pastoralists worldwide make a living in isolated, hostile areas where traditional farming is either impossible or very difficult.

Training needs assessment of pastoralists is important for pastoralists as it can increase output by optimizing nutritional strategies and herd oversight; protect community health by implementing sanitary milking protocols and effective pasteurization methods; promote animal well-being by training pastoralists in preventive health measures and disease management. Broaden market access by delivering training in cooperative governance, entrepreneurial skills, and product value-addition techniques. Maximize adoption by embedding training within pastoralists' cultural contexts and everyday landscapes. There is scanty research literature regarding training needs assessment of pastoralists. So the present study was designed to assess the training needs of pastoralists of Punjab in a comprehensive manner.

*Corresponding author: dr.rajesh.kasrija@gmail.com

MATERIALS AND METHODS

Location of study and sampling procedure

A total of 90 pastoralists were selected from three regions Majha (Group-I), Malwa (Group-II) and Doaba (Group-III) of Punjab by purposive selection method since pastoralists do not have a fixed location of residence and practice transhumance. 30 pastoralists were selected from each region, respectively. The data was collected at their doorstep in different regions of Punjab with the help of pre-structured interview schedule by personal interview method to observe knowledge level of pastoralists. The total of 8 questions for breeding, 9 questions/items feeding, 7 questions for housing and management practices, 4 questions for marketing, 5 questions healthcare practices and 7 questions for government policies were finalized in interview schedule after discussion with subject matter expert, progressive farmers, field veterinarians and carefully examining research literature.

Measurement of Knowledge Index

The various knowledge indices for various farming practices- the breeding knowledge index (KIB), feeding

knowledge index (KIF), housing and management knowledge index (KIHM), healthcare knowledge index (KIH), marketing knowledge index (KIM), government services knowledge index (KIG) - were determined by dividing the total score by the highest possible score and multiplying the result by 100.

KI = Obtained score/Maximum possible score × 100

The Knowledge index on pastoralists was also calculated by using the following-

Formula: KIP = KIB + KIF + KIHM + KIH + KIM + KIG

The different knowledge indices were ranked in to order for different regions.

Assessing Training needs

The assessment of perceived training needs was based on the premise that a person's perceived training needs would decrease with increasing KI score. Training needs were also ranked in ascending order. A region's training requirements were determined using the knowledge indexes specific to that area.

RESULT AND DISCUSSION

Ranking of Knowledge Index

The results as presented in Table 1 shows the pastoralist's knowledge index on different dairy farming practices. Findings of the study showed with Group II having the highest overall KI followed by Group-III and Group-I. Among the six dairy farming practices, breeding recorded the highest overall KI, followed by healthcare, housing and management, marketing and feeding, while government services had the lowest KI. This ranking suggests that pastoralists exhibit the most knowledge in breeding and healthcare but dearth knowledge regarding government services. In terms of regional rankings, Group-II had the highest knowledge scores across most practices, particularly thriving in healthcare and marketing. Group-III have highest in breeding, but its knowledge in feeding was significantly lower than other. Group-I showed the lowest overall knowledge, with government services knowledge being completely void.

The overall Knowledge Index highlights Group-II as the most informed group (66.73%), followed by Group-III (43.62%) and Group-I (38.85%) indicating that Malwa-based pastoralists have comparatively higher knowledge across all domains, while Majha-based pastoralists are the most disadvantaged, particularly in accessing government resources as similar with findings of Gour et al. (2015) Sabapara (2012).

Ranking of Knowledge Indices and Training needs

From Fig. 1 and Table 2; the ranking of dairy farming practices in three regions- Group-I, Group-II and Group-III according to their performance is made by rating their Knowledge Index (KI). The results show noticeable regional differences in knowledge levels, with Group-II having the highest overall KI followed by Group-III and Group-I. The training-needs rankings show a distinct hierarchy of capacity-building priorities as follows:

- Across all groups, government services have been identified as the most significant training need (Order I), indicating pervasive gaps in access to institutional frameworks for livestock management, veterinary extension and policy support.
- The fact that feeding practices came in second place (Order II) highlights the serious issue of seasonal forage shortages and nutritional deficiencies that restrict milk production in native herds.
- Marketing came in third place (Order III), emphasizing the necessity for pastoralists to improve their business organization, value-chain involvement and market connections.
- Following housing and management (Order IV), there was a moderate need for training to enhance shelter design, herd monitoring and general husbandry procedures.
- Healthcare came in fifth place (Order V), indicating the need for improved preventive medicine knowledge, mastitis management and fundamental veterinary care.
- Although breeding frequently appears prominently in other dairy-farmer assessments, it was consistently ranked lowest in training priority (Order VI), indicating that pastoralists view themselves as relatively more knowledgeable or deem it a less urgent need in this area.
- The top training needs for Group-I were identified as housing and management, which was followed by marketing, breeding, feeding, and healthcare. There is a clear gap in extension service outreach, as government services continue to be the most needed and least accessed area for intervention (Order I).
- Healthcare was the most important training need in Group-II, followed by marketing, breeding, feeding, and housing and management. Once more, the top training priority was determined to be Government Services. The group's active involvement in the livestock trade and increased awareness of disease-related losses are reflected in the strong emphasis on healthcare and market engagement.
- The highest training priority for Group-III was breeding, which also had the highest KI score. Healthcare, housing and management, marketing, and feeding came next. It is noteworthy that Government Services once more occupied the first order of need (Order I),

Table 1. Knowledge index about different practices

Parameter	Dairy farming practices	Group-I (n=30)	Group-II (n=30)	Group-III (n=30)	Total (N=90)
Knowledge index (Mean ± S.E.)	Breeding (8)	$47.08^{a} \pm 2.14$	$77.08^{b} \pm 4.02$	$67.08^{c} \pm 2.57$	$63.75^{d} \pm 2.17$
	Feeding (9)	$45.55^{a} \pm 2.57$	$76.29^{b} \pm 3.40$	$37.03^{c} \pm 1.63$	$52.96^{d} \pm 2.34$
	Housing and management (7)	$54.28^{a} \pm 2.77$	$69.53^{b} \pm 2.72$	$45.24^{c} \pm 3.22$	$56.35^{d} \pm 1.97$
	Healthcare (5)	$48.67^{a} \pm 3.13$	$80.67^{b} \pm 1.51$	$47.33^{c} \pm 2.03$	$58.89^{d} \pm 2.10$
	Marketing (4)	$37.50^{a} \pm 2.61$	$79.17^{b} \pm 2.42$	$45.00^{c} \pm 3.47$	$53.89^{d} \pm 2.52$
	Government services (7)	$0.00^{a} \pm 0.00$	$17.62^{b} \pm 3.85$	$20.01^{c} \pm 2.12$	$12.54^{d} \pm 1.73$
Overall KI (Mean ± S.E.)		$38.85^{a} \pm 2.20$	$66.73^{b} \pm 2.99$	$43.62^{c} \pm 2.51$	$49.23^{d} \pm 2.14$

Values with different superscript differ significantly at $P < 0.01$

Table 2. Ranking of knowledge indices and training needs of pastoralists for different practices

Dairy farming practice	Group-I (n=30)			Group-II (n=30)			Group-III (n=30)			Overall (N=90)		
	KI	Ranking of KI	Training needs order	KI	Ranking of KI	Training needs order	KI	Ranking of KI	Training needs order	KI of KI	Ranking needs order	Training
Breeding	47.08±2.14	III	IV	77.08±4.02	III	IV	67.08±2.57	I	VI	63.75±2.17	I	VI
Feeding	45.55±2.57	IV	III	76.29±3.40	IV	III	37.03±1.63	V	II	52.96±2.34	V	II
Housing and management	54.28±2.77	I	VI	69.53±2.72	V	II	45.24±3.22	III	IV	56.35±1.97	III	IV
Healthcare	48.67±3.13	II	V	80.67±1.51	I	VI	47.33±2.03	II	V	58.89±2.10	II	V
Marketing	37.50±2.61	V	II	79.17±2.42	II	V	45.00±3.47	IV	III	53.89±2.52	IV	III
Government services	0.00±0.00	VI	I	17.62±3.85	VI	I	20.01±2.12	VI	I	12.54±1.73	VI	I

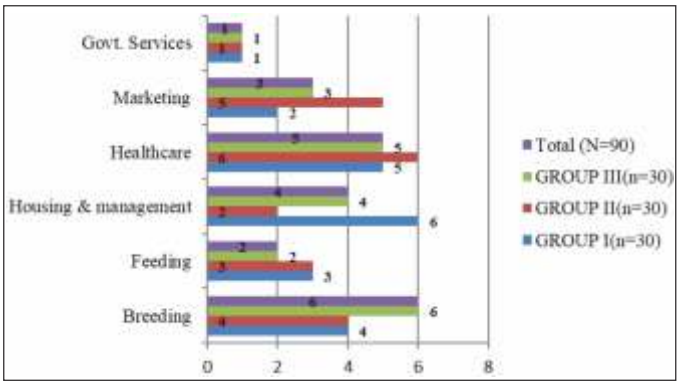

Fig. 1. Ranking of Training needs of Pastoralists of Punjab

suggesting that systemic barriers to accessing public veterinary and advisory services continue to affect even relatively better-informed groups.

CONCLUSION

According to the training requirements assessment, training about knowledge on government services should be given I ranking. The results on feeding revealed as second on the training priority list; suggesting that feeding management has lack of understanding, especially in Group-III and Group-I. The third most important area that needed training was marketing expertise. Given that breeding had the highest KI; it was ranked to be the lowest important for training. These results suggest for improving government and extension services, tackling feed-management issues and cultivating market-oriented skills should be the primary goals of capacity-building programs for pastoralists. Although they can be adjusted as secondary priorities, support for housing, health and breeding should be maintained.

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Research Article

EXPLORATORY SEROLOGICAL INVESTIGATION OF LEPTOSPIROSIS AND SCRUB TYPHUS IN FIELD RODENTS AND SHREWS OF UTTAR PRADESH, INDIA

VIBHA SINGH, SONU S. NAIR[1], ABHISHEK[1], AKASH B. MOTE, PRINCEE VERMA, HIMANGI GUPTA, M. SUMAN KUMAR and HIMANI DHANZE*

Division of Veterinary Public Health, [1]Division of Bacteriology & Mycology, Indian Veterinary Research Institute, Izatnagar-243122, Uttar Pradesh, India

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ABSTRACT

Rodent and shrew surveillance is essential for public health because these mammals act as reservoirs for several zoonotic pathogens and contribute to disease transmission through direct contact, environmental contamination and amplification of arthropod-borne infections. This preliminary study investigated the presence of *Leptospira* spp. and *Orientia tsutsugamushi* in rodents and shrews from Bareilly, Mathura and Gorakhpur districts of Uttar Pradesh, India. Fifty animals were captured and serum samples were tested for *Leptospira* antibodies using the microscopic agglutination test (MAT) and for scrub typhus antibodies using the Weil-Felix test. All samples were screened against 12 *Leptospira* serovars by MAT and none showed detectable antibodies, suggesting no serological evidence of exposure. In contrast, one sample from Bareilly and two from Gorakhpur yielded presumptive positive reactions for scrub typhus by the Weil-Felix test. Although Weil-Felix has low sensitivity and specificity, the test has been used in the present study as till date no validated serological assays are available for the detection of scrub typhus antibodies in rodents or shrews. Despite these limitations, the detection of presumptive scrub typhus antibodies is notable, particularly given reported human cases from Bareilly and Gorakhpur district. The low apparent seroprevalence may reflect heterogeneous pathogen distribution, transient antibody responses or diagnostic constraints. The absence of *Leptospira* antibodies may indicate lack of exposure, early infection or true absence of the pathogen. Further studies with larger sample sizes, wider geographic coverage and improved diagnostic methods, including ELISA, indirect immunofluorescence assay (IFA) or molecular approaches are recommended to clarify zoonotic disease dynamics.

Keywords: Chiggers, *Leptospira*, Orientia, Rodents and shrews, Zoonotic diseases

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Zoonotic diseases represent a significant global health burden, particularly in regions where human-wildlife interactions are frequent. More than 60% of infectious diseases affecting humans originate from animals, with approximately 75% of emerging infectious diseases having zoonotic origins (CDC, 2024). Among mammalian reservoirs, rodents are the most species-rich order and play a crucial role in zoonotic pathogen transmission. An estimated 10.7% of rodent species are hosts to zoonotic pathogens, carrying at least 85 known zoonotic diseases (Han *et al.*, 2015). Their ubiquitous presence in both urban and rural settings, coupled with their interactions with livestock and human settlements, makes them a significant contributor in pathogen amplification and transmission (Luis *et al.*, 2013; Dahmana *et al.*, 2020). Zoonotic transmission from rodents can occur directly through bites or inhalation of aerosols containing pathogens from rodent faeces or indirectly through contamination of water and food sources (Rabiee *et al.*, 2018). Rodents could also amplify and transfer diseases transmitted by arthropod vectors. Urbanization and deforestation have been associated with increased human-rodent interactions, elevating disease risk (Rupasinghe *et al.*, 2022).

*Corresponding author: hdhanze@yahoo.co.in

Among the numerous zoonotic diseases associated with rodents, leptospirosis and scrub typhus are of particular epidemiological importance due to their extensive geographical distribution, potential to cause both localized and large-scale outbreaks and significant morbidity and mortality. These diseases pose a major public health challenge, particularly in areas with high rodent densities and inadequate sanitation. Leptospirosis is a widespread zoonoses recognized as one of the 17 neglected tropical diseases by the World Health Organization (WHO, 2021). Leptospirosis, caused by pathogenic *Leptospira* spp., is primarily transmitted through direct or indirect contact with water or soil contaminated by rodent urine (Vihol *et al.*, 2024). Scrub typhus is a rickettsial disease caused by a pathogenic bacterium, *Orientia tsutsugamushi* and transmitted by *Leptotrombidium* chigger mites (larva of trombiculid mites) through rodents/shrews as primary animal host. Both diseases exhibit a broad spectrum of clinical manifestations, ranging from mild febrile illness to severe complications such as renal failure, meningitis and multi-organ dysfunction. Due to their significant public health impact, implementing robust surveillance systems, targeted preventive strategies and early, accurate diagnostics is crucial for effective disease control and mitigation.

The present study was designed to assess the presence of *Leptospira* spp. and *Orientia tsutsugamushi* in field rodents/shrews of Uttar Pradesh with the ultimate aim to inform control programs in order to prevent spillover of infection to humans.

MATERIALS AND METHODS

Study Design

The study was conducted in selected districts of Uttar Pradesh, India, a state characterized by substantial geographical, ecological and climatic heterogeneity between its eastern and western regions. To ensure representative coverage, districts from both regions were included. Site selection was guided by the availability of laboratory facilities, as transportation of live rodents/ shrews over long distances was not feasible. Among the three selected districts, Gorakhpur (eastern Uttar Pradesh) and Bareilly (western Uttar Pradesh) were included due to the occurrence of reported human cases in the recent past, while Mathura district was selected to broaden regional representation. This approach minimized sampling bias while balancing logistical feasibility and epidemiological relevance.

Uttar Pradesh exhibits marked agro-climatic diversity due to variations in rainfall, soil types and topography. Based on rainfall and soil characteristics, the state is divided into multiple agro-climatic zones ranging from the humid north-eastern plains to comparatively drier central and western plains. Gorakhpur district, located in the north-eastern plains, experiences relatively high annual rainfall and humid conditions, with extensive alluvial soils that retain moisture during and after the monsoon. In contrast, Bareilly district lies within the mid-western/ western plains and is characterized by moderate rainfall, a sub-humid climate and loamy to sandy loam soils with better drainage. Mathura district represents an additional agro-climatic setting within the broader plains system of the state. These differences in rainfall, soil moisture and landscape influence surface water availability and habitat conditions relevant to the environmental survival of *Leptospira* spp. and the ecology of scrub typhus vectors.

Sample Collection

The present study was conducted as a pilot investigation to generate baseline data from selected sites under logistical and ethical field constraints. A total of 50 field rodents and shrews were captured from Bareilly, Mathura and Gorakhpur districts between August and October 2024, including 17 samples from Gorakhpur, 19 from Bareilly and 14 from Mathura. No formal statistical method was employed for sample size calculation due to the exploratory nature of the study.

Trapping was carried out using Sherman collapsible live traps placed in peri-urban areas and human dwellings. Traps were baited with peanut butter and dried coconut to enhance capture efficiency and were set in the evening and checked the following morning.

Ethical Approval and Sample Processing

The study was approved by the Institutional Animal Ethics Committee (IAEC), ICAR-Indian Veterinary Research Institute (F. No. 26–1/2023–24/JD(R)/IAEC), for trapping rodents and shrews. Captured animals were anesthetized using isoflurane and blood samples were collected by cardiac puncture following standard ethical and biosafety guidelines.

Blood samples were collected in clot activator tubes and immediately placed in insulated ice boxes to maintain the cold chain (2-8 °C) during transportation to the laboratory. Serum was separated shortly after collection by centrifugation at 5,000 rpm for 10 minutes. The separated serum samples were aliquoted to avoid repeated freeze–thaw cycles and stored at -20 °C until further serological analysis. All procedures were carried out following standard biosafety and quality control practices to ensure sample integrity.

Statistical Analysis

Regional differences in the occurrence of scrub typhus antibodies among districts were evaluated using Fisher's exact test due to small sample sizes and low expected cell counts. A p-value of <0.05 was considered statistically significant.

Microscopic Agglutination Test

The microscopic agglutination test (MAT) is the reference serological method for the detection of antibodies against *Leptospira* spp. (Levett *et al.*, 2001). It employs a panel of live *Leptospira* serovars representing circulating strains and allows serogroup-level identification when an appropriate antigen panel is used.

MAT was performed following standard procedures recommended by the World Organisation for Animal Health (WOAH, 2023). Validated positive and negative control sera obtained from the *Leptospira* Laboratory, ICAR–Indian Veterinary Research Institute (IVRI), were included in each assay to ensure reliability and consistency of results. An initial serum dilution of 1:50 was prepared by mixing 20 µL of test serum with 980 µL of phosphate-buffered saline (PBS). From this dilution, 50 µL was dispensed into wells of a 96-well microtiter plate and mixed with an equal volume (50 µL) of live *Leptospira* antigen, resulting in a final screening dilution of 1:100.

A panel of 12 *Leptospira* serovars- Australis, Autumnalis, Ballum, Patoc, Pomona, Canicola, Icteroha-

emorrhagiae, Hardjo, Hebdomadis, Grippotyphosa, Javanica and Tarassoviwas used as antigens. Plates were incubated at 37 °C for 2-4 hours, after which agglutination was examined under a dark-field microscope using a 10X objective and 10X eyepiece (total magnification 100X). Samples were assessed for the presence or absence of visible agglutination at the screening dilution.

Weil-felix Test

The Weil-Felix is an inexpensive and widely available serological test. It detects antibodies to alkali-based carbohydrate antigens shared by certain *Rickettsia* spp. and *Proteus* spp. (*P. vulgaris* OX19, OX2 and *P. mirabilis* OXK). The OXK strain specifically agglutinates with sera having antibodies against *O. tsutsugamushi*, aiding in the diagnosis.

The Weil Felix test was performed using the rapid slide agglutination method with OXK antigen (Tulip Diagnostics, Goa, India) to detect antibodies specific to *O. tsutsugamushi* for scrub typhus (Rashmi *et al.*, 2015). A drop of the test serum (50 µL) was placed on a clean glass slide, followed by the addition of an equal volume of the OXK antigen. The mixture was gently rotated on the slide for 1-2 minutes to allow agglutination to occur. The presence of visible clumping indicated a positive reaction, suggesting the presence of antibodies against *O. tsutsugamushi*. Due to the pilot nature of the study and non-availability of standardized positive and negative control sera, assay interpretation was based on visible agglutination at the recommended serum dilution, as per established Weil-Felix test guidelines.

RESULTS AND DISCUSSION

A total of 50 small mammals, comprising 26 rats and 24 shrews, were captured during the study period, including 19 samples from Bareilly, 17 from Gorakhpur and 14 from Mathura. Of the 50 serum samples analysed, three (3/50) showed seroreactivity against *O. tsutsugamushi* using the Weil-Felix test (Fig. 1). Scrub typhus antibodies were detected in 6% of samples, with a 95% confidence interval of 2.0% to 16.0%. Among the positive samples, one was obtained from Bareilly district and two from Gorakhpur district. Although scrub typhus positive samples were detected in Bareilly and Gorakhpur but not in Mathura Fisher's exact test showed no statistically significant difference in the occurrence of scrub typhus antibodies among the three districts ($p = 0.5929$). Notably, human cases of scrub typhus have also been reported from these regions, highlighting the relevance of continued surveillance of reservoir hosts to prevent potential spillover of infection to humans. None of the samples tested positive for antibodies against *Leptospira* spp. when screened using the microscopic agglutination test (MAT) with a panel of 12 serovars.

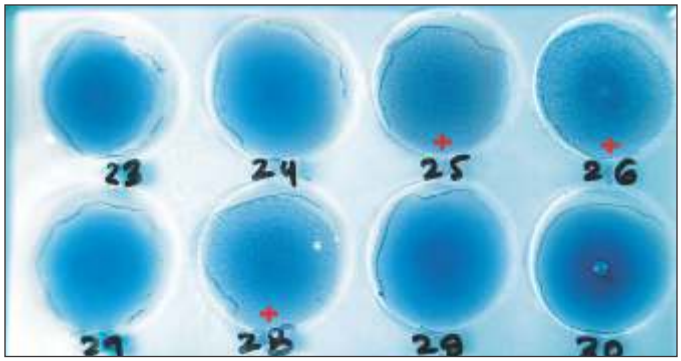

Fig. 1. Representative results of the Weil-Felix slide agglutination test showing visible agglutination (positive) and smooth suspensions (negative) with OXK antigen.
(Samples numbered 25, 26 and 28 show visible agglutination indicating positive reactions, while rest samples show negative reactions.)

Rodent and small-mammal surveillance represents a critical component of public health programs due to the role of these animals as reservoirs for a wide range of zoonotic pathogens. Their close association with human habitations, adaptability to diverse environments and high reproductive rates facilitate pathogen maintenance and transmission. Leptospirosis, caused by *Leptospira* spp., and scrub typhus, caused by *Orientia tsutsugamushi*, are important zoonotic diseases with significant public health implications in India. Serological assays are often employed for surveillance purposes as cost-effective and practical tools, particularly in resource-limited settings, although they possess inherent diagnostic limitations.

In small mammal reservoir hosts, antibody responses to certain zoonotic pathogens may be short-lived or decline rapidly over time, particularly during asymptomatic or subclinical infections (Vernel-Pauillac *et al.*, 2021). As a result, serological assays may fail to detect antibodies depending on the timing of sampling relative to exposure. Thus, the low detection rate observed in our study may be attributed to the patchy distribution of the pathogen, transient antibody responses in reservoir hosts and the inherent limitations of the Weil-Felix test, which is known to have low sensitivity (43.3%) (Prakash *et al.*, 2006). Currently, no standardized or validated serological assay is routinely available for detecting antibodies against *O. tsutsugamushi* in rodent reservoirs. Advanced diagnostic methods such as ELISA or IFA are mainly optimized for human samples and their performance characteristics in rodents/shrews remain inadequately defined. Consequently, despite its well-recognized limitations, the Weil-Felix test has historically been used in preliminary ecological and field surveillance studies involving animal hosts.

Given the limited sample size and restricted geographic coverage, the findings of the present study should be interpreted with caution and are not intended to represent statewide prevalence. In this investigation, 6%

of the sampled small mammals showed seroreactivity to *O. tsutsugamushi*. In India, outbreaks of scrub typhus in humans have been documented across northern, eastern and southern regions (Mathai *et al.*, 2001; Kamarasu *et al.*, 2007; Mahajan *et al.*, 2008; Sharma *et al.*, 2005). Despite its endemicity, scrub typhus continues to be underdiagnosed due to nonspecific clinical manifestations, limited access to advanced diagnostic facilities and inadequate awareness among high-risk populations (Bakshi, 2007).

Rodents are well-recognized reservoir hosts of *Leptospira* spp. and play a significant role in the transmission of leptospirosis to humans and domestic animals (Twigg *et al.*, 1969). Numerous studies worldwide have highlighted the importance of rodents in leptospiral ecology. For instance, a study from Vellore district, Tamil Nadu, reported seropositivity in 42% of trapped rats (Vimala *et al.*, 2014), while a systematic review by Gupta *et al.* (2023) reported exposure to rats in 67% of leptospirosis cases. In contrast, none of the small mammal sera in the present study tested positive for *Leptospira* antibodies using MAT. This absence of seroreactivity may reflect low or absent exposure in the sampled areas, early-stage infections with antibody levels below the detection threshold, or regional variation in circulating serovars. While the present findings suggest a low burden of leptospirosis in the sampled sites, broader investigations employing sensitive diagnostic tools and expanded geographic coverage are warranted.

Despite limitations related to sample size and spatial coverage, the present study underscores the importance of continuous surveillance of rodents and other small mammals for zoonotic pathogens. Even low levels of pathogen detection in localized areas may have important implications for public and animal health. Future studies should incorporate larger, statistically powered sample sizes, precise host species identification and contemporary diagnostic approaches such as ELISA, immunofluorescence assays or molecular techniques to enhance understanding of zoonotic disease dynamics and support effective prevention strategies.

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Research Article

STUDIES ON BACTERIAL ISOLATES ASSOCIATED WITH REPRODUCTIVE PROBLEMS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PROFILE IN BOVINES OF HARYANA

JINU MANOJ*, MAHAVIR SINGH and RAJESH CHHABRA
College Central Laboratory, LUVAS, Hisar-125004, Haryana

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ABSTRACT

A study for three years was carried out to know the bacterial association with reproductive problems among bovines of Haryana. The vaginal swabs or discharges were collected from bovines exhibiting reproductive issues such as repeat breeding, endometritis and retained placenta to assess the presence of pathogenic bacteria. The bacterial isolates were phenotypically identified based on their colony morphology, haemolytic pattern, biochemical tests and Gram staining pattern. A total of 857 bacterial isolates were obtained from the vaginal samples collected from 416 bovines. The major pathogenic bacterial isolates were found as *Staphylococcus* (34.89%), *Streptococcus* (30.46%) and *E. coli* (24.04%). All the isolates were analyzed for their antimicrobial susceptibility pattern. Among 26 antibiotics used in this study, the greatest resistance level was observed towards carbenicillin (93.38%). However, the isolates exhibited maximum sensitivity for gentamicin (58.31%) followed by ciprofloxacin (43.98%). The reduced sensitivity of the isolates to frequently used antibiotics for both human and animal use suggests that there is a worrisome level of antibiotic resistance across the study area. There is utmost need to focus on the judicious use of antibiotics in bovines of the States.

Keywords: AMR, Endometritis, *E. coli*, *Staphylococcus*, *Streptococcus*, Vaginal swab

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Infertility is a critical issue in the dairy sector, contributing to significant economic losses (Patel *et al.*, 2019). The reproductive issues such as repeat breeding, endometritis and retained placenta are often associated with microbial infections that can disrupt uterine and ovarian functions (Nguyen *et al.*, 2025). These infections can significantly impact fertility and overall reproductive health in bovines. Bacterial infections of the reproductive tract play a crucial role in repeat breeding among bovines. Common bacterial pathogens identified in such cases include *Staphylococcus*, *Escherichia coli*, *Klebsiella*, *Trueperella pyogenes* and *Pseudomonas*. The presence of these bacteria can lead to inflammation of the endometrium leading to endometritis characterized by mucopurulent or purulent vaginal discharge (Neelam *et al.*, 2018). This condition is often observed postpartum and can be diagnosed through clinical examination and bacteriological culture of vaginal swabs. The regular monitoring and prompt diagnosis of reproductive issues in bovines are essential for effective management and to mitigate the risks associated with reproductive tract infections (Nguyen *et al.*, 2025).

The bacterial infections of the uterus and vagina are typically treated with antibiotics. The choice of antibiotic regimen for treating reproductive tract infections in dairy animals is influenced by several factors, including the type of infection, its severity, the causative bacteria and the animal's overall health status (Moraes *et al.*, 2024). Antimicrobial resistance (AMR) in bovine reproductive infections arises primarily due to inappropriate antibiotic use and selective pressure in the farm environment. Due to the complexity of reproductive tract infections and the potential for antibiotic resistance, it is crucial to conduct the isolation of the causative agent along with antibiogram studies for an accurate diagnosis and appropriate treatment plan. Addressing AMR in bovine reproductive infections requires a multifaceted approach, including the responsible use of antibiotics in animal husbandry, improved management practices for prevention and control of infections and enhanced research and surveillance systems to ensure sustainable dairy production (Manoj *et al.*, 2024; Mekibib *et al.*, 2024). Therefore, this study was carried out to analyze the bacterial association with reproductive problems among bovines of Haryana and adjoining States.

MATERIALS AND METHODS

Sample Collection

This retrospective study analyzed the data for three consecutive years from 1 July, 2021 to 30 June, 2024. The vaginal swab/discharge received at the College Central Laboratory, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana for the bacteriological examination were used for this study. These samples were routinely submitted to the laboratory for the cultural examination and antibiotic sensitivity testing either by the dairy farmers or veterinarians of the Haryana and adjoining States. The details of each case were recorded from the owner of the animal. The bovines involved in this study were exhibiting reproductive issues such as repeat

*Corresponding author: drjinumanoj@gmail.com

breeding, endometritis or retained placenta.

Isolation of the bacteria

A total of 416 vaginal swab/discharge samples were subjected to bacteriological analysis as per the standard method (Quinn *et al.*, 2011). The bacterial isolation of both Gram positive as well as Gram negative organisms were performed separately using sheep blood agar (SBA) and Mac Conkey's Lactose agar (MLA) plates, simultaneously under aseptic conditions. These plates were incubated aerobically at 37º C. The plates were checked for bacterial growth after 16-18 hours of incubation. If no growth were observed, then the plates were reincubated upto 48 hours. The colonies developed on the SBA and MLA was identified presumptively, based on their morphological and phenotypical features such as colony size and shape, haemolytic pattern, colour development, biochemical tests and Gram staining.

***In vitro* antimicrobials sensitivity testing**

The bacterial isolates obtained were tested for their antibiotic susceptibility pattern using the Kirby-Bauer disc diffusion method according to CLSI guidelines (2020). A total of 26 different antibiotics from nine classes viz. aminoglycosides, penicillins, cephalosporins, lincomycins, macrolides, phenicol, quinolones, sulphonamides, tetracyclines were used, which include the following antibiotics, amoxicillin (10 µg), ampicillin (10 µg), amoxyclav (10 µg), amikacin (30 µg), chloramphenicol (30 µg), carbenicillin (100 µg), clindamycin (2 µg), ciprofloxacin (10 µg), cephalexin (10 µg), co-trimoxazole (25 µg), cloxacillin (30 µg), cefoperazone (75 g), ceftriaxone (30 µg), cephotaxime (30 µg), erythromycin (15 µg), enrofloxacin (10 µg), gentamicin (10 µg), kanamycin (30 µg), levofloxacin (5 µg), moxifloxacin (5 µg), neomycin (30 µg), oxytetracycline (30 µg), penicillin (10 units), streptomycin (10 µg) and tobramycin (30 µg). The isolates were classified as resistant, intermediate or sensitive towards the tested antibiotics based on the zone of inhibition developed as per manufacturer's quality control instructions (HiMedia, Mumbai, India).

RESULTS AND DISCUSSION

The major reproductive health problems affecting dairy bovines in India include anoestrus, repeat breeding, retention of foetal membranes, dystocia, abortion, uterine and vaginal prolapse as well as various uterine infections such as endometritis and pyometra (Khan *et al.*, 2016, Ashoo *et al.*, 2020). Bacterial isolation from uterine secretions of bovines with endometritis has identified a diverse array of microorganisms. Bacterial endometritis

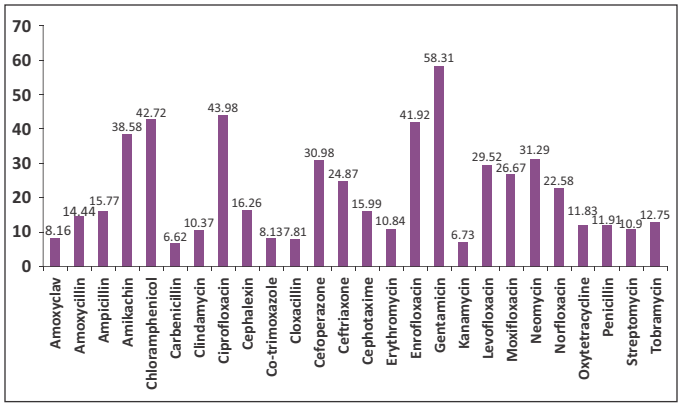

Fig. 1. *In vitro* antibiotic sensitivity pattern of bacterial pathogens isolated

cause infertility in animals as it impedes uterine involution, delays the onset of first estrus, increases the number of services per conception and extends the interval to calving (Gani *et al.*, 2008, Neelam *et al.*, 2018). Endometritis due to bacterial pathogens has been identified as a leading cause of conception failure in a significant proportion of bovines in India (Udhayavel *et al.*, 2013). Out of 416 vaginal swab samples analyzed, 380 samples (91.35%) were found as culture positive for microorganisms and a total of 857 pathogenic bacterial isolates were obtained from bovines involved in this study. Among these, 67.91% of isolates were identified as Gram positive, while 30.81% were found as Gram negative. In our study, Gram positive bacterial pathogens were more prevalent as compared to Gram negative bacterial isolates. Very few fungal isolates of *Candida* spp. (1.28%) were also recovered during this study.

In this study, the major Gram positive bacterial pathogen associated with reproductive problems in bovines were found as *Staphylococcus* species with an average occurrence rate of 34.89%. The *Streptococcus* species were also found as significant contributors to reproductive tract infections, comprising 30.46% of the total isolates in this study. *Trueperella pyogenes* (2.33%) and *Bacillus* spp. (0.2%) were also recovered from bovines under this study. Among Gram negative bacteria, *Escherichia coli* were found as the most predominant bacteria (24.04%) followed by *Klebsiella* spp. (5.02%), *Pseudomonas* spp. (1.05%) and *Proteus* spp. (0.7%) from the bovines of study area (Table 1). These results are similar to the findings from infertility cases of bovines reported by other studies (Gani *et al.*, 2008, Bhat and Bhattacharyya, 2012, Patel *et al.*, 2019). However, some studies have identified *Escherichia coli* as the most prevalent bacterial pathogen in reproductive infections in bovines (Udhayavel *et al.*, 2013, Pohl *et al.*, 2018, Mekibib

Table 1. Bacterial isolates obtained from vaginal swabs/secretions of bovines

Organisms isolated	Major mixed infections
Staphylococcus spp. (299), *Streptococcus* spp. (261), *E. coli* (206), *Klebsiella* spp. (43), *T. pyogenes* (20), *Pseudomonas* spp. (09), *Proteus* spp. (06), *Bacillus* spp. (02), *Candida* spp. (11)	*Staphylococcus* spp. + *Streptococcus* spp. (91), *Staphylococcus* spp. + *E. coli* (36), *Staphylococcus* spp. + *Streptococcus* spp. + *E. coli* (81), *Streptococcus* spp. + *E. coli* (21), *Staphylococcus* spp. + *Klebsiella* spp. (08), *Staphylococcus* spp. + *Streptococcus* spp. + *Klebsiella* spp. + *E. coli* (07), *Staphylococcus* spp. + *Streptococcus* spp.+ *Klebsiella* spp. (06), *Staphylococcus* spp. + *T. pyogenes* (06)
	Number in the parenthesis indicates number of isolates

et al., 2024). The variation in the percentage of different organisms observed may be attributed to differences in the animals' health status, the prevalence rate of organisms in a specific area and individual susceptibility among the animals (Kusum *et al.*, 2022). Some studies do not consistently identify a specific bacterium or group of bacteria as the primary cause of uterine infections in bovines, instead they recognize mixed bacterial contamination as the underlying cause (Mekibib *et al.*, 2024). In our study as well, the majority of infections were found to be of mixed nature (Table 1).

All the isolates obtained were subjected to *in vitro* antibiotic sensitivity testing. The sensitivity patterns of isolates were given in Fig. 1. Our investigation into the antibiotic susceptibility profiles of bacteria isolated from bovines revealed that the infections were of a multidrug-resistant nature, exhibiting varying levels of resistance to two or more of the tested class of antibiotics. Among 26 antibiotics used in this study, the greatest resistance level was observed towards penicillin group of antibiotics especially to carbenicillin (93.38%). However, these isolates had shown maximum sensitivity for aminoglycosides (gentamicin, 58.31%) and fluoroquinolones (ciprofloxacin, 43.98%) class of antibiotics. Mekibib *et al.* (2024) also identified gentamicin as the most effective antimicrobial agent in their study. Bacterial infections responsible for reproductive diseases in bovines generally localize within the tubular genital tract. According to Pyorala *et al.* (2014), recent research consistently indicates that routine parenteral antibiotic treatment for all bovines with reproductive disorders is not justified. The efficacy of antimicrobial agents may differ significantly depending on geographical variations, prevailing bacterial resistance patterns and the health status of individual animals (Reygaert, 2018).

CONCLUSIONS

The study identified multiple bacterial species associated with reproductive problems in bovines of Haryana State. Gram-positive organisms such as *Staphylococcus* and *Streptococcus* spp. were the most frequently isolated pathogens in the present study. Varying degrees of resistance to many of the tested antibiotics were also observed, however gentamicin proved to be effective against most of the isolates. These findings underscore the importance of responsible use of antibiotics to prevent the emergence of resistance. Promoting awareness among dairy farmers about proper herd health management and the adverse effects of indiscriminate antibiotic use is essential. In summary, AMR poses a significant threat to global health, necessitating urgent and coordinated actions across human, animal and environmental health sectors. By adopting One Health approach we can mitigate the risks associated with AMR and safeguard the effectiveness of antimicrobial treatments for animals as well as public health.

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IMPORTANT INFORMATION

- Research/Clinical articles are invited for next issue from the Scientists/Veterinarians engaged in Veterinary Profession.
- Please follow strictly the format of **'The Haryana Veterinarian'** for manuscript writing/submission.
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Editors

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Research Article

CANINE DIABETES AND ITS SUCCESSFUL MANAGEMENT

CHAUDHARI, P.P., DHOOT, V.M., BHOJNE, G.R., UPADHYE, S.V.[1] and THAKUR, K.R.*
Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, [1]Department of Surgery and Radiology,
Nagpur Veterinary College, Seminary Hills-440006, Nagpur (Maharashtra)

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ABSTRACT

Diabetes has evidence in ancient literature, though recently is being considered as most emerging disease condition in both human and companion animals. Diabetes mellitus is a common endocrine disorder in dogs, presenting with persistent hyperglycemia and clinical signs such as polyuria, polydipsia, polyphagia, and weight loss. The present study was undertaken to evaluate the blood glucose level alterations in canine diabetes mellitus and compare therapeutic management strategies with special reference to blood glucose as a diagnostic tool. Out of 642 dogs screened, 12 were diagnosed as diabetic and randomly divided into two groups. Group-I (n=6) received an alternative therapy comprising activated zinc, arginine, calcium pantothenate, L-carnitine and lettuce extract, while Group-II (n=6) was treated with recombinant insulin @ 0.5 IU/kg intramuscularly, twice daily. Dogs were monitored clinically and through haemato-biochemical parameters, including random, fasting, and post-prandial blood glucose, over 21 days. Both groups showed improvement, but insulin therapy produced more consistent and significant reductions. In Group-II, random glucose decreased from 278.33±28.93 mg/dl to 146.00±11.10 mg/dl, and fasting glucose from 230.22±17.27 mg/dl to 116.83±5.59 mg/dl, approaching normal values by day 21. Post-prandial glucose levels also improved markedly. In conclusion, canine diabetes can be effectively diagnosed and managed with proper monitoring and recombinant insulin remains the most reliable treatment for achieving normoglycemia.

Keywords: Diabetes mellitus, Dogs, Glucose

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Diabetes mellitus (DM) is one of the most common metabolic diseases affecting middle-aged to geriatric dogs, characterized by hyperglycemia, glycosuria, polyphagia, polydipsia and weight loss, resulting from an absolute or relative deficiency of insulin. The syndrome, commonly referred to as diabetes, is a frequently occurring disease in humans as well as companion animals, especially dogs. The reported incidence in dogs ranges between 0.2-1.3% of the canine population worldwide (Guptill *et al.*, 2003; Hess *et al.*, 2000).

DM exerts a significant impact on canine health, with complications that substantially reduce quality of life and survival. Common clinical manifestations include persistent polyuria, polydipsia, polyphagia, weight loss and reduced immunity (Feldman & Nelson, 2004; Nelson, 2015). Secondary complications are frequent, with bilateral cataracts reported in up to 75% of diabetic dogs within one year of diagnosis (Beam *et al.*, 1999; Kooistra and Galac, 2012). Other sequelae include peripheral neuropathy, recurrent urinary tract infections, hepatopathy, and diabetic ketoacidosis, the latter being potentially life-threatening (Feldman and Nelson, 2004; Catchpole *et al.*, 2005). Many cases progress insidiously and dogs are often presented only after significant complications have developed (Rand *et al.*, 2004).

Although insulin therapy remains the cornerstone of treatment and is generally life-saving, its limitations include the requirement for lifelong daily injections, close monitoring of blood glucose levels, risk of hypoglycemia, and variable owner compliance (Nelson, 2015; Behrend *et al.*, 2018). Alternative and adjunctive therapies, such as dietary modification and oral hypoglycemic agents, have been investigated, such as zinc and arginine help to stimulate the secretion of insulin while the lettuce extract helps to reduce gastrointestinal glucose absorption (Cheta and Trifan, 2010) but the efficacy of these agents are limited compared with insulin (Eigenmann *et al.*, 1985; Catchpole *et al.*, 2005). This underscores the importance of early diagnosis, careful monitoring, and comprehensive long-term management to optimize prognosis in canine diabetes.

Diagnosis of DM has evolved significantly over time (World Health Organization, 2011). Historically, diabetes was suspected through the sweet taste of urine and later confirmed primarily by glycosuria (Banting *et al.*, 1922). Today, diagnosis and treatment are largely based on measurement of blood glucose concentrations blood glucose concentrationsand associated biochemical parameters.

MATERIALS AND METHODS

The study was carried out at the Teaching Veterinary Clinical Complex (TVCC) and Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Nagpur Veterinary College, Nagpur on diagnostic and therapeutic management of diabetes mellitus in canine. The dogs presented at TVCC with symptoms of polyuria, polydipsia, polyphagia and obesity of 5 years or and above, irrespective of breed and sex were screened for random blood glucose. Those having random blood glucose more

*Corresponding author: kalyanithakur333@gmail.com

than 150 mg/dl (Rucinsky *et al.*, 2010) were included in the study and recently whelped bitches, bitches in estrus or cases of pseudo-pregnancy and pyometra were excluded. Complete clinical examination of the dogs was carried out proceeding with the owner's complaint, history of the patient, signalment of the patient. Further, a thorough clinical examination was undertaken by considering the relevant points like polyuria, polydipsia, polyphagia and obesity. Haemato-biochemical estimations viz. Random blood glucose, Fasting blood glucose and Post-prandial blood glucose was carried out in each group.

Dogs having blood sugar more than 150mg/dl were divided into two groups comprising of six dogs in each group. Group I dogs were treated with alternate therapy consisting combination of Syrup Activated Zinc, Activated Arginine, Calcium Pantothanate L carnitine and lettuce extract @ 1 ml/5 kg body weight. Group-II Dogs were treated with recombinant insulin, @ 0.5 IU/kg, i/m, after feeding at every 12 hrs interval.

Observations of various parameters recorded during the experiment period were tabulated and the data was statistically analysed using one way classification and student t-test by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

In the period of study, total 642 dogs were screened having polyuria, polydipsia, polyphagia and obesity. Out of which 12 dogs had random blood glucose more than 150 mg/dl and therefore were considered and evaluated for further biochemical parameters. The prevalence in this study was found to be 1.86 per cent. These findings are in accordance with Fracassi *et al.* (2004) who reported 1.33% of prevalence of Diabetes mellitus in dogs and Nelson and Reusch (2014) accounted hospital prevalence rate of 0.4 to 1.2%. Whereas Shruthi *et al.* (2017) revealed incidence of Diabetes mellitus to be 0.14% in dogs.

The blood glucose concentration was measured and the average ± S.E. of Random Blood Glucose was found to be 287.57±43.97 mg/dl in Group-I and 278.33±28.93 mg/dl in Group-II at 0^{th} day, 257.50 ±37.89 mg/dl in group-I whereas 241.50 ±24.12 mg/dl in group-II at 3^{rd} day, 230.67 ±32.97 mg/dl in group-I and 211.00 ±22.13 mg/dl in group-II at 7^{th} day, 195.17±25.47 mg/dl in group-I whereas 181.67±16.36 mg/dl in group-II at 14th day of treatment which reduces to 157.50±20.35 mg/dl and 146.00±11.10 mg/dl on 21^{th} day of treatment, respectively. Qadri *et al.* (2015) suggested that blood glucose level may go up to 700-800 mg/dl although most remain in the range of 400-600 mg/dl.

The average ± S.E. of Random Blood Glucose was found to be 287.57±43.97 mg/dl on day 0 in Group-I whereas 278.33±28.93 mg/dl in Group-II which gradually reduced to 157.50±20.35 mg/dl and 146.00±11.10 mg/dl on 21^{th} day of treatment, respectively. There was no significant difference between the days as well as between the groups for Random Blood Glucose. It indicated that the Random Blood Glucose does not significantly change during 3^{rd} day to 21^{th} day for both groups. In the present study persistent hyperglycaemia was recorded random which may be because of combination of resistance to the actions of insulin in liver and muscle together with impaired pancreatic β-cells function leading to relative insulin deficiency. However, in susceptible individuals it is likely that pancreatic β-cells are unable to sustain the increased demand for insulin. In the present subject it is likely that the excessive production of glucose in the liver and skeletal muscle result from resistance to the action of insulin.

Medicament of Group-I consisted of zinc which is an essential component of many enzymes. Zinc deficiency has been observed secondary to energy metabolism derangement. Another component of this is activated arginine as we know that proteins from main structural components of body cells and an adequate intake is essential for health. Arginine is a conditionally essential amino acid. However, incorporation of this did not appear to restore post treatment blood glucose level to physiological value.

The average ± S.E. of fasting Blood Glucose was found to be 248.67 ±44.97 mg/dl in Group-I whereas 230.22 ±17.27 mg/dl in Group-II at 0^{th} day, 219.17±34.41 mg/dl in group-I and 203.50 ±18.65 mg/dl in group-II at 3^{rd} day, 191.83±26.94 mg/dl in group-I whereas 175.17±18.96 mg/dl in group-II at 7^{th} day, 166.33±23.65 mg/dl in group-I and 147.17±11.11 mg/dl in group-II at 14^{th} day, of treatment which reduces 133.67±12.96 mg/dl in group-I whereas 116.83 ±5.59 mg/dl in group-II at 21^{st} day, respectively. Kumar *et al.* (2014) reported that in insulin treated dogs, concentration of fasting blood sugar varies between 100 and 300 mg/dl which is in accordance with the present findings of the study.

The average ± S.E. of Fasting Blood Glucose was found to be 248.67±44.97 mg/dl on day 0 in Group-I and 230.22±17.27 mg/dl in Group-II and reduced to 133.67±12.96 mg/dl in group-I and 116.83±5.59 mg/dl in group-II at 21^{st} day, respectively. There was no significant difference between the days as well as between the groups. It indicated that the fasting blood glucose does not significantly change during 3 days to 21 days for both groups. In Group-II when compared with the Group-I, the values at 21^{st} day were found within the normal reference value. Hence the effectiveness of treatment in Group-II appears to be better.

The Average±S.E. of Post-prandial Blood Glucose

Table 1. Average ± S.E. of Random Blood Glucose in dogs suffering from diabetes mellitus

Days	0 (mg/dl)	3 (mg/dl)	7 (mg/dl)	14 (mg/dl)	21 (mg/dl)
GROUP-I	287.57±43.97	257.50±37.89	230.67±32.97	195.17±25.47	157.50±20.35
GROUP-II	278.33±28.93	241.50±24.12	211.00±22.13	181.67±16.36	146.00±11.10
Result	NS	NS	NS	NS	NS

NS - non significant

Table 2. Average ± S.E. of Fasting Blood glucose in dogs suffering from diabetes mellitus

Days	0 (mg/dl)	3 (mg/dl)	7 (mg/dl)	14 (mg/dl)	21 (mg/dl)
GROUP-I	248.67±44.97	219.17±34.41	191.83±26.94	166.33±23.65	133.67±12.96
GROUP-II	230.22±17.27	203.50±18.65	175.17±18.96	147.17±11.11	116.83±5.59
Result	NS	NS	NS	NS	NS

NS- non significant

Table 3. Average ± S.E. of Post-Prandial Blood Glucose in dogs suffering from diabetes mellitus

Days	0 (mg/dl)	3 (mg/dl)	7 (mg/dl)	14 (mg/dl)	21 (mg/dl)	CD (1%/5%)
GROUP-I	303.00±46.66^{a}	252.50±35.54^{a}	222.50±31.16^{a}	198.17±27.54^{b}	160.83±16.80^{c}	94.25
GROUP-II	289.28±7.31^{a}	233.50±17.78^{b}	197.17±15.74^{c}	178.83±10.84^{cd}	149.50±5.32^{d}	48.68 /35.98

* = Significant at 5% level, NS- non significant

Different Column-wise superscripts indicate significance

Note: Rows indicate significance between the different days and it indicates using small alphabet symbol. Column indicates significance between two groups, it indicates using ** (1%) and *(5%) level of significance.

level in Group-I was 303.00±46.66, 252.50±35.54, 222.50±31.16, 198.17±27.54 and 160.83±16.80 mg/dl on 0th, 3rd, 7th, 14th and 21th day of treatment period where as 289.28±7.31, 233.50±17.78, 197.17±15.74, 178.83±10.84 and 149.50±5.32 mg/dl on respective days in Group-II.

The post-prandial blood glucose values in Group-I were 303.00±46.66 on day 0, whereas 160.83±16.80 mg/dl on 21th day of treatment period. Similarly, they were 289.28 ±7.31 mg/dl on day 0 and 149.50±5.32 mg/dl on day 21 in Group-II. The decreasing trend of post-prandial blood glucose level in both the treatment group was noticed. The difference between the intervals was significant in Group-I after 7th day. Similarly, in Group-II Post-prandial Blood Glucose level decreased consistently during 3rd to 21st day post treatment, but did not returned to normal physiological limit. These findings are in accordance with Feldman and Nelson (2004) and the Rucinsky *et al.* (2010) where insulin therapy is considered as the gold standard in canine diabetes management and effective in reducing both fasting and post-prandial glucose levels within weeks.

From the investigation, following conclusions have been drawn that the common symptoms exhibited by diabetic dogs are polydipsia, polyuria and polyphagia. There was no significant effect of treatment on fasting blood glucose levels, however, the post-prandial blood glucose levels significantly decreased between the intervals. Treatment of diabetic dogs with injections of recombinant insulin @ 0.5 IU/kg, intramuscularly twice a day resulted in significant decrease in blood glucose levels over the period of time.

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Editors/Editorial Board Members are highly thankful to all the distinguished referees who helped us in the evaluation of articles. We request them to continue to extend their co-operation and be prompt in future to give their valuable comments on the articles for timely publication of the journal.

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Research Article

HISTOMORPHOLOGICAL AND HISTOCHEMICAL STUDIES ON GOBLET CELLS, MUCOSAL AND SUBMUCOSAL GLANDS OF INTESTINE OF RABBIT

RAJESH RANJAN* and PARTHA DAS[1]
Department of Veterinary Anatomy, Co.V.Sc. & A.H., Rewa, N.D.V.S.U., 486001 India
[1]Department of Veterinary Anatomy, Faculty of Veterinary Science, WBUAFSc, Kolkata-700037, India

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ABSTRACT

The present research work was undertaken to elucidate histomorphological and histochemical characters of goblet cells, mucosal glands and submucosal glands within the rabbit intestine as these structures collectively play a role in protecting the intestinal mucosa from acidic gastric pH by secreting mucous. Since, the rabbit intestine has anatomical similarities with that of human intestine; hence, this present study was carried out in rabbits. Samples from different segments of intestine were collected, fixed, processed and stained with specific stains as per the standard protocols. The results revealed that the goblet cells and mucosal glands were present throughout the intestinal length with varying anatomical features; the submucosal Brunner's glands were limited to the duodenum only. The goblet cells and Brunner's glands were highly alcinophilic while on the other hand; the mucosal glands (Crypts of Lieberkühn) were intensely PAS-positive with scattered alcinophilic goblet cells within its secretory epithelium.

Keywords: Brunner's glands, Crypts of Lieberkühn, Goblet cells, Histochemistry, Histomorphology, Rabbit intestine

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The intestinal epithelium is protected from the adverse effects of gastric acid and enzymes by a protective mucous layer. In intestine, this mucous is secreted by goblet cells and submucosal Brunner's gland. Goblet cells are present throughout the lining epithelium of the intestine as well as mucosal glands (Crypts of Lieberkühn). The mucosal glands are present within the tunica mucosa and open at the villous base. These are simple tubular glands having goblet cells within their secretory epithelium. Brunner's gland is present specifically in the submucosa of duodenal segment of small intestine of all the mammals (Verdiglione and Montesi, 2019). They are branched tubulo-alveolar glands and have functional importance owing to mucous secretions in order to neutralize the acidic ingesta entering the small intestine from stomach and lubricating the intestinal mucosa. So, before the chyme proceeds further down, it acidity gets balanced (Zheng *et al.*, 2009). Furthermore, the rabbits are caecotrophic in nature, i.e. during the night time; they directly feed their own soft faeces. Hence, the re-ingested faeces need further protective mucous coating to protect the microbes from acidic pH of the stomach (Halls, 2008). Mucous is a gel like substance comprising of water, mucin and some electrolytes. This mucin is a high molecular weight glycoprotein that has gel forming properties which is responsible for its protective functions (Schumacher *et al.*, 2004).

Hence, this research work was designed to investigate the histological details of goblet cells, mucosal glands and Brunner's gland in rabbit, as the rabbit intestine has anatomical similarities with that of human intestine (Amiry *et al.*, 2019) that can provide the basic information for further work focused on intestinal diseases using the rabbit model for pre-clinical studies.

*Corresponding author: rajesh.ranjan837@gmail.com

MATERIALS AND METHODS

The present research work was carried out on twelve adult rabbits between body weights of 1.0 to 1.5 kg as per IAEC guidelines. The animals were euthanized and the tissue samples were collected from every 10 cm interval of intestine and quickly fixed in 10% Neutral buffered formalin (10% NBF) and Bouin's fixatives after properly washing in 0.9% normal saline solution. Processing of tissue samples was done by acetone-benzene schedule and 5-6 µm thick sections were obtained on clean glass slides. The sections were stained with Haematoxylin and eosin (H&E) for routine morphology, Masson's trichrome method for collagen fibers, Gridley's method for reticular fibers, Weigert's method for elastic fibers and PAS-AB (pH 2.5) for mucopolysaccharides contents (Luna, 1968). Micrometrical observations were recorded using Leica Qwin Image Analyzer software and microscopic photographs of stained slides were taken in Leica DM 2000 microscope. The data analysis was done using SPSS statistical software.

RESULTS AND DISCUSSION

Goblet cells

In between the columnar epithelial cells of intestine and within the mucosal glands (crypts of Lieberkühn), goblet cells were interspersed which were histologically

characterized by clear apical cytoplasm with basal nuclei (Fig. 1) which accorded with the reports of Mohammad *et al.* (2020) in sheep and rabbit. Special staining with PAS-AB (pH 2.5) indicated presence of strong alcinophilic mucinous granules within the cytoplasm of goblet cells (Fig. 2) which was in accordance with the reports of Schumacher *et al.* (2004) in cotton-tailed and domestic rabbits, Ergün *et al.* (2010) in Angora rabbits and Al-Kennany *et al.* (2012) in mice.

The average goblet cell population in the small intestine increased from oral to aboral direction which was similar with the reports of Jawad *et al.* (2019) in rabbit and rats. Within the crypts and mucosal epithelium, there was significantly highest number of goblet cells in the ileum as compared to duodenum and jejunum. The goblet cell count further increased in large intestine; however, within the mucosal epithelium the goblet cell number did not varied significantly while within the crypts of Lieberkühn, the goblet cell count in the colon was significantly high as compared to caecum and rectum (Table 1). Oliver *et al.* (2011) also reported the presence of abundant goblet cells within the colon of the African giant rat that indicated its role in increased mucosal protection and lubrication for fecal expulsion. Keskin *et al.* (2012) reported similar observation in mouse. The caecotrophic activity of rabbit has been reported by Halls (2008) which include re-ingestion of soft faeces passed out at night time. The mucous coating over the soft faeces would further prevent the caecal microbes adhered and mixed with the faeces from acidic gastric pH.

Mucosal glands (Crypts of Lieberkühn)

The mucosal glands (crypts of Lieberkühn) were present within the lamina propria of the entire length of intestine that opened between the villous bases. These glands were arranged in different layers within the lamina propria (Fig. 3). These simple branched tubular glands were lined by cuboidal to low columnar epithelium having basal spherical to oval nuclei and acidophilic cytoplasm (Fig. 4) which was in accordance with the reports of Kumar *et al.* (2013) in sheep and Mohammad *et al.* (2020) in sheep and rabbit. Special staining with PAS-AB (pH 2.5) revealed that the crypts were positive for neutral mucopolysaccharides with presence of alcinophilic goblet cells within their secretory epithelium (Fig. 2) which was similar with the reports of Al-Samawy *et al.* (2020) in rats and rabbits.

The cryptal depth in duodenum (188.13±22.361 µm) was significantly higher than that of the jejunum (95.69±8.596 µm) and ileum (103.08±9.877µm). That might be due to the reason that the crypts of Lieberkühn were present in various superficial and deeper parts in the lamina propria of duodenum (Fig. 3) and more superficially arranged in jejunum and ileum. In the large intestine, the cryptal glands within the lamina propria of caecum and rectum were relatively short and straight while, the lamina propria of colon had long and straight mucosal glands (Fig. 5) that significantly increased the mean thickness of tunica mucosa of the colon (600.84± 16.066 µm) as compared to caecum (247.35±17.536 µm) and rectum (481.83±30.938 µm). Presence of numerous goblet cells and intestinal glands in colon of the African giant had also been observed by Oliver *et al.* (2011).

Submucosal glands (Brunner's gland)

Brunner's glands were observed specifically in the submucosal layer of the duodenum (Fig. 3) only, while the remaining segments of the small intestine were completely devoid of it. Within the duodenum also, the cranial 3/4th of the total length presented densely packed Brunner's gland within its submucosa while the rest of the caudal duodenal length showed a decreasing concentration and was almost absent in the last few sections which was in accordance with the reports of Al-Samawy *et al.* (2020) in rats and rabbits.

These glands were observed to be branched tubulo-alveolar, densely packed within the submucosal connective tissue and opened at the base of the intestinal mucosal glands (crypts of Lieberkühn), due to which, the average thickness of submucosal layer of duodenum (177.14± 7.960 µm) was significantly more than that of the jejunum (24.31±1.545 µm) and ileum (44.76±5.550 µm) which accorded with the reports of Ergün *et al.* (2010) in Angora rabbit.

In routine histological staining with H & E, it was observed that the lining epithelium of glands were cuboidal to pyramidal. The nuclei of the glandular epithelium were flattened to oval, heterochromatic, basally located and strongly basophilic. The cytoplasm

Table 1. Number of Goblet cell /10,000 µm^2 area of stained slides of different parts of intestine.

Structure	Duodenum	Jejunum	Ileum	Caecum	Colon	Rectum
Cryptal epithelium	5.1±0.64^a	3.35±0.334^a	12.45±0.958^c	9.55±1.243^b	21.4±0.916^d	12.9±0.817^c
Mucosal epithelium	6.0±0.580^a	7.3±0.598^a	10.8±1.037^b	6.2±0.484^a	7.6±0.654^a	6.4±0.373^a

N.B. – Mean Values having different superscripts in a row differ significantly, where P<0.05

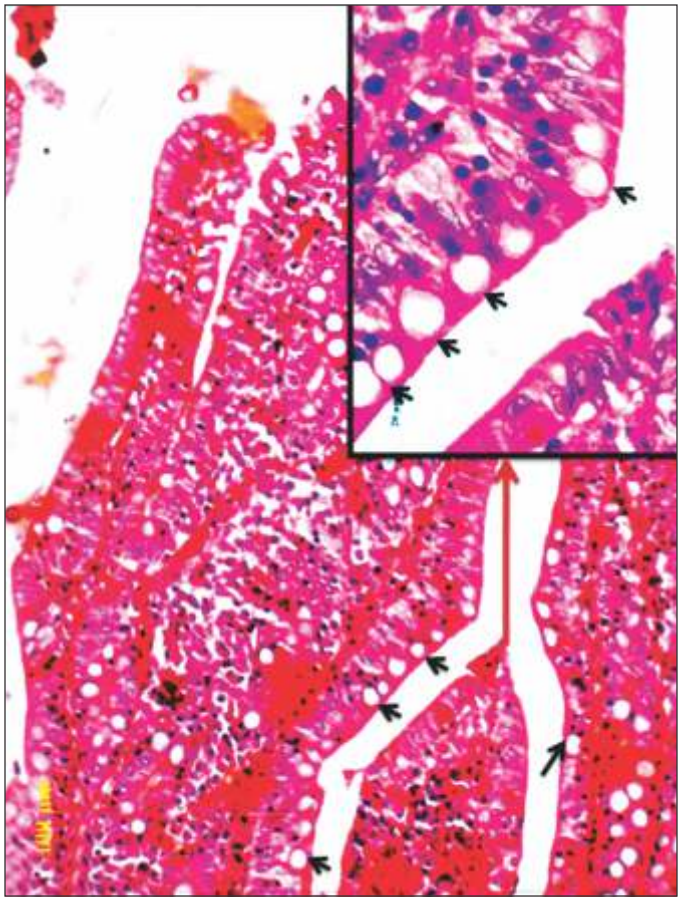

Fig. 1. Photomicrograph of rabbit ileum showing numerous goblet cells with clear apical cytoplasm (arrow) within the lining epithelium. H&E 20x. [Inset: Goblet cells. H&E 100x]

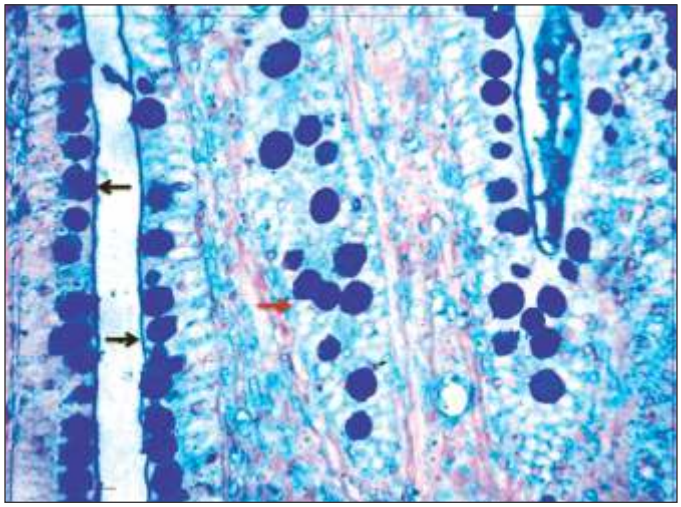

Fig. 2. Photomicrograph of rabbit ileum showing numerous alcinophilic goblet cells within the lining epithelium (black arrow) and within the crypts of Lieberkühn (brown arrow). PAS-AB 40x.

was finely granular and eosinophilic (Fig. 6) which was in accordance with the reports of Verdiglione and Montesi (2019) in bovines. Kumar *et al.* (2013) also reported varying shapes and dimensions of the acini of Brunner's glands in sheep duodenum with simple columnar glandular epithelium. The nucleus was strongly

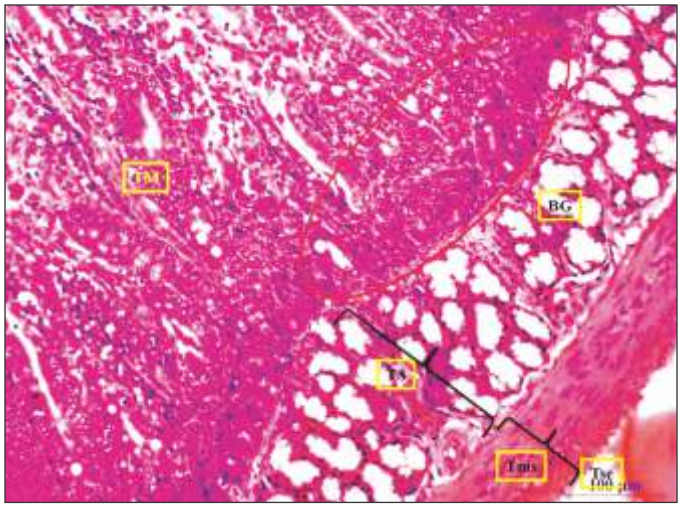

Fig. 3. Photomicrograph of rabbit duodenum showing all the tunics viz; Tunica mucosa (TM), Tunica submucosa (TS), Tunica muscularis (Tms) and Tunica Serosa (Tse). The tunica mucosa has numerous crypts of Lieberkühn (red circle) arranged in several layers. The tunica submucosa is filled with numerous Brunner's gland (BG). H&E 20x.

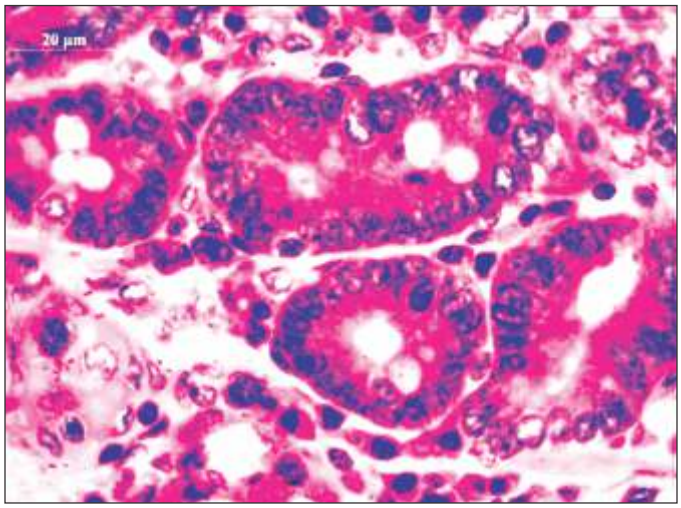

Fig. 4. Photomicrograph showing cross-sections of crypts of Lieberkühn (mucosal glands) within the lamina propria of intestine lined by cuboidal to low columnar epithelium with basal spherical to oval nucleus and acidophilic cytoplasm. H&E 100x.

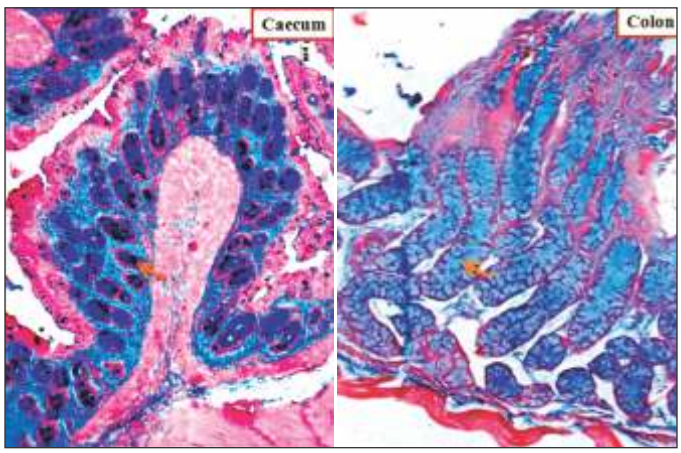

Fig. 5. Photomicrograph showing cross-sections of crypts of Lieberkühn (mucosal glands) within the lamin a propria of caecum and colon in rabbit. The cryptal glands in caecum are relatively short and straight while, very long and straight cryptal glands are present in colon. PAS-AB 10x.

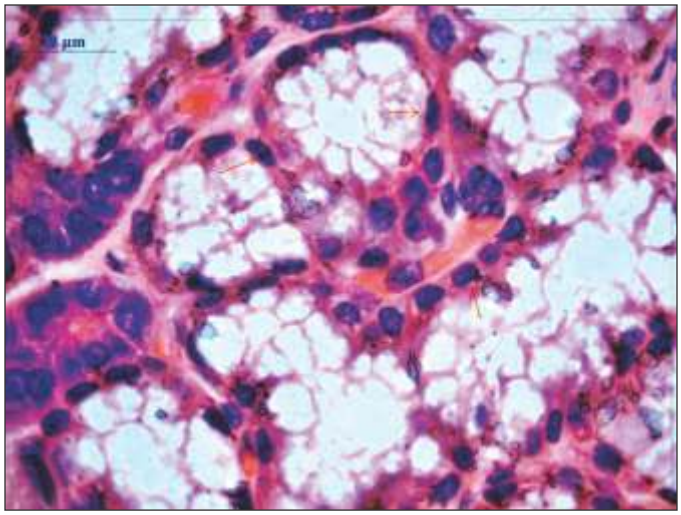

Fig. 6. Photomicrograph showing cross-sections of Brunner's glands (Submucosal glands) within the tunica submucosa of duodenum in rabbit. The nuclei of the glandular epithelium is flattened to oval, basally located and strongly basophilic (arrow). The cytoplasm is finely granular and eosinophilic. H&E 100x.

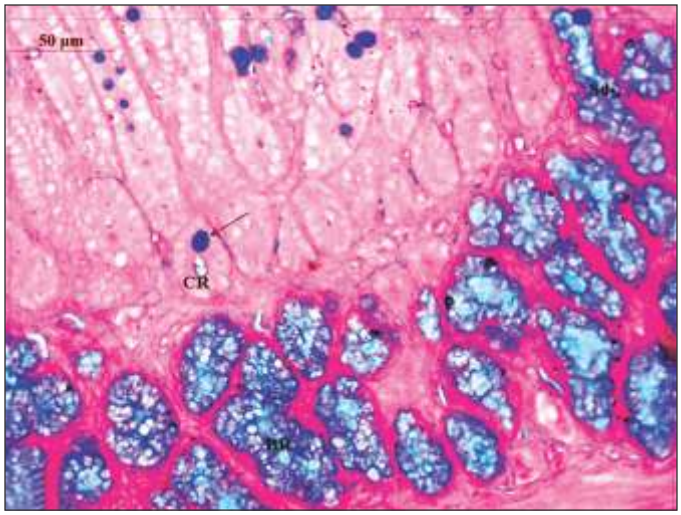

Fig. 7. Photomicrograph showing cross-sections of Brunner's glands (BR; Submucosal glands) within the tunica submucosa and crypts (CR; mucosal glands) within the lamina propria of duodenum in rabbit. The Brunner's glands and secretory duct (Sd) opening at the base of crypt are highly alcinophilic, while the crypts are positive for PAS with alcinophilic goblet cells (arrow). PAS-AB 40x.

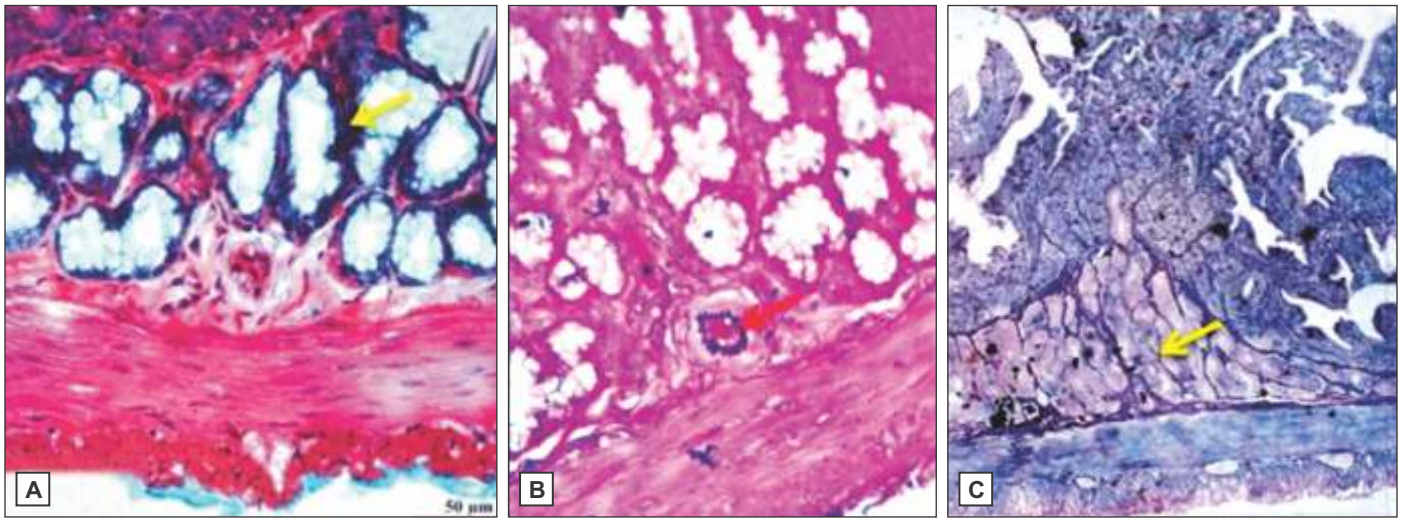

Fig. 8. Photomicrograph showing very sprase distribution of collagen fibers around Brunner's glands [arrow; (A)] elastic fibers are present in blood vessels only [arrow; (B)] and reticular fibers are present as thick bands around Brunner's glands [arrow; (C)].

basophilic, elongated and pushed towards the base. The gland concentration reduced towards the caudal part of the descending duodenum.

After specific staining with PAS-AB (pH 2.5), it was observed that these Brunner's gland consisted of strong alcinophilic mucous cells. The secretory granules within the mucous cells and the excretory duct did not react for PAS but was strongly positive for AB at pH 2.5 (Fig. 7). Very few serous cells present within the mucous acini were weakly positive for PAS. Thus it can be inferred that the mucous cells of the Brunner's gland contained acid mucopolysaccharides in a very high concentration. Krause (2000) and Verdiglione *et al.* (2002) also reported a species variation regarding mucin content of the Brunner's glands.

Upon staining for connective tissue fibers, it was evident that the submucosal layer was rich in collagen and reticular fibers along with connective tissue cells, but very sparse collagen fibers were evident around the Brunner's gland which was in conformity with the reports of Burgisser *et al.* (2024) in rabbit who also observed the presence of weak collagen staining around Brunner's gland. Elastic fibers were evident within the wall of blood vessels. Individual alveolar masses were distinctly separated from each other by thick bands of reticular fibers (Fig. 8).

CONCLUSION

The present research findings revealed a reverse relation between the goblet cell count, mucosal gland

concentration and Brunner's gland concentration in different segments of rabbit intestine. The average goblet cell population in the intestine increased from the oral to aboral direction with significantly higher count in ileum (small intestine) and colon (large intestine), while on the other hand, the submucosal Brunner's glands were present only in the duodenum, the remaining segments intestine were devoid of Brunner's gland. The mucosal glands were also present throughout the length of intestine with varying anatomical features. Within the duodenum also, the cranial 3/4th of the total length presented densely packed Brunner's gland within its submucosa while the rest of the caudal duodenal length showed a decreasing concentration and was almost absent in the last few sections. Regarding functional aspects, both the goblet cells and Brunner's glands were highly alcinophilic while, the mucosal glands (Crypts of Lieberköhn) were intensely PAS-positive with scattered alcinophilic goblet cells within its secretory epithelium indicating their role in synthesis and secretions of mucous that play definite protective role in neutralizing the acidic pH of ingesta passing from stomach to intestine, thus preventing the destruction of intestinal mucosal lining from corrosive effects of acidic pH of ingesta and other proteolytic enzymes.

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Research Article

PHYTOCHEMICAL ANALYSIS AND *IN VITRO* STUDY ON ANTIMICROBIAL, ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES OF *TRIDAX PROCUMBENS* EXTRACTS AGAINST BOVINE MASTITIS PATHOGENS

M. VENKATESAN[1]*, K.K. PONNUSWAMY[2], E. VENKATESAKUMAR [3], K. SENTHILKUMAR[4], S. JAYACHANDRAN[5], V. BHARATHI[6], R. RAGUVARAN[7], S. AKKARA[8] and A. ELANGO[9]

[1]Veterinary Clinical Complex, [3]Department of Veterinary Medicine, [9]Department of Dairy Science, Veterinary College and Research Institute, TANUVAS, Salem, Tamil Nadu - 636 112

[2]Department of Veterinary Clinical Medicine, [5]Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal- 637 002

[4]Centralized Clinical Laboratory, Madras Veterinary College, Vepery, Chennai-600 007

[6]Shrimati Indira Gandhi College, Tiruchirappalli-620002

[7]Division of Medicine, [8]Division of Bacteriology and Mycology, ICAR-IVRI, Izatnagar, Bareilly, Uttar Pradesh-243122

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ABSTRACT

This study evaluated the antimicrobial, anti-inflammatory and antioxidant properties of *Tridax procumbens* leaf extracts. The ethanolic extract demonstrated significant antibacterial activity against common bovine mastitis pathogens, including *Staphylococcus* spp., *Bacillus* spp., *Klebsiella* spp., *E. coli* and *Pseudomonas* spp. Additionally, the extract exhibited potent antioxidant properties, exceeding those of ascorbic acid, along with a notable anti-inflammatory effect. Phytochemical analysis confirmed the presence of flavonoids, phenols, tannins and other bioactive compounds. These findings indicate that *Tridax procumbens* extracts have therapeutic potential for treating bovine mastitis, warranting further investigation.

Keywords: Anti-Inflammatory, Antimicrobial, Antioxidant properties, Bovine, Mastitis, *Tridax procumbens*, Phytochemical analysis

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Mastitis, an inflammatory condition affecting the mammary gland, poses a substantial threat to the global dairy industry. This condition is marked by changes in glandular tissue and milk composition, encompassing clinical, physical, chemical, and microbiological aspects (Gera and Guha, 2011). Clinical mastitis is an economic importance, especially to small and marginal farmers due to milk discard, treatment expenses, and other related costs (Halasa *et al.*, 2007). The growing issue of antibiotic resistance among mastitis-causing pathogens, coupled with diminished treatment efficacy, has become increasingly prevalent in veterinary care (Wang *et al.* 2006). Herders often rely on antibiotics to treat and prevent bovine mastitis, but the individual use of these medications has exacerbated antibiotic resistance of common mastittogens (Srinivasan *et al.*, 2007).

Mastitis treatment is anticipated to become increasingly challenging due to the rapid rise of antibiotic-resistant pathogens that can be transmitted through foodborne infections. Currently, the primary treatment approach involves administering antibiotics via intramammary infusion, intramuscular injection, or intravenous injection (Bhosale *et al.*, 2014). However, the growing issue of antibiotic resistance has reduced the effectiveness of these treatments, and the presence of antibiotic residues in milk and dairy products poses a significant public health risk (Yang *et al.*, 2019). In search of alternative solutions, research has highlighted the potential of plant-based antibacterial agents, such as flavonoids, which exhibit therapeutic properties with minimal side effects (Lutterodt *et al.*, 1999). Notably, Tridax procumbens, a plant traditionally used in India for wound healing and other purposes, has demonstrated various pharmacological effects, including antimicrobial, anti-inflammatory and immunomodulatory activities (Taddel and Rosas-Romero, 2007). Given its potential, this study investigates the *in vitro* efficacy of *Tridax procumbens* against common pathogens causing bovine mastitis.

*Corresponding author: drsenthilkumark@yahoo.co.in

MATERIALS AND METHODS

Collection of Samples

A total of 264 milk samples were collected from cows exhibiting udder and milk abnormalities at the Veterinary Clinical Complex of Veterinary College and Research Institute, located in the Salem district of Tamil Nadu. The mastitis milk samples were aseptically collected in sterile containers from cows with mastitis and transported to the laboratory for further analysis, including

bacterial culture and isolation. Based on ABST, categorized into resistant mastitis (n=176), non-resistant (n=52) and culturally negative (n=36).

Isolation and identification of mastitogens

Each milk sample was inoculated onto Muller Hinton Agar (M/s Hi-Media Laboratories Ltd., Mumbai, India) using the streak plate method and incubated at 37 °C. Growth was monitored at 24 and 48 hours. Colony morphology was examined and bacterial smears were prepared and characterized using Gram staining. Biochemical test kits (M/s Hi-Media Laboratories Ltd., Mumbai, India) were used to further characterize the isolates. Molecular characterization of the mastitis-causing pathogens was performed using polymerase chain reaction (PCR) with microorganism-specific primers. The following mastitogens can be detected using PCR with specific primers and conditions: Staphylococcus aureus, *E. coli*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. For *S. aureus*, primers NUC1 F: ATGAAGTCAAATAAATCGCT and NUC2 R: TTTGGTGAAAAATACTTCTC are used with PCR cycling of 94°C 2 min, 40 cycles of 94°C 2 min, 55°C 2 min, 72°C 3 min and a final 72°C 10 min, yielding a 458 bp product (Gandra *et al.*, 2011). For *E. coli*, primers *uspA* F: CCGATACGCTGCCAATCAGT and R: ACGCAGACCGTAGGCCAGAT are used with PCR cycling of 94ºC 5 min, 30 cycles of 94ºC 1 min, 55ºC 1 min, 72ºC 2 min and a final 72ºC 5 min, yielding an 884 bp product (Singathia *et al.*, 2023).

For *Bacillus cereus*, primers F: AGAGTTTGATCCTGGCTCAG and R:AAGGAGGTGATCCAGCCGCA are used with PCR cycling of 95°C for 5 min, 30 cycles of 94°C 1 min, 52°C 1 min, 72°C for 90s and a final 72°C 10 min, yielding a 1500 bp product (Meng *et al.*, 2023). For *Klebsiella pneumoniae*, primers Pf: ATTTGAAGAGGTTGCAAACGAT and Pr2: CCGAAGATGTTTCACTTCTGATT are used with PCR cycling of 94°C 10 min, 35 cycles of 94°C 30s, 57°C 20s, 72°C 20s and a final 72°C 10 min, yielding a 260 bp product (Osman *et al.*, 2020). For *Pseudomonas aeruginosa*, primers PA-SS-F: GGGGGATCTTCGGACCTCA and PA-SS-R: TCCTTAGAGTGCCCACCCG are used with PCR cycling of 95°C 2 min, 25 cycles of 94°C 20 sec, 58°C 20 Sec, 72°C 40 Sec and a final 72°C 1min, yielding a 956 bp product (Spilker *et al.*, 2004).

***Tridax procumbens* plant collection**

Fresh leaves of *Tridax procumbens* were collected from the vicinity of the Veterinary College and Research Institute Campus in Salem, Tamil Nadu. The plant's taxonomic identity was confirmed by the Botany department at Rajah Serfoji Government College (Autonomous) in Thanjavur. The fresh plant material was washed with tap water, cut into small pieces, and shade-dried at room temperature for a week. The dried material was then powdered using a mechanical blender and stored in an airtight container.

Extract preparation

Aqueous and ethanolic extracts of *Tridax procumbens* were prepared using the cold maceration method at room temperature, with slight modifications to the protocols described by Monika *et al.* (2013) and Rana *et al.* (2022).

Aqueous extraction of *T. Procumbens* leaves

Ten grams of air-dried *T. Procumbens* powder was mixed with 100 mL of distilled water and kept at -20 °C for 7 days with intermittent shaking three times a day. It was filtered through eight layers of muslin cloth, then centrifuged at 5000 rpm for 10 min and filtered through Whatman No.1 filter paper. The supernatant was collected, dried and concentrated in a hot air oven at 55 °C for 12-24 hours. The extracts were collected and stored at -20 °C until further processing.

Ethanol extraction of *T. procumbens* leaves

Ten grams of air-dried *T. Procumbens* powder was mixed with 100 mL of 70% ethanol and kept at -20 °C for 7 days with intermittent shaking three times a day. It was then filtered through eight layers of muslin cloth, centrifuged at 5000 rpm for 10 minutes and then filtered through Whatman No. 1 filter paper. The supernatant was collected, dried, and concentrated in a hot air oven at 55 °C for 12 - 24 hours. The extracts were collected and stored at -20°C until further processing.

Phytochemical screening

Phytochemical analysis was conducted on the aqueous and ethanolic extracts using standard procedures to identify the phytoconstituents, following methods described by Sofowara (1993), Trease and Evans (1989), Harborne (1991) and Dharajiya *et al.* (2012).

Quantitative analysis of phytochemicals in *T. Procumbens* extracts

The total flavonoid content of the extracts was determined using a colorimetric method (Nabavi *et al.*, 2008), expressed as milligrams of catechin per gram of extract. Total phenolic content was measured using the Folin-Ciocalteu reagent method (Biglari *et al.*, 2008), with results expressed as milligrams of gallic acid equivalent

per gram of dry weight (mg GAE/g). The phenolic compound content was calculated using the formula C = A/B, where C represents the concentration (mg GAE/g dry weight), A is the equivalent gallic acid concentration from the calibration curve (mg) and B is the dry weight of the extract (g). Additionally, Thin Layer Chromatography (TLC) was used to quantify phenol and flavonoid compounds in the ethanolic extract.

Antibacterial activity of the extract of *T. Procumbens*

The antibacterial activity of aqueous and ethanolic extracts was evaluated against five mastitis-causing pathogens. For each bacterium (*Staphylococcus* spp., *Bacillus* spp., *Klebsiella* spp., *Escherichia coli* and *Pseudomonas* spp.), individually tested the antibacterial properties of the plant extracts (using the agar well diffusion method (Perez *et al.*, 1990; Nair and Chanda, 2005). Briefly, Mueller Hinton Agar plates were inoculated with 0.5 McFarland (108 CFU/mL) bacterial suspensions, and a well (0.85 cm) was created and filled with 100 µL of extract. After overnight incubation at 37°C, antibacterial activity was assessed by measuring the zone of inhibition (mm). Controls included pure solvents and Gentamicin, and each experiment was repeated three times, with results presented as mean values ± standard deviation (SD) (Parekh and Chanda, 2007).

Antioxidant property of the *T. procumbens*

The antioxidant property of *T. procumbens* leaves was evaluated using a DPPH assay kit. To a methanolic solution of DPPH (90.25 mm), an equal volume of ethanolic leaf extract of *T. procumbens* was added and made up to 1.0 mL with methanolic DPPH. An equal amount of methanol was added to the control. After 20 min, the absorbance was recorded at 517 nm in a Systronics UV-visible Spectrophotometer. Ascorbic acid was used as a standard for comparison. The percentage inhibition of free radicals by DPPH was calculated using a specific equation.

$$\% \text{ Scavenging} = \frac{\text{A Control OD - A sample}}{\text{A blank}} \times 100$$

Where A control is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound.

Anti-inflammatory activity of *T. procumbens* extracts

The anti-inflammatory activity of *T. procumbens* was evaluated using the albumin denaturation inhibition technique, based on methods described by Mizushima and Kobayashi (1968) and Sakat *et al.* (2010) and followed with minor modifications. Briefly, the reaction mixture consisted of test extracts, and a 1% aqueous solution of bovine albumin fraction was prepared, and the pH of the reaction mixture was adjusted with 1N HCl. The sample extracts were incubated at 37 ºC for 20 min and then heated to 51 ºC for 20 min, then the sample was cooled and the turbidity was measured at 660 nm with UV-Visible Spectrophotometer Model 371(M/s Elico India Ltd). The experiment was performed in triplicates.

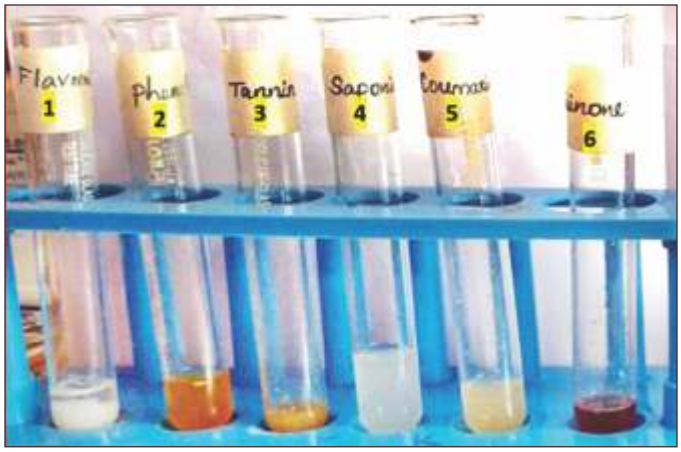

Fig. 1. Results of qualitative analysis of Aqueous and Ethanolic *Tridax procumbens* leaves, showcasing positive and negative outcomes. 1. Shinoda test (Flavonoids), 2. $FeCl_3$ test (Phenol), 3. Lead acetate test (Tannin), 4. Foam test (Saponin), 5. Sodium chloride test (Coumarins), 6. KOH test (Quinine)

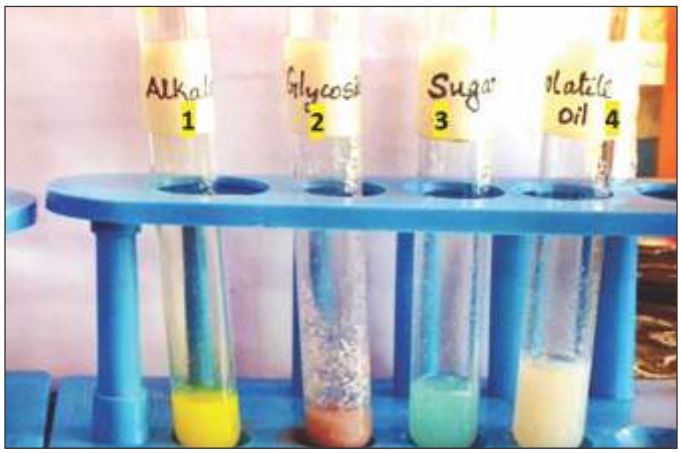

Fig. 2. Results of qualitative analysis of Aqueous and Ethanolic *Tridax procumbens* leaves, showcasing positive and negative outcomes. 1. Alkaloids, 2. Legal's test (Glycoside), 3. Sugar test, 4. Volatile oil test.

RESULTS AND DISCUSSION

The phytochemical screening results for aqueous and ethanolic extracts of *Tridax procumbens* leaves are presented in Table 1. The analysis revealed a greater diversity of phytochemicals in the ethanolic extracts, including flavonoids, phenols, tannins, coumarins and quinones, compared to the aqueous extracts, which contained tannins, quinones, alkaloids and volatile oils (Figures 1 and 2). This study found that ethanolic extracts of *T. procumbens* contained a greater variety of

Table. 1. Qualitative phytochemical evaluation of Aqueous and Ethanolic extracts of Tridax procumbens

S.No.	Phytochemical	Aqueous extract	Ethanolic Extract
1.	Flavonoids	-	+
2.	Phenol	-	+
3.	Tannin	++	+
4.	Saponin	-	-
5.	Coumarin	-	+
6.	Quinone	+	+
7.	Alkaloids	+++	-
8.	Glycosides	-	-
9.	Sugar	-	-
10.	Volatile oil	++	-

Table. 2. Antibacterial activity of Aqueous and Ethanolic extracts of Tridax Procumbens (Diameter of Zone of inhibition in mm)

Mastitogens	Aqueous extracts (n=43)	Ethanolic extracts (n=43)	Positive control Gentamicin (n=43)
Staphylococcus aureus (n=8)	4.8 ± 0.3	10.5 ± 0.3	23.4 ± 0.6
Bacillus spp. (n=8)	3.7 ± 0.4	11.4 ± 0.4	18.1 ± 0.3
E. coli (n=8)	3.3 ± 0.2	9.4 ± 0.4	21.8 ± 0.5
Klebsiella spp. (n=9)	NA	7.7 ± 0.4	19 ± 0.6
Pseudomonas spp. (n=9)	NA	6.8 ± 0.3	20.2 ± 0.6

NA – no activity

phytochemicals (flavonoids, phenols, tannin, coumarin and quinone) compared to aqueous extracts (tannin, quinone, alkaloids and volatile oil). These findings align with previous research, Syed *et al.* (2020) and Archana *et al.* (2022), reported the presence of glycosides, flavonoids, carbohydrates, polyphenols and tannins in ethanolic extracts, and glycosides, flavonoids, saponins, and polyphenols in water extracts. Similarly, Sawant and Godghate (2013) detected various phytochemicals, including saponin, steroids, diterpenes, alkaloids, flavonoids, phenols and tannins, in *T. procumbens* leave.

The extraction yield of *T. procumbens* leaves using different solvents showed that hydro-ethanolic solvent resulted in the highest yield (19.8%), followed by ethanolic solvent (12.8%) and aqueous extract (11.4%).

The aqueous extract of *T. procumbens* contained 1.037 mg/100g of phenols, with a total phenolic content of 68.65 mg GAE/g (Folin-Ciocalteu method), and 35 mg/g of flavonoids (aluminum chloride assay). The ethanolic extract contained 1.1 mg/100g of tannins. Further analysis using thin-layer chromatography (TLC) detected phenol (Rf = 0.5) and flavonoid (Rf = 0.3) in the ethanolic extract.

The antibacterial activity of *T. procumbens* extracts against common bovine mastitis pathogens showed that the ethanolic extract exhibited stronger antibacterial effects than the aqueous extract (Table 2). The minimum inhibitory concentration (MIC) of the ethanolic extract revealed that Gram-positive bacteria were more susceptible to the extract than Gram-negative bacteria, requiring a lower amount of extract for inhibition. The antibacterial properties of *T. procumbens* are attributed to the presence of phytochemicals like flavonoids, alkaloids, saponins and tannins (Mundada and Shivhare, 2010). This study found that the ethanolic extract exhibited significant antibacterial activity against common mastitis pathogens, including *Staphylococcus* spp., *Bacillus* spp., *Klebsiella* spp., *E. coli* and *Pseudomonas* spp. In contrast, the aqueous extract showed minimal activity against some pathogens and no activity against others. These findings are consistent with previous research conducted by Aniel and Naidu (2010), who reported antibacterial activity in ethanolic extracts but not in aqueous extracts. Other studies have also demonstrated the antimicrobial effects of *T. procumbens* leaf extracts against both Gram-positive and Gram-negative bacteria (Udopa *et al*., 2014; Chitra *et al.*, 2011). The present study confirms that the ethanolic extract has higher antibacterial activity than the aqueous extract, suggesting that *T. procumbens* leaves are a promising source of antimicrobial compounds (Bharathi *et al.*, 2012; Arulraj *et al.*, 2018).

The antioxidant activity of *T. procumbens* extracts was evaluated using the DPPH method. The ethanolic extract showed a higher percentage of inhibition than the standard antioxidant ascorbic acid, followed by the hydroethanolic extract and aqueous extract. This study found that the ethanolic extract of *T. procumbens* leaves exhibited higher antioxidant activity, with a greater percentage of inhibition than the standard antioxidant ascorbic acid, followed by hydroethanolic and aqueous extracts. These findings are consistent with Syed *et al.* (2020), who reported significant antioxidant activity in ethanolic extracts (61.52 ± 0.32%) and lower activity in the aqueous extracts (25.57 ± 0.38%). *T. procumbens* has been strongly proven for its medicinal and analgesic activities.

In animal studies, the protective effects are linked to the presence of flavonoids and sterols (Vinod *et al.*, 2013). *T. procumbens* is commonly used for pharmacological purposes because of its wide range of phytochemical properties. These include analgesic, anticoagulant, antileishmanial, antioxidant, anticancer, immunomodulatory, insecticidal, anthelmintic, cardiovascular, antiseptic, antimicrobial and insecticidal effects (Varsharani *et al.*, 2022). In this present study, compared to aqueous extracts, the ethanolic extracts provided better activity because of the compound's flavonoids and phenols, the presence of which was confirmed by qualitative and quantitative

screening of these compounds.

The anti-inflammatory activity of *T. procumbens* extracts was assessed using the albumin denaturation assay. The results showed that the ethanolic extract exhibited higher anti-inflammatory activity than the standard control, aspirin, followed by the hydroethanolic extract and aqueous extract.

Geographical variation significantly impacts the phytochemical composition of plants. Factors like climate, soil composition and altitude, which vary across different regions, influence the types and amounts of secondary metabolites (phytochemicals) a plant produces. Therefore, selected plants should be subjected to qualitative and quantitative fingerprinting and that should be matched with available published reports.

CONCLUSION

The ethanolic extract of *T. procumbens* leaves contains various bioactive compounds, indicating significant potential. This study demonstrates that hydroethanolic and ethanolic extracts exhibit potent antimicrobial, anti-inflammatory and antioxidant properties against common bovine mastitis pathogens. These findings suggest that *T. procumbens* extracts may serve as a valuable therapeutic option for treating bovine mastitis.

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IMPORTANT INFORMATION

- Research/Clinical articles are invited for next issue from the Scientists/Veterinarians engaged in Veterinary Profession.
- Please follow strictly the format of **'The Haryana Veterinarian'** for manuscript writing/ submission.
- **The article processing and publication charges will be revised from Rs. 1200/- to 1500/- per manuscript with effect from the next year (01.01.2027).**
- We solicit your co-operation.
- All correspondence should be addressed to the 'Editor, The Haryana Veterinarian', Department of Veterinary Parasitology, College of Veterinary Sciences, LUVAS, Hisar-125004, Haryana.

Editors

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Research Article

PARASITOLOGICAL DIAGNOSIS WITH HAEMATO-BIOCHEMICAL ALTERATION AND THERAPEUTIC MANAGEMENT OF *SCHISTOSOMA INDICUM* IN BULLOCK: A CASE REPORT

N.N. BRAHMBHATT, B.J. THAKRE, V.D. DODIA[1], A.S. PATEL*[2] and DARSHNA CHAUDHARY
Department of Veterinary Parasitology, [1]Department of Veterinary Clinical Complex,
[2]Department of Livestock Products Technology,
College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, India

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ABSTRACT

Two Kankrej bullocks, ages 3.5 and 5 years, were brought to the Veterinary Clinical Complex, Veterinary College, Kamdhenu University, Junagadh with symptoms of anorexia, brisket oedema, abdominal pain persisted for five days, reduced feed as well as water intake, bloody, watery, blackish colour diarrhoea. Clinical findings included dehydration, tenesmus, colic symptoms, pale conjunctival mucus membrane, blood mixed with black diarrhoea, and a rectal temperature of about 103ºF. Examination of the faecal sample indicated a distinctive *Schistosoma indicum* egg, with the characteristic of small spine at posterior region. The haematological analysis revealed anaemia with neutrophilia, while serum biochemical test revealed elevated values of SGPT, SGOT, BUN and hypoprotenemia. Infected bullocks were administered a single oral dose of Closantel@-10 mg/kg body weight, complemented by supportive therapies including ranitidine, metronidazole, fluid therapy, meloxicam, chlorpheniramine maleate, and haematinics. By the 15th-day post-treatment, faecal sample examinations revealed no detectable parasitic eggs, and haemato-biochemical parameters had returned to normal ranges, indicating a successful recovery. This treatment regimen aligns with documented efficacy against *Schistosoma indicum* infections in bovines.

Keywords: Dysentery, Faecal examination, Haemato-biochemical, *Schistosoma indicum*

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Schistosomiosis is a significant parasitic disease affecting both domestic and wild animals across tropical and subtropical regions of Asia (Ayanda, 2009). The sixth most prevalent helminthosis afflicting domestic animals in the Indian subcontinent is schistosomiosis (Sumanth *et al.*, 2004). According to Kumar and Burbure (1986), *Schistosoma indicum* is one of the prevalent species that causes visceral schistosomiosis in cattle. In India, the disease is common in goats, sheep, cattle, and buffaloes and it's popularly called as "blood flukes". Additionally, schistosomes are morphologically elongated, unisexual trematodes parasite that live in the blood vessels of their definitive hosts.

Adult schistosomes colonize specific blood vessels based on their species: *Schistosoma nasalis* inhabits the capillaries of the nasal mucosa; *S. haematobium* resides in the venous plexus of the urinary bladder and the intestinal and visceral species are *Schistosoma indicum*, *S. spindale* and *S. incognitum* are found within the mesenteric venules. The most prevalent infections in endemic parts of the Indian subcontinent are caused by *S. indicum*, which is subclinical and costs the farming community a lot of money in terms of growth, productivity and increased vulnerability to other parasites (Bulbul *et al.*, 2022). *Schistosoma indicum* females produce numerous oval eggs, each bearing a terminal spine and containing a fully developed miracidium upon laying (Soulsby, 2012). Animals display common clinical signs like dehydration, pale mucous membrane, significant weight loss, emaciation, and decreased production caused due to the irritation produce by the spined eggs (Bhatia *et al.*, 2010).

Clinical symptoms and an investigation of infected animal's faeces are necessary for the routine diagnosis of *S. indicum* infection. Current communication describes the diagnosis of *S. indicum* infection in bullocks using parasitological technique, haemato-biochemistry, and therapeutic management.

MATERIALS AND METHODS

Two bullocks were presented to the Department of Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Junagadh with a history of blood mixed watery black colour diarrhoea, reduced feed and water intake, anorexia, brisket oedema, 103 ºF body temperature and abdominal pain for last five days. For further diagnosis, faecal sample examination was recommended and standard sedimentation methods described by Hansen and Perry (1994) and Rana *et al.*, (2011) were used to concentrate the samples. Additionally, blood samples were drawn from the animal's jugular vein in both serum and an EDTA-coated vacutainer for hematological and biochemical analysis.

*Corresponding author: ajay.patel@kamdhenuuni.edu.in

RESULTS AND DISCUSSION

a) Clinical manifestations

Schistosomes are significant helminth parasite responsible for causing infections in both human and animals worldwide. Cattle and buffaloes, due to their habit of free grazing in waterlogged area where infective cercariae are present, are highly susceptible to *Schistsosome* infections (Rashid *et al.*, 2022). In the present case of Kankrej bullocks, clinical signs such as pale mucus membrane, bloody diarrhea, tenesmus and straining while defecation were observed. These findings are consistent with observation by Sucharitha *et al.*, (2024). Physical examination revealed blood mixed watery black colour diarrhoea, rectal temperature of around 103 ºF, pale conjunctival mucus membrane, dehydration, tenesmus and colic signs. Additional clinical findings included dull behavior, lethargy and unwilling to move. Vital parameters such as heart rate and respiratory rate were noted to be within the normal limits, through the elevated body temperature and dehydration point towards systemic involvement. Base on history and clinical signs, it was suspected that bull might be suffering from severe gastrointestinal infection (Fig.1 & 2).

b) Microscopic examination

Faecal sample examination using the sedimentation technique revealed oval, thin-shelled eggs with a terminal spine, characteristic of *Schistosoma indicum* identified based on their distinctive morphology (Figs. 3 & 4) (Bhatia *et al.*, 2010 and Chaudhary *et al.*, 2000).

Possible differential diagnosis was done for paramphistomosis, fasciolosis and coccidiosis.

The gastrointestinal tract is the main site of schistosomosis, which is caused by the genus *Schistsosoma* and results in anorexia and bloody diarrhea. Local cattle breed had a higher risk of infection for bovine schistosomosis than crossbred (Yihunie *et al.*, 2018). Diarrhoea, weight loss, anemia, hypoalbuminemia, hyperglobulinemia and pronounced eosinophilia typically develop once egg shedding begins. Animals with heavy infections often deteriorate rapidly and may die within a few months, whereas those with milder infections exhibit growth retardation and chronic illness (Yogeshpriya, 2022).

c) Haemato-biochemical parameters

Serum biochemical study indicates elevated levels of SGPT, SGOT, blood urea nitrogen and hypoproteinaemia. Haematological results were not significantly changed, but demonstrated mild anaemia, neutrophilia and an elevated total leucocyte count. As a result of parasite-induced damage to intestinal and mesenteric arteries during parasite migration and egg deposition, anaemia and leucocytosis were frequently observed. In contrast, leucocytosis is brought on by an inflammatory reaction to schistosome antigens that are present during severe and acute infections. According to serum biochemical data, migrating schistosome induced hepatic cell damage resulted in raised levels of SGOT and SGPT, while dehydration or decreased renal clearance caused an increase in BUN. Here, both bullocks exhibit hypoproteinaemia as a result of the host's intestinal injury (Table 1 & 2). When coupled, haemato-biochemical marker along with faecal sample examination aids in diagnosis.

d) Therapeutic Management

To treat schistosomosis and its related complications, a thorough treatment protocol was started. Praziquantel @ 25 mg/kg and Closantel @10 mg/kg body weight P/O were administered to the afflicted Kankrej bullocks on the first day of presentation and continued for a total of 14 days. To address dehydration and restore electrolyte balance, supportive treatment included Metronidazole @ 25 mg/kg BW IV, Streptochrome at 10 ml IM, Meloxicam @ 0.2 mg/kg BW IM, Chlorpheniramine Maleate @ 0.5 mg/kg BW IM for anti-inflammatory and allergic reaction, and Ranitidine @10 ml IM.

The animals' bloody diarrhea decreased on the second day of treatment, and the following treatments were administered on the second and third days: Metronidazole at a dose of @ 25 mg/kg BW IV, Tribivet @15 ml IM, Meloxicam @0.2 mg/kg BW, IM, Chlorpheneramine Maleate @ 0.5 mg/kg BW IM and Streptochrome @10 ml IM. After three days, both bullocks began eating normally and the faeces substance and appearance were normal. This comprehensive strategy bought to effectively promote the animal's rehabilitation, address underlying issues and reduce clinical symptoms. The recovery was evaluated based on the fact that, following the fifteenth day of treatment, no eggs were found in the faecal sample. After 15 days, regular feeding, watering and faecal consistency also indicated clinical recovery.

Schistosoma indicum females deposit ova which is oval, thin-shelled and bearing a terminal spine via the faeces of infected ruminants. Upon contact with freshwater, these eggs hatch almost immediately, releasing miracidia that actively penetrate the gastropod *Indoplanorbis exustus* a well-established intermediate host. Within the snail, the miracidium develops into a mother sporocyst, which gives rise to daughter sporocysts; the latter generate motile cercariae, the infective larval stage. These cercariae emerge and actively penetrate the skin of the definitive bovine host, subsequently transforming into schistosomula that migrate via the circulatory system to mature into adults within the mesenteric vein. Clinically, *S. indicum* infection in Kankrej bullocks' manifests with muco-haemorrhagic diarrhoea, elevated rectal temperatures, pale mucous

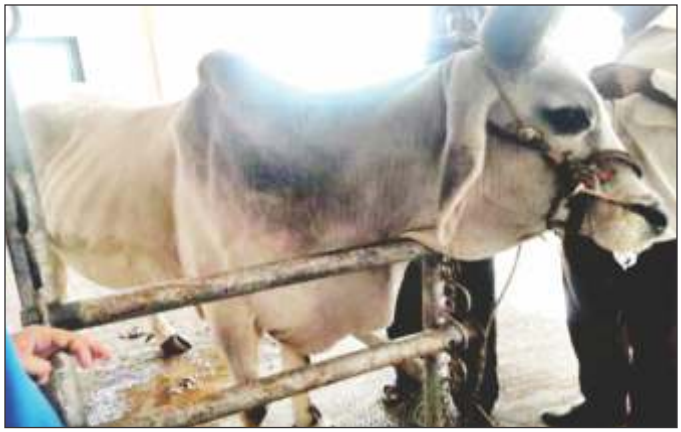

Fig. 1. Bullock with symptoms (Age 3.5 year)

Fig. 2. Bullock with symptoms (Age 5 Year)

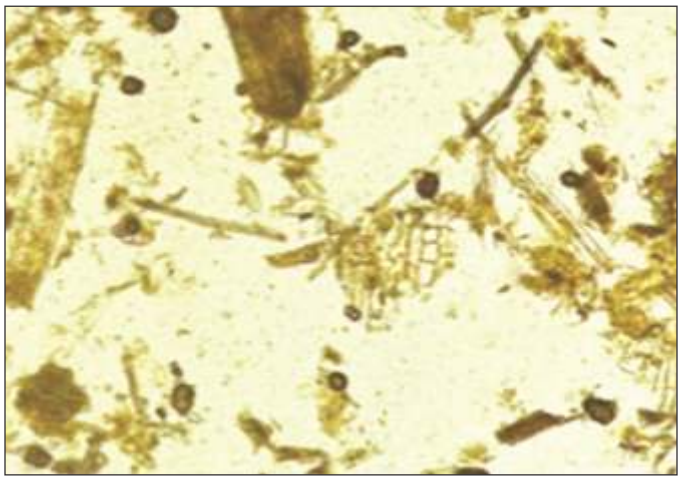

Fig. 3. Egg of Schistosoma indicum (10X)

Fig. 4. Egg of Schistosoma indicum (40X)

(Size of egg: 130-160 µm in length × 70-90 µm)

Table 1. Haematological parameters in diseased bullocks

Parameters	References ranges	Before treatment (0^{th} day)		After treatment (15^{th} day)	
		3.5 years bullock	5 years bullock	3.5 years bullock	5 years bullock
Haemoglobin (g/dL)	8-14	7.8	8.8	11.8	12.3
PCV (%)	24-44	31.56	40	40.6	39.5
RBCs ($x10^6$/µL)	5-9.5	7..84	7.57	8.2	8.9
MCV (fl)	40-60	42	53	56.2	57.0
MCH (pg)	10-17	11.6	14	15.0	16.8
MCHC (g/dl)	30-34	27.8	26	33.4	33.9
Plateles ($x10^6$/µL)	1-10	2.58	2.07	6.0	8.0
WBCs count	4000-11000	12340	14930	10232	11020
Neutrophils (%)	15-45	49.0	47.0	23.0	29.0
Lymphocytes (%)	40-75	59.0	49.0	43.0	66.0
Monocytes (%)	2-8	1.0	1.0	6.0	7.0
Eosinophils (%)	1-15	2.00	1.00	0.00	0.0
Basophils (%)	0-2	0.00	0.00	0.00	0.00

membranes, abdominal discomfort, tenesmus and anal spasms. These symptoms reflect the inflammatory and haemorrhagic damage induced by egg migration across the intestinal mucosa and mesenteric vessels.

Digraskar *et al.* (2018) and Deepak *et al.* (2022) reported similar clinical findings. Anaemia and eosinophilia were indicated by the haematological examination, which also showed a decrease in Hb, PCV, TEC and a high eosinophil count. Digraskar *et al.* (2018) and Giri *et al.* (2018) reported similar results. Examination of faecal sample showed that the egg of *S. indicum* had a terminal spine and a miracidium within (Fig. 1). Fresh faeces can be examined to diagnose hepato-intestinal schistosomosis

Table 2. Serum Biochemical parameters in diseased bullocks

Parameters	References ranges	Before treatment (0^{th} day)		After treatment (15^{th} day)	
		3.5 years bullock	5 years bullock	3.5 years bullock	5 years bullock
Creatinine (mg/dl)	0.8-2.5	1.90	2.09	1.0	1.25
Blood Urea Nitrogen (mg/dl)	6.25	27.01	32.11	13.02	17.5
Cholesterol (mg/dl)	35-160	64	71	60	69
SGOT(IU/L)	60-115	126.53	128.12	110.12	109.5
Bilirubin –Total (mg/dl)	0-1.9	0.17	0.03	0.7	0.5
Total Protein (g/dL)	5.6-7.0	4.6	5.0	6.9	7.2
Albumin (g/dL)	2.0-3.5	3.91	3.58	2.5	3.1
SGPT- (IU/ml)	6.9-35.3	40.3	39.01	20.2	30.3

(Bhatia *et al.*, 2006; Digraskar *et al.*, 2018; Giri *et al.*, 2018; Deepak *et al.*, 2022). A single oral dose of closantel @ 10 mg/kg body weight, administered alongside supportive therapy, proved effective in the treatment of *S. indicum* infection in bullocks. Closantel, a salicylanilide anthelmintic, acts by disrupting oxidative phosphorylation in the parasite's mitochondria, leading to a critical depletion of energy reserves (Sandhu, 2013). The drug interferes with the mitochondrial proton gradient, thereby inhibiting ATP synthesis, which is essential for parasite survival and motility (Pax & Bannette, 1989). This mechanism underscores its potent flukicidal activity and therapeutic efficacy in managing trematode infections.

CONCLUSION

This case report highlights the importance of a comprehensive, integrated treatment strategy in managing *S. indicum* infections in livestock. *S. indicum* infection in Kankrej bullocks presented with distinct clinical signs of anaemia, diarrhea, edema and fever, which can be effectively diagnosed through faecal sample examination to identify the characteristic eggs. Haemato-biochemical findings, including anemia, neutrophilia and hypoproteinemia, further supported the diagnosis and revealed the systemic impact of the parasitic infection. The use of anthelmintics, particularly closantel and praziquantel, proves to be effective in managing the infection by targeting the parasites' metabolic processes and causing their expulsion. Timely intervention with appropriate treatment offers a favorable prognosis, restoring health and improving the overall productivity of the affected animal. Early detection and treatment remain a key to prevent severe complications and ensuring the well-being of livestock.

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Research Article

KLEIBER RATIO IN SIROHI GOATS

VISHNU KUMAR* and R.K. NAGDA[1]
Department of Animal Genetics and Breeding
Post Graduate Institute of Veterinary Education and Research, Jaipur
[1]College of Veterinary and Animal Science, Navania, Udaipur.

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ABSTRACT

To estimate the pre and post weaning Kleiber ratios (KR) and the factors influencing them, data from 6,748 Sirohi goats in farmers' flocks were analyzed. The overall least-squares means for the Kleiber ratio from birth to three months (KR1) and from three months to twelve months (KR2) were 16.26 ± 0.06 and 4.11 ± 0.03 gm/kg of metabolic weight, respectively. The study examined the effects of sire, period, season of birth, sex of the kid, type of birth, cluster, and parity on the Kleiber ratios. Among these factors, the sire was treated as a random effect, while all others were considered fixed effects. The results indicated significant effect ($P \leq 0.05$) of all the factors on both KR1 and KR2. Heritability estimates for KR1 and KR2 were 0.65 ± 0.07 and 0.29 ± 0.05, respectively. The genetic and phenotypic correlations between KR1 and KR2 were found to be high but negative.

Keywords: Genetic correlation, Heritability, Kleiber ratio, Least-squares means, Sire, Sirohi goat, Phenotypic correlation

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Among the 41 registered goat breeds across India's diverse agro-climatic zones, the Sirohi goat holds a prestigious status due to its superior qualities, such as faster growth rates, lower disease incidences, and enhanced adaptability to dry, hot climates. The breed is predominantly found in the regions surrounding the Aravali hills in Rajasthan, particularly in the districts of Sirohi, Udaipur, Rajsamand, Pratapgarh, Chittorgarh, Nagaur and Ajmer. Additionally, its popularity has led to the expansion of its home tract into other adjoining areas also. The importance of goats in the Indian meat industry is undeniable. As a meat animal, fast growth and efficient feed conversion are key economic traits. In India, where most goat flocks are reared under extensive or semi-intensive systems, traditional metabolic trials are often not feasible for assessing feed conversion efficiency in free-range grazing conditions.

The Kleiber ratio (KR), defined as growth rate/body weight$^{0.75}$ (Kleiber, 1947), provides an indirect but reliable criterion to evaluate feed conversion efficiency. This ratio is independent of body size and does not require individual intake measurements, making it especially useful for free-range animals. Goats with a higher KR require less maintenance energy, thereby demonstrating a more efficient use of feed. (Vyas *et al.*, 2024) However, studies on the Kleiber ratio in Sirohi goats are scarce. To address this gap, the present study was undertaken to identify the factors influencing the Kleiber ratio and to estimate both the genetic and phenotypic parameters associated with this trait in Sirohi goats under field condition.

*Corresponding author: vishnukumarvijay@gmail.com

MATERIALS AND METHODS

The data used for this study were collected from Sirohi kids born between 2005 and 2017 in farmers' flocks registered under the All India Coordinated Research Project (AICRP) on Goats (Sirohi Field Unit), Livestock Research Station (LRS), Vallabhnagar, Udaipur. As per the technical protocol of the project, all registered animals were properly identified through ear tagging. Vaccination and deworming were carried out according to established guidelines, ensuring the health and welfare of the goats. To improve the genetic quality of the flock, elite Sirohi bucks were provided to farmers for breeding. The farming system predominantly followed a semi-intensive management approach, where goats were allowed to graze for 8-10 hours a day, while they were housed at night. The goats primarily fed on natural vegetation, including shrubs such as Ber (*Ziziphus mauritiana*), Ker (*Capparis decidua*) and Thor (*Euphorbia caducifolia*), as well as trees like Neem (*Azadirachta indica*), Khejri (*Prosopis cineneraria*) and Ardu (*Ailanthus excelsa*). Kids were weaned at 3 months of age.

Kleiber ratio from birth to three months age (KR1) and from three months to twelve months age (KR2) were calculated as following formulae:

KR1 = ADG from 0-3M in gms. /Metabolic weight at 3M age (3 MBWT$^{0.75}$) in Kg.

KR2 = ADG from 3-12M in gms. /Metabolic weight at 12M age (12 MBWT$^{0.75}$) in Kg.

As the data were non-orthogonal, analysis was performed to estimate the least-squares means and genetic parameters through Mixed Model Least-Squares and

Maximum Likelihood method designed by Harvey (1990). Duncan's Multiple Range Test as modified by Kramer (1957) was used to make pairwise comparison among the least squares means.

Effect of sire, period, season of birth, sex of kid, type of birth, cluster and parity were studied on Kleiber ratios. Among these, sire was considered as random effect whereas all others were as fixed effects. Year of birth was categorized into 3 periods: P1 (2005-2009), P2 (2010-2013) and P3 (2014-2017). Season of birth was classified into three categories i.e. rainy (July-October), winter (November-February) and summer (March-June). Sex was grouped as male and female whereas type of birth was categorized as single and multiples. Data were collected from six clusters that were Vallabhnagar (Udaipur), Railmagra (Rajasamand), Deogarh (Rajasamand), Nathdwara (Rajasamand), Bhadsoda (Chittaugarh), Karget (Udaipur), Bojunda (Chittaugarh) and Salumber (Salumber). Parity of dam was classified as first to fifth. Kids born in above fifth parity were pooled into 5th parity group.

RESULTS AND DISCUSSIONS

The overall least-squares means for KR1 (0-3M) and KR2 (3-12M) were 16.38±0.03 and 4.11±0.05, respectively which has shown in table 1. Higher Kleiber ratio from birth to three months age depicts the better feed conversion efficiency during pre-weaning stage which occurs due to suckling of dam's which serves as a complete nutritious food for kid. Similar performance for KR1 was observed by Barazandeh *et al.* (2012) as 15 and Mokhtari *et al.* (2019) as 15.27 in Raieni cashmere goats, Khadda *et al.* (2018) as 15.29±0.16 in Pantja goats and Thepparat *et al.* (2012) as 14.82 to 18.00 in mixed population of goats. However, the finding of KR1 in Sirohi goats was found closer to the sheep rather than goats as per available publications. KR1 was recorded by Ved Praksah *et al.* (2012a) as 16.88±0.06 in Malpura sheep, Mandal *et al.* (2015) as 16.18±0.06 in Muzaffarnagari sheep, Kumar *et al.* (2017) as 15.44±0.14 and Banger *et al.* (2018) as 16.47±0.05 in Deccani sheep, Kumar *et al.* (2018) as 15.86±0.07 in Nellore Jodipi sheep and Illa *et al.* (2019) as 16.00 in Nellore sheep. Regarding KR1, more similarity of Sirohi goats towards sheep breeds indicates its better feed conversion efficiency. Lower estimates for KR1 was reported by Vyas *et al.* (2024) as 12.89±0.03 in Marwari goats, Tesema *et al.* (2020) as 13.99±0.11 in Boer x Central Highland goat, Rashidi *et al.* (2011) as 14.1 in Markhoz goats for 0-4M age, Supakorn and Pralomkarn (2012) as 10.26±1.31 in crossbred goats for 0-5M age, Nakavisut and Thepparat (2014) in Thai native goats as 14.72, Gupta *et al.* (2016) as 14.20±0.13 in Mehsana goats and Dige *et al.* (2021) as 13.74±0.02 in Jamunapari goats. Thus, the highest value of KR1 in Sirohi goats among all the Indian goat breeds so far reported proves its supreme status regarding feed efficiency during pre-weaning stage. Regarding KR2, lower estimate was reported by Kesbi and Gholizadeh (2017) as 3.307 in Baluchi sheep.

Highly significant effect (P≤0.01) of sire was found on KR1 and KR2. Similar results were found by Khadda *et al.* (2018) in Pantja goats. The significant effect of sire on Kleiber ratio indicates the existence of additive genetic variability among these traits.

Period of birth also had highly significant effect (P≤0.01) on both Kleiber ratios. For both Kleiber ratios, significantly higher value was reported during second period (2010-2013). The difference in performance for KR over the years might be due to continuous breed improvement programme by using of selected sires, variation in climatic conditions and availability of forage as well as inclusion or exclusion of clusters over the years. Similar findings were noted by Barazandeh *et al.* (2012), Moghbeli *et al.* (2013) and Mokhtari *et al.* (2019) in Raieni Cashmere goats, Supakorn and Pralomkarn (2012) in crossbred goats, Gupta *et al.* (2016) in Mehsana goats, Bhusan *et al.* (2019) in Jakhrana goats and Vyas *et al.* (2024) in Marwari Goats. However, non-significant effect of year of birth on KRs was observed by Ved Prakash *et al.* (2012a, 2012b) in Malpura sheep.

Season of birth had highly significant effect (P≤0.01) on KR1, but showed significant (P≤0.05) effect on KR2. In both cases, kids born during summer season had higher Kleiber ratio whereas kids born during rainy season had lowest Kleiber ratio. Kids born during rainy season have more chance to be affected by many infectious diseases and face more stressed condition due to precipitation and increased population of arthropods. These factors might affect their feed conversion efficiency which leads to lower Kleiber ratio. In the month of April and May, good availability of crop residues in the post-harvest fields facilitates ad libitum feed for dams during grazing which results into higher milk production to nourish the young ones and ultimately leads to better growth of kids. Apart from this, kids born in summer season got the opportunity to graze into two harvest season i.e. both Rabi and Kharif during 3 to 12M age. That's why the Kleiber ratio was higher for kids born in summer. Similar results were reported by Supakorn and Pralomkarn (2012) in crossbred goats whereas non-significant effect of season was found by Khadda *et al.* (2018) on KR1 in Pantja goats.

Table 1. Least square means (Mean±S.E.) of Kleiber ratios (KR) in Sirohi goat

Effect		KR1 (0-3M)	KR2 (3-12M)
Overall		16.38±0.03 (6748)	4.11±0.05 (3070)
Sire		**	**
Period of birth		**	**
	P1(2005-2009)	16.33 ± 0.04^{b} (2290)	4.05 ± 0.08^{a} (1136)
	P2(2010-2013)	16.51 ± 0.04^{c} (2018)	4.24 ± 0.08^{b} (806)
	P3(2014-2017)	16.30 ± 0.04^{a} (2440)	4.05 ± 0.08^{a} (1128)
Season of birth		**	*
	Rainy (July to Oct)	16.29 ± 0.04^{a} (2651)	4.08 ± 0.05^{a} (1037)
	Winter (Nov to Feb)	16.37 ± 0.04^{b} (3115)	4.08 ± 0.05^{ab} (1470)
	Summer (March to June)	16.48 ± 0.05^{c} (982)	4.18 ± 0.06^{b} (563)
Type of birth		**	**
	Single	16.14 ± 0.03^{a} (4423)	4.00 ± 0.05^{a} (2199)
	Multiple	16.62 ± 0.04^{b} (2325)	4.22 ± 0.05^{b} (871)
Sex		**	**
	Male	16.53 ± 0.03^{b} (3366)	4.22 ± 0.05^{b} (1002)
	Female	16.22 ± 0.03^{a} (3382)	4.00 ± 0.05^{a} (2068)
Cluster		**	**
	Vallabhnagar	16.72 ± 0.08^{c} (289)	3.89 ± 0.20^{b} (54)
	Railmagra	17.66 ± 0.05^{e} (834)	3.84 ± 0.1^{b} (328)
	Devgarh	17.41 ± 0.03^{d} (3275)	3.85 ± 0.05^{b} (1905)
	Nathdwara	17.22 ± 0.10^{d} (169)	3.33 ± 0.23^{a} (40)
	Bhadsoda	17.76 ± 0.04^{e} (1333)	4.69 ± 0.08^{d} (311)
	Karget	13.79 ± 0.08^{a} (319)	5.25 ± 0.16^{e} (169)
	Bojunda	13.99 ± 0.07^{a} (436)	3.95 ± 0.09^{c} (263)
	Salumber	16.49 ± 0.14^{b} (93)	—
Parity		**	*
	1	16.46 ± 0.04^{c} (1513)	4.17 ± 0.06^{b} (802)
	2	16.39 ± 0.04^{b} (1346)	4.12 ± 0.06^{b} (632)
	3	16.46 ± 0.04^{c} (1201)	4.13 ± 0.06^{b} (537)
	4	16.33 ± 0.05^{a} (891)	4.08 ± 0.06^{a} (403)
	5 & above	16.26 ± 0.04^{a} (1797)	4.07 ± 0.06^{a} (696)

**P ≤ 0.01, *P ≤ 0.05; Subclass means with the same superscript do not differ significantly (P ≤ 0.05)

Type of birth had highly significant (P≤0.01) effect on both KR1 and KR2. For both age durations, kids born as multiples had higher Kleiber ratio than single born kids due to compensatory growth phenomenon. Similar finding was reported by Barazandeh *et al.* (2012) in Raieni cashmere goats for KR1, Khadda *et al.* (2018) in Pantja goat only up to 6M age and Vyas *et al.* (2024) in Marwari Goats. Non-significant effect of type of birth was noted by Bhusan *et al.* (2019) in Jhakhrana goats for KR1, Mokhtari *et al.* (2019) in Raieni cashmere goats for KR1 and Dige *et al.* (2021) for KR1 in Jamunapari goats.

Highly significant effect (P≤0.01) of sex was recorded for both pre- and post-weaning Kleiber ratio and male kids showed significantly higher values than females. The results indicate better feed conversion efficiency of males than females which might be due to anabolic action of androgens. Barazandeh *et al.* (2012), Moghbeli *et al.* (2013) and Mokhtari *et al.* (2019) in Raieni cashmere goats, Supakorn and Pralomkarn (2012) in crossbred goats, Khadda *et al.* (2018) in Pantja goats and Bhusan *et al.* (2019) in Jhakhrana goats found significant effect of sex on Kleiber ratio. However, Gupta *et al.* (2016) in Mehsana goats on KR1 and Vyas *et al.* (2024) in Marwari Goats on KR2 did not report any significant effect of sex. The difference may be due to spatial or temporal variation in population under study.

Highly significant effect (P≤0.01) of cluster was observed on both traits. For KR1, Railmagra and Bhadsoda cluster showed highest values where Karget had the minimum but for the KR2, Karget had the highest value which might be due to compensatory growth phenomenon in post-weaning stage. The value of KR 2 could not be estimated for cluster Salumber due to non-availability of data of 12 months age. The performance regarding pre- and

post-weaning Kleiber ratios was comparatively higher in cluster Bhadsoda. The varaiation in estimates for different clusters are due to difference in management practices, types of vegetation consumed as well as difference in genetic properties of the population. Significant effect of cluster was also reported by Khadda *et al.* (2018) in Pantja goats and Vyas *et al.* (2024) in Marwari Goats.

Parity had also highly significant effect (P≤0.01) on KR1, but showed significant (P≤0.05) effect on KR2. Kleiber ratio was higher in kids born in up to 3rd parity. The results were in agreement with Vyas *et al.* (2024) in Marwari Goats but Khadda *et al.* (2018) in Pantja goats and Bhusan *et al.* (2019) in Jhakrana goats did not find significant effect of parity on KRs.

Heritability estimates for KR1 and KR2 were 0.65±0.07 and 0.29±0.05, respectively. The heritability estimates in our present study were very close to the heritability estimate for KR1 in Marwari goats (0.66±0.28) by Vyas *et al.* (2024). However, lower estimates were reported by Gupta *et al.* (2016) in Mehsana goats for KR1 (0.28±0.13), Khadda *et al.* (2018) in Pantja goats as 0.28±0.09, Mokhtari *et al.* (2019) in Raieni cashmere goats as 0.19±0.06, Rashidi *et al.* (2011) in Markhoz goats for KR (0-4M) as 0.27±0.05 and Supakorn and Pralomkarn (2012) in crossbred goats for KR (0-5M) as 0.35±0.04, which may be due to spatial or temporal difference in the sample. The genetic correlation between two traits was -0.66±0.10 whereas phenotypic correlation was -0.50. Thus both the correlations were negative showing that kids having higher KR in 0-3 M stage had lower KR during 3-12 M stage which might be due to compensatory growth effect. Similar findings were reported by Khadda *et al.* (2018) in Pantja goats.

CONCLUSION

The appreciable Kleiber ratio observed in Sirohi goats underscores their superior feed conversion efficiency, which is one of the most desirable economic traits for meat animals. This characteristic highlights the breed's potential for enhancing productivity in the meat industry. To maximize the profitability of goat farmers, it is essential to promote the adoption of Sirohi goats and the genetic upgrading of local herds through the use of superior Sirohi bucks, particularly within the breed's home tract. Additionally, breeding programs should be tailored to optimize the season of birth, with a focus on producing more offspring during the summer months. This timing takes advantage of favorable environmental conditions and ensures a steady supply of kids for market demand. The higher Kleiber ratio observed in multiple-born kids further suggests the potential for improving prolificacy through the use of high-quality bucks and assisted reproductive technologies (ART) at the field level. Farmers in regions with lower Kleiber ratios should also consider adopting management systems and rearing practices used by clusters with higher feed conversion efficiency. These practices could include better nutrition, disease management, and breeding strategies, all of which contribute to improving growth rates and feed efficiency. Furthermore, the medium to high heritability of the Kleiber ratio offers an excellent opportunity for individual selection. This means that through targeted breeding programs, further genetic improvement in feed efficiency can be achieved, leading to more productive and sustainable farming practices.

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Research Article

HUMECTANT OPTIMIZATION OFFERS A STRATEGIC APPROACH TO ENHANCING THE PHYSICO-CHEMICAL AND SENSORY QUALITY OF GOAT MILK SOFT CHEESE

RISHI KUMAR, MEENA GOSWAMI*, VIKAS PATHAK, CHIRAG SINGH and ABHISHEK MISHRA
Department of Livestock Products Technology, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura-281001

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ABSTRACT

The present study aimed to evaluate the impact of varying concentrations of two humectants glycerol and sorbitol on the physico-chemical, textural, and sensory characteristics of goat milk soft cheese. Glycerol (G1, G2, G3) and sorbitol (S1, S2, S3) were incorporated at concentrations of 2%, 4%, and 6%, respectively, along with a control sample (C) prepared with 0.75% salt and subjected to a 15-hour incubation. Results from the glycerol-treated samples revealed that increasing glycerol levels significantly ($P<0.05$) reduced pH, moisture, fat-to-dry matter ratio, and water activity, while significantly increasing ($P<0.05$) titratable acidity, total solids, ash content, and Brix values. However, yield, protein, and overall fat content remained unaffected. Textural analysis showed a significant ($P<0.05$) increase in adhesiveness, gumminess, chewiness, and lightness (L*) with higher glycerol levels, although sensory acceptability decreased significantly ($P<0.05$), with G2 (4% glycerol) identified as the most favorable treatment. Similarly, sorbitol-supplemented cheeses showed a significant ($P<0.05$) decline in pH, moisture, fat-to-dry matter ratio, and water activity, accompanied by a significant ($P<0.05$) rise in titratable acidity, total solids, and Brix values. No significant differences were observed in yield, protein, fat, or ash content across sorbitol treatments. Among textural parameters, adhesiveness and gumminess increased significantly ($P<0.05$), while other textural and color traits remained unchanged. Sensory evaluation indicated a significant ($P<0.05$) drop in scores for S3 (6% sorbitol), whereas S2 (4% sorbitol) emerged as the optimal formulation. These findings highlight the potential of humectant optimization to enhance specific quality attributes of goat milk soft cheese.

Keywords: Glycerol, Goat milk, Humectants, Quality attributes, Soft cheese, Sorbitol

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India maintains its position as the leading global producer of milk, contributing approximately 24.76% to global output, according to the Basic Animal Husbandry Statistics (DAHD, 2024). The country produced 239.30 million tonnes of milk in fiscal year 2023-2024, growing at a compound annual growth rate (CAGR) of 5.62% during the previous ten years. Within this dynamic dairy landscape, goat milk has emerged as a significant contributor, with an estimated output of 7.59 million tonnes in 2023, approximately 3.3% of the country's total milk production. This marks a noteworthy year-on-year growth of 9.04%, reflecting a rising demand and growing prominence of goat milk in India's dairy economy. In developed nations, there is an increasing consumer shift towards milk from non-bovine sources, including goat milk, often regarded as a premium and niche product (Haenlein and Wendorff, 2006; Strzałkowska *et al.*, 2009). Goats, sometimes known as the "poor man's cow" because of their low maintenance requirements (Devendra and McLeroy, 1988; Goswami *et al.*, 2017), have long been an important part of smallholder livelihoods, particularly in environments with limited resources. Globally, the value derived from goat farming is distributed among milk (60%), meat (35%), and skin (5%). In many developing regions, goats continue to offer a cost-effective alternative for milk production, especially in areas unsuitable for large-scale bovine dairy systems (Schwarz *et al.*, 2017).

Consumer preference for functional and health-oriented foods has catalyzed a resurgence in goat milk consumption. Known for its digestibility and hypoallergenic properties, goat milk is increasingly recommended for individuals with lactose intolerance or gastrointestinal sensitivities (Mituniewicz *et al.*, 2019). A wide array of goat milk derivatives-ranging from fermented and enriched milk products to curd, frozen yogurt, butter and milk powder is now commercially available (Park, 2007). Among these, goat milk cheese, particularly the soft variety, stands out for its nutritional richness and unique organoleptic characteristics, including its soft texture, white appearance, and vitamin-enhanced flavor profile (Park *et al.*, 2017). Soft goat cheese is believed to be among the earliest forms of cheese consumed by humans, with its origins traceable to ancient Mesopotamia (Kosikowski, 1999). Soft cheese derived from goat milk has gained attention due to its superior digestibility and distinctive flavor, often preferred over cow milk-based cheeses in certain consumer segments (Gänzle, 2015). The market for goat milk-based products, such as cheese, the market for goat milk-based products in India is projected to expand steadily, with an estimated compound annual growth rate (CAGR) of 4.9% between 2024 and 2030. By the end of this period, the sector is anticipated to reach a

*Corresponding author: dr.goswami2008@yahoo.co.in

value of approximately USD 3.68 billion, highlighting growing consumer interest and market potential. However, the production of goat cheese remains constrained by several factors, including technological limitations, the absence of standardized processing protocols, and challenges related to moisture control, textural stability, and shelf life (Bielik *et al.*, 2019). Soft goat cheese, due to its high moisture content, is inherently more perishable than harder cheese variants. Its relatively short shelf life, coupled with a pronounced "goaty" flavor, can limit consumer acceptance despite its nutritional advantages. To mitigate these constraints, the incorporation of humectants such as glycerol and sorbitol has been proposed as a potential strategy to enhance product stability and sensory quality (Aung and Nam, 2024). Glycerol, a triol compound belonging to the sugar alcohol family, is known for its moisture-retention properties and ability to improve the softness and structural integrity of cheese matrices (Haug *et al.*, 2003). Similarly, sorbitol, another polyol, effectively modulates water activity and contributes to favorable textural modifications in dairy formulations (Gänzle *et al.*, 2010). Beyond physicochemical benefits, these humectants may also enhance sensory parameters such as mouthfeel and flavor perception (Ming *et al.*, 2020). While the application of humectants has been explored in various cheese types, comprehensive comparative evaluations of glycerol and sorbitol specifically in goat milk soft cheese remain scarce (O'Neill *et al.*, 2012; Amaral *et al.*, 2022). In light of this knowledge gap, this study was designed to assess the impact of adding glycerol and sorbitol at varying concentrations of 2%, 4% and 6% on the physicochemical properties, texture and sensory qualities of soft cheese made from goat milk. The primary objective of this study was to identify the optimal type and concentration of humectants that can improve the overall quality, shelf life, and consumer acceptance of soft goat cheese while preserving its nutritional profile.

MATERIALS AND METHODS

Location of Study

The experimental work was carried out in the Department of Livestock Products Technology at the College of Veterinary Science and Animal Husbandry, Uttar Pradesh Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), located in Mathura-281001, Uttar Pradesh, India.

Raw Materials

Fresh, clean, and wholesome goat milk was collected from the Department of Veterinary Physiology, DUVASU, Mathura. All ingredients and reagents used in the preparation of goat milk soft cheese, including food-grade glycerol and sorbitol, were sourced from HiMedia Laboratories Pvt. Ltd. Mumbai, a certified supplier. Granular food-grade microbial rennet, sourced from *Mucor pusillus* var. *lindt*, was acquired from Meito Sangyo Co. Ltd., Japan. A starter culture (NCDC-149), containing a mixed strain of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* was obtained from the National Dairy Research Institute (NDRI), Karnal. All cultures and enzymes were stored under recommended conditions until use to ensure viability and efficacy.

Standardization of Processing Conditions

A set of preliminary trials was undertaken to refine the formulation and processing parameters for producing goat milk soft cheese. The preparation followed the methodology outlined by Shabbir *et al.* (2019), with slight adjustments made to suit the objectives of the current study. Key processing parameters, including coagulation time, rennet concentration, pasteurization temperature and curd handling techniques, were standardized to ensure consistent textural characteristics and effective moisture retention in the final product.

Cheese Preparation and Incorporation of Humectants

Following standardization, batch trials of goat milk soft cheese were carried out. For each batch, 5 litre fresh and clean goat milk was taken and passed through a double-layer muslin cloth to eliminate any foreign particles or impurities. The milk was thermally processed at 85 °C for 30 minutes, followed by cooling to 37 ± 2 °C. Thereafter, a composite starter culture (NCDC-149) was introduced comprising *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* was added at a rate of 0.2 g/L and allowed to rest for 1-2 minutes before gentle stirring. The culture-inoculated milk was maintained at a temperature of 35-37 °C for a duration of 2 hours to facilitate fermentation. Subsequently microbial rennet was added at a concentration of 0.02 g/L after dissolving in 15 mL of distilled water. The mixture was incubated at 35-37 °C for 15 hours to facilitate Optimal coagulation. The resultant coagulated mass was cut into 5 cm cubes using a sanitized cheese knife and left undisturbed for 15 minutes to allow whey separation. The curd was then transferred to a sterile muslin cloth and hung for 12 hours to achieve optimal whey drainage. The resulting coagulum was kneaded to achieve uniform consistency and manually blended with common salt (0.75%) and humectants. Two food-grade humectants, glycerol and sorbitol were incorporated at three different levels i.e. 2%, 4%, and 6% (w/w, based on the weight of the

curd) on the basis of preliminary trials and literature available.

Storage Conditions

The formulated goat milk soft cheese was packaged into pre-sterilized thermo-rigid moulds and stored under refrigeration (4 ± 2 °C) overnight to allow the product to attain the desired consistency and shape. Samples were then analyzed at predetermined intervals to monitor changes in physicochemical, textural and sensory attributes during storage.

Analytical Methods

Physicochemical analyses, including determination of moisture, fat, protein, and ash content, were conducted by standard procedures prescribed by the Association of Official Analytical Chemists (AOAC, 2016). Textural characteristics were evaluated through Texture Profile Analysis (TPA) utilizing a TA. XT plus Texture Analyzer (Stable Micro Systems Ltd., United Kingdom) to assess parameters such as hardness, chewiness, cohesiveness, and springiness, following the protocol of Bourne (1978). Rheological properties were evaluated using a dynamic rheometer (MCR 72, Anton Paar Ltd., Austria) to determine the viscoelastic behavior of cheese samples. *Color parameters* (*L**, *a** and *b**) were determined using a Hunter Lab colorimeter (ColourTech PCM+, Colour Tech Associates Inc., Clinton, NJ, USA) at the Department of Goat Products Technology, Central Institute for Research on Goats (CIRG), Makhdoom, India.

Sensory Evaluation

The sensory characteristics were assessed by a semi-trained group comprising seven individuals, including postgraduate students and faculty members from the College of Veterinary and Animal Sciences, DUVASU, Mathura. Training was imparted to the selected sensory panelists prior to the sensory evaluation related to the type of products, sensory evaluation methods and rules of sensory evaluation. A nine-point hedonic scale (1 = extremely disliked, 9 = extremely liked) was used to assess key sensory attributes assessed included visual characteristics (color and appearance), organoleptic qualities (flavor, odor), perceived acidity, textural consistency, salt perception, and overall sensory acceptability as per the method of Keeton (1983). Total 2 bites of each sample were given to the sensory panelists for evaluation. Plain lukewarm water was provided for palate cleansing between samples. The evaluations were conducted at approximately 4:00 p.m. in a controlled environment, with panelists seated individually and prohibited from communicating.

Statistical Evaluation

All experimental data were subjected to statistical analysis using IBM SPSS Statistics software, version 20.0 (IBM Corp., Armonk, NY, USA) described by Snedecor and Cochran (1995). Each parameter was measured in duplicate across three independent replicates (n = 6). The collected data were analyzed using one-way analysis of variance (ANOVA) and significant differences among treatment means were determined through Duncan's Multiple Range Test (DMRT), with statistical significance set at $P < 0.05$.

RESULTS AND DISCUSSION

Effect of glycerol on quality characteristics of goat milk soft cheese

Table 1 presents the effects of different glycerol levels on pH, titratable acidity, moisture content, total solids, fat, protein, ash, water activity, and Brix values. The pH values exhibited a statistically significant decline ($P < 0.05$) with the incremental addition of glycerol, while titratable acidity demonstrated a corresponding significant increase ($P<0.05$). However, no significant variation was noted between the control group (C) and the G1 treatment. Significant reductions ($P < 0.05$) were observed in moisture content, fat-to-dry matter ratio and water activity across treatments, although the moisture content of C and G1 remained statistically comparable. Similarly, the fat/dry matter ratio and water activity levels in G1 and G2 did not significantly differ from those recorded in both C and G3. These findings align with the observations of Chen *et al.* (2014), who reported decreased moisture levels in various jerky products with the incorporation of glycerol or sorbitol. There were no statistically significant differences ($P > 0.05$) in yield percentage, protein concentration, or total fat content across the treatment groups. In contrast, total solids, ash content, and Brix values showed a significant increase ($P < 0.05$). However, the total solid content between G1 and G2 and the ash content between C and G1 did not differ significantly. The elevated Brix readings may be attributed to the water-binding properties of glycerol, which potentially reduce the amount of unbound water, thus lowering moisture levels in the final cheese matrix. Similar trends were reported by Torrico *et al.* (2019), who established a positive association between sugar alcohol addition and Brix values, reflecting enhanced thickness and viscosity in flavored yogurt formulations.

Texture Profile Analysis (TPA) for goat milk soft cheese formulated with varying concentrations of glycerol showed statistically significant enhancement ($P<0.05$) in adhesiveness, gumminess and chewiness was recorded as

Table 1. Physico-chemical properties (Mean±SE) of goat milk soft cheese prepared with different levels of glycerol percentage

Parameters	C	G1	G2	G3	Treatment mean
pH	$5.99^{a} \pm 0.09$	$5.95^{a} \pm 0.07$	$5.81^{b} \pm 0.15$	$5.74^{c} \pm 0.09$	5.87 ± 0.15
Titratable acidity	$0.50^{c} \pm 0.01$	$0.53^{c} \pm 0.03$	$0.58^{b} \pm 0.02$	$0.64^{a} \pm 0.02$	0.56 ± 0.01
Total Solid (%)	$37.64^{c} \pm 0.66$	$41.52^{b} \pm 0.31$	$42.51^{b} \pm 0.47$	$45.34^{a} \pm 0.29$	41.75 ± 0.44
Yield (%)	21.88 ± 0.20	22.09 ± 0.14	21.29 ± 0.26	21.59 ± 0.22	21.69 ± 0.16
Moisture (%)	$62.25^{a} \pm 1.06$	$60.48^{a} \pm 0.31$	$58.48^{b} \pm 0.47$	$54.65^{c} \pm 0.29$	58.96 ± 0.43
Protein (%)	19.81 ± 0.26	19.24 ± 0.39	19.04 ± 0.40	18.65 ± 0.39	19.18 ± 0.21
Fat (%)	19.65 ± 0.29	19.64 ± 0.26	20.11 ± 1.21	20.09 ± 0.25	19.87 ± 0.18
Fat/Dry Matter (%)	$52.14^{a} \pm 1.32$	$47.29^{ab} \pm 1.43$	$47.30^{ab} \pm 2.74$	$44.28^{b} \pm 1.78$	47.75 ± 1.07
Ash (%)	$1.73^{c} \pm 0.05$	$1.75^{c} \pm 0.08$	$1.84^{b} \pm 0.09$	$1.93^{a} \pm 0.08$	1.81 ± 0.06
Water activity	$0.95^{a} \pm 0.01$	$0.94^{ab} \pm 0.03$	$0.94^{ab} \pm 0.02$	$0.93^{b} \pm 0.04$	0.94 ± 0.02
Brix value	$23.27^{d} \pm 0.17$	$30.16^{c} \pm 0.21$	$30.80^{b} \pm 0.18$	$32.28^{a} \pm 0.34$	29.12 ± 0.72

Overall means bearing different superscripts in a row (a, b, c, d) differ significantly (P<0.05)

Table 2. Texture profile analysis (Mean±SE) of goat milk soft cheese with different levels of glycerol percentage

Parameters	C	G1	G2	G3	Treatment mean
Adhesiveness (g/sec)	$-21.89^{d} \pm 0.31$	$-22.89^{c} \pm 0.28$	$-24.66^{b} \pm 0.37$	$-26.32^{a} \pm 0.27$	-23.94 ± 0.21
Springiness(cm/mm)	0.42 ± 0.02	0.43 ± 0.04	0.42 ± 0.01	0.41 ± 0.04	0.42 ± 0.03
Cohesiveness (Ratio)	0.61 ± 0.05	0.62 ± 0.03	0.63 ± 0.01	0.64 ± 0.05	0.62 ± 0.03
Gumminess (N/cm2)	$2.54^{d} \pm 0.31$	$2.93^{c} \pm 0.33$	3.24b ± 0.50	$3.88^{a} \pm 0.80$	3.14 ± 0.30
Chewiness (N/cm)	$0.36^{d} \pm 0.02$	$0.40^{c} \pm 0.01$	0.42b ± 0.03	$0.45^{a} \pm 0.02$	0.40 ± 0.02
Resilience (Ratio)	0.14 ± 0.05	0.13 ± 0.02	0.12 ± 0.05	0.15 ± 0.01	0.13 ± 0.03

Overall means bearing different superscripts in a row (a, b, c, d) differ significantly (P<0.05)

Table 3. Color values (Mean±SE) of goat milk soft cheese with different levels of glycerol percentage

Parameters	C	G1	G2	G3	Treatment mean
Lightness (*L**)	$81.09^{b} \pm 0.11$	$81.26^{b} \pm 0.19$	$81.94^{b} \pm 0.27$	$83.81^{a} \pm 0.33$	82.02 ± 0.25
Redness (*a**)	0.75 ± 0.09	0.81 ± 0.50	0.88 ± 0.29	0.91 ± 0.28	0.83 ± 0.16
Yellowness (*b**)	13.76 ± 0.22	13.42 ± 0.30	13.99 ± 0.33	13.40 ± 0.69	13.64 ± 0.21

Overall means bearing different superscripts in a row (a, b) differ significantly (P<0.05)

Table 4. Sensory scores (Mean±SE) of goat milk soft cheese with different levels of glycerol percentage

Parameters	C	G1	G2	G3	Treatment mean
Color and appearance	$7.92^{a} \pm 0.05$	$7.62^{b} \pm 0.03$	$7.56^{b} \pm 0.06$	$6.76^{c} \pm 0.04$	7.46 ± 0.06
Acidity	$7.84^{a} \pm 0.14$	$7.77^{b} \pm 0.03$	$7.70^{b} \pm 0.03$	$5.92^{c} \pm 0.11$	7.30 ± 0.09
Flavor	$7.82^{a} \pm 0.08$	$7.73^{b} \pm 0.02$	$7.67^{b} \pm 0.48$	$5.62^{c} \pm 0.74$	7.21 ± 0.10
Body and texture	$7.65^{a} \pm 0.14$	$7.77^{b} \pm 0.02$	$7.71^{b} \pm 0.48$	$6.69^{c} \pm 0.07$	7.45 ± 0.10
Saltiness	$7.61^{a} \pm 0.07$	$7.48^{b} \pm 0.07$	$7.43^{b} \pm 0.07$	$6.55^{c} \pm 0.07$	7.26 ± 0.08
Overall acceptability	$7.79^{a} \pm 0.08$	$7.75^{b} \pm 0.02$	$7.66^{b} \pm 0.43$	$6.70^{c} \pm 0.89$	7.47 ± 0.83

Overall means bearing different superscripts in a row (a, b, c) differ significantly (P<0.05)

glycerol concentration increased (Table 2). This enhancement in textural attributes may be attributed to glycerol's hygroscopic nature and water-binding capacity, which likely contributed to a more viscous and firmer matrix in the cheese. These findings align with the study conducted by Swain *et al.* (2014), which demonstrated that sugar alcohols exert a favorable impact on the growth of *Lactobacillus* species in fermented dairy systems, potentially through mechanisms involving cytoplasmic dehydration and triggering external osmotic efflux mechanisms. ultimately leading to a firmer and more compact product texture. However, no statistically significant differences (P > 0.05) were detected in springiness, cohesiveness, and resilience among the control and treatment groups, which suggests that glycerol incorporation had a limited effect on these specific parameters.

Table 5. Physico-chemical properties (Mean±SE) of goat milk soft cheese prepared with different levels of sorbitol percentage

Parameters	C	S1	S2	S3	Treatment mean
pH	$6.01^{a} \pm 0.10$	$5.91^{b} \pm 0.07$	$5.74^{c} \pm 0.15$	$5.62^{d} \pm 0.09$	5.82 ± 0.05
Titratable acidity	$0.51^{d} \pm 0.01$	$0.53^{c} \pm 0.02$	$0.57^{b} \pm 0.02$	$0.61^{a} \pm 0.01$	0.55 ± 0.01
Total Solid (%)	$37.64^{c} \pm 1.06$	$42.65^{b} \pm 0.83$	$43.44^{b} \pm 0.63$	$45.14^{a} \pm 0.66$	42.21 ± 0.72
Yield (%)	21.91 ± 0.20	22.01 ± 0.22	22.29 ± 0.42	22.59 ± 0.44	22.20 ± 0.16
Moisture (%)	$62.15^{a} \pm 0.26$	$58.34^{b} \pm 0.23$	$57.23^{b} \pm 0.35$	$55.39^{c} \pm 0.58$	58.27 ± 0.71
Protein (%)	19.86 ± 0.26	19.34 ± 0.39	19.14 ± 0.40	18.83 ± 0.39	19.29 ± 0.21
Fat (%)	19.66 ± 0.29	19.84 ± 0.66	20.31 ± 1.20	20.11 ± 0.85	19.98 ± 0.39
Fat/Dry Matter (%)	$52.23^{a} \pm 0.32$	$46.51^{b} \pm 0.43$	$46.75^{b} \pm 0.24$	$44.55^{c} \pm 0.38$	47.51 ± 0.17
Ash (%)	1.82 ± 0.15	1.82 ± 0.10	1.89 ± 0.24	1.96 ± 0.21	1.87 ± 0.24
Water activity	$0.96^{a} \pm 0.06$	$0.95^{b} \pm 0.04$	$0.94^{b} \pm 0.05$	$0.92b \pm 0.03$	0.94 ± 0.06
Brix value	$23.30^{b} \pm 0.17$	$31.07^{a} \pm 0.34$	$31.80^{a} \pm 0.28$	$32.89^{a} \pm 0.36$	29.76 ± 0.28

Overall means bearing different superscripts in a row (a, b, c, d) differ significantly (P<0.05)

Table 6. Texture profile analysis (Mean±SE) of goat milk soft cheese with different levels of sorbitol percentage

Parameters	C	S1	S2	S3	Treatment mean
Adhesiveness (g/sec)	$-21.92^{d} \pm 0.11$	$-22.64^{c} \pm 0.05$	$-24.22^{b} \pm 0.07$	$-26.12^{a} \pm 0.02$	-23.72 ± 0.07
Springiness(cm/mm)	0.41 ± 0.02	0.44 ± 0.04	0.43 ± 0.02	0.41 ± 0.04	0.422 ± 0.00
Cohesiveness (Ratio)	0.65 ± 0.05	0.66 ± 0.04	0.67 ± 0.01	0.68 ± 0.05	0.66 ± 0.00
Gumminess (N/cm^2)	$2.63^{d} \pm 0.21$	$2.66^{c} \pm 0.12$	$2.85^{b} \pm 0.60$	$3.62^{a} \pm 0.20$	2.94 ± 0.22
Chewiness (N/cm)	0.38 ± 0.03	0.42 ± 0.04	0.43 ± 0.02	0.44 ± 0.03	0.41 ± 0.00
Resilience (Ratio)	0.15 ± 0.01	0.14 ± 0.01	0.15 ± 0.02	0.16 ± 0.04	0.15 ± 0.00

Overall means bearing different superscripts in a row (a, b, c, d) differ significantly (P<0.05)

Table 7. Color values (Mean±SE) of goat milk soft cheese with different levels of sorbitol percentage

Parameters	C	S1	S2	S3	Treatment mean
Lightness (*L**)	81.12 ± 0.18	81.47 ± 0.20	81.96 ± 0.27	83.87 ± 0.34	82.10 ± 0.30
Redness (*a**)	0.74 ± 0.17	0.88 ± 0.84	0.89 ± 0.27	0.92 ± 0.29	0.85 ± 0.21
Yellowness (*b**)	13.82 ± 0.27	13.51 ± 0.32	13.92 ± 0.36	13.96 ± 0.69	13.80 ± 0.21

Table 8. Sensory scores (Mean±SE) of goat milk soft cheese with different levels of sorbitol percentage

Parameters	C	S1	S2	S3	Treatment mean
Color and appearance	$7.92^{a} \pm 0.08$	$7.87^{a} \pm 0.03$	$7.86^{a} \pm 0.06$	$7.07^{b} \pm 0.08$	7.68 ± 0.05
Acidity	$7.81^{a} \pm 0.14$	$7.78^{a} \pm 0.03$	$7.75^{a} \pm 0.03$	$6.92^{b} \pm 0.10$	7.5 ± 0.09
Flavor	$7.78^{a} \pm 0.14$	$7.81^{a} \pm 0.08$	$7.89^{a} \pm 0.06$	$6.65^{b} \pm 0.09$	7.53 ± 0.08
Body and texture	$7.66^{a} \pm 0.10$	$7.68^{a} \pm 0.01$	$7.79^{a} \pm 0.04$	$7.18^{b} \pm 0.04$	7.57 ± 0.04
Saltiness	$7.81^{a} \pm 0.11$	$7.83^{a} \pm 0.07$	$7.94^{a} \pm 0.07$	$6.86^{b} \pm 0.06$	7.61 ± 0.08
Overall acceptability	$7.75^{a} \pm 0.11$	$7.72^{a} \pm 0.02$	$7.78^{a} \pm 0.04$	$7.08^{b} \pm 0.08$	7.58 ± 0.08

Overall means bearing different superscripts in a row (a, b) differ significantly (P<0.05)

Non-significant variations (P > 0.05) were detected in the redness (a*) and yellowness (b*) parameters among the control and glycerol-enriched cheese samples (Table 3), indicating that the incorporation of glycerol exerted a negligible effect on the intrinsic color characteristics of goat milk soft cheese. These findings are consistent with those reported by Sofyan *et al.* (2013), who observed comparable results in shrikhand fortified with white crystallized sugar syrup, indicating similar effects on the product's compositional or sensory characteristics. However, the lightness (L*) values exhibited a significant (P<0.05) increase with higher glycerol concentrations, indicating a perceptible enhancement in the brightness of the cheese matrix.

As the concentration of glycerol increased, all sensory attributes, namely color and appearance, acidity, flavor, body and texture, saltiness and overall acceptability declined significantly (P<0.05), as shown in Table 4.

However, no significant differences were observed between the G1 and G2 treatments for any of the sensory parameters. The decline in sensory scores at higher glycerol levels may be attributed to its ability to mask the natural acidic flavor of goat milk soft cheese by imparting a mildly sweet aroma. Additionally, the lower texture scores recorded for G3 could be associated with increased stickiness and dryness, likely due to reduced moisture content. These findings are consistent with the observations of Khanam (2017), who reported a decline in sensory acceptability with the incremental addition of glycerol (0.5% to 1.5%) in chicken spread formulations.

Effect of sorbitol on quality characteristics of goat milk soft cheese

Table 5 outlines the physico-chemical properties of goat milk soft cheese developed using different concentrations of sorbitol. The pH of goat milk soft cheese declined significantly ($P<0.05$) with increasing concentrations of sorbitol, while titratable acidity and total solids exhibited a significant rise ($P<0.05$). Nonetheless, total solids did not differ significantly between the S1 and S2 treatments. Moisture content and fat content on a dry matter basis also decreased notably ($P<0.05$) with higher sorbitol levels, though the reduction between S1 and S2 was not statistically meaningful. No significant variations were observed across treatments in yield percentage, protein, fat or ash content. These findings align with those of Rai *et al.* (2017), who reported stable proximate composition in Rabri formulated with 4%, 6%, and 8% sugar alcohols in cow, buffalo, and mixed milk. Water activity decreased significantly ($P<0.05$), whereas Brix values increased ($P<0.05$) as sorbitol concentration rose; however, differences among S1, S2, and S3 were not statistically significant for either parameter. Comparable patterns have been observed by Syed and Babar (2018) in sheerqurma, as well as by Anurag and Chawla (2016) in studies involving milk-based sweets formulated with bottle gourd where sugar alcohols enhanced sweetness and moisture regulation without markedly altering basic composition.

Among texture profile analysis (TPA) a statistically significant ($P<0.05$) increase in adhesiveness and gumminess was observed with the progressive addition of sorbitol (Table 6), likely attributable to the higher solute content, which may enhance the viscosity and structural firmness of the cheese matrix. A moderate increase in adhesiveness and gumminess is generally desirable in soft cheeses because it contributes to a creamier, smoother mouthfeel and better spreadability-attributes typically preferred by consumers (Cai *et al.*, 2024).

Similar findings were documented by Nurhartadi *et al.* (2017), who reported increased textural integrity and viscosity in cheese whey yogurt when supplemented with sugar alcohol syrups at levels of 8%, 10%, and 12%. Soukoulis *et al.* (2007) also suggested that increased acidity throughout the fermentation can strengthen casein-calcium ion interactions, thereby promoting gel firmness and viscosity. In contrast, other parameters such as springiness, cohesiveness, chewiness and resilience did not exhibit significant variation across treatment and control groups, suggesting that these textural traits were not substantially affected by sorbitol addition.

As shown in Table 7, the chromatic parameters of goat milk soft cheese, including lightness (L^*), redness (a^*), and yellowness (b^*), did not exhibit any statistically significant differences ($P>0.05$) between the control group and the samples supplemented with sorbitol. This observed stability in color can likely be attributed to the chemically inert and thermally stable nature of sorbitol, which does not undergo degradation or participate in Maillard browning reactions under typical processing conditions. Nezzal (2000) similarly noted the resistance of sugar alcohols like sorbitol to thermal decomposition and non-enzymatic browning pathways. Conversely, Broyart *et al.* (1998) identified a significant ($P<0.05$) correlation between lower sugar content and higher lightness in baked goods, where Maillard reaction was the key driver of color formation. Additionally, Bates *et al.* (1998) emphasized that changes in pH can influence color dynamics in dairy products through non-enzymatic browning, often leading to decreased hue angles and a shift from yellow to reddish tones. In contrast, such color transitions were not evident in the present study, suggesting that sorbitol inclusion had minimal impact on the visual quality of goat milk soft cheese.

The sensory attribute scores, including color and appearance, acidity, flavor, body and texture, saltiness and overall acceptability, declined significantly ($P<0.05$) with increasing sorbitol concentration (Table 8), particularly in the S3 treatment (6% sorbitol). However, no significant differences ($P>0.05$) were observed among the control (C), S1 (2% sorbitol) and S2 (4% sorbitol) across any sensory parameter, including overall acceptability. These results align with the findings of Manalo and Benedicto (2014), who noted that sorbitol incorporation had a limited impact on the sensory properties of ready-to-eat tocino, indicating that sugar alcohols may preserve product palatability without markedly altering consumer perception. Furthermore, the enhanced spreadability and overall acceptability identified in the current study resonate with the findings of Prajapati *et al.* (1991), who documented improvements in the organoleptic characteristics of low-fat cheese spreads following the incorporation of sugar

alcohols. Among the evaluated formulations, the S2 variant exhibited the highest overall acceptability score, likely due to its balanced sweet–acidic flavor profile and desirable firmness. As a result, the goat milk soft cheese containing 0.75% salt and 4% sorbitol (S2) was identified as the most suitable formulation in this study based on comprehensive sensory evaluation.

CONCLUSION

This study assessed the effects of glycerol and sorbitol, used as humectants, on the physicochemical, textural, and sensory characteristics of goat milk soft cheese. The findings revealed that both additives significantly influenced the overall quality profile of the cheese. Among the glycerol-enriched formulations, the sample containing 4% glycerol (G2) demonstrated improved physicochemical and textural parameters. However, its sensory attributes particularly flavor and overall acceptability, were comparatively lower than those observed in the sorbitol-enriched variants. In contrast, the incorporation of sorbitol at a 4% concentration (S2) produced the most favorable outcomes, enhancing not only texture and flavor but also overall consumer acceptability. The sweet–acidic balance and pleasant aroma introduced by sorbitol were especially well-received by the sensory panel, without detracting from key quality parameters such as appearance or acidity. Collectively, these results indicated that incorporating 4% sorbitol into goat milk soft cheese is a promising approach for optimizing product quality. It effectively enhances organoleptic appeal while maintaining desirable structural and compositional attributes, thereby presenting a viable formulation strategy for improved consumer satisfaction.

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IMPORTANT INFORMATION

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Research Article

COMPLETE CASE STUDY OF *HEPATOZOON CANIS*: CLINICAL, HAEMATO-BIOCHEMICAL, MOLECULAR ANALYSIS AND THERAPEUTIC MANAGEMENT

N.N. BRAHMBHATT*[1], A.S. PATEL[3], V.D. DODIA[2], B.J. THAKRE[1] and SUHANA GAMIT[1]
[1]Department of Veterinary Parasitology, [2]Department of Veterinary Clinical Complex,
[3]Department of Livestock product and Technology,
College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

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ABSTRACT

A three-year-old GSD dog arrived at the Veterinary Clinical Complex, Veterinary College, Junagadh, with a history of malnutrition, a significant tick infestation and severe thigh pain. Physical exams showed that the animal was hyperthermic (104.5°F) and mildly dehydrated. During the examination, a blood sample was taken for hematology, microscopic analysis and PCR for diagnosis of hemoprotozoan infection and tick samples were taken for morphological identification. Additionally, a serum sample was also collected for biochemical analysis. When a thin blood smear was made and stained with Giemsa stain, it revealed that the neutrophil contained a gamont of *Hepatozoon canis*. Using morphological traits, ticks were recognized as female *Rhipicephalus sanguineus*. Haematological investigation shows neutrophilia, lymphocytopenia, thrombocytopenia, and severe anemia. Biochemical investigation showed an elevated AST/SGOT and AKP value and a low level of total protein, hypoalbuminemia. Whole blood PCR analysis revealed amplification at 666 bp for *H. canis* infection, but all other hemoparasites, including *B. vogeli*, *B. gibsoni*, *T. evansi* and *E. canis* were negative. Following the injection of doxycycline and imidocarb dipropionate, the dog's clinical condition was improved. Drugs were administered intramuscularly (IM) at a dose of 5-6 mg/kg; imidocarb dipropionate is repeated every 14 days. Doxycycline is frequently recommended in combination with imidocarb because of its broad-spectrum antibacterial qualities and capacity to lower parasitemia. This combined therapy lowers the organism burden, enhances health and manages the clinical indications of hepatozoonosis.

Keywords: Anemia, Haemato-biochemical, *Hepatozoon canis*, PCR, *Rhipicephalus sanguineus*

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Canine hepatozoonosis is an important tick-borne and widely prevalent disease of dogs caused by *H. canis*, and transmission of *H. canis* to dogs occurs by ingestion of an infected tick, *Rhipicephalus sanguineus* (Baneth *et al.*, 2001). The brown dog tick, *R. sanguineus*, is a globally prevalent ectoparasite, particularly common in tropical and subtropical regions. In India, this tick species is widespread and poses significant concerns for canine health. In Gujarat, research highlights a significant prevalence of *R. sanguineus* among the canine population. A study conducted in the middle zone of Gujarat found that 58.11% of dogs harbored clinical tick infestations, with *R. sanguineus* being the predominant species (Raval *et al.*, 2014). These findings underscore the tick's widespread presence in Gujarat, necessitating regular monitoring and control measures to safeguard canine health. Ingestion of infected ticks or tick parts containing mature oocysts with infectious sporozoites through the oral route is the main cause of *H. canis* infection (Aktas *et al.*, 2013). Dog leucocytes are impacted by this internal parasite, which resembles malaria. In peripheral blood smears, leucocytes harboring *H. canis* gamonts are typically observed in neutrophils (Hendrix, 2006; Dhankar *et al.*, 2011).

The widespread presence of *R. sanguineus* in India's tropical and subtropical regions facilitates the maintenance and transmission of *H. canis* among the canine population (Agnihotri *et al.*, 2012). While *H. canis* infections in India are typically subclinical or mild, dogs may exhibit clinical signs such as intermittent fever, anemia, lethargy and weight loss in cases with heavy parasite loads or concurrent infections. The immune response to the parasite involves inflammation and granuloma formation in affected tissues, which can contribute to pathology. PCR analysis for confirmation and blood smear analysis for gamont detection are essential for an accurate diagnosis. Implementing suitable control methods to lessen the burden of this disease on dogs requires an understanding of the occurrence, transmission and pathophysiology of *H. canis* in India (Singh *et al.*, 2013; Gondal & Kaur, 2014). In this study, a dog infected with *H. canis* was conventionally and molecularly diagnosed along with haemato-biochemical analysis. Additionally, control measures were also explained.

MATERIALS AND METHODS

A three-year-old, female German shepherd breed dog was brought to the Veterinary Clinical Complex, Veterinary College, Junagadh during the month of May 2025 with the history of high fever, tick infestation, having pain at thigh region and anorexia. Clinical examination of the dog revealed that pyrexia (104.5 °F), pale conjuctival and oral mucous membranes. The animal was suspected

*Corresponding author: nnbrahmbhatt@kamdhenuuni.edu.in

for the haemoprotozoan infection based on the clinical examination. A thin peripheral blood smear, whole blood, serum sample and ticks were received to the clinical diagnostic laboratory for the parasitological examination. During collection, the animal was thoroughly examined, physically and clinically and details such as age, breed, sex and history were recorded. Before sample collection, owner consent was taken. Standard collection procedure was followed for the collection of blood sample from animals following bio-safety measures. Blood sample was collected from the dog in EDTA coated vaccutainer & serum vaccutainer. Animals were thoroughly examined for tick infestations, illness and clinical signs and then ticks were also collected in vial for identification and sent to Department of Veterinary Parasitology for identification.

RESULTS AND DISCUSSION

Microscopic Examination

The thin blood smear was methanol-fixed, stained with 1:20 diluted Giemsa for 40-45 minutes, washed, dried and examined under oil immersion. Microscopy examination revealed rectangular, capsule-like bodies within neutrophils, indicating the infection (Soulsby, 1982) as shown in Figs. 1. & 2. Canine hepatozoonosis is a disease that is currently very common in India. The infection is known to be caused by the species *H. canis* (Palanivel *et al.*, 2010). In this case, the haemato-biochemical alterations and molecular detection of other haemoparasites were detected. The results indicate that *H. canis* is responsible for causing pathogenicity. The variations observed in the haemogram and leukogram parameters as well as in serum biochemical parameters of the infected dog may be related to parasitaemia and response of animal.

Tick Identification

Tick samples were also collected from infected dog. Collected ticks were identified under stereo-zoom microscope based on morphology using standard identification key (Walker *et al.*, 2003) at department of Veterinary Parasitology, Veterinary College, Kamdhenu University, Junagadh as shown in Figs. 3 & 4.

Haemato-biochemical analysis

Haematological analysis of the blood sample was done by using Automatic Whole Blood Analyzer (Mindray BC-2800 Vet) and biochemical analysis of the serum samples was done by Serum Auto-analyzer (MicroLab-300) with commercial kit (Randox, UK) at the College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh. haematological investigation shows

Table 1. Key points for identification of tick at genus level.

Sr. No.	Major tick genera of dog	Main identification points
1.	*Rhipicephalus* (*Boophilus)*	Bravirostrate, Festoon absent, Coxae 1 have small paired spurs.
2.	*Rhipicephalus sanguineus*	Bravirostrate, eye present, Hexagonal basis capitulum, Festoon present.
3.	*Haemaphysalis*	Bravirostrate, Eye absent, Ventral plates are absent, Festoon present.

Table 2. Haematological finding of the present case.

Sr. No.	Parameters	Result of positive case	Reference Value
1.	Hb (g/dl)	3.0	10-16
2.	RBCs $\times 10^6$	2.0	05-08
3.	WBCs	13400	5500-19500
4.	Neutrophils (%)	85	35-70
5.	Eosinophils (%)	00	0-1.0
6.	Basophils (%)	00	0-1.0
7.	Lymphocytes (%)	17	20-55
8.	Monocytes (%)	07	1.0-04
9.	Platelates $\times 10^5$	01	03-07
10.	PCV (%)	14.0	30-50
11.	MCV (fl)	70.0	55-75
12.	MCH (pg)	19.0	19-24
13.	MCHC (g/dl)	27.0	30-36

Table 3. Result of serum biochemistry of the present case.

Sr. No.	Parameters	Result of positive case	Reference Value
1.	ALT/SGPT	58.28	25-92
2.	AST/SGOT	124.34	10-52
3.	AKP	103.4	10-94
4.	Total protein	4.42	5.0-7.0
5.	Albumin	0.91	2.5-4.0
6.	Urea nitrogen (mg/dl)	35.68	8.0-25
7.	Cholesterol (mg/dl)	97	110-250

neutrophilia, lymphocytopenia, thrombocytopenia and severe anemia. The severity of the infection may have contributed to the dog's anemia in this investigation. According to several experts, the most prevalent and primary haematological indication seen in the majority of cases is anemia (Baneth, 2006). The main findings of this study was anemia. This could be the result of a persistent blood loss-induced iron shortage. Neutrophilia, lymphocytopenia, and thrombocytopenia were found in the study, which is in line with the results of other researchers who regularly documented these alterations (Voyoda *et al.*, 2004). These changes could result from the parasite's multiplication in the animal's organs, which

Table 4. PCR primers used for detection of haemoparasites of dogs in Gujarat

Sr.No.	Name of Parasite	Primer information (5′ → 3′)	Expected amplicon size	Gene	Reference
1.	*B. vogeli*	5' gtgaaccttatcacttaaagg3' 5' caactcctccacgcaatcg3'	610 bp	18S *rRNA*	Kumar *et al.*, 2022
2.	*B. gibsoni*	5' aagccaacatcaaggaaagc3' 5' ttctggtatgcggcagtgta3'	679 bp	*TRAP*	
3.	*T. evansi*	5' gcacaaatgccgacggta 3' 5' gtcgttgccggttattgct 3'	200 bp	RoTat1.2	Kumar *et al.*, 2021
4.	*H. canis*	5' atacatgagcaaaatctcaac 3' 5' cttattattccatgctgcag 3'	666 bp	18S *rRNA*	Kumar *et al.*, 2018
5.	*E. canis*	5' caattatttatagcctctggctctggctatagga3' 5' tataggtaccgtcattatcttccctat'	389 bp	16S *rRNA*	Murphy *et al.*, 1998

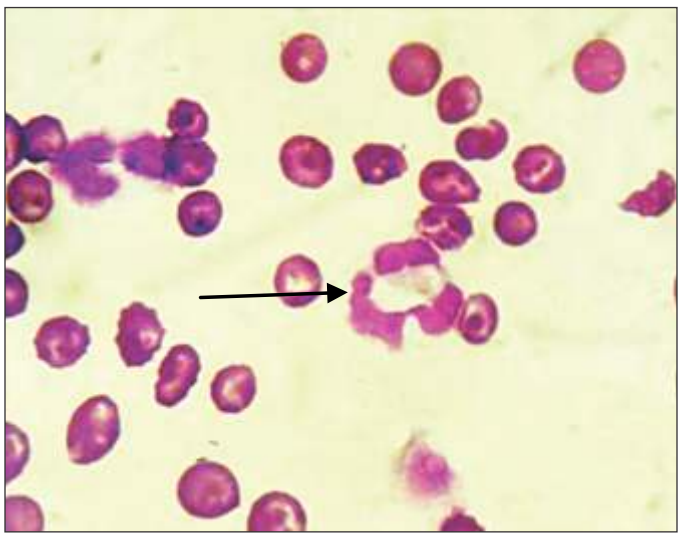

Fig. 1. *H. canis* gamonts in neutrophils (100X)

Fig. 2. Clinical presentation of GSD dog

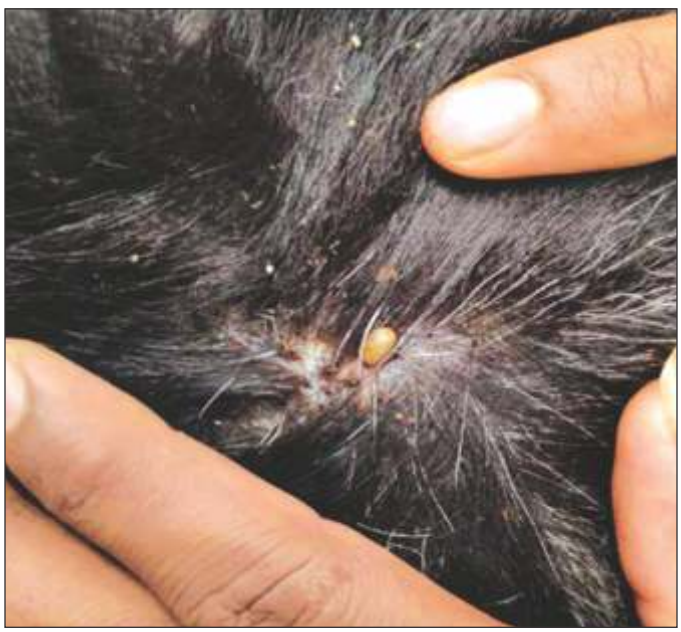

Fig. 3. Infestation of ticks on body coat

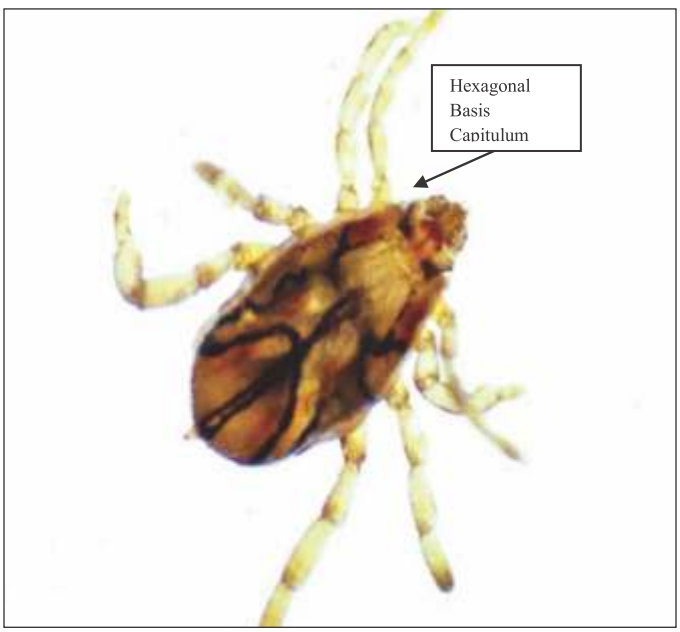

Fig. 4. Rhipicephalus sanguineus tick

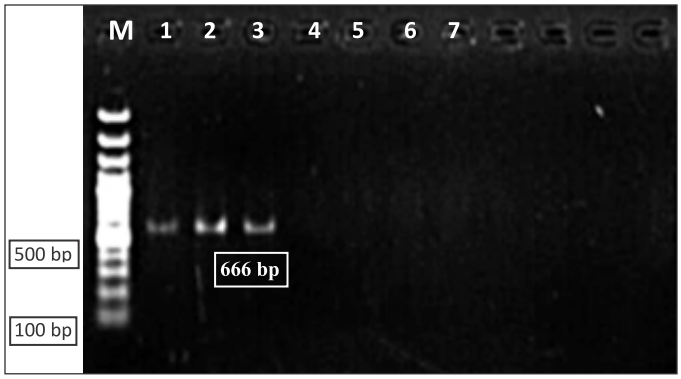

Fig. 5. Amplification of 18S *rRNA* of *H. canis* (666 bp) in dog
M= Marker 100 bp; L1 & L2= Positive control; L3= Sample positive for *H. canis* (666 bp); L4 to L7= Negative for other haemoparasites (*B. vogeli*, *B. gibsoni*, *T. evansi* & *E. canis*)

triggers an inflammatory reaction that could be made worse by the existence of other bacterial diseases. Necrosis and inflammation in the spleen, lymph nodes, liver and lungs are probably the cause of the neutrophilia and anemia (Roopali *et al.*, 2017). Thrombocytopenia was also detected throughout this investigation. While the precise cause of *H. canis*-related thrombocytopenia is uncertain, it may be connected to general thrombocytopenia causes or concomitant infections (Pasa *et al.*, 2009) & it might be also because *H. canis* gamonts cause bone marrow suppression, which could result in decreased platelet production.

Biochemical investigation showed an elevated AST/SGOT & AKP value and a low level of total protein, hypoalbuminemia. Elevated AST/SGOT and AKP values, as well as a low level of total protein and hypoalbuminemia, were the only changes identified by serum biochemistry in this sick dog; however, this could be explained by the dogs' severe tick infestation. Increased osteoblastic activity, hepatic lesions, or cholestasis are all suggested by elevated liver enzymes. Elevated serum AKP activity has been reported in dogs with *H. canis* because of increased osteoblastic activity (Baneth *et al.*,1997). Elevated AST values in the current study show liver & kidney function impairment linked to *H. canis* merogony in different organs. This study found hypoalbuminemia and decreased total protein levels, which have been previously described by Singh *et al.* (2017) and Gavazza *et al.* (2003). These findings could be caused by lower protein intake, chronic inflammation, or protein-losing nephropathy. Lower protein levels in afflicted dogs could have been caused by renal failure (Pasa *et al.*, 2009).

Molecular analysis

Whole blood genomic DNA was extracted from 200 µL blood sample using commercially available Blood DNA isolation kit (Thermo Scientific, Lithuania; Quigen, Germany) as per the manufacturer's protocol. To rule out the common haemoparasitic infection in dogs, in-house standardized PCR assays was used for detection of *B. vogeli*, *B. gibsoni*, *T. evansi*, *H. canis* & *E. canis*. The PCR reaction was conducted at optimized conditions in Thermal Cycler (Applied BioSystem, USA). The PCR products were resolved in 1% agarose gel and amplicons were visualized under UV light. Genomic DNA isolated from blood sample found microscopically positive for *Hepatozoon* gamont in WBCs of dog was used as positive control. Species specific PCR primers were synthesized for molecular identification of different haemoparasites of dogs (Table 4). In PCR, the sample was positive only for *Hepatozoon canis* (666 bp), while all other haemoparasites tested negative on agarose gel. Thus, the sample was confirmed as a single infection of hepatozoonosis. (Fig. 5). According to Abd Rani *et al.* (2011), the most prevalent canine TBD pathogen infecting dogs in India was *H. canis*, which was followed by *E. canis*, *M. haemocanis* and *A. platys*. The most prevalent tick species in this study was *Rhipicephalus*, which was followed by *Haemaphysalis*. The first study on the molecular detection of *H. canis* in spontaneously infected dogs from Kerala was published by Lakshmanan *et al.* (2018).

Therapeutic Management

Based on microscopic and molecular findings, the dog was diagnosed with *Hepatozoon canis* infection. The appropriate treatment was given as a combination therapy, including a single dose of Imidocarb dipropionate at 5-6 mg/kg intramuscularly and Doxycycline at 5 mg/kg orally for 28 days along with supportive therapy such as fluids, antiemetics and hematinics. Hepatozoonosis is usually chronic, with low-grade parasitemia. Short courses of drugs are ineffective; prolonged therapy is needed to reduce parasite load. Moreover, Imidocarb dipropionate may not be effective or well tolerated in all cases. Hence, Doxycycline (5 mg/kg PO BID for 28 days) is commonly used in field conditions, especially in endemic areas. Additionally, to control ticks, Protector Spot On (Fibronil) was administered topically. Following a four-week course of medication, the dog's condition significantly improved. Once more, an examination of the peripheral blood smear under a microscope revealed no *H. canis*. According to studies by Vijayasarathi *et al.* (2024) and Thakur *et al.* (2018), imidocarb dipropionate and doxycycline combined therapy is effective in treating canine *H. canis* infections.

CONCLUSION

In order to prevent drug resistance today, a thorough case study with a confirmative diagnosis is required. In

order to treat dogs with a confirmed positive case of *H. canis* during their acute stage and stop additional losses, early diagnosis is essential. Additionally, a haemato-biochemical test can aid in the disease's diagnosis. Since microscopic diagnosis might be ambiguous, PCR serves as an excellent tool for both preclinical and confirmative diagnosis for any haemoparasitic condition. The treatment of dogs infected with *H. canis* may benefit from the combination of supportive care and imidocarb dipropionate and doxycycline.

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Research Article

ACUTE FASCIOLOSIS IN A SHEEP – A DETAILED STUDY ON HEPATIC HISTOPATHOLOGY AND PARASITOLOGICAL FINDINGS

SRINIVASA NAIK H., CHARITHA GNANI V[1]*, JYOTHI C. and YUGANDHAR C.
Department of Veterinary Pathology, [1]Department of Veterinary Parasitology,
College of Veterinary Science, Proddatur, Sri Venkateswara Veterinary University, SVVU, Tirupati, Andhra Pradesh

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ABSTRACT

Acute fasciolosis, a zoonotic trematode infection caused by *Fasciola hepatica* and *Fasciola gigantica*, poses a significant challenge to ovine health and productivity, particularly in regions transitioning from non-endemic to endemic status. This report details an unanticipated outbreak in a flock of 1,000 sheep under extensive management in the arid Proddatur region of Andhra Pradesh, India, investigated in May 2024. Postmortem examination of a representative four-year-old male sheep revealed hemoperitoneum, extensive hepatic migratory tracts, and immature flukes, corroborated by histopathological evidence of severe parenchymal necrosis and hemorrhage of liver. Parasitological analysis confirmed the presence of juvenile *F. gigantica*. The emergence in a typically dry zone underscores the perils of suboptimal anthelmintic regimens and the introduction of infected stock from waterlogged endemic areas. These findings advocate for robust, integrated parasite control strategies to mitigate disease dissemination in vulnerable agroecological zones.

Keywords: Acute fasciolosis, *Fasciola gigantica*, hepatic histopathology, ovine health, zoonosis, parasite management, Proddatur

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Parasitic infestations constitute a perennial threat to livestock health, particularly in agrarian economies where they cause substantial economic losses and compromise food security. Among these, fasciolosis, induced by the liver flukes *Fasciola hepatica* and *Fasciola gigantica*, stands out due to their zoonotic potential and impact on ruminant productivity (Mas-Coma *et al.*, 2005). These trematodes rely on lymnaeid snails, notably *Galba truncatula*, as intermediate hosts, restricting their prevalence to moisture-rich environments conducive to snail proliferation (Smith *et al.*, 2024). Acute fasciolosis, occurring 2-3 weeks post-infection, is characterized by extensive hepatic damage from migrating juvenile flukes, whereas chronic fasciolosis results from adult flukes residing in bile ducts, leading to protracted morbidity (Fairweather, 2011).

Sheep exhibit heightened susceptibility to acute fasciolosis due to their limited innate immunological resilience compared to cattle, which demonstrate greater tolerance (Mezo *et al.*, 2013). Goats, conversely, are less affected, likely due to selective grazing habits minimizing exposure to contaminated pastures (Charlier *et al.*, 2008). The arid Proddatur region of Andhra Pradesh, traditionally considered non-endemic for fasciolosis, experienced a sudden mortality in five sheep from a flock of 1,000 under extensive management. This investigation, undertaken by the Department of Veterinary Pathology and Parasitology at Sri Venkateswara Veterinary University, elucidates the pathological and parasitological characteristics of a representative case, offering critical insights into the epidemiology and control of fasciolosis in atypical ecological settings.

*Corresponding author: dr.charithagnani@gmail.com

MATERIALS AND METHODS

A four-year-old male sheep exhibiting acute clinical signs (anorexia, weakness and icterus) was selected from the affected flock following sudden death. Necropsy was performed within 6 hours at the College of Veterinary Science, Proddatur, adhering to institutional ethical guidelines (IAEC/SVVU/2024/05). Gross lesions were photographed and tissue samples (liver, spleen, intestines) were collected in 10% Neutral Buffered Formalin (NBF) for histopathological analysis.

Fixed liver samples were processed using standard paraffin embedding techniques, sectioned at 5 µm and stained with hematoxylin and eosin (H&E). Additional sections were subjected to Masson's trichrome staining to assess fibrosis. Slides were examined under a light microscope (Olympus BX53) at 10× and 40× magnifications, with digital images captured for documentation. Liver tissue was minced and examined under a stereomicroscope (Leica S8 APO) to identify immature flukes. Fecal samples from the carcass were processed using the sedimentation technique to identify fluke eggs.

Flock history was reviewed, including deworming practices, water sources and recent animal introductions. Meteorological data from May 2024 (India Meteorological Department) confirmed atypical rainfall (150 mm, 120% above average), potentially facilitating snail habitat development.

RESULTS AND DISCUSSION

The post-mortem examination revealed a bloated carcass distended, with pale mucous membranes and subcutaneous tissue changes suggestive of significant blood loss due (Fig. 1). Upon opening the abdominal cavity, approximately 2 liters of dark red blood were

Fig.1. Bloated carcass with pale visible mucous membranes

Fig. 2. Haemorrgaic peritoneum and blood in the abdominal cavity

Fig. 3. Blood clot on the diaphragmatic surface of the liver

Fig. 4. Cut section of liver showing adult flukes and migratory track on visceral surface

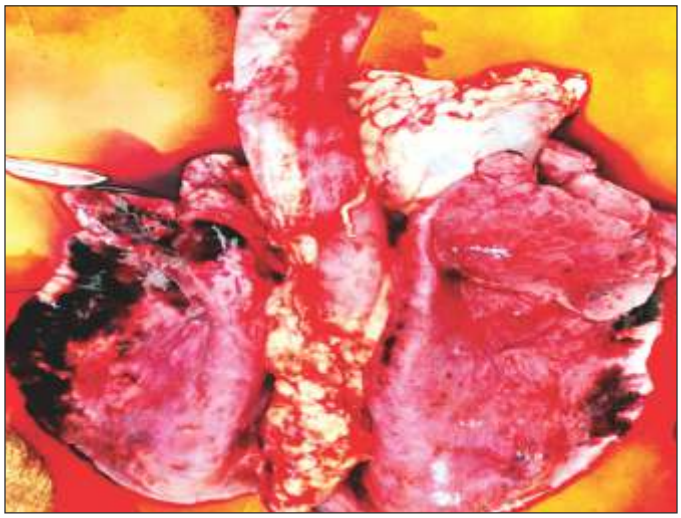

Fig. 5. Diaphragmatic lobes of lungs showing dark consolidation

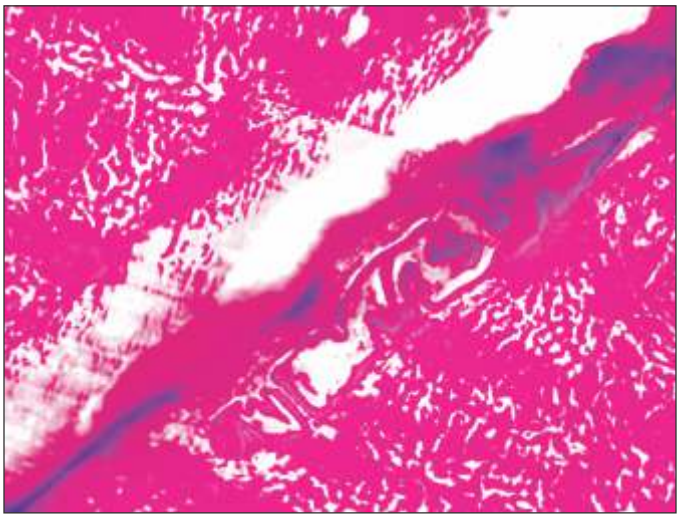

Fig. 6. Liver histopathology section showing presence of the parasite (H&E 10X)

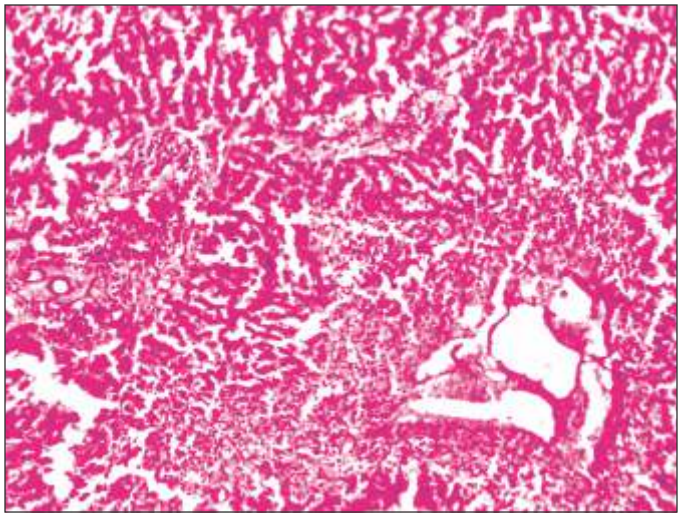

Fig. 7. Liver section showing severe infiltration of inflammatory cells (H&E 10X)

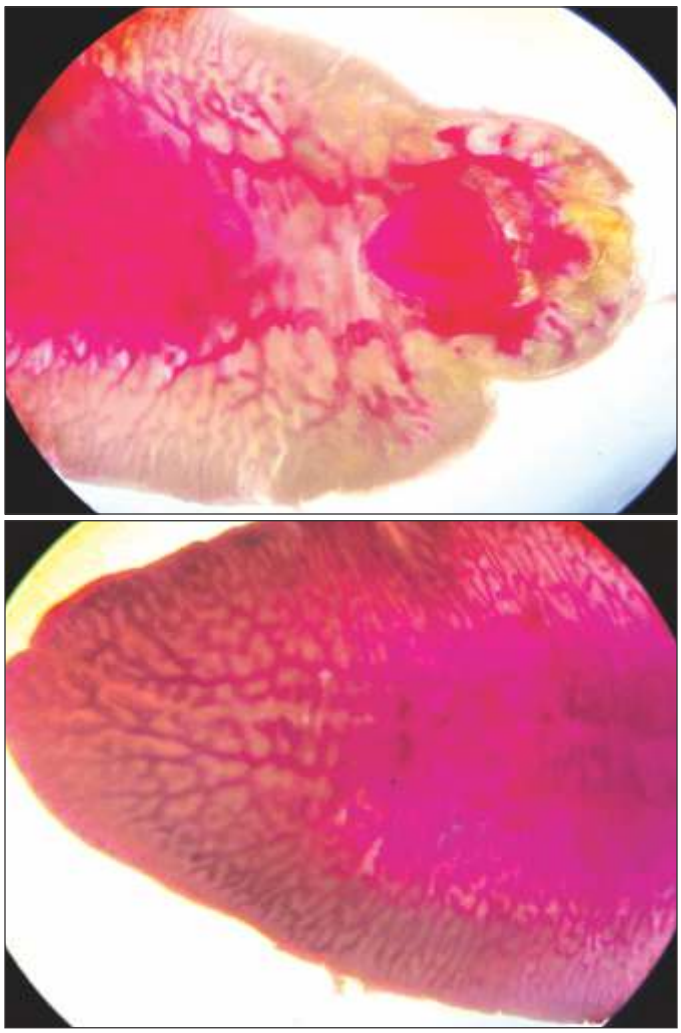

Fig. 8-9. Anterior and posterior end of carmine stained *Fasciola giagntica* worm with intestinal caecae showing internal diverticulations (5X)

observed, indicating hemoperitoneum (Fig. 2). A clotted mass of blood, weighing around 400 to 500 grams, was found on the diaphragmatic surface of the liver (Fig. 3). The visceral surface exhibited migratory tracks with focal

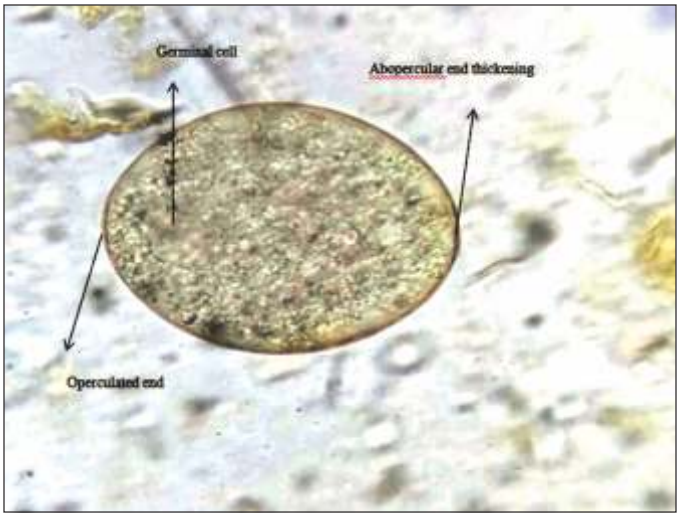

Fig. 10. *Fasciola* egg- note the large size (~ 160 µm long) thin wall with small operculum and abopercular end with thickening (40x)

necrotic and hemorrhagic areas. Mild hepatomegaly with moderately distended gallbladder was also noted. On the cut section, numerous leaf-like immature or juvenile *Fasciola* worms, approximately 3 cm in size, were observed (Fig. 4). Consolidated areas were present in the diaphragmatic and apical lobes of the lungs, and the cut sections revealed immature flukes (Fig. 5). Frothy content was noted in the trachea and lungs, while the heart appeared pale with endocardialhemorrhages and scanty blood clots. These gross lesions are consistent with the findings of Ashoor and Wakid (2023) and Elmonir *et al.* (2015).

Histopathological examination of the liver revealed migratory tunnels associated with hemorrhagic areas and necrosis with fibrin deposition. Cross-sections of the parasite were visible, along with degenerative changes in hepatocytes and dilated, congested blood vessels. Multifocal areas showed severe infiltration of mononuclear cells, such as macrophages and lymphocytes, with disruption of hepatic cords. Bile ductul hyperplasia and mononuclear cell infiltration were evident (Figs. 6 & 7). Further, the worms collected in NBF were subjected to parasitological examination wherein permanent mounting with borax carmine staining was performed as per the methodology of Singh and Srivastava (1977) and the worms (Figs. 8 & 9) were identified as *Fasciola gigantica* based on morphological features as described by Soulsby (1982). Further, sedimentation of fecal samples revealed characteristic *Fasciola* eggs (Fig. 10).

The highly pathogenic parasitic disease, fasciolosis, affects both humans and livestock is caused by liver flukes, of the genus *Fasciola*. Despite the global distribution of *Fasciola hepatica* and *Fasciola gigantica*, infections caused by these parasites are among the most neglected zoonotic diseases (Mas Coma *et al.*, 2005). The presence of standing

water and suitable snail hosts allows *Fasciola* spp. to sustain their life cycle, as these conditions promote the development and dissemination of metacercariae. (Jaja *et al.*, 2017; Martin and Cabrera, 2018). However, Proddatur in the Kadapa district, a dry and arid region, has reported no cases in the past five years, with only 1% of cases documented (Jalajakshi and Varaprasad, 2023) (Reference). The current outbreak might be due to purchase and transport of fifty sheep by the farmer from the waterlogged Guntur district. Although fasciolosis typically occurs in autumn and summer, infection can occur anytime of the year when sheep graze on contaminated plants (Jaja *et al.*, 2017; Nyirendra *et al.*, 2019). The favorable ecological conditions for snails and the parasite likely contributed to the disease's occurrence. Furthermore, sheep confined to sheds during winter and fed hay contaminated with metacercaria in summer, without proper deworming, are at a higher risk of infection (Valero *et al.*, 2018; Sah *et al.*, 2020). Sheep with fasciolosis may exhibit acute, subacute, or chronic symptoms. Acute fasciolosis, common in sheep, presents as severe anemia and sudden death, often without prior signs. The cut section of the liver reveal multiple immature liver flukes. The migrating flukes releases proteolytic enzymes, causing extensive liver tissue damage and hemorrhage (Derso and Genet, 2015; Yusuf *et al.*, 2016). The extensive migration of juvenile flukes can rupture the liver capsule, leading to blood leakage into the peritoneal cavity, peritonitis and death due to traumatic hepatitis (Abdisa 2017). The inflammatory response triggered by hemorrhagic and necrotic lesions attracts immune cells (Zafra *et al.*, 2013). The secretion of cytokines and chemotactic factors perpetuates this process, resulting in sustained cellular infiltration (Molina *et al.*, 2015). Histopathological examination of the liver showed severe infiltration of inflammatory cells and migratory tracts, accompanied by fibrous connective tissue proliferation, consistent with the findings of Dow *et al.* (1967) and Smith *et al.* (1972).

Despite their close morphological resemblance and overlapping geographical occurrence, these two species exhibit distinct morphological, biological and epidemiological characteristics that influence the clinical presentation and transmission dynamics of the disease (Mas-Coma *et al.*, 2005). India, being a tropical country, reports a predominance of *F. gigantica* infections in cattle and buffaloes, with *F. hepatica* occurring sporadically in high-altitude temperate zones (Lalrinkima *et al.*, 2021). The carmine stained specimens (Figs. 8 and 9) were identified based on distinct morphological characteristics viz., leaf-shaped, dorsoventrally flattened body with anterior cephalic cone with less prominent shoulders, branching pattern of intestinal caeca, and the configuration of reproductive organs and posteriorly tapering end, consistent with the morphology of *Fasciola gigantica* in accordance with the diagnostic keys proposed by Soulsby (1982). In addition, coprological examination of fecal samples from infected animals was conducted using the sedimentation technique. The analysis revealed the presence of characteristic *Fasciola* eggs with large, operculated, ellipsoidal ova with a distinct yellowish shell confirming active infection in the herd (Fig. 10). The concurrent detection of adult flukes in bile ducts and eggs in fecal samples provided conclusive evidence of acute fasciolosis, corroborating the pathological and clinical observations recorded during the study.

CONCLUSION

This investigation elucidates the pathological and parasitological profile of acute fasciolosis in an unexpected ovine outbreak in Proddatur, driven by ecological and management factors. Effective control of fasciolosis requires a multifaceted and evidence-based approach that combines chemotherapy, environmental management and host-targeted interventions. Comprehensive strategies such as, regular deworming, quarantine of introduced stock and snail habitat management are imperative to prevent recurrence. Moreover, draining swampy areas and using molluscicides can effectively control the intermediate host population and reduce indirect economic losses.

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THE HARYANA VETERINARIAN

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Research Article

PROTECTIVE ROLE OF SILIBININ AGAINST BISPHENOL A INDUCED SUB-ACUTE TOXICITY IN WISTAR RATS

SWANAND SANJAY KULKARNI, VIJEYTA TIWARI*, VINAY KANT, ARUN SINGH and MANEESH SHARMA[1]

Department of Veterinary Pharmacology and Toxicology, [1]Department of Veterinary Clinical Complex, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004, Haryana

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SUMMARY

Bisphenol A (BPA) is a widely used industrial chemical compound with recognized endocrine disrupting property, whereas, Silibinin is a flavonolignan component of plant *Silybum marianum* shown to have protective antioxidant potential in various toxicological animal models. This study aimed to evaluate the ameliorative efficacy of silibinin against BPA-induced systemic toxicity in male Wistar rats. A total of 50 male Wistar rats were randomly divided into experimental groups, with 10 animals per group. All animals were administered treatments via oral gavage once daily for 28 days. Clinical signs were monitored and scored daily using a standardized scale. Body weight was recorded at weekly intervals. BPA exposure led to distinct clinical signs including hyporexia, piloerection, hunched posture and pasty faeces, along with a significant reduction in body weight gain. Haematological analysis revealed elevated haemoglobin, TEC and PCV, suggestive of haemoconcentration, while serum biochemistry showed significantly increased creatinine and reduced cholesterol levels, indicating renal stress and altered lipid metabolism. Co-treatment with silibinin, particularly at 200 mg/kg, mitigated of these alterations, reflected by reduced clinical severity scores, improved weight gain, stabilization of haematological parameters to normal levels and partial restoration of serum creatinine and cholesterol levels.

Keywords: Bisphenol A, Silibinin, Sub-acute toxicity, Wistar rats

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Bisphenola (BPA) is recognized as an endocrine-disrupting chemical (EDC) due to its ability to bind to estrogen receptors and interfere with hormonal balance (Iwamoto *et al.*, 2021). Silibinin, a major flavonolignan component of *Silybum marianum* (milk thistle), is well-documented for its distinct hepatoprotective and some other pharmacological activities i.e. neuroprotective, anti-cancerous, antioxidant, anti-inflammatory etc. (Kittur *et al.*, 2002; Polyak *et al.*, 2010; Polachi *et al.*, 2016; Tuli *et al.*, 2021; Liu *et al.*, 2023). Some recent studies have described silibinin as a potent natural polyphenol with strong anti-inflammatory and antioxidant properties due to its five hydroxyl (OH) groups, three of which (5− OH, 7− OH and 20− OH) exhibit phenolic properties and can scavenge free radicals and reactive oxygen species (ROS) (Tuli *et al.*, 2021). Psotová *et al.* (2002) studied the iron chelator activity of silibinin. Owing to its strong free radical scavenging activity via multiple hydroxyl groups, silibinin has shown potential in mitigating oxidative stress and cellular apoptosis (Kim *et al.*, 2009; Liu *et al.*, 2023). However, its potential role in protecting against BPA-induced systemic toxicity, particularly in relation to growth performance and hematobiochemical alterations, remains underexplored. Therefore, the present study was undertaken to evaluate the protective effect of silibinin against BPA-induced subacute toxicity in male Wistar rats, with emphasis on relative body weight gain, clinical signs, relative organ weight changes, haematological and biochemical parameters.

*Corresponding author: dr.vijeytatiwari@luvas.edu.in

MATERIALS AND METHODS

The study was carried out at Department of Veterinary Pharmacology and Toxicology, College of Veterinary Sciences, LUVAS, Hisar from the period between July 2024 to April 2025.

1. Chemicals

Bisphenol A (BPA, purity 97%) and silibinin (purity >98%) were procured from Sigma-Aldrich (Merck Co., USA). Corn oil (Korn Health, India) was used as the vehicle for oral administration.

2. Animals

The experiment was conducted on 50 healthy male Wistar rats (6-8 weeks old, 130-170 g) obtained from the Disease-Free Small Animal House (DFSAH), LUVAS, Hisar. All procedures were approved by the Institutional Animal Ethics Committee (IAEC) (Protocol No. IAEC/LUVAS/31/01, Letter No. VCC/IAEC/2024/480-99 dated 06/07/2024) and the study adhered to CPCSEA guidelines.

3. Experimental Design

Rats were acclimatized for seven days under standard laboratory conditions. Animals were randomly divided into 5 groups (n = 10 per group) and treated once daily by oral gavage for 28 days as follows:

- Group 1 (Control): Corn oil @ 1ml/100 g b.wt.
- Group 2 (BPA): BPA @ 325 mg/kg b.wt.
- Group 3 (SLB): Silibinin @ 200 mg/kg b.wt.
- Group 4 (BPA + SLB low): BPA @ 325 mg/kg + SLB @ 100 mg/kg b.wt.
- Group 5 (BPA + SLB high): BPA @ 325 mg/kg + SLB @ 200 mg/kg b.wt.

Silibinin was administered 30 minutes prior to BPA in co-treatment groups i.e. group 4 and 5. Doses were prepared freshly each day in corn oil and administered at 1 ml/100 g body weight using an 18G metal gavage needle.

4. Clinical Observations, Body Weight and organ weight

Animals were observed daily for clinical signs of toxicity, behavioural changes and mortality. A semi-quantitative scoring system (0: absent, ±: rare, +: occasional, ++: frequent, +++: persistent) was used to assess clinical signs. Body weight was recorded weekly and relative body weight gain and organ weights were calculated by using following formula:

$$\text{Relative body weight gain} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

$$\text{Relative organ weight} = \frac{\text{Organ weight (g)}}{\text{Body weight (g)}} \times 100$$

5. Blood Collection

On day 29, animals were anaesthetized with thiopental sodium (200 mg/kg b.wt.) and blood was collected via retro-orbital plexus using heparinized capillary tubes. Blood was collected in separate vials for haematology (EDTA-coated vials) and serum biochemistry (clot activator coated vials) and sent immediately for analysis.

6. Haematological Analysis

Parameters such as haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, total leukocyte count (TLC)and differential leukocyte count (DLC) were analysed using an automatic haematology analyzer (MS4Se, Melet Schloesing Laboratories, France).

7. Serum Biochemistry

Serum levels of ALT, AST, ALP, total protein, triglycerides, cholesterol, BUN and creatinine were estimated using commercial diagnostic kits (Erba Mannheim,

Table 1. Clinical signs and behavioral changes due to 28-days oral dosing of Bisphenol A (BPA), silibinin and their co-treatment

Clinical Signs & Symptoms	G-1	G-2	G-3	G-4	G-5
Hyporexia	0	+++	0	+	±
Ruffled & Dull Hair coat	0	+++	0	++	+
Pasty Faeces	0	+++	0	++	±
Piloerection	0	+++	0	++	+
Hunched Posture	0	+++	0	±	0
Perinasal Erythema	0	++	0	+	±
Foul Odour & Bedding Discoloration	0	++	0	±	0
Bradypnea (Open-Mouth Breathing)	0	++	0	±	0
Mortality	0	0	0	0	0

G-1: Vehicle Control, G-2: BPA (325 mg/kg), G-3: SLB (200 mg/kg), G-4: BPA + Low dose SLB (100 mg/kg), G-5: BPA + High dose SLB (200 mg/kg).

Table 2. Effect of 28-days oral dosing of Bisphenol A (BPA), silibinin and their co-treatment on relative organ weights (g/100g b. wt.) in male Wistar rats

Group	Left Testes	Right Testes	Liver	Left Kidney	Right Kidney	Spleen	Brain	Left Epididymis	Right Epididymis
G-1	$0.73^{a} \pm 0.03$	$0.75^{a} \pm 0.03$	$4.30^{a} \pm 0.12$	$0.35^{a} \pm 0.01$	$0.36^{a} \pm 0.01$	$0.24^{a} \pm 0.01$	$1.06^{a} \pm 0.04$	$0.31^{a} \pm 0.01$	$0.32^{a} \pm 0.01$
G-2	$0.73^{a} \pm 0.04$	$0.74^{a} \pm 0.04$	$4.67^{a} \pm 0.14$	$0.34^{a} \pm 0.02$	$0.32^{a} \pm 0.01$	$0.20^{a} \pm 0.01$	$1.02^{a} \pm 0.03$	$0.28^{a} \pm 0.01$	$0.29^{a} \pm 0.01$
G-3	$0.74^{a} \pm 0.03$	$0.76^{a} \pm 0.03$	$4.56^{a} \pm 0.10$	$0.42^{a} \pm 0.03$	$0.41^{a} \pm 0.02$	$0.25^{a} \pm 0.01$	$1.28^{a} \pm 0.07$	$0.32^{a} \pm 0.03$	$0.33^{a} \pm 0.03$
G-4	$0.74^{a} \pm 0.03$	$0.76^{a} \pm 0.03$	$4.61^{a} \pm 0.35$	$0.39^{a} \pm 0.02$	$0.40^{a} \pm 0.02$	$0.23^{a} \pm 0.01$	$1.17^{a} \pm 0.04$	$0.31^{a} \pm 0.02$	$0.31^{a} \pm 0.02$
G-5	$0.70^{a} \pm 0.04$	$0.72^{a} \pm 0.04$	$4.37^{a} \pm 0.11$	$0.36^{a} \pm 0.01$	$0.37^{a} \pm 0.01$	$0.26^{a} \pm 0.02$	$1.07^{a} \pm 0.04$	$0.32^{a} \pm 0.03$	$0.33^{a} \pm 0.03$

Values are expressed as Mean ± SE (n = 10/group). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Different superscripts (a, b, c, etc.) within a column indicate significant ($p \leq 0.05$) differences.

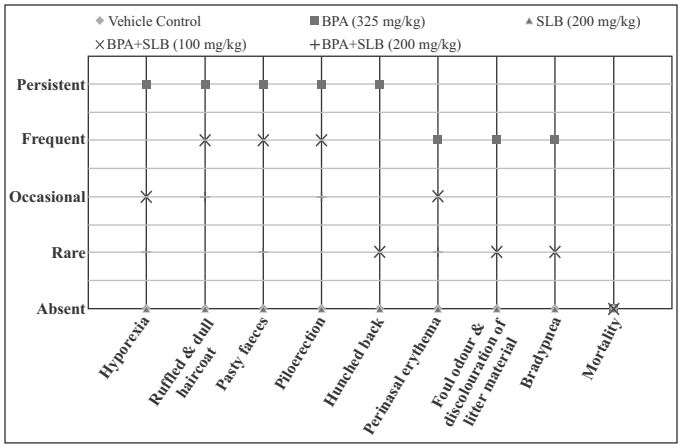

Fig. 1. Scatter plot for clinical signs and behavioral changes due to 28-days oral dosing of Bisphenol A (BPA), silibinin and their co-treatment

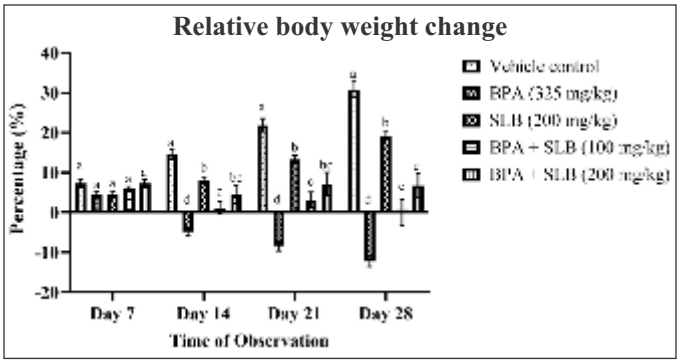

Fig. 2. Effect of 28-days oral dosing of Bisphenol A (BPA), silibinin and their co-treatment on relative body weight change (%) in male Wistar rats. Values are expressed as mean ± SEM (n=10/group). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Different superscripts (a, b, c, etc.) on bars indicate significant ($p \leq 0.05$) difference between groups.

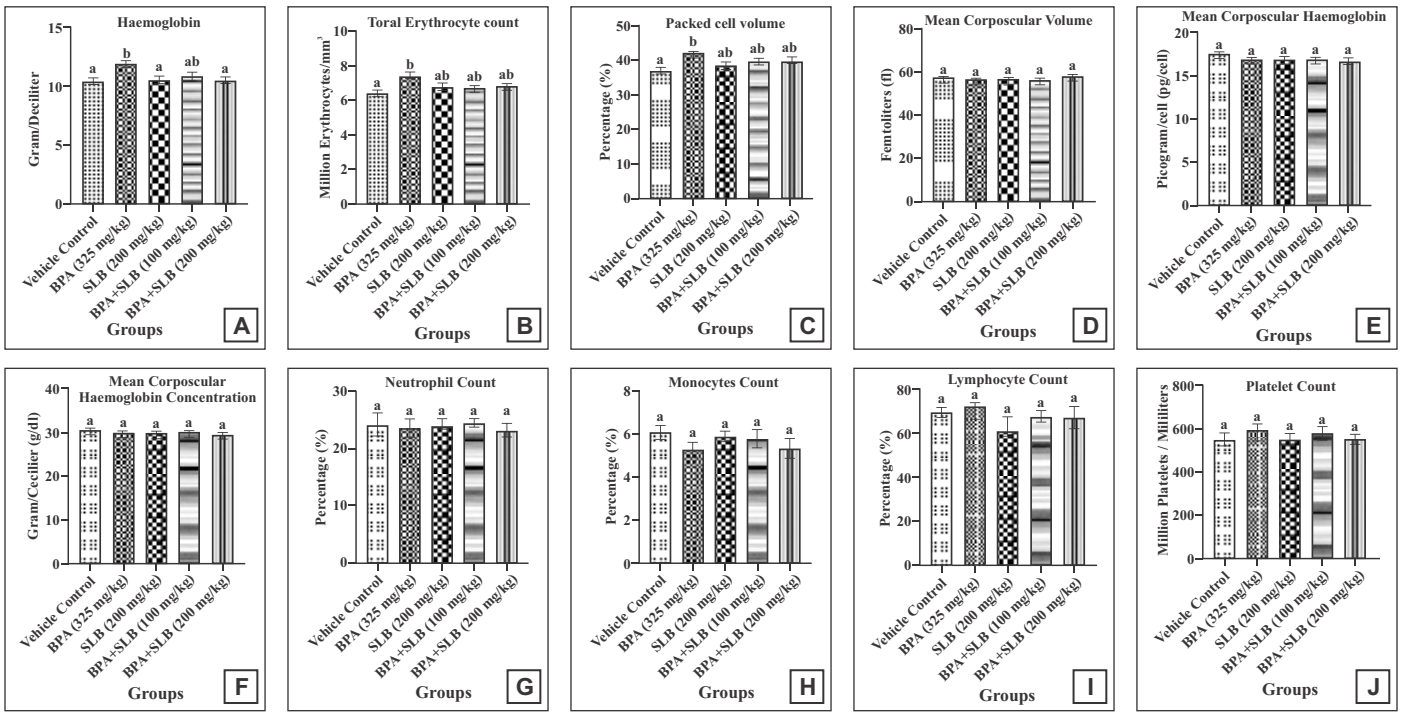

Fig. 3. (a-k): Effect of 28-days oral dosing of Bisphenol A (BPA), silibinin and their co-treatment on various haematological parameters in male Wistar rats **(a)** Haemoglobin (g/dl); **(b)** Total erythrocyte count (Mil/mm3); **(c)** Packed cell volume (%); **(d)** Mean corpuscular volume (fl); **(e)** Mean corpuscular haemoglobin (pg/cell); **(f)** Mean corpuscular haemoglobin concentration (g/dl); **(g)** Neutrophil count (%); **(h)** Monocyte count (%); **(i)** Lymphocyte count (%); **(j)** Platelet count (Mil/ml); **(k)** Total leucocyte count (Mil/mm^3). Values are expressed as Mean ± SE (n=10/group). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Different superscripts (a, b, c etc.) on bars indicate significant ($p \leq 0.05$) difference between groups.

Germany) on an automated biochemistry analyzer (EM 200, Transasia Bio-Medicals Ltd., India).

8. Statistical analysis

The data obtained from various experimental parameters were subjected to statistical analysis using GraphPad Prism version 10.4.2 (San Diego, CA, USA). One-way ANOVA was applied and Tukey's post-hoc test was used to test the differences between the means and significance level was set at $P<0.05$.

RESULTS AND DISCUSSION

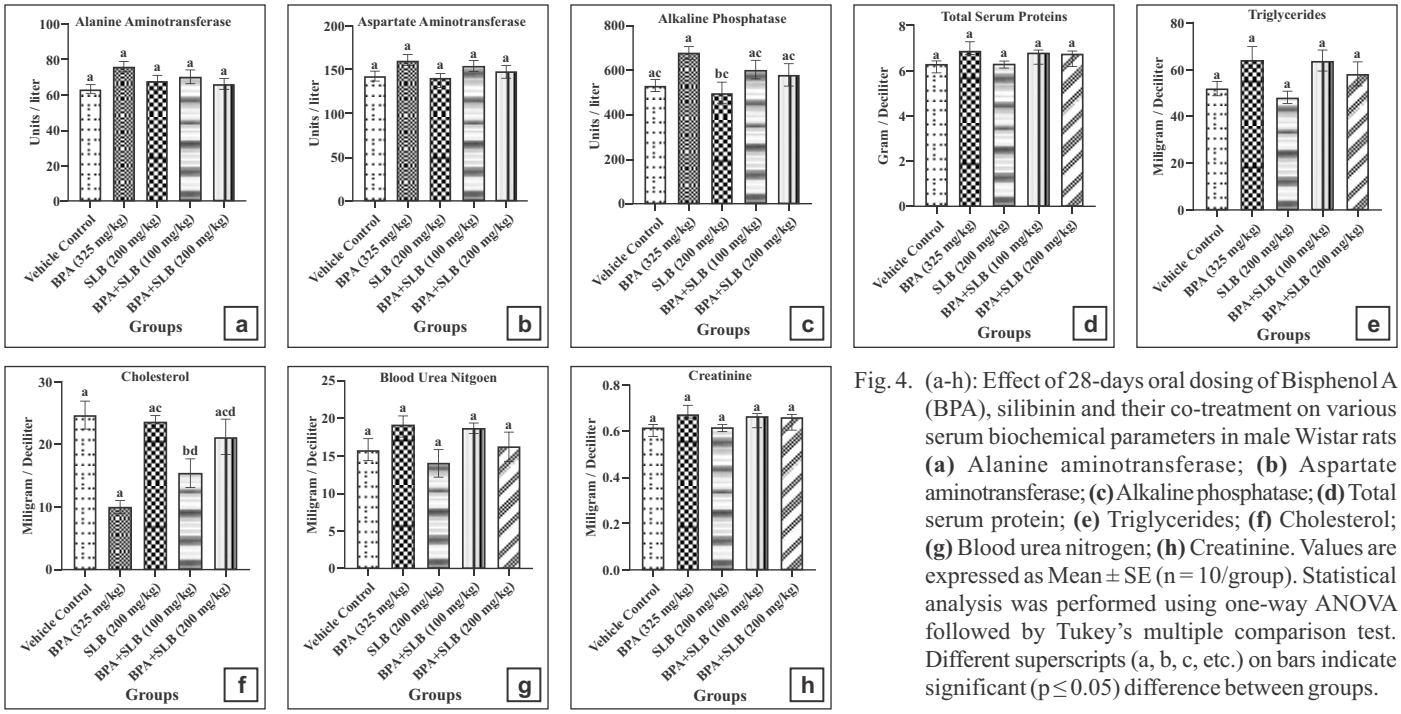

Fig. 4. (a-h): Effect of 28-days oral dosing of Bisphenol A (BPA), silibinin and their co-treatment on various serum biochemical parameters in male Wistar rats **(a)** Alanine aminotransferase; **(b)** Aspartate aminotransferase; **(c)** Alkaline phosphatase; **(d)** Total serum protein; **(e)** Triglycerides; **(f)** Cholesterol; **(g)** Blood urea nitrogen; **(h)** Creatinine. Values are expressed as Mean ± SE (n = 10/group). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Different superscripts (a, b, c, etc.) on bars indicate significant ($p \leq 0.05$) difference between groups.

1. Clinical Signs

BPA-treated rats exhibited persistent clinical signs including hyporexia, piloerection and hunched posture, whereas control and silibinin-alone groups remained asymptomatic. Co-treatment with silibinin reduced the severity and frequency of these signs in a dose-dependent manner, with minimal manifestations at 200 mg/kg. No mortality was observed (table 1; Fig. 1). These signs are indicative of systemic toxicity and are consistant with the findings of Karnam (2003) and Prakash (2010), who reported a similar spectrum of BPA induced behavioural and physical changes in rats.

2. Relative body weight gain

BPA caused significant suppression of body weight gain, whereas silibinin co-treatment, particularly at 200 mg/kg, significantly restored growth trends toward normal, indicating mitigation of BPA-induced metabolic stress (Fig. 2). These findings are in line with the reports of Molayousefian et al. (2024) and Grami et al. (2020). who observed similar body weight reductions following BPA exposure.

3. Relative organ weight

No significant differences were observed in the relative weights of liver, kidney, brain, spleen, testes or epididymis among the experimental groups, indicating absence of gross organ hypertrophy or atrophy (table 2).

4. Haematological parameters

BPA exposure resulted in significant increases in haemoglobin, TEC and PCV, suggestive of haemoconcentration likely due to dehydration and gastrointestinal disturbances. These changes were partially normalized by silibinin co-treatment, supporting its protective role against BPA-induced hematological imbalance (Fig. 3). Similar findings were reported by Alabi *et al.* (2021) who demonstrated the hematoxic effects of BPA in mice. Likewise, Esam *et al.* (2022) observed BPA induced sub-acute toxicity of Wister rats, characterized by alterations in hematological parameters.

5. Serum biochemistry

BPA exposure significantly elevated serum creatinine and alkaline phosphatase levels, along with a reduction in serum cholesterol, indicating renal stress, altered lipid metabolism and possible hepatobiliary disturbance. The elevated ALP levels may indicate early hepatobiliary disturbances, which is plausible given that BPA is known to be excreted through bile (Pattenger *et al.*, 2000). These results also align with those of Abdul Hassan and Hussein (2022), who reported elevated serum creatinine following BPA exposure in albino rats. The decreasing trands in serum cholesterol levels were consistent with Higashihara *et al.* (2007). Co-administration of silibinin attenuated these biochemical alterations, particularly at 200 mg/kg, suggesting a protective effect. These findings are consistent with earlier reports on BPA-induced renal and metabolic toxicity and the documented nephroprotective and lipid-modulatory properties of silibinin (Fig. 4).

CONCLUSIONS

Sub-acute oral exposure to bisphenol A induced systemic toxicity in male Wistar rats, evidenced by adverse clinical signs, impaired body weight gain, haematological

alterations and renal dysfunction. Co-administration of silibinin, particularly at 200 mg/kg body weight, significantly mitigated these toxic effects. The protective influence of silibinin is likely mediated through its antioxidant and anti-inflammatory properties. The findings support the potential use of silibinin as a natural ameliorative agent against BPA-induced systemic toxicity.

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Research Article

MOLECULAR PREVALENCE OF *THEILERIA* INFECTION IN *HYALOMMA ANATOLICUM* INFESTING CATTLE IN DIFFERENT AGRO CLIMATIC ZONES OF INDIA

DEVENDRA PRASAD PATEER, HIRA RAM*, RASMITA PANDA, SHOBHA YADAV and RAJAT GARG
Division of Parasitology, ICAR-IVRI, Izatnagar-243122 (Uttar Pradesh), India

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ABSTRACT

The present study was conducted to determine the molecular prevalence of *Theileria* organism in vector ticks infesting bovines in four different agro-climatic zones of India. Partially engorged ticks were collected randomly from a total of 100 cattle out of 235 screened. Sampling was done from different age group of animals during the period from November 2021 to October 2022. A pool of two to five ticks collected from individual cattle was processed for the extraction of genomic DNA. Two sets of primers were used in the study for molecular detection of *Theileria* organism in the tick vectors: one set targeting the genus-specific 18S ribosomal RNA (18S *rRNA*) gene, and another set targeting the *Theileria annulata* merozoite surface antigen-1(*Tams*1) gene specific to *T. annulata*. The present study revealed a total tick infestation prevalence of 42.55%, with the Upper Gangetic Plain exhibiting the highest infection rate at 54.5%, followed by the Trans Gangetic Plain (45.0%), Western Dry Region (44.4%) and Western Himalayan Region (22.9%). Using an 18S *rRNA* gene-based PCR test, the study revealed a total molecular prevalence of *Theileria* organisms in a vector tick population of 23.0%. However, the *Tams*1 gene-based PCR assay for *T. annulata* revealed a prevalence of 21.0%. Agroclimatic zone-specific analysis of PCR findings revealed that the Western Dry Region showed the highest prevalence of *Theileria* infection (31.25%), followed by the Trans Gangetic Plain (25.93%), Upper Gangetic Plain (16.67%) and Western Himalayan Region (9.09%). Seasonal studies revealed that the monsoon season had the highest prevalence (28.94%), followed by summer (19.51%) and winter (19.04%). Adult cattle showed the highest incidence (24.48%), followed by calves (20%) and heifers (19.44%). Data generated in the present study provides key information about the prevalence and distribution of *T. annulata* in selected agroclimatic zones of the country, which may be helpful in developing and strategizing effective control measures against this parasite.

Keywords: Cattle, Molecular detection, *Theileria annulata*, Tick, PCR assay

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Bovine Tropical Theileriosis (BTT) caused by *Theileria annulata* is an extremely fatal and debilitating tick-borne disease affecting cattle and is transmitted by *Hyalomma anatolicum* (Ixodid tick), having a complex lifecycle in both vertebrate and invertebrate hosts. This disease is manifested by pyrexia, lymphoid hyperplasia, and digestive disorders and associated pathology due to destruction of erythrocytes. The conventional method of diagnosing *Theileria* organism in the tick vector is based on the staining of the salivary gland by various chemicals including Methyl Green-Pyronin, Giemsa and Feulgen (Voigt *et al.*, 1995). However, the detection by staining is not specific and is unable to differentiate between sporoblasts of different *Theileria* species. Therefore, in recent years, application of molecular diagnostic assays such as polymerase chain reaction (PCR) have been explored as an epidemiological and diagnostic tool for the detection and identification of haemoparasites both in the vertebrate hosts and tick vectors (Bhat *et al.*, 2017; Dehuri *et al.*, 2022). These tools are more sensitive and specific than the other common methods by virtue of amplifying even the minute quantity of parasitic DNA (Mans *et al.*, 2015). Of the many target genes, the18s *rRNA* gene, consisting of both conserved and variable regions is suitable marker for distinguishing closely related species of *Theileria* organisms (Chaisi *et al*., 2011). Conversely, the *Tams*1 gene is widely recognized for its high specificity and sensitivity in identifying *T. annulata*, which makes it a helpful diagnostic market (Jongejan *et al.*, 1994). The diverse climatic zones of India are vastly conducive for survival and propagation of vectors and vector-borne pathogens (Bhattacharjee and Sarmah, 2013). Data pertaining to the epidemiology of tick-borne diseases, such as dynamics of pathogen transmission by the tick vector, is imperative for devising effective control strategies. Keeping in view the importance of tick-borne diseases in bovines, the aim of the present study was to investigate the molecular prevalence of *Theileria* organism in vector ticks i.e. *Hyalomma anatolicum* collected from cattle maintained in different agro-climatic zones of India.

MATERIALS AND METHODS

Study area and duration

The present study was conducted in four agro-climatic zones of India viz. Upper Gangetic Plain (Bareilly, Uttar Pradesh and Udham Singh Nagar, Uttarakhand), Trans Gangetic Plain (Hisar and Ambala; Haryana), Western Dry Region (Bikaner, Rajasthan) and Western Himalayan Region (Nainital, Uttarakhand) during the period from November 2021 to October 2022.

*Corresponding author: hiraram.35@gmail.com

Screening of animals for tick infestation and sample collection

A total of 235 cattle were screened carefully for tick infestation from different agro-climatic zones of India (Table1) and ticks were collected from a total of 100 animals of different categories (15 calves, 36 heifers and 49 adult) and season of the year (41-summer, 38-monsoon and 21-winter). Stratified random sampling was made keeping in view the probability of animal movements from one place to another.

Collection of ticks from animals

Ticks were manually removed from the body surface of cattle taking precaution not to break any appendages with the help of fine forceps. Ticks were temporarily paralyzed with cotton soaked in ethanol or chloroform for this purpose. A total of 5-10 fully engorged or semi engorged female ticks per animal were collected in sample collection tubes. Collected samples were labelled properly and brought to the laboratory for morphological identification and further downstream application.

Morphological identification and isolation of genomic DNA from *Hyalomma* sp.

Ticks collected from individual animals were identified in laboratory using stereozoom microscope considering the morphological features described by Soulsby (1982). Genomic DNA was extracted from *Hyalomma* spp. using Presto™ DNA/RNA/Protein kit (Geneaid). Partially or fully engorged female ticks recovered from individual cattle were cut in two pieces and pooled (2-5 ticks per sample) before DNA extraction. Each pooled sample was homogenized using the pestle and mortar and after that 400 µL of DR buffer and 4 µl of ß-mercaptoethanol was added and again homogenized. Tissue lysate was sheared through 20-G needle syringe by passing 10 times followed by the incubation step at room temperature of 5 min. Thereafter, sample was centrifuged at 13000 rpm for 2 min and supernatant was transferred in GD column and all other steps were performed according to the protocol provided with kit. In the end, DNA was eluted in a total of 50 µl final volume and it was stored at -20°C till further use.

Optimization of PCR assay:

PCR protocol for detection of *Theileria* organism was optimized in the laboratory using positive DNA of *T. annulata* and two sets of primers (Table 2). PCR reaction was performed in a total volume of 25 µL and reaction mixture was prepared using the following reagents; 10x PCR buffer-2.5 µL, forward and reverse primers (20 pmol/µL)-0.5 µL each, dNTPs-0.5 µL, genomic DNA-3.0 µL, Dream Taq DNA polymerase-0.25 µL. and nuclease free water-17.75 µL. Reaction was run in a thermal cycler (S1000 Gradient Thermal Cycler, Bio-Rad) with the cycling conditions as an initial denaturation of 95°C for 5 min, followed by 34 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 45 sec (18S *rRNA* gene) or 58°C for 1 min (Tams1 gene) and extension at 72 °C for 45 sec, and a final extension at 72 °C for 10 min. Thereafter, gDNA samples extracted from ticks (n=100) were screened at the optimized PCR condition as described above. The PCR amplified products were run in 1.2% agarose gel using TAE (Tris-acetate-EDTA) buffer. GeneRulerTM 100 bp Plus DNA ladder (Thermo Scientific, USA) was used to determine the size of amplified PCR product. Amplification was visualized in gel documentation system under the UV transilluminator (GeNei).

Statistical analysis of data:

Agroclimatic zone, season and age wise prevalence data was analyzed using Chai square (χ^2) test (https://www.socscistatistics.com/tests/chisquare/default2.aspx) to determine the significance of data generated in the study.

RESULTS AND DISCUSSION

In the present study, screening of a total of 235 animals revealed overall 42.55% tick infestation rate. The highest infestation rate was recorded in the Upper Gangetic Plain (54.5%), followed by the Trans Gangetic Plain (45.0%), Western Dry Region (44.4%), and Western Himalayan Region (22.9%). According to an estimate, about 84.0% of the world cattle populations are exposed to tick infestation (FAO, 1984). Further, high tick infestation rate in cattle ranging from 58.06% to 86.15% had already been reported from different part of India (Patel *et al.*, 2013; Singh and Rath, 2013; Balasubramanian *et al.*, 2019).

The ticks collected were morphologically identified as *Rhipicephalus* spp. and *Hyalomma* spp. Genomic DNA extracted from the partially or fully engorged *Hyalomma* spp. (n=100) and subjected for PCR amplification targeting the 18S *rRNA* and *Tams*1 gene revealed positive amplification (Figs. 1 & 2) in a total of 23 and 21 samples, respectively. Agroclimatic zone-wise analysis of PCR results (18S *rRNA* gene based) indicated highest prevalence of *Theileria* infection in the Western Dry Region (31.25%) followed by the Trans Gangetic Plain (25.93%), Upper Gangetic Plain (16.67%) and Western Himalayan Region (9.09%) (Table 3 & Fig. 3). Likewise, seasonal analysis revealed highest prevalence during the monsoon season (28.94%) followed by summer (19.51%) and winter season (19.04%) (Fig. 4). Age wise analysis revealed higher prevalence in adult cattle (24.48%) followed by calves

Table 1. Agro climatic zone wise prevalence of *Hyalomma* sp. infestation in cattle

S. No.	Agro climatic zone	No. of cattle screened	No. of cattle positive (% prevalence)
1.	Upper Gangetic Plain	55	30[a] (54.5%)
2.	Trans Gangetic Plain	60	27[a] (45.0%)
3.	Western Dry Region	72	32[a] (44.4%)
4.	Western Himalayan Region	48	11[b] (22.9%)
	Total	235	100 (42.55%)

Note: Superscript (a) denoter non-significant correlation, whereas superscript(b) denotes significant correlation at P<0.05.

Table 2. Primers used for detection of *Theileria* infection in tick vectors

Target gene	Oligomer name and sequences (5'-3')	Amplicon Size	Reference
18S *rRNA*	Theil 18S-For: GCTGCATTGC TTGTGTCCCTCT Theil18S-Rev: CAAGGTGCT GAAGGAGTCGTAAAAAC	440bp	Self-designed
Tams1	Tamulti-For: CCGTTAATGC TGCAAATGAGGAGG Tamulti-Rev: GAGGCGAAGA CTGCAAGGGGAG	751bp	Kundavae *et al.* (2018)

Table 3. Agro-climatic zone wise prevalence of *T. annulata* infection in cattle tick

S. No.	Agro-climatic zone	No. of DNA samples (tick) examined	No. of DNA samples (tick) positive
1.	Upper Gangetic Plain	30	5[a] (16.67%)
2.	Trans Gangetic Plain	27	7[a] (25.93%)
3.	Western Dry Region	32	10[a] (31.25%)
4.	Western Himalayan Region	11	1[a] (09.09%)
	Total	100	23[a] (23.00%)

Note: Superscript (a) denotes non-significant correlation (P<0.05)

(20%) and heifers (19.44%) (Fig. 5). Statistical analysis of agroclimatic zone, season and age wise PCR results could not witness any significant difference (P >0.05).

The present study was performed to determine the molecular prevalence of *Theileria* organism in vector ticks collected from 4 different agroclimatic zones of the country. Study recorded overall 23.0% molecular prevalence of *T. annulata* by using 18S *rRNA* gene based PCR assay and 21.0% prevalence by *Tams*1 gene based PCR assay in vector ticks. Above difference in the detection rate of both PCR assay(s) could by justified by the fact that 18S *rRNA* gene based PCR has ability to amplify *Theileria* genus specific DNA, whereas *Tams*1 gene based PCR is specific for *T. annulata* only. Detection of pathogens in the tick vector utilizing PCR assay may prove beneficial in larger epidemiological investigations.

In the study, molecular prevalence of *T. annulata* has been found reasonably high in Upper Gangetic, Trans Gangetic and Western dry region (16.67% to 31.25%) in

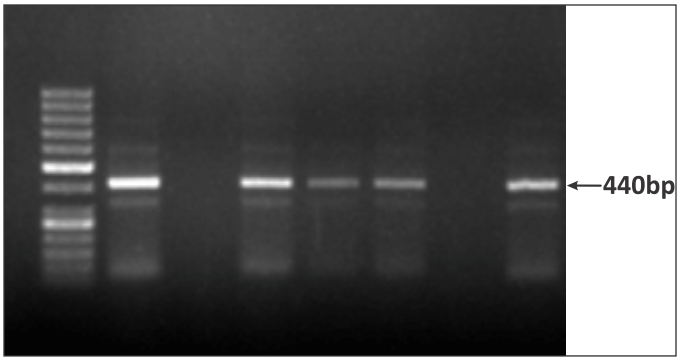

Fig. 1. PCR amplification of 18S *rRNA* gene of *Theileria* from vector tick M- 50 bp plus DNA ladder, L1- positive control, L2- Negative control, L3 to L7- Tick DNA samples

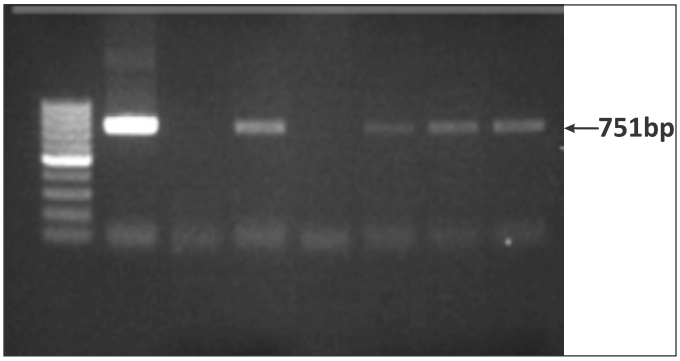

Fig. 2. PCR amplification Tams1gene of *Theileria annulata* from vector tick (M- 100 bp plus DNA ladder, L1- positive control, L2- negative control, L3 to L7- tick DNA samples)

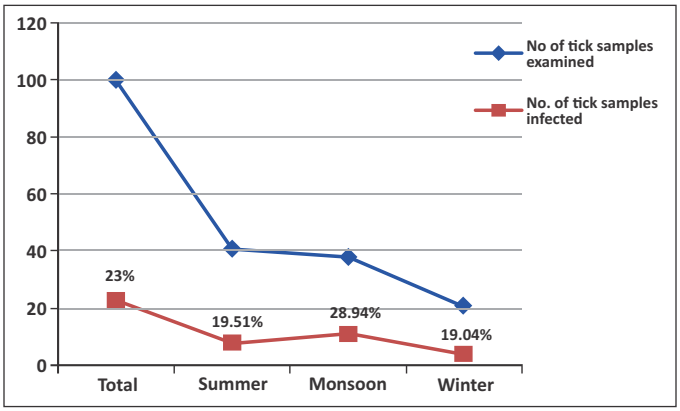

Fig. 4. Season wise prevalence of *Theileria* infections in cattle tick

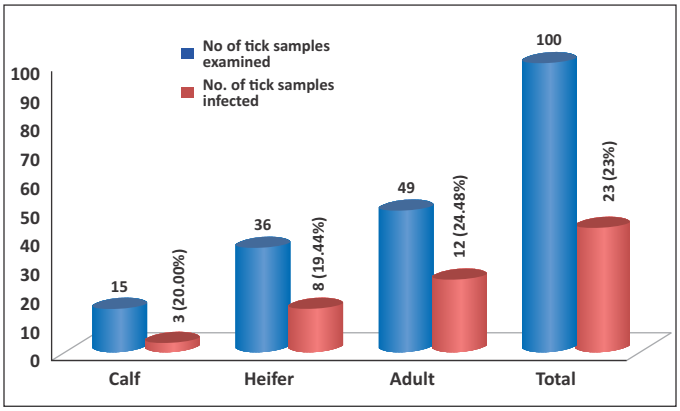

Fig. 5. Age wise prevalence of *Theileria* infections in cattle tick

comparison to the Western Himalayan region (9.09%). Therefore, an in-depth epidemiological investigation is required in these areas involving appropriate sample size of cattle population. Molecular studies conducted by other workers have also reported variable prevalence rate of this parasite in different states of India. Tiwari *et al.* (2015) tested DNA samples extracted from *H. anatolicum* collected from healthy cattle in Punjab and recorded 20.0% prevalence of *T. annulata* using primary PCR and 60.0% prevalence by nested PCR. Another study from Chhattisgarh reported overall 34.24% intra-acarine haemoprotozoan (*Theileria* and *Babesia*) infection (Jadhao *et al.*, 2021). Recently, Dehuri *et al.* (2022) employed a nested PCR assay targeting the *Tams*1 gene and recorded an overall prevalence of 14.6% *T. annulata* infection in vector ticks from Odisha.

In the study, highest prevalence of *T. annulata* was recorded in ticks collected from western dry region, while it was lowest in the Western Himalayan region. Such difference recorded in the prevalence rate in tick vectors seems to be influenced by the prevailing climatic condition in both the region. Hot and humid climate usually favors the survival of *Hyalomma anatolicum*, which is prevailing in Western dry region. Seasonal dynamics revealed highest prevalence rate in vectors during the monsoon season followed by summer and winter seasons. Lower seasonal temperature of the area seems to be detrimental for the life cycle stages of the ixodid ticks, which ultimately results in the low vector population during winter season. Based on the findings of the molecular studies performed in other countries, where theileriosis is endemic, a high prevalence of *Theileria* infection was reported in *Hyalomma* sp. (Khodabandeh and Razmi, 2015). Sporadic reports are also available on the presence of *Theileria* sp. infection in vector tick other than *Hyalomma* sp. (Ionita *et al.*, 2013; Jadho *et al.*, 2022).

CONCLUSION

Molecular detection of *Theileria* organism in vector ticks by PCR assay has been a powerful tool for epidemiological investigation. In the study, *18S rRNA* gene based PCR was found more useful in comparison to the *Tams*1 gene based PCR. Further, agroclimatic zones having considerably high prevalence rate of this parasite in vectors may be targeted as a priority for detail epidemiological investigations. Data generated in the present study provides key information about the distribution of *T. annulata* in the targeted agroclimatic zones, which may be helpful in developing and strategizing effective control measures against this parasite.

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Research Article

HEMATOLOGY AND PHENOTYPIC CHARACTERIZATION OF MUDHOL HOUND

SURAJ KOUJALAGI*, CHANDRAKANT GALDHAR, RAJIV GAIKWAD, DHANANJAY PAWALKAR[1], SHAILESH INGOLE[2], RAHUL DESHMUKH[3], VIVEK KASRALIKAR[4], DISHANT SAINI[5], ANIL SINDHUR[6] and PRASHANT KURBET[7]

Dept. of Veterinary Clinical Medicine, Ethics & Jurisprudence, [1]Dept. of Veterinary Epidemiology and Preventive Medicine, [2]Dept. of Veterinary Physiology, [3]Dept. of Veterinary Animal Genetics and Breeding, [5]Dept. of Veterinary Surgery and Radiology, Mumbai Veterinary College, Parel, Mumbai-400012, MAFSU, Nagpur (Maharashtra)

[4]Dept. of Veterinary Clinical Complex, Veterinary College Bidar-585401, Karnataka

[6]Government Veterinary Polyclinic, Koppal-583231, Karnataka; [7]Veterinary dispensary-Avaradi-591312, Karnataka

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ABSTRACT

In the present study, phenotypic characterization and haemato-biochemical studies of Mudhol Hound, a popular dog breed of Karnataka and Maharashtra was undertaken. Mudhol hound is also known as the Maratha Hound, and the Caravan Hound. During the survey, 14 physical morphometric traits were recorded of 30 adult KCI registered Mudhol Hounds (aged above 1 year and less than 10 year). Thirty Mudhol Hound owners were contacted in order to get information about animal routine management. Mudhol Hounds are compact, medium to large sized, with a slender and slim body, skin coloured nostrils and eyelids, dangled ears, a straight to arced tail and a straight upper line. The average body weight was around 27.7 ± 1.2 kilogram for males and 26.2 ± 0.8 kilogram for females and was obtained from owner's recent records. The values were analysed by Bars and Whiskers. The mean height at wither was 73.1± 0.88 cm, mean length of head was 28.2± 0.33 cm, mean girth of chest was 68.2 ± 1.27 cm and mean length of tail was 57.55 ± 0.81 cm. The mean haemoglobin values of Mudhol Hounds was found to be 14.76 ± 0.46 gram percent and that of mean platelet count was recorded to be $3.83 \pm 0.36 \times 10^6$/cmm. The hounds were fed with both vegetarian and non-vegetarian food and were fed thrice a day. The diet consists of roti/chapati soaked in milk, rice, boiled meat, boiled egg and mixed grain diet. Hounds were regularly dewormed and vaccinated as per standard schedule. The Mudhol Hounds are used for guarding in the farm house, chasing out the vertebrate pests from farms. Mudhol hound being a part of Border Security Force, recently involved in security of Prime Minister and its popularity, there is a need of recording of data and documentation.

Keywords: Haematology, Hound, Morphometry, Mudhol

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Dog (*Canis lupus familiaris*) is known to be the first animal domesticated by humans. Popular Indian dog breeds include the Rampur Hound, Kanni, Himalayan sheep dog, Chippiparai, Rajapalayam, Mudhol Hound, and Bhutia sheep dogs (Gandhi 2010). These Indian dog breeds were primarily used for hunting, shepherding and guarding. Mudhol Hound is one among the three NBAGR registered Indain dog breeds with the accession number "INDIA_DOG_0800_MUDHOL HOUND_19003". Mudhol is town situated in Bagalkot district of North Karnataka. Different body confirmation and with differing functional relationship has been widely observed in different breeds (Latorre *et al.*, 2011, Yakubu *et al.*, 2010, Zaitoun *et al.*, 2005,). A hound is a breed of dog used for hunting, particularly one that can follow scent. Originally utilized for hunting, the Hound breeds were known for their strong sense of scent, exceptional speed, or both. Over the years, a number of researchers have drawn attention to the potential connection between morphometric characteristics and the reason a population was bred. However, morphometric characterization and recording of native dog breeds have not received enough attention. In order to characterize the Karnataka Mudhol Hound and create breed-specific morphometric descriptions, the current study was conducted.

MATERIALS AND METHODS

A questionnaire was created to gather data on the breed, management techniques, morphometric and physical characteristics, and the breed's usefulness in its natural environment. The study on Mudhol hound population was undertaken in and around Mudhol town of Bagalakote district in Karnataka state. The data was gathered using a random sample technique. A total of 14 morphometric measurements on 30 adult animals (15 male and 15 female) were recorded during the study. The weight of the dogs was noted from owner and the Kennel Club of India's registration certificates were used to determine the animals' ages. The morphometric measurements used in this investigation were in accordance with research by Gonzalez *et al.* (2011) and Sutter *et al.* (2008). A graded measuring tape was used to measure the animals' morphometric characteristics as they stood on a level surface. Body length, height at withers, height at rump, chest girth, abdominal girth, head length, tail length, snout

*Corresponding author: surajck11@gmail.com

length, face length, ear length, front pastern, rear pastern elbow to withers, and height at elbow in centimetres were among the morphometric characteristics being studied. (Fig. 1). The hemogram was done using auto analyzerDiatronAbacus.The data were analysed using bars and whiskers in Microsoft excel 2019.

Fourteen discrete body measurements were collected from each of 30 dogs depicted in Fig. 1 serially and their Morphologic descriptions are as below.

1. Height at withers- The linear distance from the ground to scapula (withers).

2. Height at Rump- The linear distance from the ground to the dorsal-most point of rump.

3. Snout length- The distance along the skull from the rostral end of the planum nasale to dorsal plane between eyes.

4. Head length- The distance along the skull from thetop to the external occipital protuberance to dorsal plane between the eyes.

5. Face length- Head length + snout length.

6. Body length- The length of the body between the pin bone and the anterior portion of the sternum.

7. Chest girth- The circumference of the deepest part of the thoracic cavity.

8. Abdomen girth- The circumference of the body at abdomen.

9. Fore limb pastern diameter- The circumference of the fore limb at the pastern region.

10. Hind limb pastern diameter- The circumference of the hind limb at the pastern region.

11. Ear length- Length from base to tip of ear pinna.

12. Tail length- Length from base to tip of tail.

13. Height at elbow- Length from ground to elbow joint.

14. Elbow to withers- Distance from withers to elbow joint.

RESULTS AND DISCUSSION

Face Length

The mean length of face of Mudhol hound was recorded to be 28.28±0.33 cm. Fig. 2 depicts the box plot of length of face of Mudhol hounds, where 'T bars' represent the range from 24 to 32 cm, interquartile range, 25th to 75th percentile, as 27.37 to 29.62 cm with median as 28.50 cm. Gonzlez *et al.* (2014) reported the face length of Spanish sight hounds as 23.58±0.15 cm in males and 22.31±0.16 cm in females. Milivoje *et al.* (2020) published the face length of Turkish sighthounds as 23.27±0.11 cm and 22.29±0.13 cm for males and females, respectively. Raja *et al.* (2017) measured the face length of Rajapalayam hound and noted to be 21.30±0.35 cm in male hounds and 19.41±0.34 cm in the female hounds.

Snout / Muzzle Length

The mean length of snout of Mudhol hound was recorded to be 11.41±0.12 cm. Fig. 3 depicts the box plot of length of snout of Mudhol hounds, where 'T bars' represent the range from 10 to 13 cm, interquartile range, 25th to 75th percentile, as 11 to 13 cm with median as 11 cm. Gonzlez *et al.* (2014) reported the snout length of Spanish sight hounds as 10.57±0.14 cm in males and 9.89±0.14 cm in females. Milivoje *et al.* (2020) published the snout length of Turkish sighthounds as 7.78±0.10 cm and 7.35±0.13 cm formales and females, respectively. Raja *et al.* (2017) measured the snout length of Rajapalayam hound and noted to be 10.73±0.25 cm for male hounds and 10.44±0.18 cm for the female hounds.

Head / Frontal Length

The mean length of head of Mudhol hound was recorded to be 16.85±0.26 cm. Fig. 4 depicts the box plot of length of head of Mudhol hounds, where 'T bars' represent the range from 15 to 19 cm, interquartile range, 25th to 75th percentile, as 16.5 to 18 cm with median as 17 cm. Gonzlez *et al.* (2014) reported the face length of Spanish sight hounds as 13.59±0.15 cm and 12.81±0.14 cm for males and females, respectively.

Chest Girth

The mean girth of chest of Mudhol hound was recorded to be 68.28±1.27 cm. Fig. 5 depicts the box plot of girth of chest of Mudhol hounds, where 'T bars' represent the range from 54 to 78 cm, interquartile range, 25th to 75th percentile, as 62 to 75.12 cm with median as 68 cm. Gonzlez *et al.* (2014) reported the chest girth of Spanish sight hounds as 70.21±0.57 cm in males and 66.93±0.45 cm in females. Milivoje *et al.* (2020) published the chest girth of Turkish sighthounds as 64.96±0.54 cm and 61.22±0.49 cm for males and females, respectively. Raja *et al.* (2017) measured the chest girth of Rajapalayam hound and noted to be 67.85±0.60 cm for male hounds and 63.38±0.63 cm for the female hounds.

Abdomen Girth

The mean girth of abdomen of Mudhol hound was recorded to be 43.66±0.30 cm. Fig. 6 depicts the box plot of girth of abdomen of Mudhol hounds, where 'T bars' represent the range from 40 to 46 cm, interquartile range, 25th to 75th percentile, as 42 to 45 cm with median as 44 cm. Raja *et al.* (2017) measured the abdomen girth of Rajapalayam hound and noted to be 48.95±1.02 cm and 46.55±0.71 cm for males and females, respectively. Mudhol hound was observed to be comparatively slim at

abdomen when compared to Rajapalayam hound.

Body Length

The mean length of body of Mudhol hound was recorded to be 73.65±0.88 cm. Fig. 7 depicts the box plot of length of body of Mudhol hounds, where 'T bars' represent the range from 65 to 84 cm, interquartile range, 25th to 75th percentile, as 71 to 78 cm with median as 72 cm. Gonzalez *at al.* (2014) reported the body length of Spanish sight hounds as 67.83±0.53 cm in males and 65.02±0.55 cm in females. Milivoje *et al.* (2020) published the body length of Turkish sighthounds as 61.73±0.33 cm and 58.64±0.41 cm for males and females, respectively. Mudhol hound comparatively measures lengthier than other two hounds. Raja *et al.* (2017) measured the body length of Rajapalayam hound and noted to be 58.95±0.49 cm for male hounds and 55.95±0.65 cm for the female hounds.

Table 1. Haematological values of Mudhol Hound

Sr. No.	Parameters	Mean ± SE values of Mudhol Hound	Reference Range (Merck's Veterinary Manual, 2018)
1.	Hemoglobin (g/dl)	14.76 ± 0.46	12-18
2.	Packed Cell Volume (%)	44.4 ± 2.15	37-55
3.	Total Erythrocyte Count (x 10^6/cmm)	7.06 ± 0.18	5.50-8.50
4.	Total Leucocyte Count (x 10^3/ cmm)	12.24 ± 0.60	6-17
5.	Neutrophils (%)	73.53 ± 2.00	62-87
6.	Lymphocytes (%)	22.4 ± 2.12	12-30
7.	Monocytes (%)	1.3 ± 0.18	2-10
8.	Eosinophils (%)	2.5 ± 0.37	3-10
9.	Basophils (%)	0.23 ± 0.09	0-0
10.	Platelets (x 10^5/cmm)	3.83 ± 0.36	2-5

Table 2. The Mean ± SD Haematological values of different Hounds

Sr. No.	Parameters	Present study Mudhol Hound	Uhrikova *et al.* (2013)			
			Grey Hound	Saluki Hound	Sloughi Hound	Pharaoh Hound
1.	Hemoglobin (g/dl)	14.76 ± 2.52	19.1 ± 1.5	18.5 ± 2.2	18.9 ± 1.7	17.7 ± 2.0
2.	Packed Cell Volume (%)	44.4 ± 11.80	56.20 ± 4.0	55.0 ± 5.7	56.5 ± 4.9	52.80 ± 4.8
3.	Total Erythrocyte Count (x 10^6/cmm)	7.06 ± 1.01	7.7 ± 0.5	8.0 ± 0.8	8.1 ± 0.9	7.5 ± 0.7
4.	Total Leucocyte Count (x 10^3/ cmm)	12.24 ± 3.33	5.4 ± 1.8	7.6 ± 1.6	9.8 ± 1.7	8.8 ± 1.9
5.	Mean Corpuscular Volume (fL)	70.60 ± 11.88	73.4 ± 1.6	68.9 ± 2.6	70.2 ± 1.9	70.4 ± 2.8
6.	Mean Corpuscular Haemoglobin (pg)	22.23 ± 4.21	24.9 ± 0.8	23.2 ± 1.6	23.5 ± 1.2	23.6 ± 1.8
7.	Mean Corpuscular Haemoglobin Concentration (g/l)	33.54 ± 10.40	33.9 ± 1.2	33.5 ± 1.5	33.5 ± 1.5	33.5 ± 2.0
8.	Platelets (x 10^5/cmm)	3.83 ± 1.99	1.73 ± .49	2.11 ± 0.81	2.63 ± .92	3.02 ± 0.15

Tail Length

The mean length of tail of Mudhol hound was recorded to be 57.55±0.81 cm. Fig. 8 depicts the box plot of length of tail of Mudhol hounds, where 'T bars' represent the range from 50 to 65 cm, interquartile range, 25th to 75th percentile, as 54 to 62 cm with median as 57.5 cm. The Rajapalayam hound's tail length was measured by Raja *et al.* (2017) and found to be 41.55±0.62 cm and 38.20±0.43 cm for male and female hounds, respectively. Mudhol hound tail was comparatively lengthier than other hounds.

Height at Withers

The mean height at withers of Mudhol hound was recorded to be 73.10±0.88 cm. Fig. 9 depicts the box plot of height at withers of Mudhol hounds, where 'T bars' represent the range from 64 to 82 cm, interquartile range, 25th to 75th percentile, as 70 to 77.25 cm with median as 72.50 cm. Gonzalez *et al.* (2014) reported the height at withers of Spanish sight hounds as 68.40±0.34 cm in males and 64.98±0.33 cm in females. Milivoje *et al.* (2020) published the height at withers of Turkish sight hounds as 62.02±0.30 cm and 58.38±0.34 cm for males and females, respectively. Raja *et al.* (2017) measured the height at withers of Rajapalayam hound and noted to be 63.10±0.91 cm and 58.91±0.57 cm for male and female hounds, respectively.

Height at Rump

The mean height at rump of Mudhol hound was recorded to be 73±0.86 cm. Fig. 10 depicts the box plot of height at rump of Mudhol hounds, where 'T bars' represent the range from 64 to 81 cm, interquartile range, 25th to 75th percentile, as 70 to 77.25 cm with median as 72.50 cm. Height at rump was found to be same as that of height at withers. Gonzalez *et al.* (2014) reported the height at rump of Spanish sight hounds as 66.57±0.39 cm in males and 64.19±0.34 cm in females. Milivoje *et al.* (2020) published the height at rump of Turkish sight hounds as 61.75±0.35 cm and 58.70±0.33 cm for males and females, respectively.

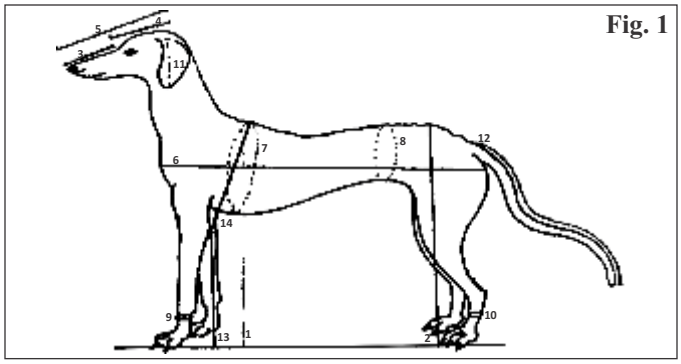
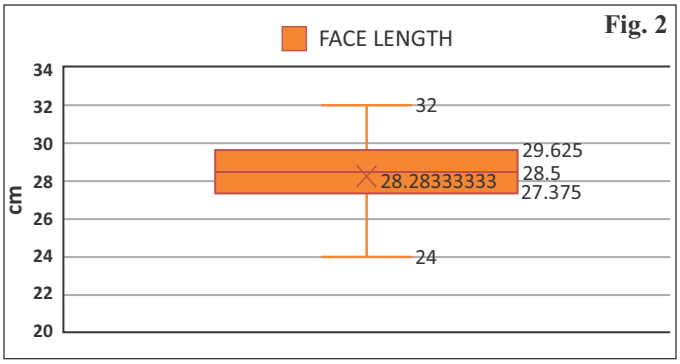


Fig. 1



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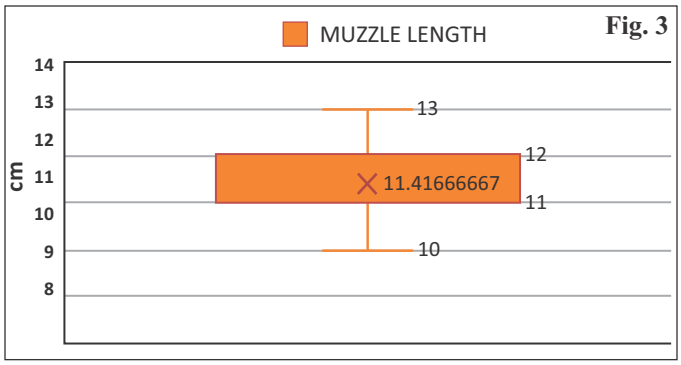

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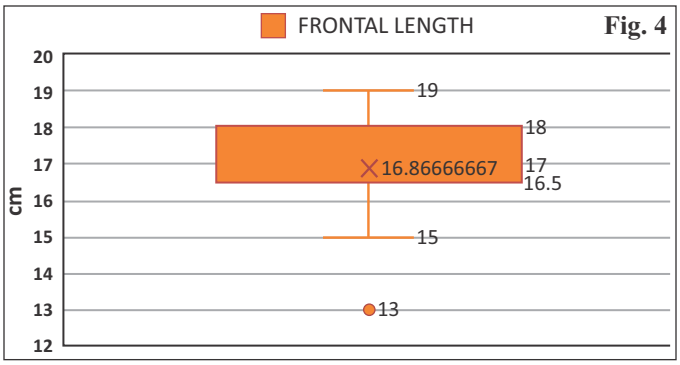

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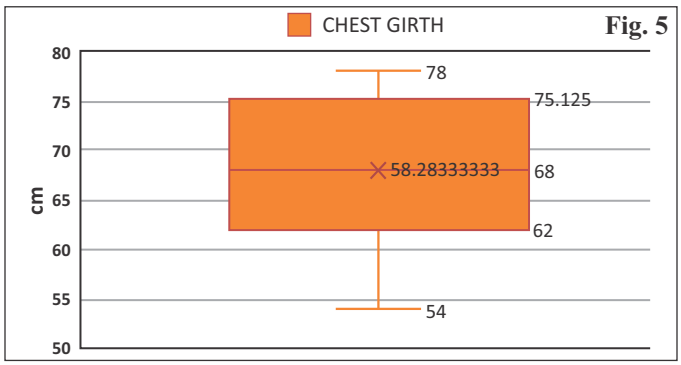

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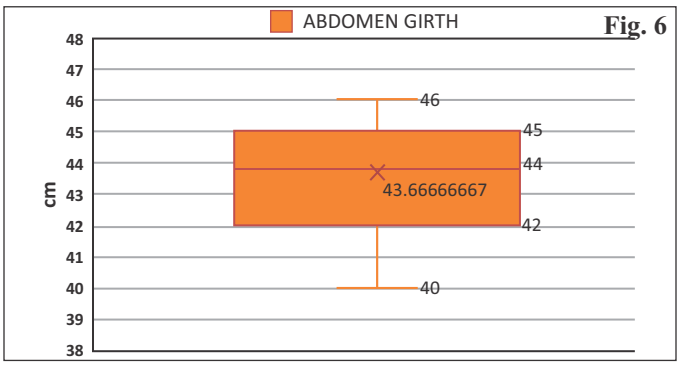

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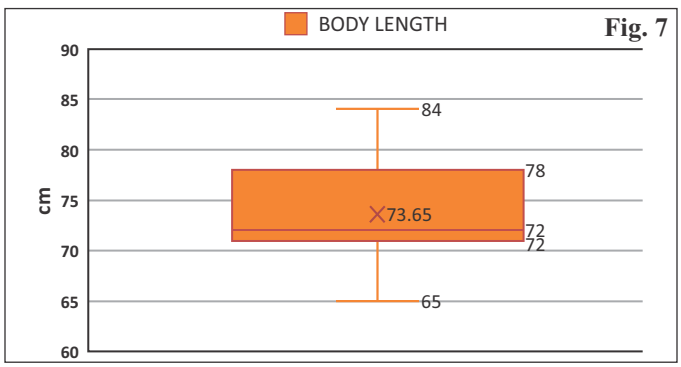

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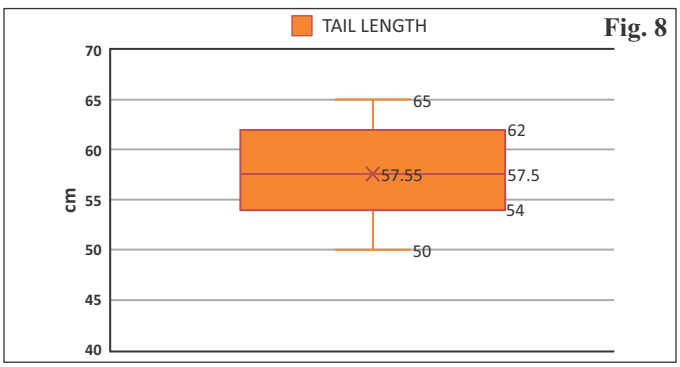

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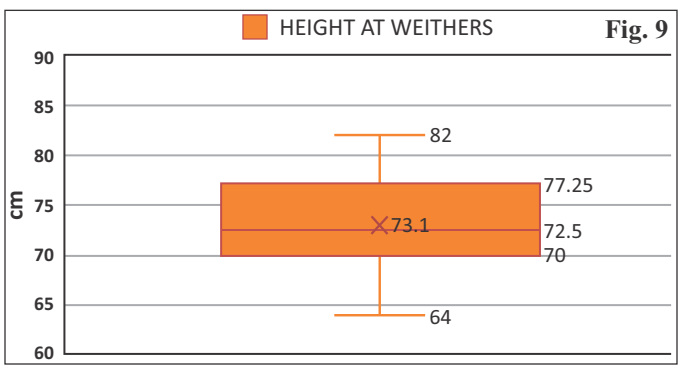

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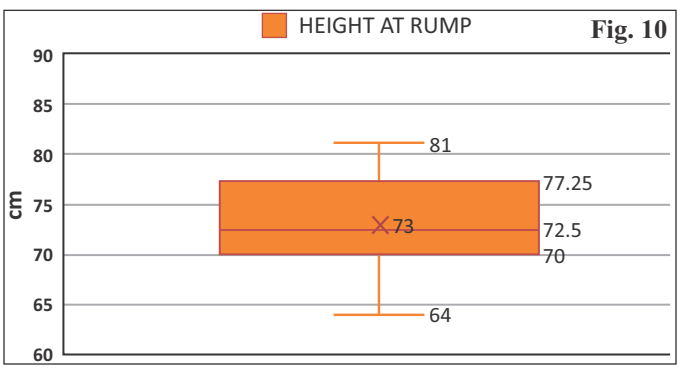

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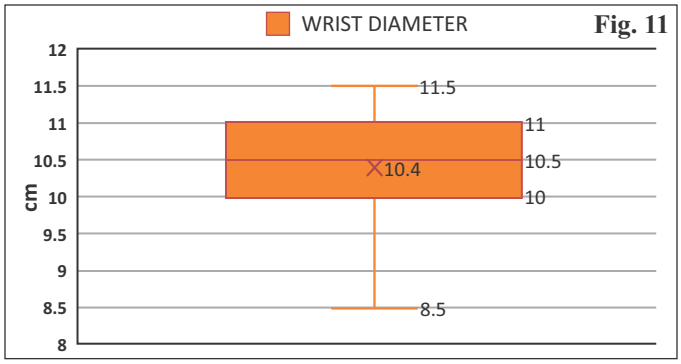

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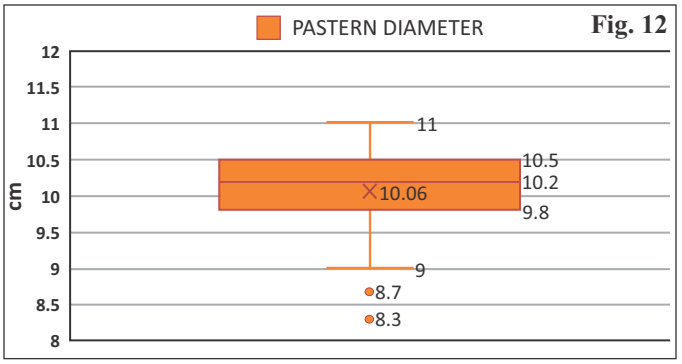

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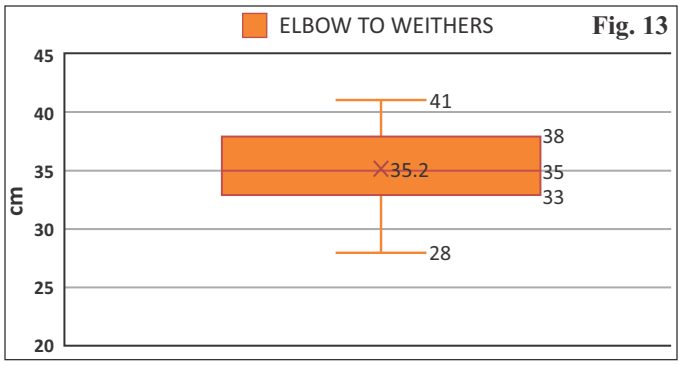

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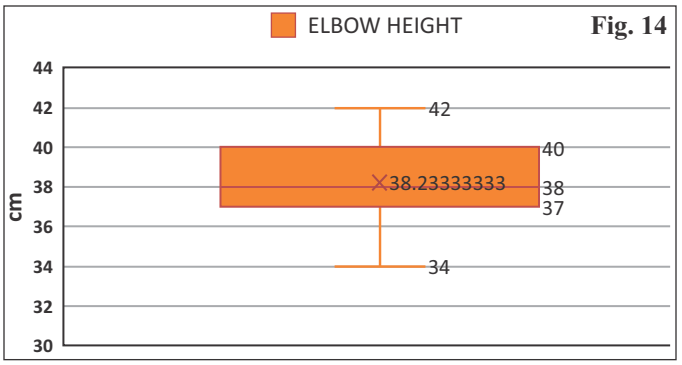

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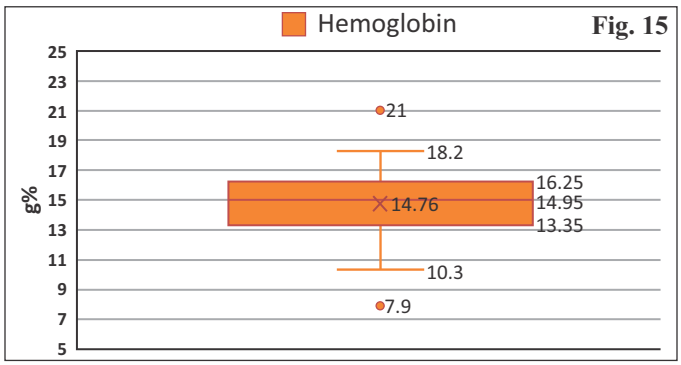

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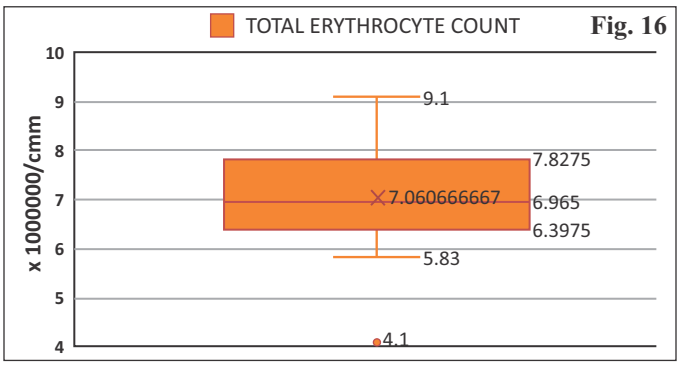

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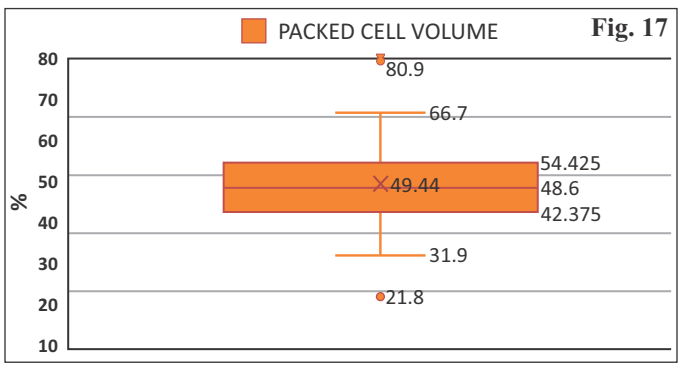

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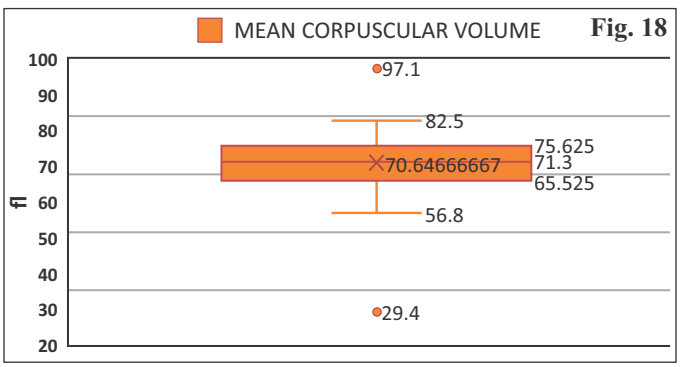

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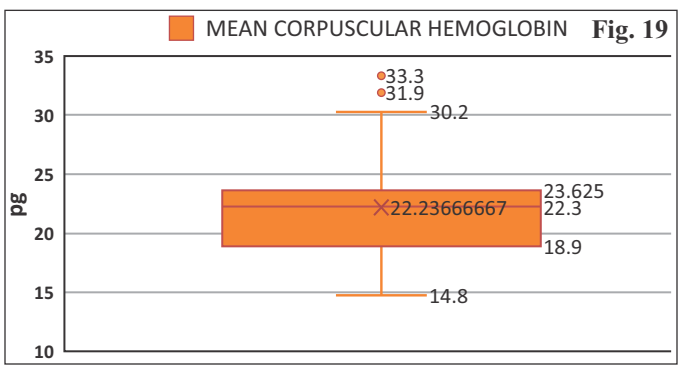

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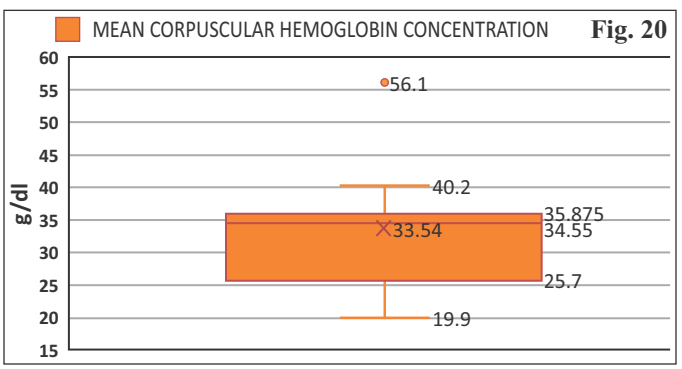

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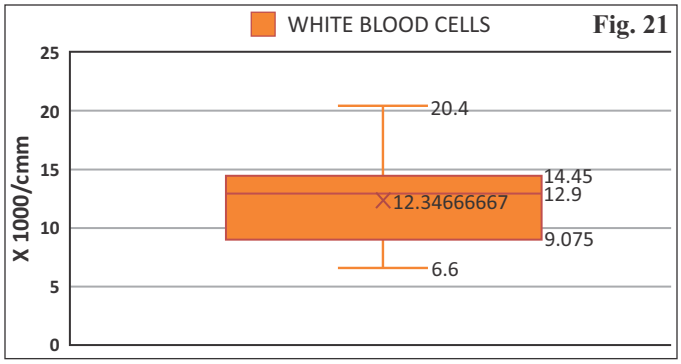

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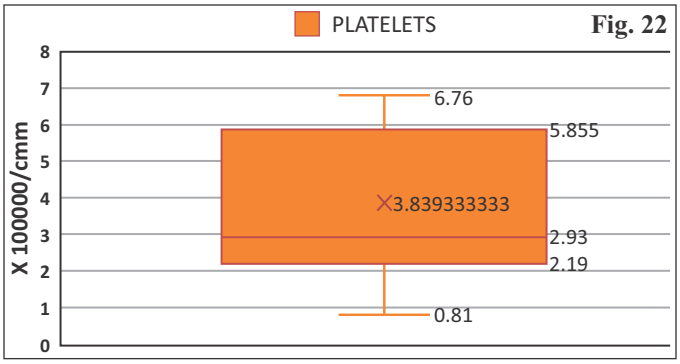

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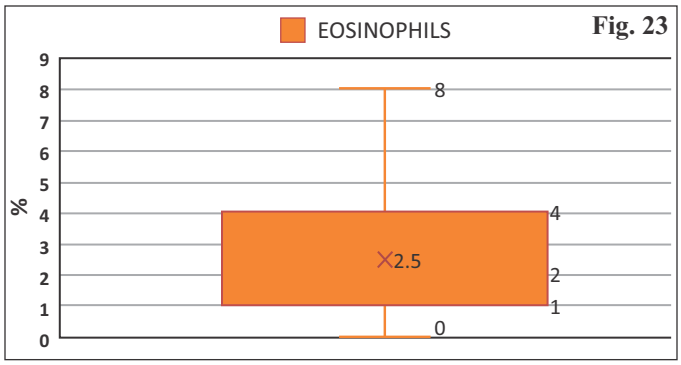

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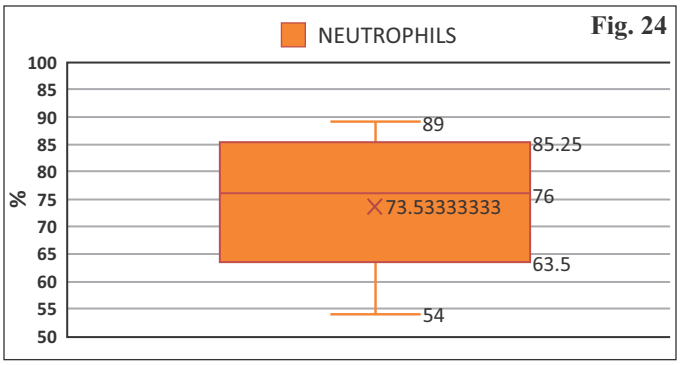

Fig. 24

Fore Limb Pastern Diameter

The mean fore limb pastern diameter of Mudhol hound was recorded to be 10.4±0.11 cm. Fig. 11 depicts the box plot of fore limb pastern diameter of Mudhol hounds, where 'T bars' represent the range from 8.5 to 11.5 cm, interquartile range, 25th to 75th percentile, as 10 to 11 cm with median as 10.5 cm. Gonzalez *et al.* (2014) reported the forelimb pastern diameter of Spanish sight hounds as 9.75±0.11 cm and 9.19±0.14 cm for males and females respectively. Milivoje *et al.* (2020) published the forelimb pastern diameter of Turkish sight hounds as 9.71±0.08 cm and 9.24±0.09 cm for males and females, respectively.

Hind Limb Pastern Diameter

The mean hind limb pastern diameter of Mudhol hound was recorded to be 10.06±0.11 cm. Fig. 12 depicts the box plot of hind limb pastern diameter of Mudhol hounds, where 'T bars' represent the range from 8.3 to 11 cm, interquartile range, 25th to 75th percentile, as 9.8 to 10.5 cm with median as 10.2 cm. The mean fore limb pastern diameter was comparatively bigger than mean hind limb pastern diameter of Mudhol hound. Gonzalez *et al.* (2014) reported the hindlimb pastern diameter of Spanish sight hounds as 9.17±0.09 cm and 8.69±0.13 cm in male and female hounds, respectively.

Elbow To Withers Length

The mean distance between elbow to withers of Mudhol hound was recorded to be 35.20±0.52 cm. Fig. 13 depicts the box plot of length of distance between elbow to withers of Mudhol hounds, where 'T bars' represent the range from 28 to 41 cm, interquartile range, 25th to 75th percentile, as 33 to 38 cm with median as 35 cm.

Height at Elbow

The mean height at elbow of Mudhol hound was recorded to be 38.23±0.41 cm. Fig. 14 depicts the box plot of height at elbow of Mudhol hounds, where 'T bars' represent the range from 34 to 42, interquartile range, 25th to 75th percentile, as 37 to 40 cm with median as 38 cm. Milivoje *et al.* (2020) published the height at elbow of Turkish sight hounds as 32.99±0.40 cm and 31.88±0.21 cm in male and female hounds, respectively.

Ear Length

The mean length of ear of Mudhol hound was recorded to be 12.70±0.18 cm. Milivoje *et al.* (2020) published the ear length of Turkish sighthounds as 12.00±0.92 cm in males and 11.31±0.71 cm in females. Raja *et al.* (2017) measured the ear length of Rajapalayam hound and noted to be 12.33±0.23 cm and 11.46±0.14 cm in male and female hounds, respectively. All the mean haematological values of Mudhol Hound were in in accordance with the mean values of Mercks' Veterinary Manual presented in table 1.

Table 2 shows that Mudhol hounds had lower mean values of hemoglobin, total erythrocyte count and packed cell volume than other hounds, whereas, the total leucocyte counts and platelets were comparatively higher in Mudhol Hounds.

Haemoglobin (g/dl)

The mean ± SE haemoglobin value of Mudhol hound was recorded to be 14.76±0.46 g/dl. Fig. 15 depicts the box plot of haemoglobin values of Mudhol hounds,

where 'T bars' represent the range from 10.3 to 18.2 g/dl, interquartile range, 25th to 75th percentile, as 13.35 to 16.25 g/dl with median as 14.95 g/dl.

Total Erythrocyte Count (x 10^6/cmm)

The mean ± SE total erythrocyte count value of Mudhol hound was recorded to be 7.06±0.18 × 10^6/cmm. Fig. 16 depicts the box plot of total erythrocyte count values of Mudhol hounds, where 'T bars' represent the range from 10.3 to 18.2×10^6/cmm, interquartile range, 25th to 75th percentile, as 13.35 to 16.25×10^6/cmm with median as 14.95×10^6/cmm.

Packed Cell Volume (%)

The mean ± SE packed cell volume of Mudhol hound was recorded to be 44.4±2.15 %. Fig. 17depicts the box plot of packed cell volume values of Mudhol hounds, where 'T bars' represent the range from 31.9 to 66.7%, interquartile range, 25th to 75th percentile, as 13.35 to 16.25% with median as 48.6%.

Mean Corpuscular Volume (fl)

The mean ± SE mean corpuscular volume of Mudhol hound was recorded to be 70.64±2.17 fl. Fig. 18 depicts the box plot of mean corpuscular volume values of Mudhol hounds, where 'T bars' represent the range from 56.8 to 82.5 fl, interquartile range, 25th to 75th percentile, as 65.5 to 75.62 fl with median as 70.64 fl.

Mean Corpuscular Haemoglobin (pg)

The mean ± SE mean corpuscular haemoglobin of Mudhol hound was recorded to be 22.23±0.76 pg. Fig. 19 depicts the box plot of mean corpuscular haemoglobin values of Mudhol hounds, where 'T bars' represent the range from 14.8 to 30.2 pg, interquartile range, 25th to 75th percentile, as 18.9 to 23.62 pg with median as 22.3 fl.

Mean Corpuscular Haemoglobin Concentration (g/dl)

The mean ± SE mean corpuscular haemoglobin concentration of Mudhol hound was recorded to be 33.54±1.89 g/dl. Fig. 20 depicts the box plot of mean corpuscular haemoglobin concentration values of Mudhol hounds, where 'T bars' represent the range from 19.9 to 40.2 g/dl, interquartile range, 25th to 75th percentile, as 25.7 to 35.87 g/dl with median as 34.93 g/dl.

White Blood Cells/ Total Leucocyte count (x 1000/cmm)

The mean ± SE total leucocyte count of Mudhol hound was recorded to be 12.34±0.60 × 1000/cmm. Fig. 21 depicts the box plot of total leucocyte count values of Mudhol hounds, where 'T bars' represent the range from 6.6 to 20.4 × 1000/cmm, interquartile range, 25th to 75th percentile, as 9.07 to 14.45 × 1000/cmm with median as 12.9 × 1000/cmm.

Platelets (x 100000/cmm)

The mean ± SE total leucocyte count of Mudhol hound was recorded to be 3.83±0.36 × 100000/cmm. Fig. 22 depicts the box plot of platelet values of Mudhol hounds, where 'T bars' represent the range from 0.81 to 6.76×100000/cmm, interquartile range, 25th to 75th percentile, as 2.19 to 5.85 × 100000/cmm with median as 2.93×100000/cmm.

CONCLUSION

Results of 14 morphometric measurements reveal that the values of Mudhol hound are in similar range as that of Spanish sight hound and Turkish sight hound. As several researchers have mentioned the importance of relationship between morphometric traits and the purpose of dog, such results are helpful in setting the standards for particular breed.

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Research Article

CLINICAL WOUND HEALING ACTIVITY OF *ALOE VERA* GEL IN DOGS

A.P. UPASE, N.D. JADHAV*, S.R. RAJURKAR, A.G. SHINDE, G.M. CHIGURE[1], P.R. RATHOD[2] and S.D. CHEPTE[3]

Department of Veterinary Pharmacology and Toxicology, [1]Department of Veterinary Parasitology, [2]Department of Veterinary Pathology, [3]Department of Veterinary Surgery and Radiology, College of Veterinary and Animals Sciences, Parbhani, Maharashtra Animal and Fishery Sciences University, Nagpur (Maharashtra)

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ABSTRACT

Aloe vera is a very important and effective plant with multiple health applications and barely any part of human or animal body remain uninfluenced by its healing medicinal use. It acts as a natural fighter against all classes of infection. Considering the major property of *Aloe vera* plant and their easy availability, *Aloe vera* plant leaves were selected to assess their dermal safety in rats and clinical wound healing activity in dogs. In the acute dermal toxicity, no evidence of erythema and oedema were noted in any of the wistar rats exposed to 5% *Aloe vera* gel formulation. Even no sign of clinical and behaviour abnormalities was noted in the exposed rats. The clinical evaluation of 5% *Aloe vera* gel formulation on wound was carried in the dogs reported at Veterinary clinical complex. Twelve dogs with wound were divided into two groups, one applied with povidone iodine solution and other with *Aloe vera* gel. There were no significant changes observed in haematological, biochemical and in wound contraction in both the groups. On the basis of results, it is concluded that, *Aloe vera* gel was found safe for dermal application and accelerates the rate of percent wound contraction in cases of dogs.

Keywords: Acute dermal toxicity, *Aloe vera* gel, Wounds, Wound healing

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India's pet ownership statistics show that 60% of the population owns at least one pet, with dogs being the most popular choice. Number of pet dogs in 2023 was over 31 million, expected to reach 43 million by 2026. Although, due to their playful nature or jumping over the fences, fighting or being dragged across a rough surface, biting dogs often get wound (Elad, 2024).

Wound is considered as an interruption of cell, anatomical and functional continuity of a living tissue caused by physical, chemical, thermal, microbial or immunological harm to the body tissue. Clinical management of wound is one of the commonly encountered conditions among Veterinarians in their routine practice. Wound caused by various reasons such as accidental injuries, maggots, dog bites, etc. These wounds, based on the extent and cause can be detrimental to the dog to the extent of being life threatening and hence, faster recovery is of paramount importance. Wound if left untreated may lead to further complications such as secondary bacterial infections, pus formations, abscess development and maggot infestation, (Zinat *et al.*, 2020). In recent years, new herbal medications are developed by using medicinal plants as a primary source of drug development. India is making progress in the health care sector by advancing the traditional medical system of AYUSH (Ayurveda, Unani, Siddha and Homoeopathy) through international networks (Shakya, 2016). Plant based therapy not only accelerate wound healing process but also maintain the aesthetics. More than 70% of wound healing pharmaceutical products are plant based, 20% are mineral based and remaining containing animal products as their base material (Kumarasamyraja, 2012). There are more than 5000 species of plantmaterials shows promising pharmacological action. Among them Aloe vera has a long history as a medicinal plant and considered to be a more potent in wound healing (Saleem *et al.*, 2022).

MATERIALS AND METHODS

Fresh *Aloe vera* leaves were collected from surrounding areas of Vasantrao Naik Marathwada Krishi Vidyapith (VNMKV), Parbhani and were cleaned with fresh tap water and scraped off to collect pulp. This pulp was thoroughly mixed in an electric grinder and the 5% *Aloe vera* gel was prepared by using carbopol and 98% tri-ethanol amine.

Preparation of *Aloe vera* gel formulation:

Aloe vera gel formulation was prepared by using the 20 gm of carbopol (@ of 2% concentration), 900 ml of distilled water and 5 ml of tri-ethanolamine (@ 0.5% concentration).

In the procedure 20 gm of carbopol was mixed in the 900 ml of distilled water and kept the solution for 8-10 hrs for proper dissociation of carbopol in the distilled water. After 10 hrs, poured 5 ml of tri-ethanol amine drop by drop in the prepared mixture with continuously stirring and then added the 5% concentration of *Aloe vera* pulp in the

*Corresponding author: nitinjadhav@mafsu.ac.in

prepared mixture and the volume of the prepared mixture was adjusted to 1000 ml by adding the required distilled water.

Acute dermal toxicity of *Aloe vera* gel in Wistar rats:

The acute dermal toxicity was evaluated in wistar rats of 250-300 gm weight. The Institutional Animal Ethical Committee (IAEC) approval was accorded with the resolution number IAEC/127/23 dated on 15/12/23. All the rats were maintained as per the CCSEA guidelines. In the safety evaluation the prepared *Aloe vera* gel was applied on the dorsal skin areas of rats and were monitor for the skin lesions, behavioural patterns, adverse clinical signs, mortality, body weight and feed intake up to 14 days. Evaluation of skin reaction is observed by erythema and edema formation by using Draize criteria (Yonezawa *et al.*, 2015). At the end of the study the rats were sacrificed for necropsy and histopathology of vital organs.

Table 1. Scoring pattern for erythema and edema according to Draize criteria

Parameter	No. of Rats	Observation period (hrs)		
		24 hrs	48 hrs	72 hrs
Erythema	Rat 1	0	0	0
	Rat 2	0	0	0
Edema	Rat 1	0	0	0
	Rat 2	0	0	0

Table 2. Mean values of wound contraction (%) in group A and group B

Groups	Days interval					F cal	CoV
	0^{th}	7^{th}	14^{th}	21^{st}	28^{th}		
Group A	$0^{d} \pm 0$	$37.71^{c} \pm 3.15$	$75.9^{b} \pm 4.53$	$93.09^{a} \pm 2.9$	$99.7^{a} \pm 0.21$	257.35	9.73
Group B	$0^{d} \pm 0$	$41.2^{c} \pm 3.33$	$79.7^{b} \pm 4.13$	$98.08^{a} \pm 0.8$	$100^{a} \pm 0$		

Treatments found to be significantly different at 1% and 5% level, CD (0.01) = 9.408, CD (0.05) = 7.057

Table 3. Haematological analysis at different intervals in group A and group B

Parameters	Groups	Days interval		F cal	CoV
		Day 0	Day 14		
Haemoglobin (gm/dl)	Group A	15.06 ± 0.53	15.14 ± 0.52	1.54	8.37
	Group B	13.96± 0.45	14.11 ± 0.47		
Packed cell volume (%)	Group A	43.88 ± 1.04	44.56 ± 1.11	1.002	7.06
	Group B	45.66 ± 1.44	46.86 ± 1.54		
Red Blood Cells ($\times 10^{6}$/cu.mm)	Group A	6.25 ± 0.26	6.35 ± 0.26	2.47	9.99
	Group B	6.89 ± 0.29	7.14 ± 0.25		
White Blood Cells ($\times 10^{3}$/cu.mm)	Group A	10.29 ± 0.81	10.31 ± 0.81	1.22	17.18
	Group B	8.95 ± 0.51	9.06 ± 0.49		

Table 4. Biochemical analysis at different intervals in the group A and group B

Parameters	Groups	Days interval		F cal	CoV
		Day 0	Day 14		
Aspartate aminotransferase (IU/L)	Group A	24.30 ± 2.72	23.65 ± 2.57	0.11	35.43
	Group B	26.43 ± 4.41	25.10 ± 4.45		
Alanine transaminase (IU/L)	Group A	36.33 ± 2.52	34.67 ± 2.44	2.43	21.48
	Group B	29.83 ± 3.27	26.83 ± 2.89		
Total Protein (mg/dl)	Group A	6.36 ± 0.21	6.21 ± 0.21	1.50	8.32
	Group B	6.11 ± 0.22	5.76 ± 0.19		
Blood Urea Nitrogen (mg/dl)	Group A	17.27 ± 1.13	17.16 ± 1.13	0.006	21.71
	Group B	17.45 ± 7.44	17.23 ± 1.83		
Serum Creatinine (gm/dl)	Group A	0.89 ± 0.10	0.84 ± 0.10	1.04	25.5
	Group B	0.91 ± 0.06	0.71 ± 0.06		

Clinical wound healing evaluation of *Aloe vera* gel in dogs:

The clinical efficacy of 5% *Aloe vera* gel was evaluated in the dogs presented at the Veterinary Clinical Complex (VCC), COVAS, Parbhani. A total of 12 clinical cases of wound in dogs were equally divided into group A and group B for the evaluation of the *Aloe vera* gel formulation. Group A received, the standard treatment, involving the topical application of (7.5% w/v) povidone iodine twice daily until complete healing. Group B received, topical application of *Aloe vera* gel formulation (5% w/v) twice daily till complete recovery. The Visual parameters (wound swelling, wound colour, exudation from wound, pain sensation and wound irritation) and

percent wound contraction were observed and scored on day 0, 7 and 14 of healing. In addition, hematological (Hb, PCV, TEC, TLC and DLC) and biochemical (AST, ALT, TP, BUN and Serum creatinine) parameters were analyzed on day 0 and day 14. Blood samples were collected from the cephalic vein of dogs using EDTA vials on both days for both treatment groups.

Statistical analysis:

Statistical analysis was carried out by using Web Agri Stat Package version 2.0 (WASP 2). All the parameters were analyzed by using completely randomized design (CRD) statistical method.

RESULTS AND DISCUSSION

Acute Dermal toxicity:

There were no adverse skin reactions (erythema and edema) was observed in rats applied with that *Aloe vera* gel during after 24, 48 or 72 hours (Table 1). Score for erythema and edema formation of skin was recorded zero in the rats according to Draize criteria, 1959 (Yonezawa *et al.*, 2015).

Based on observations, it was found that *Aloe vera* gel did not show any sign of dermal lesion on exposed skin area of rats @2000 mg/kg body weight. Even no any adverse behavioural patterns, clinical signs, mortality, body weight and feed intake were observed in the exposed rats. There were no histo-architectural alterations observed in collected organ/tissue in the exposed rats. Acute dermal toxicity study of *Aloe vera* gel formulation revealed that the 5% *Aloe vera* gel was safe to use topically.

Evaluation of clinical wound healing efficacy of *Aloe vera* gel in dogs

Visual observations of wounds:

Clinical wound healing efficacy of *Aloe vera* gel in dogs were evaluated by visual observations of wound swelling, pain, irritation, exudation, change in wound colour on weekly basis till complete wound healing in both group A and B. Quality of wound healing was measured as percent wound area contraction.

The percent wound contraction in group A at 0, 7th, 14th, 21st and 28th days were 0d, $37.71^{c} \pm 3.15$, $75.9^{b} \pm 4.53$, $93.09^{a} \pm 2.9$, $99.7^{a} \pm 0.21$, respectively. In group B it was 0d, $41.2^{c} \pm 3.33$, $79.7^{b} \pm 4.13$, $98.08^{a} \pm 0.8$, $100^{a} \pm 0$, respectively.

In present investigation, wounds in group B, showed non significantly faster wound contraction compared to group A from 7th to 28th day (Table 2). Results of visual wound parameters and percent wound contraction suggested that *Aloe vera* gel formulation shown better wound healing activity than povidone iodine in dogs. Similarly, Attah *et al.* (2016) and Kayode (2017) reported that 5% of *Aloe vera* gel shown greater wound contraction rate. Yadav (2012) reported that, the increased wound contraction in Aloe vera gel treatment may be due to active elements like vitamins, minerals, glycoproteins, growth factors and enzymes.

Haematological analysis

Mean haematological parameters such as haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC) and white blood cells (WBC) in group A and B were analysed on day 0 and 14 and recorded in (Table 3).

The haemoglobin count in group A was 15.06 ± 0.53 and 13.96 ± 0.45 in group B on 0 day and get increased to 15.14 ± 0.52 and 14.11 ± 0.47 on 14 days, respectively. The PCV, RBC and WBC count in group A and B were 43.88 ± 1.04, 45.66 ± 1.44; 6.25 ± 0.26, 6.89 ± 0.29 and 10.29 ± 0.81, 8.95 ± 0.51, respectively on 0 day of reporting at VCC campus, but there was increased in the PCV, RBC and WBC count were 44.56 ± 1.11, 46.86 ± 1.54; 6.35 ± 0.26, 7.14 ± 0.25 and 10.31 ± 0.81, 9.06 ± 0.49, respectively on 14 days of *Aloe vera* application.

The study found non-significant increase in haemoglobin values in both the groups on 14 days. However, Ani (2018) found that after oral administration of 500 mg/kg *Aloe vera* gel in anaemic rats, haemoglobin values was increased and they confirmed that, it might be due to presence of antioxidants, vitamins and iron in *Aloe vera* and their resultant role in haemopoietic process. There were non-significant increases in packed cell volumes after 14 days was observed in both the groups. Ekanade (2015), found a similar significant increase in PCV and RBC in rats administered with *Aloe vera* extract for 24 hours. They concluded that, this increased PCV count attributed to stimulation of haematopoiesis. Study found no significant increase in total erythrocyte count in both the groups on 14 days, similar observations were noted by Iji (2010), Udefa (2018) and Nku (2018). Similar non significantly increased total leukocyte count were observed in *Aloe vera* treatment group by Perveen (2023) and Nku (2018) indicates the activation of defence mechanisms and immune system in infection and wound cases.

Biochemical analysis

Biochemical parameters include aspartate amino-transferase, alanine transaminase, total protein, blood urea nitrogen and serum creatinine in group A and B were evaluated on day 0 and day 14 and mentioned in Table 3.

In group A and B the aspartate aminotransferase, alanine transaminase, total protein, blood urea nitrogen

and serum creatinine values were 24.30 ± 2.72, 26.43 ± 4.41; 36.33 ± 2.52, 29.83 ± 3.27; 6.36 ± 0.21, 6.11 ± 0.22; 17.27 ± 1.13, 17.45 ± 7.44; and 0.89 ± 0.10, 0.91 ± 0.06 on 0 day, respectively, however these values were get reduced to 23.65 ± 2.57, 25.10 ± 4.45; 34.67 ± 2.44, 26.83 ± 2.89; 6.21 ± 0.21, 5.76 ± 0.19; 17.16 ± 1.13, 17.23 ± 1.83; and 0.84 ± 0.10, 0.71 ± 0.06, respectively on 14 days.

There was non-significant decrease in aspartate aminotransferase levels was observed in both the groups on 14 days of *Aloe vera* gel application. Madhav (2011) and Sharma (2013) also reported the similar observations in the diabetic rats treated with *Aloe vera* gel. There were also non-significantly decreased alanine transaminase values in both the groups on day 14 values. Similarly, Iji *et al*. (2010) found a significant reduction in alanine transaminase value in diabetic rats after oral administration of *Aloe vera* gel at 100, 250 and 500 mg/kg concentrations for four weeks. Even, there were non-significant decrease in total protein values were recorded by Chatterjee (2012), after administration of aqueous leaf extract of *Aloe vera* gel in gentamicin-induced nephrotoxic rats. In both the groups there was non-significant changes in blood urea nitrogen on 0 day and 14 dayswere observed, Iji (2010) and Chatterjee (2012) also noticed the similarly observations. Even, there were non-significant decrease in serum creatinine values were observed which was confirmed by Iftikhar (2015), who found similarly non significant decrease in serum creatinine values treated with *Aloe vera* gel at 200, 400 and 600 mg/kg in diclofenac induced nephrotoxicity in rabbits at different for 15 days.

CONCLUSION

Based on the observations of the present study, it can be concluded that the prepared 5% *Aloe vera* gel formulation found to be safe in rats exposed to acute dermal toxicity. In clinical cases of dogs, the wound healing activity (in terms of wound contraction) was higher in the *Aloe vera* gel applied group as compared to the routine treatment with povidone group. Overall, Aloe vera gel formulation did not exhibit any adverse reactions and might be considered as a safer and possibly the alternative for treating wounds.

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Short Communication

NITRATE-NITRITE TOXICITY IN SOUTH-WEST REGION OF PUNJAB: A REPORT

AFROZ JAHAN* and M. K. LONARE
Department of Veterinary Pharmacology & Toxicology, College of Veterinary Science, Rampura Phul-151103, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab

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SUMMARY

A study was undertaken to determine the effect of season and type of fodder causing nitrate and nitrite toxicity in cattle population of south-west region of Punjab. Diphenylamine spot test was performed for the screening of the samples. The samples were positive during the month of September to February because drought cloudy or cold weather, excessive rain, frost, moisture affect nitrate contents in plants to a great extent.

Keywords: Nitrate toxicity, Cattle, Diphenylamine, Punjab

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Nitrate-nitrite toxicity can be an important cause of production losses in the cattle industry. It occurs naturally in the environment as a part of nitrogen cycle. During periods of draught, the amount of nitrate in the soil increases due to reduced leaching, reduced nitrogen uptake by plants, and decomposition of organic matter. Consequently, when such a draught breaks by rain, plants resume their growth and uptake of nitrate rise sharply, particularly in the first week after rainfall. Consumption of such draught stricken fodders results in poisoning (Sarah, 2007).

Many species are susceptible to nitrate and nitrite poisoning but most commonly cattle are affected. Nitrate poisoning in cattle is caused by the consumption of an excessive amount of nitrate or nitrite from grazing crops, hay, silage, weeds, drinking water, lubricating oil, fertilizer, etc however consumption of nitrate containing plants (oats, sorghum, maize, rye, wheat, barley) remains the most important source of poisoning in ruminants. In ruminant animals such as cattle, sheep, and goats, nitrate is converted to nitrite by bacteria in the rumen. This nitrite is then changed to ammonia. Excess ammonia is absorbed by the blood and passed in the urine as urea. This normal process occurs when the nitrate breakdown system is in balance and no surplus of nitrites accumulate (Aiello, 2016; Hall, 2018).

The present study was undertaken to determine the effect of season and type of fodder causing nitrate and nitrite toxicity in cattle population of South-west region of Punjab. Study period, type of sample, study area test procedure and differentiation test were taken into consideration.

The study was conducted for a period of two years i.e. from February, 2023 to February, 2025 from the cases presented to VCC, COVS, Rampura Phul from villages nearby to Rampura Phul, Manasa and Bhatinda. A total of 100 samples were received with a history of diarrhoea and vomiting, salivation, abdominal pain, rapid pulse and dyspnoea or death of animals in the herd. Diphenylamine spot test was performed for the screening of the samples. The positive samples were further confirmed quantitatively by nitration of salicylic acid procedure (Lastra, 2003). The sample were differentiated from cyanide poisoning. The

*Corresponding author: afrozvet@gmail.com

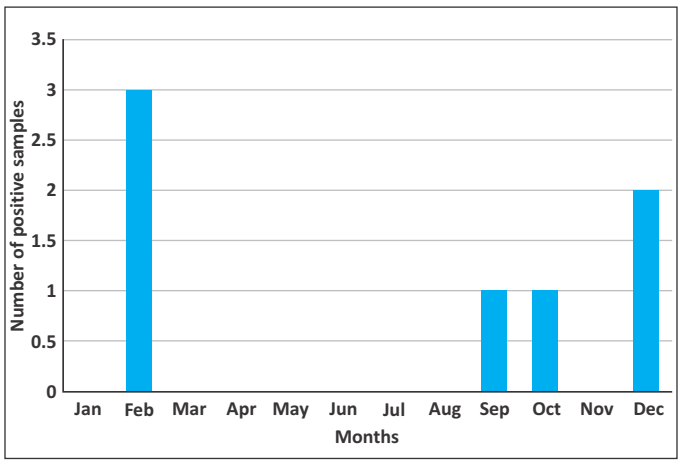

Fig. 1. Number of positive samples during different month of the year

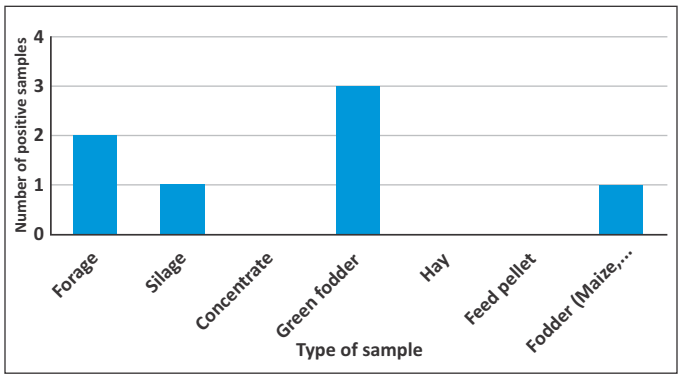

Fig. 2. Different type of sample screened and total number of positive samples

differentiation test performed for cyanide was sodium picrate paper test as per the standard protocol (Bradbury and Egan, 1992 & 1994).

Out of 100 samples screened, a total of 7 samples were found positive for nitrate-nitrite. The samples were positive during the month of September to February as during this period the climate usually hot, humid and thereafter become cold in south west Punjab (Fig.1). This extreme weather condition is responsible for the nitrate accumulation in the fodders and subsequently contributes to the toxicity in ruminants. 1 sample each of dry fodder (wheat straw, paddy straw, hay etc.) and freshly prepared silage, 3 green fodder (Maize, Sorghum, Berseem etc.) and 2 forage samples (soyabean, grass, rye) were positive for nitrate-nitrite toxicity (Fig. 2).

The screened samples were positive during the month of September to February because drought, cloudy or cold weather, excessive rain, frost, moisture affect nitrate contents in plants to a great extent. Similar findings are reported by Premalatha and Ramya (2016). Further the fodder, green forages, silages were positive for nitrate-nitrite because the content of nitrate is higher in these samples.

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IMPORTANT INFORMATION

- Research/Clinical articles are invited for next issue from the Scientists/Veterinarians engaged in Veterinary Profession.
- Please follow strictly the format of **'The Haryana Veterinarian'** for manuscript writing/ submission.
- **The article processing and publication charges will be revised from Rs. 1200/- to 1500/- per manuscript with effect from the next year (01.01.2027).**
- We solicit your co-operation.
- All correspondence should be addressed to the 'Editor, The Haryana Veterinarian', Department of Veterinary Parasitology, College of Veterinary Sciences, LUVAS, Hisar-125004, Haryana.

Editors

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Short Communication

LAPAROSCOPIC CHOLECYSTECTOMY BY SUBSEROSAL LAYER DISSECTION TECHNIQUE IN CANINES: A REPORT OF EIGHT CASES

JOG MUGDHA, GAURI FISKE*, S.B. AKHARE, B.K. BHADANE, V.S. UPADHYE, A.P. GAWANDE, M.S. PATIL and JITENDRA HAZARE
Department of Veterinary Surgery and Radiology, Nagpur Veterinary College,
Nagpur Maharashtra Animal & Fishery Sciences University, Nagpur-440001, Maharashtra, India

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SUMMARY

A total of eight laparoscopic cholecystectomy procedures were carried out on patients with varied clinical signs suggestive of hepatobiliary disorder during a period of January 2024 to December 2024. All the patients were subjected to ultrasonographic examination. The patients primarily suffering from biliary conditions like cholecystoliths, chronic cholecystitis and gallbladder mucocele were selected for the procedure. Laparoscopic cholecystectomy was carried out by subserosal dissection, starting at the fundus. The gall bladder was dissected by separating the inner serosa from the outer serosa; keeping the outer serosa attached to the hepatic parenchyma. Endoclips as well as ligatures were used for sealing of the biliary duct. The study indicated that laparoscopic cholecystectomy by Subserosal Layer Dissection Technique served as an ideal and minimally invasive technique for management of affected gallbladder in canines.

Keywords: Cholecystectomy, Gallbladder, Laparoscopy

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Minimally invasive surgery is a comparatively recent advancement in veterinary practice with laparoscopy having numerous advantages over conventional laparotomy. Cholecystectomy is indicated in case of any pathogenic conditions like cholecystitis, cholecystoli-thiasis, gallbladder mucocele, gallbladder sludge, gallbladder polyps or neoplasia, gallbladder rupture or any other inflammatory conditions of the hepatobiliary tract causing obstruction (Kanai *et al.*, 2022; Chae *et al.*, 2023).

Ultrasonography is extensively employed to visualize the liver and gallbladder in dogs and cats to diagnose such conditions. It is currently the gold standard diagnostic tool for biliary affections (Kumar *et al.*, 2012). Certain cases of hepatobiliary affections can be managed conservatively while most require surgical intervention. Cholecystectomy is the treatment of choice in acute cholecystitis and gallstone disease. Laparoscopic cholecystectomy is preferred over open cholecystectomy due to better operative safety (Mayhew *et al.*, 2008). Laparoscopic cholecystectomy can be performed in a variety of ways. The subserosal layer dissection method has been shown to be safer than the Fundus First Technique, with a lower conversion rate to open cholecystectomy, fewer cases of bile duct damage, and fewer post-operative complications, even though retrograde laparoscopic cholecystectomy is the accepted practice protocol for dogs. The technique involves antegrade blunt dissection of the gallbladder after incising the outer subserosal layer (Kondo *et al.*, 2023).

Pre-operative abdomino-pelvic sonography was performed on every patient. The required areas were clipped and shaved for effective scanning and visualization of abdo-pelvic organs. Scans were carried out in ventro-dorsal, left lateral and right lateral positions to detect abnormalities in the abdomino-pelvic region related to intra-abdominal conditions.

Patients exhibiting signs of anorexia, vomiting, nausea, lethargy, diarrhoea, weight loss, pyrexia, abdominal pain etc. were taken into consideration. Those patients which could not be managed conservatively underwent laparoscopic procedures. Preoperative CBC and biochemical parameters like Serum alkaline phosphatase (IU/L), serum alanine aminotransferase (IU/L), serum aspartate aminotransferase (IU/L), serum total protein (gm/dL), serum albumin (gm/dL) and serum globulin (gm/dL), serum total bilirubin (mg/dL), serum direct bilirubin (mg/dL), serum indirect bilirubin (mg/dL), serum gamma-glutamyl transferase (mg/dL), serum cholesterol (mg/dL), serum creatinine (mg/dL) and serum blood urea nitrogen (mg/dL) were conducted.

Patients exhibiting signs of anorexia, vomiting, nausea, lethargy, diarrhea, weight loss, pyrexia, abdominal pain etc. were taken into consideration. Those patients which could not be managed conservatively underwent laparoscopic procedures. Preoperative CBC and biochemical parameters like Serum alkaline phosphatase (IU/L), serum alanine aminotransferase (IU/L), serum aspartate aminotransferase (IU/L), serum total protein (gm/dL), serum albumin (gm/dL) and serum globulin (gm/dL),

*Corresponding author: drgauriifiske@gmail.com

serum total bilirubin (mg/dL), serum direct bilirubin (mg/dL), serum indirect bilirubin (mg/dL), serum gamma-glutamyl transferase (mg/dL), serum cholesterol (mg/dL), serum creatinine (mg/dL) and serum blood urea nitrogen (mg/dL) were conducted. A total of eight dogs diagnosed with hepatobiliary conditions like gall bladder sludge, cholecystitis, cholecystolithiasis and gall bladder mucocele were included in the study.

A total of eight dogs diagnosed with hepatobiliary conditions like gall bladder sludge, cholecystitis, cholecystolithiasis and gall bladder mucocele were included in the study.

Preoperative hematological parameters in all eight cases remained within the normal range. Specific biochemical indicators of biliary affection remained elevated in all the cases. Serum ALP (494.92±250.08 IU/L), serum total bilirubin (0.91±0.28 mg/dl) and serum GGT (9.73±3.93 IU/L) were found to be elevated in all the cases. Hypoalbunemia was found in all canines, attributed to inappetence and gastric symptoms. Laparoscopic cholecystectomy was performed within 7 days after diagnosis by ultrasonography. Post sedation and anaesthetic induction of the patient, endotracheal intubation as well as urinary catheterization was performed. The patient was placed in Trendelenburg position with the head facing the laparoscopic monitors as indicated by Kirpekar *et al.* (2023). Four port method was employed for the surgical procedure. The first port or the umbilical port was placed about 10mm caudal to the umbilicus. The operator's right-hand port was positioned at the left subcostal margin of the patient, approximately one-third of the distance laterally from the midline. Similarly, the operator's left-hand port was placed at the right subcostal margin, about two-thirds of the distance laterally from the midline. Additionally, an assistant port was located on the outer caudal side of the operator's left-hand port, to the right side of the patient (Fig. 1).

A small 5 mm skin incision was taken caudal to the umbilicus using a 11 Bard Parker blade through which the first port was placed after establishment of pneumoperitoneum. Veress needle was inserted through the incision. The hanging drop technique was used to ensure entry into the abdomen. CO_2 insufflation was performed via the Veress needle, successfully creating pneumoperitoneum in all dogs. The CO_2 insufflation parameters were set to maintain intra-abdominal pressure between 7 and 13 mmHg, with a flow rate of 1.5 to 6.6 L/hr. Subsequent ports were inserted under telescopic guidance by making small skin incisions and using a trocar with a cannula to pass through the muscle and peritoneum layers.

Subserosal dissection was carried out as described by Kondo *et al.* (2023) with the exception that initial dissection was carried out using a KARL STORZ SE & Co. KG Autocon 200 laparoscopic cautery instead of a laparoscopic scissor. The hepatobiliary system was identified and gallbladder carefully visualized, and grasped with atraumatic forceps. The gall bladder was retracted and manipulated so that the subserosal layer was visible (Fig. 2). Dissection of the gall bladder was initiated from the fundus in all the cases. Dissection was done so that the outer subserosal layer remained adherent to the hepatic parenchyma while the inner subserosal layer remained adherent to the gallbladder (Fig. 3). Along the downward dissection, the cystic duct was located and isolated (Fig. 4). Either metallic endoclips alone (Fig. 5) or a combination of endoclips and sutures were used for ligating the cystic duct right below the neck of the gallbladder before its transection (Fig. 6). The gallbladder was retrieved through one of the ports. Haemorrhage or any spillage was checked after removal of the gall bladder. In case of spillage, the abdominal cavity was lavaged using Normal Saline and the contents suctioned out. The abdomen was completely deflated. The muscle layer was sutured usingpolyglactin 910 No. 1 or 0, depending on the size of the dog, and the skin incision was closed with polypropylene No. 2-0. The operative time for all of the eight surgeries was between 120 and 180 minutes. A steep learning curve was associated with the process. The operative time improved significantly in the later surgeries; reducing by as much as 60 minutes.

Ultrasonographic examinations prior to the procedure revealed cholecystitis in all the eight cases to a varying severity. In one case of Mongrel suffering from severe cholecystitis and acute abdominal pain, the gallbladder wall was found to be thickened to the extent of 7.7 mm (Fig. 7). Tamborini *et al.* (2016) stated that the gallbladder wall was considered thickened or inflamed if the thicknessis greater than 3 mm.

In cases of gallbladder wall edema, Kumar *et al.* (2012) observed a similar double rim effect of the gallbladder. In a different instance, a Spitz with less severe symptoms than others had a double wall appearance (Fig. 8). Cholecystoliths were found in four cases. Multiple gallstones of varied sizes along with gallbladder sludge were found in all the cases (Fig. 9). According to Kumar *et al.* (2012), on ultrasound imaging, cholecystoliths appeared as bright, echogenic structures within the gallbladder lumen; they vary in size and number and often demonstrated acoustic shadowing, characterized by a shadow extending behind the stones caused by the blockage of the ultrasound

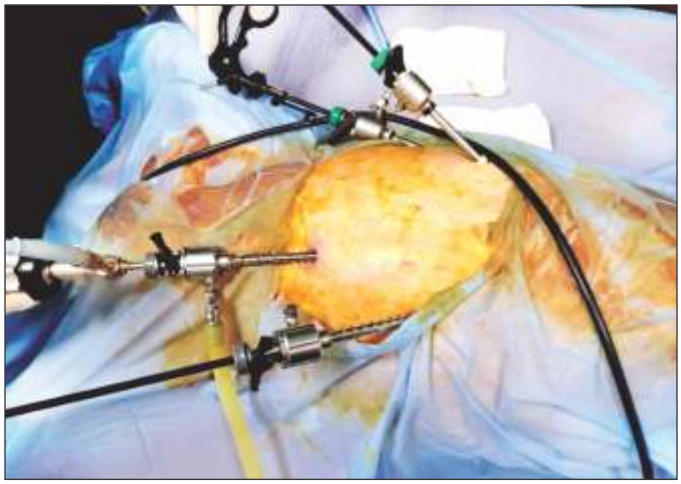

Fig. 1. Image showing placement of ports

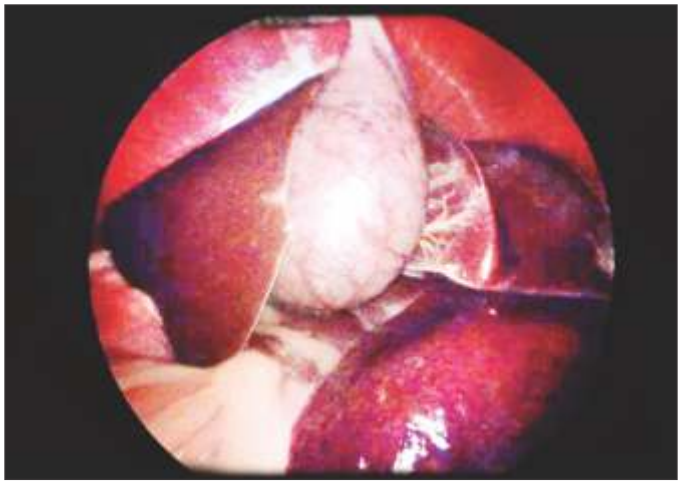

Fig. 2. Image showing retraction of the gallbladder

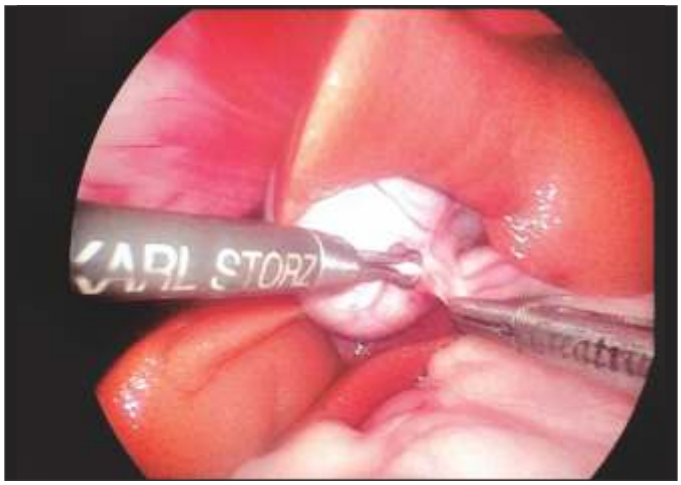

Fig. 3. Image showing dissection of the subserosal layer.

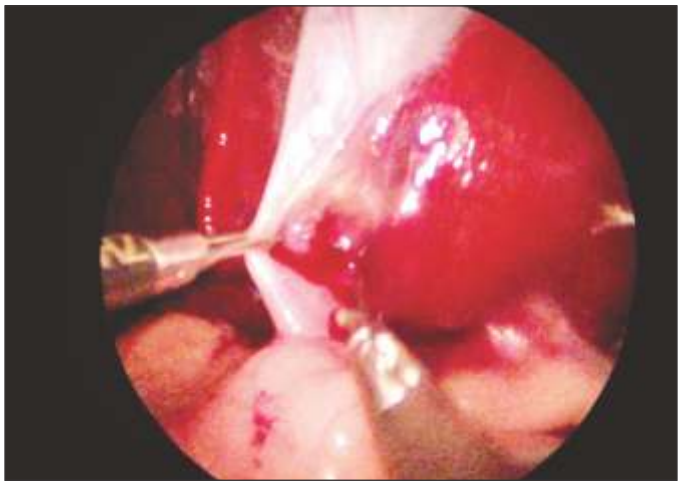

Fig. 4. Image showing isolation of the cystic duct.

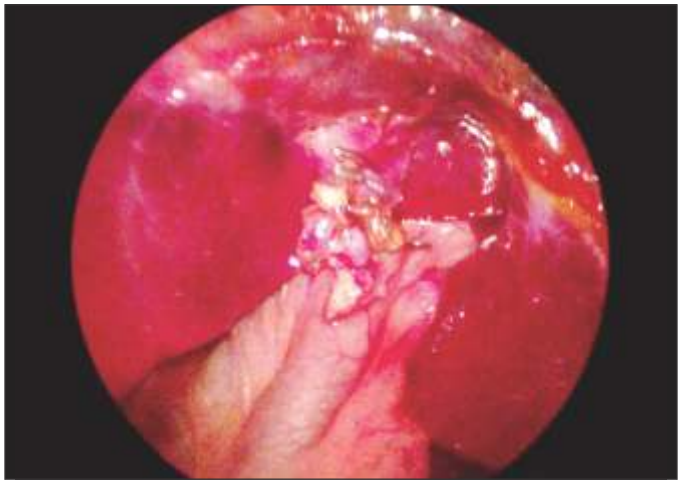

Fig. 5. Image showing Use of endoclips alone.

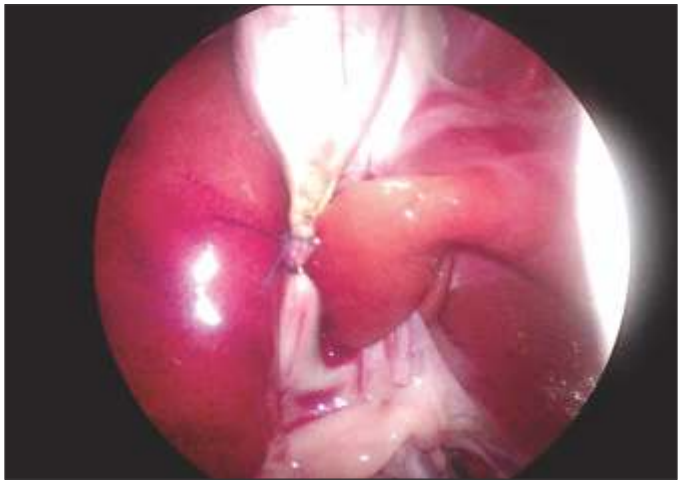

Fig. 6. Image showingSealing of the cystic duct using a combination of endoclips and ligature.

beam. Onultrasonography, sludge is visualized as low-intensity echoes within the gallbladder lumen, often forming layers or shifting position in response to gravity. In the present study, immobile and gravity independent sludge was found in two cases (Fig. 10).

Only one case of gallbladder mucocele was diagnosed and consequently operated (Fig. 11). Physical examination of the patient revealed acute abdominal pain on palpation with yellowish mucous membranes indicating onset of jaundice. Upon further biochemical investigation, SAP and GGT values were found to be severely elevated. The patient was diagnosed to be

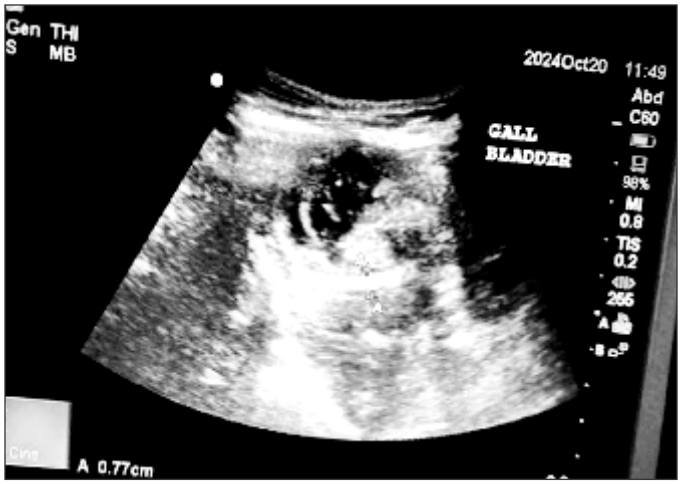

Fig. 7. Ultrasonographic image showing thickened gall badder wall (0.77cm) indicating cholecystitis.

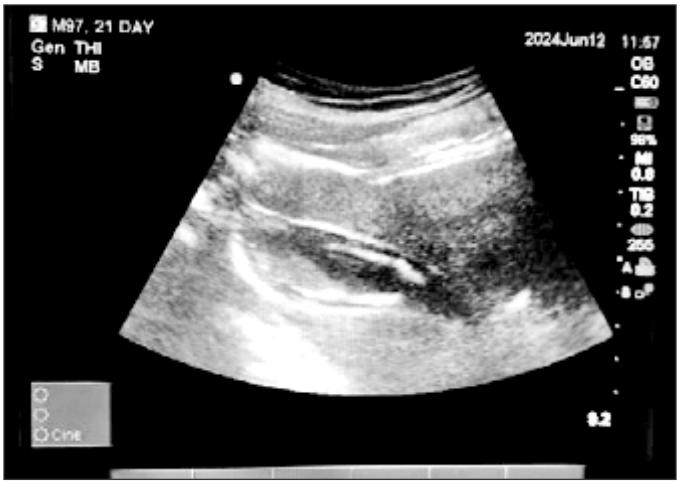

Fig. 8. Ultrasonogram showing double wall appearance indicating oedema of gall bladder wall.

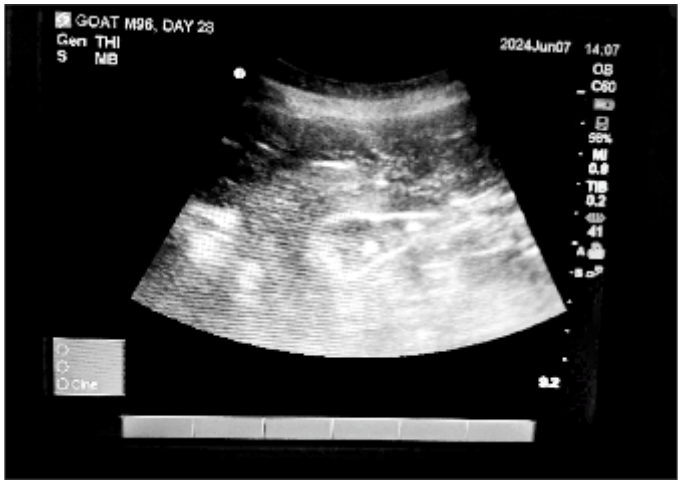

Fig. 9. Ultrasonographic image showing cholecystitis and 2 cholecystic calculi.

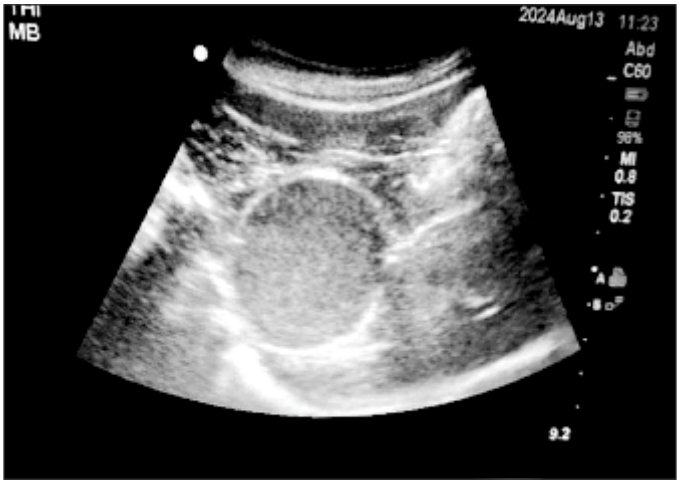

Fig. 10. Ultrasonographic image showing severe distension of gall bladder due to sludge.

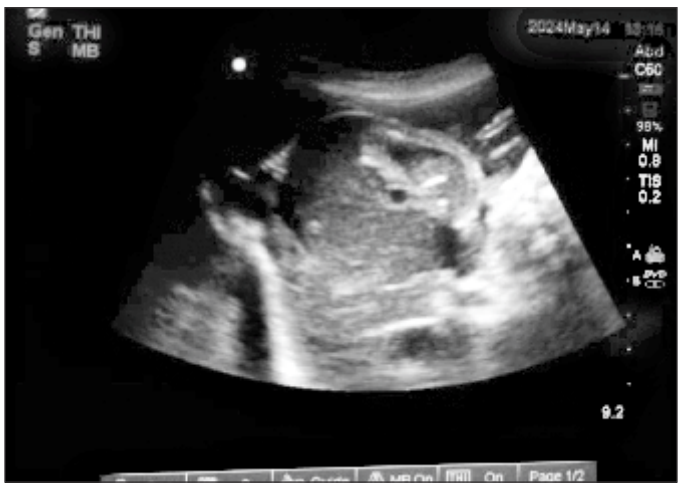

Fig. 11. Ultrasonographic image showing gall bladder mucocele.

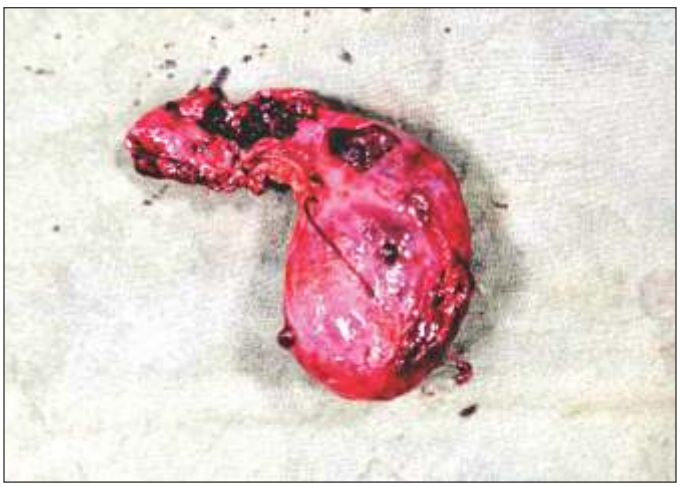

Fig. 12. Retrieved gall bladder showing extensive necrosis of the gall bladder wall.

suffering from biliary obstruction and deemed to be in an advanced stage of hepatobiliary disease.

Abdominal pain on palpation with yellowish mucous membranes indicating onset of jaundice. Upon further biochemical investigation, SAP and GGT values were found to be severely elevated. The patient was diagnosed to be suffering from biliary obstruction and deemed to be in an advanced stage of hepatobiliary disease. Ultrasonographically, gall bladder mucoceles were presented with diverse appearances. The classic

pattern resembled a "kiwi fruit", characterized by hyperechoic striations radiating from a central point. Other variations included irregular or striated, non-gravity-dependent contents, or a stellate pattern with a distinct hypoechoic rim, which is thought to result from mucin accumulation between the gallbladder wall and the organized structure (Rademacher, 2011).

Laparoscopic cholecystectomy was performed within 7 days after diagnosis by ultrasonography. The patients were premedicated using Inj. Atropine Sulphate at a dosage of 0.04 mg/kg BW, Inj. Ranitidine at 0.2 mg/kg BW, and Inj. Meloxicam at 0.2 mg/kg BW. Inj.Amoxicillin was administered intraoperatively at 15 mg/kg BW. The patients were sedated using Inj. Xylazine HCl at 1.1 mg/kg BW. Induction of anaesthesia was done using a combination of Inj. Diazepam at 0.5 mg/kg BW and Inj. Ketamine at 5 mg/kg BW intravenously through Normal saline. Anaesthesia was maintained using 1-3% Isoflurane. After induction, endotracheal intubation was performed with the assistance of a laryngoscope. The patient was placed in Trendelenburg position with the head facing the laparoscopic monitors. Foley's catheter or an infant feeding tube was placed and secured to evacuate the bladder.

Subserosal dissection was successfully performed in seven out of eight cases. In the eighth case, separation of the outer serosa from the inner serosa was not possible due to extensive cholecystitis and necrosis of the gallbladder wall which could have led to a gallbladder rupture (Fig. 12). In this case, the fundus first approach was followed where the entire gallbladder was dissected out from its bed starting from the fundus to the hepatic fossa. This inclusion criterion was set as directed by Kondo *et al.* (2023) who discouraged the selection of complicated cases for subserosal dissection. The infundibulum of the gallbladder was separated from the hepatic fossa in all the cases. In two cases, endoclips were applied to prevent leakage while in the remaining four cases, a combination of endoclips as well as ligature using polyglactin 910 vicryl no. 2-0 was employed. The deciding factor for the use of endoclips or ligatures was the thickness and the size of the infundibulum. In cases where the gallbladder was severely distended or inflamed, a combination was used to ensure that no leakage occurs. Only endoclips were used in cases where the thickness of the infundibulum was small enough to prevent leakage from the use of endoclips alone.

No major complications were encountered during any of the procedures. Minor hemorrhage was observed in one case during dissection of the gallbladder which was managed effectively without requiring the need for conversion. Kondo *et al.* (2023) advocated the use of the subserosal layer dissection technique for lesser intraoperative and consequently, post operative complications. Kanai *et al*. (2018) switched from dissection from the hepatic fossa, which is the conventional dissection technique for laparoscopic cholecystectomy to the subserosal layer dissection technique with notable decline in complication rates. The author concluded that while retrograde dissection is an ideal technique in humans, dogs have a lesser working space in the abdomen which makes the antegrade dissection technique favourable. Furthermore, this technique was suitable for uncomplicated cases where the general condition of the patient was relatively good.

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Short Communication

ASSESSMENT OF ECONOMIC LOSS DUE TO GASTROINTESTINAL NEMATODE (GIN) INFECTION IN GOAT OF JABALPUR DISTRICT, MADHYA PRADESH

ALOK KUMAR SINGH* and G. DAS
Department of Veterinary Parasitology, College of Veterinary Science & Animal Husbandry, (NDVSU), Jabalpur, Madhya Pradesh, India
#Present Address: Department of Veterinary Parasitology, College of Veterinary Science & Animal Husbandry, (NDVSU), Rewa, Madhya Pradesh (M.P.), India

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SUMMARY

Gastrointestinal nematode (GIN) infection is prevalent throughout the world, including India and is one of the important causes for poor performance in goats. The goal of the current study was to evaluate the financial burden caused by GIN infection in goats at Ghana village of Jabalpur district, Madhya Pradesh. A total of 60 kids approximately 3-4 months of age were screened for GIN infections by qualitative faecal examination method. Faecal samples positive for strongyle infections were subjected to modified McMaster technique. Twenty goats with ≥ 300 EPG were selected for this study. Two groups of ten goats each were randomly selected, and one group received treatment with an appropriate anthelmintic medication (fenbendazole @7.5 mg/kg body weight), while the other group was left as an untreated control group. At 9 months, the infected groups had the greatest faecal egg count (1233.33) with a mean EPG of 629.68. Body weight decreased significantly (P<0.05) in untreated control group as compared to the treated group with an estimated loss of 3.385 kg body weight along with monetary loss of Rs. 744.70 per goat per year. The present studied shows that, GIN infections can cause a significant reduction in weight, leading to economic loss in goats rearing.

Keywords: Economic loss, Gastrointestinal nematodes, Goat, Madhya Pradesh

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In the rural Indian economy, small ruminants like goats are important since they make up a large portion of marginal farmers and labourers without land (Singh *et al.*, 2015). Numerous helminthic parasites cause significant losses in afflicted goats because of illness, mortality, and decreased productivity. Goats are susceptible to a number of parasitic infections that not only compromise their general health but also significantly reduce their total output (Sanyal, 1996; Jas *et al.*, 2007); gastrointestinal parasitism (GIPs) is a major cause of this kind of ailment in India. Gastrointestinal nematodes cause poor performance in infected goats (Knox and Steel, 1996; Dhamdar, 2020). The gastrointestinal parasites cause anaemia, emaciation, oedema, weakness, diarrhoea and death in case of untreated infected animals (Lutu, 1983). Jas *et al.*, 2007 reported economic losses in small ruminants in India. The goal of the current study was to calculate the economic loss resulting from GIN infections in goats by calculating the conversion of body weight gain losses into monetary loss per goat.

The present investigation was organized on a local goat breed, naturally infected with GINs at Ghana village of Jabalpur district, Madhya Pradesh (M.P.). The current study was carried out over a 12-month period, starting in January and ending in December of 2012. The selected animals were kept in a semi-intensive management regime with irregular deworming procedures. A total of 60 young goats of either sex were screened coprologically for the presence of GIN parasites by qualitative faecal examination method and positive samples of strongyle infection were subsequently examined by modified McMaster technique (Mines, 1977). Twenty goats approximately 3-4 months old with faecal egg counts of >300 were selected for whole study period and divided randomly into two different groups; ten animals in each. These selected animals were identified individually by using proper tags and their body weight gains recorded at monthly interval. The goats of one group (n=10) were treated with fenbendazole @ 7.5 mg/kg body weight once at the start of the study after faecal examination, when the faecal egg counts were observed as >100 and the other kept as untreated control group. Both the groups were allowed to graze during day time in groups and fed the traditional hay and fodder by the farmers during night. Furthermore, the efficacy of the fenbendazole was checked by faecal egg count reduction test (FECRT) at the monthly interval. The body weight increases of both groups were noted at the time of faecal samples in order to evaluate the impact of GIN infection on goat chevon production. The Statistical Package for the Social Sciences (SPSS), Version 14.0, was used to compare the body weight gains of the two groups and analyse the data. At the 5% (p<0.05) level, the significance of the p value was recorded.

The losses brought on by helminth parasite infections are catastrophic, and they are typically asymptomatic and

*Corresponding author: alok122@gmail.com

Table 1. Particulars of monthly body weights (kg) of goats in treated and untreated groups.

Month (post-treatment)	Body weights (kg)			
	Treated group (n=10) (Body weight)	EPG	Untreated group (n=10) (Body weight)	EPG
0	7.70±0.63	342.23	7.50±0.56	415.38
1st	8.60±0.60	371.43	8.10±0.56	469.23
2nd	9.35±0.57	344.44	8.70±0.57	438.46
3rd	10.60±0.51	437.50	9.70±0.52*	484.62
4th	11.70±0.48	366.67	10.60±0.50*	446.15
5th	12.92±0.44	387.50	11.40±0.49*	530.77
6th	14.14±0.41	437.50	12.25±0.52*	569.23
7th	15.27±0.42	711.11	13.25±0.57*	723.08
8th	16.16±0.43	944.44	14.15±0.61*	1330.77
9th	17.41±0.43	1233.33	14.95±0.64*	1092.31
10th	18.71±0.45	1022.22	15.65±0.65*	853.85
11th	19.84±0.47	777.78	16.54±0.68*	569.23
12th	20.96±0.45	522.22	17.38±0.68*	660.26
Difference in weight gain	13.265±0.24		9.88±0.29*	
Loss in body weight (kg)			3.385	
Loss in chevon production (kg)			1.693	
Loss due to weight @ Rs. 440/- per kg			Rs. 744.92	

Values expressed as Mean ± SE; Values with different superscripts differ significantly (rows) ($p<0.05$)

persistent in character. Monthly egg per gram (EPG) counts of nematodes were measured in all infected goats, and the resultant faecal egg count was utilized as a gauge for the severity of GIN infections. The highest EPG was recorded during 9th month (1233.33) with a mean EPG of 629.68 in the treated group. The estimation of impact of naturally occurring GIN infections in goats were determined by compared to body weight gains of both groups i.e. treated and untreated. The ultimate loss in body weight of infected goats might be due to presence of GI nematodes and in term of considered as production loss i.e., chevon. An average pricing of Rs. 440/-per kilogram of chevon in Jabalpur, Madhya Pradesh, was used to convert the final loss in chevon production into Indian rupees (INR) (Table 1). When compared to the recorded weight prior to treatment, the body weight gains of the treated goats showed a substantial increase ($P<0.05$).

When the body weight gains of the treated and untreated goat groups were compared, it was found that the treated goats had greater body weight growth ($P<0.05$) than the untreated goats. In the end, the goats in the treated and untreated groups gained an average of 13.265 kg and 9.880 kg, respectively; the mean losses in body weight growth were 3.385 kg (Table 1). The mean loss of chevon production in goats was 1.693 kg, with considering 50% as the dressing percentage in goats. Therefore, economic losses in chevon production were calculated Rs. 744.92 (Rs. 440 × 1.693 kg), considering the average local market rate of chevon as Rs. 440/-. Although there is little research on the financial damages GIN parasites bring to goats in India, there is some indication that they are to blame for these productivity losses (Jas and Ghosh, 2009; Murhaban *et al.*, 2017).

According to the current study, untreated goats' body weight gain is significantly ($P<0.05$) lower than that of the treated group when they have GIN infections. Since the young goats were chosen for the current study, the decrease in the body weight gain of untreated goats suggests that GIN infections have a detrimental effect on growth rate. The current study's findings about the reductions in body weight gains brought on by GIN parasites were consistent with previous findings (Githigia *et al.*, 2001; Jas *et al.*, 2007). Worm load causes the growth rate of infected animals to decrease, and even a subclinical infection can cause the body weight increase to decrease (Soulsby, 1965). Even though the current study's mean EPG was 629.68, the untreated group's decreased body weight increase was still noticeable and in good agreement with previous findings by Ilangopathy *et al.* (2019). Reduced feed intake and low feed conversion efficiency brought on by nematode infection may be the cause of the untreated group's decreased body weight gain. Reduced body weight gain in the untreated group is thought to be caused by a variety of factors, including abdominal pain,

changes in pH, inflammation in the gut, changes in the flow rate of digesta, changes in the protein to energy ratio of absorbed nutrients, and the secretion of the hormone cholecystokinin (Martin *et al.*, 1996). The exact mechanism underlying this reduced feed intake is unclear. It is estimated that reduced feed utilization contributes about 25g of body weight per day, and this could be another reason for the untreated group's lower body weight growth (Soulsby, 1976). In addition to lowering feed intake, GIN infections cause a decrease in feed conversion efficiency, which increases protein catabolism and impairs protein digestion (Soulsby, 1976). In conclusion, GIN infections result in a large financial loss for goats raised for meat. Using the right anthelmintic to manage mental techniques for GI parasite infection control will increase goat farming output.

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Short Communication

EFFECT OF GRADED LEVELS OF BEE PROPOLIS ON FERMENTATION CHARACTERISTICS OF MAIZE FODDER BASED RATION: AN *IN VITRO* ASSAY

SRISTI DHURIA, JASMINE KAUR*, R.S. GREWAL and J.S. HUNDAL
Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141004, Punjab, India

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SUMMARY

An *in vitro* assay was carried out to evaluate the fermentation parameters of supplementing graded levels of bee propolis to maize fodder based ration. Maize fodder based total mixed ration (TMR) was supplemented with bee propolis at 0, 0.025, 0.05, 0.1, 0.15 and 0.2% (on DM basis). The addition of graded levels of bee propolis had no significant effect on the fermentation parameters, such as net gas production, partitioning factor, digestibility of nutrients (OM, NDF, DM), microbial mass production, efficiency of microbial mass production, production of short chain fatty acids, and availability of metabolizable energy. However, at 0.2% (DM basis) inclusion level of bee propolis, ammonia-N (NH_3-N) production decreased significantly ($P \geq 0.05$) in the *in vitro* media. It was concluded that adding bee propolis at 0.2% level (DM basis) in the maize fodder based diet effectively reduced NH_3-N production, thereby, indicating its potential to decrease ruminal NH_3-N nitrogen during fermentation in animals.

Keywords: Ammonia-N, Bee propolis, *In vitro* evaluation, Maize fodder based ration

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The growing global human population puts enormous pressure on livestock farmers to increase productivity, maintain animal health, and improve feed to meet the growing demand for meat, dairy, and eggs; this pressure is further accentuated by concerns about environmental sustainability and animal welfare. These objectives have long been met by the use of synthetic feed additives and antibiotics; however, rising awareness about antimicrobial resistance and persistence of chemical residues in livestock based products and consumer preference for natural and organic food sources have fuelled the search for safer, sustainable and organic alternatives for animal feed supplementation. In this context, bee propolis has gained significant attention of scientists as a potential feed additive, owing to its rich chemical composition and biological properties (Abd El Ghany, 2024).

Propolis is composed of resins and bees wax gathered by the honey bees from various plants and its parts like flowers and leaf buds; and plays protective role by sealing the cracks and crevices in the hive (Bogdanov, 2017). As propolis naturally contains potent bioactive compounds, especially flavonoids, phenolic acids, steroids, alcohols, fatty acids and ketones, it has antimicrobial, antimeth-anogenic, anti-inflammatory, immunomodulatory, anticarcinogenic and antiparasitic properties (Morsy *et al.*, 2016). Considering the broad spectrum of useful properties of bee propolis, an *in vitro* study was conducted to investigate its impact on various fermentation parameters.

An *in vitro* assay was carried out at Department of Animal Nutrition, GADVASU (Ludhiana, Punjab). All the experimental protocols and procedures described here were approved by the guidelines of the Institutional Animal Ethics Committee. The bee propolis was sourced from local market.The roughage to concentrate ratio in the TMR was 65:35, with the roughage part consisting of wheat straw and maize fodder in 50:50 ratio. Graded levels of propolis at 0, 0.025, 0.05, 0.1, 0.15, and 0.2% (DM basis) were added to maize fodder-based TMR. Bee propolis and maize fodder-based TMR were analyzed for proximate (AOAC, 2005) and cell wall components (Van Soest *et al.*, 1991).

Ruminal fluid was collected from male buffaloes having rumen fistula, that were maintained on ration comprising of 2 kg conventional concentrate mixture, 15 kg fresh green forage and 3 kg wheat straw. *In vitro* evaluation was carried out by gas production technique (Menke *et al.*, 1979; Menke and Steingass, 1988). The ratio of substrate actually degraded *in vitro* (mg) to the amount of gas produced (ml) by it was used to calculate the partitioning factor (PF) (France *et al.*, 1993). The ME value of the substrate was determined using the equation given by Menke *et al.* (1979). Simple ANOVA was used to analyze the data generated throughout the investigation using SPSS version 21. Tukey's b was used to test the mean differences.

The bee propolis used in the study contained 95.63% organic matter, 4.37% total ash, 2.51% crude protein, 1.10% ether extract, 0.60% crude fibre and 92.02% total carbohydrates. The ingredient composition of TMR is

*Corresponding author: sachdeva_jasmine@rediffmail.com

given in Table 1. In the TMR, the roughage portion was made up of wheat straw and maize fodder in 1:1 ratio. TMR contained 12.40% crude protein and 2.85% ether extract (Table 2).

Table 1. Ingredient composition of TMR used in the *in vitro* experiment (% DM basis)

Ingredient	Percent
Wheat straw	32.5
Maize fodder	32.5
Concentrate Ingredients	
Maize	12.25
Soybean meal	5.25
Mustard cake	5.25
Wheat Bran	3.15
Rice polish	2.45
Deoiled rice bran	5.425
Mineral mixture	0.7
Common salt	0.35
Urea	0.175

Table 2. Chemical composition of maize fodder based TMR used in the *in vitro* experiment (% DM basis)

Parameter	Percent
Organic matter	93.12
Total ash	6.88
Crude protein	12.40
Ether extract	2.85
Neutral detergent fibre	56.90
Acid detergent fibre	31.15
Hemicellulose	25.75
Cellulose	21.70
Acid detergent lignin	4.35
Total carbohydrates	77.87

The *in vitro* fermentation parameters of maize fodder based TMR are presented in Table 3. There was no significant difference in net gas production (NGP) among the various levels of bee propolis tested in the TMR. Our results are similar to those reported by Nascimento *et al.* (2020), who stated that addition of propolis extraction residue (100 g/cow/day) into bovine diets (with varying concentrate levels viz. 25, 50 and 75% DM) *in vitro* had no significant effect on total volume of gases produced in the treatments as compared to control group (without propolis extraction residue). Moreover, Morsy *et al.* (2015) compared the effects of increasing levels of ethanolic extracts of Brazilian red propolis and Egyptian brown propolis on ruminal degradation of nutrients and methane productionusing a semi-automatic *in vitro* gas production system and found no variation in NGP at various propolis levels. Further, Morsy *et al.* (2011) found no significant (P>0.05) difference in gas production between Brazilian green propolis extract (BGP) and Brazilian alamopropolis extract (BAP) at various levels (125, 250 and 500 µg/75 ml culture fluid) in comparison to control.

The partitioning factor (PF) in the current study varied non-significantly across treatments. Our results are in line with those of Morsy *et al.* (2015), who compared the effects of increasing levels of ethanolic extracts of Brazilian red propolis and Egyptian brown propolis on ruminal degradation of nutrients and methane production using a semi-automatic *in vitro* gas production system, and reported no significant difference in the PF values across the propolis treatments. In addition, Morsy *et al.* (2011) observed no significant difference in the PF values among both Brazilian propolis extracts-BGP and BAP at different levels (125, 250 and 500 µg/75 ml culture fluid) in comparison to control. The PF value of ruminant ration should lie in the range of 2.71 to 4.41 (Blümmel *et al.*, 1997). The PF value (mg/ml) in the present study ranged from 3.33 to 3.57, which is within the suggested range.

The digestibility of nutrients (OM, NDF and DM) varied non significantly across various levels of bee propolis supplementation in the TMR. Our results are consistent with those of Mahmood *et al.* (2022), who used RUSITEC to study the effects of different moringa by-products and raw propolis on ruminal fermentation, and reported that there wasno significant (P>0.05) variation in the degradation of nutrients across the treatments (control diet without any feed supplement and the control diet added with moringa byproducts or raw propolis). Further, Nascimento *et al.* (2020) investigated the effects of adding propolis extraction residue (100 g/cow/day) into bovine ration with varying levels ofconcentrate on in vitro rumen fermentation, and reported that addition of propolis extraction residue into bovine ration had no significant effect on digestibilityof DM at variouslevels of concentrate as compared to control (without propolis extraction residue).

The supplementation of graded levels of bee propolis in to TMR did not have any significant effect on microbial mass production (MMP) and efficiency of microbial mass production (EMMP). Our results are in line with that of Valero *et al.* (2015), who studied the substitution of propolis for monensin in the diet of feedlots bulls, and reported that both MMP and EMMP were unaffected (P>0.05) by the addition of monensin or propolis. Further, Aguiar *et al.* (2014) investigated the effect of inclusion of three variations of propolis based products (PBP) as PBP B1, PBP C1 and PBP C3 (varying in the concentrations of phenolic compounds as 3.81 mg/kg, 3.27 mg/kg and 1.93 mg/kg of ingested dry matter,

Table 3. Effect of level of bee propolis on the *in vitro* gas production and digestibility of nutrients in maize fodder based TMR (24 h)

Parameter	Level of bee propolis (% DM basis)						SEM
	0	0.025	0.05	0.1	0.15	0.2	
NGP (ml/g DM/24h)	169.33	168.67	167.33	172.67	175.33	174.67	1.03
PF (mg/ml)	3.57	3.39	3.45	3.33	3.48	3.33	0.03
OMD (%)	64.92	61.34	62.05	61.77	65.63	62.48	0.62
NDFD (%)	42.59	36.73	37.90	37.43	43.76	38.61	1.01
MMP (mg)	86.99	75.04	78.64	73.24	84.54	74.09	1.99
EMMP (%)	38.35	35.03	36.29	33.94	36.82	33.96	0.58
DMD (%)	68.27	65.47	66.00	66.13	69.60	67.07	0.54
SCFA (mmole)	0.75	0.74	0.74	0.76	0.77	0.77	0.00
ME (MJ/kg DM)	7.68	7.66	7.62	7.78	7.86	7.84	0.03
NH3-N (mg/dl)	20.39^{ab}	21.55^{b}	20.97^{ab}	20.68^{ab}	20.82^{ab}	19.37^{a}	0.22

NGP- Net gas production, PF- Partitioning factor, D- Digestibility, OM- Organic matter, NDF- Neutral detergent fibre, MMP- Microbial mass production, EMMP- Efficiency of microbial mass production, DM- Dry matter, SCFA- Short chain fatty acids, ME- Metabolizable energy, NH3-N- Ammoniacal nitrogen, R:C ratio was 65:35 on dry matter basis
Means bearing different superscripts in a row differ significantly ($P \geq 0.05$)

respectively) to the diets of Holstein cattle and reported that there was no significant ($P>0.05$) variation in the microbial protein synthesis as well as microbial efficiency on addition of propolis-based products in the cattle ration as compared to control. Moreover, De Paula *et al.* (2016) studied the effects of offering propolis-derived phenolic compounds to water buffaloes at various doses (0, 16.95, 33.9 and 50.85 mg/d), and revealed that there were no significant variations ($P>0.05$) in MMP as well as EMMP among control and treatment groups.

Adding graded levels of bee propolis to TMR had no significant effect on the production of short chain fatty acids (SCFA).Our results are similar to those of Mahmood *et al.* (2022), who used RUSITEC to study the effects of various moringa by-products and raw propolis on ruminal fermentation and reported that propolis treatment had no significant ($P>0.05$) effect on the concentration of total SCFA as compared to control (devoid of feed additive). In the present study, adding graded levels of bee propolis to TMR had no significant effect on ME availability. Our results resembled with those of Da Silva *et al.* (2025) who explored the effects of supplementation of increasing levels (0, 6, 12, 18 and 24 ml/day) of green propolis extract (GPE) in ration of feed lot rams for 85 days and reported that ME in the rams' diet was not affected ($P > 0.05$) by the supplementation of GPE across treatment groups. However, Coşkuntuna *et al.* (2023) carried out an *in vitro* study to investigate effects of adding 0.05% propolis extract to sorghum cultivars (Es8z102, Albanus, Sugar Drip, GülŞeker and Csr9303) having varying levels of tannins and reported that availability of ME increased significantly ($P = 0.000$) with the addition of propolis extract to all the sorghum cultivars in comparison to control (without propolis extract).

However, *in vitro* NH_3-N (mg/dl) varied significantly ($P \geq 0.05$) across the various levels of propolis with the highest ($P \geq 0.05$) value (21.55 mg/dl) recorded at 0.025% level of propolis and lowest ($P \geq 0.05$) value (19.37 mg/dl) recorded at 0.2% level of propolis. The results of the our study corroborated with those of Ehtesham *et al.* (2018), who used an *in vitro* gas production system to explore the effects of increasing levels of phenolic compounds found in Iranian propolis (IP) extracts (25, 50 and 75 g of propolis in 100 ml of 70% ethanol) on rumen fermentation. They revealed that addition of 75% IP in ration significantly reduced ($P<0.0001$) levels of NH_3-N in comparison to control as well as other propolis levels (25 and 50 g of propolis). Lower rumen NH_3-N levels in ruminants can offer several advantages, including improved nitrogen utilization by rumen microbes and reduced nitrogen waste excretion. This enhances feed efficiency, improves animal health and lowers environmental pollution. Lower rumen NH_3-N levels decrease energy expenditure on excretion of ammonia as urea, thereby, improving energy efficiency and animal performance (Chanu *et al.*, 2020).

CONCLUSION

Net gas production, nutrient digestibility (OM, NDF and DM), microbial mass production,and availability of ME were similar across different bee propolis levels used in maize fodder-based TMR, suggesting that bee propolis levels had no negative impact on nutrient digestibility. However, at a 0.2 % (DM basis) inclusion level of bee propolis, NH_3-N production decreased significantly ($P \geq 0.05$) in the *in vitro* medium.

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Short Communication

PANCREATIC ELASTASE-1 ELISA FOR THE DIAGNOSIS OF EXOCRINE PANCREATIC INSUFFICIENCY AND ITS MANAGEMENT WITH ENZYME REPLACEMENT THERAPY: A CASE REPORT

ABHIJITH, S.P.*, KSHAMA, M.A., LATHAMANI, V.S. and ABDUL KALAM, A.
Department of Veterinary Medicine, Veterinary College, Bengaluru

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SUMMARY

Exocrine pancreatic insufficiency (EPI) is a mal-absorptive syndrome caused by insufficient secretion of digestive enzymes from pancreatic acini. The most common causes of EPI in dogs and cats are pancreatic acinar atrophy and chronic pancreatitis. The primary cause of EPI in canines is pancreatic acinar atrophy (PAA), an immune-mediated condition that results in the progressive loss of pancreatic acinar cells responsible for enzyme secretion. A four-year old intact male German shepherd dog (GSD) was presented with the complaints of progressive weight loss, lethargy, polyphagia, abnormal stools with soft consistency and flatulence. The complete blood count, hepatic and renal biochemical tests, along with abdominal ultrasound and fecal elastase testing, were performed. Based on the clinical signs, physical examination, hematology, biochemical finding and fecal elastase levels, the condition was diagnosed as Exocrine Pancreatic Insufficiency (EPI). Pancreatic Enzyme Replacement Therapy (PERT) was initiated, accompanied by cobalamin supplementation. Within two weeks, there was a noticeable reduction in clinical signs, and over the subsequent eight weeks, the dog gained 6 kg of body weight, with fecal consistency returning to normal.

Keywords: Elastase, ELISA, Exocrine pancreatic insufficiency, German Shepherd

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Exocrine pancreatic insufficiency (EPI) is characterized by inadequate production of digestive enzymes by the pancreatic acini, resulting in poor digestion, mal-absorption, and mal-nutrition (Soetart *et al.*, 2019, Westermarck *et al.*, 2012).

Exocrine pancreatic insufficiency has a worldwide distribution. The most well-documented breed pre-disposition is the German Shepherd Dog (GSD), and while any breed can be affected, Rough-coated Collies, Cavalier King Charles Spaniels, Cairn Terriers, Chows, Cocker Spaniels, Eurasier dogs, and West Highland White Terriers are reported to be at increased risk of EPI (Batchelor *et al.*, 2007; Moeller *et al.*, 2002; Proschowsky *et al.*, 2007; Wiberg *et al.*, 1999). Clinical signs associated with EPI are caused by defective digestion and absorption of dietary macromolecules (protein, fat, and starch) (Pongprasobchai 2013, Owens *et al.*, 2007).

The most observed clinical signs are polyphagia, parorexia, coprophagia, soft stools, steatorrhea, increased fecal volume, borborygmus, flatulence, weight loss, seborrhea, and muscle atrophy (Westermarck *et al.*, 2012). EPI is diagnosed based on measuring decreased pancreatic secretion capacity by pancreatic function tests. (Freudiger 1971). The exocrine pancreas has a large reserve secretory capacity, and clinical signs of maldigestion do not occur until 90% of secretory capacity is lost (Dimagno *et al.*, 1973). In dogs with EPI, the hydrolase activities of the pancreas are only 1/50,000th of those of healthy dogs (Westermarck *et al.*, 1980). The diagnosis of EPI is most accurately determined through the measurement of serum trypsin-like immunoreactivity (TLI), with lower levels indicating the presence of the condition. However, in specific situations, such as in developing countries like India where financial constraints may limit access to TLI testing, an alternative diagnostic approach like patient-side ELISA for fecal pancreatic elastase 1 can be used as a supportive tool, which is also internationally accepted as a diagnostic tool in diagnosing EPI.Management of EPI typically involves oral supplementation with pancreatic enzymes to compensate for the deficient digestive enzymes, thereby promoting better nutrient absorption. Additionally, dietary adjustments-such as providing a highly digestible, low-fiber diet-can further improve therapeutic outcomes.

A four-year-old intact male German Shepherd Dog (GSD) was presented with a history of progressive weight loss, lethargy, polyphagia, abnormal defecation characterized by soft, voluminous stools (Fig. 1.1) and excessive flatulence. Vital parameters, including mucous membrane color, capillary refill time, rectal temperature, heart rate, and respiratory rate, were within normal physiological limits. Routine fecal examination was negative for endoparasitic infections. On physical examination, the patient was alert and responsive, exhibiting a body condition score indicative of emaciation to cachexia, with evidence of mild muscle wasting, brittle hair coat, mild dehydration, and signs of abdominal discomfort. Palpation of the right epigastric region elicited borborygmi.

Complete blood count, serum biochemistry, fecal

*Corresponding author: abhijithsp560@gmail.com

elastase-1 assay, and abdominal ultrasonography were performed. The hematological and biochemical alterations included mild neutrophilic leucocytosis, macrocytic anemia suggestive of nutritional deficiency, hypoproteinaemia and hypophosphatemia. Ultrasonographic examination revealed hepatomegaly and a mildly enlarged pancreas with increased echogenicity (Fig. 1.2), indicative of pancreatopathy. Exocrine pancreatic insufficiency (EPI) is diagnosed primarily by measuring species-specific serum trypsin-like immunoreactivity (cTLI), which directly correlates with the functional mass of pancreatic acinar tissue. Currently, the only validated alternative to cTLI for diagnosing EPI in dogs is a canine-specific patient-side ELISA for fecal pancreatic elastase-1 (cE1; ScheBo Biotech AG, Germany). This assay, validated for canine fecal samples, interprets concentrations >20 µg/g as excluding EPI, while concentrations <20 µg/g are diagnostic of EPI in dogs exhibiting compatible clinical signs (Spillmann *et al.*, 2000).

Based on the patient's clinical history, breed predisposition, clinical examination findings, ultrasonographic abnormalities, hematobiochemical profile, and markedly reduced fecal pancreatic elastase-1 concentration (Fig. 1.3), a definitive diagnosis of Exocrine Pancreatic Insufficiency was established.

EPI is treated by Pancreatic Enzyme Replacement Therapy (PERT), nutritional management (low-residue diets with moderate fat content) and supplementation of cobalamin. Pancreatic enzymes in the form of coated capsules (Tab Pancresolve-DS [1-0-0], Vivaldis, India) were provided 20 minutes before food in the initial phase of treatment along with proton pump inhibitor, Pantoprazole (Tab Acidfre-20 mg [1-0-1], Freossi, India). The proton pump inhibitors were used only for first 14 days as gastric pH decreases the enzymatic activity of lipase and trypsin.

To control the small intestinal bacterial overgrowth, antibiotic Metronidazole (Metrogyl Suspension, JB Chemicals & Pharmaceutics, India) was used. For supplementation of vitamins and minerals, multivitamin syrup (Multistar Pet [5 mL-0-5 mL], Mankind Pharma, India) and gastrointestinal diet was added along with pre & probiotics.

Over a period of 8 weeks, digestion, the consistency

Fig.1.1. Voluminous stool due to indigestion before Pancreatic Enzyme Replacement Therapy

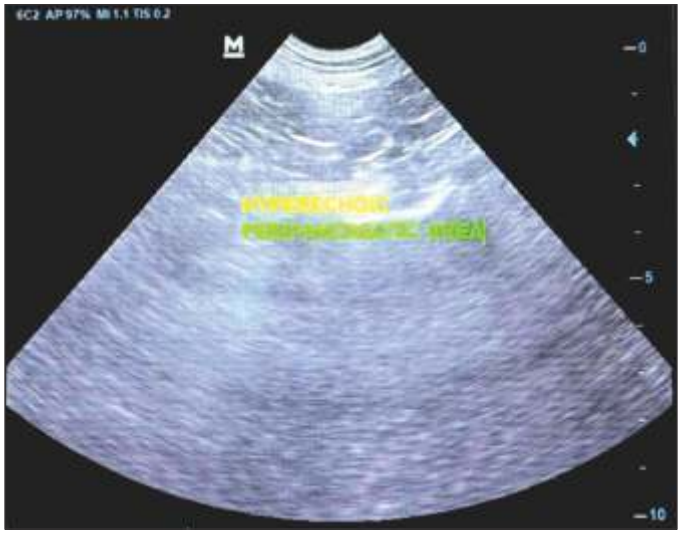

Fig.1.2. Hyperechoic Per-pancreatic region

Fig.1.3

Fig.1.4

Fig.1.5

Fig.1.3. ScheBo PE-1 Quick ELISA Kit [No line seen in "T" indicates: means no detectable pancreatic elastase-1 → suggests exocrine pancreatic insufficiency (EPI)]. Fig.1.4. ScheBo® Pancreatic Elastase-1 (PE-1) stool test Kit. Fig. 1.5. Stools after PER Therapy

of faeces and weight gain was assessed and the dog gained 6 kg of weight during this period. The owners were advised to avoid feeding any junk food products and to maintain strict monitoring of the feeding schedule. Over time, the fecal consistency improved from a 'cow dung-like' appearance to a normal, brown-coloured, segmented form (Fig 1.4).

Underlying pathologic processes that may result in clinical signs of EPI in dogs are pancreatic acinar atrophy (PAA), chronic pancreatitis and pancreatic neoplasia. PAA is reported to be by far the most common cause of severe EPI in dogs. Characteristic of PAA is a selective destruction of the digestive enzyme–producing acinar cells, which may lead to almost complete loss of secretory capacity. Chronic pancreatitis is a common cause of EPI in cats and human beings (Westermarck *et al.*, 2003). Trypsin-like immunoreactivity measures serum trypsin and trypsinogen concentrations and is considered the test of choice for EPI. Serum TLI assays are highly sensitive for the diagnosis of EPI (Williams *et al.*, 1988).

Animals with EPI do not produce sufficient trypsin, and as a result, diagnosis is made through the measurement of serum trypsin-like immunoreactivity (TLI), a test with high specificity and sensitivity. For dogs, the reference value of TLI is between 5.0 and 32.0 ng/mL and values below 2.5 ng/ mL confirm the disease (Matilda *et al.*, 2011, Westermarck *et al.*, 2012, Nelson *et al.*, 2015, Soetart *et al.*, 2019).

Gastric pH decreases the enzymatic activity of lipase and trypsin. Therefore, the administration of proton pump inhibitors such as omeprazole, one to two times a day for continuous use, is necessary (Nelson *et al.*, 2015; Matilda *et al.*, 2011). The use of antibiotics such as metronidazole aims to prevent intestinal bacterial overgrowth caused by the loss of antimicrobial factors in pancreatic juice or the increased availability of undigested substrates in the intestine (Nelson *et al.*, 2015, German 2012). Decreased or absent intrinsic factor causes hypocobalaminemia, so Vitamin B12supplementation should also be part of the treatment (Nelson *et al.*, 2015, Soetart *et al.*, 2019).

The dietary recommendation for dogs with EPI consists of diets based on hydrolysed protein with high digestibility, low fiber content, and a higher quantity of B-complex vitamins (Nelson *et al.*, 2015). Low-fat content was previously indicated, but it has been found to directly interfere with the animal's weight gain and is therefore only used as a last resort to control steatorrhea (German 2012). Lifelong adherence to enzyme supplementation and dietary management is typically necessary to maintain the dog's health and quality of life.

CONCLUSION

This case report describes the clinical presentation of exocrine pancreatic insufficiency in a German shepherddog, highlighting the diagnostic application of patient-side ELISA for fecal pancreatic elastase-1, and evaluating the treatment response and effectiveness of oral pancreatic enzyme replacement therapy.

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Short Communication

TRANSVAGINAL HYBRID ENDOSCOPIC OVARIECTOMY IN DOG: A PRELIMINARY CLINICAL STUDY

KOMAL GAIKWAD, S.B. AKHARE*, S.V. UPADHYE, M.V. KAMBLE, V.M. DHOOT[1], GAURI FISKE and B.K. BHADANE
Department of Surgery & Radiology, [1]Department of Clinical Medicine,
Nagpur Veterinary College, Nagpur
Maharashtra Animal and Fishery Sciences University, Nagpur-440001, Maharashtra

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SUMMARY

The transvaginal hybrid endoscopic ovariectomy in dog is a new approach for ovariectomy. In the present study, the surgeries were performed on clinically healthy female dogs using rigid endoscope (Laparoscope). Ultrasonography was done to rule out the pregnancy, if any. The female dogs were positioned in a Trendelenburg position. Sedation was achieved using xylazine, followed by induction with diazepam and ketamine, while anaesthesia was maintained with Isoflurane. Laparoscopic procedure was performed using transvaginal access through the fornix and one hybrid port was used at umbilicus. Both the ovaries were removed from the either ports. The haemato-biochemical parameters were recorded on day 0 and 7th in all the female dogs and the changes were found statistically non-significant. It indicates that the surgical procedure does not affect haemato-biochemical parameters. Surgical time was noted intraoperatively and the mean surgical duration during the study was 133.67±6.55 minutes. Pain score was calculated up to four days postoperatively using Glasgow pain scale. The mean pain score of the bitches showed a declining trend from 24 to 96 hours, indicating a reduction in pain. Transvaginal Hybrid Endoscopic Ovariectomy is a type of NOTES (Natural Orifices Transluminal Endoscopic Surgery) technique in which the vaginal access is used as a surgical approach along with one hybrid port at the umbilicus. This is minimal invasive surgery with fewer scars as comparative to conventional open and laparoscopic surgeries. This surgery involves less surgical trauma, lesser complications and early recovery.

Keyword: Dog, Flexible endoscope, Minimally Invasive Surgery, Ovariectomy, Transvaginal

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Natural Orifices Transluminal Endoscopic surgery (NOTES) is minimally invasive surgical procedure that uses natural orifices as a route to enter the peritoneal cavity. NOTES is a recent advancement in minimally invasive surgery that shows encouraging outcomes compared to traditional and other endo-surgical techniques (Freeman *et al.*, 2009; Brun *et al.*, 2011). NOTES technique utilizes natural body openings such as the mouth, anus, nostrils, vagina, and urethra to reach luminal organs, where their walls are then punctured to enter the peritoneal cavity. One of the main natural orifice used in NOTES includes the vagina (Arntz, 2019).

Notes can be done using the hybrid technique, which combines laparoscopic access with natural orifices. The benefit of the hybrid technique is that it allows the transvaginal port to be positioned with direct visualization, which significantly reduces the risk of accidental injury to abdominal and pelvic organs during the procedure (Linhares *et al.*, 2019). The three primary rationales for NOTES are enhanced cosmetic outcomes, reduced post-operative discomfort and pain (Swain, 2008). The transvaginal approach for laparoscopic ovariohysterectomy (OVH) is a viable method that offers the benefit of reducing the need for an additional abdominal port (Bakhtiari *et al.*, 2012). The present study was conducted on eight healthy female dogs over two years of age presented at the Department of Veterinary Clinical Complex, Nagpur Veterinary College, during 2024-2025. The primary objective of the present study was to assess the clinical applicability of laparoscopic transvaginal ovariectomy in female dogs. Comprehensive signalment and detailed anamnesis were recorded for each subject, including breed, age, body weight, coat colour and identifying markings. A complete clinical history was obtained, covering previous illnesses, vaccination and deworming status, prior surgical interventions, dietary patterns, exercise regimen, and any observed behavioral or physiological abnormalities. All animals underwent a thorough physical examination, which included the evaluation of rectal temperature, heart rate, respiratory rate, mucous membrane colour, and general demeanor. Abdominal palpation and ultrasonographic assessment were conducted to rule out pregnancy and detect any underlying abdominal abnormalities. Preoperative haematological and biochemical analyses were performed to evaluate systemic health status and determine suitability for anaesthesia and surgical intervention.

Blood samples of about 5-7ml were collected for haemato-biochemical testings. All parameters were

*Corresponding author: sandeepakhare@yahoo.co.in

measured on the day of surgery (0 day) and again on the 7th post operative day. Haematological studies include haemoglobin, total leukocyte count, packed cell volume, total erythrocyte count, differential leukocyte count, and platelet count, using an automated haematolgyanalyzer (Vetscan 5 Hm, Abaxis Company). Biochemical evaluation involved assessment of blood urea nitrogen, serum creatinine, AST, and ALT using a semi-automatic biochemistry analyzer (Vetscan 2, Abaxis Company). The anaesthetic protocol includes premedication using Atropine Sulphate (0.04 mg/kg body weight), Ranitidine (0.2 mg/kg body weight), amoxicillin and cloxacillin (20 mg/kg body weight) and meloxicam (0.2 mg/kg body weight). Sedation was achieved using Xylazine HCl at a dose of 1.1 mg/kg body weight. Anaesthesia was induced with an intravenous combination of Diazepam (0.5 mg/kg body weight) and Ketamine (5 mg/kg body weight), administered intravenously. After successful induction, the dogs were intubated and anaesthesia was maintained using 1.5-3% Isoflurane delivered through an anaesthesia machine equipped with a vaporizer. A multiparameter monitor was used throughout the procedure to monitor heart rate, respiratory rate, temperature, oxygen saturation and ECG activity.

In all dogs, food was withheld for10 hours and water was withdrawn for 6 hours prior to the surgery. The soap water enema was given 2 hours prior to surgery. The ventral abdomen was shaved, scrubbed with iodopovidone (povidone iodine 10%) and prepared aseptically. A urinary catheter was placed and intravenous access was secured. Laparoscopic instruments were sterilized in 2% glutaraldehyde for 30 minutes and rinsed with sterile water before use. The animals were positioned in dorsal recumbency with the pelvic limbs extended and secured caudally (Fig. 1). A Veress needle was inserted in the midline between the umbilicus and xiphoid process and proper positioning was confirmed using the Hanging Drop Test (Fig. 2). Carbon dioxide was insufflated to create pneumoperitoneum, maintaining intra-abdominal pressure between 7 and 13 mmHg at a flow rate of 1.5 to 6.6 L/min A 30° laparoscope was inserted through the first port. The vaginal canal was accessed using a speculum, and a 5 mm cannula (Fig. 3) was introduced and guided against the abdominal wall. Under telescopic visualization, a blunt trocar was inserted through the cannula into the peritoneal cavity (Fig. 4).

Using grasping forceps, the ovaries were identified, manipulated, cauterized using unipolar cautery. The cauterised ovary was held against lateral abdominal wall of respective ovarian side (Fig. 5). Ovaries were removed either through the vaginal or laparoscopic port, depending on their size. The surgical site was inspected for haemorrhage, and once haemostasis was confirmed, the pneumoperitoneum was released. The muscular layer was sutured with Vicryl (No. 1 or 0) and the skin was closed with polypropylene (No. 0). The vaginal incision was left unsutured as suggested by (Arntz, 2019).

Postoperative care included oral administration of Ranitidine (2 mg/kg body weight) and amoxicillin and cloxacillin (22 mg/kg body weight) for five days. Pain was assessed using the Glasgow Pain Scale, and analgesics were administered accordingly. Topical Pendistrin ointment was applied to the vaginal site for five days. Dogs showing anorexia were given supportive fluid therapy. Owners were instructed to ensure the use of an Elizabethan collar for at least five days to prevent self-trauma. Haematological parameters remained within normal physiological range and the changes were statistically non-significant (using student t-test). Similar results were reported by Fernandez-Martin *et al.* (2022). During the study, haemoglobin levels increased slightly from 14.35/ g/dL to 15.02/ g/dL and platelet counts rose from 350 to 396 lakh/cumm, reflecting stable systemic responses. Similarly, the differential leukocyte counts, including neutrophils, lymphocytes, monocytes and eosinophils, showed no clinically meaningful changes. These findings indicated that the surgical procedure did not induce haematological stress or inflammatory responses. Biochemical parameters such as serum AST, ALT, creatinine, and BUN were also assessed on days 0 and 7. AST remained nearly constant (25.92 to 25.33/ IU/L) and similar results were noted by Kumari *et al.* (2018) where AST values remain same throughout observation period. The ALT showed a mild, non-significant rise from 26.42 to 29.46/ IU/L. Serum creatinine levels increased slightly from 0.61 to 0.81/mg/dL and BUN remained unchanged. These results confirmed the absence of hepatic or renal compromise following surgery. Collectively, the haematological and biochemical stability supports the safety of the transvaginal hybrid endoscopic ovariectomy in terms of physiological impact.

The mean surgical time required in the study was 133.67 ± 6.55 min. The time required for the first and second surgery was nearly same. Third surgery took more time than first and second operation. Subsequently, the surgical time in remaining five surgeries were in descending order indicating the more feasibility of technique than before stated by Yang *et al.* (2019). Pain scores assessed via the Glasgow Composite Pain Scale showed a declining trend from 6.17 at 24 hours to 0.67 at 96 hours postoperatively, with only three dogs requiring analgesia. Similar observations were recorded by Freeman

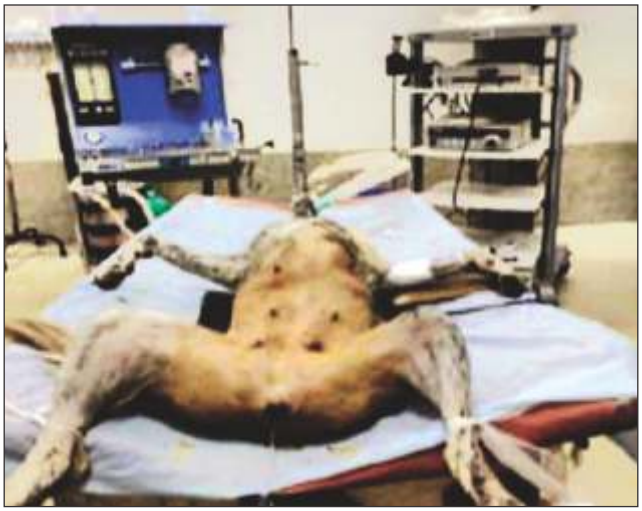

Fig. 1. Trendelenburg positioning and aseptic preparation of the dog

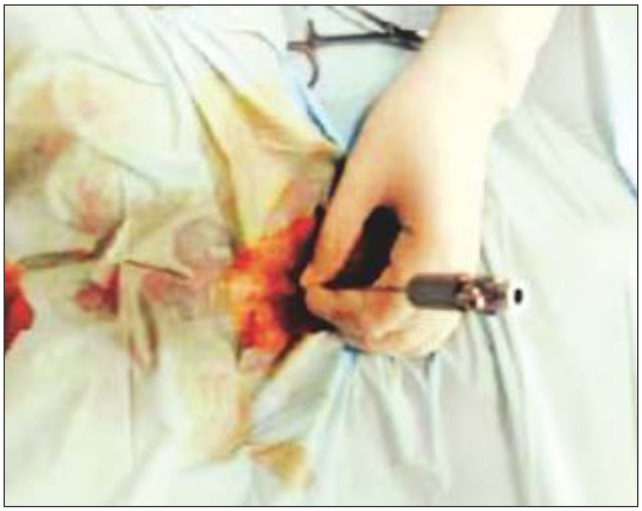

Fig. 2. Veress needle insertion for pneumoperitoneum at umbilicus.

Fig. 3. Introduction of transvaginal port using vaginal speculum

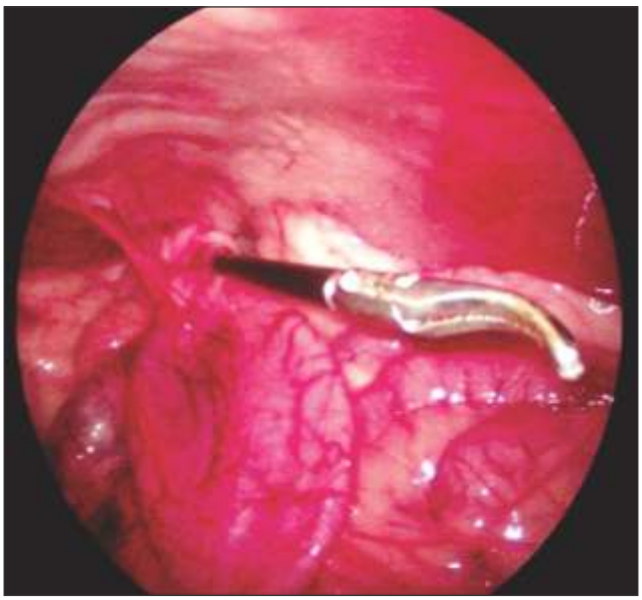

Fig. 4. Entry of grasping forceps through transvaginal port

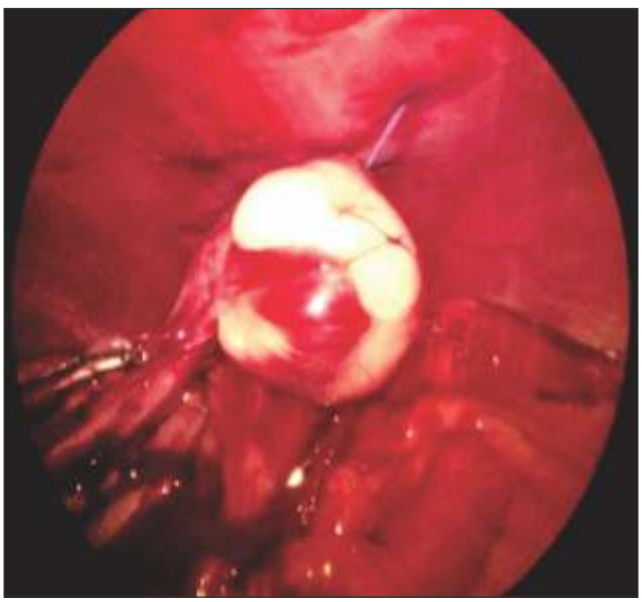

Fig. 5. Ovary tied to lateral abdominal wall of the respective side

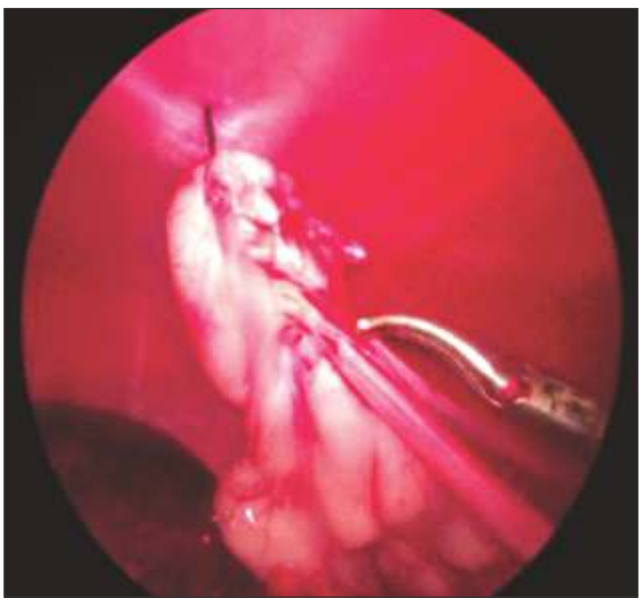

Fig. 6. Ovarian cauterisation using unipolar cautery

et al. (2010) as they compared postoperative pain in oophorectomy procedures performed via NOTES, laparoscopy and open surgery. They further opined that the NOTES group consistently experienced the lowest pain scores across all postoperative intervals.

Minor complications were noted which include difficulty in inserting the transvaginal port in obese dogs and ovary retrieval which was managed by increasing the incision. Overall, the procedure proved to be a safe, minimally invasive, and effective neutering option with excellent cosmetic and clinical outcomes.

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Short Communication

HISTOLOGICAL STUDIES ON THE MYENTERIC PLEXUS IN RABBIT

RAJESH RANJAN* and PARTHA DAS[1]
Department of Veterinary Anatomy, College of Veterinary Science & Animal Husbandry, Rewa-486001
Nanaji Deshmukh Veterinary Science University (NDVSU), Madhya Pradesh
[1]Department of Veterinary Anatomy, Faculty of Veterinary Sciences & Animal Husbandry, Kolkata-700037
West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal

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SUMMARY

The present research work was planned to demonstrate the histological characters of myenteric plexus in rabbit. It was present within the tunica muscularis of intestine between the longitudinal and circular smooth muscle layers. Histologically, it appeared in the form of ganglions that comprised of ganglionic neurons, glial cells and nerve fibers surrounded on periphery by telocytes. Thin layer of collagen fibers ensheathed the whole ganglia.

Keywords: Histology, Intestine, Myenteric plexus, Rabbit

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The enteric nervous system (ENS), a part of autonomic nervous system (ANS) covers the entire length of the mammalian gastro-intestinal tract and play an important role in coordinating the activities of gastro-intestinal tract such as absorption, secretion, motility and microvasculature. Two basic plexuses of the ENS are interconnected and arranged within the layers of the intestine. The first is the myenteric plexus, also known as Auerbach's plexus that lies within the tunica muscularis specifically between the longitudinal and circular smooth muscle layers. The second is the submucosal plexus (Meissner's plexus) located within the submucosal layer. Myenteric plexus has been reported toregulate the intestinal motility (Mandic *et al.*, 2016). Diseases of the ENS may lead to severe altered bowel motility due to changes within the structure of the myenteric plexus. Hence, the present work was designed to study the basic histological details of myenteric plexus in rabbit as the rabbit intestines are anatomically similar to the human gastrointestinal tract (Amiry *et al.*, 2019) that can provide the basic information for researchers focusing on the diseased state of the intestine using the pre-clinical rabbit model.

The present work was carried out on six adult rabbits between body weights of 1.0 to 1.5 kg as per the IAEC guidelines. The animals were euthanized and the tissue samples were collected from intestine and quickly fixed in 10% Neutral buffered formalin (10% NBF) and Bouin's fixatives after properly washing in 0.9% normal saline solution. Processing of tissue samples was done by acetone-benzene schedule and 5-6 µm thick sections were obtained on clean glass slides. These tissue sections were stained with Haematoxylin and eosin to identify the cellular details, Masson's trichrome stain for collagen fibers, Gridley's stain for reticular fibers and Weigert's stain for elastic fibers (Luna, 1968). Photography of the stained slides was done in Leica DM 2000 microscope.

In the present study, the myenteric plexus was found in the tunica muscularis layer of the intestine, specifically between the outer longitudinal and inner circular smooth muscle layers (Fig. 1). It appeared in the form of ganglions that comprised of ganglionic neurons, glial cells and nerve fibers surrounded on periphery by telocytes (Fig. 2). Similar observations were reported by Verres *et al.* (2022). Theganglia were irregular in shape and ensheathed by thin layer of collagen fibers (Fig. 3) which was in accordance with the reports of Serenini *et al.* (2020). The reticular and elastic fibers were not observed around or within the ganglia.

The ganglionic cell bodies had prominent, relatively euchromatic, eccentric, spherical nuclei with prominent nucleoli and granular eosinophilic cytoplasm. These ganglionic cell bodies were surrounded by glial cells. The nerve fibrils formed a hazy network around the cells. The glial cells (ganglionic gliocytes) surrounding the neuronal cell bodies had ovoid heterochromatic nuclei. On the periphery of the ganglions, were present, very thin cells known astelocytes with long filamentous telopodes with spindle shaped and darkly stained nuclei (Fig. 2) which was in accordance with the reports of Verres et al. (2022). Rosa et al. (2021) reported presence of telocytesas newly identified interstitial cells having telopodesas thin and long cytoplasmic processes and considered to be a part of

*Corresponding author: rajesh.ranjan837@gmail.com

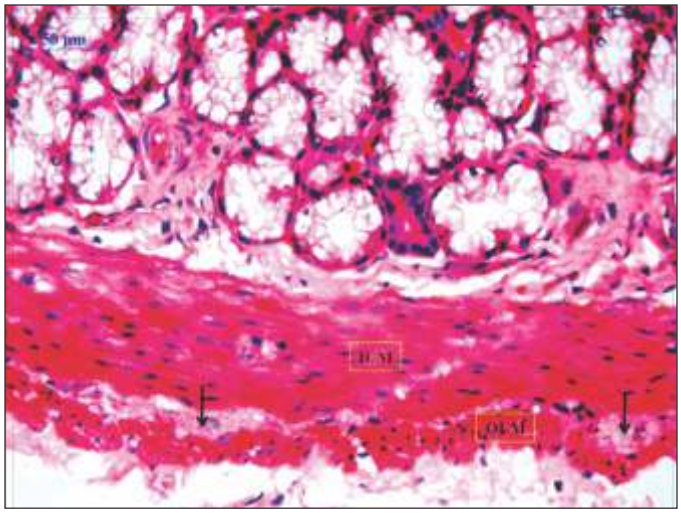

Fig. 1. Photomicrograph of rabbit intestine showing presence of myenteric plexus (arrow) within the tunica muscularis specifically between the inner circular (ICM) and outer longitudinal (OLM) smooth muscle layers. H&E 40x.

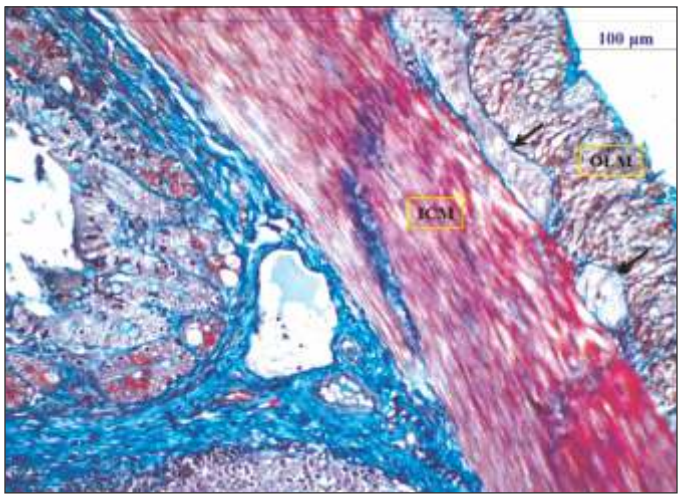

Fig. 3. Photomicrograph of rabbit intestine showing presence of myenteric plexus within the tunica muscularis specifically between the inner circular (ICM) and outer longitudinal (OLM) smooth muscle layers and ensheathed by a thin layer of collagen fibers (arrow). MT 20x.

stem cell niches in the intestine. They have been described in the myenteric plexus region where they surround gangliaand enteric nerves and among the smoothmuscle bundle layers.

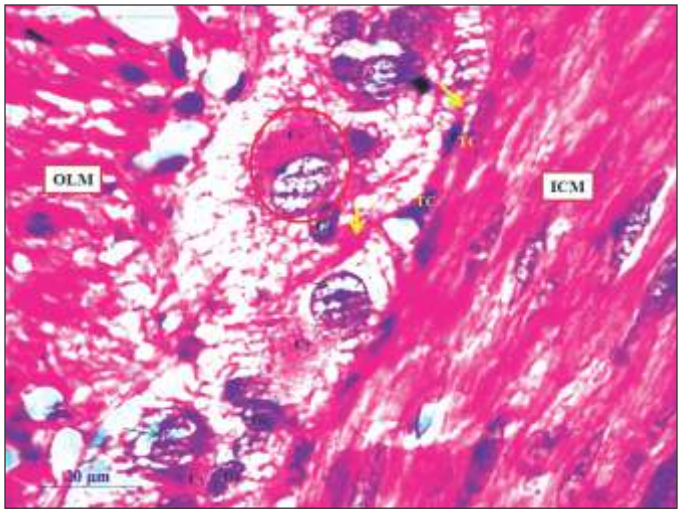

Fig. 2. Photomicrograph of a ganglion of myenteric plexus with ganglionic neuronal cell body (red circle), having a prominent eccentric, euchromatic, spherical nucleus (arrow) and granular eosinophilic cytoplasm (Cy). The neuronal cells are surrounded by gliocytes (Gc) with oval, heterochromatic nucleus. The neuronal fibres are hazy in appearance. The telocytes (Tc) are present at the periphery of the ganglion with thin telopodes (yellow arrow). The smooth muscle layers are marked as ICM and OLM. H&E 100x.

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Clinical Article

SURGICAL MANAGEMENT OF PERINEAL HERNIA IN MEHSANA BUFFALO

R.K. GOSAI*, A.M. PATEL, J.B. PATEL and SURESH CHAUDHARY
Dr. V.M. Zala Clinical Complex, Deesa
College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardar Krushinagar-385506, Gujarat

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SUMMARY

A 12 year old Mehsana buffalo was brought at Dr. V. M. Zala Clinical Complex, Deesa. The buffalo was found with history of right unilateral swelling on vulvar lip and difficulty in micturition since last two months. Neutrophilia was noted in complete blood count. Ultrasonography shown a hyper echoic hernia sac encircling the urinary bladder. The case was diagnosed as perineal hernia and herniorraphy was carried out under epidural anesthesia. The buffalo showed subcutaneous seroma as minor complications. The buffalo recovered uneventfully.

Keywords: Buffalo, Herniorraphy, Perineal hernia, Urinary bladder

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Perineal hernia is the protuberance of organs of the abdominal cavity through the pelvic diaphragm, which supports the rectal wall. This condition primarily arises due to a weakened pelvic diaphragm, resulting in the abnormal displacement of pelvic organs into areas surrounding the perineal region (Prasad *et al.*, 2015). Predisposing factor to perineal hernia was external injuries, rupture to the pelvic diaphragm and increased abdominal pressure during staining, which can lead to the herniation of the urinary bladder and abdominal organs (Shridhar, 2011). It was more commonly seen in male dogs which not castrated (Kumar *et al.*, 2016) and is a rare condition in large ruminants. This present paper a successful surgical operative for unilateral perineal hernia in a Mehsana buffalo.

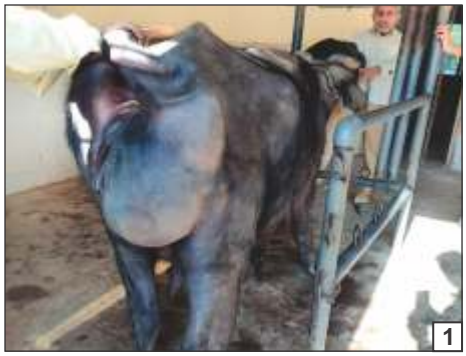
A 12 year old Mehsana buffalo, with a weight of 575 kg, suffered from right unilateral perineal swelling and difficulty in urinating for the past two months (Fig. 1). Blood parameters were found within normal range with slightly elevated neutrophils, suggestive of inflammatory changes. The swelling gradually increased when the bladder was full, leading to challenges during micturition. On palpation, a soft, reducible, and painless unilateral swelling was observed on right side of vulvar lip. Manipulation leads to voiding of urine suggestive of herniated bladder. A distinct hernial ring was palpated in the perineal area, measuring approximately 5 cm in diameter. All the physiological parameters were found to be normal. Ultrasonography confirmed presence of anechoic fluid in urinary bladder with hyperechoic bladder wall (Fig. 2). No any other structures like intestine, uterus and mesentery was observed in scan. It was confirmed as right unilateral perineal hernia and planned for surgical correction.

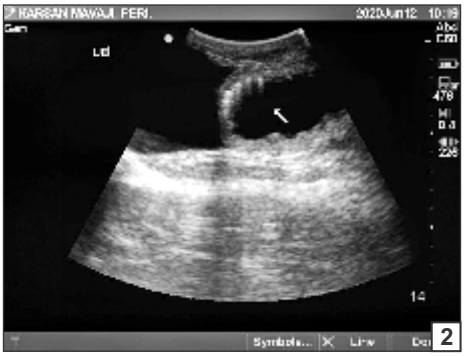
The buffalo was kept off feed for 24 hours prior to the surgery. After shaving and scrubbing the surgical site was prepared aseptically. The surgery was performed with the animal in a standing position under caudal epidural anesthesia, administered with 5 ml of 2% Lignocaine hydrochloride. A tampon of cotton was put in the anus to prevent the contamination. A dorso-ventral linear skin incision was made directly over the hernial swelling in perineal region, near from base of the tail to end of swelling. After separation of skin and fascia, adhesion was removed manually then hernia content (bladder) was identified. The content were urinary bladder and retroperitoneal fat. The hernial ring was closed with using of No. 2 silk by overlapping suture after reposition of urinary bladder (Fig. 3). Herniorraphy was performed by suturing the external anal sphincter and the levatorani, coccygeus muscles. The subcutaneous suture was taken by chromic catgut No. 2. The skin incision was united by horizontal mattress suture pattern using silk no. 2. Subcutaneous seroma around the surgical site was noted (Fig. 4). The seroma was drained and recovered uneventfully after one month (Fig. 5).

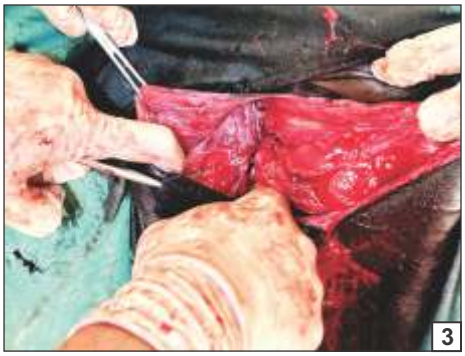
Post-operatively, the animals were treated with Strepto-penicillin @10,000 IU I/M, Meloxicam @0.2 mg/kg B.W. and Chlorpheniramine maleate @ 30-50 mg I/M for five days. Sutures were removed 12 days after surgery.

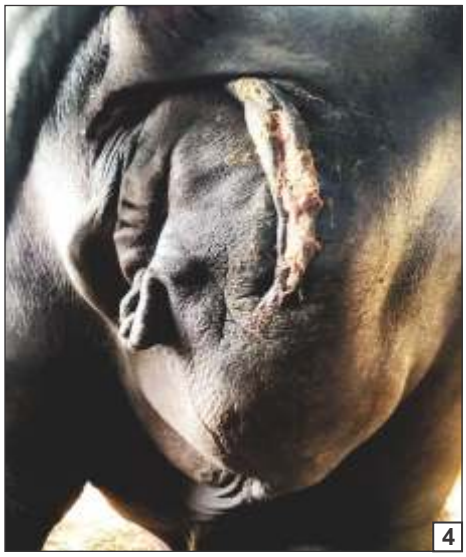
In present case, unknown etiology for development of unilateral hernia but it might be due to external trauma in perineal region. The bladder hernia in perineal region was also reported by Mathur (2003) in buffalo and Shridhar

*Corresponding author: rohit89dr@kamdhenuuni.edu.in









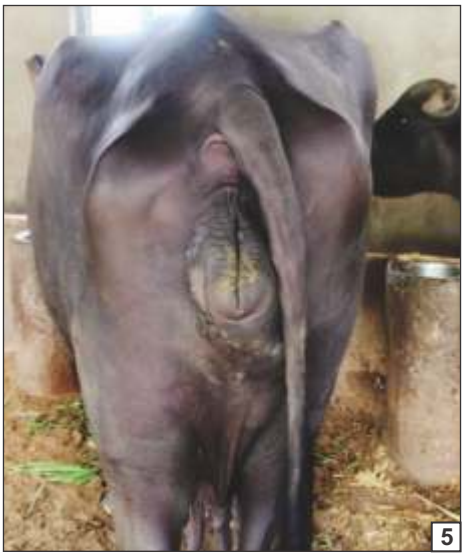


Fig. 1. Unilateral perineal hernia

Fig. 2. Hyperechoic hernia sac containing the urinary bladder

Fig. 3. Sutured hernial ring by Silk no. 2

Fig. 4. *Subcutaneous seroma* formation post-operatively complication.

Fig. 5. Post operatively recovery

(2011) in a cow. Perineal hernia in non-pregnant buffaloes was also documented by (Prasad *et al.*, 2015; Vadalia *et al.*, 2017). Similarly, Kumar *et al.* (2021) observed hyperechoic hernia sac enclosing the retroflexed urinary bladder and motile loop of large intestine with homogenous hypoechoic contents. Unilateral perineal hernias in female Mehsana buffalo can be successfully repaired with herniorraphy.

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Haryana Veterinarian (June, 2026) **65(1),** 125-128 ISSN 0033-4359 (P)

Clinical Article

SUCCESSFUL SURGICAL MANAGEMENT OF COLIC DUE TO CONGLOMERATE PLASTIC BEZOAR IN JEJUNUM IN A MARE

JOAN CYNTHIA, A.*, P. TAMILMAHAN, M. BHARATHIDASAN, A. ARUN PRASAD, D. PRUTHVI RAJ and P.V. JEEVIKA
Madras Veterinary College Teaching Hospital, Department of Veterinary Surgery and Radiology, Madras Veterinary College, Vepery, Chennai- 600 007, Tamil Nadu

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SUMMARY

A three year old Marwari mare, weighing approximately 250 kg, was presented to the Large Animal Surgery Unit, Madras Veterinary College Teaching Hospital, Vepery, with a clinical history of anorexia, signs of abdominal discomfort characterized by restlessness and repeated kicking at the ventral abdomen, progressive abdominal distension and frequent but unsuccessful attempts at defecation. Clinical examination revealed tachycardia, sweating on abdomen region. Per-rectal examination revealed scanty hard, dry fecal contents, distended small intestinal loops. On trans-rectal USG examination, absence of intestinal motility and fecal stasis was noticed. A mid-ventral laparotomy was performed under Xylazine-Ketamine-Butorphanol (induction) maintenance with Triple drip. The exploration of the abdomen revealed a jejunal impaction and multiple perforations noticed in the jejunum of about 1×1 cm^2. The impaction was resolved through jejunal enterectomy and end-to-end anastomosis after ligation of mesenteric vessels. Mid ventral laparotomy incision was closed as per the standard surgical technique. The horse recovered uneventfully.

Keywords: Colic, Enterectomy, Impaction, Triple drip

How to cite: Cynthia, A.J., Tamilmahan, P., Bharathidasan, M., Prasad, A.A., Raj, D.P. and Jeevika, P.V. (2026). Successful surgical management of colic due to conglomerate plastic bezoar in Jejunum in a mare. *Haryana Veterinarian.* **65(1)**: 125-128.

Colic is a primary cause of death for horses globally and one of the most common equine health issues. Some types of colic require surgical intervention for a favorable outcome. However, the majority of colic episodes are minor and resolve with medical care alone. Horses can develop acute abdominal obstruction of the small intestine, with the ileum being the most often implicated part (Fleming and Mueller, 2011).

Foreign body obstruction within the jejunal segment of the small intestine may occasionally manifest as intestinal distension detectable on transrectal palpation, accompanied by gastric reflux. In the majority of jejunal impaction cases, exploratory laparotomy is indicated to relieve the obstruction. In the present case, a three-year-old Marwari mare with an approximate body weight of 250 kg was admitted to the Large Animal Surgery Unit, Madras Veterinary College Teaching Hospital, Vepery. The clinical history included anorexia, restlessness, repeated abdominal kicking, progressive abdominal distension, and frequent but unproductive attempts to defecate. Localized sweating over the flank region was also reported. On clinical examination, the mare exhibited tachycardia, tachypnea and congested mucous membranes, with a capillary refill time exceeding two seconds. Auscultation and percussion of the abdomen revealed absence of borborygmi, suggestive of reduced gastrointestinal motility. Per-rectal palpation revealed distended intestinal loops containing gas along with the presence of scant, desiccated fecal material. Transabdominal ultrasonography demonstrated a complete absence of small intestinal peristaltic activity, which is strongly indicative of intestinal obstruction or strangulating lesions. Ultrasonography of the abdomen is considered a valuable diagnostic modality in differentiating small and large intestinal pathologies, including obstructive and strangulating disorders. Initial medical management included intravenous fluid therapy with Ringer's lactate (10 ml/kg b.wt.), 25% dextrose (10 ml/kg b.wt.), and flunixin meglumine (1.1 mg/kg b.wt., I/V) for analgesia and anti-inflammatory support. Following stabilization, nasogastric intubation was performed and 2 liters of liquid paraffin were administered, followed by 400 g of magnesium sulfate as a laxative. However, due to the lack of clinical improvement after 24 hours of intensive medical therapy, an exploratory laparotomy was indicated. For anesthesia, the mare was premedicated with xylazine (1.1 mg/kg, I/M), ketamine (2.2 mg/kg, I/M), and butorphanol (0.02 mg/kg, I/M). General anesthesia was maintained using a triple-drip infusion consisting of xylazine (1.1 mg/kg), ketamine (2.2 mg/kg), and glyceryl guaiacolate ether (25 g/500 ml) at 1 ml/kg/hr. The animal was positioned in dorsal recumbency and the surgical field was prepared aseptically. A caudal mid-ventral laparotomy was performed through the skin, subcutaneous tissue and linea alba, extending from the umbilicus to the pubis.

During exploratory laparotomy, the affected jejunum was identified. Several perforations were observed during the exteriorization of the impacted jejunum. The jejunum was isolated using moistened laparotomy sponges and

*Corresponding author: joancynthia1999@gmail.com

impermeable drapes. An incision was made on the antimesenteric border of the jejunum, and multiple sharp nails and phytobezoars were extracted. The jejunal vessels within the mesentery were ligated using PGA no. 2. An end-to-end anastomosis was performed using PGA no. 2 in a Cushing's pattern. A leak test was subsequently conducted.

Abdominal lavage was performed using 5 litres of normal saline and 2 litres of Inj. Metronidazole. The linea alba was sutured with PGA no. 2 using a simple continuous pattern, and reinforced sutures were applied. The subcutaneous layer was closed with PGA no. 2, and the skin incision was approximated with non-absorbable polyamide no. 1-0 suture material. The animal's recovery from anaesthesia was uneventful.

Following surgery, the patient was maintained on nil per os (NPO) for 72 hours to allow adequate gastrointestinal recovery. Supportive therapy consisted of intravenous administration of Ringer's lactate (4 L/day) for fluid and electrolyte balance. Broad-spectrum antimicrobial coverage was instituted using metronidazole (15 mg/kg, I/V), ceftriaxone (10 mg/kg, I/V), and gentamicin (4 mg/kg, I/V). Flunixin meglumine (1.1 mg/kg, I/V) was administered for analgesia and anti-inflammatory support, while pantoprazole (1 mg/kg, I/V) was used to prevent stress-induced gastric ulceration. Tribivet (10 ml, I/V) was incorporated as a multivitamin supplement. This regimen was continued for one week postoperatively. The patient was subjected to continuous monitoring of vital parameters and gastrointestinal motility. Spontaneous defecation was observed within 24 hours following surgery, indicating the resumption of intestinal function. Oral intake of feed and water was gradually reintroduced after 72 hours, with no recurrence of colic symptoms. The surgical wound was managed with aseptic dressing and topical application of Drez ointment at regular intervals, which promoted satisfactory healing. The postoperative recovery was uneventful, and the mare returned to normal feeding behavior and activity within a week.

Pus accumulation noticed in the wound site after one week of treatment. ABST revealed the sensitivity to amikacin. For the drainage of accumulated pus alternate sutures were removed and lavaged with metronidazole and normal saline. The abdominal incision healed within a week, and no colic episodes have occurred since the surgery. The animal recovered smoothly.

Digestive disorders, including colic, diarrhoea, and enterotoxaemia, account for 50% of the medical conditions that lead to mortality in adult horses (Baker and Ellis, 1981). Colic continues to be a major cause of morbidity and mortality in equines, often requiring immediate clinical intervention. In the present case, the three-year-old Marwari mare exhibited classical clinical signs of abdominal pain, including restlessness, repeated kicking at the abdomen, flank sweating, abdominal distension, and frequent but unproductive attempts to defecate. These observations are consistent with earlier reports describing flank watching, pawing, rolling, and attempts to urinate or defecate as hallmark indicators of equine colic (Cohen *et al.*, 1999).

Physiological derangements such as tachycardia, tachypnea, congestion of mucous membranes, delayed capillary refill time, and absence of intestinal borborygmi in the present case further emphasized the severity of intestinal compromise. The absence of gastrointestinal sounds on auscultation and percussion, coupled with the ultrasonographic finding of absent small intestinal motility, strongly suggested jejunal obstruction. This aligns with previous studies that highlight the importance of ultrasonography in differentiating obstructive and strangulating lesions of the equine gastrointestinal tract. The exact etiology of intestinal impactions remains multifactorial and not fully understood. However, several risk factors have been documented, including sudden changes in diet, type of forage, climatic variations, irregular deworming, and prior episodes of colic or abdominal surgery (Cohen *et al.*, 1999). In the present case, although no prior history of colic or abdominal surgery was reported, the animal had undergone recent dietary changes and weather fluctuations, which could have contributed to the onset of jejunal impaction. Prompt surgical intervention remains the treatment of choice in non-responsive impaction cases, as medical management alone is often insufficient in resolving complete jejunal obstruction. The timely exploratory laparotomy performed in this case ensured decompression and correction of the obstruction, thereby preventing progression to ischemic necrosis and systemic endotoxemia. The post-operative management, which included aggressive fluid therapy, multimodal analgesia, broad-spectrum antimicrobial coverage and gradual reintroduction of oral feeding, contributed to a favorable outcome. This case underscores the importance of early recognition, accurate diagnosis and prompt surgical management of jejunal obstruction in horses. Furthermore, it highlights the role of risk factor assessment and preventive strategies, such as gradual dietary transitions, routine deworming, and careful monitoring during environmental changes, in minimizing the incidence of colic in equines. On rare occasions, horses may ingest indigestible materials, resulting in blockages within the digestive tract. If such foreign materials obstruct

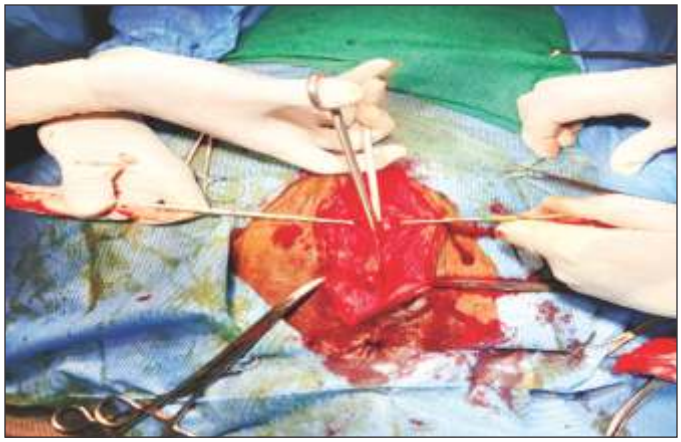

Fig. 1. Dissection of muscular layer after skin incision (ventral abdomen)

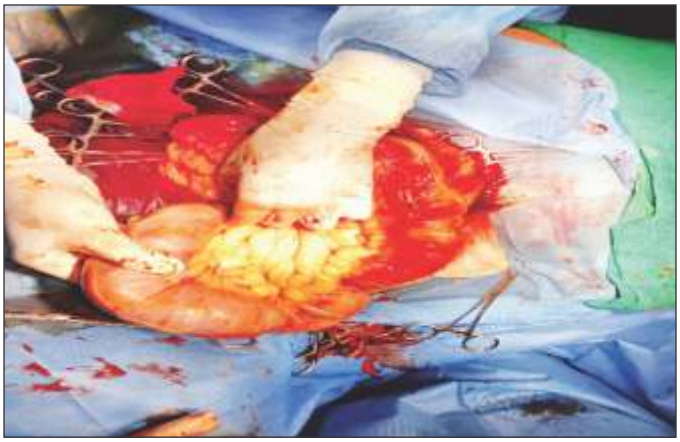

Fig. 2. Exteriorised jejunum containing impacted mass

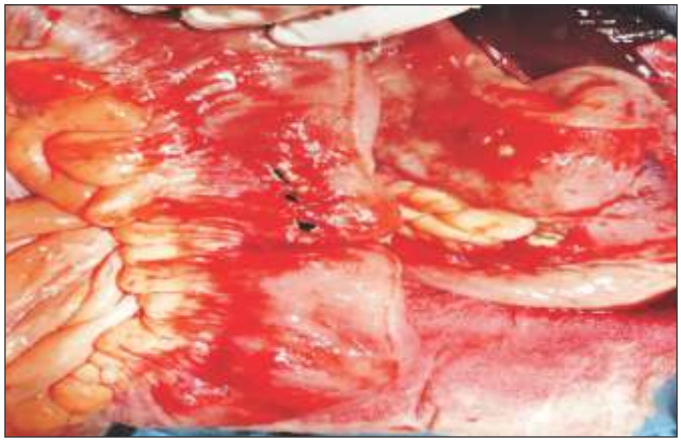

Fig. 3. Perforations in the jejunal wall

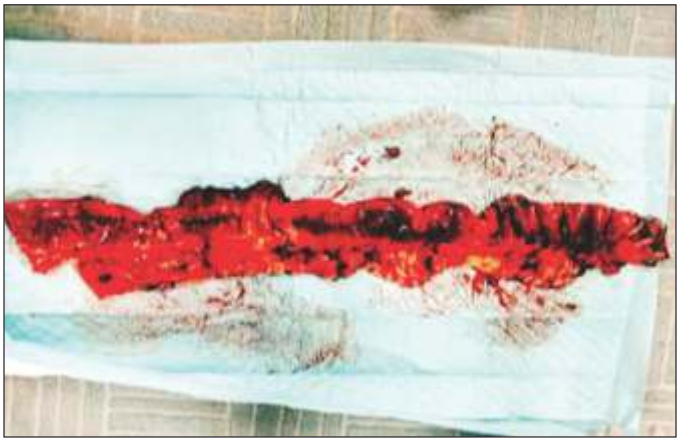

Fig. 4. Enterectomy

Fig. 5. Phytobezoars, sharp nails and enterolith removed

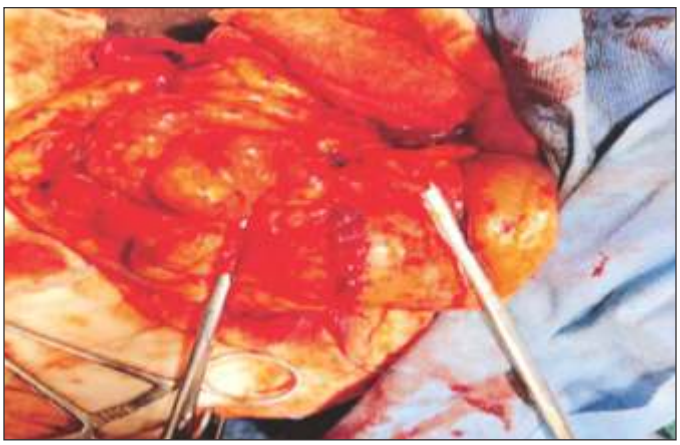

Fig. 6. End-to-end anastomosis was performed

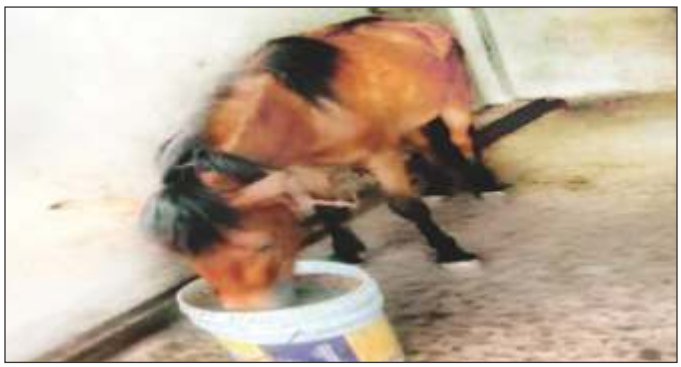

Fig. 7. After three months

narrow segments of the gastrointestinal tract, colic may ensue (Boles and Kohn, 1977). The occurrence of impaction in the jejunum is atypical. Jejunal impaction is rare and typically occurs due to underlying disease or trauma (White and Dabareiner, 1997). The management of gastrointestinal impactions in equines depends largely on the location of the obstruction and the degree of intestinal compromise resulting from distension. In many cases, impactions can be successfully managed with analgesics, cathartics, and oral or intravenous fluid therapy, which aid in softening and rehydrating the impacted ingesta

(Nathaniel *et al.*, 1997). Medical treatment typically carries a more favorable prognosis compared to cases that require surgical intervention. Supportive care may also involve withholding feed until the impaction resolves and providing analgesics as necessary. However, in severe or refractory cases, exploratory laparotomy is warranted to relieve the obstruction (Van der Linden *et al.*, 2003). Persistent, unrelenting abdominal pain that fails to respond to medical therapy necessitates immediate surgical management to prevent further intestinal compromise and systemic deterioration (David, 2011). The present case demonstrates the use of elective exploratory laparotomy as both a diagnostic and therapeutic tool in a horse with non-resolving colic. Despite its effectiveness, it should be noted that a significant proportion of colic-related fatalities occur postoperatively, primarily due to complications such as endotoxemia, peritonitis, or adhesion formation (Ihler, 2004). Prevention plays a crucial role in minimizing the incidence of colic. Dietary management strategies, such as restricting feeds rich in simple carbohydrates (e.g., molasses-based concentrates), providing clean and good-quality forage and water, avoiding ingestion of dirt or sand by using elevated feeding troughs and maintaining a consistent feeding schedule, are essential. Additionally, routine deworming, regular dental care and gradual dietary transitions are recommended preventive measures to reduce the risk of gastrointestinal impactions.

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IMPORTANT INFORMATION

- Research/Clinical articles are invited for next issue from the Scientists/Veterinarians engaged in Veterinary Profession.
- Please follow strictly the format of **'The Haryana Veterinarian'** for manuscript writing/ submission.
- **The article processing and publication charges will be revised from Rs. 1200/- to 1500/- per manuscript with effect from the next year (01.01.2027).**
- We solicit your co-operation.
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Editors

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Clinical Article

MANAGEMENT OF DYSTOCIA IN A MARE THROUGH FETOTOMY

CHANDRA SHEKHER SARSWAT*, SUMIT PRAKASH YADAV, KRISHNA NAND BANSAL, ABHAY KUMAR MEENA, NEHA TRIVEDI, WANJARI PARAS, CHANDNI MEENA and ANITA
Department of Veterinary Gynaecology & Obstetrics, Post Graduate Institute of Veterinary Education and Research, RAJUVAS, Jobner, Jaipur-302031, Rajasthan, INDIA

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SUMMARY

Delivery of dead male fetus in a pluriparous mare suffering from dystocia due to left lateral head and neck flexion with carpal flexion using partial fetotomy procedure is reported.

Keywords: Dystocia, Partial fetotomy, Pluriparous mare

How to cite: Sarswat, C.S., Yadav, S.P., Bansal, K.N., Meena, A.K., Trivedi, N., Paras, W., Meena, C. and Anita (2026). Management of dystocia in a mare through fetotomy. *Haryana Veterinarian.* **65(1)**: 129-130.

Dystocia in mares is perhaps one of the most challenging conditions faced by equine practitioners (Purohit, 2011). Fetotomy operations are performed on the dead fetus for the purpose of reducing its size by either division or removal of certain parts of the dead fetus (Kebede *et al.*, 2017). According to Frazer (2001) only 4 percent is the incidence of dystocia in Thoroughbred mares. The effective management during dystocia will be the deciding factor for the survival of the mare and foal with subsequent fertility of the mare (Pynn, 2014). Different types of obstetrical manoeuvers are used to resolve dystocia in various farm animals and in mares (Frazer, 2001). As per Vandeplassche (1987), partial fetotomy is effectively utilized in 80 percent of dystocia cases.The present case report describes a rare case of fetal malposture with left lateral head and neck flexion with carpal flexion in a pluriparous mare resulting in dystocia, which was successfully resolved by using partial fetotomy (subcutaneous and percutaneous), repulsion and traction techniques.

An eight year old Marwari mare at her full term pregnancy of fourth parity was presented to the Gynaecology section of Veterinary Clinical Complex (VCC), Post Graduate Institute of Veterinary Education and Research (PGIVER), Jaipur with the history of intense and continuous straining with failing of foal delivery since last evening. The first sac (allantochorionic sac) was ruptured 12 hours before but there was no progression in foal delivery. General examination revealed alert and active animal with 102º F of body temperature, 64-68 of heartbeat and 24-26 per min of respiratory rate. The birth canal was moist enough with sufficient dilation of the cervix.

On the basis of clinical examination, the case was diagnosed as dystocia in the mare due to left lateral head and neck flexion with carpal flexion of the dead foal. The malposture was handled by obstetrician failing which the partial fetotomy was decided to be carried out. Prior to manipulation, sedation of the animal was achieved in order to reduce the straining and restrained the animal by administering 8 ml of Xylazine intravenously along with epidural anaesthesia with 2 percent lignocanine hydrochloride 05 ml. The mare was stabilized by fluid therapy Dextrose 5 percent 07 liter and Ringer lactate 07 liter with supportive therapy of antibiotics Inj Intacef 4.5 gm I/V and analgesics Inj Melonex® 25 ml I/M with Inj. Anistamin® 15 ml I/M and Tetanus Toxoid 5 ml I/M. The perineal region was thoroughly washed and cleaned with antiseptic solution before the obstetrical operation. With the help of paraffin lubricant, the malposture was manipulated and traction was applied on the flexed carpals to extend. By this manipulation technique, both forelimbs were extended out from the birth canal. Afterwards, the manipulation was carried out on the deviated head and neck region but due to the extended both forelimbs limits the manipulation procedure through the birth canal. Partial fetotomy was carried out on the extended forelimbs by the subcutaneous method in which snares were applied on the forelimbs below the fetlock joint at the pastern joint. The skin was dissected with a scalpel blade and the muscles with fascia were separated with the help of fingers in order to expose the underneath carpal bones. Disarticulation of the metacarpal bones from the fetlock joint and elbow joint was accomplished without severing the skin. By applying traction on the metacarpal bones, the decorticated limbs were detached and removed (Fig. 1). The same procedure was repeated for the other fore limb. Afterwards, tried to reach the deviated head and neck, but again the birth canal

*Corresponding author: drsaraswat83@gmail.com

Fig. 1. Partial fetotomy operations in mare

space was still insufficient. So, it was decided to perform percutaneous fetotomy on the left side forelimbs. By using thygesons fetotome set, the left side forelimb was dissected at scapular region without damaging the birth canal. Afterwards, sufficient space was created with in the birth canal but failed to extend the deviated neck and head. Percutaneous fetotomy was carried out on the neck region by using the Thygeson's fetotome and decapacitation was done. Later on, sufficient traction on the right forelimb and on the neck region by the help of snares and Krey-schotler hook, respectively leading to the successful expulsion of dead male foal along with placenta (Fig. 2). Mare recovered uneventfully on the next day.

Dystocia in mares is very challenging, especially in the case of a dead fetus and should be handled by an expert obstetrician, otherwise it is potentially dangerous (Higgins and wright,1999). Duration of vaginal intervention should be kept to a minimum even if the fetus is dead. Similar case was also reported by Sutaria *et al.* (2014), Singh *et al.* (2017), Hasssan *et al.* (2020). Bawane *et al.* (2022) and Dutt *et al.* (2024) performed partial fetotomy in mare to relieve the dystocia due to fetal abnormal presentation, position and posture.

Fig. 2. Dead foal delivered after partial fetotomy

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Clinical Article

DYSTOCIA DUE TO VENTRAL HERNIA WITH MACERATED FETAL BONE PROTRUDED THROUGH UDDER IN A SALEM BLACK DOE- A CASE REPORT

S. MANOKARAN[1], S. SHANMATHI, T. SARATH*, A. RESHMA, A. THANGAMANI and R. RAJKUMAR
Department of Veterinary Gynaecology and Obstetrics, [1]Veterinary Clinical Complex,
Veterinary College and Research Institute, Salem,
Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu-636112

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SUMMARY

A pluriparous doe was presented with the history of swelling in the right-side ventral abdomen, foul smelling serous discharge and small fetal bones being protruded from the swollen area of the udder. On vaginal examination, the cervix was full hand dilated but was not able to reach the uterus. Radiographic examination showed irregular mass of fetal bones in the uterine horns. Conclusively, the condition was reported to be a hysterocele sequel to maceration of fetus. Local anaesthesia obtained by 2% Lignocaine hydrochloride on the surgical site and exposed portion was incised which showed ruptured uterus and maceration of fetus. The macerated fetus was carefully removed. The uterus was cleaned and closed as per standard surgical procedure. The animal showed uneventful recovery after 10 days of post-operative care.

Keywords: Caesarean section, Maceration, Salem black doe, Ventral hernia

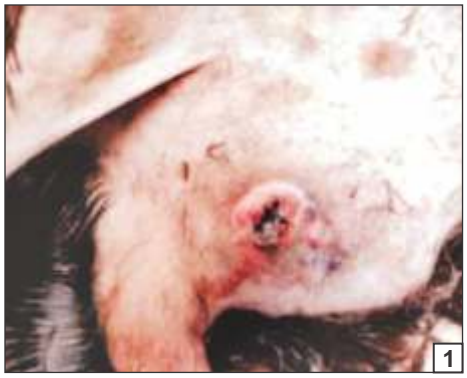
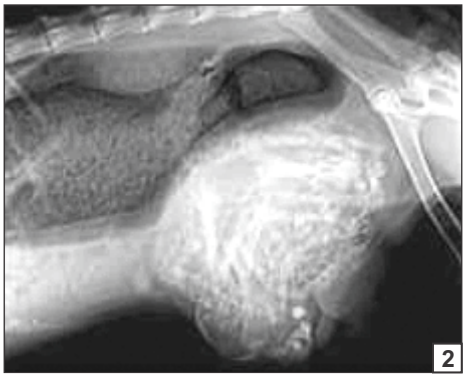
How to cite: Manokaran, S., Shanmathi, S., Sarath, T., Reshma, A., Thangamani, A. and Rajkumar, R. (2026). Dystocia due to ventral hernia with macerated fetal bone protruded through udder in a Salem black doe- A case report. *Haryana Veterinarian.* **65(1)**: 131-132.

Ventral hernia is the protrusion of viscera pass through the abdominal wall and it can occur abdominal cavity from iliac crest to lateral side of abdominal cavity above the stifle joint (Al-Sobayil and Ahmed, 2007; Jettennavar *et al.*, 2010).Ventral hernia of gravid uterus is generally due to blunt trauma, abdominal wall abscesses, extreme abdominal distension of gravid uterus due to multiple fetus, weakening of abdominal muscles during labouror due to rupture of prepubic tendon in advance stage of gestation. The ventral hernia is generally not considered as the obstetrical emergency by the farmers unless they result in serious symptoms (Rangasamy *et al.*, 2021). The ventral hernia during late gestation leads to trapping of gravid uterine horn into the herniated sac and are prone for difficulty of kidding. Moreover, culminate to adhesions of uterine horns to the abdominal muscles and other viscera (Kalaiyarasan *et al.*, 2024). Long standing case of herniation uterus may result in dead of fetuses (Vijayanand *et al.*, 2012) and moreover failure of expulsion of dead fetus through cervix may leads to either mummification (Monica *et al.*, 2018a) or maceration (Monica *et al.*, 2018b) in small ruminants. In this case report, ruptured ventral hysterocele with progressive maceration of fetus in a Salem black doe was described.

A 4 year old pluriparous Salem Black Doe was presented to emergency unit of Veterinary Clinical, VCRI, Salem with a history of in appetence since last 3 days. The owner reported that the doe was met with an automobile accident one month ago. Since then, a swelling has been present near to the udder region. However, no veterinary attention was sought, as the animal's feeding habits were normal. Fetal bones began protruding near the udder region through the opening, accompanied by a foul-smelling purulent discharge from the vagina (Fig. 1). On general physical investigation the goat appeared dull and congestion of conjunctival mucous membrane. A large ventral hernia was palpable in the right caudal abdominal region with fistula near the udder through which fetal bones were protruding. Radiographic examination revealed irregular mass of fetal bones in the uterine horn and fistulous tract was also clearly visualized. (Fig. 2). Based on the history, clinical, vaginal and radiographic examination the case was diagnosed as ventral hernia complicated with maceration of foetus.

The animal was placed in the left lateral recumbency. The operative site was prepared aseptically. Epidural anaesthesia of 1.5 ml of 2% lignocaine hydrochloride was injected into lumbosacral region along with local infiltration of 2% lignocaine hydrochloride injection to the surgical site. An oblique skin incision of about 12cm was made over the swollen region and the abdominal muscles and peritoneum was incised and then the gravid uterine horn was exposed. After careful incision of the uterus, the macerated fetus embedded to the uterus along with disintegrated fetal bones was separated and removed carefully (Fig. 3). The uterus was thoroughly cleaned and sutured with Cushing followed by Lembert pattern by using PGA1 suture material. After that peritoneal lavage with 2 litres of NS and 100 ml of Metrogyl® was performed. The muscle and skin were sutured by using PGA-1, Polyamide 2-0, respectively. Post-operative care included fluid therapy with Ringers Lactate 200 ml for 3 days, ampicillin and cloxacillin @ 10 mg/kg b.wt, meloxicam

*Corresponding author: drsarathvet96@gmail.com

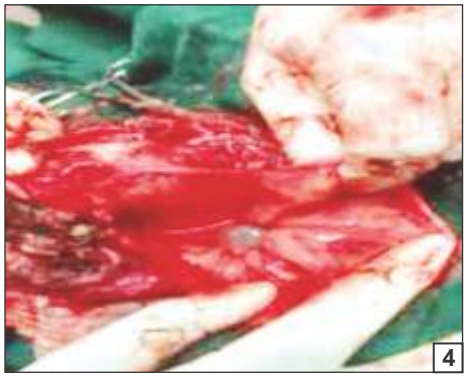



Fig. 1. (i) Ventral region-Udder, (ii) Protruded bone

Fig. 2. Irregular mass of fetal bones in the uterine horn

Fig. 3. Macerated fetus relived after C-Section

Fig. 4. Adhesion of the uterus removed bluntly and sutured

Fig. 5. Uneventful recovery of doe after C-section

@0.5 mg/kg b.wt, chloropheneramine @ 0.5 mg/kg b.wt for 7 days. Animal had an uneventful recovery.

Ventral hernia of gravid uterus is rare in case of goats. Extreme abdominal distension due to pregnancy, weakness of the abdominal muscles in advanced with age and accidental trauma are the predisposing factors for development of herniation of uterus (Singh *et al.*, 2022). Hernia is not considered as emergency in ewes as they may give birth spontaneously but in delayed cases it may leads to loss of muscle mobility, adhesion between other visceral organs or subcutaneous tissue (Peker *et al.*, 2019). Hysterocele condition should be addressed immediately to save the life of dam as well as fetues and further herniorhaphy is useful in case of large hernial opening (Vijayanand *et al.*, 2012). In advanced pregnancy caesarean section is considered as the best treatment for ventral hysterocele. In the present case, goat met with automobile accident during late gestation which might be the reason for hysterocele and progressive to fetal maceration due to death of the fetus.

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Clinical Article

ENDOSCOPIC RETRIEVAL OF THORACIC OESOPHAGEAL FOREIGN BODY IN A 4-MONTH-OLD TOM CAT

T. SAI KUMAR, K.S. KAMALESH KUMAR, ROHIT KUMAR*, MAHESH KUMAR, BHANU PRATAP SINGH, SHIVANSH MEHRA and NAGELLA NAVEEN[1]

Division of Surgery, [1]Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar (IVRI), Bareilly-243122 (U.P.), India

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SUMMARY

A tom cat was presented to Referral Veterinary Polyclinic (RVP), ICAR-IVRI, Izatnagar, after being treated for clinical signs of inappetence, retching, and abdominal discomfort for 3 days with no improvement in condition. Clinical examination and palpation revealed discomfort in the oesophageal and abdominal regions. A survey radiographic examination of chest and abdomen revealed the presence of a tiny, radiopaque foreign body at the level of the base of the heart in the thoracic oesophagus. Endoscopic examination confirmed it as a ball-point pen. Subsequently, the pen was carefully retrieved using a Doyen clamp, avoiding damage to surrounding tissues. Post-operatively, the cat recovered uneventfully. The use of endoscopy has proved to be highly effective for retrieving the foreign body in the cat.

Keywords: Endoscopy, Foreign body, Oesophagoscopy, Oesophagus

How to cite: Kumar, T.S., Kamalesh Kumar, K.S., Kumar, R., Kumar, M., Singh, B.P., Mehra, S. and Naveen, N. (2026). Endoscopic retrieval of thoracic oesophageal foreign body in a 4-month-old Tom cat. *Haryana Veterinarian.* **65(1)**: 133-134.

In veterinary practice, encountering stomach and esophageal foreign bodies (GFBs and OFBs, respectively) is quite common (Deroy *et al.*, 2015). These foreign bodies can vary widely, but the most frequently identified objects include needles, fish bones, and small toys, though various other types have also been detected (Hayes, 2009). To manage these cases, endoscopic retrieval is frequently employed as a minimally invasive technique to retrieve esophageal and gastrointestinal foreign bodies (Deroy *et al.*, 2015). The success rates for endoscopic removal are notably high, ranging from 78-94% for gastric foreign bodies and 68-88% for esophageal foreign bodies, illustrating its efficacy and reliability (Wood and Gallagher, 2021). This report discusses the diagnosis and management of the endoscopic removal of an esophageal foreign body in a non-descript tom cat, highlighting the practical application and benefits of this technique in a real-world scenario.

A 4-month old male non-descript kitten, weighing 2.2 kg, was presented to the Referral Veterinary Polyclinic (RVP) at ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, U.P., exhibiting clinical signs of inappetence, retching and abdominal discomfort for three days. The owner reported that the cat had been treated for the same condition over the past three days, but there was no noticeable improvement. On clinical examination, the cat displayed signs of dullness, depression, and significant discomfort upon palpation of the esophageal and abdominal regions.

Orthogonal thoracic abdominal radiographs, including lateral and ventro-dorsal views, confirmed the presence of a small, 9 mm radiopaque foreign body at the level of the base of the heart in the thoracic esophagus (Fig. 1). Subsequently, an emergency endoscopy was performed under general anesthesia using a flexible endoscope (Karl Storz Endoskope®) to provide a more precise diagnosis and to visualize and facilitate the removal of the foreign object. The kitten was premedicated with butorphanol (0.2 mg/kg BW I/V) and Diazepam (0.2 mg/kg BW I/V). Anaesthesia was induced and maintained with ketamine (11 mg/kg BW, IV) and diazepam (0.2 mg/kg BW, IV) in 1:1 ratio.

Endoscopic examination revealed mild esophageal inflammation and the presence of a ballpoint pen, with its tip lodged in the mid-thoracic esophagus and the remaining portion extending into the gastric fundus. Skillful endoscopic retrieval under left lateral recumbency, facilitated by the use of a Doyen intestinal clamp, effectively extracted the foreign object from the animal without injuring the surrounding tissue (Fig. 2). The cat recovered well from anesthesia after the procedure (Fig. 3).

Post-procedure of endoscopy, the kitten was administered Inj. Meloxicam @ 0.2 mg/kg BW, Inj. Ceftriaxone @ 15mg/kg BW, Inj. Ondansetron @ 0.2 mg/kg BW and Inj. Pantoprazole @ 1 mg/kg BW for 3 days, respectively. Oral food intake was resumed on the second postoperative day. The cat was reportedly normal at the last follow-up conversation with the owner 7 months later. The animal recovered uneventfully immediately after the procedure without any complications. The cat immediately regained its diet and exhibited normal behavior following the retrieval.

Foreign bodies commonly get lodged in the esophagus at three constricted anatomical sites: the cricopharyngeal sphincter, the base of the heart and the esophageal hiatus in the diaphragm. They can also stuck at the thoracic inlet, where soft tissues may impede further movement

*Corresponding author: drrohits.singh@gmail.com

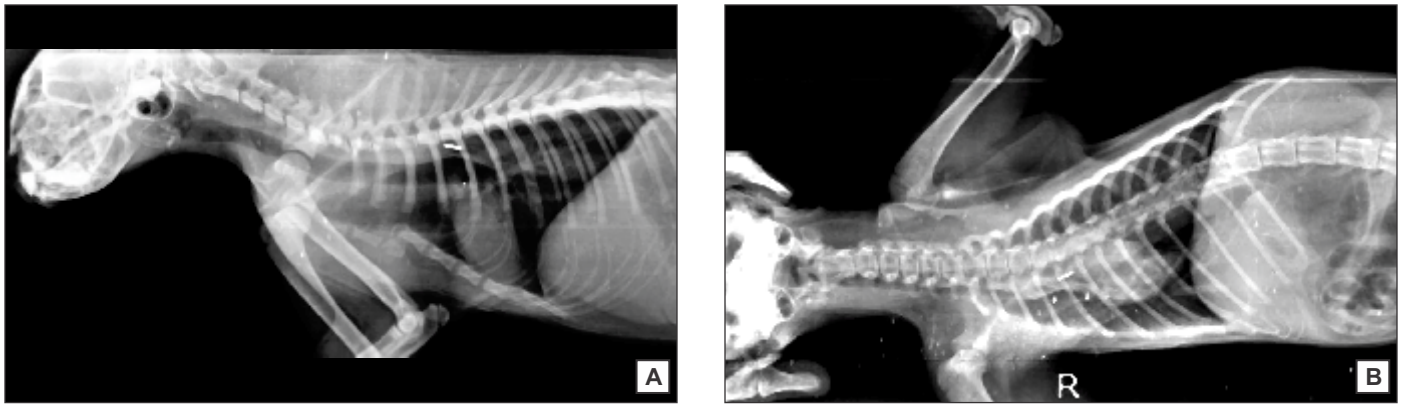

Fig. 1. Thoracic abdominal radiographs, including lateral (A) and ventrodorsal views (B), showing radiopaque foreign body located at the base of the heart in the thoracic esophagus

Fig. 2. Endoscopic retrieval of ball point pen with Doyen clamp

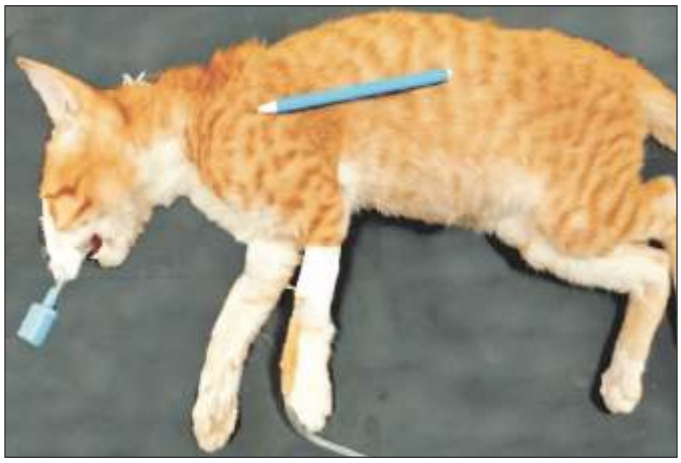

Fig. 3. Cat after endoscopic retrieval of foreign body

(Gualtieri, 2001). Potential complications from esophageal foreign bodies include aspiration pneumonia, strictures, inflammation, and esophageal perforation (Sterman *et al.*, 2018). Radiography provides valuable information about both the location of the foreign body and potential complications (Tams, 2003). Flexible endoscopy has become widely popular for its dual role in diagnosing (Zoran, 2005) and facilitating the extraction of foreign objects. Due to the radiolucent properties of the plastic pen, determining the nature of the foreign body through radiographs was challenging. However, the final diagnosis was made through endoscopy.

The removal of esophageal foreign bodies is classified as an emergency procedure because prolonged retention of an object increases the risk of aspiration and esophageal wall injury due to pressure necrosis (Bebchuk, 2002). In this case, no complications were observed, possibly due to the smooth nature of the pen.

Endoscopic retrieval of esophageal foreign bodies proves to be a highly effective and minimally invasive technique by significantly reducing the need for surgical intervention, thereby minimizing pain, shortening hospitalization time, and lowering the risk of complications. Endoscopic retrieval not only facilitates the safe removal of foreign objects but also allows for the assessment of mucosal damage, providing a comprehensive approach to treatment.

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Clinical Article

SURGICAL AND HISTOPATHOLOGICAL ANALYSIS OF SUBCUTANEOUS FIBROMA IN A PET RAT

MAHESH KUMAR, RAVI DABAS[1], BHANU PRATAP SINGH, T. SAI KUMAR, KIRANJEET SINGH*, ASWATHY GOPINATHAN, UTTAM KUMAR SAHU[2] and KAMALESH KUMAR, K.S.
Division of Surgery, [1]Division of Medicine, [2]Division of Animal Reproduction, ICAR-IVRI, Izatnagar, Bareilly 243122

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SUMMARY

Fibromas are benign tumors of fibrous connective tissue, seldom seen in pet rats. This case report details the diagnosis and surgical management of a fibroma in a 7-month old male Indian white pet rat .The rat presented with a rapidly growing, hard subcutaneous lump on the right abdomen. The tumor was excised under xylazine-ketamine anesthesia using electrocautery. Histological analysis showed a well-defined tumor with spindle-shaped fibroblasts and mature collagen fibers, devoid of aberrant cells or invasive growth. The absence of mitotic activity and cellular pleomorphism confirmed its benign nature. This case highlights the rare occurrence and successful surgical management of a subcutaneous fibroma in a rat.

Keywords: Fibroma, Histopathology, Indian white pet rat, Tumor

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Fibroma is a rare benign neoplasm composed of mature fibrous connective tissue whichcan be congenital, developmental or acquired in origin. Typically, the tumors are made up of interlacing bundles of uniform, fusiform spindle-shaped cells that produce varying amounts of collagen (Mohr, 1992). In rats, a fibroma of desmoid type (also known as fibromatosis-type) has been described as postoperative lesion in Bhd gene mutant rats (Kouchi *et al.*, 2008). This form of fibroma is occasionally seen in conventional strains as well. Fibromas have also been reported to develop in Wistar rats at the site of repeated injection of low doses of iron dextran solution (Roe and Carter, 1967). Fibromas are more commonly seen in larger animals and humans (Cullen *et al.*, 2002); however, their occurrence in rats offers valuable insights into the tumors pathology, treatment options and prognosis (Mumba *et al.*, 2013). In this study, we describe a case of fibroma with a distinctly fluctuating appearance, found in the subcutis near the right side of abdomen of an Indian white rat.

A 7-month old male Indian white pet rat was presented with a noticeable abdominal mass. The mass had been growing progressively over several weeks, leading to discomfort and reduced activity in the rat. Upon clinical examination, the mass was firm, irregular and fixed to the underlying tissues (Fig. 1A). Upon physical examination rat exhibited signs of discomfort and lethargy, with a palpable abdominal mass. On ventro-dorsal radiograph there was no evidence of metastasis. The mass was not attached to the abdomen (Fig. 1B).

The animal was anesthetized with a combination of inj. Xylazine (5 mg/kg body weight I/V, Xylaxin, Indian Pharmaceuticals Limited, Hyderabad, Telangana, India) and inj. Ketamine (50 mg/kg body weight I/V, AESKENT, Aespire Formulations Pvt. Ltd., New Delhi, India) administered intramuscularly with a ten-minute interval between injections (Amarpal *et al.*, 2010). The surgical excision of the tumor was performed using electrocautery (Fig. 1C). Postoperatively, the animal received the antibiotic inj. Ceftriaxone (Intacef, Intas Pharmaceuticals Ltd., Ahmedabad, Gujarat, India) at a dose of 20 mg/kg body weight, administered intramuscularly for 5 days. An analgesic, inj. Meloxicam (Melonex, Intas Pharmaceuticals Limited, Gujarat, India), was given at a dose of 0.3 mg/kg body weight via the subcutaneous route for 3 days. Antiseptic dressing with a 1:10 dilution of povidone-iodine was applied on alternate days. The patient was closely monitored for any signs of complications and follow-up assessments were conducted regularly to evaluate the surgical site and ensure proper healing. Follow up assessment was done upto one month and proper healing or recovery of the rat was achieved during this period.

The histopathological findings revealed a dense, poorly cellular fibrous mass that compresses adjacent tissues with Haemotoxyline and eosin (H & E) staining. It consisted of interwoven bands of mature collagen and interlacing bundles of well-differentiated fibroblasts. The fibroblasts were fusiform with elongated, tapered, hyperchromatic or vesicular nuclei and one or more nucleoli. Rare mitotic figures were observed; in some areas, pale blue myxomatous regions containing stellate cells were also present. The presence of well-differentiated fibroblasts and collagen bundles showed infiltration of surrounding musculature, but without cytological features indicative of malignancy.

A fibroma features a dense, sparsely cellular fibrous mass composed of well-differentiated fibroblasts that compresses surrounding tissue. It displays interwoven bands of mature collagen, scarce mitotic figures and minimal to no leukocyte infiltration (Greaves *et al.*, 2013). It can also originate from visceral organs, including the ovary, uterus, stomach and intestine (Sundberg and Nielsen, 1980). Cutaneous fibromas are common in dogs, while they are uncommon in other animals (Goldschmidt and Hendrick, 2002). The tumors aggressiveness necessitates prompt surgical intervention. Histological examination remains the

*Corresponding author: ksuppli@yahoo.co.in

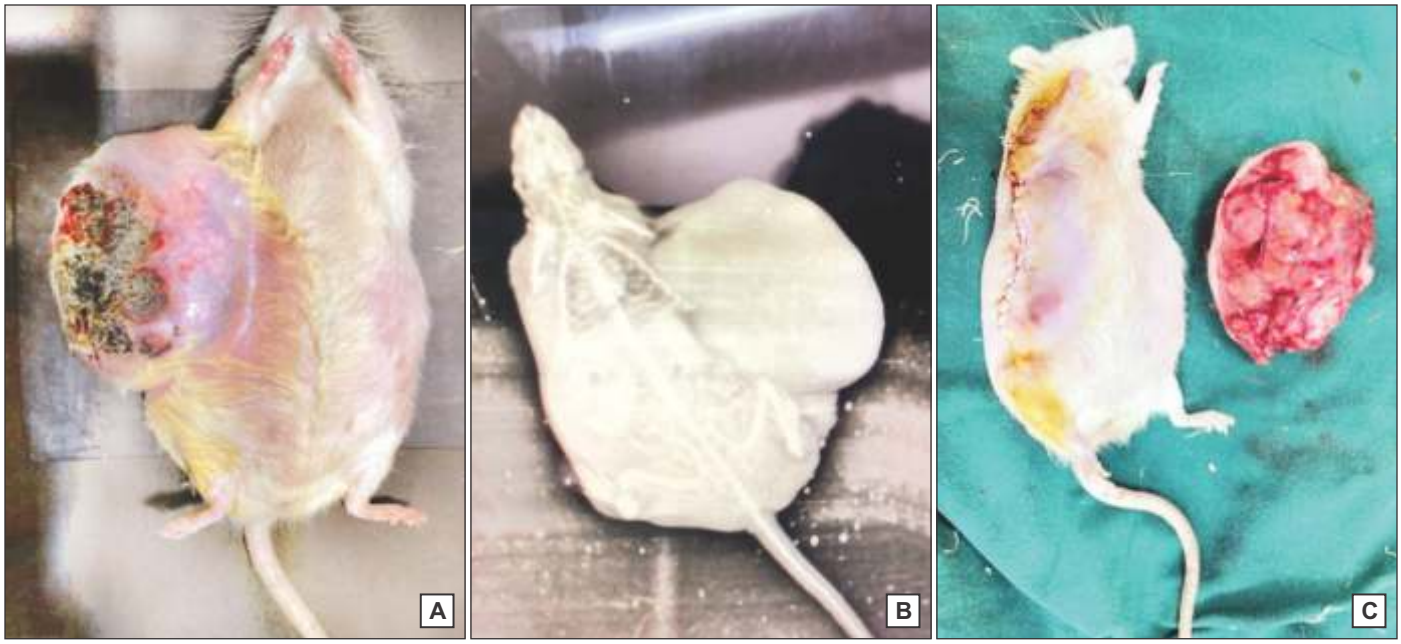

Fig. 1. Tumorous growth attached onventro-lateral abdominal wall (A),ventro-dorsal radiograph taken to rule out metastasis (B) and image taken post-surgery of the rat (C)

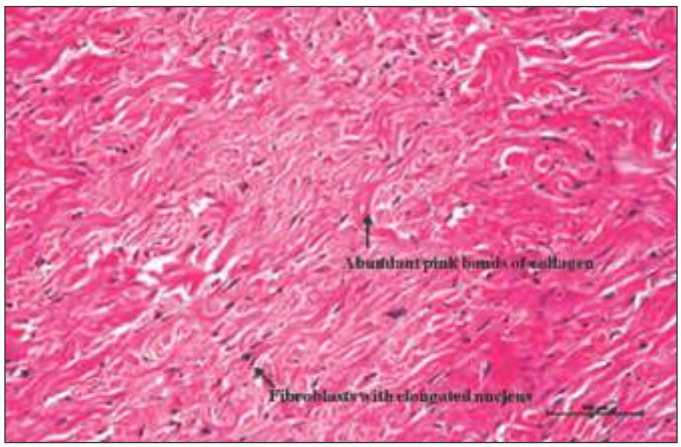

Fig. 2. Fibroma showed well differentiated fibroblasts with elongated nucleus, abundant pink bands of collagen in interwoven patterns. Overall, a poor cellular collagen rich fibroma is evident (H & E 40x).

gold standard for confirming the diagnosis and providing information on the tumors grade and potential behavior. In this case, the surgical approach was deemed effective and the tumor was removed with adequate margins. The histological findings are consistent with a fibroma due to the uniformity of cellular and nuclear appearance, absence of mitotic figures, organized growth pattern and previous descriptions of well-defined spindle-shaped cells and collagen fibers (Sastry and Rao, 2002). Blood vessels were congested, mitotic figures were absent and collagen was found in a bluish mucinous stroma, consistent with features of fibroma and fibromyxoma (Sastry and Rao, 2002). No recurrence or metastasis was reported for the three fibromas and two fibrosarcoma for which post-surgical clinical information was available (Parker and Picut, 1993).

CONCLUSION

Early detection, followed by prompt surgical intervention, is crucial for managing fibroma in pet rats. Histopathological analysis remains essential for accurate diagnosis and prognosis of a case.

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Clinical Article

SUCCESSFUL THERAPEUTIC MANAGEMENT OF CONGENITAL BILATERAL GOITRE IN A FEMALE CALF

TARINEE RANJAN PRADHAN[1],VINITA RAJPUROHIT[1], SIRAJ ANSARI[1]* and HİMANSHU BEHERA[2]
[1]Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India
[2]Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, India

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SUMMARY

Congenital goitre is a metabolic disorder in neonates, primarily caused by iodine deficiency during pregnancy, leading to thyroid hormone insufficiency. This case reports an 18-day-old female calf presenting with progressive neck swelling, inspiratory dyspnoea and signs of hypothyroidism such as weakness, unthriftiness and poor suckling reflex. The dam grazed on a mixed pasture without mineral supplementation was likely iodine deficient. Clinical examination revealed bilateral thyroid gland enlargement. Diagnostic tests including serum free T4 levels, radiography and ultrasonography confirmed congenital goitre and thyroid gland enlargement which was compressing the trachea. Haematological analysis revealed no major abnormalities. The calf was treated with Levothyroxine sodium 0.01- 0.02 mg/kg body weight orally once daily for first month of treatment and supportive therapy with prednisolone acetate@ 0.5-1 mg/kg intramuscularly for 5 days every 24 hrs and iodine supplementation through iodized salt and tincture iodine for the calf and dam. Significant clinical improvement was observed within 30 days with reduced thyroid gland size, normalization of serum free T4 levels and restored suckling reflex. This case highlights the importance of iodine in prenatal nutrition, early diagnosis and treatment with thyroid hormone replacement therapy can effectively manage congenital goitre preventing long-term morbidity in affected animals.

Keywords: Calf, Congenital goitre, Iodine deficiency, Levothyroxine

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Thyroid hormones are general metabolic hormones that neonates require to develop their immunological competence and perform other homeostatic functions. The thyroid hormones triiodothyronine (T3) and thyroxine (T4) control calorigenesis, basal metabolic rate (BMR) and the metabolism of fats and carbohydrates (Capen and Martin, 2003). Additionally, they impact the animal's immunological, gastrointestinal, reproductive, cardiovascular, neuromuscular, haematological and endocrine systems and they may serve as markers of nutritional and metabolic health in animal (Riviere and Papich, 2017). The major causes responsible for thyroid gland enlargement include iodine deficient diets-primary goitre; goitrogenic substances that disrupt thyroxinogenesis-such as brassica plants, soybean byproducts, and water with high calcium and nitrate content - are the main causes of thyroid gland enlargement. Cruciferous vegetables, such as cabbage, kale, cauliflower, broccoli, turnips and rapeseed, contain glucosinolates, whose metabolites compete with iodine for thyroidal uptake (Zimmermann, 2020; Radostits *et al.*, 2017). In rare instances, secondary goitre may result from an excess of dietary iodine and genetically determined hereditary enzyme abnormalities that affect the synthesis of thyroidal hormones (Agrawal *et al.*, 2015). Congenital goitre is a fatal thyroid metabolic disease marked by low thyroid hormone levels, excessive secretion of thyroid-stimulating hormone (TSH) from pituitary gland and compensatory thyroid gland enlargement (Nourani and Sadr, 2023). It is frequently seen in newborn animals to dams on iodine deficient diet, in-utero deficiency of iodine, ingestion of goitrogenic plants and hereditary (Ankita *et al.*, 2023). Iodine deficient diet is reported to be the most common cause of congenital diffuse hyperplastic goitre in calves (Homerosky *et al.*, 2019). Thyroid gland enlargement results in tracheal compression, dyspnoea and cardiovascular abnormalities leading to severe economic losses than in adults (Singh and Beigh, 2013). The inotropic and chronotropic effects of the thyroxine could be responsible for the major cardiovascular abnormalities (Naveen *et al.*, 2024). Calves are commonly weak necessitating assistance to stand and nurse for several days after birthor are stillborn (Seimiya *et al.*, 1991). Retardation of foetal development and dead or weak neonates with goitre are adverse effects of iodine deficiency during pregnancy (Zimmermann, 2020). The condition is described as 'neonatal animal hypothyroidism' with colloidal goitre, alopecia, unthriftiness, thyroid thrill and a high rate of mortality, associated with decreased serum T3, T4 levels and increased TSH activity (Hassan *et al.*, 2013; Singh *et al.*, 2003). Ramakrishna *et al.* (1992) reported that goitre as an emerging mineral disorder in animal grazed on iodine deficient endemic areas. The clinical survey in 13 districts of Uttar Pradesh and Uttarakhand revealed 58.23%, prevalence of goitre in goats. The prevalence was highest in Bareilly (69.04%)

*Corresponding author: sirajansari1998@yahoo.com

Fig. 1. Broad based stance and dyspnoeic calf

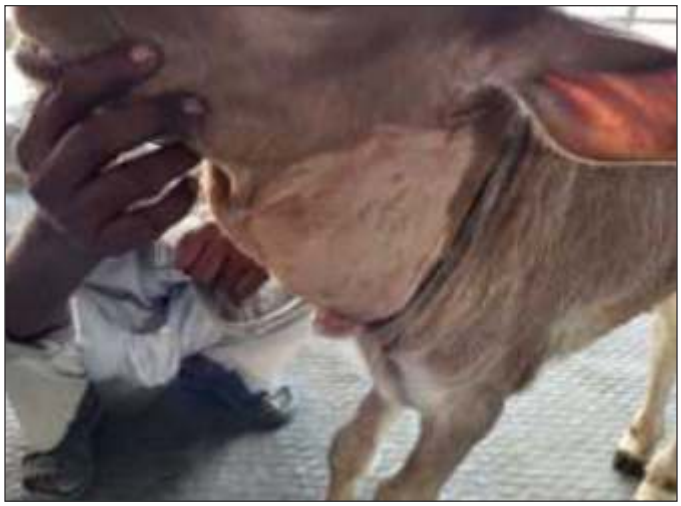

Fig. 2. Enlarged thyroid glands on presentation

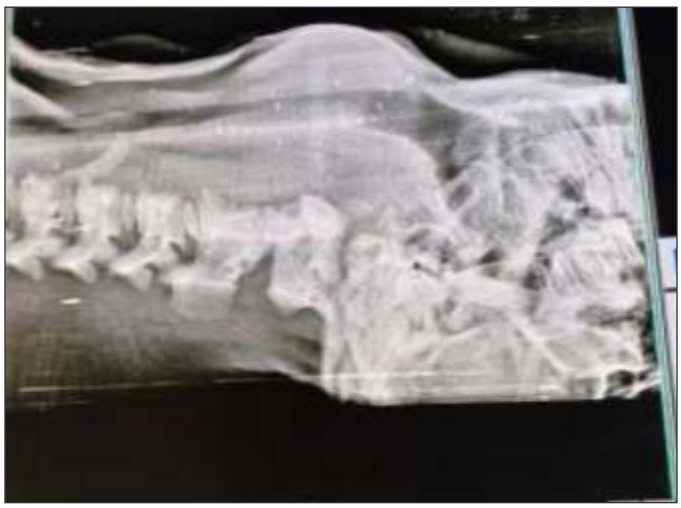

Fig. 3. Radiography of cervical region with swelling ventral to trachea

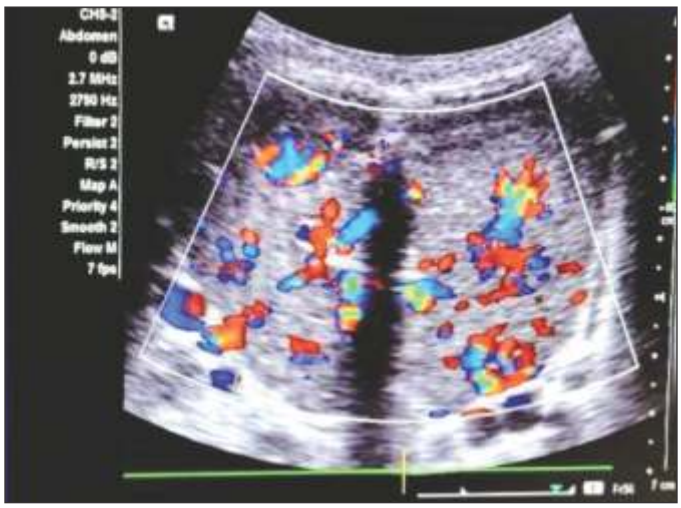

Fig. 4. Profuse central and peripheral circulation in thyroid gland

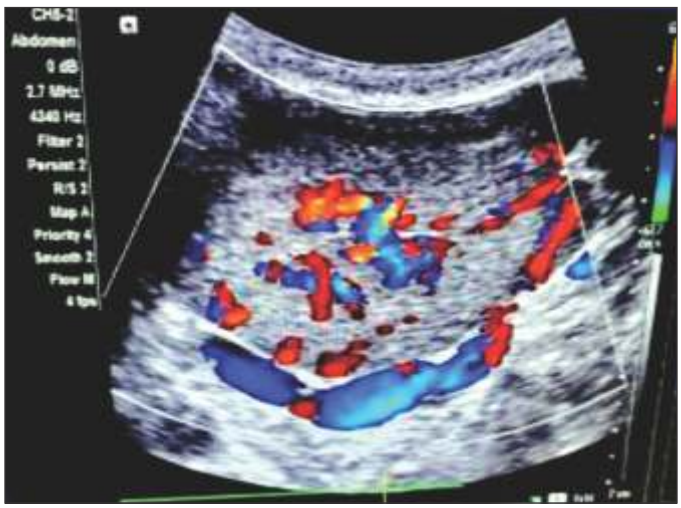

Fig. 5. Complete recovery after thyroxine medication

Fig. 6. USG revealed bilobed thyroid gland with isthmus

can be designated as goitre centre (Kumar *et al.*, 2011). The aim of the present report is to describe therapeutic management of congenital goitre in animals on endemic iodine deficient area.

A non-descript female calf weighing 32 kg of aged 18 days was brought to the Referral Veterinary Polyclinic, ICAR-Indian Veterinary Research Institute, Bareilly with progressive enlargement of throat swelling from birth,

Parameter	Day 0	Day 30	Reference Range
Haemoglobin (g/dl)	12.6	10.8	11.3 ± 1.02
PCV (%)	41.5	38.7	35 ± 3
RBC count (millions/mm^3)	8.5	8.3	8.86 ± 0.68
Total WBC count (10^3 cells/mm^3)	9.3	8.8	4.9-12
Neutrophil (%)	36	34	18–63
Lymphocyte (%)	55	57	16–56
Eosinophil (%)	4	5	0-9
Monocytes (%)	3	4	0-8
Basophils (%)	2	2	0-1
Platelet count (lakhs/mm^3)	4.7	4.6	2-6.5
Free T_4 (ng/dl)	0.09	3.1	3.3

"Table" (Haematology and serum free T4 level)
Reference Ranges are from Constable, P.D., Hinchcliff, K.,W., Done, S.H. and Grünberg, W. Veterinary Medicine: A Textbook of The Diseases of Cattle, Horses, Sheep, Pigs and Goats, Eleventh Edition, 2017.

inspiratory dyspnoea since last 5days with abdominal respiration and shallow breathing with discomfort and extension of neck, sunken eyeball and dull haircoat. Dam of affected calf appeared clinically normal and in adequate body condition. A full hair coat and expected calving date indicated the calf was likely term birth but failed to respond to the suckle reflex test. Calf seemed to have difficulty in raising the feet and the limbs were spread out so as to form a broad base of support (Fig. 1).

A feeding history was obtained from the farmer regarding Dam which grazed on mixed pasture and no free choice of loose mineral was offered. On clinical examination rectal temperature was found 103.2° F, heart rate was 50 beats per minute, respiratory rate was 66 per minute, and mucous membrane was pinkish. Animal had no history of deworming and no abnormality was detected in Defecation and Urination. There was bilateral swelling in antero-ventral region of neck below trachea which fluctuates on palpation and no pain or pits on pressure was evident. The size of thyroid gland measured as 7.6 cm × 5.3 cm × 2.5 cm (Length × Width × Height) (Fig. 2).

For investigation blood sample is taken aseptically from jugular vein and collected in EDTA vial and Clot activator vial for haematological analysis and serum free T4 test. There was no significant changes in haematology of calf. Serum free T4 level was found to be reduced from reference range to a level of 0.09 ng/dl. A lateral cervical radiograph was taken and mass of 6-7 cm that was compressing the lumen of trachea was found (Fig. 3). Ultrasonographic examination of the affected area revealed homogenous echotexture in ventral neck region ventral to carotid artery and jugular vein. There was profuse peripheral and central circulation. Both lobes were evident with presence of a stalk/isthmus between both lobes (Figs. 4 and 5).

Based on the history, clinical signs, laboratory diagnosis, the current case was diagnosed as Congenital Goitre. Calf was treated with levothyroxine sodium tablets at a dose of 0.01-0.02 mg/kg body weightorally for first month of treatment and prednisolone acetate at a dose of 0.5-1 mg/kg intramuscularly for 5 days every 24 hrs as anti-inflammatory to reduce respiratory distress. The owner was also instructed to paint cow's teat with tincture iodine and also paint throat of affected calf regularly. Iodised salt was provided orally to calf. Treatment continued for one month and marked improvement was noticed with visible decrease in size of glands and positive suckle reflex with normal appetite (Fig. 6).

Serum free T4 level was measured after one month and found to be 3.1 ng/dl which is within normal reference range. We advised owner to add potassium iodate to the feed if the dams are fed cabbage when they are pregnant. Levothyroxine sodium (Commonly used in human medicine) was found effective for treating goitre in kid with high T3 and low T4 levels (Ozmen *et al.*, 2005). Significant clinical response was observed after 15 days in tentatively diagnosed calf with goitre when treated with thyroxine with complete recovery in 20-25 days. (Kashyap *et al.*, 2015). The major pathogenic mechanisms responsible for development of thyroid hyperplasia include iodine deficient diets (Paulíková *et al.*, 2002). An iodine-deficient diet resulting in decreased thyroxine and subsequent secretion of excess thyroid-stimulating hormone (TSH) from the pituitary is the most common cause of congenital diffuse hyperplastic goitre in calves (Homerosky *et al.*, 2019; Radostits *et al.*, 2017). Dietary iodine deficiency is suspected in the current case due to the lack of mineral supplementation throughout mid to late gestation. Mountainous regions, low-height regions far from the sea, and areas with low soil iodine levels can result in iodine deficiency (Nyström *et al.*, 2016).

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THE HARYANA VETERINARIAN

Editors/Editorial Board Members are highly thankful to all the distinguished referees who helped us in the evaluation of articles. We request them to continue to extend their co-operation and be prompt in future to give their valuable comments on the articles for timely publication of the journal.

Haryana Veterinarian (June, 2026) **65(1),** 141-143 ISSN 0033-4359 (P)

Clinical Article

INCIDENCE OF A FETAL CYCLOPS MONSTER WITH PROGNATHISM AND PEROSOMUS HORRIDUS IN A NON-DESCRIPT STILLBORN KID

J.V. SUBASH CHANDRA BOSE, S. PRAKASH, S. RAJA, K. RAGUL, V. UMANATH and N. ARUNMOZHI1*
Department of Veterinary Gynaecology and Obstetrics,
Veterinary College and Research Institute, Orathanadu, Thanjavur-614 625
[1]Department of Clinics, Madras Veterinary College, TANUVAS, Chennai-600 007

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SUMMARY

A two years old, full term pregnant, primiparous non-descriptive doe was presented with the history of colic like symptoms and difficulty in parturition. On vaginal examination twisting or rotation of the vaginal walls toward the right side was observed. Based on the obstetrical examination, the case was diagnosed as dystocia due to right side post cervical uterine torsion and the fetal monster was delivered by performing caesarean section under inverted L block and the dam had an uneventful recovery.

Keywords: Doe, dystocia, fetal monster, Perosomus horridus, Prognathism

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Congenital malformations are physical or functional defects that develop during fetal growth and are present at the time of birth that may affect individual organs, entire systems, or multiple systems at once. In some cases, these defects can result in dystocia during parturition (Singh *et al.*, 2013; Kavitha *et al.*, 2018). Cyclops is characterized by severe form of holoprosencephaly. Some environmental teratogenic agents that cause developmental abnormalities during the early stages of fetal development (organogenesis) are considered potential causes of holoprosencephaly. These factors may interfere with the normal division of the embryonic forebrain (prosencephalon), leading to severe congenital malformations like cyclopia, where the brain fails to divide into two hemispheres, resulting in facial abnormalities such as a cyclops (Rashed *et al.*, 2014). Cyclops has been previously reported in goats (Rashed *et al.*, 2014; John *et al.*, 2020,) cattle (Nourani *et al.*, 2014), buffalo (Patel *et al.*, 2019), pigs (Karthikeyan *et al.*, 2011) and mare (Singh *et al.*, 2012). The current report emphasizes a rarely observed condition or occurrence of a fetal cyclops monster with prognathism and Perosomus horridus in a still born kid.

A two years old full term pregnant nulliparous non-descript doe was presented to teaching hospital with the history of straining and difficulty in parturition. On visual observation discharge from vagina was noticed and the cranial stretching of the vulva towards dorsal commissure on its right side was evident. On vaginal examination, cervix was not palpable. On ultrasonographic examination c shaped placentomes and foetal parts could be visualised but foetal heart beat could not be visualised. The case was determined to be dystocia resulting from a right-sided post cervical uterine torsion with a non-viable foetus based on clinical and diagnostic evaluations.

Modified Schaffer's method of rotation was adopted on the right side with an effort to relieve the torsion. After two rotations the torsion was not corrected and hence caesarean section was opted. Caesarean section was performed (Fig. 1) under epidural and inverted L block by administering 2% lignocaine and a dead female cyclops monster was relieved. Following caesarean section, adhesion of the uterine horns with intestinal loops was also noticed. The incisions on the uterus was sutured using a Cushing pattern followed by lembert using PGA no. 1 and the muscle was closed by ford interlocking with PGA no. 2 and the skin was sutured using cross mattress with silk. Following Caesarean section, the doe was treated with Inj. Enrofloxacin 5 mg/kg BW, Inj. Meloxicam 0.5 mg/kg BW, Inj.Chlorpheniramine maleate 0.5 mg/kg BW intramuscularly, Inj.Ringer's Lactate 10 ml/kg BW Intravenously and ecbolic suspension Involon 25 ml BID orally. The same treatment was continued for five days, and the dam showed steady improvement with no adverse events.

A comprehensive evaluation of the malformed fetus revealed single eyeball in its orbit, absence of eyelids, rudimentary nose, smaller sized skull, prognathic mandible which clearly indicated Cyclops (Fig. 2). On further examination it revealed shorter spine, "S" shaped curvature in vertebral column, ankylosis of limbs and generalized muscle contractures (Fig. 3). Radiographic evaluation identified prognathism along with an S-shaped spinal deformity, suggestive of Perosomus horridus fetal monster (Fig. 4). Thus, two monstrosities were observed in

*Corresponding author: drarunmozhivet@gmail.com

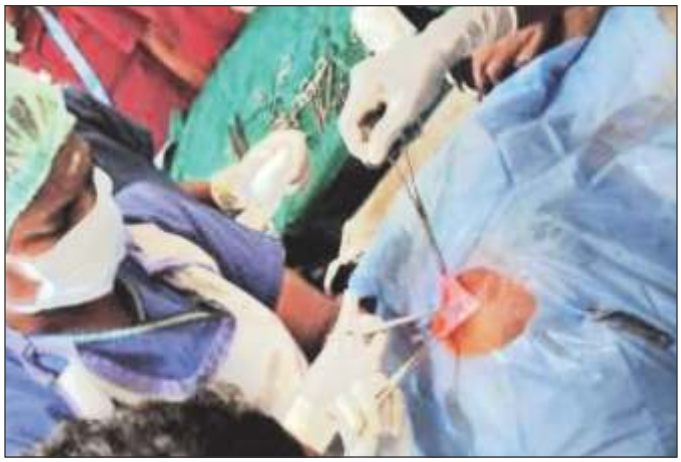

Fig. 1. Caesarean section performed under epidural anaesthesia 2% lignocaine and inverted L block to relieve the fetus.

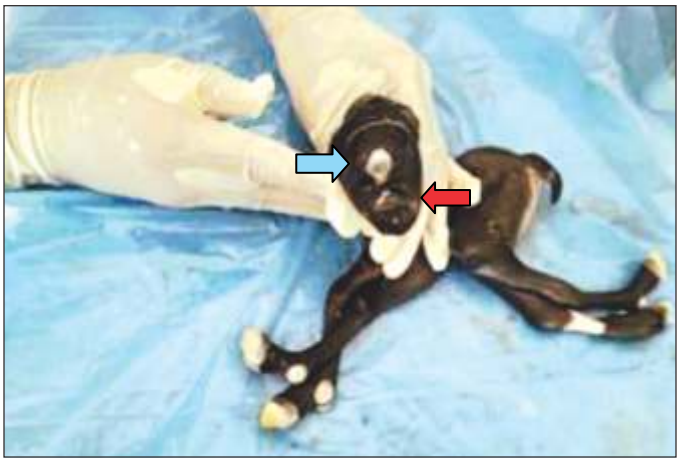

Fig. 2. Cyclops fetal monster with single eyeball in its orbit with absence of eyelids (blue arrow) and prognathic mandible (red arrow).

a single fetus. Cyclopia, also termed cebocephalus, is an uncommon congenital disorder defined by the abnormal development of facial structures, typically resulting in a single central orbital cavity, which may contain a single, seemingly normal eye or underdeveloped or absent eyes. The eyelids are often rudimentary or missing, and the nose is typically absent or appears as a tubular appendage located above the centrally positioned eye. This underdeveloped nose does not connect to the pharynx. The skull is generally smaller in size, with the lower jaw being longer than the malformed upper jaw (Dutt *et al.*, 2018). Similar findings of absence of eyelids, rudimentary nose and prognathic mandible was observed in the present case. Partial or complete absence of the anterior cerebral structures, corpus callosum, cranial bones, and cranial nerves, indicating severe developmental abnormalities is observed (Noakes *et al.*, 2019) and the current case also

Fig. 3. Lateral view of Cyclops foetal monster with *Perosomus horridus* (red arrow) indicating "S" shaped curvature in spine and ankylosis in the hind limb (yellow arrow).

had small sized skull and skull abnormality as revealed in the radiographic picture (Fig. 4).

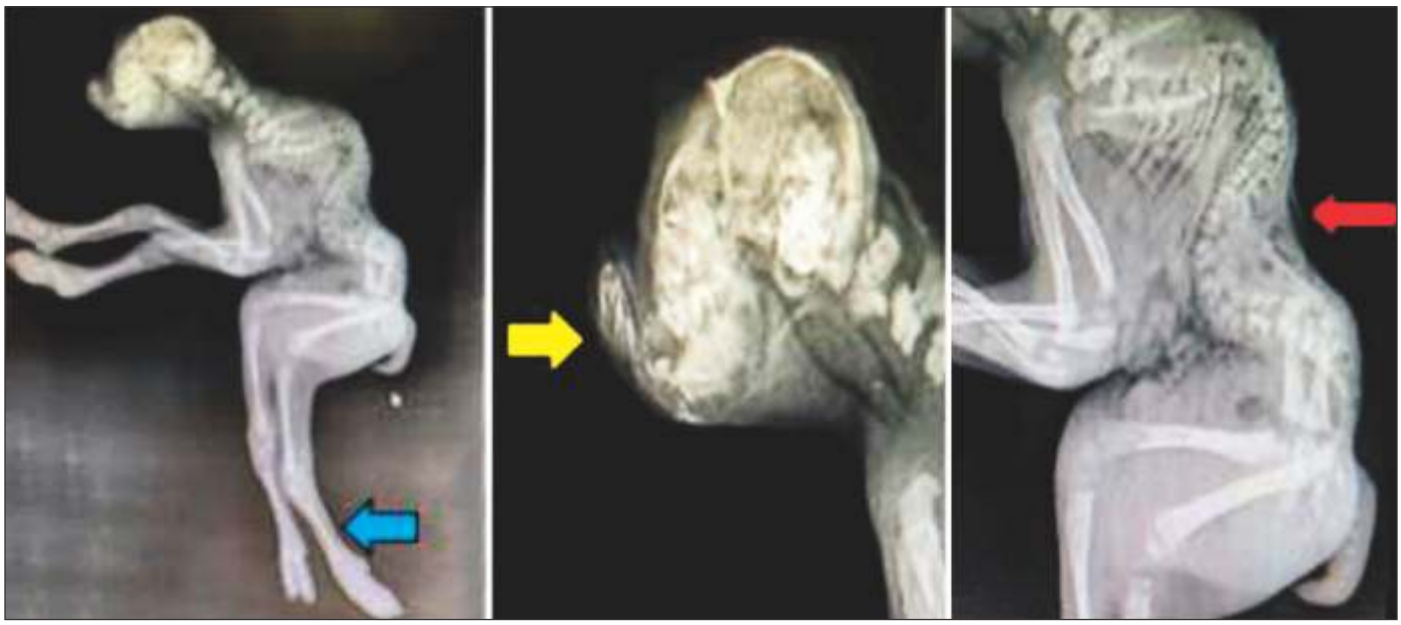

Fig. 4. Radiography revealing ankyloses (blue arrow), prognathic mandible (yellow arrow), S" shaped curvature of the vertebral column (red arrow).

Various teratogenic environmental factors during organogenesis are believed to contribute to the development of holoprosencephaly. In newborn lambs, cyclopic malformations have been observed when pregnant ewes consume Veratrum californicum between the 8th and 17th days of gestation, with the 14th day being the most critical period. This plant contains three primary steroidal alkaloids cyclopamine, cycloposine and jervine -that can interfere with neural development in lambs. Several other factors such as hypovitaminosis, radiation may also cause this defect (Rashed *et al.*, 2014).

Perosomus horridus is marked by extensive joint stiffness (ankylosis) and severe muscle tightening, causing multiple sideways and downward curvature of the spine from the back of the head (occiput) to the tailbone (sacrum), forming a characteristic "S"-shaped deformity. The affected fetus has clearly abnormal vertebrae that are shortened and fused together, along with malformed limbs, neck and tail (Roberts, 1986). The incidence of *Perosomus horridus* was documented in cow (Dutt *et al.*, 2018) and buffalo (Napolean *et al.*, 2008; Sarath *et al.*, 2022).

Developmental abnormalities in the ovum, sperm, zygote, embryo, or fetus can lead to structural malformations in the fetus, commonly known as monstrosities (Singh *et al.*, 2011). These conditions can cause significant economic losses for livestock owners. Therefore, there is a growing need for the development and implementation of advanced diagnostic techniques for the early detection of fetal abnormalities during pregnancy (Singh *et al.*, 2020). Foetal abnormalities or monstrosities are observed in less than 1% of all congenital defects in bovine and caprine species. Perosomus horridus monster can sometimes result in dystocia due to misalignment of the extremities (Sarath *et al.*, 2022). Since adhesions of the uterus with intestinal loops were noticed which might have hindered the correction of torsion when modified Schaffer's method of rotation was opted. Caesarean section is the preferred and safer method in cases of fetal malformations to ensure the survival of the dam and to retain future fertility of the dam.

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Clinical Article

SUCCESSFUL SURGICAL MANAGEMENT OF SPLENIC HEMANGIOMA BY TOTAL SPLENECTOMY IN A ROTTWEILER

S. KOKILA, A. KUMARESAN, S. KATHIRVEL, K. GOPAL, M. SUMATHI, M. MANIKANDAN and S. DHARMACEELAN*
Department of Veterinary Surgery and Radiology,
Veterinary College and Research Institute, Namakkal-637 002 (Tamil Nadu)

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SUMMARY

A nine year old male Rottweiler was presented to the Veterinary Clinical Complex, Namakkal with the history of intermittent fever since past one week. Clinical examination revealed hyperthermia. Upon abdominal palpation, mass was palpated in mid ventral portion of abdomen. Ultrasonographic examination of abdomen revealed mixed echogenicity at the mid splenic area. The condition was tentatively diagnosed as space occupying mass in the spleen and decided to intervene surgically by total splenectomy. Animal was prepared for surgery after initial stabilization with appropriate fluid therapy and antibiotics. Under general anaesthesia, total splenectomy was performed. Splenic mass was submitted for histopathological examination which revealed cavernous hemangioma. The dog recovered uneventfully, highlighting the significance of timely surgical intervention in managing hemangioma of spleen.

Keywords: Dog, Hemangioma, Splenectomy

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Spleen is a secondary lymphoid organ that can be affected by both neoplastic and non-neoplastic nodules. Splenic neoplasia can arise from any of the splenic tissues viz., vascular, lymphoid tissue, smooth muscle, connective tissues, myeloid and adipose tissue. Splenic tumors may be benign (hemangioma) or malignant (hemangiosarcoma) (Bettini *et al.*, 2001). Splenic tumors were commonly reported in old and large breed dogs viz., Labradors, German shepherd, Golden retrievers, Boxers etc. (Ziogaite *et al.*, 2024). Clinical signs are typically vague, non-specific and include enlarged abdomen, anorexia, weight loss, lethargy, depression, vomition and diarrohea.

Several splenic lesions like hematoma, hemangioma and hemangiosarcoma have similar ultrasound and gross appearance. Prognosis for splenic masses cannot be determined without histology which necessarily requires surgery. Ultrasound guided FNAC is not usually recommended for splenic tumours as the mass is highly friable and there is increased risk of haemorrhage. Splenectomy is the treatment of choice for splenic tumors when there is no evidence of metastasis.

A nine year old male Rottweiler weighing around 52 kg was presented to Veterinary Clinical Complex with the history of intermittent fever for the past one week. The animal looked dull and depressed, temp. 40.5° C with congested conjunctival mucus membrane. Abdominal examination revealed non painful palpable mass in the cranial ventral abdomen. Ultrasonographic examination revealed mixed echogenicity at the mid splenic area (Fig. 1). Haematological evaluation revealed leucocytosisand neutrophilia whereas serum biochemical values were within normal physiological range (Table 1).

The case was diagnosed as splenic mass and decided to perform splenectomy. The ventral abdomen was aseptically prepared for surgery. Premedication done using Inj.Dexmeditomidine @ 5 µg/kg i/m and Inj.Diazepam 0.5 mg/kg i/v. General anaesthesia was induced using Inj. Ketamine @ 5 mg/kg i/v and maintained using Isoflurane under variable vapour pressure settings. Preoperative antibiotic Inj.Ceftriaxone @ 10 mg/kg i/v and analgesic Inj. Tramadol @ 2mg/kg i/v was administered.

A midline ventral skin incision was made from the xiphoid, extending caudal to the umbilicus. Spleen was identified and slowly exteriorised out of the abdomen (Fig. 2). Four vessels namely short gastric arteries, dorsal artery, main splenic artery terminating in the mid portion of the spleen and caudal omental branch coursing along the tail of the spleen to the greater omentum were ligated by applying single ligature at the abdominal side of each vessel while a mosquito forceps was applied on the splenic side using 2-0 monfilament absorbable suture leaving a small vessel stump on the abdominal side to prevent suture slippage (Figs. 3 and 4). Total splenectomy was done and the total length of the spleen was found to be 30 cm and the tumour mass at the center measured approximately length -9.2 cm and Width -7.4 cm (Fig. 5). Abdominal muscle was closed using polyglycolic acid 1 in cross mattress pattern, subcutaneous sutures using PGA 1-0 and skin sutured using polyamide 0.

Post-operative fluid therapy (Ringers Lactate and Dextrose normal saline) for 3 days, Inj.Ceftriaxone for 7

*Corresponding author: saranyathangave100@gmail.com

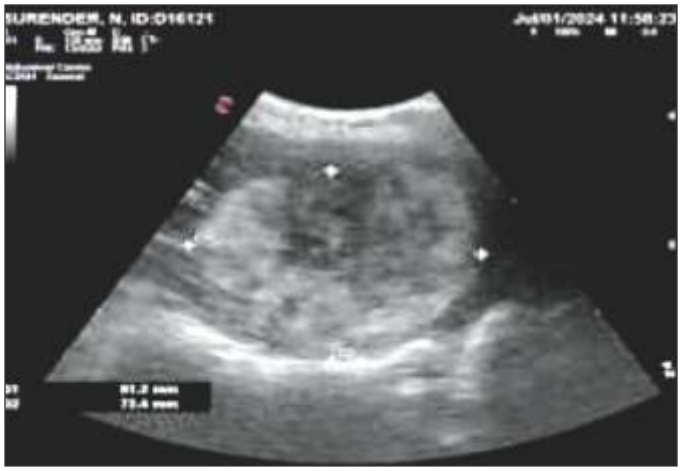

Fig. 1. Mixed echogenicity in spleen

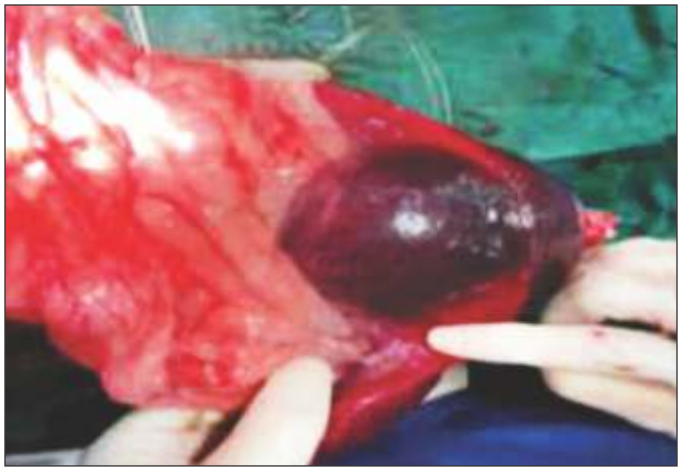

Fig. 2. Exterioration of spleen

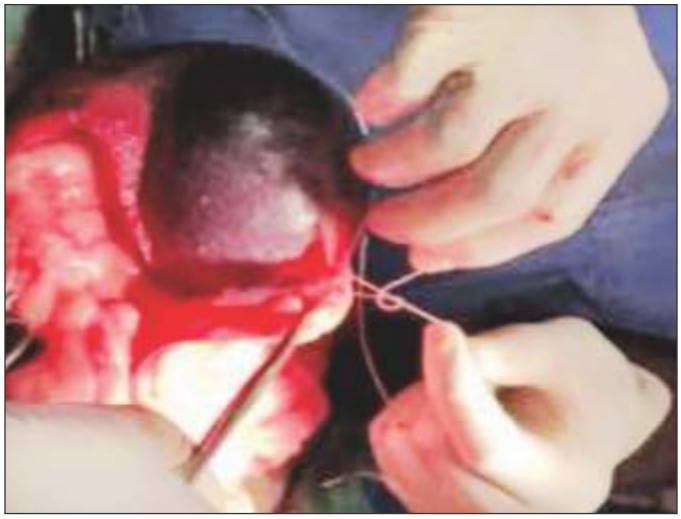

Fig. 3. Ligation of gastro splenic ligament

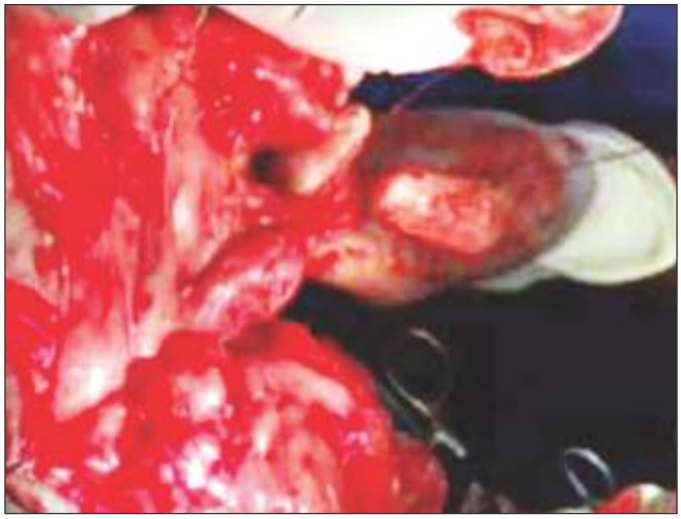

Fig. 4. Ligation of caudal omental branch

Fig. 5. Spleen showing mass at the center

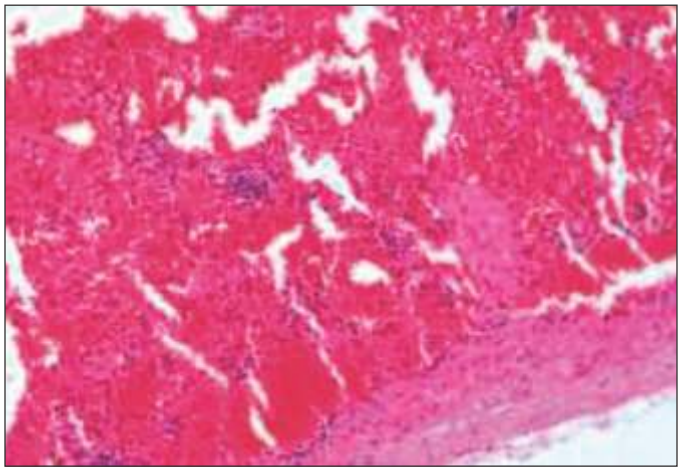

Fig. 6. Spleen-Wide blood filled vascular structures lined by single layer of endothelial cells. H & E x 400

days and Inj.Tramadol for 2 days were followed. Animal started taking liquids on 3rd postoperative day and solid food on 4th post-operative day. Surgical site was dressed on alternate days and sutures were removed on 12th postoperative day. Post operative blood profile is mentioned in table 2.

Histopathological findings revealed multiple vascular channels organized in a lobular pattern, lined by a single layer of flat to plumpy epithelial cells and supported by thin fibrous septa. The channels were filled with erythrocytes (Fig. 6). Based on the microscopic lesion the case was

Table 1. Pre-operative hematobiochemical analysis

Parameters	Value	Parameters	Value
Haemoglobin (g/dl)	12	Total Protein (mg/dl)	7
PCV (%)	36	Albumin (g/dL)	2.6
RBC (10^6/µl)	5.4	ALT (U/L)	112
WBC (10^3/µl)	23.11	SAP (U/L)	261
Neutrophils (%)	83	Bilirubin–Total(mg/dL)	1
Lymphocyte (%)	14	Bilirubin-Direct(mg/dL)	0.4
Monocyte (%)	3	BUN (mg/dL)	16
Platelet (10^3/µl)	4.8	Creatinine (mg/dL)	1.5

Table 2. Post-operative hematobiochemical analysis

Parameters	Value	Parameters	Value
Haemoglobin (g/dl)	12.4	Total Protein (mg/dl)	7.2
PCV (%)	41.6	Albumin (g/dL)	3.4
RBC (10^6/µl)	5.76	ALT (U/L)	20
WBC (10^3/µl)	14.3	SAP (U/L)	479
Neutrophils (%)	81	Bilirubin–Total(mg/dL)	0.7
Lymphocyte (%)	15	Bilirubin-Direct(mg/dL)	0.2
Monocyte (%)	4	BUN (mg/dL)	20
Platelet (10^3/µl)	8.2	Creatinine (mg/dL)	1.1

diagnosed as cavernous hemangioma.

According to Eberle *et al.* (2012) the splenic tumours affects the senior dogs (83%), with a median age of 10 years, which coincides with our present case whose age is 9 years. In the present case the diagnosis was challenging as there was only intermittent fever. This was in accordance to the findings of (Eberle *et al.*, 2012) and (Anoop *et al.*, 2023) who reported clinical signs associated with splenic tumors are non specific. Clinical signs vary according to the advancement of the disease. The haematological parameters are also normal except forneutrophilia. This coincides with (Wrigley *et al.*, 1989) who stated that haemato-biochemical parameters non-specific for splenic masses.

Splenectomy is the treatment of choice for both cancerous and non cancerous spleen because it is diagnostic as well as therapeutic. Post-operative complications of splenectomy include haemorrhage, arrhythmias, damage to the left pancreatic lobe and gastric wall because of devascularisation and acute infection. Wendelberg *et al.* (2014) in his study on risk factors for perioperative death in dogs undergoing splenectomy for splenic masses of 539 cases reported that 41 dogs (8%) died or were euthanized during or following surgery. Mortality in (24%) of these dogs was associated with hemorrhage and hemostatic dysfunction, while post-operative thrombosis was suspected in (32%) dogs that experienced unexpected peri-operative deaths. In the present case there was no such intraoperative and postoperative haemorrhage. The animal was closely monitored for 3 months and showed no postoperative complications.

CONCLUSION

The case study implies that dogs with a normal haemato-biochemical profile and no particular clinical symptoms may develop splenic tumors. The prognosis is favourable for splenic masses that show no signs of bleeding or metastases. The case's success was made possible by early diagnosis, prompt surgical intervention, and suitable postoperative care.

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Clinical Article

SURGICAL INTERVENTION AND MANAGEMENT OF HORN CONDITIONS IN CATTLE

A.R. NINU*, N. KRISHNAVENI, R. THANGATHURAI[1] and V. KUMAR[1]
Department of Veterinary Surgery and Radiology, [1]Department of Veterinary Pathology, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Tirunelveli-627358, India

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SUMMARY

Horn amputation is done as a preventive/therapeutic procedure in cattle. The therapeutic conditions that need amputation of horn are horn fractures, septic horn, and squamous cell carcinoma. The aim of this case report is to describe and document the surgical techniques of unilateral horn amputation and its outcome in three cattle. All the procedures were done under xylazine-ketamine sedation and cornual nerve block in lateral recumbency. Two cases of horn fracture involving cornual process of right and left side along with one case of septic horn was included in the present study. The septic horn case was treated with multiple drugs by local veterinarians but the case didn't respond to treatment until horn amputation was done.

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Horn base fractures are the most common fractures encountered in the horn of cattle (Patil *et al.*, 2017). Incomplete fracture of horn is treated by immobilization with plaster cast and wiring (Sama *et al.*, 2016), whereas, complete horn fracture of horn base is treated by horn amputation. Disbudding can be beneficial in aspects like prevention of injuries inflicted by horn, more housing space, easy to transport, for treating self-inflicted horn injuries and as a treatment for several horn affections (Alvarez *et al.*, 2015; El-hawari *et al.*, 2015; Prasad *et al.*, 2016 and Mulatu *et al.*, 2021).

Case I: A five year old Kangayam breed of bullock that was brought to Veterinary Clinical Complex, Tirunelveli with a left horn base fracture (Fig. 1). The horn was in normal position but was loose. The incident happened after a fight with another bullock a day back. Clinical examination revealed crepitation and swelling at the horn base. There was emphysema at the base of the head, ear and the forehead on palpation. Feeding and voiding habits were normal. X-ray revealed a complete transverse fracture of the base of the left horn (Fig. 2). Initially horn bandage was applied and inj. Meloxicam @ 0.5 mg/kg b. wt. was given for one month and three days, respectively. The emphysema subsided during the second follow up after one month. Under cornualnerve block (5 ml of 2 % lignocaine) and xylazine (@ 0.1 mg/kg b.wt. intramuscularly) - ketamine(@ 2mg/kg b.wt. intramuscularly) general anaesthesia, the fractured part of the horn was excised. The skin flaps were sutured using silk no. 2. Post-operatively, Bolus Amoxirum forte was advised orally bid @ 5 mg/kg b.wt. for five days, Bolus Melonex plus® @ 1½ bolus daily for three days and BoliEcotas 2 BID for five days. The bullock recovered uneventfully.

Case II: An one year old non-descript bull was brought with fracture at the base of the right horn and its loosening following a collision with a vehicle and hitting the horn on a nearby wall as an after impact. Amputation of the right horn was done undercornual nerve block with 2 % lignocaine, xylazine premedication and ketamine induction. Post-operatively Inj. Enrofloxacin was given @ 5 mg/kg b.wt. intramuscularly for five days and Inj. Meloxicam @ 0.5 mg/kg b.wt. intramuscularly for three days. The case recovered uneventfully in a month.

Case III: A 7 year old female Kankrej cow was brought with an incomplete fracture of horn and accumulation of purulent discharge inside the horn (Fig. 3). The case was treated locally for one month before presenting. The biopsy of the cornual diverticulum revealed pyogranuloma (Fig. 4). The sample sent for isolation and culture revealed the presence of *Proteus* species and *Bacillus* species. The ABST revealed intermediate sensitivity to ceftriaxone alone and resistant to other antibiotics. The ceftriaxone injection @ 10 mg/kg b.wt. intramuscularly by the authors didn't show any results . Hence amputation of the horn was done under cornualnerve block (7 ml of 2 % lignocaine hydrochloride) along with Inj. Xylazine (@ 0.1 mg/Kg b. wt. i/m) - Inj. Ketamine (@ 2 mg/Kg i/v) anaesthesia. Two elliptical incisions were made around the horn base, then two linear incisions were made, one from base of horn towards nuchal crest and other from base of horn towards frontal crest. Skin flaps were made by careful undermining. The horn was separated from frontal bone by using angle grinder. Bleeding was controlled by ligation of superficial temporal artery using chromic catgut no. 2 and cauterization of the stump of the horn core using potassium

*Corresponding author: ar.ninu@gmail.com

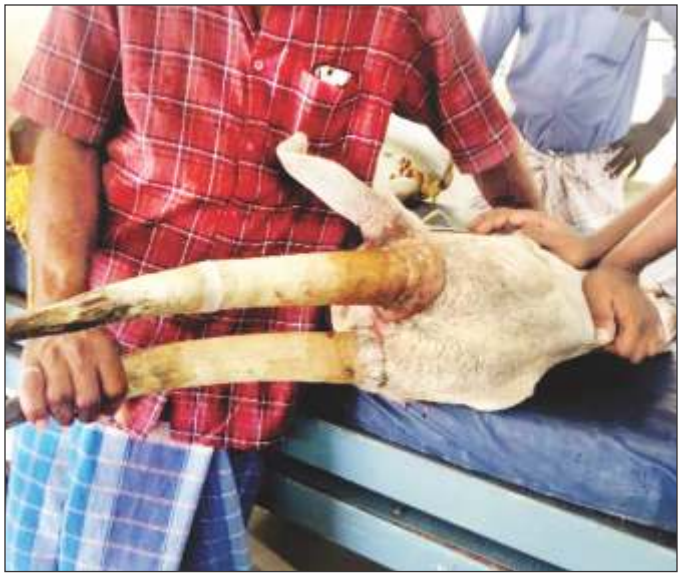

Fig. 1. Case I- Kangayam breed of cattle brought for horn amputation in right lateral recumbency showing signs of mild swelling at the left horn base

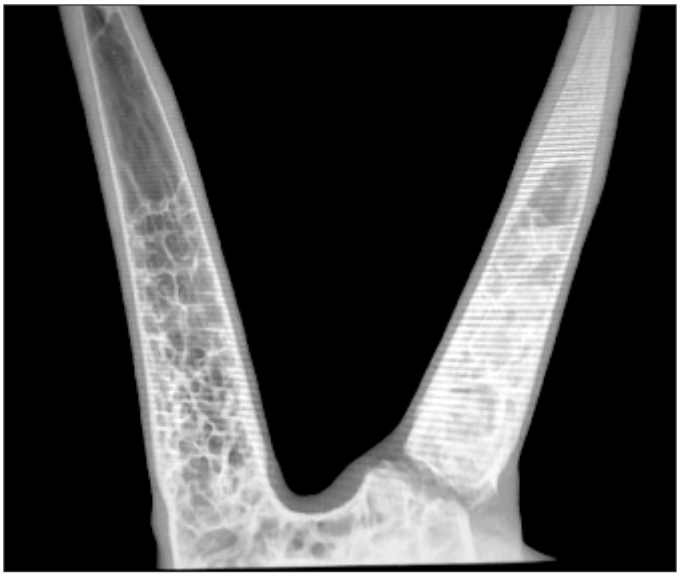

Fig. 2. Radiograph of the horn revealing the complete transverse fracture of the left horn base in the Kangayam bullock

Fig. 3. Five year old female Kankrej cow with incomplete fracture of the left horn

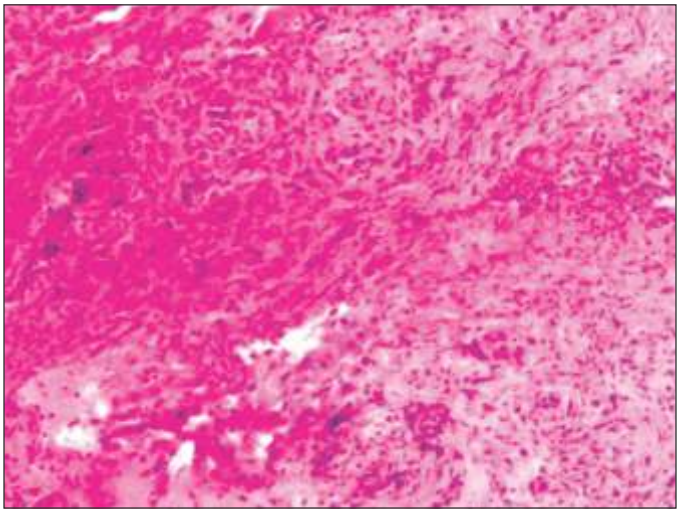

Fig. 4. The biopsy of the septic horn showing the presence of numerous neutrophils

permanganate crystals. The frontal sinus was irrigated thoroughly with normal saline. The skin flaps were trimmed to keep the stump open. Open wound healing of the stump and partial apposition of the skin around the base of the stump using silk no. 2 suture material in simple interrupted pattern was advocated, followed by bandaging with adhesive crepe bandage.

Post-operatively, daily antibiotic injections for five days and analgesic (Inj. Flunixin @ 2mg/kg b. wt. I/v) for three days. Long acting Inj. Enrofloxacin@ 7.5 mg/kg b. wt. i/m was given three times at an interval of 72 hours to which a positive response was obtained. Though Enrofloxacin was found to be resistant, long acting Inj. Enrofloxacin was found to be sensitive. The lavaging of the cornual diverticulum and the frontal sinus with 1: 1000 potassium permanganate solution and application of topical antibiotic spray was also advised. The septic horn could be treated successfully by combined surgical management and antibiotic therapy in a time period of nine days post-operatively without any complications.

Complete fracture of the horn base was treated by horn amputation and septic horn cases by horn amputation leaving the stump open (Patil *et al.*, 2017; Dehury *et al.*, 2017). There are reports of horn base fractures due to bull fight. Majority of the animals undergoing horn amputation were restrained in lateral recumbency (Sharma *et al.*, 2021). The horn was amputated from frontal bone by using hacksaw blade, hammer and mallet by Kamalakar *et al.* (2016) whereas in our case in the heavy horn of the Kankrej cow was amputated using angle grinder at the base. The septic horn responded to drugs after providing drainage by amputation. The resistance to short acting

Enrofloxacin and sensitivity to long acting Enrofloxacin could be due to the optimized Pharmacokinetic/ pharmacodynamics ratios in long acting formulations allow them to overcome higher minimum inhibitory concentrations that short acting antibiotics might fail to reach (Gutierrez *et al.*, 2025).

Horn amputation in cattle affected by horn fracture and septic horn are discussed in this report. Non-healing horn base fractures can cause discomfort to the animals and hence, amputation may provide pain relief and ease the stress during grazing. Septic horn in massive horned breeds like Kankrej may be an early symptom of horn cancer as these light-coloured breeds are predisposed to squamous cell carcinoma of horn (Prasad *et al.*, 2016). So horn amputation can help in healing of the condition and prevent horn cancer in future.

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IMPORTANT INFORMATION

- Research/Clinical articles are invited for next issue from the Scientists/Veterinarians engaged in Veterinary Profession.
- Please follow strictly the format of **'The Haryana Veterinarian'** for manuscript writing/ submission.
- **The article processing and publication charges will be revised from Rs. 1200/- to 1500/- per manuscript with effect from the next year (01.01.2027).**
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Editors

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Clinical Article

SURGICAL MANAGEMENT OF HYSTEROCELE IN A PREGNANT DOE USING POLYPROPYLENE MESH HERNIOPLASTY: A CASE REPORT

PRARTHANA, R.*, ASWATHY GOPINATHAN, KAMALESH, K.S.[1], RAKESH YADAV, B.P. SINGH, CHANDAN KUMAR and ROHIT KUMAR

Division of Surgery, ICAR- Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India- 243122

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SUMMARY

The herniation of gravid uterus through a large defect in the abdominal wall is an uncommon but potentially life-threatening condition in small ruminants. This case report describes the successful surgical management of an advance-gestation doe presented lately with a large ventrolateral abdominal swelling. Upon clinical examination and ultrasonography confirmed the presence of non-viable foetus within the herniated sac. Following aseptic preparation and epidural anaesthesia, the foetus was removed and gravid uterus was repositioned into the abdominal cavity, and the abdominal wall defect (18.2 cm×12.5 cm) was reinforced using a sterilized polypropylene mesh (15 cm × 10 cm × 10 cm) 693 sutured via outlay technique. No recurrence or wound complications were observed during a three-month follow-up. This report highlights the effectiveness of polypropylene mesh hernioplasty as a safe, economical and reliable method for correcting large abdominal wall defects.

Keywords: Doe, Gestation, Hernioplasty, Hysterocele, Polypropylene mesh

How to cite: Prarthana, R., Gopinathan, A., Kamalesh, K.S., Yadav, R., Singh, B.P., Kumar, C. and Kumar, R. (2026). Surgical management of hysterocele in a pregnant doe using polypropylene mesh hernioplasty: A case report. *Haryana Veterinarian.* **65(1)**: 150-152.

In animals, ventral hernia primarily occurs as a consequence of traumatic incidents such as kicks, blows, horn thrusts or falls on blunt objects, as well as rupture of the prepubic tendon (Preethi *et al.*, 2018). In ruminants, goats are considered more susceptible to abdominal injuries and subsequent hernia formation compared to cattle. This increased predisposition is attributed to their relatively small body size and docile temperament. Previous studies have reported that trauma accounts for approximately 71.4% of abdominal hernia cases in small ruminants (Sharun *et al.*, 2021). Furthermore, the type and nature of hernial contents are largely determined by the anatomical location of the herniation. But, they may result in serious complications, particularly during advanced pregnancy (Ali *et al.*, 2019). Hysterocele, or herniation of the gravid uterus through a defect in the abdominal wall, is a rare but life-threatening condition in does (Vijayanand *et al.*, 2012). It predisposes the dam to dystocia, uterine rupture, circulatory impairment, and even foetal mortality if left untreated. Conservative management using bandaging or slings provides temporary support but is often inadequate in advanced cases (Jettennavar *et al.*, 2010). Surgical correction remains the treatment of choice. Among surgical options, hernioplasty with synthetic meshes such as polypropylene has been widely adopted in both veterinary and human medicine due to its tensile strength, biocompatibility, inertness, and minimal tissue reaction (Seifalian *et al.*, 2023). This article presents the surgical management of a hysterocele in a pregnant doe using polypropylene mesh hernioplasty and discusses the clinical outcome.

A 4.5 old multiparous pregnant doe in its late gestation was presented with a history of progressive swelling in the right ventrolateral abdominal region over the past 4 weeks. The swelling increased in size with advancement of pregnancy (Fig. 1A) and was associated with difficulty in ambulation and recumbency during rest. On physical examination, the swelling was reducible, soft to firm in consistency and enlarged upon straining. Palpation revealed gravid uterus within the hernial sac. Ultrasonography confirmed the presence of non-viable foetus within the herniated portion of the uterus might be due to late presentation of case. Hematobiochemical parameters were within normal limits, making the animal fit for surgery.

Preoperative prophylaxis included administration of broad-spectrum antibiotics (Ceftriaxone @ 10 mg/kg, IM) and NSAIDs (Meloxicam @ 0.2 mg/kg, IM). The surgical site was shaved, scrubbed, and aseptically prepared. Epidural anaesthesia at lumbosacral region, using 2% lignocaine hydrochloride was administered. The animal was restrained on dorsal recumbency, Atransverse skin incision was made over the hernial swelling. An emergency C-section was performed and the non-viable foetus was removed (Fig. 1C) and inversion (cushing followed by Lembert) sutures were placed over uterus using polyglactin 910 size 1-0 (Kumari and Dutt, 2020) (Fig. 1B). The gravid uterus was repositioned into the abdominal cavity with minimal manipulation to avoid uterine trauma. The large hernial rent, measuring approximately 18.2 cm × 12.5 cm,

*Corresponding author: prarthanaravindra1998@gmail.com

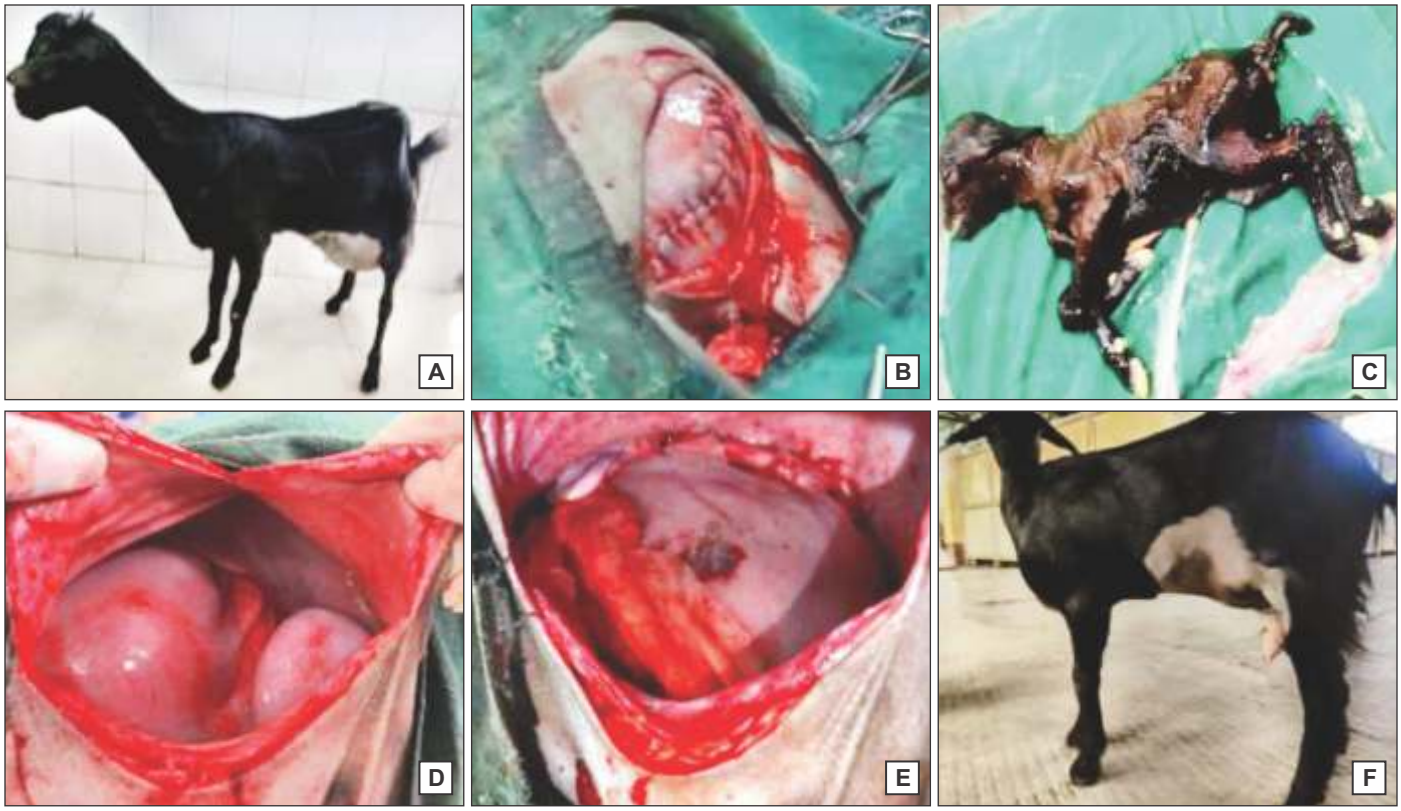

Fig. 1. **A.** Doe presented with ventral hernia; **B.** emergency C-section performed and uterine closure by inversion; **C.** Non-viable foetus was removed; **D.** Large hernia rent measuring 18.2 cm ×12.5 cm (Hysterocele); **E.** Hernioplasty done using polypropylene mesh; **F.** Post-surgery presentation (12th day) after suture removal.

was identified (Fig. 1D). A sterilized polypropylene mesh (15 cm × 10 cm × 10 cm) was used to cover the defect via outlay technique. The mesh was sutured to the healthy abdominal wall margins were secured using Nylon size 1-0 byhorizontal mattress pattern (Fig. 1E). Subcutaneous tissue was closed using absorbable suture material (polyglactin 910) in continuous pattern. Skin was closed with non-absorbable nylonby horizontal mattress sutures. Post-operatively Antibiotics (Ceftriaxone) were continued for 5 days. Analgesics (Meloxicam) were given for 3 days. Supportive therapy included multivitamins and fluid supplementation. The doe recovered uneventfully following surgery with no evidence of wound infection or dehiscence. Skin sutures were removed after 12 days (Fig. 1F). The traumatic ventral hysterocele was successfully reduced in the goat after performing an emergency caesarean section and hernioplasty using polypropylene mesh, which provided effective reinforcement of the abdominal wall and maintained integrity throughout the healing period. No recurrence of hernia was observed during the 3-month follow-up period. The surgical site showed firm healing, and the animal resumed normal feeding and locomotor activity within a week.

A hernia occurring ventral to the stifle skin fold is classified as a ventral hernia, whereas hernias located elsewhere along the abdominal wall are referred to as lateral hernias (Sharun *et al.*, 2021). Lateral or ventral hernias are most frequently encountered along the costal arch, in the ventral abdominal wall, within the flank region either high or low, and in the intercostal space between the last few ribs (Kumar *et al.*, 2018). Hernias during late gestation in does pose significant risk to both dam and foetus. In this case, the defect was large and incapable of being corrected by herniorrhaphy alone. Therefore, polypropylene mesh hernioplasty was employed, which provided tensile strength and resisted abdominal pressure during the remaining gestation (Khan *et al.*, 2018). The size and shape of the swelling associated with a hernia can vary considerably. The diagnosis of a hernia is primarily established through a combination of case history, characteristic clinical signs, and thorough physical examination (Sharun *et al.*, 2019). Among the diagnostic features, precise identification of the hernial ring remains the most crucial step in confirming the presence of a hernia. Goats carrying multiple foetuses are particularly predisposed to developing abdominal hysterocele, with the condition being more frequently observed during the advanced stages of pregnancy (Ali *et al.*, 2019; Manjusha *et al.*, 2020). This increased susceptibility is primarily associated with the progressive stretching and weakening

of the abdominal musculature, which compromises its structural integrity and predisposes to herniation. In addition to physiological strain, external factors such as a sudden impact or trauma to the abdominal wall may also precipitate the occurrence of ventral hernia involving the gravid uterus in goats (Sharun *et al.*, 2021).

Polypropylene mesh has been reported as effective one because of exhibiting following properties like minimal tissue reaction, resistance to infection, and inertness (Seifalian *et al.*, 2023). The successful outcome in this case corroborates earlier reports of mesh hernioplasty in cattle, buffaloes and small ruminants (Kassem *et al.*, 2014). Since an "ideal mesh" has not yet been developed, the use of polypropylene mesh in this case was associated with minor local complications. Mild inflammation was observed at the surgical site following the procedure. However, these signs resolved within one week with the administration of appropriate antibiotics and NSAID drugs, similar to studies by Kassem *et al.* (2014). The favourable pregnancy outcome indicated that surgical correction during late gestation is feasible if performed with careful handling of the gravid uterus and adequate postoperative management. Early surgical intervention prevents further complications such as rupture of uterus, adhesions, or circulatory compromise.

In the present case, it is presumed that the gravid uterus contributed to weakening of the abdominal musculature following violent trauma caused by a blunt object during an automobile accident. The resulting abdominal tear, measuring approximately 19.6 cm in length, was successfully repaired through Polypropylene mesh hernioplasty, which is an effective, economical and reliable method for correction of large hysterocele in pregnant does. The technique ensures restoration of abdominal wall integrity, successful completion of gestation, and prevention of recurrence. Timely diagnosis, gentle intraoperative handling of gravid uterus, and diligent postoperative care are critical for successful outcome.

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Clinical Article

UTERINE TORSION IN A PREGNANT CAT - A CASE REPORT

ADITYA V. SINGH[1] and K.S. ADEY*
Department of Veterinary Surgery and Radiology, Nagpur Veterinary College,
Maharashtra Animal and Fishery Sciences University, Nagpur-440010
[1]Veterinary Surgeon, Vets for Life, Bhupeshnagar, Nagpur-440013 (Maharashtra)

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SUMMARY

An eight-month old female Persian cat was presented with history of lethargy, inappetence and anaemia from last two-days. The cat had delivered three kittens (two alive and one stillbirth) 24 hour earlier. Ultrasonography revealed two foetuses with no cardiac activity. Urgent surgical intervention was deemed necessary. The left uterine horn appeared dark red during surgery, and its base had turned round in a clockwise direction at 360 degrees. An ovariohysterectomy was then carried out. The cat showed full recovery postoperatively.

Keywords: Cat, Ultrasonography, Uterine torsion

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Uterine torsion, which is more frequently seen in multiparous females, is described as the rotating of the entire uterus or either one or both horns along its longitudinal axis. Even though cattle and buffalo are commonly observed to have this problem, in cats and dogs, it has rarely been reported. Pregnancy is frequently associated with uterine torsion, which can develop from mid to late in the gestational period (Thilagar, 2005). The exact etiology for uterine torsion is unknown. However certain contributing factors are associated with uterine torsion. It may be related to uterine ligament laxity, foetal movements, premature contractions of uterus, uterine walls flaccidity, long and/or sagging mesometrium, and possibly an insignificant quantity of foetal fluids; excessive physical activity during pregnancy, obstetric operations during dystocia, and variations in the proper ovarian ligament's length and mobility (Stone, 2007).

Torsion of either one or both uterine horns has been documented, with torsion degrees varying from 180° to 360° (Freeman, 1988). Torsion compromises uterine vasculature, resulting abruption of placenta and uterine tissue, alongwith haematological and metabolic disturbances (Misumi *et al.*, 2000 ; Ridyard *et al.*, 2000) that could result in endotoxemia, peritonitis and disseminated intravascular coagulation (DIC) (De La Puerta *et al.*, 2008).

An eight-monthold 3.5 kg female Persian cat was presented with a history of recent parturition 24 hrs earlier. On physical examination, the cat demonstrated signs of systemic compromise including lethargy, mild dehydration (5%), pale mucous membrane with a prolonged capillary refill time of 3 seconds and an acute abdomen with palpable large tubular structure in the mid abdominal cavity. The patient demonstrated dullness with preserved responsiveness to environmental stimuli. No vaginal discharge was observed. The vital parameters such as rectal temperature (100.00 F), heart rate (150 beats/min) and respiratory rate (25 breaths/min) were within the normal physiological range. Haematological assessment revealed anaemia, with haemoglobin levels significantly reduced at 6.8 g/dL, RBC (3.7×10^{12}/L) leukocytosis (25×10^{9}/L), PCV (29%), platelets (240×10^{3}/μL). Serum creatinine, blood urea nitrogen, ALT and AST were within the normal range. Ultrasound examination was performed which showed two fetuses with no cardiac activity. The cat displayed no signs of straining after delivery of two kittens for 24 hrs. As a result, it was decided to opt forimmediate surgical intervention.

Prior to the procedure, the cat was premedicated using atropine sulphate (0.04 mg per kg) subcutaneously and buprenorphine HCl (0.02 mg per kg intravenously; Leptan [Otsuka Pharmaceuticals]). Sedation was achieved with the combination of triflupromazine HCl (2 mg/kg, Siquil [Zydus Animal Health]) and ketamine HCl (20 mg/kg, Ketmin [Themis Medicare]) intramuscularly. Propofol (5 mg per kg intravenously; Neorof [Neon]) was administered to induce general anaesthesia and maintained at the same dose till effect. A routine ventral midline laparotomy was performed. Intraoperatively, scant amount of mild dark bloodcoloured fluid was noted in the peritoneal cavity. The right uterine horn appeared empty whereas the fetuses were stuck in left horn due to torsion. The affected left horn of the uterus appeared distended, congested and friable (Fig. 1) which underwent a 360-degree clockwise rotation at its. The right uterine horn exhibited anemia-related pallor

*Corresponding author: khushbooadey7@gmail.com

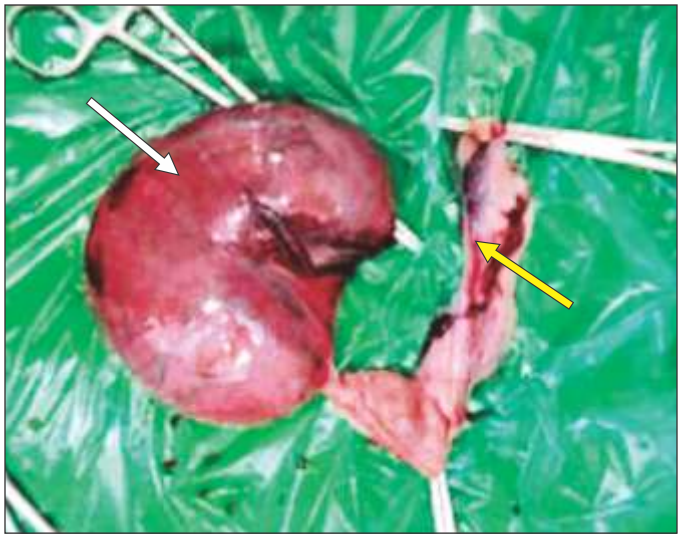

Fig. 1. Post operative photograph of uterine torsion. The left uterine horn (yellow arrow) is dark in colour. The right uterine horn is pale because of anemia (blue arrow).

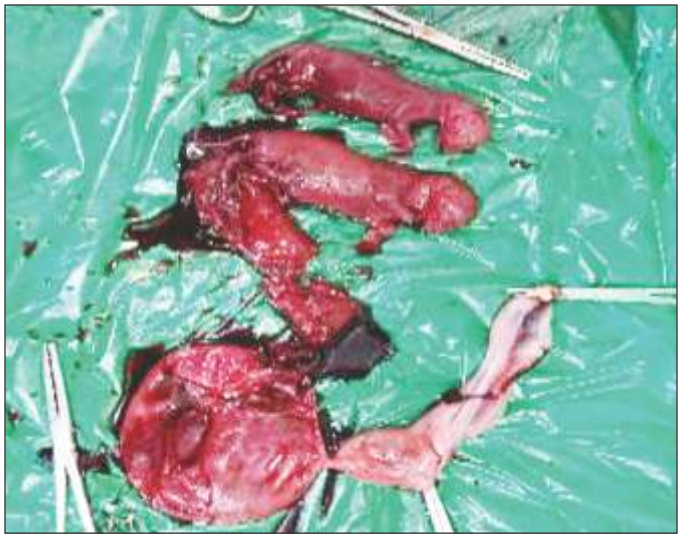

Fig. 2. Post operative two dead fetuses were observed from the affected uterine horn.

while remaining in normal anatomical position. (Fig. 2). As no fetal cardiac activity was noted on ultrasonogram, ovariohysterectomy was carried out using standard technique without performing caesarean section. The left and right ovaries were identified and grossly appeared normal. The torsion was left unresolved to prevent endotoxin release into the systemic circulation. Abdominal lavage with warm saline solution was performed. The surgical incision was closed using routine closure techniques.

Post-operatively, the cat was given intravenous fluid therapy, antibiotic (ceftriaxone [Wocef, Vetoquinol] 12.5 mg/kg every 12 hours), analgesic (Tramadol; [Contramal, Abbott], 2 mg/kg every 8 to 12 hours) for five days. The cat was advised to use Elizabethan collar for seven days. The cat was bright and alert and urinated normally with no evidence of discharge from the vulva. The cat recovered uneventfully.

The incidence of uterine torsion in cats is low, with sparse reporting in clinical cases (Thilagar *et al.*, 2005; Matsumoto *et al.*, 2009). In all domestic animal species, uterine horn torsions can occur during mid to late gestation or right before delivery (Pratt, 1983), however, in cats, it usually happens just before parturition or in late pregnancy (Kudale *et al.*, 1972). In the present case, torsion in left uterine horn was noted at the time of parturition while the fetuses present within the right uterine horn were delivered normally without any assistance. Uterine torsion has been represented with the clinical sign of acute abdomen (Misumi *et al.*, 2000; Ridyard *et al.*, 2000; De La Puerta *et al.*, 2008) which is also noted in the present case where the cat was presented with abdominal distension and pain. Cats mat experience uterine torsion typically involving a rotation of 180 to 360 degrees (De La Puerta *et al.*, 2008). Prior studies have demonstrated that this factor substantially impacts the severity, clinical sign duration and prognostic outcomes (Thilagar *et al.*, 2005). Cats from earlier reports had immediate, serious clinical symptoms such as peritonitis, septicemia, disseminated intravascular coagulation, and endotoxemia (De La Puerta *et al.*, 2008). In the present case, the uterine torsion was of 3600 and was reported early that resulted in uncomplicated recovery of the queen. The results of the hematological tests revealed anemia that is consistent with loss of blood, attributing to the significant blood volume in uterus and haemostasis of the uterine vasculature as a result of torsion (Ridyard *et al.*, 2000; Thilagar *et al.*, 2011).

Although the identification of fetal death and fluid accumulation necessitates abdominal ultrasonography, the precise diagnosis of uterine torsion could only be made by an exploratory laparotomy as reported by Dal-Bo *et al.* (2013) and Niwas *et al.* (2023). As soon as the left uterine horn's torsion was detected, ovariohysterectomy was carried out without the uterus being repositioned as advised by Stanley and Pacchiana (2008) to curtail the translocation of inflammatory mediators and endotoxins into systemic circulation.The research indicates that a favourable outcome for cats is dependent on early surgical intervention, adequate perioperative medical support and extent of uterine torsion (De La Puerta *et al.*, 2008). Similarly, Ridyard *et al.* (2000) stated that with early intervention, supportive critical care and ovariohysterectomy

(Niwas *et al.*, 2023), the prognosis for uterine torsion ranges from favourable to good.

CONCLUSION

Cats seldom have uterine torsion and is mostly diagnosed at the time of exploratory laparotomy. It is an emergency condition and a delay in surgical intervention, which mostly accounts for ovariohysterectomy could be life threatening.

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