

UTERINE LAVAGE, LEVAMISOLE, CLOPROSTENOL AND ITS COMBINATIONS IMPROVE PLASMA PROTEINS AND FERTILITY OUTCOMES IN ENDOMETRITIC BUFFALOES

NARENDRA SINGH, BHOOPENDRA SINGH¹ and RAJESH KUMAR^{1*}

Department of Veterinary Clinical Complex, R.R. College of Veterinary and Animal Science,
Deoli, Tonk, RAJUVAS, Rajasthan-304 804, India

¹Department of Veterinary Gynaecology & Obstetrics, College of Veterinary Science and AH,
ANDUAT, Ayodhya-224 229 (UP), India

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ABSTRACT

The study was aimed to assess comparative efficacy of uterine lavage, levamisole subcutaneously, PGF2 α (Cloprostenol) intramuscularly and their combinations on biochemical parameters and treatment outcomes in buffaloes with endometritis. Total thirty-six repeat breeder buffaloes with endometritis were allocated as untreated control (G0, n=6), G1 (Uterine Lavage), G2 (Levamisole, s/c), G3 (Cloprostenol, i/m), G4 (Uterine Lavage+Levamisole, s/c) and G5 (Uterine Lavage + Cloprostenol, i/m), each group comprised of 6 animals. After treatment total protein, albumin and globulin concentration were increased significantly ($p<0.05$) in treated buffaloes. The highest clinical cure was recorded with uterine lavage plus levamisole (83.33%) and uterine lavage plus Cloprostenol (83.33%) treated buffaloes followed by G2 (66.67%) and G3 (66.67%), G1 (50.00%) and G0 (33.33%) group. The highest conception rates was in group G4 and G5 (66.67% each) followed by G2 and G3 (50% each), G1 (33.00%) and G0 (16.67%). In conclusion, endometritic buffaloes can be treated using lavage of uterus with physiological saline in combination with parenteral Levamisole or Cloprostenol.

Keywords: Buffalo, Cloprostenol, Endometritis, Levamisole, Uterine lavage

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Subclinical endometritic and non-specific infections are most common cause of repeat breeding syndrome in buffaloes. Clinical endometritis is defined as purulent vaginal discharge (21 days or more postpartum) or a muco-purulent vaginal discharge (after 26 days postpartum). while subclinical endometritis is characterized by absence of muco-purulent or purulent vaginal discharge (Sheldon *et al.*, 2006) of uterine origin. Subclinical endometritis is often undiagnosed (Dutt *et al.*, 2017), hence remain untreated and results in subfertility. Transrectal and ultrasonographic examination of uterus are usually performed to diagnose the disease under field conditions (Barlund *et al.*, 2008).

The therapeutic regimen used to treat infected bovine uterus should effectively clear uterine infection without compromising its local immunity and adulterating the milk or meat. Many therapeutic agents have been tried and tested in treatment of infected uterus (Singh *et al.*, 2018; Ahmadi *et al.*, 2019). In view of development of antimicrobial resistance and various side effects with use of antibiotics; the levamisole may be used to substitute or supplement antibiotic therapeutics (Singh *et al.*, 2017). In equines, the infection of uterus can be treated with physiological saline solution as uterine lavage. The uterine lavage results in removal of bacteria, non-functional neutrophils, hasten myometrial contractility which in turns

causes evacuation of uterine content and influx of fresh neutrophils in uterine lumen (Brinsko *et al.*, 2011). Thus, the present work was planned with the hypothesis that lavage of uterus along with administration of Levamisole or Cloprostenol parenterally, would cure subclinical endometritis and improve reproductive outcomes of such buffaloes. Thus, this study was carried out to assess aforesaid approaches on plasma proteins and treatment outcomes in repeat breeder buffaloes with endometritis.

MATERIALS AND METHODS

Referral cases presented at Veterinary Clinical Complex, C.V.Sc. & A.H, ANDUAT, Ayodhya, were screened for study following approval from ethical committee of university (No. IAEC/CVSc/2019/P-02). Based on animal breeding history and records, trans-rectal palpation of genitalia and White side test, total 36 repeat breeder buffaloes were assigned in six groups, each comprised of 6 buffaloes *viz.* G0 (untreated control), G1: Uterine lavage (UL) with 300 ml NSS in divided doses, 50 mL each time, as slow IU infusion), G2: Levamisole (LEV) @ 2.5 mg/kg body weight subcutaneously, three occasions i.e. 0, 7th and 14th day of the cycle; G3: Cloprostenol or CP @ 250 μ g intramuscularly, on day-10 of the cycle; G4: (G1+G2 combined i.e. UL+LEV) and G5 (G1+G3 combined i.e. UL+CP). Six repeat breeder buffaloes were served as untreated control. For biochemical analysis, 6 ml of blood was collected via jugular vein puncture in

*Corresponding author: drrajesh25@gmail.com

clean sterile vials and centrifuged at 1200xg for 15 min at 4°C. Thereafter, the plasma was separated and preserved at -20 °C until analysis. The plasma was analyzed for estimation of total protein, albumin, globulin, using diagnostic kits as per manufacturer instructions. Blood sample was collected twice, first at commencement of experiment (BT, before therapy) and second at consequent estrus following treatment (AT, after therapy). The double insemination was done at 12 h interval using frozen thawed semen in estrus buffaloes. The animals were inseminated again at second and third subsequent estrus, if returned to estrus. In order to diagnose pregnancy, trans-rectal palpation was done at 45-60 days after AI. The data were analyzed using Duncan's MRT and complete randomized design at significance of $p < 0.05$ level (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

TOTAL PROTEIN: The total protein (gm/dl) (mean \pm SE) elevated significantly in treated buffaloes (G1 to G5) than their corresponding values before treatment, however in untreated animals, the values were did not differ significantly (Table 1). Similar to present observations, lower serum protein levels were recorded in endometritic buffaloes (Patil, 2010) and cows (Sivaraman *et al.*, 2003) but there is also evidence to contrary findings (Ahmad *et al.*, 2004; Sharma, 2018). The significant increase in the serum protein level of endometritic buffaloes following therapy with UL might be due to dilution of bacteria and elimination of micro organisms, debris and products of uterine inflammation that improved general body condition of the animal and thereby increased serum protein level in the animal after treatment. Our findings are corroborated well with earlier studies (Biswal *et al.*, 2014; Singh *et al.*, 2017). To date, no literature cited regarding effect of CP on serum protein level in endometritic buffaloes. So, our results could not be compared. The significant increase in the serum protein level of endometritic buffaloes after treatment with UL+LEV and UL+CP, might be due to combined effect of uterine lavage with LEV/CP. Scanning of literature revealed that no citation available concerning effect of uterine UL+LEV and uterine UL+CP on serum protein level of endometritic buffaloes. So, our results could not be compared.

ALBUMIN: The Albumin (gm/dl)(mean \pm SE) levels were elevated significantly in treated buffaloes (G1 to G5) than their corresponding pretreatment values, whereas the values did not differ significantly among untreated control buffaloes (Table 1). Like present findings, earlier workers also recorded lower level of serum albumin in endometritic cattle (Sivaraman *et al.*, 2003; Amle *et al.*, 2014) and buffaloes (Patil, 2010; Sharma, 2018). In present study, intrauterine infusion of saline may cause dilution of infection and elimination of microorganisms,

debris and inflammatory exudates from the uterus that improved general body condition of the animal and thereby increased serum albumin level in the animal after treatment. Our findings are consistent with earlier reports on UL and LEV subjected endometritic animals (Patil, 2010; Biswal *et al.*, 2014; Singh *et al.*, 2017). The significant increase in the serum albumin level of endometritic buffaloes after treatment with uterine UL+LEV and UL+CP might be due to combined effect of uterine lavage and levamisole. Scanning of literature revealed that no citation available concerning effect of Cloprostenol, uterine UL+CP and UL+LEV on serum albumin level in endometritic buffaloes. So, our results could not be compared.

The significant increase in the serum albumin level of endometritic buffaloes after treatment with levamisole might be due to its immunomodulatory effect. The concentration of albumin is negatively correlated with acute phase proteins i.e. inflammation and infection results in declined albumin level (Eckersall and Bell, 2010) and elevation of albumin concentration following levamisole therapy was might be due to its immune-potentiating properties. The significant increase in the serum albumin level of endometritic buffaloes after treatment with CP might be due to its luteolytic effect (which in turns results in declined progesterone concentration and hence improved uterine immunity), stimulation of myometrial contractility, phagocytic activity of uterine polymorphonuclear, hence improved general body condition of the buffaloes thereby increased plasma albumin level of animals.

GLOBULIN: The Globulin level (gm/dl) (mean \pm SE) elevated significantly in treated buffaloes (G1 to G5) than their corresponding values before treatment, whereas the values did not differ significantly among control buffaloes (Table 1). Similar to our observations, lower serum globulin levels were reported in endometritic cattle (Sivaraman *et al.*, 2003; Magnus and Lali (2009) and buffaloes (Patil, 2010). Our findings are consistent with earlier reports on UL and LEV treated endometritic animals (Patil, 2010; Singh *et al.*, 2017). The significant increase in the serum globulin level of endometritic buffaloes following therapy with lavage of uterus with normal saline might be due to elimination of uterine debris, microorganisms, dilution of inflammatory contents as well as infection, which in turns results in improved body condition of the animal and thereby increased serum globulin level in the animal after treatment. Furthermore, the significant increase in the serum globulin level of endometritic buffaloes after treatment with levamisole might be due to immunomodulatory property of levamisole. The significant increase in the serum globulin level of endometritic buffaloes after treatment with Cloprostenol CP might be due to its luteolytic effect (which

Table 1. Effect of different treatment on plasma proteins and fertility outcomes in endometritic buffaloes

Groups (n=6)	Total Protein (g/dL)		Albumin (g/dL)		Globulin (g/dL)		Fertility outcomes	
	BT	AT	BT	AT	BT	AT	RR	CR
G0 (Untreated)	6.07±0.05	6.16±0.05 ^D	2.74±0.02	2.81±0.03 ^C	3.33±0.06	3.35±0.05 ^C	33.33 ^D (2/6)	16.67 ^D (1/6)
G1 (UL)	6.19±0.08 ^a	7.39±0.08 ^{Cb}	2.68±0.04 ^a	2.91±0.07 ^{Bb}	3.51±0.09 ^a	4.48±0.11 ^{Bb}	50.00 ^C (3/6)	33.33 ^C (2/6)
G2 (LEV)	6.18±0.09 ^a	8.10±0.08 ^{Ab}	2.66±0.04 ^a	3.02±0.03 ^{Ab}	3.52±0.06 ^a	5.07±0.07 ^{Ab}	66.67 ^B (4/6)	50.00 ^B (3/6)
G3 (CP)	6.17±0.07 ^a	7.90±0.05 ^{Bb}	2.62±0.04 ^a	2.95±0.06 ^{Bb}	3.53±0.05 ^a	4.95±0.06 ^{Ab}	66.67 ^B (4/6)	50.00 ^B (3/6)
G4 (UL+LEV)	6.13±0.07 ^a	8.20±0.05 ^{Ab}	2.64±0.03 ^a	3.12±0.03 ^{Ab}	3.48±0.09 ^a	5.08±0.10 ^{Ab}	83.33 ^A (5/6)	66.67 ^A (4/6)
G5 (UL+CP)	6.20±0.06 ^a	8.16±0.06 ^{Ab}	2.69±0.04 ^a	3.09±0.05 ^{Ab}	3.50±0.09 ^a	5.07±0.09 ^{Ab}	83.33 ^A (5/6)	66.67 ^A (4/6)

Means with different superscripts within group (a, b) and between groups (A, B, C) differ significantly ($p < 0.05$); BT (Before therapy); AT (After therapy); RR (Response rate); CR (Conception rate).

in turns results in declined progesterone concentration and hence improved uterine immunity), stimulation of myometrial contractility, phagocytic activity of uterine polymorphonuclear, hence improved general body condition of the buffaloes thereby increased serum globulin level of animals. The significant increase in the serum globulin level of endometritic buffaloes after treatment with uterine lavage plus Levamisole and uterine lavage plus Cloprostenol might be due to combined effect of uterine lavage and levamisole. To date, no literature is cited regarding effect of Cloprostenol, Lavage of uterus with saline plus Levamisole and Lavage of uterus with saline plus Cloprostenol on serum globulin level in endometritic buffaloes. So, our results could not be compared.

CONCLUSION

In conclusion, Levamisole and Cloprostenol alone were found to be more effective than the uterine lavage therapy. The higher cure rate was recorded in uterine lavage along with parenteral Levamisole or Cloprostenol treated buffaloes as compared to any regimen alone; as evidenced by maximum improvement in plasma protein level, recovery rate and conception rate was observed in levamisole plus uterine lavage and Cloprostenol plus uterine lavage group. The sub-clinical endometritis in buffaloes can be treated using Levamisole (@ 2.5 mg/kg body weight via s/c route on three occasions at 7 days interval), furthermore, it can be used as a substitute or supplement to antibiotic therapy.

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EFFECT OF TRIFLUPROMAZINE AND BUTORPHANOL ON CANINE GASTROINTESTINAL TRACT EMPTYING TIME USING BARIUM SULPHATE CONTRAST MEAL

PRIYANKA PANDEY*, H.R. BHARDWAJ, ANKUR SHARMA and ASHOK KUMAR

Division of Veterinary Surgery and Radiology, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-J, R.S. Pura-181102, Jammu, J&K, INDIA

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ABSTRACT

The gastrointestinal contrast study is an important diagnostic tool used in the identification of gastrointestinal diseases in small animals. In a randomized crossover design study, eighteen healthy adult mongrel dogs (12-24 months, 12-20 kg) were selected for barium meal contrast study. The animals were randomly divided into three groups of 6 animals each. In group-I normal transit time of canine gastrointestinal tract barium contrast meal was administered without giving any sedation, group II triflupromazine hydrochloride @2 mg/kg b.wt. I/M and in group III Butorphanol @ 0.2 mg/kg b.wt. I/M was administered to evaluate the effect of sedation on canine gastrointestinal tract emptying time using barium sulphate contrast meal. In summary, the total gastrointestinal tract emptying time was significantly prolonged by use of an triflupromazine hydrochloride and butorphanol in dogs as compare to non-sedated dogs. The study was useful to evaluate the effect of sedatives on the total GIT emptying time once a control value has been established.

Keywords: Barium sulphate, Butorphanol, Dog, Gastrointestinal tract, Triflupromazine

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The gastrointestinal contrast study is an important diagnostic tool used in the identification of gastrointestinal diseases in small animals. Gastric emptying time is a complicated process controlled by neural and hormonal mechanisms. Pressure differences and the degree of resistance at the gastroduodenal junction have a direct influence on the rate of gastric emptying in dogs. Abnormalities in gastric emptying times can indicate serious pathologic issues such as pyloric outlet obstruction, hypertrophic gastritis, protruding neoplasia and nerve dysfunction (Burns and Fox, 1986).

Barium sulphate is the most commonly used contrast medium for gastrointestinal tract, which comes in paste, liquid or powder form which is mixed with water (Kang *et al.*, 2020). Barium sulphate is an inert substance and is neither diluted nor digested during its passage through the gut, thus providing excellent mucosal detail. If it leaks out of the gut through a perforation, it causes granuloma and adhesion formation in the mediastinum or peritoneum, so if a perforation is suspected, a water soluble iodine-based contrast medium should be used instead of barium sulphate (Murphy, 2021).

Several non-invasive techniques have been proposed to evaluate stomach emptying and gastrointestinal tract transit of solid food in dogs. Barium sulphate suspension mixed with food is a simple and inexpensive method (Hogan and Aronson, 1988). Gastric emptying and transit times can be affected by anticholinergic drugs, various sedatives and tranquilizers, and psychic influences, such as

stress, fear, pain, anxiety, and rage (Mickael *et al.*, 2002). Oral administration of contrast medium is required, and animals in unfamiliar environments may become anxious, fractious, and difficult to handle. In these instances, sedation may be required to perform the examination. The ideal sedative is one that produces adequate restraint and affects transit time minimally, so that each segment of the gastrointestinal tract can be properly examined.

The purposes of this study were to evaluate complete gastrointestinal tract emptying time of healthy adult dogs using barium meal without sedation to compare and evaluate effects of triflupromazine hydrochloride and butorphanol on transit time of barium meal. Practicality of this technique is to detect alterations in total gastric emptying time in clinical situations in sedated and non-sedated dogs.

MATERIALS AND METHODS

In a randomized crossover design study, eighteen healthy adult mongrel dogs aged between 12-24 months, weighing 12-20 kg were selected for barium meal contrast study. Prior to being admitted to study, normal physical examination (Rectal Temperature, heart rate, respiration rate and colour of mucus membrane) and haematological parameters were examined. The animals were randomly divided into three groups with 6 animals each. In group I barium contrast meal was administered without giving any sedation, in group II triflupromazine hydrochloride @ 2mg/kg b.wt. I/M and in group III butorphanol @ 0.2 mg/kg b.wt. I/M was administered with barium sulphate contrast meal.

*Corresponding author: itsmepriyankapandey@gmail.com

Dogs were fasted for 12 hours prior to the study and water was offered adlib. Enemas were not given, so the study would more closely resemble general practice. On the day of examination, dogs were brought to radiology area 1 hour prior to the study. Commercially available barium sulphate powder (80% w/v) was orally administered at a dose rate of 7 ml/kg b.wt in all the animals of group I, group II and group III. Right lateral and ventro-dorsal radiographs were taken at 0 min. (immediately after administering barium sulphate), 30 mins, 60 mins, 120 mins, 240 mins. and after 12 hrs. in all the groups, until contrast medium reached the colon. The animals were restrained manually after the sedative effect was over. Gastrointestinal tract emptying time was considered as the time from administration of contrast medium to the point when contrast medium reached the descending colon.

RESULTS AND DISCUSSION

The gastrointestinal tract emptying time during each study were noted and mean values determined for sedated and non-sedated dogs. There was no statistical difference observed in the body weight (kg) (16.17 ± 1.15 , 16.33 ± 1.39 and 16.67 ± 1.26) and age (months) (17.17 ± 2.04 , 16.50 ± 1.15 and 16.33 ± 1.12) in all the three groups, respectively (Table 1, Fig. 1). On the basis of radiographic interpretation there was a significant difference was observed in gastrointestinal tract emptying time (Hrs) in all the three groups ($3.92^a \pm 0.35$, $10.00^b \pm 0.65$ and $9.25^b \pm 0.89$, respectively) (Table 1, Fig. 1). Lower doses of butorphanol are used for Barium Sulphate Contrast studies (Spillmann and Candido, 2019). Hence the total gastrointestinal tract emptying time was significantly prolonged by use of triflupromazine hydrochloride and butorphanol in dogs.

In the present study no statistical difference was observed in the body weight (kg) and age (months) in all the three groups. Bourreau *et al.* (2022) reported that gastric emptying rate is inversely related to body weight in dog breeds of different size and age. On the basis of radiographic interpretation there was a significant difference in gastrointestinal tract emptying time (hrs) in all the three groups. Burns and Fox (1986), Patricia *et al.* (1988) and Kumar *et al.* (2012) also observed the prolong effect of triflupromazine, acepromazine and butorphanol on positive-contrast upper gastrointestinal tract transit time in dogs. In this study normal GIT emptying time was 3.92 ± 0.35 hrs. Funkquist and Garmer (1967), Obrien (1978) and Miyabayashi *et al.* (1986) also reported that triflupromazine and butorphanol were used to compare the gastrointestinal tract emptying time using barium sulphate contrast meal in dogs with non-sedated dogs and reported total emptying time in the normal dog is 4.7 hrs. Study

Table 1. GIT emptying time of barium sulphate contrast meal

Parameters	Group-I	Group- II	Group- III
Body Weight (Kg)	16.17 ± 1.15	16.33 ± 1.39	16.67 ± 1.26
Age (Months)	17.17 ± 2.04	16.50 ± 1.15	16.33 ± 1.12
GIT Emptying time (Hours)	$3.92^a \pm 0.35$	$10.00^b \pm 0.65$	$9.25^b \pm 0.89$

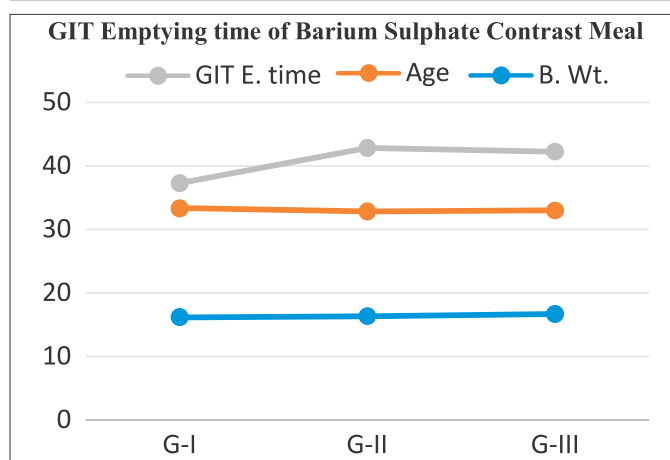


Fig. 1. GIT Emptying time of Barium Sulphate Contrast Meal in Different Groups

interpreted that if sedation is required low dose of butorphanol (0.05 mg/kg) may be used for barium sulphate contrast study. Scrivani *et al.* (1998) observed the same results. Evans and Laufer (1981) also recorded ventro-dorsal and right lateral recumbency radiographic views to evaluate total GIT emptying time in dogs.

CONCLUSION

The triflupromazine hydrochloride and butorphanol administration prolonged the GIT emptying time, decreased GI motility, and facilitated non-manual restraint for duration of the examination. Therefore, recommendations have been made to avoid the use of tranquilizers and sedatives when performing gastrointestinal contrast procedures. When animals in unfamiliar to environments may become anxious, fractious, and difficult to handle sedation may be required. For oral barium contrast medium study low dose of Butorphanol are used because it provides less GIT emptying time as compare to Triflupromazine hydrochloride. In summary, the total gastrointestinal tract emptying time was significantly prolonged by use of and triflupromazine hydrochloride and butorphanol in dogs as compare to non-sedated dogs. The study was useful to evaluate the effect of sedatives on the total GIT emptying time once a control value has been established.

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