SUCCESS STORY

OF

PROJECT UNDER RASHTRIYA KRISHI VIKAS YOJNA (RKVY)

ON

Project name: Establishment of Modern Pathological Laboratory with Special Reference to Animal Tumour Diagnosis

Department of Veterinary Pathology, COVSc

Lala Lajpat Rai University of Veterinary and Animal Sciences

Hisar-125004 (Haryana)

1. **Project title:** Establishment of Modern Pathological Laboratory with Special Reference to Animal Tumour Diagnosis [**Scheme No.:** 4016-C (g)-VPT-1-OA (RKVY)]

2. **Category:** Animal Husbandry (Specific innovative scheme)

3. **Challenges:**

Cancers are number two killer disease in humans being secondary to cardio-vascular disorders. About one million new cancer cases are being reported in India every year as per report of Indian Council of Medical Research. Such data are not available for animal cancer cases in the country owing to lack of advance diagnostic facilities for diagnosis of tumours in animals. However, the domestic animal tumor cases recorded and diagnosed on the basis of histopathology at Department of Veterinary Pathology, LUVAS, Hisar for the last more than 25 years have shown rise in tumors in animals. It seems that systematic study at regional or national level to find out the specific pattern of tumor occurrence in animals is sparse in India. But considering the frequency of reports on animal neoplasms in the recent past, including horn cancer, papilloma, fibroma, leiomyoma, lymphosarcoma, canine transmissible venereal tumour, mammary tumour and several others, one can discern the indications of the ascending tendency of tumours in animals. The frequency of clinical cases of tumours in different species of animals is 5-6 per week at Department of VCC, LUVAS, Hisar. The mammary tumors are also causing production losses in the domestic animals that exert a negative impact on milk quality, quantity, animal health, and thus may reduce profits to individual farmers and dairy industry. Therefore, the diagnosis of tumours will immensely benefit the livestock farmers. Unlike humans, evolution and application of advanced diagnostic techniques for early detection of tumors in animals have rarely been attempted, though molecular techniques in the diagnosis and prognosis of animal neoplasms are widely used in Western countries. Therefore, early diagnosis of tumours in animals and research on molecular characterization of neoplastic cells will be of clinical help to take corrective or remedial measures for prevention and finding assessment of cancer therapies in animals.

4. **Initiative:** Following activities were undertaken in the project to address the above mentioned challange

   i. Histopathological/Cytological examination based on light microscopy of tissue biopsies/cytological specimens to diagnose benign and malignant tumours

   ii. Immuno-histochemical techniques on neoplastic cells such as immunoperoxidase

   iii. Biochemical analysis of blood samples of tumour affected animals from diagnostic/prognostic points of view

   iv. Maintenance of tumour registry and repository regarding various tumours prevalent in Haryana State

   v. Imparting training to Veterinary Surgeons of Haryana state Animal Husbandry Department to strengthen the state diagnostic units with particular reference to animal tumours conditions

5. Key results/insight/interesting facts:

Facilities generated:

a. The renovation of the modern pathological laboratory i.e. PG Lab-1 & II, Histo pathology laboratory, Media room and Associated Faculty rooms (@Rs. 8.82 lakhs) was completed successfully in the year 2015-16.

b. Strengthening of diagnostic services primarily related to immunohistochemistry

Instruments Purchased under the project:

Sr. No	Name of the equipment/instrument		Use
1.	**Five Header Research Microscope with accessories (Now Deca-headed)**		For interpretation of histopathological slides
2.	**Thermocycler (Now transferred to FMD Laboratory)**		For diagnosis of tumors and infectious diseases by making multiple copies of DNA by Polymerase chain reaction
3.	**GelDoc system**		For photography of Polymerase chain reaction
4.	**Refrigerated centrifuge**		For centrifuge of blood/tissue homogenate samples
5.	**Electophoresis units** (Both Horizontal and Vertical)		To run the Gel for diagnosis of tumors and infectious diseases
6.	**Spinix/microcentrifuge and Vortex mixture**		For centrifugation and mixing of solutions

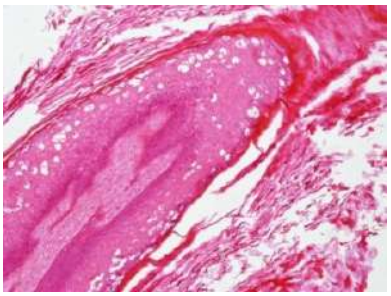
9.	**Quartz distillation unit**		For preparing deionized and high purity distilled water
10.	**Deep freezer (-20^{o}C)**		For storing the specimens
12.	**Electronic pH meter**		For testing pH
13.	**Electronic weighing balance**		For weighing chemicals
14.	**Air Conditioner with stabilizer (Two)**	-	To maintain constant temperature for the laboratories

Research Findings: (with good quality pictures**)**

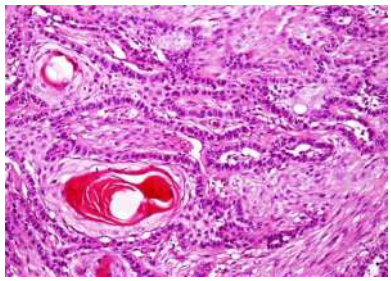
- A total of **103 different tissue biopsies in different species of animals** (Dog - 41 cases, Cow - 14 cases, Buffalo - 38 cases, Horse/Mule/Mare - 10 cases) suspected of tumour conditions were processed up to year 2015-16 for histopathological/cytological examinations to diagnose benign and malignant tumours. Major tumor conditions found were transmissible venereal tumour, ovarian haemangiosarcoma, squamous cell carcinoma and lipoma in dogs; squamous cell carcinoma, papilloma, leiomyoma, fibroma and ameloblastoma in bovines and squamous cell carcinoma, fibrinomatous epulis in equines. The incidence of epithelial tumours was higher as compared to mesenchymal tumours. Malignancy was more severe in ocular squamous cell carcinoma. The results were communicated to the concerned farmers/owners/diagnostic laboratories.
- The haemosiderin laden macrophages were demonstrated by Pearl's staining as blue granules in ovarian haemangiosarcoma. Varying amount of collagenous stroma between vascular channels was demonstrated by Masson's trichrome staining in ovarian haemangiosarcoma.
- AgNOR staining was done in squamous cell carcinoma and it revealed the presence of dark brown dots exclusively localized throughout the nucleolar area.
- Toluidine blue staining was performed on Transmissible Venereal Tumour (TVT) case to differentiate it from mastocytoma.
- Experimental work on following was undertaken

1) Pathobiological studies on bovine neoplasms with special reference to epithelial tumours and findings were

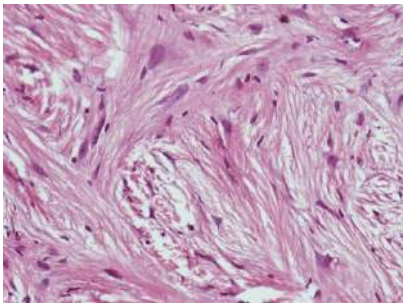
- ✓ Buffalo was most affected species as compared to cattle regarding the occurrence of epithelial tumours. The clinical examination revealed that buffalo and cattle of 5-8 years of age and that also female were mostly affected. The clinical examination revealed that buffalo and cattle of 5-8 years of age and that also female were mostly affected. Clinical signs observed were anorexia, weakness, epistaxis, swelling in cases of oral tumours. Bleeding, purrulant conjunctivitis, swelling and apparent blindness were recorded in cases of eye tumour. Swelling, ulceration, bleeding was recorded in rest of the clinical cases. Buffalo was most affected as compared to cattle
- ✓ Impression smears from tumour biopsy were prepared and they were stained with Giemsa /Leishman/Papanicolau staining methods. Impression smears examination revealed pleomorphic cells/nuclei, inflammatory cells in case of malignant tumours like squamous cell carcinoma.
- ✓ Histopathological examination of biopsies revealed presence of various types of tumours of epithelial as well as mesenchymal tissues. Epithelial tumours include Papilloma (6), Squamous Cell Carcinoma (7), Adenoma (2) and Ameloblastoma (2). Mesenchymal tumours include Fibroma (3), Leiomyoma (4), Mastocytoma (1), Hemangioma (1), Hemangiopericytoma (1), Myxoma (1) and Fibromatous Epulis (1). A case of Sertoli Cell tumour was also diagnosed.



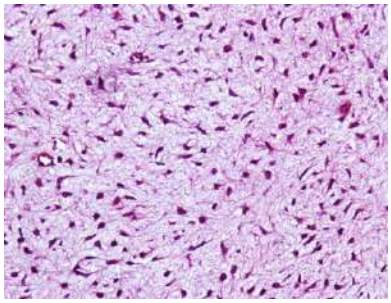
Papilloma (H&E X 400)



Squamous cell carcinoma (H&E X 400)

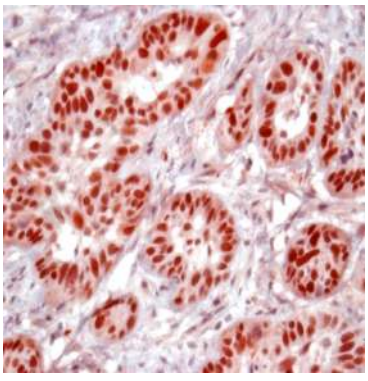


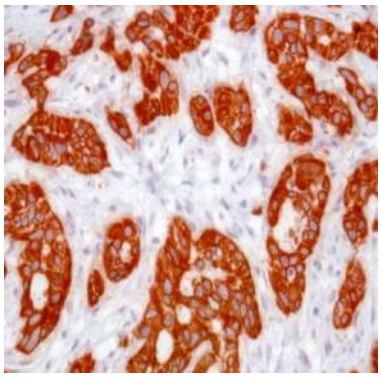
Leiomyoma (H&E X 400)



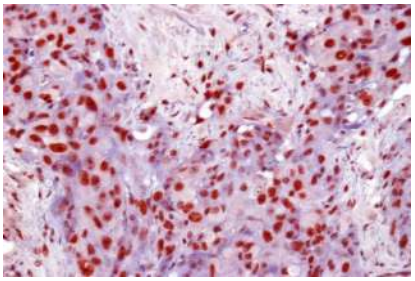
Myxoma (H&E X 400)

- ✓ Squamous cell carcinoma shows strong nuclear reaction for p53 (DO-7) and strong cytoplasmic reaction for pan-cytokeratins (pck-26). Whereas, few cases of papilloma shows cytoplasmic reaction for p53 (DO-7) and for pan-cytokeratins (pck-26).

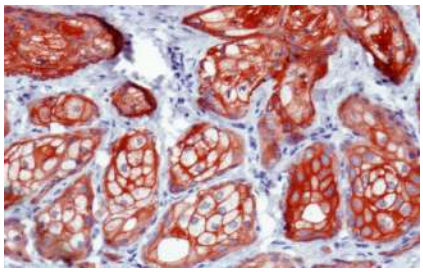




Squamous cell carcinoma (eye)
Intense nuclear p53 mmunoreactivity
(X400)



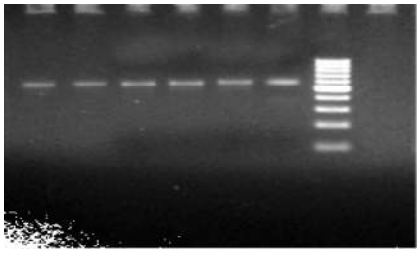
Squamous cell carcinoma (eye)
Strong cytoplasmic PCK immunoreactivity
(X400)



Sertoli cell tumour
Moderate to intense nuclear p53 immunoreactivity
(X400)

Sebaceous adenoma
Intense cytoplasmic PCK immunoreactivity
(X400)

2) Isolation of the genomic DNA from the selected biopsy samples that were confirmed as epithelial tumours by histopathological/immunohistochemistry methods was done by using kit method (Quigen). Quality check of isolated DNA was done by Nanodrop & agarose gel electrophoresis method. Polymerase Chain Reaction for p53 tumour suppressor gene was performed. Visualization of amplified product by Agarose Gel Electrophoresis revealed specific target products of p53 genes.



PCR amplification of p53 specific target products of p53 gene of malignant tumours.

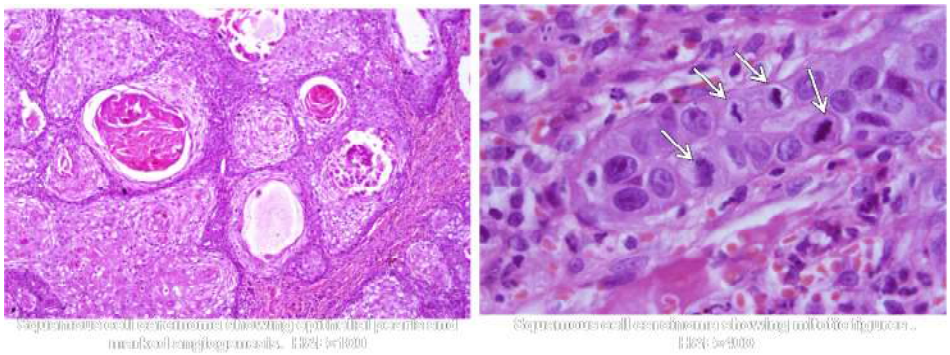
- ✓ Studies on incidence of tumours: One hundred and eleven different cases of tumours were recorded from the past ten years data available in the department of Veterinary Pathology (2005-2014). Most affected species was found to be buffalo followed by cattle. Sexwise females were found to be more affected with tumour conditions in comparison to males. Fibroma was the most prevalent tumour followed by Squamous cell carcinoma, Myxoma, Leiomyoma and Adenoma. Incidence of tumours affecting the oral cavity were highest followed by eye, udder, horn and genital organs.

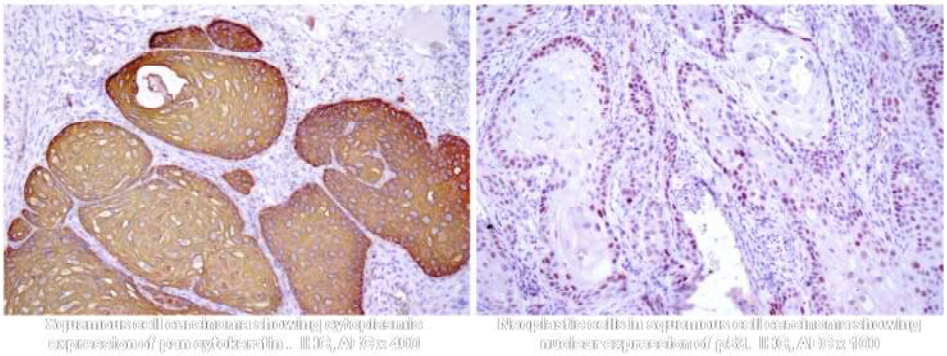
3) Molecular and Immunopathological Studies on Canine Mammary Tumours with Special Reference to Role of Heavy Metals in Carcinogenesis it was observed that:

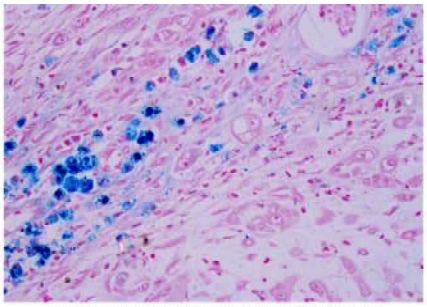
- ✓ 50 different biopsy samples of revealed tumours of different types viz. transmissible venereal tumour, ovarian haemangiosarcoma, squamous cell carcinoma and lipoma in dogs; papilloma and fibroma/leomyoma in bovines and fibromatous epulis in equines were diagnosed.
- ✓ Gross and histopathological examination of canine mammary tumours revealed simple adenocarcinoma, adenocarcinoma papillary type, adenocarcinoma tubulo-papillary type, adenocarcinoma-spindle cell variant, complex carcinoma, adenoma, lipoma, carcinosarcomas and osteosarcomas.
- ✓ One case of transmissible venereal tumour was also noticed in mammary gland.

- ✓ Canine mammary tumours (CMTs) were mainly noticed in German Sephered, Spitz and Labrador breeds in middle age group of 6-10 years.
- ✓ Two cases (carcinosarcoma and lipoma) were also noticed in male Labrador dogs. Size and age did not reveal any correlation with tumour type and prognosis.
- ✓ Radiographical findings revealed distant metastasis in about 60% CMT and were categorised as Stage V indicating poor prognosis.
- ✓ Histopathological classification revealed 44.7% cases of carcinosarcomas, 26.3% carcinomas, 10.5% sarcomas, 15.7% benign tumours and one case of transmissible venereal tumour (TVT) indicating higher incidence of malignant CMT tumours (81.5%) mostly belonging to Grade II category.
- ✓ Solid and anaplastic carcinomas showed severe malignancy characterized by undifferentiated neoplastic cells. Special variants forms of carcinomas like lipid rich, spindle, mucinous and ductal carcinomas were also observed.
- ✓ The mean AGNOR count was significantly lower in benign than the malignant tumours with the highest count in Grade III followed by Grade II and Grade I. Serum sex steroid hormones in both benign and malignant tumours affected animals were significantly higher as compared to tumour free animals, though within normal peak range.
- ✓ Mean values of iron, zinc, mercury, cadmium and copper were significantly higher in tumour tissues and serum samples as compared to the values of tumour free mammary dogs.
- ✓ Positive nuclear immunoreactivity of ERα of varying intensity was observed in 52.6% cases of CMT. ERα reactivity score was more in benign tumours and decreased with increasing grade and malignancy.
- ✓ Over-expression of mutant p53 protein by IHC and RT-qPCR was found in osteochondrosarcomas and carcinosarcomas of higher grade indicating the significant role of p53 mutation in mesenchymal origin CMT.
- ✓ Positive immunoreactivity for PCK was found in epithelial origin tumours with intense reactivity in the majority of cases.

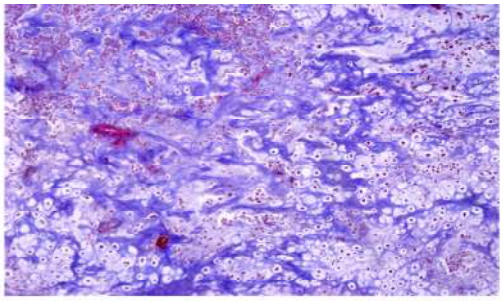
- ✓ Cytokeratin expression was found to be useful to differentiate luminal epithelial, myoepithelial and mesenchymal tumours and decreased with increased malignancy/grade.



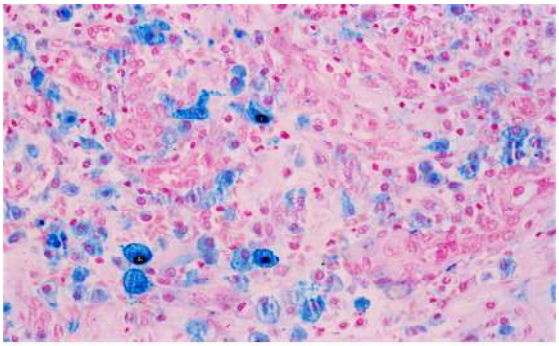




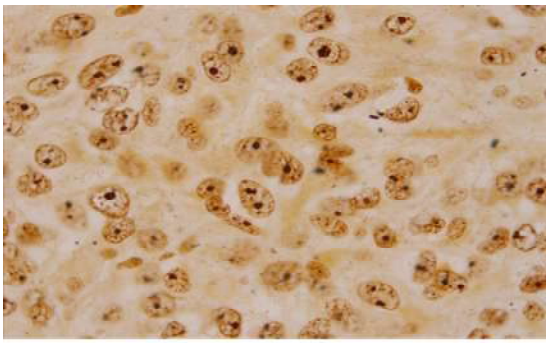
PEARL'S STAIN: OSTEOSARCOMA (MAMMARY GLAND)



MASSON'S TRICHROME STAIN: OSTEOSARCOMA (MAMMARY GLAND)



HAEMAGIOSARCOMA (MAMMARY GLAND): PEARL'S STAIN



CARCINOSARCOMA(MAMMARY GLAND): AGNOR STAINING

- ✓ Retrospective studies of 88 biopsies revealed maximum tumours in dogs were of epithelial origin (mammary tumours and squamous cell carcinoma) followed by mesenchymal tumours (fibroma/fibrosarcoma) and TVT in the middle age groups with equal distribution in both male and female.

6. Impact:

i. Physical

a) The renovation of the modern pathological laboratory i.e. PG Lab-1 & II, Histo pathology laboratory, Media room and Associated Faculty rooms (@Rs. 8.82 lakhs) was completed successfully in the year 2015-16.

b) Strengthening of diagnostic services primarily related to immunohistochemistry

ii. Social and economical impacts

Social:-

a. Maintenance of tumour registry and repository regarding various tumours prevalent in Haryana State

b. Imparted one training to Veterinary Surgeons of Haryana state Animal Husbandry Department to strengthen the state diagnostic units with particular reference to animal tumours condition. Training was imparted to fifteen veterinary Surgeons nominated by Department of Animal Husbandry and Dairying, Haryana from 26-30 May, 2014 in the Department of Veterinary Pathology. A training manual and CD was also released for the participants.

Participants along with Chief Guest, Worthy Vice Chancellor Maj Gen (Retd.) Dr. Shri Kant Sharma; Director of Research Dr. Ravindra Sharma; Dean COVSc. Dr. Gurdial Singh; Prof. & Head Department of Vety. Pathology- cum- Training Director Dr. K.K. Jakhar; Training Coordinators Dr. R. P. Gupta and Dr. Vikas Nehra in the five days training on "Postmortem Examination, Dispatch of Specimens and Pathological Diagnostic Techniques with Special Reference to Tumours" was organized by the Department of Veterinary Pathology, LUVAS, Hisar from 26-30 May, 2014

Economical impacts:-

Specific diagnosis of tumours with prognosis will help farmers by the timely treatment of the animals, accordingly thereby saving their precious animals and money.

7. Lessons Learned:

a. Regular maintenance of tumour registry and repository regarding various tumours prevalent in Haryana State should be continued for the better presentation of the tumour conditions affecting animals

b. Work on tumour markers should be continued so that specific tumour markers of diagnostic and prognostic significance can be identified in tumours of animal origin.

8. Supporting quotes and Images: Nil

9. Additional Information:

Total Budget of the project: Rs. 108.50/- lacs

First Instalment: From office of Director Agriculture, Haryana vide endst. No. 1073-76 dated 16-08-2013 and further endorsed by Director of Research, LUVAS, Hisar vide Endst. No. DR/CR/2013/1932-37 dated 29-08-2013 (Rs. 35,75,000/-)

Second and subsequent Instalments: Not Released

Time duration: Three years (From 2013-14 to 2016-17)- Project completed in March, 2016

Total No. of Beneficiaries: Beneficiaries were mainly pet and domestic animal owners whose number was 106. Contact nos. of selected beneficiaries along with other details is as under:

S. No.	Name & Father's name	Address	District	Phone/ Mobile
1.	Satbir (10-3274)	Hansi	Hissar	9416216807
2.	Bimmi (1-5656)	Hissar	Hissar	8950494404
3.	Vinod (1-5917)	Fatehabad	Fatehabad	9466639483
4.	Diwan Chand (2-6079)	Bhabalpur, Hisar	Hisar	9466329531
5.	Vijay S/o Satbir (6-9947)	Rohtak	Rohtak	9992638549
6.	Maman Saloni (9-2410)	Jhajjar	Jhajjar	8689051940
7.	Bhupender (9-2529)	Delhi Police	Delhi	9958198822
8.	Rajesh (10-210)	-		-
9.	Rajesh (10-3026)	Bhiwani	Bhiwani	9992672690
10.	Umed Singh (8-1986)	Piranwali, Sirsa	Sirsa	9466373941
11.	Yogesh (9-2694)	Sirsa	Sirsa	9812000713
12.	Ombir (9-3403)	Alwar	Alwar	88104532171
13.	Krishan Chander Chaudhary (9-3736)	Hisar	Hisar	8684908619
14.	Rahul (1-6117)	Kirmara, Hisar	Hisar	8816006801
15.	Roshan (3-7751)	Chater, Jind	Jind	9671481872
16.	Dr. Divya	TVCC, Hissar (One case of dog)	Hissar	8398051249

Work Contribution by P.I. and Co-P.I.s:

Name	Designation	Work done
Dr. R. P. Gupta	P.I	a) Overall monitoring of the project. b) Histopathological/Cytological examination based on light microscopy of tissue biopsies/cytological specimens (for two months) to diagnose benign and malignant tumours c) Experimental work as major advisor on "Pathobiological studies on bovine neoplasms with special reference to epithelial tumours" d) Experimental work as major advisor on "Molecular and Immunopathological Studies on Canine Mammary Tumors with Special Reference to Role of Heavy Metals in Carcinogenesis" e) Acted as Training Coordinator in one of the training imparted to the field veterinarians and compiled the training manual of the same
Dr. K. K. Jakhar	Co-P.I	a) Histopathological/Cytological examination based on light microscopy of tissue biopsies/cytological specimens to diagnose benign and malignant tumours b) Acted as Training Director in one of the training imparted to the field veterinarians
Dr. Vikas Nehra	Co-P.I	a) Histopathological/Cytological examination based on light microscopy of tissue biopsies/cytological specimens to diagnose benign and malignant tumours b) Helped the P.I. in above mentioned experimental work c) Acted as Training Coordinator in one of the training imparted to the field veterinarians and compiled the training manual of the same d) Prepared all the monthly progress reports and RDMIS Performa, Success story etc. related to the project.

Dr. (Mrs.) Deepika	Co-P.I	a) Histopathological/Cytological examination based on light microscopy of tissue biopsies/cytological specimens to diagnose benign and malignant tumours b) Conducted her Ph.D. research work on "Molecular and Immunopathological Studies on Canine Mammary Tumors with Special Reference to Role of Heavy Metals in Carcinogenesis" c) Helped the P.I. in above mentioned experimental work d) Maintained tumour registry and repository e) Helped in organization of one of the training imparted to the field veterinarians
Dr. Prem Singh (From Department of Veterinary Surgery and Radiology)	Co-P.I	a) Provided the much needed samples of different timorous conditions after their extraction from the affected clinical cases b) Helped in organization of one of the training imparted to the field veterinarians

Acknowledgement

Dr. Babu Lal Jangir and Dr. (Mrs.) Chandratre Gauri A., Assistant Professors of Department of Veterinary Pathology were also associated with the research scheme and performed the following duties:

a) Histopathological/Cytological examination based on light microscopy of tissue biopsies/cytological specimens to diagnose benign and malignant tumours
b) Helped the P.I. in above mentioned experimental work.
c) Helped in organization of one of the training imparted to the field veterinarians

10. Checklist:

No.	Question to consider	Yes	No
1.	Is the story interesting to the target audience of the project/activity report?	Yes	-
2.	Does the story explain what new insights the project brings? What is the main lesson learned from the story? Does the story describe a key insight on what works and what doesn't and something that future projects could build on	Yes	-
3.	Does the story describe the outcomes the project produced and the people who are benefitting? What changes-in skills, knowledge, attitude, practice or police has the project brought about and who is benefitting from these changes?	Yes	-
4.	Does the story make a compelling point that people will remember? Does the story show how the project makes a difference to improving livelihoods and lessening poverty?	Yes	-
5.	Does the story provide an interesting fact that people will remember? For example, how yields increased, how many hectares of land could become more productive from the innovation or technology?	-	-
6.	Does the story explain what kind of impact this innovation or technology could have if scaled up?	Yes	-
7.	Does the story show which partners contributed and how?	Yes	-

8.	Does the story include quotes from stakeholders or beneficiaries?	-	-
9.	Have I proved links to other media (journal articles, website news, newsletter blogs, annual reports of other Programme/project) that also feature this story?	-	-
10.	Have I provided contact details of people who can provide more information?	Yes	-

P. I.
(Dr. R. P. Gupta)

Prof & Head

SUCCESS STORY

OF

PROJECT UNDER RASHTRIYA KRISHI VIKAS YOJNA (RKVY)

ON

Project name: Establishment of immunopathological diagnostic laboratory to provide quality services to the livestock and poultry owners

Department of Veterinary Pathology, COVSc

Lala Lajpat Rai University of Veterinary and Animal Sciences

Hisar-125004 (Haryana)

1. **Project title:** Establishment of immunopathological diagnostic laboratory to provide quality services to the livestock and poultry owners [**Scheme No.:** 4026-C (g)-VPT-2-OA (RKVY)]

2. **Category:** Animal Husbandry (Infrastructure and Assets Development (Setting up of laboratories and testing Facilities)

3. **Challenges:**

Department of Veterinary Pathology has a mandatory responsibility to study pathology, pathogenesis and diagnosis of the diseases of livestock and poultry healths that are reared by their owner's which belongs primarily to small and marginal farmers. Immunopathological laboratory facilities extended to them plays an integral part in this. The present project will be helpful to the farmer's in in developing wide range of clinical immunologic testing procedures for diagnosis and monitoring of diseases where the pathogenesis involves components of the immune system and will deliver high quality, responsive services to clinicians and farmers. Cost-effective immuno-pathological diagnostic services and in turn economic benefits to farmers. Quickly provide with accurate and efficient results to the veterinarians and diseased animal caretakers especially farmer's. It will aim for the sharpening diagnosis and understanding the pathogenesis of immunopathological diseases. It will provide wide range of technical services to the different departments of the University like immunohistochemical (IHC) techniques including immunoperoxidase and immunoflourescence. The project was planned with the following objectives:

1. To establish immunopathology laboratory and generate immunopathological diagnostic techniques in the department.
2. To provide diagnostic services to the livestock and poultry owners regarding immunopathological disorders/diseases

4. **Initiative:**

Following activities were undertaken in the project to address the above mentioned challange:

i. To generate the facilities and techniques for the prompt diagnosis of immunopathological disorders/diseases.
ii. To identify immunopathological disorders in which immunological tests can be used for diagnosis of the diseases of livestock and poultry.
iii. To provide diagnostic services to the Teaching Veterinary Clinical Complex of the University and also to provide services and support for research done in the other Departments of the University.
iv. To conduct the preliminary studies on the effects of certain immunomodulating agents in poultry/laboratory animals after prior permission from institutional animal ethics committee so that it can be directly linked to the improvement of livestock health.
v. Maintenance of record of prevalence of immunopathological diseases in Haryana state.
vi. To provide training to field veterinarians for awareness about immunopathological disorders

5. **Key results/insight/interesting facts:**

Facilities generated:

a. Immuno-pathological laboratory was established (at cost of Rs. 3.25 lacs)
b. Purchase of equipments worth Rs. Rs. 24,19,617/- were undertaken
c. Strengthening of diagnostic services primarily related to immunopathological techniques, immunohistochemical techniques and fluorescent microscopic examination of infectious/non-infectious diseases/disorders of the animals

Instruments purchased under the project:

✓ Purchase of equipments worth Rs. Rs. 24,19,617/- were undertaken

S. No.	Name of the Equipment	Quantity
i.	Fluorescent microscope along with accessories	One
ii.	B.O.D. incubator	One
iii.	Shaker	One
iv.	Magnetic stirrer	One
v.	Micro pipettes	Two
vi.	Research microscopes	Two
vii.	Refractometer	One
viii.	Centrifuge machine	One
ix.	Blood cell counter	One
x.	Digital Vernier Calliper	One
xi.	Air Conditioner with Stabilizer	One
xii.	Microscope with battery	One
xiii.	Refrigerator	One
xiv.	Tissue floatation water bath	One
xv.	Colony counter	One

Fluorescent microscope

Shaker and Magnetic stirrer

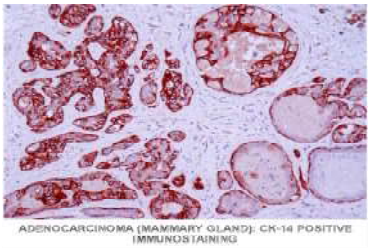
BOD Incubator

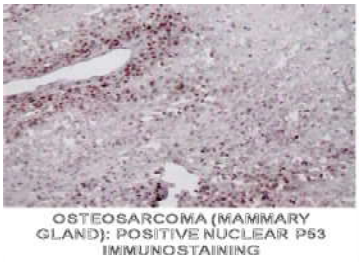
Refrigerator

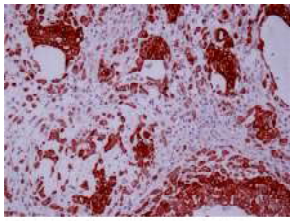
Research Findings: (with good quality pictures**)**

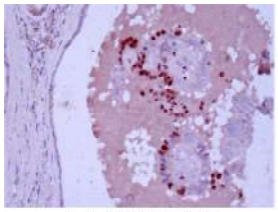
✓ One hundred and sixty two (162) serum/plasma samples were processed for examining their immune status and accordingly advice was given to the farmers/owners. These samples revealed hyperproteinemia, hyperglobulinemia and hypoproteinemia, respectively.

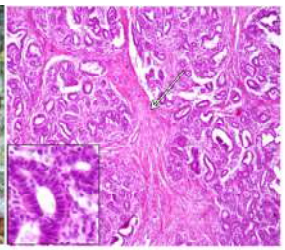
- ✓ *Tinospora cordifolia* (Giloy) extract (@1 gm/kg feed) supplementation enhanced humoral immunity in chicks suggesting its immunomodulatory effect. Probiotic *Bacillus subtilus* (@10^9 CFU/bird/day) supplementation also showed immunomodulatory effect. *Tinospora cordifolia* extract and probiotic supplementation enhanced cell mediated immunity in chicks.
- ✓ Vitamin C (@100 mg/kg b. wt.) supplementation enhanced the humoral immune response and ameliorated the oxidative stress due to imidacloprid toxicity in chickens.
- ✓ *Emblica officinalis* (Amla) dry fruit supplementation (@10 gm/kg feed) enhanced humoral immunity in chicks suggesting the immunomodulatory effect of amla extract. Delayed type hypersensitivity response against Dinitrochlorobenzene was significantly higher in amla supplemented groups as compared to non-supplemented groups indicating enhanced cell mediated immune response due to amla supplementation.
- ✓ Thiacloprid toxicity (@1/5th MTD) lead to impairment of cell mediated immunity and increased the severity of the *Salmonella* Gallinarum infection in broiler chicken


ADENOCARCINOMA (MAMMARY GLAND): CK-14 POSITIVE IMMUNOSTAINING

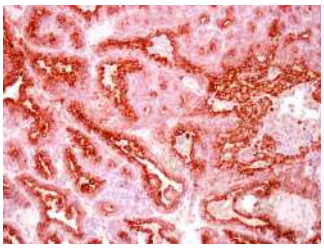

OSTEOSARCOMA (MAMMARY GLAND): POSITIVE NUCLEAR P53 IMMUNOSTAINING

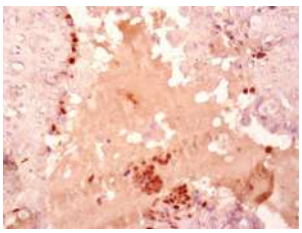

OSTEOSARCOMA(MAMMARY GLAND): PCK STAINING


ADENOCARCINOMA (MAMMARY GLAND): POSITIVE NUCLEAR ESTROGEN ALPHA IMMUNOSTAINING



- ✓ Immunohistochemical studies on 38 canine mammary tumours revealed that estrogen receptor alpha (Erα) immunoreactivity was found to be more in benign tumours and decreased with increased grade and malignancy, indicating loss of estrogen receptors in malignant CMT which indicates its usefulness as prognostic factor.
- ✓ Immunohistochemical staining & real time PCR analysis of p53 gene revealed role of p53 gene in pathogenesis of mesenchymal origin CMT, but limited role in epithelial tumours.
- ✓ Immunohistochemical expression of cytokeratins (PCK and CK14) was useful in diagnosis of epithelial origin metastatic tumours as well as in complex and undifferentiated malignant tumours.





Low immunoreactivity of CK-14 in anaplastic carcinoma of mammary	ERα nuclear immunoreactivity in epitheial cells of mammary gland

6. Impact:

iii. Physical

Immune-pathological laboratory was established (at cost of Rs. 3.25 lacs)
Purchase of equipments worth Rs. Rs. 24,19,617/- were undertaken

Newly established Immune-pathological laboratory (@ Rs. 3.25 lacs)

iv. Social and economical impacts

Social:-

- ✓ Five days training on "*Postmortem Examination, Dispatch of Specimens and Pathological Diagnostic Techniques with Special Reference to Immunopathological Diseases*" was organized for 20 Veterinary Surgeons. Dr. K.K. Jakhar, Prof. & Head acted as Training Director whereas Dr. Vikas Nehra and Dr. Babu Lal Jangir acted as Training Coordinators. This training had provided 'Hands on Training" to Field Veterinarians for postmortem examination and dispatch of specimens along with updating their knowledge on pathological diagnostic techniques with special reference to immunopathological diseases. The Training manual and CD was also released for the participants.

Trainee Veterinarians with Worthy VC LUVAS

Maj Gen (Dr) Shri Kant VC, LUVAS addressing the Trainees

Economical impacts:

A. Establishment of such a laboratory will be helpful in developing wide range of clinical immunologic testing procedures for diagnosis and monitoring of diseases where the pathogenesis involves components of the immune system thereby delivering high quality, responsive services to clinicians and farmers.
B. Cost-effective immune-pathological diagnostic services and in turn economic benefits to farmers

7. Lessons Learned:

i. Research on clinical specimens with the aims of diagnosis and understanding the pathogenesis of immunopathological diseases will be helpful to the clinicians for treatment of animals and farmers by saving their animals through specific treatments.
ii. To provide wide range of technical services to the different departments of the University like immunohistochemical (IHC) techniques including immunoperoxidase and immunoflourescence.

8. Supporting quotes and Images: Nil

9. Additional Information:

Total Budget of the project: Rs. 38.50 lacs

First Instalment: Rs. 34,00,000/-

Second Instalment: Rs. 4,50,000/-

Time duration: Three years i.e. from 2014-15 to 2016-17 (Project completed on March, 2017)

Total No. of Beneficiaries: Beneficiaries were mainly pet and domestic animal owners whose number was 162. List of beneficiaries along with other details is as under:

Sr. No.	Name of owner & Department of VCC case no.	Address
1.	Bajrang Kanar (4/9368)	Bhadra, Distt. Hanumangarh, Rajasthan
2.	Naresh (4/9375)	Birdana, Distt. Jhajjar
3.	Abhishek (4/9377)	Distt. Rohtak
4.	Vijay Pal (4/9379)	Mamanpura, Distt. Hisar
5.	Rishipal (5/10332)	Distt. Kaithal
6.	Rajapal (5/10333)	Distt. Bhiwani
7.	Rajesh (5/10335)	Distt. Jind
8.	Bhagwan (6/11495)	Dokla, Distt. Jhajjar
9.	Vikash Kumar (6/11497)	Chobara, Distt. Fatehabad
10.	Sarif (6/11499)	Rajli, Distt. Hisar
11.	Ramesh (6/11526)	Miran, Distt. Bhiwani
12.	Vakil (6/11620)	Distt. Mansa, Punjab
13.	Vikram Kajal (6/11626)	-
14.	Harashvardhan (6/11629)	Tosham, Distt. Bhiwani
15.	Rambir (7/919)	Distt. Sonepat
16.	Kapoor (7/920)	Distt. Jind
17.	Sakhbir (7/921)	Distt. Bhiwani
18.	Rajan (7/925)	Narwana
19.	Sanjay (7/927)	Distt. Jhajjar
20.	Ramchander (8/156)	Khustipura, Distt. Reawri
21.	Rattan Singh (8/2218)	Chikanwas, Distt. Hisar
22.	Hawa Singh (8/2220)	Distt. Rohtak
23.	Sunil Kumar (8/2223)	Distt. Hisar
24.	Chiranji (8/2226)	Badchapper, Distt. Hisar
25.	Vijay (8/2227)	Hansi, Distt. Hisar
26.	Satbir (9/3557)	Sahapur, Distt. Hisar
27.	Wazir (9/3558)	Bhuna, Distt. Fatehabad
28.	Ram Niwas (9/3560)	Distt. Hisar
29.	Krishan Singh (9/3561)	Sangrur
30.	Mahender (9/3562)	Distt. Bhiwani

31.	Lilu Ram (9/235)	Bhadra, Distt. Hanumangarh
32.	Mukesh (10/4888)	Distt. Rohtak
33.	Rajesh (10/4891)	Bahlamba, Distt. Rohtak
34.	Sohan Lal (10/4892)	Nohar
35.	Bajrang (10/4895)	Distt. Churu, Rajasthan
36.	Sandeep (10/4897)	Distt. Jhajjar
37.	Sachin (10/4899)	Distt. Hisar
38.	Ramesh (11/5823)	Distt. Bhiwani
39.	Naresh (11/5824)	Distt. Fatehabad
40.	Ramawtar (11/5829)	Distt. Bhiwani
41.	Mehwa Singh (11/5832)	Distt. Bhiwani
42.	Sanjay (11/5834)	Mohila, Distt. Bhiwani
43.	Dara Singh (11/5837)	Distt. Hanumangarh, Rajasthan
44.	Rakesh (12/6457)	Distt. Bhiwani
45.	Satish (12/6821)	Uchana Mandi
46.	Ramkesh (12/6826)	Distt. Hisar
47.	Rajinder (12/6828)	Bhagana, Distt. Hisar
48.	Mahender Singh (12/6896)	Bhadra, Distt. Hanumangarh, Rajasthan
49.	Narender (12/6907)	Distt. Rohtak
50.	Narender (12/6909)	Distt. Rohtak
51.	Virender (1/7112)	Malek, Distt. Sonepat
52.	Samsher (1/7118)	Distt. Sonepat
53.	Monu Ram (1/7121)	Distt. Sirsa
54.	Inder Singh (1/7454)	-
55.	Ajmer (1/7455)	Distt. Sonepat
56.	Pawan Kumar (1/7456)	Distt. Hisar
57.	Krishan (1/7462)	Kharod, Distt. Jind
58.	Manvindra (1/7466)	Distt. Bhiwani
59.	Rohtash (1/7477)	Talava, Distt. Jind
60.	Bhawer Ram (2/8560)	-
61.	Dharam Pal (2/8567)	Tohana
62.	Mange Ram (3/9410)	Distt. Hisar
63.	Baljeet (3/9413)	Barampur, Distt. Jind
64.	Ram Mehar (3/9414)	Distt. Hisar
65.	Ram Mehar (3/9415)	Distt. Hisar
66.	Dharambir (3/9423)	Distt. Hisar
67.	Sandeep (3/9426)	Milakpur, Distt. Hisar
68.	Vinod (3/9430)	Distt. Hisar
69.	Krishan (3/9435)	Distt. Hisar
70.	Sanjay (3/9439)	Distt. Jind
71.	Krishan (3/9440)	Kaimari, Distt. Hisar
72.	Naresh (4/10434)	Distt. Jind
73.	Kuldeep (4/10436)	Sangrur
74.	Balraj (4/10445)	Distt. Jind
75.	Vikram (4/10448)	Distt. Hisar
76.	Rajesh (4/10450)	Distt. Hisar
77.	Jitender Singh (5/11503)	Distt. Hisar
78.	Subash Chander (5/11573)	Distt. Hisar

79.	Satish (5/11578)	Distt. Jind
80.	Sandeep (5/11580)	Distt. Bhiwani
81.	Ram Niwas (5/11587)	Distt. Bhiwani
82.	Manphool (5/11692)	Ateli, Mahendragarh
83.	Madan Singh (5/11693)	Distt. Jind
84.	Mahender (5/11697)	Distt. Hisar
85.	Dev Karan (5/11704)	Dabri, Distt. Hanumangarh, Rajasthan
86.	Satbir (6/12648)	Distt. Jind
87.	Kitab Singh (6/12687)	Khurd, Distt. Jind
88.	Himanshu (6/12691)	Distt. Hisar
89.	Subhash ((6/12717)	Distt. Hanumangarh, Rajasthan
90.	Charan Singh (6/12719)	Distt. Hanumangarh, Rajasthan
91.	Krishan Kumar (6/12722)	-
92.	Rajender (6/12723)	Distt. Jind
93.	Ajit (6/12728)	Rakhi Khar
94.	Dharambir Singh (6/12731)	Kaimari road, Distt. Hisar
95.	Omprakash (6/12738)	Distt. Hisar
96.	Rajesh Jaglan (7/717)	-
97.	Sunil (7/748)	Pilani, Distt. Jhunjhunu, Rajasthan
98.	Kashmir Singh (7/769)	Distt. Fatehabad
99.	Yogesh (7/771)	Distt. Jind
100.	Balbir (7/776)	Udaypur, Distt. Jind
101.	Ishwar Singh (7/777)	Kalirawan
102.	Umed Singh (7/783)	Dabra, Distt. Hisar
103.	Surendra (7/914)	Distt. Bhiwani
104.	Surendra (7/920)	Distt. Jind
105.	Amandeep (7/925)	Punjab
106.	Rohtas (7/929)	Distt. Sonepat
107.	Satish (7/935)	Distt. Sonepat
108.	Deyanand (8/2134)	Distt. Jind
109.	Deepak (8/2136)	Distt. Rohtak
110.	Bhupendra (8/2139)	Balamba, Distt. Rohtak
111.	Anil Kumar (8/2141)	Distt. Bhiwani
112.	Sandeep (8/2142)	Distt. Hisar
113.	Sarvan (8/2145)	M. P. Rohi, Fatehabad
114.	Hari Singh (9/3461)	Distt. Churu, Rajasthan
115.	Hanuman (9/3471)	Distt. Hisar
116.	Ramawtar (9/3473)	Distt. Bhiwani
117.	Satish (9/3533)	-
118.	Ramji (9/3539)	Distt. Jind
119.	Inder Singh (9/3545)	Distt. Hisar
120.	Bhajan Lal (9/3549)	Tohana
121.	Sanjay (10/4355)	Jind
122.	Sohan (10/4373)	Kharkadi, Bhiwani
123.	Naresh Kumar (10/4376)	Badale, Hisar
124.	Suresh (10/4380)	Hisar
125.	Kapil (10/4412)	Kiathal
126.	Naresh (10/4413)	Sonepat

127.	Sanjay (10/4415)	Ladwa, Hisar
128.	Balwant (10/4416)	Hisar
129.	Ram Niwas (10/4422)	Hisar
130.	Lakhwinder Singh (10/4423)	Sirsa
131.	Vinod (11/4899)	-
132.	Balbir (11/5322)	Kaimari, Hisar
133.	Prem Chand (11/5323)	Sirsa
134.	Satbir (11/5346)	Rohtak
135.	Satbir (11/5364)	Hisar
136.	Vinod (11/5377)	Atela, Bhiwani
137.	Ajmer (12/7018)	Jind
138.	Kuldeep (12/7048)	Mahendergarh
139.	Hawa Singh (12/7059)	Hisar
140.	Ravikant (12/7062)	Fatehabad
141.	Ramesh Kumar (12/7065)	Manwali
142.	Dilip Singh (1/6804)	Narwana, Jind
143.	Subhash (1/6806)	Kaithal
144.	Tadhiya (1/6807)	Kaithal
145.	Sohi (1/6810)	Hisar
146.	Manjeet (1/6812)	Rohtak
147.	Anil (1/6819)	Hisar
148.	Jai Kishan (1/6820)	Hisar
149.	Raj Kumar (2/7837)	Balsamand, Hisar
150.	Harichand (2/7845)	Nunak, Sangrur
151.	Umed Singh (2/7847)	Bhiwani
152.	Kehar Singh (2/7853)	Neta Khera, Sirsa
153.	Sitaram (2/7854)	Fatehabad
154.	Hansraj (2/7860)	Hansi
155.	Sandeep (2/7862)	Jind
156.	Samsher (2/7863)	Dakal, Narwana
157.	Mahavir (3/8937)	Rishi Nagar, Hisar
158.	Baldev (3/8940)	Fatehabad
159.	Shamsher (3/8948)	-
160.	Ramesh (3/8953)	Churu, Rajasthan
161.	Kuldeep (3/8954)	Sonepat
162.	Ramswaroop (3/8962)	-

Work Contribution by P.I. and Co-P.I.s:

Name	Designation	Work done
Dr. K. K. Jakhar	P.I	f) Overall monitoring of the project. g) Immune-pathological laboratory was established (at cost of Rs. 3.25 lacs). h) Purchase of equipments worth Rs. Rs. 24,19,617/- were undertaken i) Monitored experimental research regarding immune-pathological work j) Acted as Training Director in one of the training imparted to the field veterinarians and compiled the training manual of the same

Dr. Vikas Nehra	Co-P.I	a) Helped the Principal Investigator in the above activities b) Acted as Training Coordinator in one of the training imparted to the field veterinarians and compiled the training manual of the same
Dr. Babu Lal Jangir	Co-P.I	a) Helped the Principal Investigator in the above activities b) Acted as Training Coordinator in one of the training imparted to the field veterinarians and compiled the training manual of the same
Dr. Chandratre Gauri A.	Co-P.I	a) Helped the Principal Investigator in the above activities b) Helped in organization of one of the training imparted to the field veterinarians

Acknowledgement

Dr. R. P. Gupta, Professor and Dr. (Mrs.) Deepika, Assistant Professor of Department of Veterinary Pathology were also associated with the research scheme and performed the following duties:

a. Helped in experimental research work regarding immuno-pathological work.

b. Helped in organization of one of the training imparted to the field veterinarians

10. Checklist:

No.	Question to consider	Yes	No
11.	Is the story interesting to the target audience of the project/activity report?	Yes	-
12.	Does the story explain what new insights the project brings? What is the main lesson learned from the story? Does the story describe a key insight on what works and what doesn't and something that future projects could build on	Yes	-
13.	Does the story describe the outcomes the project produced and the people who are benefitting? What changes-in skills, knowledge, attitude, practice or police has the project brought about and who is benefitting from these changes?	Yes	-
14.	Does the story make a compelling point that people will remember? Does the story show how the project makes a difference to improving livelihoods and lessening poverty?	Yes	-
15.	Does the story provide an interesting fact that people will remember? For example, how yields increased, how many hectares of land could become more productive from the innovation or technology?	-	-
16.	Does the story explain what kind of impact this innovation or technology could have if scaled up?	Yes	-
17.	Does the story show which partners contributed and how?	Yes	-
18.	Does the story include quotes from stakeholders or beneficiaries?	-	-
19.	Have I proved links to other media (journal articles, website news, newsletter blogs,	-	-

	annual reports of other Programme/project) that also feature this story?		
20.	Have I provided contact details of people who can provide more information?	Yes	-

P. I.
(Dr. K. K. Jakhar)

Prof & Head

SUCCESS STORY

OF

PROJECT UNDER RASHTRIYA KRISHI VIKAS YOJNA (RKVY)

ON

Project name: Strengthening of clinical pathology and post-mortem facilities to provide effective and high quality diagnosis services to the farmers

Department of Veterinary Pathology, COVSc

Lala Lajpat Rai University of Veterinary and Animal Sciences

Hisar-125004 (Haryana)

1. **Project title:** Strengthening of clinical pathology and post-mortem facilities to provide effective and high quality diagnosis services to the farmers [**Scheme No.:** 4042-C (g)-VPT-03-OA (RKVY)]

2. **Category:** Animal Husbandry (Infrastructure and Assets Development (Setting up of laboratories and testing Facilities)

3. **Challenges:**

In modern Veterinary Medicine, the availability of clinical pathological laboratory along with quality post-mortem facilities are important to the clinician as are the history and physical examination of the animal and thereby providing an excellent diagnostic facility to the farmers/livestock and poultry owners. In some instances, such laboratories are more important, as the information derived through different procedures may provide absolute evidence regarding the physiological alterations resulting from a pathological condition/disease process. The importance of clinical pathology in disease diagnosis can be judged from the fact that a number of diagnostic laboratories have come up which are employing latest technologies in disease investigation. Many of these laboratory tests have found a place in veterinary clinical laboratory, whereas others remain as unknown in terms of their reliability with respect to the diagnosis of animal diseases. In the past decades, there has been tremendous advancement in the understanding and application of clinical pathological, bacteriological, biochemical, toxicological and parasitological techniques as an aid to the diagnosis of animal diseases. As a result, there has been marked increase in the accuracy of clinical diagnosis and many of these laboratory procedures have become a part of the routine clinical practice.

Veterinary Clinical Pathology Laboratory along with good post-mortem facilities has proved a great asset in reaching to the final diagnosis with fair degree of success. It provides basis for judging the:

- Correct diagnosis of disease /Nature of disease in live livestock/poultry and better diagnosis of the cause of death in livestock/poultry
- Correct prognosis of disease in live livestock/poultry
- Efficacy of treatment given by clinician to livestock/poultry
- Physiological alterations resulting from a pathological condition in livestock/poultry
- The extent of injury to tissues/organs of the body in livestock/poultry

By conducting some of the clinical pathological tests regularly in the herd, a large number of diseases can be prevented. A test schedule for important infectious/contagious, parasitic diseases and metabolic disorders should be prepared and regular testing should be done. The reactors/ diseased animals can be segregated on the basis of various clinical pathological tests and post-mortem procedures and the diseased livestock/poultry can be given appropriate preventive medication.

Due to above reason, every veterinary college/institution should have at least a small clinical pathological laboratory and good post-mortem facilities where routine diagnostic specimens/samples could be analyzed through the standard procedures and post-mortem can be done in more efficient way. In addition, should be supported by a well equipped veterinary clinical pathology laboratory. Recognizing the importance of clinical pathology in disease diagnosis, the

Veterinary Council of India has incorporated veterinary laboratory diagnosis as a part of the curriculum for the veterinary graduates. The project was planned with the following objectives:

1. To strengthen and develop effective, high quality, clinical pathology laboratory services particularly responsive to the needs of farmer's/livestock and poultry owners.

2. To strengthen and develop effective, high quality, post mortem services particularly responsive to the needs of farmer's/ livestock and poultry owners.

4. Initiative:

Following activities were undertaken in the project to address the above mentioned challange:

i. Provided accurate and timely clinical, consultancy and diagnostic services to the farmers and Veterinary Clinical Complex (VCC) particularly related to the diseased animals.

ii. Prompt histopathological/cytological examination based on light microscopy of tissue biopsies/cytological specimens using special staining techniques (if any) to diagnose the disease conditions.

iii. Provided training to field veterinarians

iv. Experimental studies:

Experiment 1: Immunopathological studies on transmissible venereal tumour in dogs.

Experiment 2: Pathological and immunohistochemical studies on neoplasms in dogs with special reference to skin epithelial tumours

5. Key results/insight/interesting facts:

Facilities generated:

d. Clinical pathology laboratory was renovated (at cost of Rs. 8.95 lacs)

e. Renovation of post-mortem hall and associated Seminar Hall of the Department is also under progress at cost of Rs. 10.11 lacs.

f. Purchase of equipments worth Rs. 9.30 lacs were undertaken

Instruments purchased under the project:

✓ Purchase of equipments worth Rs. 9.30 lacs were undertaken

Sr. No.	Name of the Equipment	Quantity
i.	Autopsy table	Three
ii.	Automatic tissue processor	One
iii.	Weighing balance	One
iv.	Microscopes with battery backup	Two
v.	Air conditioner	Two
vi.	Fly catchers	Four
vii.	Stabilizer power system	One
viii.	LED display	One
ix.	Autopsy case	One
x.	Deep freezer	One
xi.	Urine analyzer	One
xii.	Renovation of the cooling system equipment	One
xiii.	Exhibit panel with acrylic cover	Two
xiv.	Embedding rings	-

Necropsy table in Postmortem hall

Automatic Tissue Processor

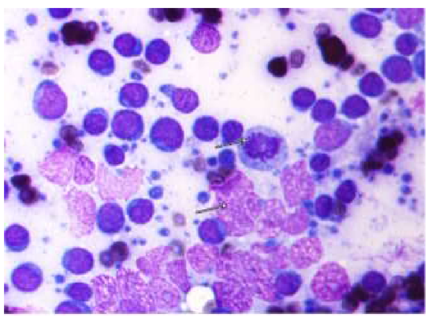
Urine Analyzer

Deep Freezer

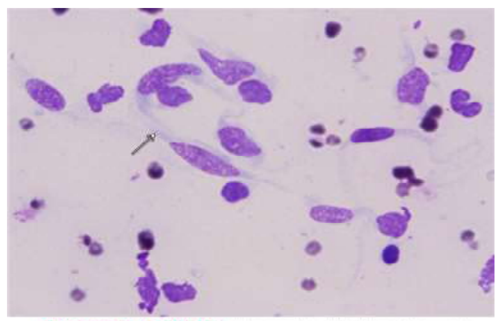
Research Findings: (with good quality pictures**)**

2015-16

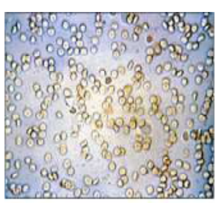
- ✓ Post mortem examination of 321 different animals (including *31 buffaloes, 16 cattle, 31 buffalo calves, 16 cattle calves, 26 sheep, 22 goat, 26 lambs*, *13 kids*, *87 nilgai*, *37 black buck*, *6 dogs*, *5 pigs, 2 equine* and one each of *camel*, *rabbit* and *monkey*) and 1338 birds was undertaken.
- ✓ 35 different clinical samples were estimated and revealed anaplasmosis, theileriosis, microfilariasis, anaemia, jaundice, mastocytoma, mixed infection of haemoprotozoan parasites, *Balantidium coli*, increased BUN, Creatinine, ALT, hypproteinemia and pus cells in urine.



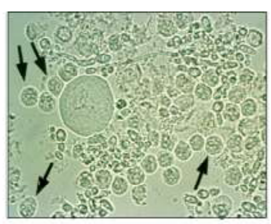
Lymphosarcoma In Dog: [illegible]



Fibrosarcoma In Buffalo: [illegible]

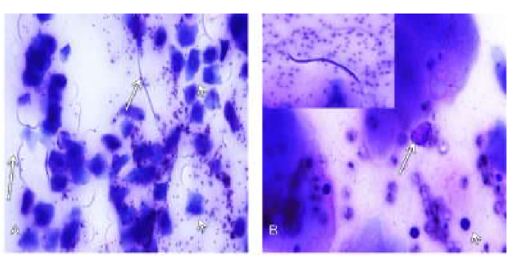


RBCs in urine



Pus cells and epithelial cells in urine

[illegible] urinary bladder



A- [illegible]

B- [illegible] **Inset :** [illegible]

2016-17

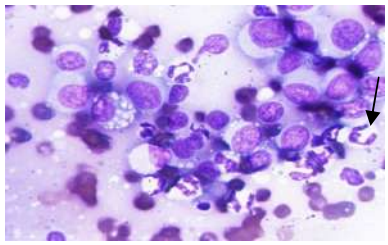
- ✓ Post mortem examination of 376 carcasses of different animals (including *33 buffaloes, 26 cow, 36 buffalo calves, 19 cow calves, 33 sheep (both young and adult), 25 goat (both young and adult), 153 nilgai*, *33 black buck*, 05 *chinkara*, *03 dog*, 03 *equine*, 0*2 rabbit, 01 owl and 01 piglet)* was done during the period from 1st July, 2016 to 30th June, 2017.
- ✓ Analysis of 147 different clinical samples including blood, serum, urine samples were examined which mainly revealed mixed infection of anaplasmosis, theileriosis, babesiosis, jaundice, anaemia, nervous form of trypanosomiasis in buffalo, increased BUN, creatinine, ALT, hypoproteinemia and pus cells in urine.

- ✓ **Experimental studies:**

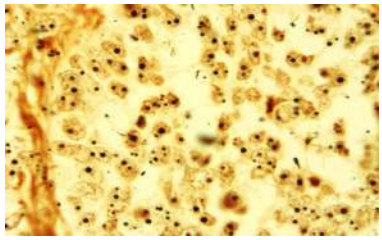
i. Immuno-pathological studies on transmissible venereal tumour in dogs revealed:

- Transmissible venereal tumours were more common in 2-4 years of age mainly in females affecting the external genitalia and occasionally the internal genital organs.
- Clinical history revealed variable size ranging from 4-25 cm with cauliflower or nodular/ irregular shape having varying colours. Tumour growth was associated with bleeding, soiling of the floor, ulceration, inappetance and depression in the tumour affected dogs.
- Haematological studies revealed normocytic normochromic type of anaemia showing significant decrease in values of Hb, PCV, TEC, TLC, absolute lymphocyte count; absolute neutrophil count and thrombocyte count whereas significant increase in the values of ESR in the tumour affected dogs.
- Biochemical studies revealed significant increase in the values of AST, ALT, globulin, creatinine and calcium and significant decrease in albumin and glucose. However no significant changes were found in the values of total protein, ALP, BUN and phosphorus in the tumour affected dogs as compared to tumour free dogs.
- Oxidative stress study revealed significant decrease in the values of SOD, catalase and GSH whereas significant increase in the values of LPO was found in the tumour affected dogs as compared to tumour free dogs.
- ANAE staining revealed significant decrease in the values of T lymphocytes whereas non significant decrease in the values of B-lymphocytes in the tumour affected dogs as compared to tumour free dogs.
- Cytological studies revealed that TVT cells had vacuolated cytoplasm and high nucleo-cytoplasmic ratio with hyperchromatic nuclei. AgNOR staining revealed that TVT cells had low proliferative capacity or the affected dogs were treated with antimitotic agents like vincristine, vinblastine etc. whereas sertoli cell tumour had moderate to high proliferative capacity.
- Special stain with Masson's trichrome revealed that in TVT, minimal proliferation of connective tissue was seen (mainly collagen fibres) whereas in sertoli cell tumour more proliferation of connective tissue was seen.
- Histopathological examination revealed that TVT cells had round to polyhedral shape and interspersed with delicate stroma. The tumour cells had a high nucleus to cytoplasm ratio with prominent nucleoli, cytoplasmic vacuolation and numerous mitotic figures.
- Immunohistochemical studies revealed that in TVT cells, moderate type of immunoreactivity was observed with alpha anti-trypsin suggesting that TVT had histiocytic origin. On other hand, no immunoreactivity was observed in sertoli cell tumour with alpha anti-trypsin.

Transmissible Venereal Tumour (TVT) in dog showing cauliflower like reddish tumour mass attached



Photomicrobar diagram of TVT showing tumour cells with coarse to reticulate chromatin and punctate basophilic cytoplasm (arrow), high vacuolation and neutrophils. (Field's stain,

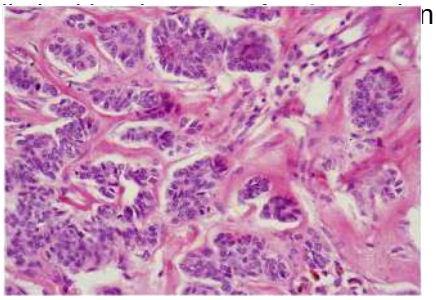


Photomicrobar diagram of sertoli cell tumour showing AgNOR dots in nucleus in tumour cells. (AgNOR staining, X400)

ii. Pathological and immunohistochemical studies on neoplasms in dogs with special reference to skin epithelial tumours revealed:

- The study was undertaken on 36 tumour biopsies collected from dogs over the period of about one year. In dogs, the tumours were observed in 3-12 years age group.
- Histopathologically, cutaneous tumours diagnosed were papilloma, squamous cell carcinoma, basal cell carcinoma, sebaceous carcinoma, perianal adenoma, fibroma, fibrosarcoma, haemangioma, histiocytoma, mastocytoma and malignant melanoma. Mammary tumours were adenoma, myxochondroma, complex carcinoma, carcinoma-mixed tumour and carcinosarcoma.
- All cases of squamous cell carcinoma were of grade II. Basal cell carcinomas were of grade II or grade III. Sebaceous carcinoma was of grade III whereas fibrosarcoma and malignant melanoma was of grade I.
- Regarding mammary gland tumours, mammary complex carcinoma was of grade I or grade III, carcinoma-mixed tumour were of grade I or grade II and carcinosarcoma was of grade I.
- Results of staging revealed that sebaceous carcinoma was of stage 3 and all other malignant tumours were of stage 2. Regarding mammary malignant tumours, one case of complex carcinoma was of stage 3 and all other malignant tumours of mammary gland of stage 5.
- Immunohistochemical analysis revealed that cytoplasmic PCK reactivity was noticed in both benign and malignant epithelial tumours but the score and colour intensity were variable. However, in mammary gland tumour, benign tumour showed more colour intensity as compared to malignant.
- Two cases of mammary complex carcinoma, two cases of carcinoma-mixed tumour and one case of mammary carcinosarcoma showed immunoreactivity of CK14 showing myoepithelial involvement in tumour formation. Ki67 immunoreactivity was mainly noticed in malignant tumours particularly those exhibited severe anaplastic features. Over expression of p53 protein was noticed only in one case of squamous cell carcinoma.
- Retrospective study of 90 tumour cases of dogs from last two years (2015 and 2016) revealed different types of benign and malignant tumours but the incidence of tumours in both the years was almost equal.

- It was concluded that most prominent cutaneous diagnosed was mastocytoma. PCK immunohistochemical staining was useful to identify undifferentiated tumours. There was very [illegible] in tumourigenesis of squamous cell carcinoma.



Basal cell carcinoma showing solid sheets of neoplastic cells showing arrangement of nuclei perpendicular to surrounding connective tissue in periphery and hapazardous in centre.

6. Impact:

v. Physical

a. Clinical pathology laboratory was renovated (at cost of Rs. 8.95 lacs)

Newly renovated clinical pathology laboratory

b. Post-mortem hall and associated Seminar Hall of the Department was also renovated at cost of Rs. 10.11 lacs

c. Purchase of equipments worth Rs. 9.30 lacs were undertaken

vi. Social and economical impacts

Social:-

- ✓ A five days training on "Clinicopathological diagnostic techniques, postmortem examination and dispatch of specimens" was organized by the Department of Veterinary Pathology, LUVAS, Hisar from 08-12 May, 2017. The training was organized for twenty Veterinary Surgeons that were nominated by Dr. G. S. Jakhar, Director General, Department of Animal Husbandry and Dairying, Haryana and also for Post graduate students of the Department of Veterinary Pathology. Dr. K.K. Jakhar, Prof. & Head acted as Training Director whereas Dr. Vikas Nehra and Dr. (Mrs.) Chandratre Gauri acted as Training Coordinators. This training had provided 'Hands on Training" to Field Veterinarians for postmortem examination and dispatch of specimens along with updating their knowledge on Clinicopathological diagnostic techniques. A Training Manual on "Clinicopathological diagnostic techniques, postmortem examination and dispatch of specimens" along with CD for the participants was also released.

Trainee Veterinarians with Worthy Dean, COVS, LUVAS

Worthy Dean, COVS and Dr. R.S. Sheokand, DEE, LUVAS releasing the training CD along with training director and training coordinators

Economical impacts:

C. Renovation of such a laboratory and post mortem hall will be helpful in developing wide range of clinical pathological testing procedures and prompt post mortem examination for diagnosis and monitoring of diseases where the pathogenesis involves clinical components thereby delivering high quality, responsive services to clinicians and farmers.

D. Cost-effective clinic-pathological diagnostic services and in turn economic benefits to farmers

7. Lessons Learned:

iii. Research on clinical specimens with the aims of diagnosis and understanding the pathogenesis of diseases will be helpful to the clinicians for treatment of animals and farmers by saving their animals through specific treatments.

iv. To provide wide range of technical services to the different departments of the University like different clinical techniques including prompt post mortem examination.

8. Supporting quotes and Images: Nil

9. Additional Information:

Total Budget of the project: Rs. 45.0 lacs

Budget allotted in the first and second instalments: - Rs. 45.0 lacs

Time duration: Three years i.e. Three years i.e. from 2015-16 to 2017-18 (Project completed in March, 2018)

Total No. of Beneficiaries: Beneficiaries were mainly pet and domestic animal owners whose number is 226 till December, 2017. List of some of the beneficiaries along with other details is as under:

Sr. No	Name of owner (case no of TVCC)	Address	District
1	Ravi (4-9672)	Rohtak	Rohtak
2	Omprakash Rawal (4-9743)	Kalan	-
3	Nikhil (4-9752)	Bhiwani	Sirsa
4	Vikram (4-9851)	Sirsa	Sirsa
5	Pawan (4-9889)	hisar	hisar
6	Pyrelal (4-9902)	Hisar	Hisar
7	Commandent BSF(4-9901)	Hisar	Hisar
8	Sunil Nagpal (4-9955)	Hisar	Hisar
9	Naveen (5-10657)	Gorakhpur	Gorakhpur
10	Ravinder (5-10719)	Hisar	Hisar
11	Teluram (5-10745)	Hisar	Hisar
12	Kuldeep (E-5941)	Bhiwani	Bhiwani
13	Gaurav Kumar (5-10608)	Hisar	Hisar
14	Sonu Sharapur(5-11051)	Hisar	Hisar
15	Subhash chander (5-11573)	Hisar	Hisar
16	Satpal Gole (5-11530)	Bhiwani	Bhiwani
17	Jai singh (5-11526)	Jhajjar	Jhajjar
18	Kapil(5-11422)	Balsamad	Hisar
19	Haswir (5-11454)	Hisar	Hisar
20	Suresh nansana (5-11734)	Rohtak	Rohtak
21	Ankit (6-11871)	Hisar	hisar
22	Nitish (6-11939)	Hisar	hisar
23	Mukeash (6-12062)	Jhajjar	Jhajjar
24	Sunil kumar (6-12551)	Hisar	hisar
25	Kapil ()6-12427	Hisar	hisar
26	Krishan (6-12420)	Hisar	hisar
27	Ravi (6-12645)	Hisar	hisar
28	Yogesh(6-12486)	Hisar	hisar
29	Parvinder teran (6-12414)	Bhiwani	Bhiwani
30	Dinesh (6-12663)	Hisar	hisar
31	Pardeep (7-88)	hisar	hisar
32	Paleram (7-263)	hisar	hisar
33	Satbir (7-253)	Bhiwani	Bhiwani
34	Atter singh(7-111)	Bhiwani	Bhiwani
35	Vikram (7-123)	hisar	hisar
36	Mahender (E-7-16)	Jhujhnu	Jhujhnu
37	Sandeep(E-7-14)	hisar	hisar
38	Ashok (7-74)	Jind	Jind
39	Atter singh (7-66)	Bhiwani	Bhiwani
40	Madan (7-92)	Jhajjar	Jhajjar
41	Amit (7-157)	Sat road Hisar	hisar
42	Vinay (7-220)	Fatehbad	Fatehbad
43	Ramkumar bhari (7-421)	Bahu akbarpur	Rohtak
44	Pardeep singh (7-302)	Bhiwani	Bhiwani
45	Amit (7-413)	Sirsa	Sirsa

46	Balbir kumar(7-363)	Hisar	Hisar
47	Navaldeep (7-319)	Hisar	Hisar
48	Vicky (7-448)	Kaithal	Kaithal
49	Surinder singh (7-866)	Bhiwani	Bhiwani
50	Satish (7-935)	sonipat	Sonipat
51	Surrender (7-935)	Hisar	Hisar
52	Sajjan (7-830)	Hisar	Hisar
53	Sandeep (7-831)	Jind	Jind
54	Tanuj (7-732)	Hansi	Hansi
55	Sunil (7-748)	Pilani	Pilani
56	Ravi (7-682)	Karnal	Karnal
57	Commandant BSF (7-686)	Hisar	Hisar
58	Sunita suri (7-659)	Hisar	Hisar
59	Sahabram (7-695)	Hisar	Hisar
60	Satish (7-525)	Bhiwani	Bhiwani
61	Balwant (8-1734)	Bhiwani	Bhiwani
62	Gurmed singh (8-1720)	Jind	Jind
63	Ramswaroop (8-1747)	Hisar	Hisar
64	Aseen (8-1750)	Jawahar nagar Hisar	Hisar
65	Mandeep (8-1739)	Jind	Jind
66	Dildar sarhra (8-1681)	Hisar	Hisar
67	Sumit (8-1648)	Hisar	Hisar
68	Jaswinder singh (8-1612)	Tohana	Fatehabad
69	Jai singh (8-1557)	Bhiwani	Bhiwani
70	Mahender singh (8-1555)	Cant Hisar	Cant Hisar
71	Balkar (8-1596)	Fatehabad	Fatehabad
72	Barinder (8-1593)	Uchana	Uchana
73	Kuldeep (8-1532)	Jind	Jind
74	Sandeep (8-1346)	Mirchpur hisar	Hisar
75	Anand Bhagara (8-1343)	Hisar	Hisar
76	Satnam singh (8-1344)	Fatehabad	Fatehabad
77	Surajmal (8-1345)	Habalpur	Hisar
78	Rajbir (8-1100)	Hisar	Hisar
79	Virender (8-1149)	Hisar	Hisar
80	Ranbir singh (8-1156)	Bhiwani	Bhiwani
81	Navdeep (8-1058)	Hisar	Hisar
82	Commandant BSF (8-1033)	Hisar	Hisar
83	Suresh (9/3436)	Ralwar Khurd	
84	Pawan (9/3408)	Ghaskhurd	Jind
85	Manjeet Singh(9/3399)	Hisar	Hisar
86	Kuldeep (9/3330)	Hisar	Hisar
87	Bhagirath(9/3331)	Malkuha	
88	Bhagirath(9/3332)	Malkuha	
89	Sushant (/93324)	Sonipat	Sonipat
90	Pardeep (E 356)	Hisar	Hisar
91	Aman (9/3023)	Jind	Jind
92	Hari ram (9/3286)	Muklan	
93	Hanuman singh (9/3471)	Rawalvas khurd	
94	Ram krishan (9/2695)	Hisar	Hisar
95	Deepak (9/2864)	Hisar	Hisar
96	Rudrash (9/2873)	Hisar	Hisar
97	Mahender singh(9/3083)	Rajgarh	Churu
98	Vinod ludana (9/2985)	Jind	Jind
99	Anil (9/2924)	Hisar	Hisar
100	Moyu (9/3182)	Hisar	Hisar

101	Sarachand (9/3184)	Sirsa	Sirsa
102	Anand (10/3561)	Hisar	Hisar
103	Baljeet singh (10/3568)	Hisar	Hisar
104	Sonu (10/3452)	Hisar	Hisar
105	Rajpal (10/3591)	Shikharpur	Hisar
106	Ramkumar (10/3710)	Narwana	Jind
107	Jai singh (10/3737)	Bhiwani	Bhiwani
108	Raja (10/3935)	Narwana	Jind
109	Akshay (10/3971)	Jind	Jind
110	Rajnesh (10/4018)	Hisar	Hisar
111	Sunil (10/4071)	Hisar	Hisar
112	Vivek (10/4012)	Bhiwani	Bhiwani
113	Ravinder (10/4217)	Bhiwani	Bhiwani
114	Ankit (10/4276)	Hisar	Hisar
115	Ramniwas (10/4422)	Hisar	Hisar
116	Manoj (11/4638)	Rohtak	Rohtak
117	Satish (11/4630)	Rohtak	Rohtak
118	Rakesh	Bhiwani	Bhiwani
119	Deepak (11/4984)	Hisar	Hisar
120	Kiran singh (11/4801)	Hisar	Hisar
121	Ranvir (11/4937)	Hisar	Hisar
122	Kuldeep (11/5337)	Hisar	Hisar
123	Sunny (12/575)	Bhiwani	Bhiwani
124	Lalaram (12/5452)	Hansi	Hansi
125	Suresh (12/5457)	Hisar	Hisar
126	Rampal (12/5468)	Bhiwani	Bhiwani
127	Biker singh(12/5495)	Fatehabad	Fatehabad
128	Pralhad (12/5752)	Hisar	Hisar
129	Birsingh (12/5756)	Dabri	
130	Anmol (12/5859)	Hisar	Hisar
131	Rajkumar (12/5863)	Kaithal	Kaithal
132	Ramkrishan (1/6256)	Jhajjar	Jhajjar
133	Mukesh (1/6268)	Jhajjar	Jhajjar
134	Naveen (1/6283)	Hisar	Hisar
135	Dilip singh(1/6804)	Narwana	Jind
136	Randhir (2/7848)	jind	jind
137	Ramesh (2/7891)	Hisar	Hisar
138	Vijay (2/8134)	Bhiwani	Bhiwani
139	Sujay (2/8136)	Hisar	Hisar
140	Kishan (2/7541)	Hisar	Hisar
141	Viraj (3/7121)	Hisar	Hisar
142	Sujay (3/5134)	Bhiwani	Bhiwani
143	Vijay (3/7191)	Hisar	Hisar
144	Kishan (3/6145)	Bhiwani	Bhiwani
145	Ramkrishan (3/7914)	Jhajjar	Jhajjar
146	Vikas (3/7196)	Hisar	Hisar
147	Rajbir (4/3147)	Hisar	Hisar
148	Ramkumar (4/5194)	Fatehabad	Fatehabad
149	Vinod (4/5196)	Kaithal	Kaithal
150	Rajesh (4/6123)	Bhiwani	Bhiwani
151	Suyash (4/5184)	Hisar	Hisar
152	Vir (4/5174)	Kaithal	Kaithal
153	Mohan (5/3126)	Sirsa	Sirsa
154	Vikram (5/3214)	Hisar	Hisar
155	Ayush (5/3213)	Hisar	Hisar

156	Vicky (5/3417)	Bhiwani	Bhiwani
157	Ramnaresh (5/3514)	Hansi	Hansi
158	Ram (5/3216)	Hansi	Hansi
159	Kiran singh (6/7191)	Bhiwani	Bhiwani
160	Deepak (6/6141)	Hansi	Hansi
161	Kuldeep (6/6138)	Bhiwani	Bhiwani
162	Anmol (6/5141)	Hisar	Hisar
163	Lalaram (6/7184)	Hisar	Hisar
164	Srajmal (7/6146)	Kaithal	Kaithal
165	Naveen (7/6251)	Bhiwani	Bhiwani
166	Suresh (7/6291)	Hisar	Hisar
167	Vinay (7/6292)	Hisar	Hisar
168	Pardeep (7/5191)	Kaithal	Kaithal
169	Pramod (7/4124)	Fatehbad	Fatehbad
170	Vishwas (7/6285)	Hisar	Hisar
171	Ramprit (8/9126)	Bhiwani	Bhiwani
172	Vinod (8/7114)	Hisar	Hisar
173	Virchand (8/2279)	Hisar	Hisar
174	Vivek (8/3114)	Fatehbad	Fatehbad
175	Vijay (8/2115)	Hisar	Hisar
176	Dharmpal (8/2110)	fatehabad	Fatehbad
177	Himanshu (8/2068)	Hisar	Hisar

Work Contribution by P.I. and Co-P.I.s:

Sr. No.	Name	Designation	PI/or Associate	Work done/Activity profile
1.	Dr. K. K. Jakhar	Professor	Principal Investigator	a) Overall monitoring of the project b) Clinical pathology laboratory was renovated (at cost of Rs. 8.95 lacs) c) Renovation of post-mortem hall and associated Seminar Hall of the Department is also under progress at cost of Rs. 10.00 lacs d) Purchase of equipments worth Rs. 9.30 lacs were undertaken e) Monitored experimental research regarding clinico-pathological work f) Provided accurate and timely clinical, consultancy and diagnostic services to the farmers and Veterinary Clinical Complex (VCC) particularly related to the diseased animals. g) Prompt histopathological/cytological examination based on light microscopy of tissue biopsies/cytological specimens using special staining techniques (if any) to diagnose the disease conditions. h) Supervised the experimental research work of on “Immuno-pathological studies on transmissible venereal tumour in dogs”

				i) Acted as Training Director in one of the training imparted to the field veterinarians and compiled the training manual of the same
2.	Dr. R. P. Gupta	Professor	Co-Investigator	a) Helped the Principal Investigator in the above activities b) Supervised the experimental research work of on "Pathological and immunohistochemical studies on neoplasms in dogs with special reference to skin epithelial tumours" c) Helped in organization of one of the training imparted to the field veterinarians
3.	Dr. Vikas Nehra	Scientist	Co-Investigator	a) Helped the Principal Investigator in the above activities b) Acted as Training Coordinator in one of the training imparted to the field veterinarians and compiled the training manual of the same
4.	Dr. Babu Lal Jangir	Assistant Professor	Co-Investigator	a) Helped the Principal Investigator in the above activities. b) Helped in organization of one of the training imparted to the field veterinarians
5.	Dr. (Mrs.) Chandratre Gauri A.	Assistant Professor	Co-Investigator	a) Helped the Principal Investigator in the above activities. b) Acted as Training Coordinator in one of the training imparted to the field veterinarians and compiled the training manual of the same
6.	Dr. (Mrs.) Deepika	Assistant Professor	Co-Investigator	a) Helped the Principal Investigator in the above activities. b) Helped in organization of one of the training imparted to the field veterinarians
7.	Dr. Ramesh Kumar	Assistant Disease Investigation Officer (Deptt. Of VPHE)	Co-Investigator	a) Helped the Principal Investigator in the above activities. b) Helped in organization of one of the training imparted to the field veterinarians

Acknowledgement

Dr. Maneesh Sharma, Assistant Professor, Department of Veterinary Clinical Complex was also associated with the research scheme and performed the following duties:

c. Helped the Principal Investigator in the above activities.
d. Helped in organization of one of the training imparted to the field veterinarians

10. Checklist:

No.	Question to consider	Yes	No
21.	Is the story interesting to the target audience of the project/activity report?	Yes	-
22.	Does the story explain what new insights the project brings? What is the main lesson learned from the story? Does the story describe a key insight on what works and what doesn't and something that future projects could build on	Yes	-
23.	Does the story describe the outcomes the project produced and the people who are benefitting? What changes-in skills, knowledge, attitude, practice or police has the project brought about and who is benefitting from these changes?	Yes	-
24.	Does the story make a compelling point that people will remember? Does the story show how the project makes a difference to improving livelihoods and lessening poverty?	Yes	-
25.	Does the story provide an interesting fact that people will remember? For example, how yields increased, how many hectares of land could become more productive from the innovation or technology?	-	-
26.	Does the story explain what kind of impact this innovation or technology could have if scaled up?	Yes	-
27.	Does the story show which partners contributed and how?	Yes	-
28.	Does the story include quotes from stakeholders or beneficiaries?	-	-
29.	Have I proved links to other media (journal articles, website news, newsletter blogs, annual reports of other Programme/project) that also feature this story?	-	-
30.	Have I provided contact details of people who can provide more information?	Yes	-

P. I.
(Dr. K. K. Jakhar)

Prof & Head

SELF IMPACT ASSESSMENT REPORT

OF

PROJECT UNDER RASHTRIYA KRISHI VIKAS YOJNA (RKVY)-RAFTAAR SCHEME

ON

Project name: **Establishment of Immuno-histo-chemical laboratory and strengthening of Immuno-technology laboratory for diagnosis of diseases of animals and poultry**

Scheme:- **4060-C(g)-VPT-04-O.A. (RKVY)**

Principal Investigator:

Dr. Gulshan Narang, Principal Scientist
Department of Veterinary Pathology
LUVAS, Hisar

Co-Investigator(s): Multidisciplinary Project

Department of Veterinary Pathology	Collaborating Departments		
	Veterinary Anatomy	Veterinary Public Health and Epidemiology	Department of Veterinary Microbiology
Dr. Vikas Nehra Scientist Dr. (Mrs.) Deepika Assistant Professor Dr. Babu Lal Jangir Assistant Professor Dr. (Mrs.) Chandratre Gauri A. Assistant Professor	i) Dr. Pawan Kumar Professor & Head Dr. Parveen Kumar Gahlot, Scientist Dr. Amandeep Singh Assistant Professor Dr. Tej Parkash Assistant Professor	i) Dr. Naresh Jindal Sr. DIO and All ADIOs at Hisar and Outstation	i) Dr. Jagveer Rawat Associate Professor ii) Dr. Akhil Kumar Gupta, Scientist iii) Dr. Anshul Lather Assistant Professor

Department of Veterinary Pathology, COVSc

Lala Lajpat Rai University of Veterinary and Animal Sciences

Hisar-125004 (Haryana)

11. Project title: Establishment of Immuno-histo-chemical laboratory and strengthening of Immuno-technology laboratory for diagnosis of diseases of animals and poultry

12. Scheme No.: 4060-C(g)-VPT-04-O.A. (RKVY) (A Multidisciplinary Project)

13. Grant received: ***Project sanctioned in the year 2018-2019***

First Installment (2018-2019): From Comptroller, LUVAS Letter No. CVU/B-1/2018/2787-89 dated 25.09.2018 (Budget sanctioned and released through Director of Agriculture & Farmers Welfare, Panchkula, Haryana order Endst. No. 1227-1230/ADO- (RKVY) dated 03.08.2018) (Rs. 37,65,000/-)

Second Installment (2018-2019): From the office of Director Agriculture and Farmers Welfare, Haryana, Panchkula vide Endst. No. 346-349/ADO-(RKVY) dated 01.03.2019 further endorsed by Director of Research, LUVAS, Hisar email letter dated 05.03.2019 (Rs. 87,85,000/-)

In the year 2018-2019:- *Spent*- Rs. 36,77,650/-; *Balance Unspent*- Rs. 88,72,350/-

Afterwards in the year 2019-2020:- Amount of Rs. 89,49,847/- (***after revalidation***) was received in the department vide Comptroller Office letter no. LUVAS/CVU/B-1/2019/3181-83 Dated 08.07.2019 under different SOEs.

14. Total Budget of the project: Rs. 1,26,27,497/- as 1st and 2nd installments and after revalidation as mentioned above

15. Time duration: Three years (from 2018-2019 to 2020-2021) - Project will be completed in March, 2021.

6. Objectives/Activities/Targets:

I. Establishment of Immunohistochemical laboratory along with facilities and procurement of chemicals, equipments etc.
II. Standardization of immunohistochemical techniques for diagnosis of important infectious diseases and tumour conditions prevalent in the state of Haryana.
III. Standardization of immunohistochemical techniques on healthy organs for comparative purpose.
IV. Analysis of tissue samples employing special standard histochemical staining procedures.
V. Upgradation and modernization of the Immunotechnology laboratory.
VI. Maintenance of Phage Display Library and characterization of already isolated and selected single domain antibody clones from the Phage Display Library.

7. Physical facilities generated: Instruments Purchased (with their cost) in 2018-2019 and 2019-2020 under the project:

In Department of Vety. Pathology:

Sr. No.	Name of the equipment/instrument	Quantity	Cost (in Rs.)
2.	Oven Universal 224 L 605 X 605 X 605 MM (NSW) (Narang Scientific Works Nsw-143 OUS-S)	One	79,850/-
2.	Hot Plate 300X455mm (NSW – 255)	One	43,000/-
3.	Leica Microtome (Model- Histo Core MULTICUT)	One	10,65,750/-
4.	Thermocycler/PCR system (Proflex 3x32 Well PCR system)	One	5,22,199.50/-
5.	Deep freeze (Single Solid Door Upright Freezer Vestfrost)	One	74,479/-

6.	Digital Electronic Balance (Analytical balance ME 204, Mettler Toledo)	One	1,05,000/-
7.	Horizontal Electrophoresis System with power supply (Cleaver and MSCHOICERIO WITH POWER PRO 300)	One	1,14,500/-
8.	Vertical laminar flow cabinet (NSW-202)	One	4,04,250/-
9.	Vertical autoclave (40 L NSW-227)	One	95,820/-
10.	Micro centrifuge (1000- Spinwin Micro-centrifuge)	One	9,649/-
11.	Binocular Microscope (MLXi Plus Freedom with Magcam)	One	1,17,410/-
12.	Air conditioner (Hitachi 2.0 ton)	Two	1,18,916/- (for two)
13	Double Distillation unit	One	46,900/-
14	UPS 5.0 KVA/Automatic Voltage Stabilizer for AC 5.0KVA	Two	14,349/- (for Two)
15	1.0 KVA Inverter (Luminous UPS ECO 1050 +) with battery 12V 150 AH and trolley	One	14,900/-
16	PCR Series workstation with voltage stabilizer and UV Lamp	One	2,67,330/-
17	Spare sealing for Rotor (of Refrigerated centrifuge)	One	24,426/-

<u>In Department of Vety. Microbiology</u>:

Sr. No.	**Name of the equipment/instrument**	**Quantity**	**Cost (in Rs.)**
1	Thermocycler/PCR system (Proflex 3x32 Well PCR system)	One	5,22,199.50/-
2	Deep freeze (Single Solid Door Upright Freezer Vestfrost)	One	74,479/-
3	Table top centrifuge (Sigma 2-16 KL)	One	7,45,500/-
4	Horizontal Electrophoresis System with power supply (Cleaver and MSCHOICERIO WITH POWER PRO 300)	One	1,14,500/-
5	Micro centrifuge (1000- Spinwin Micro-centrifuge)	One	9,649/-
6	BOD incubator (NSW-152 Super deluxe model)	One	2,74,113/-
7	Air conditioner (Hitachi 2.0 ton)	Two	1,18,916/- (for two)
8	Vertical electrophoresis system (Cleaver Scientific single moulded)	One	64,739/-
9	PCR Series workstation with voltage stabilizer and UV Lamp	One	2,67,330/-
10	Biosafety cabinet with Accessories (Class II A2) with temperature control unit and UV Lamp	One	4,75,887/-

<u>In Department of Vety. Anatomy</u>:

Sr. No.	**Name of the equipment/instrument**	**Quantity**	**Cost (in Rs.)**
1	Leica Microtome (Model- Histo Core MULTICUT)	One	10,65,750/-
2	Digital Electronic Balance (Analytical balance ME 204, Mettler Toledo)	One	1,05,000/-
3	Digital pH meter (pH 510)	One	33,980/-
4	Vacuum oven (NSW-251B)	One	1,58,025/-
5	Air conditioner (Hitachi 2.0 ton)	Two	1,18,916/- (for two)
6	Vortex shaker (Spinix Vortex shaker 3020)	One	11,202/-
7	Magnetic stirrer with hot plate (Spinot TM Digital magnetic Stirrer Hot Plate 6040)	One	24,950/-

8. Material and Supply SOE "M&S"

a. Purchased different chemicals/kits/antibodies/materials of Rs. 14,23,300 in the year 2018-2019 for Vety. Pathology and Vety. Anatomy.

b. Purchased different chemicals/ kits/antibodies/materials of Rs. 28,55, 655/- in the year 2019-2020 for Vety. Pathology and Vety. Microbiology.

Major chemicals/kits/antibodies/materials in Department of Vety. Pathology, Microbiology and Anatomy

Polyclonal and Monoclonal primary and secondary antibodies against infectious diseases and tumors, Primary antibodies against CD4,CD8, CD14, CD45, IgA, IgE etc., PCR Master mix, Mouse Anti-Human Cytokeratin, Paraffin wax, Ethanol, Methanol, Xylene, Acetone, Silver nitrate, Microtome blades , Sheep Blood agar plate, Poly freeze (R) tissue freezing, microscope slides, Complete and Incomplete Freunds adjuvant, Erba, Kits, Proteinase K, Spectra P3 3.5K, Anti-HIS (CTERM) HRP50, VItek kits, Saline solution, Latex bead deep blue dye, Dialysis Tubing, Phusion High-fidelity PCR master Mix with GC buffer, Filter Tips, cryogenic vials etc. etc.

9. Other budget details:

a. Utilized Rs. 49,678/- of OE (NR)

b. Temporary Advance of Rs. 9,95,000/- under SOE "OC(I)" has been drawn and deposited in the account of DSW-cum-Estate Officer, LUVAS. Hisar for the renovation work of different Laboratories of the Department of Veterinary Microbiology and Veterinary Pathology under the scheme.

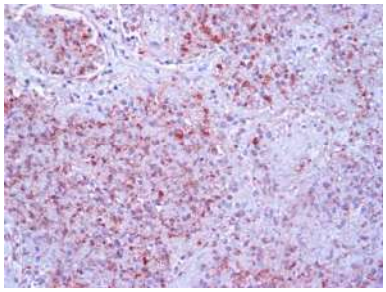
10. Brief Research Outputs:-

In Department of Vety. Pathology

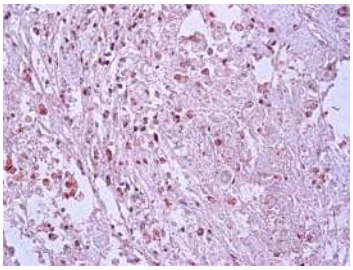
A. Brief findings of the immunohistochemical examination of different samples after proper standardization are as under:

a. Immunohistochemical staining for detection of Mycobacterium bovis in formalin fixed tissue sections collected from cattle revealed:

- ✓ Positive results were seen as brick red coloured reaction in the giant cells, macrophages, epitheloid cells and in caseous necrotic tissue present in lungs. However, no positive reaction was observed in the lymph node.
- ✓ Positive results were observed at dilution of 1: 200 and 1:400 after standardization of the IHC using various dilutions.
- ✓ However, intensity of the reaction was better at 1:400 dilution of the polyclonal antibody.



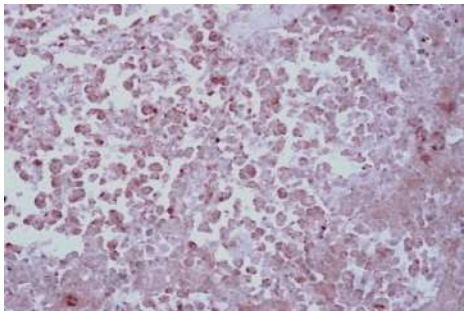
Granuloma showing intracytoplasmic brick red coloured staining for *Mycobacterium bovis* (Adult cattle lung). IHC 400X



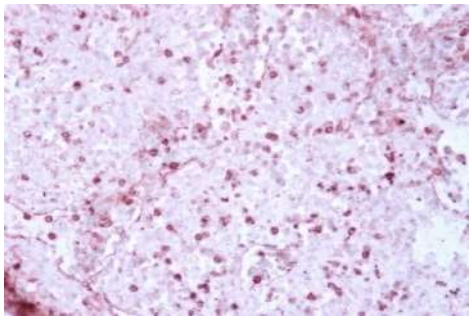
Granuloma showing intracytoplasmic brick red coloured staining for *Mycobacterium bovis* (Adult cattle lung). IHC 400X

b. Immunohistochemical studies on neoplastic conditions in poultry suspected of MD/LL revealed:

- ✓ The immunohistochemical staining for CD3 antibody (T cell marker) gave reddish brown positive reactivity in the cytoplasm as well as on the cell membrane in 80% pleomorphic neoplastic lymphoid cells in almost all the cases indicating their T-cell origin.
- ✓ No immunoreactivity was observed for CD79 alpha antibodies in pleomorphic lymphoid cells in all the cases except in few cases where mild reactivity was observed in few large lymphoblasts present particularly at perivascular locations.
- ✓ However, in mixed infection cases with lymphoid leukosis, neoplastic cells showed positive immunohistochemical reactivity to CD79 alpha in 30-50% cells. In the myelocytomatosis case, tumour cells did not revealed any immunoreactivity to both CD79 alpha and CD3 marker.



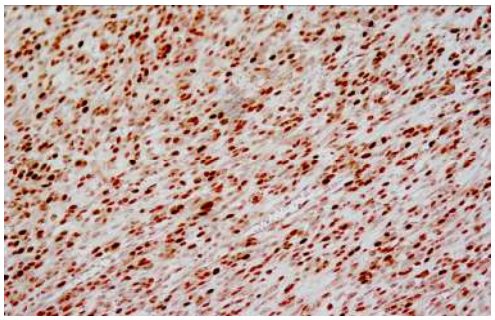
Positive membranous and cytoplasmic immunoreactivityto CD3 in 60% pleomorphic neoplastic cells (MD+LL infection). IHC stain 400X



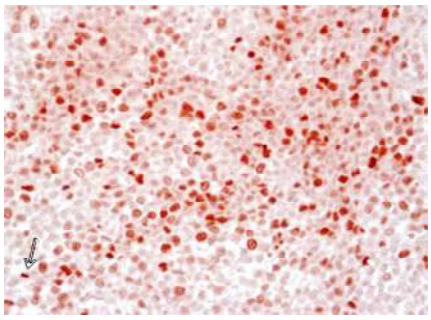
Positive membranous and cytoplasmic immunostainingto CD79 alpha in 20 % neoplastic lymphoid cells (MD+LL mixed infection). IHC stain 200X

c. Immunohistochemical expression of PCNA, Ki-67 and COX-2 in tumours of dogs particularly genital and mammary tumours revealed:

- ✓ Immunohistochemical expression of proliferating cell nuclear antigen (PCNA), Ki67 and COX-2 were higher in malignant in comparison to benign tumours indicating their importance in prognosis.
- ✓ α-SMA helped in diagnosis of smooth muscle tumours, vimentin and PCK were useful in determining the origin of the tumours.CK-14 was useful in identification of myoepithelial cells in mammary tumours and thus helped in the classification of mammary tumours.



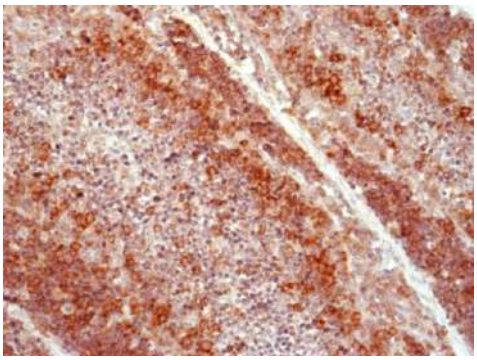
Malignant melanoma: Moderate to strong nuclear immunoreactivity for PCNA in proliferating spindle cells. IHC×400



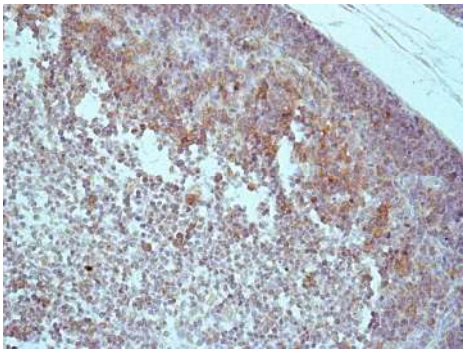
Extra genital transmissible venereal tumour (thigh region): Mild to intense nuclear immunoreactivity for Ki67 in proliferating cells and mitotic figure (arrow). IHC×400

d. Immunohistochemical detection of CD4+ and CD8+ T cells in experimental chickens vaccinated with IBD vaccines revealed:

- ✓ Bursa of Fabricius revealed immunopositive reactivity for CD4+ and CD8+ T cells in experimental chickens vaccinated with IBD vaccines.



Bursa of Fabricius revealed immunopositive reactivity for CD8+ T cells in experimental chickens vaccinated with IBD vaccines.
IHC×400



Bursa of Fabricius revealed immunopositive reactivity for CD8+ T cells in experimental chickens vaccinated with IBD vaccines.
IHC×400

e. Immunohistochemical detection of *P. multocida* antigen in formalin fixed tissues collected from experimental rabbits: IHC served as important technique which detected the immunolocalization of *P. multocida* antigen in different organs. Immunolocalization mainly in blood vessels indicated that the pathogenic effects produced by the bacteria may be mediated by involvement of blood vascular system. So, IHC can be used as a potential tool to study the detailed pathogenesis of bacteria. Since the bacterial antigen of *P. multocida* could be detected in lungs and trachea of all the rabbits by IHC, these can serve as a primary organ for detection and identification of *P. multocida* in addition to bacteriological and molecular tests during disease outbreak investigations

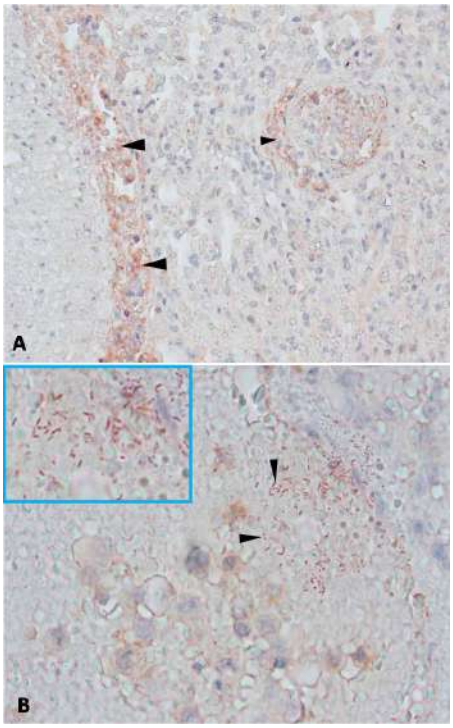


Fig. : Immunohistochemical detection of *Pasteurella multocida* antigen in formalin fixed tissue sections of lungs in experimentally infected rabbits.

A: Brick red coloured immunopositive reaction in the wall of pulmonary blood vessels (arrow heads). IHC×400

B: Brick red coloured immunopositive reaction in the bipolar organisms (arrow heads) in the lumen of blood vessel and enlarged view in inset. IHC×1000

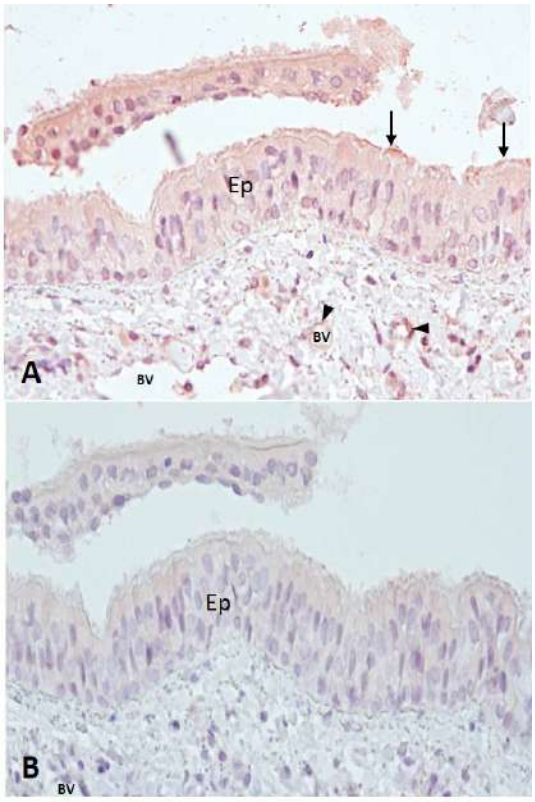


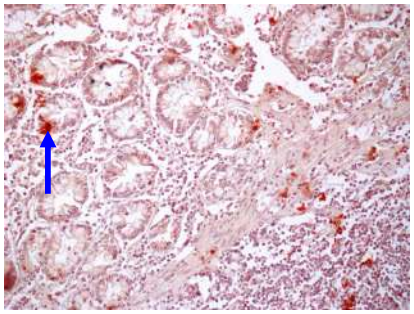
Fig. : Immunohistochemical detection of *Pasteurella multocida* antigen in formalin fixed tissue sections of trachea in experimentally infected rabbits.

A: Brick red coloured immunopositive reaction in tracheal epithelium (Ep) (arrows) and endothelium of blood vessels in lamina propria (arrow heads). IHC×400

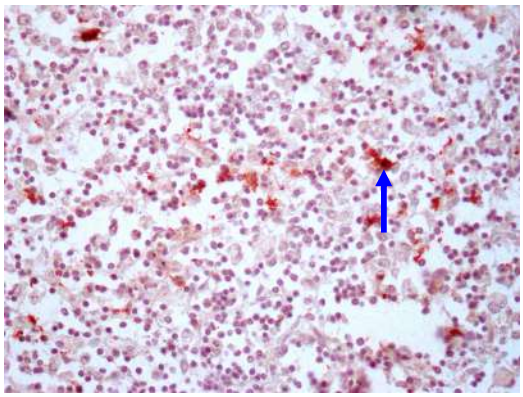
B: No immunoreactivity in tracheal epithelium (Ep) and endothelium of blood vessels (BV) in lamina propria in the negative control. IHC×400

f. Immunohistochemical studies on *Mycobacterium avium* subsp. *paratuberculosis* (MAP):

Molecular investigation revealed confirmation of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) in 23.1% cases by conventional PCR method using IS900 primers with detection of 778 bp PCR product. Immunohistochemical staining using anti-MAP polyclonal antibody revealed presence of MAP antigen in formalin fixed tissue samples of intestine and mesenteric lymph nodes indicating usefulness of immunohistochemical technique for paratuberculosis diagnosis.



Tissue section of intestine showing brick red coloured staining (arrow) in cytoplasm of macrophages in mucosa and lamina propria (IHC 200X) (Adult cattle, *MAP* positive)



Tissue section of mesenteric lymph nodes showing brick red coloured staining in cytoplasm of macrophages (IHC400X)(Adult cattle, *MAP* positive)

In Department of Vety. Microbiology

i) Phage display library having >10^7 clones was revived and cryopreserved in liquid nitrogen for further use
ii) Already isolated clones (dAb Cl16, Cl18, Cl23, Cl26) were revived on LB/amp (100µg/ml) agar plate
iii) These Clones were further characterized by VHH-PCR using A6E and FR4 primer for the presence of VHH insert. After conformation, these were cryopreserved for continuous supply of the reagent
iv) The dAb Cl26 was expressed in *E. coli* BL21(DE3) cells under IPTG (1mM) induction. The figure showed band of approx. 17 kDa size with good level of expression.
v) The expressed dAb Cl 26 was detected using Anti-His HRPO antibody in Direct ELISA.

Fig.: Growth of dAb clone VHH-pET303/CT E. coli BL21(DE3) transformants on Luria-Bertani/Amp. agar plate

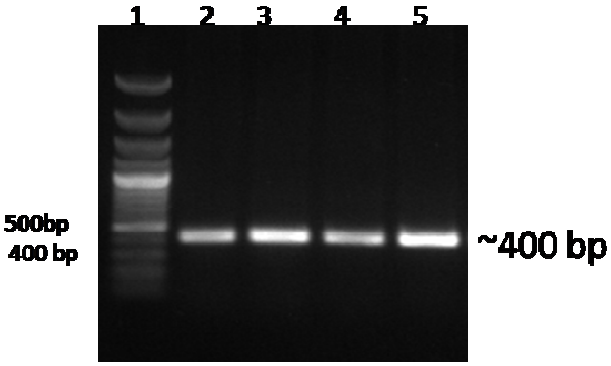


Fig.: VHH-PCR of VHH-pET303/CT dAb Clone transformed in E. coli BL21 (DE3) using A6E and FR4 primer [Lane 1: 100 bp DNA ladder; Lane 2-5: PCR product from four selected dAb Clones; dAb Cl16, Cl18, Cl23, Cl26]

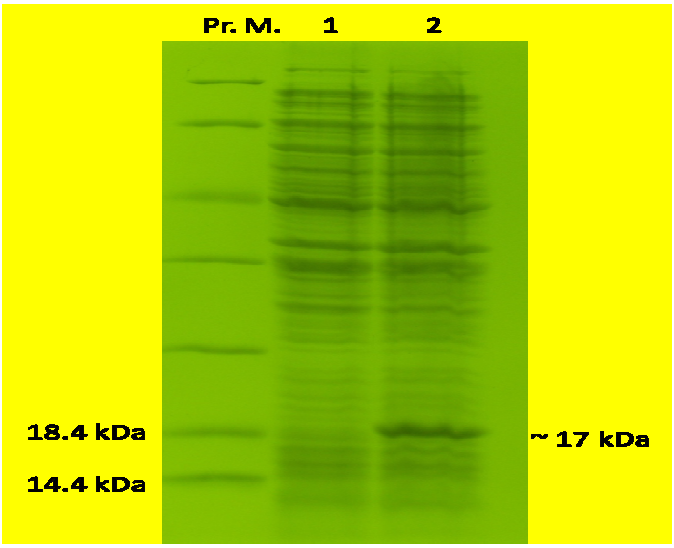
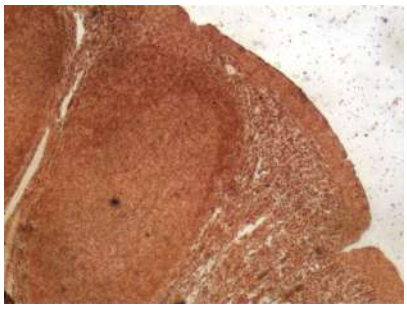


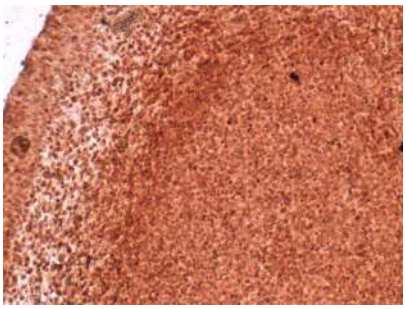
Fig. : SDS-PAGE followed by Coomassie's brilliant blue R-250 staining of 15% resolving gel to show protein profiles of uninduced and IPTG-induced bacterial lysates of dAb Cl26 [Lane Pr. M: Protein molecular marker; Lane 1: uninduced bacterial lysate; Lane 2: IPTG-induced bacterial lysate after 10 hours of growth]

In Department of Vety. Anatomy

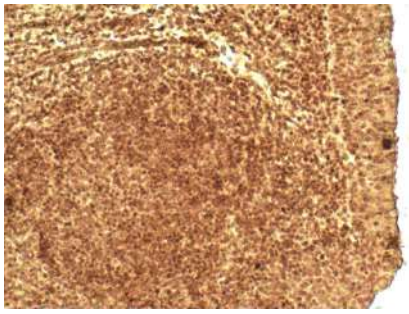
i) Different antibodies have been demonstrated in tonsils of the sheep

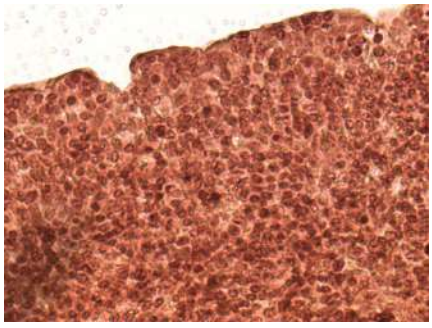
Distribution pattern of CD4 in tonsils of sheep



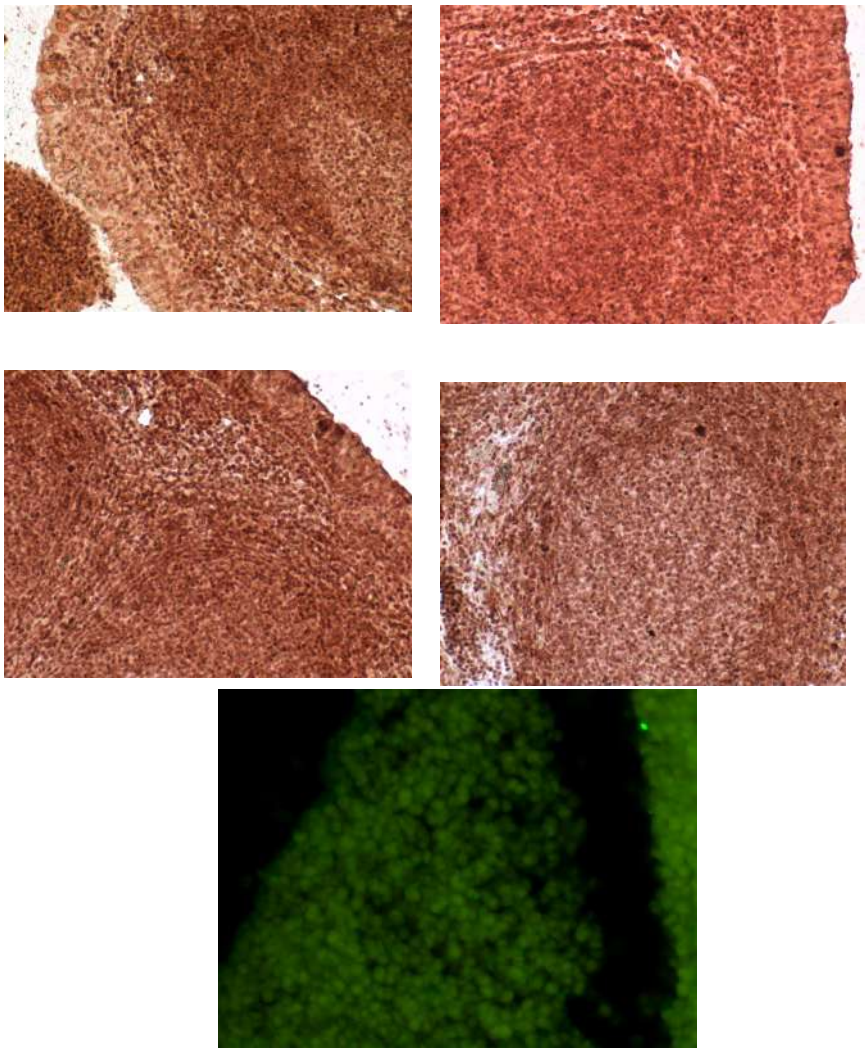


Distribution pattern of CD8 in tonsils of sheep





Distribution pattern of IgA in tonsils of sheep



The antibodies are showing some background on frozen sections

ii) The antigen retrieval technique is under standardization

11. Technology developed:

Standardized the immunohistochemical staining method for detection of:

i) ***Mycobacterium bovis*** **(Tuberculosis)**

ii) **Marek's disease/Lymphoid leucosis**- CD3 antibody (T cell marker) and CD79 alpha antibodies in tissues for neoplastic conditions in poultry

iii) **Infectious Bursal Disease vaccines** - Detection of CD4+ and CD8+ T cells in experimental vaccinated chickens

iv) **Genital and mammary tumours-** immunohistochemical expression of PCNA, Ki-67 and COX-2 in tumours of dogs

v) ***Pasturella multocida (Galghotu/HS)***- immunohistochemical detection of antigen in formalin fixed tissues collected from experimental rabbits

vi) ***Mycobacterium avium*** **subsp.** ***paratuberculosis*** **(MAP)/ Jhone's disease (JD)**

Summary of total budget allotted, utilized, remained unspent and different physical/financial targets achieved:							
Sr. No.	**Name of Project alongwith Department / Implementing Agency**	**Year (In which project approved)**	**Unit of Measurement**	**Targets of the project approved by SLSC**		**Cumulative achievement till the reporting March, 2021 Month from April, 2018 including number of beneficiaries (Total beneficieries-67)**	
				Physical	**Financial**	**Physical**	**Financial**
1.	**Project:-** "Establishment of Immuno-histo-chemical laboratory and strengthening of Immuno-technology laboratory for diagnosis of diseases of animals and poultry" Scheme:- 4060-C(g)-VPT-04-O.A. (RKVY) Department:- Veterinary Pathology (First installment sanctioned in	2018-2019	NA	Equipment/ Instruments (M&E) and chemicals/Kits/Reagents (M&S) and other laboratory infrastructure work related "OC(I)"	1. First Installment: On dated 03.08.2018- Rs. 37,65,000/- 2. Second Installment: On dated 01.03.2019 Rs. 87,85,000/- **(remained unspent and revalidated in the next year sanctioned budget)** 3. Amount of Rs. 89,49,847/- (after revalidation) was received in the department vide Comptroller Office letter no. LUVAS/CVU/B-1/2019/3181-83 Dated 08.07.2019 under different SOEs	1. **Out of Rs. 37,65,000/-** ✔Purchased three equipments (Oven, Hot plate and Leica Microtom es) of Rs. 22,54,350/- under SOE 'M & E' ✔Purchased different chemicals/kits/antibodies/materials of Rs. 14,23,300/- under SOE 'M & S' in the year 2018-2019. **2. Out of Rs. 89,49,847/- (after revalidation)** ✔ Purchased different chemicals/ kits/antibodies/materials of Rs. 28,55, 655/- under SOE 'M & S'. ✔ Purchased following equipments costing Rs. 50,49,514/- under "M&E" ❖ Thermocycler/PCR system-02 ❖ Deep freeze-02 ❖Table top centrifuge-01 ❖Digital Electronic Balance-02 ❖ Horizontal Electrophoresis System with power supply-02 ❖ Vertical laminar flow cabinet-01 ❖ Vertical autoclave-01 ❖ Digital pH meter-01	Spent:- Rs. 3677650/- +Rs. 8949847/-= Rs. 12627497/- Balance Unspent:- Rs. **Nil**

	August, 2018)					❖ BOD incubator-01 ❖ Binocular Microscope-01 ❖ Vacuum oven-01 ❖ Split air conditioner-06 ❖ Double Glass Distillation apparatus-01 ❖ Vertical electrophoresis system-01 ❖ UPS/Voltage stabilizer 5KVA for AC-02 ❖ 1.0 KVA Inverter (Luminous UPS ECO 1050 +) with battery 12V 150 AH and trolley-01 ❖ Vortex shaker-01 ❖ Magnetic stirrer with hot plate-01 ❖ Microcentrifuge-02 ❖ PCR Series workstation-02 ❖ Biosafety cabinet-01 ❖ Spare sealing for Rotor-01 ✔ Utilized Rs. 49,678/- of OE (NR) ✔ Temporary Advance of Rs. 9,95,000/- under SOE "OC(I)" has been drawn and deposited in the account of DSW-cum-Estate Officer, LUVAS. Hisar for the renovation work of different Laboratories of the Department of Veterinary Microbiology and Veterinary Pathology under the scheme.	

Annexure-I

Project name: **Establishment of Immuno-histo-chemical laboratory and strengthening of Immuno-technology laboratory for diagnosis of diseases of animals and poultry**

Scheme:- **4060-C(g)-VPT-04-O.A. (RKVY)** **RKVY-RAFTAAR SCHEME**

PHOTOGRAPHS OF THE MAIN EQUIPMENTS/INSTRUMENTS

Biosafety cabinet with Accessories (Class II A2) with temperature control unit and UV Lamp

BOD incubator (NSW-152 Super deluxe model)

Annexure-I

Project name: **Establishment of Immuno-histo-chemical laboratory and strengthening of Immuno-technology laboratory for diagnosis of diseases of animals and poultry**

Scheme:- **4060-C(g)-VPT-04-O.A. (RKVY)** **RKVY-RAFTAAR SCHEME**

Thermocycler/PCR system (Proflex 3x32 Well PCR system)

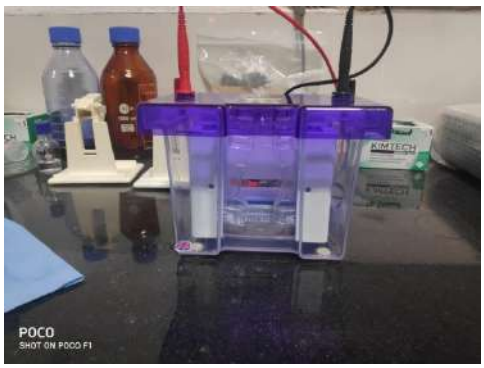
Leica Microtome (Model- Histo Core MULTICUT)

Annexure-I

Project name: **Establishment of Immuno-histo-chemical laboratory and strengthening of Immuno-technology laboratory for diagnosis of diseases of animals and poultry**

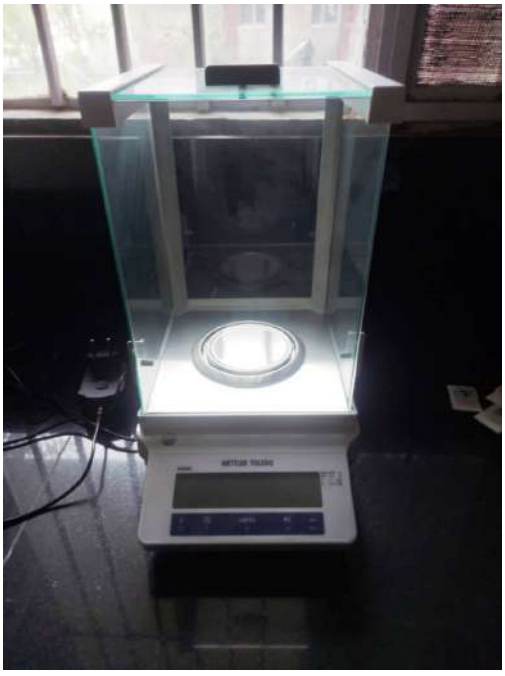
Scheme:- **4060-C(g)-VPT-04-O.A. (RKVY) RKVY-RAFTAAR SCHEME**

Horizontal Electrophoresis System with power supply (Cleaver scientific and MSCHOICERIO WITH POWER PRO 300)



Vertical electrophoresis system (Cleaver scientific single moulded)

Table top centrifuge (Sigma 2-16 KL)



Digital Electronic Balance (Analytical balance ME 204, Mettler Toledo)

Annexure-I

Project name: **Establishment of Immuno-histo-chemical laboratory and strengthening of Immuno-technology laboratory for diagnosis of diseases of animals and poultry**

Scheme:- **4060-C(g)-VPT-04-O.A. (RKVY) RKVY-RAFTAAR SCHEME**

Deep freeze (Single Solid Door Upright Freezer Vestfrost)

Vertical autoclave (40 L NSW-227)

Hot Plate 300X455mm (NSW – 255)

Oven Universal 224 L 605 X 605 X 605 MM (NSW) (Narang Scientific Works NSW-143

Annexure-I

Project name: **Establishment of Immuno-histo-chemical laboratory and strengthening of Immuno-technology laboratory for diagnosis of diseases of animals and poultry**

Scheme:- **4060-C(g)-VPT-04-O.A. (RKVY)** **RKVY-RAFTAAR SCHEME**

Vortex shaker (Spinix Vortex shaker 3020)
and
Magnetic stirrer with hot plate (Spinot TM Digital magnetic Stirrer Hot Plate 6040)

Vacuum oven (NSW-251B)

Micro centrifuge (1000- Spinwin Micro-centrifuge)

Annexure-I

Project name: **Establishment of Immuno-histo-chemical laboratory and strengthening of Immuno-technology laboratory for diagnosis of diseases of animals and poultry**

Scheme:- **4060-C(g)-VPT-04-O.A. (RKVY) RKVY-RAFTAAR SCHEME**

Impact Evaluation Study of RKVY-RAFTAAR Scheme

MINISTRY OF AGRICULTURE, GOVERNMENT OF INDIA

PROJECT UNDER RASHTRIYA KRISHI VIKAS YOJNA (RKVY)-RAFTAAR SCHEME

ON

Project name: **Establishment of Immuno-histo-chemical laboratory and strengthening of Immuno-technology laboratory for diagnosis of diseases of animals and poultry**

Scheme:- **4060-C(g)-VPT-04-O.A. (RKVY)**

LIst of Beneficiary Post Graduate Students

Name of the beneficiary student	Title of the thesis	Admn no.	MVSc./PhD.	Thesis submitted in the year	Major Advisor	Contact No.
Sushma	Sequential Immunopathological Studies on Infectious Bursal Disease Vaccine in Broiler Chickens	2016V20D	PhD.	2019	Dr. Gulshan Narang	7082226572
Shobhna Singh	Aetio-pathological studies on respiratory tract disorders in small ruminants with special reference to *pasteurellosis* and *Pestes des Petits* ruminants	2017V33M	MVSc	2019	Dr. Chandratre Gauri A.	9034330066
Shailja Pandey	Pathobiological studies on poultry mortality due to hepatic disorders with emphasis on neoplastic conditions	2017V32M	MVSc	2019	Dr. Deepika	7651999497

Annexure-I

Project name: **Establishment of Immuno-histo-chemical laboratory and strengthening of Immuno-technology laboratory for diagnosis of diseases of animals and poultry**

Scheme:- **4060-C(g)-VPT-04-O.A. (RKVY)** **RKVY-RAFTAAR SCHEME**

Charlie Sharma	Pathobiological studies on bovine respiratory disorders with emphasis on infectious diseases	2017V31M	MVSc	2019	Dr. Vikas Nehra	8219169038
Shreya Bahl	Pathomorphological and Immunohistochemical Studies on Tumours in Dogs with Special Reference to Genital and Mammary Tumours	2017V34M	MVSc	2019	Dr. Babu Lal Jangir	8178463730
Diwakar Singh Rana	Pathological studies on experimentally induced *Pasteurella multocida* infection in rabbits	2018V42M	MVSc	2020	Dr. Gulshan Narang	8307121759
Subhash (Thesis submitted)	Pathobiological studies on gastrointestinal tract disorders in ruminants with emphasis on paratuberculosis	2018V44M	MVSc	2021	Dr. Deepika	9466592856