DEPARTMENT OF VETERINARY PATHOLOGY
COLLEGE OF VETERINARY SCIENCES
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MAJOR RESEARCH ACHIEVEMENTS OF THE DEPARTMENT

1. **Brucellosis:** Diagnostic tests like dot enzyme linked immunosorbent assay, counter immunoelectrophoresis technique, coagglutination assay have been developed for the serodiagnosis of brucellosis.

2. **Listeriosis:** *Listeria ivanovii* has been isolated for the first time in India, from cases of abortion in sheep.

3. **Degnala disease:** Degnala disease in buffaloes and cattle has been found to be associated with feeding of fungus-infested (*Fusarium equiseti*) rice straw. On the basis of these findings, preventive measures have been suggested to the farmers and field veterinarians.

4. **Horn cancer:** Bovine horn cancer, a squamous cell carcinoma of the horn core epithelium, has been found to metastasize to various lymph nodes and internal organs like lungs, heart and meninges of brain. Treatment of the animals with a saline phenol extract of the cancer tissue and levamisole gave encouraging results in the restoration of the depressed CMI status and help in recovery in early cases.

5. **Hydropericardium syndrome:** Litchi disease (Hydropericardium syndrome), a highly contagious disease of broiler chicks, was experimentally reproduced and studied. A formalized liver suspension can be used for its prevention under field conditions. Significant research has been carried out in the department to study the effect of various parameters on the pathology and pathogenesis of the disease. These findings include:
   - Supplementation of Vitamin E @ 150 mg/kg of feed, Zinc sulphate @ 700 ppm or Ascorbic acid @ 330 ppm reduced the severity as well as recovery period of HPS infection suggesting their protective role in limiting the pathology and pathogenesis of HPS infection in broiler chickens. Secondly, Vitamin E supplementation enhanced the Cell mediated immune response suggesting its immunomodulatory activity.
   - Ochratoxin-A @ 1.5 ppm in feed causes the immuno-suppression and an increased severity of Hydropericardium syndrome (HPS) in broiler chicks.
   - Studies on administration of Cyclosporin A and Cyclophosphamide (important T- Cell suppressors) in experimentally affected HPS broiler chickens revealed that the T-Cell and B-Cell plays a significant role in pathogenesis and pathology of disease.
   - The effect of Infectious bursal disease (IBD) vaccine and levamisole was also studied on the pathology and pathogenesis of experimentally induced HPS and it was found that severity of HPS was increased in IBD vaccinated chickens whereas there was a therapeutic effect of levamisole on the basis of clinical signs, mortality and patho-biochemical studies.
   - A comparative study to investigate the efficacy of oil emulsion and formalin killed vaccines (inactivated vaccines) against hydropericardium syndrome in broiler chickens revealed that formalin killed vaccine is more effective than the oil adjuvant vaccine.

6. Etio-pathological and Patho-biological studies:

Etio-pathological studies on bovine calf mortality revealed that:

- ✓ Main cause of death of bovine calves is bacterial infections mainly *Escherichia coli* associated with pneumonia and enteritis and hepatitis.
- ✓ Various serotypes of *Escherichia coli* were isolated and one of the serogroup O114 has significance from public health point of view.
- ✓ *In vitro* drug sensitivity and resistance studies revealed that *Escherichia coli* isolates were most sensitive to combination of ceftriaxone and sulbactum followed by gentamicin and most resistant against oxytetracycline and streptomycin.
- ✓ Plasmid profiling of *Escherichia coli* isolates revealed that out of 19 *Escherichia coli* isolates, 18 *Escherichia coli* isolates possessed plasmids of molecular weights ranged from 4 to 20 Kb. The serotypes which were resistant to one or more than four antibiotics were found to possess plasmid of high molecular weight i.e. 20 Kb.

Pathobiological studies on poultry mortality due to hepatic disorders with emphasis on neoplastic conditions revealed that:

- ✓ Colisepticemia was the most prevalent disease condition observed in chicken (33.7%) affecting mainly 1-6 wks age group indicating *Escherichia coli* as the major pathogenic agent, followed by neoplastic conditions particularly diagnosed as Marek's disease (12.1%) in adult birds.
- ✓ *E. coli* isolates showed maximum sensitivity to gentamicin, streptomycin and polymyxin B, while *Staphylococcus aureus* isolates showed maximum sensitivity to oflaxacin and polymyxin B. So, these drugs may be used as the drug of choice for treating clinical cases. About 91.3% *E. coli isolates* showed MDR pattern; while all the isolates of *Staphylococcus aureus* were showing MDR pattern.
- ✓ Marek's disease cases were mainly found affecting layer breed with severe involvement of liver, spleen and lungs. Visceral form of the Marek's disease was much prevalent compared to classical or nervous form as none of the cases revealed nerve involvement.
- ✓ TaqMan probe real time PCR was more sensitive than nested PCR for diagnosis and screening of field samples for MDV oncogenic gene in vaccinated flocks.
- ✓ Immunohistochemical studies indicated usefulness of CD79 alpha and CD3 differentiation markers in differentiating Marek's disease lesions from other B cell lymphomas.

Pathobiological studies on bovine respiratory disorders with emphasis on infectious diseases revealed that:

- ✓ In different bovines, mortality due to respiratory system involvement was more in adults suggesting that more care and attention need to be paid to the management and treatment of the adult bovines.
- ✓ Sex-wise mortality was more in females as compare to males.
- ✓ Sero-fibrinous bronchopneumonia was the most common type of pneumonia present in all the bovines.
- ✓ Granulomatous pneumonia was most common type of pneumonia among adult cattle.
- ✓ Different bacterial species isolated from bovines include *E. coli* (35 strains), *Klebsiella pneumonia ssp. pneumoniae*, *Salmonella enterica enterica*, *Staphylococcus spp*. (6 strains), *Providencia struatii*, *Acinetobacter spp*. (2 strains), *Aerococcus viridians* (3 strains) and *Enterobacter cloacae dissolvens*.

- ✓ Most of the bacterial species were highly sensitive to cefoperazone/sulbactum and highly resistant to cloxacillin in bovines.
- ✓ *E. coli* serotype O157 and *Salmonella welteverden* requires special attention because of its zoonotic importance.
- ✓ Both, *Mycobacterium bovis* and *Mycobacterium tuberculosis* were responsible for granulomatous pneumonia in adult cattle thereby also raising the potential zoonotic concern to the public health.
- ✓ Infectious Bovine Rhinotracheitis (IBR) caused by BoHV-1 was the only viral disease detected in the present study.

Aetio-pathological studies on respiratory tract disorders in small ruminants with special reference to *pasteurellosis* and *Pestes des Petits* ruminants revealed that:

- ✓ Maximum mortality in small ruminants was found to be associated with bacteria isolated in present study and virus interaction, *Pasteurella multocida* was main pathogen which flared up due to compromised immune system as a result of PPR viral infection.
- ✓ Amikacin, Gentamycin and Tobramycin were most effective drugs against respiratory infection caused by gram negative bacteria isolated in present study.
- ✓ *Sphingomonas paucimobilis* was identified as a pathogenic bacteria in association with PPR virus, responsible for hemorrhagic pneumonia in goats
- ✓ Sandwich ELISA can be used as screening test in outbreak situation to diagnose the PPR since it is economical, easy to perform and simple to interpret in field conditions.

Etio-pathological studies on poultry mortality showed that:

- ✓ Gastrointestinal disorders are the main cause of poultry mortality. Severity of gross and microscopic lesions in cases of Salmonella spp. and *Escherichia coli* infections indicate that both are still major pathogens of poultry. Majority of isolates were sensitive to ceftrioxone, cefuroxime, amoxyclav, chloramphenicol, gentamycin and amoxycillin. So, these drugs may be used as the drug of choice for treating clinical cases of these bacterial infections.

7. Studies on *Pasteurella multocida*:

- In the experimental study of pathogenicity and preliminary vaccine trial in mice on field *Pasteurella multocida* isolated from post mortem cases it was observed that Saponin administration @200 µg/mice caused death and cannot be advised as an adjuvant in the mice with killed *Pasteurella multocida* serotype B.
- In the experimental study of induced *Pasteurella multocida* infection in rabbits; *P. multocida* serotype B:2, the causative agent of HS in large animals was found to be highly fatal in rabbits and rabbits could serve as a good laboratory animal model for the pathogenic study of the pasteurellosis.

8. Studies on deficiency diseases:

- Zinc deficiency which is quite common in Haryana state has been found to cause immuno-suppression (both cellular and humoral) and increases the severity of Salmonella infection and foot and mouth disease in guinea-pigs.

- Outbreaks of rheumatism-like syndrome in buffaloes in different parts of Haryana were successfully investigated and were found to be associated with phosphorus deficiency. The affected animals responded favourably to phosphorus.

9. Immuno-pathological studies on herbal immunomodulators/nano-formulations etc. on various poultry/laboratory animal diseases/conditions:

- Supplementation of *Ocimum sanctum* (Tulsi) leaves powder @ 5g/kg feed was found to have protective effect and immune modulatory action in experimental *Salmonella gallinarum* and HPS infections in broiler chickens.
- Supplementation of Neem leaf extract (10%) reduced the severity of HPS and *Salmonella gallinarum* infection and enhanced the cell mediated immune response, suggesting the protective role of Tulsi and Neem leaves in limiting the pathology and pathogenesis of infections in broiler chickens.
- Vitamin C @ 100mg/kg b. wt. may be incorporated as a supplement in water to reduce the severity of imidacloprid toxicity.
- *Emblica officinalis* (Amla) dry fruit extract supplementation @ 10gm/kg feed may be incorporated as a supplement in feed to reduce the severity and recovery period of colibacillosis in chickens.
- *Tinospora cordifolia* (Giloy) extract @ 1gm/kg feed and probiotic *Bacillus subtilus* @10^9 CFU/bird/day may be safely used as a supplement in feed to reduce *Escherichia coli* infection in chicken.
- *Withania somnifera* (Ashwagandha) root extract @ 20ml/litre of water and *Aloe vera* (Amla) leaf extract @ 20ml/litre of water may be used as a supplement in water to reduce *Escherichia coli* infection in chicken.
- Pathobiological and immunological studies on alpha cypermethrin toxicity and its interaction with *Salmonella* Gallinarum infection in broiler chicken revealed that alpha-cypermethrin intoxication @ 63.938 mg/kg b. wt. and 31.969 mg/kg b. wt. enhanced the severity of the *Salmonella* Gallinarum infection.
- Pathobiological and immunological studies on deltamethrin toxicity and its interaction with *Escherichia coli* infection in broiler chicken revealed that deltamethrin intoxication @ 8.75 mg/kg b. wt. and 4.375 mg/kg b. wt. enhanced the severity of the *Escherichia coli* infection.
- Effect of Spirulina feeding on sequential pathology of Infectious bursal disease vaccine in broiler chicken revealed that Spirulina supplementation @ 1 g/100 gm of feed reduced the immunosuppressive effect of infectious bursal disease vaccine as evidenced by clinical signs, biochemical parameters, immunological response and pathological lesions. There was increase in growth performance as evidenced by body weight gain.
- During toxicopathological investigations on doxorubicin induced cardiotoxicity and genotoxicity in Swiss albino mice and its amelioration by eugenol it was revealed that:-
  - ✓ Doxorubicin was found to be potentially cardiotoxic, genotoxic and neurotoxic besides causing varying degrees of pathological changes in the liver, and kidney of Swiss albino mice when administered @25mg/kg b.w single and cumulative dose (5mg/kg alternate day upto 25mg/kg) intraperitoneally.
  - ✓ Oxidative stress and inflammation were found to play a major role in causing doxorubicin induced cardiotoxicity as evidenced by high activity of nitric oxide and

lipid peroxidation along with upregulation of inflammatory cytokines TNFalpha, IL-1 and iNOS in the heart of Swiss albino mice.

- ✓ Cardiotoxic and neurotoxic lesions were more prominent in acute toxicity of doxorubicin whereas genotoxic lesions were more prominent in repeated dose toxicity of doxorubicin in bone marrow as evidenced by high frequency of micronuclei formation and significant DNA damage to bone marrow cells.
- ✓ Eugenol supplementation provided efficient protection against DOX-induced cardiotoxicity and genotoxicity by enhancing activity of antioxidant enzymes; Super Oxide Dismutase and catalase, downregulation of inflammatory cytokines and mitigation of DOX-induced apoptosis by prevention of DNA damage in bone marrow suggesting ameliorating potential of eugenol in reducing doxorubicin induced toxicity.
- ✓ Cardioprotective, genoprotective, anti-oxidant and anti-inflammatory potential of eugenol against doxorubicin induced toxicity was found to be more when eugenol was administered prior to doxorubicin treatment as compared to concomitant treatment.

- During pathomorphological studies on wound healing potential of quercetin nanoparticles in Wistar rats it was found that: -
  - ✓ Crystalline nature of the quercetin is transformed into amorphous in quercetin nanosuspension along with small size leads to increased solubility in aqueous medium, better absorption and increased bioavailability
  - ✓ Quercetin nanoparticle at a concentration of 0.3% showed significant improvement in the healing of diabetic wound as compared to the diabetic control group by improving inflammatory and proliferative phase of wound healing
  - ✓ Quercetin nanoparticles @ 30mg/kg b.wt. had significantly improved gastric ulcer healing and leads to regeneration of mucosal epithelium with increased mucus production and increased expression of COX-2 involved in prostaglandin synthesis

10. Immuno-histochemical studies on animal tumors: -

During molecular and immunopathological studies on canine mammary tumours with special reference to role of heavy metals in carcinogenesis findings were:

- ✓ 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.

During pathobiological studies on bovine neoplasms with special reference to epithelial tumours findings were:

- ✓ 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).
- ✓ 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.

During pathological and immunohistochemical studies on tumours in dogs with special reference to mesenchymal tumours findings were:

- ✓ Pancytokeratin (PCK) and vimentin expression helped in diagnosis, explaining the origin of tumour, differentiation of epithelial and mesenchymal tumours particularly undifferentiated types.
- ✓ CK-14 expression was found to be useful in differentiation of luminal epithelial and myoepithelial cells in CMT, thus their role in tumour formation.
- ✓ PCK expression decreased with increased malignancy/grade in CMT.
- ✓ PCNA (Proliferating Cell Nuclear Antigen) expression was higher in sarcomas and malignant epithelial tumours as compared to benign tumours.

Pathomorphological and Immunohistochemical Studies on Tumours in Dogs with Special Reference to Genital and Mammary Tumours revealed that:

- ✓ Evaluation of mitotic index, AgNOR counts, immunohistochemical expression of PCNA, Ki-67, COX-2, α-SMA, vimentin, PCK and CK-14 are important adjunct tools besides routine cytological and histopathological examination for determining the origin of neoplastic cells and biological behaviour of tumours.

11. Brief findings of the immunohistochemical examination of different samples for disease diagnosis after proper standardization are as under:-

Standardized the immuno-histochemical 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)**

12. **Vaccine development: -**

- **Generation of a genetically modified equine herpes virus (EHV-1) vectored vaccine candidate for infectious bursal disease and to study its protective efficacy in broiler chickens revealed that: -**
  - ✓ Deleted mutant vector in BAC as developed by NRCE particularly the two deletion mutant (2**Δ**-EHV-1-BAC) showed stable and adequate attenuation after gene insertion of VP2 gene of IBDV as assessed through reduced plaque size without any substantial loss in the replication efficiency in *in- vitro* studies.
  - ✓ 2**Δ**-EHV-1-BAC vector served as excellent vector for protein expression of the VP2 gene of IBDV as evident from the stable and stronger protein expression studies on the vector vaccine candidate using time based IFAT assay.
  - ✓ 2**Δ**-EHV-1-BAC-VP2 vector vaccine candidate caused no adverse bursal lesions proving as strong vaccination candidate comparative to present vaccines employed in field for management and control of IBDV in flocks.
  - ✓ 2**Δ**-EHV-1-BAC-VP2 vector vaccine candidate elicited stable humoral and cellular immune response and maintained the immune status of the chicks post IBDV challenge.
  - ✓ 2**Δ**-EHV1-BAC-VP2 vector vaccine candidate induced a specific neutralizing-antibody-mediated immune response that confered very high protection against IBDV challenge, and also hampered the replication of virus inside lymphoid organs thereby providing effective viral clearance from the lymphoid organs.
  - ✓ The interspecies use of EHV-1-BAC vector has not been conducted henceforth, the present study proved vector efficacy, thereby providing a channel for EHV-1-BAC to be used as vector for vaccine production in avian species.
  - ✓ The ELISA titers for serum samples were negative using commercial kits, indicating that the delivery system of 2**Δ**-EHV-1-BAC-VP2 is different from other vaccines. The present study indicated that immunization with EHV1- BAC vectored vaccine in chicks induced specific and unique antibodies compensating for stronger and better protection not provided by conventional IBD vaccines. Henceforth, it is of great practical significance for IBD prevention and control. Although, further studies are required to elaborate on the major mechanisms of immune protection utilizing 2**Δ**-EHV1-BAC vector