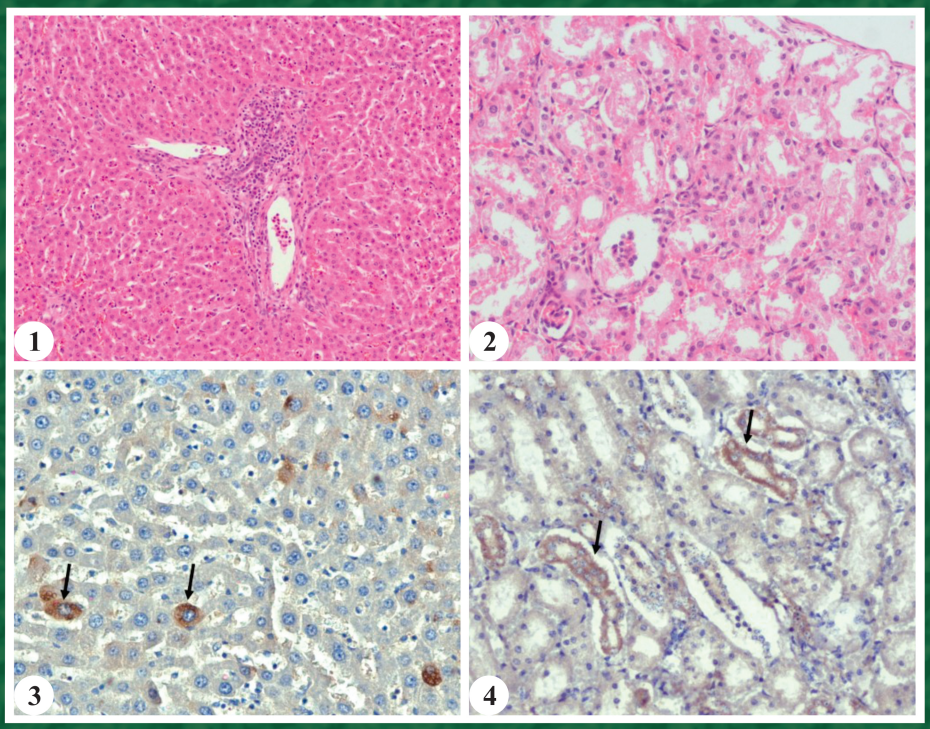
Vol.: 49(3)
September, 2025
ISSN: 0250-4758
Online ISSN: 0973-970X

IJVP-2025

INDIAN JOURNAL OF VETERINARY PATHOLOGY



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Vol. 49 (3)
September, 2025
ISSN: 0250-4758

INDIAN JOURNAL OF VETERINARY PATHOLOGY

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Cover Page Photo (Clockwise) : FMD-Photographs showing histopatholiogical lesions in FMD affected calf as periportal hepatitis with infiltration of mononuclear cells in the portal triad in liver (Left top), Necrosed glomerular tuft with degenerative changes in the tubules in kidneys (Right top), Immunohistochemical demonstration of FMDV antigens in hepatoctes (Left bottom) and Renal tubes in kidneys (Right bottom).

Indian J. Vet. Pathol., 49(3) : 199-204, 2025: DOI: https://doi.org/10.56093/xhp1wv66

REVIEW ARTICLE

Gut Health: The Foundation of Resilience in Food Producing Animals

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Received: 4.8.2025; Accepted: 10.8.2025

ABSTRACT

Optimal gut health is of vital importance to the performance of animals. Gut is responsible for regulating physiological homeostasis that provides the host the ability to with stand infectious and non-infectious stressors. The gut microbiota confers health benefits to the host, including aiding in the digestion and absorption of nutrients, contributing to the construction of the intestinal epithelial barrier, development and function of the host immune system and competing with pathogenic microbes to prevent their harmful propagation. Modulating gut health in animals involves manipulating the gut microbiome to improve overall animal health and productivity. This can be achieved through various strategies like dietary adjustments, prebiotics, probiotics, postbiotics and even faecal microbiota transplantation. These methods aim to shift the balance of gut microbes toward a more beneficial composition, thereby enhancing nutrient utilization, boosting immunity and reducing the risk of disease.

Keywords: Faecal microbiota transplantation, gut health, intestinal epithelial barrier, postbiotics, prebiotics, probiotics

INTRODUCTION

According to Hippocrates 460-370 BC, "all disease begins in the gut and health is determined by the microbiota in the gut!". The intestine represents one of the largest interfaces of the animal body with the external environment. The gastrointestinal tract is responsible for regulating physiological homeostasis that provides the host the ability to with stand infectious and non-infectious stressors[1]. Most of the studies addressing health and animal production have been focused on gut microbiota, which is justified by the crucial role of these microorganisms in nutrition, fitness and performance traits[2]. Public concerns about the use of growth-promoting antibiotics (AGPs) in animal agriculture have led to significant policy changes. The European Union has banned AGPs, while the United States is reassessing their use. These actions stem from growing evidence that AGP use contributes to antibiotic resistance, posing a threat to both animal and human health[3]. In India, several antibiotics are banned and some are restricted for use in livestock and poultry, primarily to combat antimicrobial resistance. Removal of AGPs from animal feeds results in an increase in enteric disorders, infections as described[4,5]. The ban on AGP has triggered a renewed scientific interest in the intestinal health of animals. While in the past, the focus of gut health research was almost exclusively on the veterinary aspects of pathogenic organisms invading the intestine and/or intestinal tissues, causing severe damage to the host mucosa and resulting in clinical symptoms of disease[6]. The current focus is on the fundamental aspects of the numerous complex and subtle interactions between the host mucosa, the intestinal content and all organisms residing in the intestinal tract.

Gut health is defined as "a steady state where the microbiome and the intestinal tract exist in symbiotic equilibrium and where the welfare and performance of the animal is not constrained by intestinal dysfunction"[7]. In animals raised for food, gut health is closely related to animal health and is directly related to the animals' growth and performance. A damaged gut can have a negative impact on feed conversion ratio, digestion and nutrient absorption, which can result in financial loss and increased susceptibility to disease[8,9]. However, a healthy gut is essential for the well-being of companion animals, and alterations in gut microbiota have already been linked to a number of illnesses, including allergies, cardiovascular disease and inflammatory bowel disease (IBD)[10,11].

How to cite this article : Kaore, M., Hedau, M., Sonkusale, P.M. and Kurkure, N.V. 2025. Gut Health: The Foundation of Resilience in Food Producing Animals. Indian J. Vet. Pathol., 49(3) : 199-204.

Recently, it has also been shown that there is extensive communication between the brain and the microbiota *via* the brain-gut-microbiome axis. Through this bidirectional communication, signals from the brain can influence the motor, sensory and secretory functions of the gut and visceral messages from the gut can influence brain function[12]. Similarly, the gut-kidney axis involves the interplay between the gut microbiome, intestinal barrier, microbial metabolite production and renal physiology[13].

The intestine is the site of the highest antigen load caused by microbial and feed antigens in the gut lumen. The intestine is made up of an epithelium, a robust and diverse immunological system that contains most of the body's immune cells and commensal bacteria, which outnumber the host cells overall. Understanding of the interaction between all these interrelated components of the gut is what cumulatively makes the gut the basis for the health of animals[9].

Intestinal barrier

The intestinal homeostasis is determined by the intestinal epithelium, gut microbiome and host immune system. This functional unit is entirely dependent on the integrity of the gut epithelium, which is maintained by junctional proteins such as adherent junctions, tight junctions and desmosomes that join neighbouring epithelial cells and provide a physical barrier together with the lamina propria[14]. The glycocalyx on the surface of intestinal epithelial cells contributes to barrier function by preventing exogenous molecules and live bacteria from gaining access to the epithelial brush border membrane[15]. Key cell types in the physical barrier are absorptive enterocytes, Paneth cells and goblet cells. The epithelial cells in the small intestine form a continuous layer and the space between the cells is sealed by tight junctions. These tight junctions are a critical element of the gut barrier. Although Paneth cells produce antimicrobial peptide AMPs, goblet cells have a key role in barrier function by producing gel-forming (MUC2 and MUC6) or transmembrane (MUC3, MUC12, MUC13, MUC15 and MUC17 in the small intestine; MUC4, MUC20 and MUC21 in the large intestine) mucins. The mucus layer which prevents bacterial adhesion. Lysozyme and secretory IgA are key factors of the chemical barrier[16].

Changes in the expression level and functioning of tight junctions cause gut leakage, characterized by body fluids leaking into the intestinal lumen, which may ultimately result in diarrhoea[17]. In this context, the organised intestinal barrier prevents uncontrolled microbial induced immune reactions in the gut. Disruptions of the intestinal barrier result in substantial alterations to the delicate equilibrium between luminal antigens and the local immune system. Consequently, a leaky gut permits the translocation of bacteria, other microorganisms and luminal antigens into the bowel wall, thereby inducing an overwhelming proinflammatory mucosal immune response[18,19].

Intestinal mucosa maintains immune tolerance to a wide array of antigens while also inducing appropriate immune responses to external pathogens[20]. To maintain the health of intestine, its mucosa contains variety of innate and adaptive immune cells, including innate lymphoid cells, granulocytes, dendritic cells, macrophages, B cells and both α-β and γ-δ T cells. These cells can support barrier function through direct killing of invading pathogens, production of soluble mediators, such as cytokines (IL-10, IL-17 and IL-22), neutrophil extracellular trap formation or the local induction of protective immune responses against antigens, which form an immune barrier towards invading antigens and pathogens[21]. Innate and adaptive immune responses in the intestine are constrained by the local production of anti-inflammatory cytokines (e.g. IL-10 and TGFβ), which suppress effector functions of multiple immune cell lineages and promote the population expansion of regulatory T cell responses[22]. This homoeostatic cytokine balance is crucial for preventing excessive inflammatory responses in the intestine.

Intestinal barrier dysfunction

Dysfunction of intestinal barrier and alterations in intestinal permeability is also known as "leaky gut." The effects of pathogenic organisms on host intestinal epithelial cells are complex. These primary pathogen-host interactions may result in disturbances in the normal intestinal barrier, activation of the inflammatory cascade and alterations of normal fluid and electrolyte secretion. Enteric pathogens can bind to the cell surface and induce changes in the expression of tight junction proteins[23]. In addition, the production of toxins by pathogens can promote cellular damage through disruption of intracellular protein interactions, leading to increased cellular permeability and ultimately triggering cell death[24].

IBD affect both human and animal patients and are associated with gastrointestinal dysfunction due to infiltration of the mucosa, submucosa or lamina propria with abnormal populations of immune cells. In dogs with IBD compared with normal controls, the expression of the protein E-cadherin was lower in the villus epithelium, suggesting the role of this protein in the pathogenesis of IBD in dogs. In horses with large intestinal disease, a significant difference in TNF-α expression was found in diseased mucosa, suggesting a possible role for this cytokine in the pathogenesis of equine IBD. TNF-α increases myosin light chain kinase phosphorylation, which may alter paracellular permeability through its association with actin and myosin. Myosin light chain kinase expression and enzymatic activity are increased in cases of IBD and correlated with disease activity[25].

Gut microbiota

The gut microbiome of domestic animals is a complex community of microorganisms; viruses, bacteria, fungi, protozoa and other microbes residing in their digestive tracts, with each region harbouring distinct microbial populations. The intestinal microbiota contributes to several physiological, protective (pathogen displacement, nutrient competition, receptor competition, production of antimicrobial factors), structural (GIT barrier fortifi-

cations, induction of IgA, apical tightening of tight junctions, immune system development) and metabolic functions (ferment non-digestible dietary residue and endogenous epithelial-derived mucus, synthesize vitamins, control intestinal epithelial cell differentiation and proliferation, ion absorption)[26-29]. Several of the metabolites produced by the microbiota also stimulate the neuroendocrine cell in the GIT and therefore, the microbiota plays an important role in the endocrine regulation of gastrointestinal functionality[7,30].

The microbiome is dynamic and changes depending on things including nutrition, age and environment. The makeup of this microbiome can affect many facets of an animal's health, such as immunity, digestion and even behaviour[31]. The gut microbiome of cattle and sheep is dominated by Bacteroidetes and Firmicutes[32]. The dominant bacterial phyla in the poultry gut microbiome are Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria. Lactobacilli are predominant in the upper and middle GIT of poultry[33]. The canine gut microbiome is primarily composed of three dominant bacterial phyla; Firmicutes, Bacteroidetes and Fusobacteria. These phyla, along with others like Actinobacteria and Proteobacteria, contribute to a diverse and dynamic gut ecosystem[34].

The microbiome has a direct effect on the development and function of the mucosal immune system. The gut microbial alterations in animal gastrointestinal system or the differences in gut microbiome composition and function have been associated with a variety of diseases, ranging from metabolic conditions and gastrointestinal inflammation leading to colitis and respiratory illnesses[35,36]. Age, gender and species are important internal factors that influence the composition and structure of the gut microbiota[37]. Additionally, external factors such as heavy metals, antibiotics and pesticides can markedly disrupt the gut microbiota composition, leading to dysbiosis[38]. Moreover, the effects of the gut microbial community extend beyond the gastrointestinal system and can cause other systemic diseases[36].

From eubiosis to dysbiosis

Eubiosisis the balance of the intestinal microbial environment, which has positive impacts on the animal as a whole. Overall, healthy gut microbial communities are characterized by high taxa diversity, high microbial gene richness and a stable functional core of microbiome[39]. Gut dysbiosis is defined as an imbalance in the composition of the gut microbiota that may result in modifications to the transcriptome, metabolome or proteome of micro-organisms[40].

Neonatal calf diarrhoea is the leading cause of neonatal morbidity and mortality globally. The bacterial pathogens associated with calf diarrhoea include *E. coli*, *Salmonella* spp., *Clostridium perfringens* and *Clostridium difficile*. The two main viruses implicated in calf diarrhoea are bovine coronavirus and bovine rotavirus (BRoV). Calves with rotaviral diarrhoea had a lower relative abundance of Firmicutes and Bacteroidetes and a high abundance of Proteobacteria compared to their healthy counterparts[41]. At the genus level, the genera *Escherichia*, *Clostridium* and *Streptococcus* increased in BRoV-infected calves, while *Blautia*, *Bacteroides*, *Lactobacillus* and *Coprococcus* decrease[42]. Irrespective of the causative agent responsible for the onset of calf diarrhea, there are significant changes in bacterial communities of the gut microbiota[43]. During diarrhoea there is a shift from obligate anaerobes to facultative anaerobes in the GIT, resulting in dysbiosis[44]. The abundance of *Faecalibacterium prausnitzii*, *Lachnospiraceaesp.* and *Ruminococcacea sp.* bacteria associated with gastrointestinal health decreases significantly during calf diarrhoea[45]. Concurrently, an increase in *Lactobacillus*, *Streptococcus* and Enterobacteriaceae, especially *E. coli* is observed[46]. It is frequently noted that diarrheal calves have higher levels of *Enterobacteriaceae* bacteria[47]. Dysbiosis associated with inflammation results in alterations in the metabolites available to and originating from bacteria in the GIT of calves, resulting in an environment that favours the growth of *Enterobacteriaceae*. *Salmonella* spp. and *E. coli* benefit from the production of ethanolamine, lactate, glucarate/galactarate 1, 2, propanediol, succinate and L-serine during dysbiosis[48]. Infection with *Cryptosporidium parvum* in calves results in a reduction in the microbial diversity, and this reduction is proportional to the number of oocytes detected in the feces. Furthermore, an increase in the fecal abundance of *Fusobacterium* is reported in diarrheic calves infected with *C. parvum* compared to uninfected calves[49,50].

Rumen acidosis is one of the most prevalent gastrointestinal diseases affecting cattle, significantly threatening their health and growth performance. Rumen acidosis can induce alterations in the composition and diversity of the gut microbiota in calves. Notably, the levels of certain beneficial bacteria, such as Prevotella, Succinivibrio and Succinivibrionaceae decreased significantly. These substantial changes in intestinal composition and abundance may serve as critical driving factors for the development of rumen acidosis[51].

In pigs, *Enterotoxigenic Escherichia coli* (ETEC) induced diarrhoea is associated with a decrease in the Bacteroidetes/Firmicutes ratio. ETEC-induced diarrhoea in piglets decreases the microbial diversity in the jejunum and lowers the abundance of *Prevotella* compared to healthy counterparts. ETEC in piglets is also associated with an increased abundance of *Lactococcus* in the jejunum and *Escherichia Shigella* in the feces[52].

In poultry husbandry systems, coccidiosis is an

economically significant protozoan disease caused by an intracellular parasite that significantly impacts production. *Eimeria acervulina*, *Eimeria maxima* and *Eimeria brunetti* can reduce the abundance of Eubacterium, Lactobacillus and Ruminococcus in the cecum. Conversely, Eimeria infection can increase the abundance of bacteria like Bacillus, Enterococcus and Escherichia in the cecum[53]. Changes in the microbiota due to coccidiosis can affect the immune system's ability to respond to the infection. *E. tenella* infection alters the composition and diversity of caecal microbiota, significantly reducing Proteobacteria and Firmicutes *(Enterococcus)*[54]. Alteration induced by *Eimeria tenella* infection in abundance of the bacterial community may contribute to the severity of pathology and variation observed in tissue damage[55].

In canine, short-term changes in the intestinal environment, such as in cases of acute diarrhea, affect the microbial composition. Acute diarrhoea results in decrease in microbial diversity, with lower numbers of *Bacteroidetes* and *Faecablibacterium* and higher numbers of *Clostridium sp*[56]. Intestinal dysbiosis is linked to several chronic GIT illnesses, including IBD and Mucosa-adherent Proteobacteria genera *(E. coli)*[57].

Modulation of gut health

Modulation of gut health can play a key role in reducing the dependence on antimicrobials for protecting animals from diseases and maintaining production. Modulation of barrier function may be a promising path for the treatment of a wide range of intestinal and extraintestinal diseases. Currently, numerous novel therapeutic concepts are being explored to directly or indirectly enhance barrier function. Currently, there are a few methods for modifying the gut microbiome, including dietary modifications, use of prebiotics, probiotics, synbiotics and postbiotics.

Prebiotics are substances that are selectively utilized by host microorganisms, contributing a health benefit[58]. Inclusion of prebiotics in livestock and poultry feed has shown the capability to improve host health and productivity through the selective stimulation of beneficial gut microbiota[58,60].

The potential benefits of probiotic are diverse and may include immune system activation and modulation, enhanced mucosal barrier function, competitive exclusion of pathogens and decreased risk of infection through production of antimicrobial substances including lactic and acetic acids[61]. Probiotics have been used in the treatment and prevention of IBDs, diarrhea, irritable bowel syndrome and gastroenteritis. Although several organisms have been studied, commonly used species include *Bifidobacterium*, *Lactobacillus* and *Saccharomyces*[62,63].

Plant-derived compounds, such as polyphenols, alkaloids, flavonoids and essential oils exhibit various bioactive properties that improve gut microbiota composition, support immune function and improve nutrient absorption by influencing gut morphology and digestive enzyme activity. Their antioxidant, anti-inflammatory and antimicrobial properties help to maintain and improve overall performance and lower the prevalence of diseases related to gut and intestinal integrity[64].

One novel approach to regulate gut microbiota in animals to re-establish the recipient's intestinal microbiome is faecal microbiota transplantation (FMT). Faecal microbiota transplantation refers to an approach whereby faeces are transferred from a healthy donor to the gut of an unhealthy recipient through multiple methods. FMT is helpful in treating a number of different gastrointestinal and non-gastrointestinal disorders that areclosely linked to dysbiosis[65].

Metagenomics for the identification of gut microbiome composition

Metagenomic analysis has the potential to provide information about the detection of microbial composition of the gut and diversity, novel genes, microbial pathways, functional dysbiosis, antibiotic resistance genes and the determination of interactions in the gut[45].

Methods for testing gut permeability and other markers of intestinal barrier disruption

One of the issues with determining dysfunction of the gut barrier is the lack of specific biomarkers. When testing for intestinal permeability, a variety of parameters can be evaluated. Moreover, the fact that permeability varies along the GIT must be considered with being the small intestine being more permeable than the large intestine[66]. Briefly, methods for testing gut permeability *in vivo* involve the administration of a tracer molecule by oral gavage or intestinal instillation. Tracers commonly used are non-digestible sugars such as lactulose or mannitol, PEG, fluorescently labelled dextrans and[51] Cr-EDTA, which can be later quantified in urine or blood. The size of a tracer can indicate the probable route of permeability. To obtain comprehensive information regarding epithelial leakness, it is recommended that *in vivo* and *ex vivo/in vitro* tests of permeability are used in combination with the detection of permeability associated biomarkers[67].

CONCLUSION

Several complex mechanisms are involved in GIT functionality and health. Gut microbial comparison and analysis have the potential to benefit the understanding of the pathogenesis of various animal gut-linked diseases and the development of corresponding strategies to decrease the collateral damage. It is crucial to deepen our understanding of these interactions so that strategies for the modulation of GIT functionality and health, in

the context of improved animal performance can be developed.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 49(3) : 205-214, 2025: DOI: https://doi.org/10.56093/fx7pkh37

RESEARCH ARTICLE

Pathological findings and distribution of viral antigen in the FMDV-induced hepatorenalopathy in naturally infected cattle calves

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Received: 30.5.2025; Accepted: 22.6.2025

ABSTRACT

Foot and mouth disease (FMD), the "Risk Group 4" animal pathogen, is causing huge economic loss to the livestock owner due to high morbidity and mortality in young calves. Besides vesicular and cardiac lesions, limited studies are available regarding the FMDV induced hepatorenal dysfunction in the naturally infected cattle. Therefore, the present study was undertaken to investigate the hepatorenalopathy in calves naturally infected with FMDV. The serum from the ailing calves (n=12) were assayed for the estimation of hepatorenal function tests and tumour necrosis factor (TNF)-α. The liver and kidneys from the necropsied calves (n=28) were investigated for pathological, immunohistochemical and molecular investigation along with the virus induced apoptotic changes. The affected calves showed clinical signs of high fever, salivation, vesiculo-ulcerative lesion in the buccal mucosa and skin of hoof cleft. The clinically ailing calves showed elevated serum levels of enzymes in the liver and kidneys (alanine transaminase, aspartate aminotransferase, alkaline phosphatase, blood urea nitrogen, urea, creatinine) and cytokine TNF-α. Post-mortem observations showed the classical lesions of acute necrotizing myocarditis, vesicular/ulceration lesions in the buccal mucosa and clefts of hooves, comparable with the FMD virus infection. In addition to that, variably enlarged livers with rounded borders, centrilobular haemorrhage/necrosis with lobulation, multifocal hepatitis and fibrosis along with congested/hemorrhagic and oedematous kidneys were observed in majority of the calves. Microscopically, the classical lesions of vesicular inflammatory oral lesions, acute necrotizing myocarditis and skeletal muscle necrosis (tongue) were prominent. In addition to that, liver showed prominent multifocal centrilobular necrosis and haemorrhage, surrounded by degenerated fat-laden hepatic cells (like hypoxic changes), multifocal periportal infiltration of mononuclear cells, variably bridging fibrosis and increased activity of Kupffer cells in the sinusoids. The kidneys showed vascular changes such as congestion, oedema and haemorrhages, vasculitis, glomerulitis, interstitial nephritis and tubular degeneration. The presence of viral antigens in the hepatocytes and kidney tubular epithelial cells by immunohistochemistry along with associated elevated serum enzymes support the role of FMDV, causing hepatorenal injury/dysfunction. The TdT-mediated dUTP nick-end labeling (TUNEL) assay confirmed that the death of the hepatocytes and tubular epithelial cells is due to apoptosis. The viral genome in both the liver and kidneys was confirmed to be type A FMDV in multiplex-PCR assay. These results provide insights into novel tissue tropisms in the liver and kidneys of young calves with natural FMDV infections. Therefore, therapeutic intervention should be directed to boost the liver and kidneys for better management of the infected cases.

Keywords: Apoptosis, calves, FMD, hepatorenal dysfunction, immunohistochemistry, MP-PCR, pathology, serotype-A, serum biochemical analyses

INTRODUCTION

Foot and Mouth disease (FMD) is an acute, highly contagious, transboundary viral disease of cloven-hoofed animals. It is caused by FMDV under the genus *Aphthovirus* belonging to family *Picornaviridae*. India is endemic to FMDV like other developing countries and suffers with a huge economic burden, which negatively impacts the livelihood of farmers due to high morbidity in adults, reduced production efficiency and heavy mortality in young and high-yielding crossbred animals[1]. The economic loss due to FMD in India is estimated to be USD 2,768 million for severe, USD 237 million for moderate and USD 133 million for mild outbreaks[2]. The recent report showed the financial loss per infected animal during the disease episode is estimated to be approximately Rs. 10,149.64, excluding other concurrent expenses among the dairy farmers of the highlands in Jammu and Kashmir during the FMD outbreak[3]. Out of 7 serotypes, 3 serotypes (O, A and

How to cite this article : Sahoo, M., Singh, R., Patel, S., Thakor, J.C., Das, T., Behera, J., Dash, B., Sahoo, P.K., Sahoo, N.R., Mohanty, R.K. and Kerry, R.G. 2025. Pathological findings and distribution of viral antigen in the FMDV-induced hepatorenalopathy in naturally infected cattle calves. Indian J. Vet. Pathol., 49(3) : 205-214.

Table 1. Biochemical changes (Mean ± SEM values) in naturally infected calves with FMD virus serotype A and control calves.

Parameters	FMDV infected calves	Control calves
Total protein (g/dl)	6.07 ± 0.23	8.11 ± 0.07
Albumin (g/dl)	3.75 ± 0.06	4.59 ± 0.16
Glucose (mg/dl)	98.4 ± 3.82	47.35 ± 1.65
ALT (U/L)	73.77 ± 2.94	59.19 ± 2.36
AST (U/L)	83.94 ± 1.47	47.28 ± 1.97
ALP (U/L)	119.61 ± 4.20	86.12 ± 2.72
BUN (mg/dl)	20.47 ± 0.49	18.91 ± 0.76
Urea (mg/dl)	51.89 ± 2.16	27.86 ± 1.89
Creatinine (mg/dl)	1.67 ± 0.05	1.53 ± 0.12

Highly significant at p<0.001, Statistically significant at p<0.05

Asia 1) are frequently reported to be associated with the disease outbreaks in India[4]. Of which, serotype O is the predominant one, followed by serotype A associated with frequent outbreaks in India. The disease is characterized by high fever, salivation, lameness, vesicular lesions on the mouth, tongue, feet, snout and teats of infected animals. The morbidity rate is very high (upto 100%), but the mortality rate is low in adults (5%), while the high mortality rate (>50%) is observed in young calves due to acute necrotizing myocarditis[5,6]. Sometimes, healed cardiac muscles in heart do not work properly under stress leading to sudden death in adult animals[5]. Generally, the diagnosis of FMD is based on clinical signs followed by confirmation by laboratory tests. Among the various diagnostic assays, multiplex PCR (MP-PCR) is preferred worldwide due to the serotype differentiation with experimental simplicity, greater cost effectiveness besides decreased effort and shorten time[1]. Despite extensive research carried out over the years, the pathogenesis of the disease still remains unexplored. The recent paper from our lab demonstrated the novel pathological findings along with the distribution of viral antigen in various non-target organs such as thyroid, adrenal glands, pancreas, tonsils, lymphoid organs, lungs, trachea and intestine of calves, in addition to target organs[7]. However, pathology and virus distribution in the liver and kidneys remained untouched. The earlier published reports showing the higher levels of alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine levels in the serum of naturally infected cattle and buffaloes suggest the FMDV induced hepatorenal dysfunction. Being the epitheliotropic virus, FMDV is expected to replicate in the hepatocytes of the liver and tubular epithelial cells of the kidney, causing hepatorenal injury. The recent published report showed FMDV-induced hepatic lesions in naturally infected buffaloes[8]. FMDV is reported to excrete and persist in the urine of infected animals for 39 days and helps in the transmission of the disease to the naïve population[9]. However, the detailed pathological findings, distribution of FMDV antigens along with the apoptotic changes in the liver and kidneys of cattle calves naturally infected with FMDV have not been investigated. Therefore, the present study was intended to find out the hepatorenal function tests by serum biochemical assays, levels of pro-inflammatory cytokine tumour necrosis factor (TNF)-α, gross and histopathological changes, immunohistochemical detection of viral antigens by immunohistochemistry along with the associated apoptotic changes by TUNEL assay in the liver and kidneys of calves naturally infected with FMDV.

MATERIALS AND METHODS

Animals

In the organized dairy herd of ICAR-Indian Veterinary Research Institute (IVRI) consisting of 590 dairy cattle, a total of 177 (adults-118, calves-59) animals were affected due to the outbreak of FMD. The ailing calves showed the clinical signs of high fever (39.2-41.7^{O}C), increased heart rate (110-121/min) and increased respiratory rate (39-48/min). The animals showed excessive salivation, catarrhal stomatitis, vesicles on lips, cheeks, gums, hard palate, dental pad, rostrum of the dorsum of the tongue and interdigital skin, dyspnoea, panting, mouth breathing and lameness that were suggestive of FMD. However, a few calves (n=4) died acutely without showing any clinical vesicular lesions. The calves (n=59), in particular, were severely stressed and prostrated, of which 28 crossbred (female-16, male-12) aged <6 months succumbed to the disease and were subjected to the postmortem investigations at the Division of Pathology,

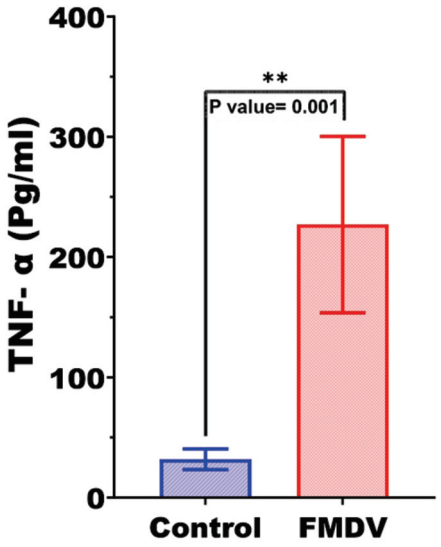

Fig. 1. The level of TNF-α level in the serum is showing significantly higher in FMDV infected calves as compared to control calves. Mean with indicates statistically significant values (P<0.001) as compared to the control calves. Error bars represent ± SEM.

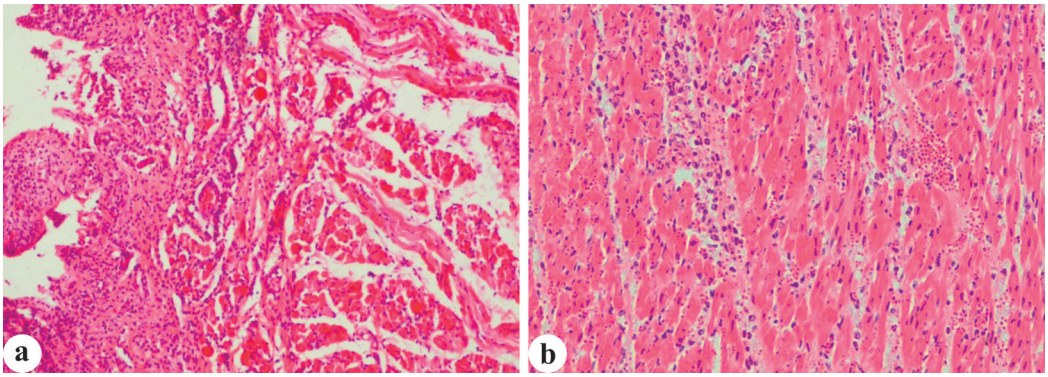

Fig. 2. Histopathological lesions in calves naturally infected with FMDV showing **a.** Marked infiltration of mononuclear cells in the SSE of the tongue. **b.** Acute necrotizing myocarditis with lymphocytic and macrophage infiltration (H&E x100).

ICAR-IVRI, Bareilly, India. The remaining 31 calves were recovered after the treatment by the clinician. The routine vaccination of animals at the farm was being practiced on a 6 monthly basis with the commercially available inactivated FMDV trivalent vaccine (serotypes A, O and Asia 1) (Indian Immunologicals Ltd). All the carcasses were systematically necropsied on their submission and gross lesions were recorded.

The representative tissues (5x10x20 mm) from the tongue, heart, liver and kidneys were collected in 10% neutral-buffered formalin for each case. After 48 hours of fixation, tissue pieces were trimmed for histotechnique. Thin, unfixed tissue pieces from the corresponding sites of organs were collected on ice and later kept at -20^{O}C for molecular detection of FMDV. The liver and kidney pieces without showing any appreciable gross lesions from 2 healthy calves during the routine necropsy unrelated to the present episode were included in the study for comparison purposes.

Serum biochemical analyses

Jugular vein blood was collected into BD Vacutainer rapid serum tubes from FMDV - infected calves (n=12) and FMDV seronegative controls (n=4) [confirmed by virus neutralization test (VNT) assay]. The serum was harvested and then stored at -20^{O}C. The levels of serum concentration of total protein, albumin, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine were determined using commercial kits (Coral Diagnostics, Perkin Elmer, USA) by Celltac-Biochemical analyzer (MEK-6550, Germany).

Estimation of TNF-α levels in serum

The levels of TNF-α were estimated in the sera of FMDV-infected calves (n=12) and FMDV seronegative calves (n=4), using a commercially available bovine TNF-α ELISA kit (MBS 2609886, My Biosource, USA) based on the double antibody sandwich technique. The concentrations were expressed in pg/ml.

Histopathology

The NBF-fixed tissues were routinely processed for the preparation of 4-5 µm thick paraffin tissue sections and stained with Haematoxylin and Eosin (H&E) stains[10]. The histopathological tissue sections were evaluated and photographed under the Medicus pro T microscope (Helmut Hund Gmbh, Germany) with the attached ToupTek camera (C-Mount adapter 0.5X). The lesion scoring was done based on the degree of pathological changes in different constituents of the liver compartments (portal triad, hepatic cords, terminal/central vein) and kidney compartments (renal cortex and medulla). The changes were assigned on a scale of 0-3. The histopathological lesion scoring in the liver and kidney was assigned to 0 if there was no damage/normal, a score of 1 if the damage was mild, a score of 2 if the damage was moderate and a score of 3 if the damage was extensive. The maximum score was taken as the

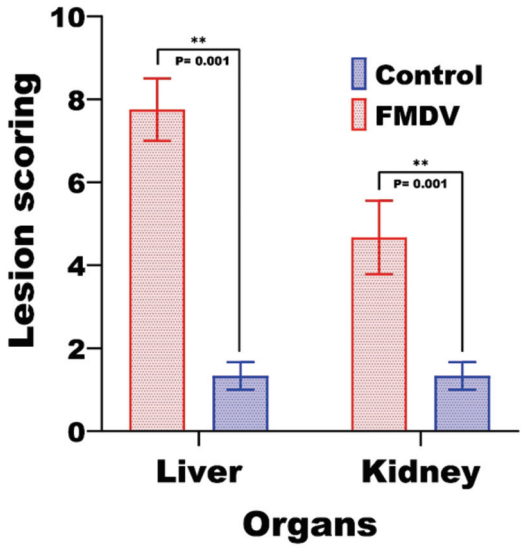

Fig. 3. Histopathological lesion scoring of liver and kidneys of FMDV infected calves showing significantly higher pathological lesions as compared to control calves. Mean with indicates statistically significant values (P<0.001) as compared to the control calves. Error bars represent ± SEM.

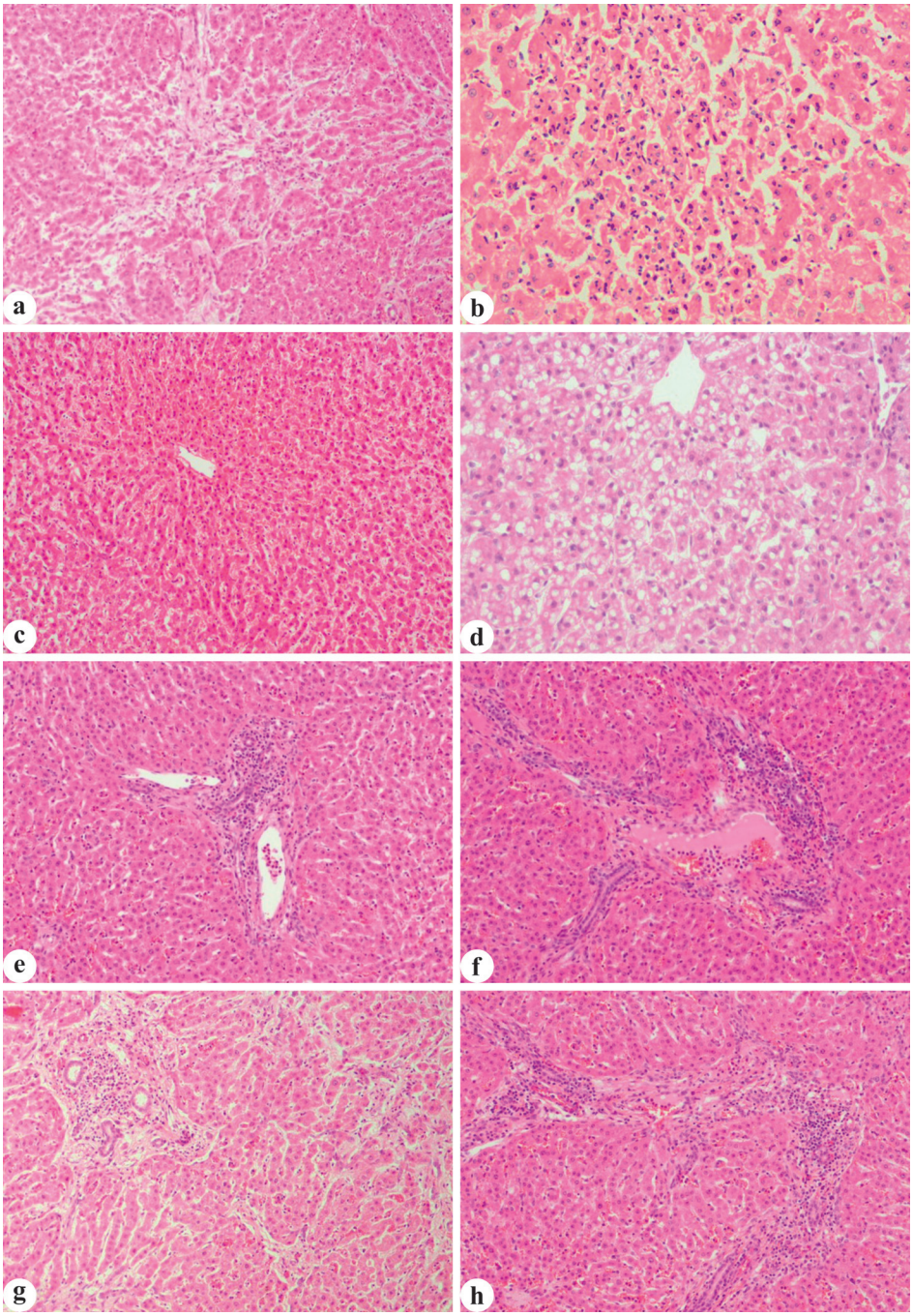

Fig. 4. Histopathological lesions in the liver of calves naturally infected with FMDV showing **a.** Centrilobular necrosis. **b.** Increased Kupffer cells activity. **c.** Passive venous congestion. **d.** Fatty change in zone. **e & f.** Periportal hepatitis with infiltration of mononuclear cells within the portal triad. **g.** Portal fibrosis and infiltration of mononuclear cells. **h.** Bridging portal fibrosis and hepatitis/necrosis (H&E x100).

most severe inflammatory/pathological changes. The lesion scoring of various organs was done in 10 infected and 2 control calves. The lesion scoring was done blind folded by 2 independent pathologists to avoid the bias and reliability of the observations.

Molecular detection of FMDV

The total tissue RNA was extracted from the frozen tissues of the tongue, heart, liver and kidney of 28 calves using the RNeasy kit (Qiagen, Germany) according to the manufacturer's instructions. The cDNA synthesis was performed using 100 units of M-MuLV reverse transcriptase enzyme (Promega, USA) and 1 µg of oligo (dT) primer (Thermo Scientific) per reaction. The identification of the specific serotype involved was done by MP-PCR using three serotype-specific forward primers consisting of DHP 13, DHP 15 and DHP 9 targeting the serotypes O, A and Asia 1, respectively,

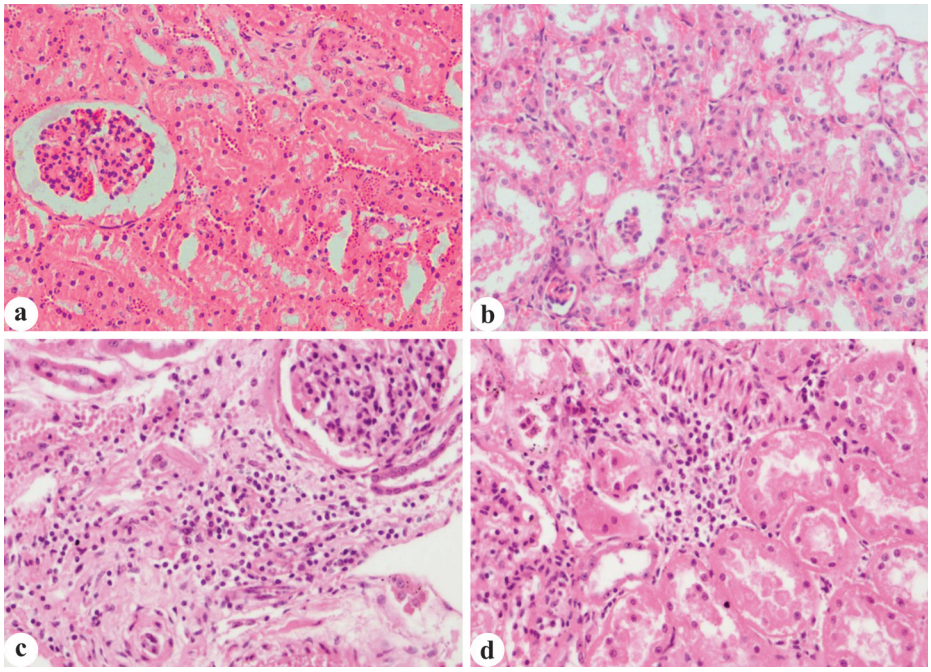

Fig. 5. Histopathological lesions in kidneys of naturally infected calves showing **a.** Vascular congestion in glomerulus and tubules (H&E X100). **b.** Necrosed glomerular tuft with degenerative changes in the tubules (H&E X100). **c.** Peri-glomerulitis (H&E X200). **d.** Interstitial nephritis (H&E X200).

and the universal FMDV-specific NK-61 primer (5'-GACATGTCCTCCTGCATCTG-3') as mentioned in the previous report[11]. Further, cDNAs were tested to rule out the intercurrent viral pathogens such as Bovine Viral Diarrhoea virus (BVDV)[12], Malignant Catarrhal Fever (MCF)[13], Bluetongue Virus (BTV)[14] and Vesicular Stomatitis Virus[15].

Distribution of FMDV antigen

The paraffin tissue sections of the liver and kidneys that were positive to FMDV by MP-PCR assay were mounted on poly-L-lysine-coated slides from FMDV affected calves (n=10) and processed by indirect immunoperoxidase technique (IPT) for the distribution of viral antigen in the cells of different compartments. After deparaffinization and rehydration, endogenous peroxidase activity was quenched with BLOXALL (Vector labs, USA) for 30 min, followed by washing in phosphate-buffered saline-Tween (PBST, 0.05 M, pH 7.6) thrice for 5 min each. The antigen retrieval was done by heating the slides in a microwave oven with the citrate-based antigen unmasking solution (Vector labs, USA) for 25 min. The sections were thoroughly washed in PBST followed by incubation in a humidifier chamber with prediluted 2.5% normal horse serum blocking solution (Vector labs, USA) at 37^{O}C for 1 h to block non-specific sites. Afterwards the sections were incubated with rabbit polyclonal anti-FMDV Polyprotein (3D polymerase) primary antibody (bs-4524R, Bioss antibodies 1:100 optimal working dilution) and incubated overnight at 4^{O}C. Sections were thoroughly washed in PBST three times to remove unbound antibody. Afterwards the sections were incubated with prediluted ImmPRESS® HRP universal (Horse Anti-Mouse/Rabbit IgG) antibody polymer agent (Vector labs, USA) at 37^{O}C for 1 h. Then the sections were washed thrice with 5 min each wash with PBST. The sections were treated with a freshly prepared solution of 1 drop of ImmPACT DAB Reagent with 1 ml ImmPACTDAB 3-3'-diaminobenzidine tetrahydrochloride (DAB) diluent (ImmPACT® DAB Substrate kit, Vector labs, USA) for the 30s for the development of brown colour. Afterwards the slides were counterstained with Mayer's haematoxylin for the 30s. The sections were washed and mounted with CC/Mount™ (Sigma-Aldrich, USA). For the negative controls, the primary antibody was substituted with rabbit IgG isotype control (Novus Biologicals, USA) and with PBS with 1% BSA to rule out false positive staining.

***In-situ* detection of apoptosis**

The labeling of apoptotic cells in tissue sections of the liver and kidneys of FMDV-infected calves (n=10) was performed with TUNEL (transferase-mediated dUTP nick end labeling) assay kit using the manufacturer's instructions.

Statistical analyses

The data were expressed as means ± standard error of mean and subjected to statistical analyses using independent t-test for all the parameters in GraphPad Prism 8.0.2 except for histopathological lesions scoring. The histopathological lesion scoring of the livers and

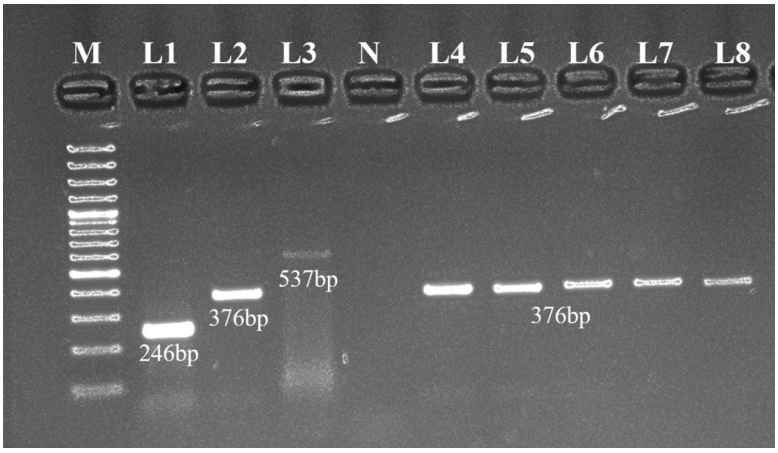

Fig. 6. Molecular detection of FMDV. M: Marker (100 bp), L1: Type O positive control (246 bp), L2: Type A positive control (376 bp), L3: Asia 1 positive control (537 bp), N: Negative control, L4: Tongue epithelium, L5: Heart, L6: Liver, L7: Liver, L8: Kidney.

kidneys of FMDV-infected and control calves was analyzed using the Mann-Whitney U test. Statistical significance was set at $p<0.05$ for significant and $p<0.001$ for highly significant.

RESULTS

Serum biochemical investigation

The FMDV-infected calves showed significantly reduced values of total protein concentrations and albumin levels ($p < 0.001$) and significantly higher ($p < 0.001$) glucose concentrations as compared to controls. The FMDV-infected calves showed significantly higher levels of ALT, AST and ALP liver enzymes ($p < 0.05$) as compared to controls. Similarly, the FMDV-infected calves showed significantly higher serum levels of BUN and urea ($p < 0.05$), whereas creatinine levels, although increased failed to show any significant change as compared to control calves.

Estimation of TNF-α level

The serum of FMDV - infected calves showed significantly higher levels of TNF-α ($p < 0.001$) as compared to controls (Fig. 1).

Gross and histopathological lesions

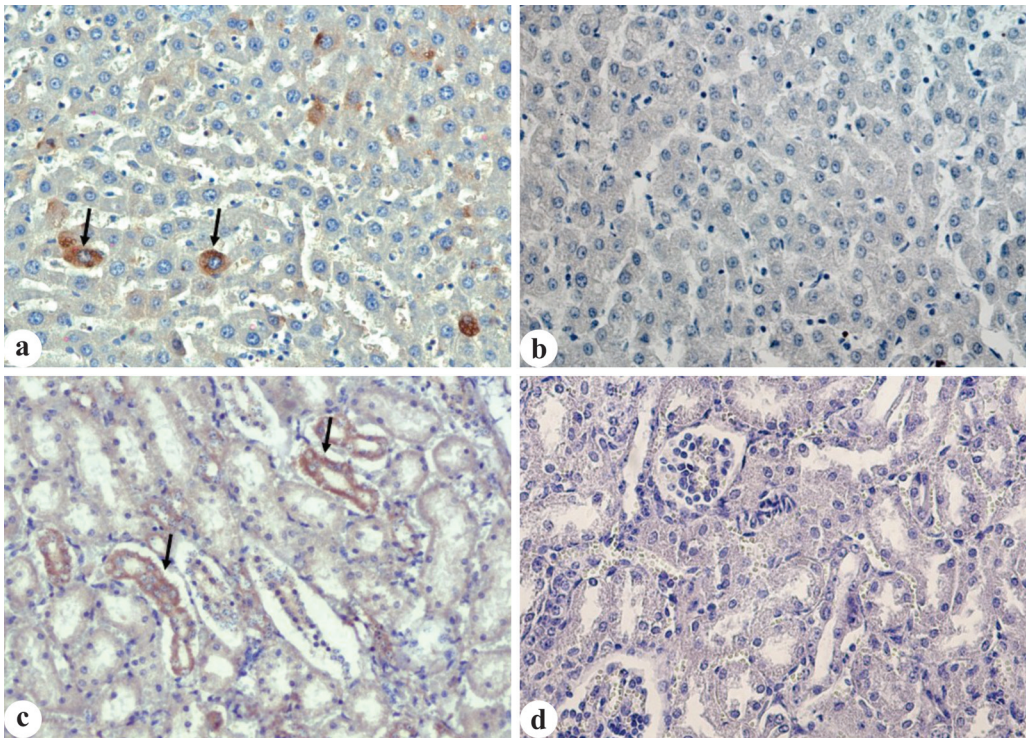

Fig. 7. Immunohistochemical detection of FMDV antigens. **a.** Hepatocytes (arrow). **b.** Absence of any immunoreactivity, negative control, liver. **c.** Renal tubules (arrow). **d.** Absence of immunoreactivity in the negative control section, kidney (IHC x100).

There were consistent vesicular lesions in the buccal mucosa, the interdigital skin and in addition, the necrotizing myocarditis (tigroid heart) in the majority of the cases. The buccal mucosa of 24 calves showed few to many vesicles (intact/ruptured) of varying sizes over the tip, lateral border and dorsum of the tongue, cheeks, gums, dental pad, lower lip and muco-cutaneous junction of the exterior nares. The heart in all the cases (n=28) showed grey to pale foci/stripes of necrosis of varying sizes alternating with red myocardium, resembling tiger stripes (Tigroid heart appearance). Microscopically, the stratified squamous epithelium (SSE) of the tongue showed marked infiltration with mononuclear inflammatory cells (Fig. 2a). Below the epithelium layer, the connective tissue, striated muscles, adipose tissue and salivary glands showed vascular congestion with perivascular infiltration of mononuclear cells. The affected heart showed acute necrotizing interstitial lymphocytic myocarditis (Fig. 2b).

Out of 28 cases, 16 calves showed gross liver lesions. The livers were swollen, mottled with distended gall bladder, of which, 12 livers were mild to moderately congested and 6 had whitish necrotic areas on its surface. Further, out of 28 cases, 19 cases showed moderately congested cortex and medulla of the kidneys. Microscopically, the liver and kidneys of the FMDV-infected calves showed significant ($p < 0.001$) pathological alterations as compared to control (Fig. 3). The liver showed centrilobular necrosis of hepatic cells with infiltration of mononuclear cells in 19 cases (Fig. 4a), vasculitis, swollen endothelial lining cells of blood vessels with hyperplasia of Kupffer cells in 14 cases (Fig. 4b). The liver of 16 calves showed passive venous congestion (Fig. 4c), oedema and infiltration of inflammatory cells within the hepatic sinusoids. The liver of the infected calves showed multifocal areas of periportal hepatic necrosis accompanied with the infiltration of inflammatory cells. A total of 12 cases showed fatty change around the central vein (Fig. 4d) characterized by the presence of variable sized fatty vacuoles in the hepatocytes. The liver showed non-suppurative hepatitis characterised by the infiltration of mononuclear cells around the portal triad and the central/terminal vein in 11 cases (Fig. 4e & f). Portal-central bridging hepatitis (Fig. 4g) and portal fibrosis (Fig. 4h) were observed in 8 and 5 cases, respectively. The kidneys showed severe vascular changes characterized by congestion and oedema in the capillary plexuses of the glomerulus and tubules of 12 cases (Fig. 5a). The glomerular necrosis (Fig. 5b) was observed in 8 cases, and 6 cases showed mesangial cell proliferation. The tubules showed degenerative changes with presence of proteinaceous exudates within the lumen (Fig. 5b). The periglomerulitis (Fig. 5c) was observed in 7 cases. The interstitial nephritis (Fig. 5d) characterized by infiltration of mononuclear cells was observed in the interstitial space of 5 cases.

Molecular detection of FMDV

In MP-PCR assay, the heart and tongue of all the

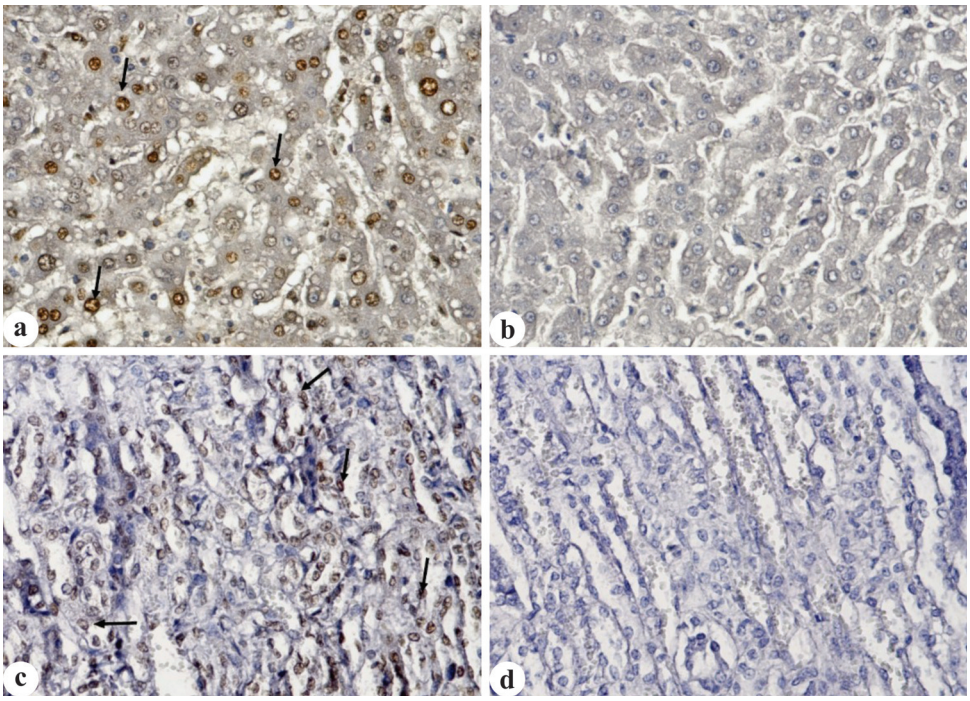

Fig. 8. Detection of apoptosis in calves naturally infected with FMDV. **a.** Apoptotic nuclei (arrow), hepatocytes, liver. **b.** Absence of immunoreactivity, negative control, liver. **c.** Apoptotic nuclei (arrow) in renal tubular cells of renal medulla. **d.** Absence of immunoreactivity in negative control, kidney (TUNEL x200).

cases showed the presence of serotype A by amplifying 376 bp. Out of 28 calves tested, the presence of viral genome was detected in 19 and 13 cases of liver and kidneys, respectively (Fig. 6). The heart, tongue, liver and kidneys failed to show the amplification for other viral differentials such as MCF, BTV, BVDV and VSV.

Distribution of viral antigen

The liver (n=19) and kidneys (n=13) of MP-PCR positive cases showed the presence of viral antigen in the hepatocytes and tubules of 11 and 8 cases, respectively (Fig. 7).

***In-situ* detection of apoptosis**

In TUNEL assay, significantly increased number of apoptotic cells were observed in the hepatic lobules of the FMDV-infected calves (Fig. 8a). The inflammatory cellular exudates within the central vein also showed apoptosis. The negative control section failed to show any immunoreactivity for apoptotic nuclei (Fig. 8b). The medullary tubules of the kidney showed the increased number of apoptotic nuclei (Fig. 8c), whereas negative control sections failed to show any immunoreactivity (Fig. 8d).

DISCUSSION

The FMDV-infected calves had higher physiological values as compared to control calves, which are in agreement with the earlier observations[8,16]. The vesicular and necrotizing myocarditis lesions were documented as reported in earlier reports[7,17-19]. The FMDV-induced necrotizing myocarditis is the primary reason for the death and high mortality in calves. The reduction in serum total protein and albumin levels that might be attributed to the starvation due to oral lesions, hepatic and renal damage due to virus replication and passive congestion due to the myocarditis as reported in earlier studies[16,19-22]. The liver damage and protein-losing enteropathy due to FMDV might be the reason for hypo-proteinemia and hypoalbunemia[20]. The higher blood glucose level in FMD infected calves might be due to lower insulin level resulting due to replication of FMDV in the beta Langerhans cells of pancreas. The similar findings are in congruent with the earlier observations[7,23]. The increased level of glucose might be another reason for the decrease in protein concentrations observed in this study[21,24]. The elevated levels of the ALT, AST and ALP liver enzymes suggest the hepatic cells damage leading to the subsequent release of hepatic enzymes into the circulation. Similar results are reported in the FMD cases in various reports[16,20,25]. The high rise of AST level is frequently regarded as a sensitive indicator of hepatocyte damage, even in cases of subclinical infection[25]. Moreover, high rise of AST levels can be due to myocardial damaged cause by FMDV resulting from acute necrotizing myocarditis. The high rise of BUN, urea and creatinine levels in the serum of FMDV infected calves suggest the renal impairment. This might lead to development of prerenal azotemia due to reduced rate of glomerular filtration rate leading to high levels of creatinine in the serum. The high rise of BUN, urea and creatinine levels in case of FMDV-infected cattle and buffaloes were reported in earlier studies[16,20]. The higher levels of TNF-α in the sera of FMDV infected calves might suggest the continued stimulation of the immune cells producing this inflammatory cytokine due to potential consequence of virus replication causing systemic inflammatory reaction[26,27]. The presence of TNF-α might act as host defense mechanism against FMD infection[28].

The tongue lesions of coagulative necrosis involving the epithelial and subepithelial structures and vesicle formation containing fluid with infiltration of inflammatory cells are consistent with the earlier observations[7,17,18]. The heart showing acute necrotizing interstitial lymphocytic myocarditis is in agreement with previous studies[5,6,29]. The liver showing multifocal hepatic necrosis and congestion of central and portal vein are in congruent with the earlier observations[8,18]. The fatty change in the affected liver might be due to disruption of the liver's ability to properly metabolize fats, leading to the accumulation of triglycerides in the liver cells. The marked infiltration of mononuclear cells around the portal triad might be due to the entry of viral toxin through the portal tract causing the hepatic damage. The passive venous congestion in liver might be linked with the myocarditis due to impaired venous drainage[8]. The portal fibrosis developed in the present case might be due to continued periportal necroinflammation along with ischemic changes induced by myocarditis lesions might initiate the fibrogenesis destroying the periportal parenchyma in liver. The portal fibrosis in case of FMDV-infected calves has been documented in the earlier report[8]. The kidney showing vascular and inflammatory changes in the glomerulus and tubules suggest the FMDV induced renal dysfunction. The degenerative changes in the tubules might be due to impaired end organ perfusion due to myocarditis lesions. Further, the increased serum levels of BUN, urea and creatinine in the present study support our findings[16,20]. The presence of viral antigen in the tubules suggest FMDV induced renal damage due to replication of virus in the tubules. The earlier report showing the replication of FMDV in continuous bovine kidney cell line supports our findings[30]. The excretion of the virus in the urine of the infected animals might be due to the replication of the virus in the tubular epithelial cells. Further, in MP-PCR assay, the detection of viral nucleic acid in the liver and kidney confirm the association of FMDV with histopathological alterations in liver and kidney. The detection of apoptosis in the hepatocytes and renal tubules suggest the FMDV induced

hepatorenal damage mediated through apoptosis in liver and kidney respectively. Apoptosis is a tightly regulated physiologic and pathologic process that plays a crucial role in the disposal of unwanted cells as well as in maintaining the homeostasis of the immune system[31]. FMDV has been reported to induce apoptosis in tongue and hoof of naturally infected swine[28] and heart showing myocarditis lesions in lamb[32]. The hepatorenal damage in the present study might have association with the FMDV induced myocarditis in calves that restricts the flow of the blood to the liver and kidney causing degenerative changes. The higher apoptotic cells in the liver and kidney of calves, suggesting the key role of apoptosis in FMDV-induced hepatorenal dysfunction. The different viral proteins of FMDV such 2C protein[33], 3C protease[34] and VP1[35] of FMDV are reported to induce apoptosis. The higher level of TNF-α might be responsible for induction of high rate of apoptosis. The association of TNF-α with apoptosis in the tongue of pigs infected with FMD has been reported in previous report[28].

The present study documents the FMDV-induced hepatorenalopathy besides the classical lesions of the FMD. This was accomplished with the higher serum levels of ALT, AST, ALP, BUN, urea and creatinine in the ailing calves, histopathological alterations with the presence of viral antigen and molecular detection of viral genome in the liver and kidney. It remains unresolved whether the findings described herein are specific to the FMDV-type A or irrespective of any serotypes that need to be investigated. Understanding these diverse pathological spectrum in other organs is essential for the accurate diagnosis and effective management of hepatorenalopathy associated with FMDV.

ACKNOWLEDGEMENTS

Thanks are due to Director, IVRI, Izatnagar for all necessary facilities and support for research work.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 49(3) : 215-218, 2025: DOI: https://doi.org/10.56093/t7a28g13

RESEARCH ARTICLE

Isolation and detection of *Pasteurella multocida* from clinical hemorrhagic septicaemia cases of buffaloes

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Received: 9.4.2025; Accepted: 27.5.2025

ABSTRACT

Hemorrhagic septicaemia (HS) is an economically important disease of livestock affecting various species of animals. The present investigation was conducted to detect and isolate the causative organism from the HS suspected cases of the buffaloes in two different outbreaks. Total 24 samples (14 nasal swabs, 6 lung swabs and 4 blood samples) from the suspected HS cases were processed for the bacterial isolation and DNA was extracted for PCR detection. Culture and biochemical assays from two samples showed characteristics of the *Pasteurella multocida* organism. The KMT1 gene was targeted as the marker gene for *P. multocida* for the PCR assay confirmation. Out of 24 samples, four were confirmed to be positive for *P. multocida*. In conclusions, *P. multocida* associated respiratory illness was common in buffaloes during stressful climatic conditions and to minimize the spread, the animal owners should ensure appropriate medication, vaccination programs and good hygienic practices in crowded areas.

Keywords: Buffaloes, isolation, *Pasteurella multocida*, PCR

INTRODUCTION

Hemorrhagic septicaemia (HS) is a per acute and devastating disease of buffaloes caused by *P. multocida*, a commensal bacterium of upper respiratory tracts of several livestock, poultry and domestic pet animal species resulting into high morbidity and mortality[1]. Under the influence of stress factors such as poor nutrition, unfavorable weather, long distance travelling, dehydration, nasopharyngeal settlement and concurrent infections of other bacteria, viruses and parasites, the organism leads to more profuse destruction to the host animal[2]. *P. multocida* is a small, gram-negative, non-spore-forming, coccobacillus bacterium with bipolar staining characters[3].

In Himachal Pradesh, HS is being reported regularly despite vaccination and even considered as one of the major bacterial killer disease among buffaloes. However, a number of studies were carried out in the state for the identification of *P. multocida* from clinical affected cattle, buffaloes, sheep, goats, rabbits and poultry[4-6]. For the detection and characterization of P. multocida, cultural and serological procedures are adopted and in recent times PCR is merely being preffered[7]. This study was carried out to detect the presence of P. multocida from clinically infected buffaloes showing symptoms of HS.

MATERIAL AND METHODS

Outbreak history

Fifteen buffaloes of age group (1 to 5 years) were presented with history of high fever (105-106^{O}F) followed by respiratory distress, septicaemia, mucopurulent nasal discharge, inappetance, oedematous swelling of the brisket region and recumbency leading to death of six animals. During post-mortem examination, subcutaneous edema in the brisket and mandibular regions and petechial-to-echymotic hemorrhages along with congestion of lungs, fibrinous pneumonia and pericarditis were recorded. The outbreaks have been reported during the period of heavy rainfall between Mid-July to September.

How to cite this article : Bhardwaj, M., Verma, S., Barsain, S., Dhar, P. and Chahota, R. 2025. Isolation and detection of *Pasteurella multocida* from clinical hemorrhagic septicaemia cases of buffaloes. Indian J. Vet. Pathol., 49(3) : 215-218.

Sample collection

A total of 24 samples (14 nasal swabs, 6 lung swabs and 4 blood samples) were collected randomly from clinical cases following outbreaks of HS in buffaloes. The samples were transported to Department of Veterinary Microbiology using transport medium (Normal Saline Solution on ice) and then processed for bacterial isolation.

Isolation of bacterial organisms

The clinical samples were put in tryptic soy broth (Himedia, Mumbai) and incubated at 37^{O}C for 18-24 hrs. Then swabs were plated onto 5% sheep blood agar (Himedia, Mumbai) and incubated at 37^{O}C overnight[8]. The isolates were identified by cultural and morphological

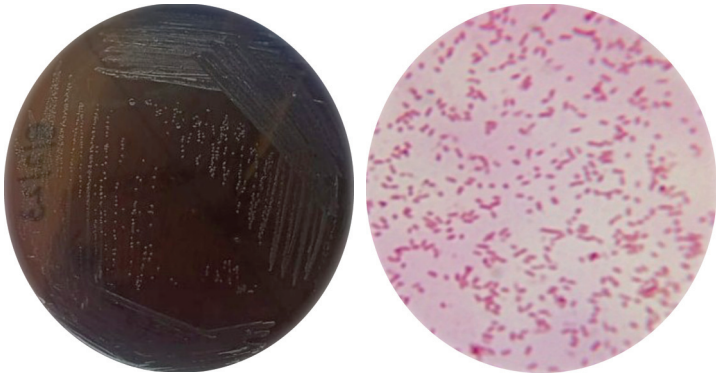

Fig. 1. Non-hemolytic colonies of *Pasteurella multocida* on 5% sheep blood agar; **Fig. 2.** Gram's stained *Pasteurella multocida* organisms.

features. Further a smear was prepared from single isolated colony and grams' staining was performed to demonstrate its morphological characteristics[9].

Biochemical methods

Biochemical tests catalase, oxidase, indole, VP, urea, citrate and motility were used as described by[9] with some modifications. The result found were recorded and compared for verification of the isolates.

Detection of *P. multocida* by PCR

All the clinical samples and bacterial isolates were subjected to DNA isolation using DNeasy blood and tissue kit (Qiagen, Germany) following the manufacturer's instructions. Conventional PCR was carried out to detect *P. multocida* in GeneAmp® PCR System 9700 thermocycler (Applied Biosystems, California, USA) targeting the KMT1 gene amplified using species specific primers[10] (Forward: KMT1SP6, 5′-GCTGTAAACGAACTCGCCAC-3′; Reverse: KMT1T7, 5′-ATCCGCTATTTACCCAGTGG-3′). The components for the DNA amplification master mix (25 µl) for *P. multocida* comprised of nuclease-free water 8.7 µl, 5 x PCR buffer 5 µl, 10 mM dNTPs 2 µl, 10 µM forward primer 1µl, 10 µM reverse primer 1 µl, 3 mM $MgCl_2$ 2 µl and Taq DNA polymerase 3 units 0.3 µl. The PCR was performed with thermal conditions of initial denaturation at 95^{O}C for 2 min followed by 30 cycles of denaturation at 95^{O}C for 1 min, annealing at 45^{O}C for 1 min, extension at 72^{O}C for 30 sec, final extension at 72^{O}C for 5 min[10].

Gel electrophoresis

A 5 µl aliquot of the PCR product was resolved on a 1.5% agarose gel and observed under UV in a Gel Documentation system (Alphaimager, BioRad, USA). Using PM-PCR assay, *P. multocida* isolates were identified based on the amplification of an approximately 460 bp fragment.

RESULTS

Cultural characteristics of *P. multocida*

Culturing of total 24 samples (blood and swab), it was observed that only two (8.3%) isolates were showing the cultural characteristics of *P. multocida* (small, glistening, mucoid with no haemolytic effect) on blood agar (Fig. 1). The *P. multocida* isolates were coccobacilli with rounded ends, stained negative by Gram staining (Fig. 2).

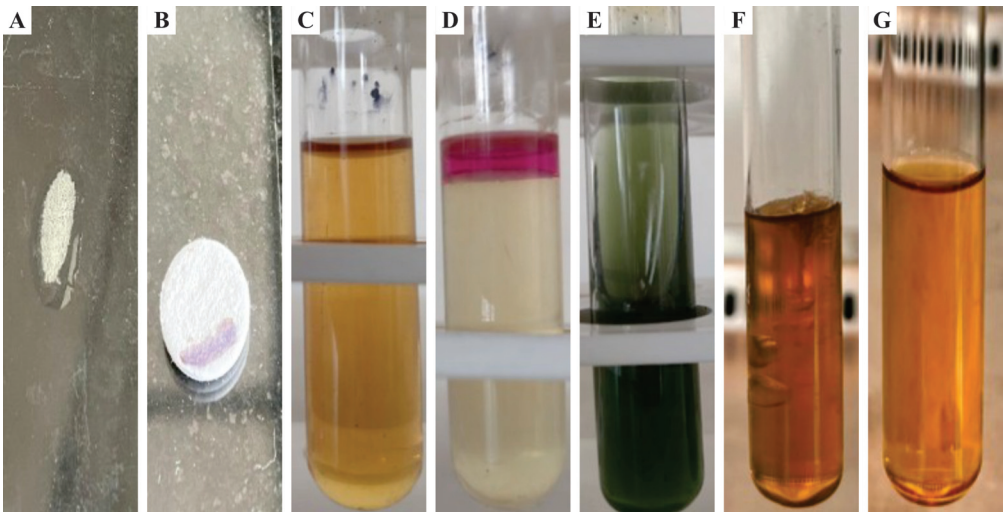

Fig. 3. Biochemical identification of *Pateurella multocida*. **A:** Catalase Positive, **B:** Oxidase Positive, **C:** Urea Negative, **D:** Indole Positive, **E:** Citrate Negative, **F:** Non-Motile (SIM), **G:** VP Negative.

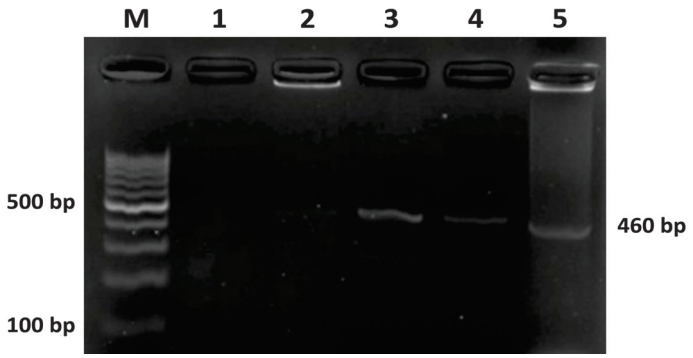

Fig. 4. PCR products (460 bp) in 1.5% agarose gel. Lane M: DNA marker, Lane 1: Negative Control, Lanes 2, 3: Clinical samples amplified using primer KTT1T7 & KMT1SP6, Lane 4: *P. multocida* isolate amplified using primer KTT1T7 & KMT1SP6, Lane 5: Positive Control.

Biochemical characteristics of *P. multocida*

The isolates of *P. multocida* were found oxidase, indole and catalase positive. Further, isolates did not produce hydrogen sulfide, urease and motility, did not have hemolytic effect and were negative in VP reaction (Fig. 3).

Molecular identification

Overall, 16.7% (4/24) of the samples from the two outbreaks were positive by PM-PCR (Fig. 4). Based on the sample type the highest positivity of *P. multocida* was recorded in the nasal samples with 20.0% (3/15) and only 16.7% (1/6) for the lung swabs. The bacterial isolates from both outbreaks were also confirmed as *P. multocida* by species specific PM-PCR. As expected, buffaloes having a HS-like disease were found to be infected with *P. multocida*.

DISCUSSION

P. multocida is an opportunistic pathogen causing a number of diseases in various animal species. In buffaloes, HS is one of the most important disease causing heavy economic losses to the farmers. This study was under taken on the samples collected from two outbreaks aimed to isolate and detect *P. multocida* resulting to HS by conventional methods and PCR. In the present study, two *P. multocida* isolates were showing characteristic features of the organism. In the previous studies, similar results were recorded for the identification of *P. multocida* as gram-negative, coccobacillus, with no haemolysis on blood agar[11,12]. Also, number of studies have stated similar results for biochemical characteristics such as positive reaction for oxidase, catalase, indole tests and negative for H_2S, urea and motility[13-15]. In the study, the overall isolation rate of *P. multocida* from the total number of clinical samples was recorded as 8.3%. Shuka *et al.*[16] reported 3.39% isolation rate of *P. multocida* from blood and swab samples of cattle which stands less in comparison to our findings. In contrast, a study carried out on calves in the region of Zambia, a higher percentage (17.5%) of *P. multocida* bacterial isolates were reported[17]. In HP, Kapoor *et al.*[18] screened 470 samples from different animal species from different districts to assess the prevalence of HS. According to the study, they were also able to isolate fewer number of *P. multocida* isolates and found isolation of *P. multocida*, a tedious and cumbersome process. This kind of ambiguity in the isolation rate of organism might be due to the uneven number of animals screened for the diagnosis for the HS like disease.

For confirmation of *P. multocida* as the principal cause of HS the PCR test was done based on Townsend *et al.* by using universal and specific primers[10]. Although, in the previous studies the organism has been found as causative agent of HS in HP. Likely, in this study 16.7% of the samples were found positive for *P. multocida* by PM-PCR using the amplification of an approximately 460 bp fragments which agree with the findings of the previous studies. The two *P. multocida* isolates procured from positive nasal swabs were also confirmed by PM-PCR. In a previous study, eighteen isolates of *P. multocida* were isolated from 138 bovine samples (nasal swabs, heart blood, spleen, lung and liver tissues) and confirmed by species specific PM-PCR[6]. Similarly, 23 *P. multocida* isolates from 335 clinically healthy and diseased cattle were confirmed by PM-PCR and further screened for the presence of virulence associated genes[5]. In HP, pasteurellosis is enzootic and has been an extended standing delinquent occurring mostly on the seasonal basis. In a comprehensive study carried out by Chaudhary *et al*., HS is the major bacterial disease with highest mortality rate in animals in HP[19]. Also in another study, HP along with other states of northern India, showed irregular occurrence of HS outbreaks between the periods 1995-2000 and 2006-2008 with overall 5.6% proportion of outbreaks[20]. Nevertheless, the vaccination is followed regularly in the state but despite of that emergence of new strains for ineffectiveness of the vaccines and increase in outbreak rates of HS cannot be ignored. The study has confirmed that the buffaloes in the outbreak were infected by *P. multocida*. Further research on *P. multocida* genetic diversity and the molecular epidemiology is required to development improved immunoprophylactics.

ACKNOWLEDGEMENTS

The authors are thankful to the Head, Department of Veterinary Microbiology, DGCN COVAS, Palampur for providing the necessary facilities for carrying out this work.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that

there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 49(3) : 219-223, 2025: DOI: https://doi.org/10.56093/p52axr40

RESEARCH ARTICLE

A pathological study of ovarian tumours in dogs

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Received: 12.5.2025; Accepted: 6.6.2025

ABSTRACT

Ovarian tumours reflect a unique and complex challenge in oncology, distinguishing themselves from other forms of abnormal cellular growth such malformation growth, reparative growth and hyperplastic growth. While these latter processes involved cellular proliferation that is typically regulated and reversible, ovarian tumours exhibit uncontrolled, often irreversible growth with distinct biological behaviours. The present study aimed to assess occurrence and histopathology of different neoplasms of ovary of dogs in Jaipur city of Rajasthan, India. In the present study, tissue samples of genital tract from 121 female dogs were collected from different Animal Birth Control (ABC) programme and different clinics in Jaipur. Gross examinations of these samples showed 141 various lesions in different parts of genital tract out of which 15 cases of neoplasms of ovaries (15/141) with occurrence (15/141) 10.64 percent. Most commonly recorded neoplasm of ovary was granulosa cell tumour followed by cystadenoma, cystadenocarcinoma, dysgerminoma and luteoma. Histopathologically, granulosa cell tumour showed pathognomonic Call-Exner bodies consisting of a small central round, to oval space with eosinophilic follicular fluid. Cystadenoma showed papillary projections lined by single layer of cuboidal epithelium and at some places tightly packed tall columnar epithelium. Cystadenocarcinoma showed acini formation with proliferating cells having hyperchromatic and more pleomorphic nucleus and mitotic figures. Dysgerminoma showed polyhedral cells which had a moderate quantity of transparent eosinophilic cytoplasm. Luteoma showed proliferation of luteal neoplastic polyhedral cells with abundant, vacuolated cytoplasm and round nuclei forming multi lobulation. Study of canine ovarian tumours provides better understanding, diagnosis and treatment aspect of these tumours which is crucial for improving the overall health and wellbeing of canines.

Keywords: Dog, genital tract, ovary, pathology, tumours

INTRODUCTION

Dogs holds a significant position in the society by its multi fascinating roles such as guide for the disabled persons, in nursing homes and hospitals therapy to encourage patients toward recovery, sniffers or police work, in searching for drugs and explosives, locating missing people, finding crime scene evidence and to provide friendship, companionship, unconditional love and affection because of the impersonal suburban lifestyle brought on by nuclear families.

Ovarian tumours and cystic ovaries seem to be the most prevalent ovarian disorders[1]. Study of canine tumours is essential to provide timely diagnosis, prognosis and treatment of neoplasms by veterinarians. Early detection and treatments are challenging since these illnesses are frequently asymptomatic until they reach severe stages[2]. An ovarian tumour is one kind of tumour that arises from the ovary's cells multiplying uncontrollably and disorderly[3]. In neoplasm, cellular multiplication is limited in amount and duration by more or less clearly specifiable factors[4]. Present study will help in better understanding of canine ovarian neoplasms to provide timely diagnosis, in research to develop effective treatment strategy and to improve survival rates. Canine ovarian tumours are important because these tumours can affect a dog's health and fertility and while not common, they can be aggressive and may require prompt diagnosis and treatment. Canine ovarian tumours are classified into different types based on their origin (epithelial, germ cell, etc.) and understanding these differences is crucial for accurate diagnosis and treatment. Research into canine ovarian tumours can help improve early detection, treatment strategies and understanding of the disease's underlying mechanisms, ultimately benefiting dog owners and veterinarians. This research work was undertaken with the objectives to study the gross and histopathology of various ovarian tumours along with their classification and occurrence.

How to cite this article : Khandelwal, P., Dadhich, R., Galav, V., Agrawal, M., Meena, S., Mehra, A. and Longesha, F. 2025. A pathological study of ovarian tumours in dogs. Indian J. Vet. Pathol., 49(3) : 219-223.

MATERIALS AND METHODS

In the present study, tissue samples of the genital tract from 121 female dogs *(Canis familiaris)* irrespective of age and breed were collected from different animal birth control (ABC) program

hospitals and different clinics in Jaipur from September to December, 2024. Gross examinations of these samples showed 141 lesions in different parts of the genital tract out of which 15 cases of neoplasms of ovaries with an occurrence 10.64 percent were recorded. Tissue samples were also collected from the ovario hysterectomized mass and submitted to the Department of Veterinary Pathology, Postgraduate Institute of Veterinary Education and Research, Jaipur. The samples showing frank macroscopic lesions were collected for further histopathological examination in 10 per cent neutral buffered formalin. The histopathological examination was done after processing by acetone and benzene technique[5,6]. The tissue sections of 4-6 micron thickness were cut and further processed for routine staining by H&E method[7,8] for histopathological examination.

RESULTS

Granulosa cell tumour

This condition was recorded in 6 (4.25 percent) cases out of total 141 samples. Grossly, the tumour had both solid and cystic areas. They were large, lobulated, ovoid or spherical and usually encapsulated (Fig. 1). Microscopically, there was proliferation in neoplastic granulosa cells of the ovary in various patterns like follicular pattern, Sertoli cell-like pattern and sheets of cells forming cystic spaces. A pathognomonic Call-Exner bodies consisting of a small central round, to oval space with eosinophilic follicular fluid surrounded by a collar of radially arranged granulosa cells were observed (Fig. 2). The neoplastic cells were polyhedral with foamy cytoplasm and round hyperchromatic nucleus resembling normal granulosa cells with mitotic figures. Tumour cells showed papillary infoldings simulating papillary cystadenocarcinoma.

Cystadenoma

This condition was found in 4 (2.84 percent) cases out of 141 samples. Grossly, cauliflower-like masses and several thin-walled cysts containing clear, watery fluid in ovaries were observed. Ovarian surface showed the presence of dark reddish areas. Microscopically, cyst wall consisted of variably sized fibrovascular papillary projections producing a labyrinth of slit like space. Papillary projections lined by a single layer of cuboidal epithelium and in some places, by tightly packed tall columnar epithelium were recorded. The ovarian tissue consisted of tumour cells with acini formation (Fig. 3). The stroma was sparse and composed of a vascular framework. The section showed the presence of severe congestion too. Tumour cells exhibited hyperchromatic nuclei.

Cystadenocarcinoma

This condition was recorded in 2 (1.42 percent) cases out of 141 samples. Grossly, it had a cauliflower-like

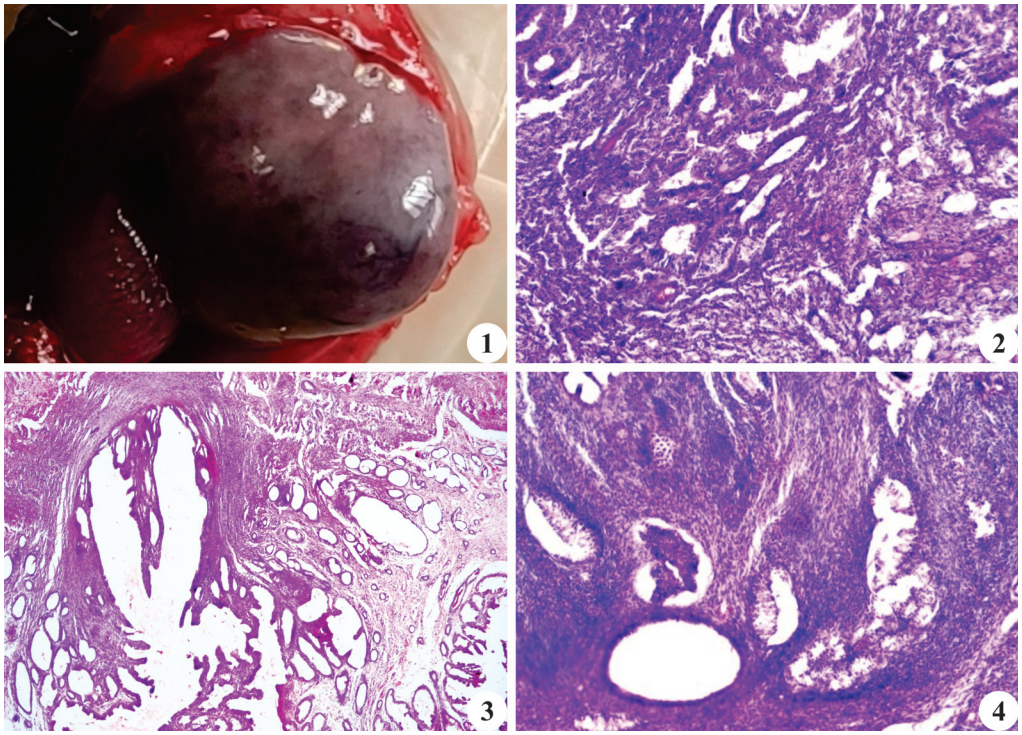

Fig. 1. Gross photograph of ovary having granulosa cell tumour; **Fig. 2.** Microphotograph of section of ovary having granulosa cell tumour showing neoplastic granulosa cells forming Call Exner bodies with eosinophilic follicular fluid (H&E 100X); **Fig. 3.** Section of ovary having cystadenoma showing papillary projections with cystic spaces (H&E 40X); **Fig. 4.** Microphotograph of cystadenocarcinoma of ovary showing multibranched papillary projections in cystic lumen proliferating cells replacing ovarian tissue (H&E 100X).

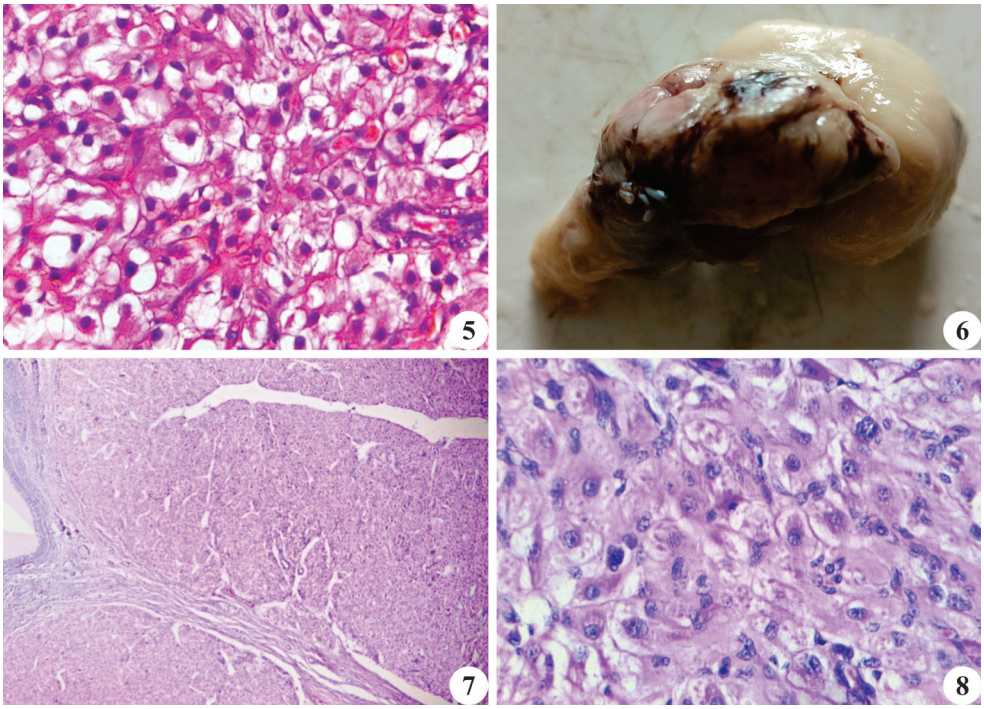

Fig. 5. Microphotograph of ovary having dysgerminoma showing polyhedral cells with markedly vacuolar cytoplasm (H&E 400X); **Fig. 6.** Gross photograph of ovary having luteoma; **Fig. 7.** Microphotograph of ovary having luteoma showing lobules of large polyhedral luteal cells (H&E 40X); **Fig. 8.** Microphotograph of ovary having luteoma showing delineated border of neoplastic luteal cells with hyperchromatic nuclei (H&E 400X).

appearance, with transparent fluid and dark haemorrhagic patches. Microscopically, long multibranching papillary projections were present in cystic lumen. At some places, the cystic lumen tightly filled with proliferating papillae. A shaggy, spongy mass of tumour tissue had largely replaced the ovarian tissue. The stroma was infiltrated by neutrophils, macrophages and plasma cells. Larger portion of the tumour consisted of sheets of tumour cells with acini formation. The tumour tissue showed hyperchromatic and more pleomorphic nucleus with mitotic figures in some places (Fig. 4). Congestion in thin-walled newly formed blood vessels was present.

Dysgerminoma

This condition was found in 2 (1.42 percent) cases out of 141 samples. Grossly, ovary had lobulations and uneven surfaces. Microscopic findings revealed a non-encapsulated, ill-defined neoformation consisting of lobules separated by strands of connective tissue. Polyhedral cells had a moderate quantity of transparent eosinophilic cytoplasm, with some cells having a markedly vacuolar cytoplasm (Fig. 5). Such cells revealed an eosinophilic cytoplasm, a high nucleus to cytoplasm ratio, a central big nucleus, sometimes multiples, with pronounced anisokaryosis and mild pleomorphism, granular chromatin and one or more conspicuous nucleoli.

Luteoma

This condition was found in 1 (0.71%) case out of 141 samples. Grossly, a nodular structure, a neoplastic mass was present on the surface of the ovary (Fig. 6). Microscopically, proliferation of luteal neoplastic polyhedral cells was observed along with abundant, vacuolated cytoplasm and round nuclei forming multilobulations. The tumour lobules were separated by the well-vascularized connective tissue stroma. The ovary was full of tumour cells obliteration and compression on cortex remaining in thin layer (Fig. 7 & 8).

DISCUSSION

In this study, the frequencies of histopathological findings in ovarian neoplasms obtained from ABC program hospitals and different clinics undergoing elective OHE were examined. Granulosa cell tumour, cystadenoma, cystadenocarcinoma, dysgerminoma and luteoma were recorded in 4.25%, 2.84%, 1.42%, 1.42% and 0.71% of cases respectively.

Granulosa cell tumour (GCT) was recorded in 6 (4.25 percent) cases out of 141 samples and almost similar incidence was recorded as 5.88 percent[9]. Much higher incidence was reported as 18.75 percent[10]. Presence of large, lobulated, ovoid or spherical and usually encapsulated masses is in close approximation was recorded as salient gross findings[11,13]. A pathognomonic Call-Exner bodies consisting of a small central round, to

oval space with eosinophilic follicular fluid surrounded by a collar of radially arranged granulosa cells were observed[12,14,15]. There was presence of polyhedral neoplastic cells with foamy cytoplasm and round hyperchromatic nucleus resemble normal granulosa cells with mitotic figures[4,10,13,16]. Granulosa cell tumours are the most frequently diagnosed ovarian tumour in bitches, representing up to 50% of tumour in female dogs and arise from the granulosa cells in the tertiary follicles[17].

Cystadenoma was found in 4 (2.84 percent) cases but a much higher incidence was recorded as 29.41 per cent in dogs in Alexandria, Egypt[9] and 9.37 percent in dogs in Rajasthan[10] respectively. Microscopically, cysts wall consists of variably sized fibrovascular papillary projections, lined by single layer of cuboidal epithelium producing a labyrinth of slit like space. The ovarian tissue consisted of tumour cells with acini formation[9,10,18].

Cystadenocarcinoma was recorded in 2 (1.42 percent) cases. Almost similar incidence was recorded as 1.10 percent[19]. Higher incidence was reported as 6.25 percent in dogs in Rajasthan during study undertaken in 2004[10]. Microscopically, the cystic lumen showed long multi-branching papillary projections and ovarian tissue was replaced by a shaggy, spongy mass of tumour tissue. The tumour tissue showed hyperchromatic and more pleomorphic nuclei with mitotic figures at some places[10,19,20].

Dysgerminoma was found in 2 (1.42 percent) cases. Higher incidence was recorded as 6-12 percent[21]. Gross findings of lobulations on the ovary and an uneven surface were recorded in this study conducted at Jaipur in the 2024-25[22]. Microscopic findings recorded in dysgerminoma were ill-defined neoformation consisting of lobules separated by strands of connective tissue[22]. Polyhedral cells revealed an eosinophilic vacuolar cytoplasm, a high nucleus to cytoplasm ratio, a central big nucleus, sometimes multiples, with pronounced anisokaryosis and mild pleomorphism, granular chromatin and one or more conspicuous nucleoli[4,14,22,23].

Luteoma was found in 1 (0.71%) case[24,26]. Microscopically, proliferation of luteal neoplastic polyhedral cells with abundant, vacuolated cytoplasm and round nuclei forming multi lobulation were described in present study. The ovary was full of tumour cells obliteration and compression on cortex remaining in thin layer. The tumour lobules separated by the well vascularized connective tissue stroma[24,25,26,27].

CONCLUSION

The present study provides valuable insight into the occurrence and histopathological characteristics of ovarian tumours and also as an important reproductive pathology in bitches. Through gross and histopathological examination, various types of ovarian neoplasms were identified, with granulosa cell tumours and cystadenomas being the most common whereas luteoma as the rare tumour. Histopathological examination is an inexpensive and efficient method for diagnosing ovarian tumours in dogs. The findings revealed that ovarian tumours, although relatively infrequent, often remaining asymptomatic until advanced stages. Histopathology proved essential for definitive diagnosis, allowing differentiation between different type of neoplasms and identifying associated degenerative or proliferative changes. These results underscore the importance of routine reproductive examination and histopathological evaluation in suspected cases to aid early diagnosis and appropriate clinical management. To enhance diagnostic accuracy and better understand tumour origin, behaviour and differentiation, further studies incorporating immunohistochemical (IHC) markers are strongly recommended.

ACKNOWLEDGEMENT

The facilities extended by the Post Graduate Institute of Veterinary Education and Research, Jaipur for carrying out this study are highly appreciated.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 49(3) : 224-231, 2025: DOI: https://doi.org/10.56093/psszp883

RESEARCH ARTICLE

Ameliorative potential of *Punica granatum* peel extract against Fipronil induced neuronal toxicity in Male Wistar Albino rats

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Received: 16.4.2025; Accepted: 26.6.2025

ABSTRACT

Fipronil is a second-generation broad spectrum phenyl pyrazole insecticide used in veterinary, households, topical pet care products and agricultural practices. Indiscriminate and improper use of this insecticides leads to a range of harmful effects in both humans and animals. So the present study was carried out to explore the effect of fipronil toxicity in brain of wistar albino rats and to check the potential ameliorative effect of pomegranate peel extract *(Punica granatum)* in it. For this study 24 male Wistar albino rats that were randomly assigned to 4 groups with 6 rats in each group. Fipronil was gavaged @ 10 mg/kg b.wt. orally using distilled water as a vehicle to group II animals. Peel extract of *Punica granatum* @ 200 mg/kg b.wt. was fed to group IV along with fipronil for exploring the ameliorative effects for a period of 6 weeks. Group I and III served as vehicle control and *Punica granatum* control respectively. Oxidative stress estimation in brain tissue revealed significant increase ($P<0.05$) in the levels of lipid peroxidation (LPO) and decreased levels of superoxide dismutase, catalase, reduced glutathione and glutathione peroxidase in fipronil treated rats. Histopathological examination of same group showed sub-meningeal hemorrhages, capillary proliferation, neuronal degenerative changes like shrinkage, central chromatolysis, satellitosis and neuronophagia, gliosis, spongiosis in the cerebral cortex and rounding, shrinkage and loss of Purkinje cells of the cerebellum were also observed. Co-administration of *Punica granatum* (pomegranate peel extract) along with fipronil resulted in restoration of oxidative stress parameters and histological architecture of brain near to normal.

Keywords: Amelioration, fipronil, male Wistar albino rats, oxidative stress, *Punica granatum*

INTRODUCTION

The use of pesticides in modern agriculture is a double-edged sword, boosting crop yields and ensuring food security, yet also presenting serious threats to both environmental and human health[1]. Pesticides play a vital role in reducing substantial agricultural losses, which are estimated to exceed 45 percent annually due to pest infestations[2], while also playing an important role in controlling vector borne diseases. Beyond agricultural applications, they are also found in various consumer products[3]. These chemicals are categorized according to their intended use and chemical structure, allowing for targeted pest control that promotes productivity and supports food security in a cost-effective manner. In the realm of modern agriculture and pest management, pesticides are commonly divided into four primary groups based on their chemical structure and mechanism of action: organochlorines, organophosphates, carbamates and pyrethroids[4].

Fipronil, a commonly used insecticide in agriculture, is effective in managing pests and maintaining public hygiene[5]. Classified under the phenyl pyrazole group, it demonstrates efficacy against over 250 insect species. However, extensive use of fipronil has been linked to negative impacts on animal health, human well-being and plant systems[6]. Fipronil has been shown to exert various toxic effects on both humans and animals, including cytotoxicity, reproductive toxicity, liver damage, neurotoxicity and neurodegenerative outcomes across both vertebrate and invertebrate species[7]. These effects are primarily linked to its action on γ-aminobutyric acid (GABA) receptors which are key components in the central nervous system and the main inhibitory neurotransmitter in mature vertebrates. There are two major subtypes of GABA receptors: GABAA and GABAB[8]. GABAA receptors act as ligand-gated ion channels that allow chloride ions to enter the neuron upon activation by GABA, thereby contributing to inhibitory synaptic transmission[9]. Fipronil disrupts this process by blocking GABA-gated chloride channels, particularly those associated with GABAA receptors, leading to nervous system excitation,

How to cite this article : Nakul, P., Sujatha, K., Kumar, A.A., Lekshmi, S. and Vijayalakshmi, S. 2025. Ameliorative potential of *Punica granatum* peel extract against Fipronil induced neuronal toxicity in Male Wistar Albino rats. Indian J. Vet. Pathol., 49(3) : 224-231.

neuronal hyperactivity, paralysis and ultimately death in insects[10]. Although both vertebrates and invertebrates possess GABA-regulated chloride channels, fipronil exhibits a significantly higher binding affinity for insect GABA receptors than for those in vertebrates, especially the GABAC subtype, which it binds with much lower affinity[11]. This selective binding profile contributes to its heightened insecticidal activity and offers a broader safety margin for mammals.

Recent findings suggest that glycine receptors in vertebrates may represent an additional target through which fipronil exerts its toxic effects. These receptors, which are ionotropic and responsive to the neurotransmitter glycine, mediate inhibitory neurotransmission by allowing chloride ions to flow into neurons. They are predominantly located in the brainstem and spinal cord and play a crucial role in maintaining neural inhibition within the central nervous system[12]. Fipronil binds strongly to several human glycine receptor subtypes such as α1, α1β, α2 and α3 which effectively inhibiting their function[13]. These subtypes share structural and functional similarities with vertebrate GABAA receptors, suggesting that all isoforms of human glycine receptors may contribute to fipronil's toxic potential in humans[13].

Fipronil may enhance oxidative stress, which leads to the generation of reactive oxygen species and is related to its toxic effects in different organs. Oxidative stress occurs when the generation of reactive oxygen species (ROS) exceeds the usual antioxidant capability of target cells[14]. Thus, measuring antioxidant indicators such as catalases, superoxide dismutase, glutathione peroxidase and lipid peroxidase can assist in determining the extent of oxidative damage to various tissues[15].

Recently, herbal medicine got more attention in seeking their complete potential in curing various toxicity cases in humans and animals[16]. Plant-derived antioxidants are composed of both essential nutrients with proven free radical-scavenging properties and a range of non-vitamin, non-mineral compounds. Alongside well-known antioxidants like alpha-tocopherol (vitamin E), ascorbic acid (vitamin C), carotenoids and zinc, many herbal remedies also contain bioactive compounds such as flavonoids, polyphenols and flavoproteins. Additionally, certain individual plants or specific herbal combinations used in traditional formulations may function as antioxidants by neutralizing superoxide radicals or by enhancing the activity of superoxide dismutase (SOD) in various tissues[17].

Pomegranate (*Punica granatum*) is known for its abundance of bioactive compounds that exhibit strong antioxidant properties[18]. In recent years, growing evidence has highlighted its wide range of health benefits, leading to increased scientific interest in exploring its therapeutic potential. The health-promoting effects of pomegranate pulp are largely credited to its rich content of phenolic compounds, including gallic acid and the distinctive polyphenols punicalagin α and β, the latter being unique to this fruit[19]. Punicalagin, a type of hydrolysable ellagitannin, is particularly noted for its antioxidant, anti-inflammatory and antiproliferative effects[20]. Most of the research explored the protective effect of fruit, leaves and seed part of pomegranate, hence current study aimed to explore the ameliorative effect of peel extract of pomegranate in neurotoxic effects induced by fipronil in wistar rats.

MATERIALS AND METHODS

Purchase of lab animals, fipronil and pomegranate peel extract

A total of 24 male Wistar albino rats were procured from Sri Venkateswara Enterprises, Bangalore, for this study. Following a two-week acclimatization period, the animals were randomly assigned to groups and housed in standard polypropylene cages. They were maintained under controlled environmental conditions at 25 ± 1^{O}C with a 12:12 hour light/dark cycle throughout the 6-week experimental period. Standard laboratory hygiene practices were followed, and the rats were provided with laboratory animal feed and water *ad libitum*. Institutional Animal Ethics Committee (IAEC) approval was obtained prior to the commencement of the experiment (Approval No. 281/go/ReBi/S/2000/CPCSEA/CVSc/TPTY/010/ Veterinary Pathology/2023, dated 08.05.2023). Technical-grade fipronil (99% pure; Batch No. FIP92B5266) was obtained from Gharda Chemicals Ltd., Mumbai. *Punica granatum* (pomegranate) peel extract (Product No. Dadim LC23030077) was procured from Chemiloids Life Science Pvt. Ltd., Vijayawada, Andhra Pradesh.

Experimental design

Groups	No. of Rats per group	Doses
Group I: Negative control	6	Adlibitum feed & distilled water
Group II: Fipronil control	6	Fipronil @ 10 mg/kg body weight (daily)
Group III: *Punica granatum* control	6	*Punica granatum* orally in distilled water @ 200 mg/kg body weight (daily)
Group IV: Fipronil + *Punica granatum*	6	Fipronil @ 10 mg/kg body weight orally in distilled water + *Punica granatum* orally in distilled water @ 200 mg/kg body weight (daily)

Table 1. Mean and SD values of LPO (nM MDA/g of tissue), SOD (U/mg of protein), Catalase (nM of H_2O_2 decomposed/min/mg of protein), Reduced glutathione (µmoles GSH/g of tissue) and Glutathione peroxidases (U/mg of protein) in brain of rats of different experimental groups.

	At the end of experimental Period (6th week)			
Oxidative stress parameters in brain	**GROUP I**	**GROUP II**	**GROUP III**	**GROUP IV**
LPO (nM MDA/g of tissue)	166.15 ± 0.94^{c}	348.84 ± 3.16^{a}	171.53 ± 1.88^{bc}	174.61 ± 1.60^{b}
SOD (U/mg of protein)	12.3 ± 0.21^{a}	8.8 ± 0.30^{c}	12.2 ± 0.33^{a}	11.2 ± 0.10^{b}
Catalase (nM of H_2O_2 decomposed/min/mg of protein)	0.23 ± 0.04^{a}	0.09 ± 0.02^{b}	0.21 ± 0.02^{a}	0.19 ± 0.01^{a}
Reduced glutathione (µmoles GSH/g of tissue)	18.64 ± 2.62^{a}	10.11 ± 0.98^{b}	17.89 ± 0.93^{a}	16.22 ± 0.84^{a}
Glutathione peroxidase (U/mg of protein)	24.89 ± 1.41^{a}	16.35 ± 0.75^{c}	22.36 ± 0.71^{ab}	20.09 ± 0.47^{b}

A total of 24 male Wistar albino rats were randomly assigned into four groups with six animals in each group.

Parameter studied

Oxidative stress

Tissue pieces of brain were minced and homogenized in 0.05 M ice cold phosphate buffer (pH 7.4) by using a Virtis homogenizer to make 10% homogenate. 0.2 ml of the homogenate was used for lipid peroxidation assay[21]. The remaining part of homogenate was mixed with 10% trichloroacetic acid in the ratio of 1:1, centrifuged at 5000 rpm for 10 min at 4^{O}C and supernatant was used for estimation of reduced glutathione[22]. The remaining part of the homogenate was further centrifuged at 15000 rpm for 60 min at 4^{O}C and the supernatant obtained was used for estimation of superoxide dismutase[23], catalase[24] and glutathione peroxidase[25] in brain of all rats in all groups.

Gross and histopathology

A detailed post-mortem examination was conducted on all the sacrificed rats in all the experimental groups. The gross lesions were recorded and representative tissue pieces from brain was collected and preserved in 10% neutral buffered formalin for histopathological studies. Fixed tissues were processed by routine paraffin embedding technique. Sections of 5-6 (µ) thickness was cut and stained with routine Hematoxylin and Eosin method (H&E)[26].

Statistical analysis

The results were statistically analysed by performing one-way ANOVA[27].

RESULT

Oxidative stress

Lipid peroxidation (LPO)

The mean brain LPO values in Group I to IV were 166.15±0.94^{c}, 348.84±3.16^{a}, 171.53±1.88^{bc} and 174.61±1.60^{b} (nM of MDA/g of tissue) respectively, and are given in Table 1 and Fig. 1a. A significant increase (P<0.05) in the brain LPO values was observed in fipronil treated rats

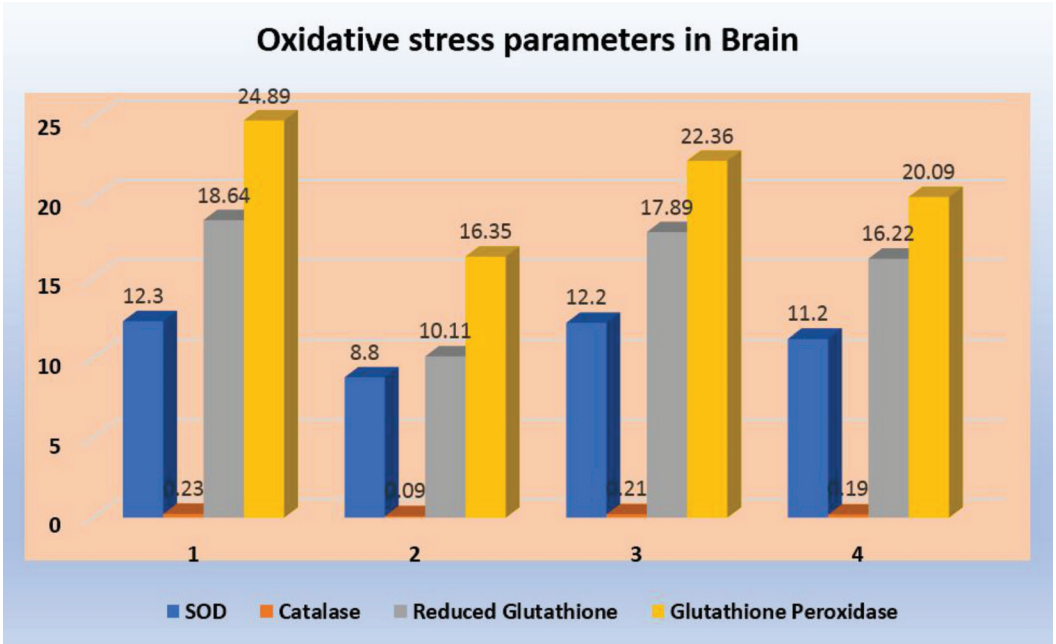

Fig. 1a. Mean and SD values of SOD (U/mg of protein), Catalase (nM of H_2O_2 decomposed/min/mg of protein), Reduced glutathione (µmoles GSH/g of tissue) and Glutathione peroxidases (U/mg of protein) in brain of rats of different experimental groups.

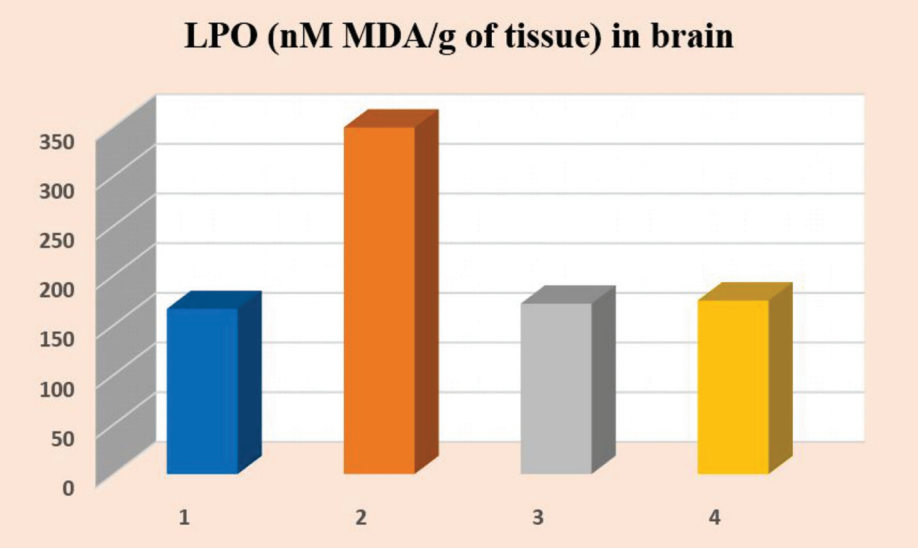

Fig. 1b. Mean and SD values of LPO (nM MDA/g of tissue) in brain of rats of different experimental groups. (Created separate graph for LPO as the LPO value ranges from 170-345 much higher compared to other parameters hence found difficulty to keep all in single graph, as Y axis value ranges differ in different parameters).

compared to the control group. However, no significant difference was noticed in LPO values among *Punica granatum* treated rats (Group III) compared to control rats (Group I). At the same time, a significant decrease in LPO values was noticed in ameliorated group rats compared to fipronil treated rats.

Superoxide dismutase

The mean SOD values in Group I to IV rats were 12.3 ± 0.21^{a}, 8.8 ± 0.30^{c}, 12.2 ± 0.33^{a} and 11.2 ± 0.10^{b} (U/mg of protein) respectively and are given in Table 1 and Fig. 1b. A significant ($P<0.05$) decrease in Group II (fipronil treated) rats was recorded in the SOD values of the brain when compared to control rats (Group I) and *Punica granatum* ameliorated (Group IV) rats. There was no significant difference in SOD values of *Punica granatum* treated rats (Group III) compared to control rats (Group I).

Catalase

The mean brain catalase values were 0.23 ± 0.04^{a}, 0.09 ± 0.02^{b}, 0.21 ± 0.02^{a} and 0.19 ± 0.01^{a} (nM of H_2O_2 decomposed/min/mg of protein) in Group I to IV respectively, and shown in Table 1 and Fig. 1b. Statistically, a significant ($P<0.05$) decrease was observed in catalase values in fipronil treated rats (Group II) when compared to control rats (Group I). A Significant ($P<0.05$) increase was observed in *Punica granatum* ameliorated rats (Group IV) when compared to fipronil treated rats. There was no significant difference in the catalase level among *Punica granatum* treated (Group III) rats compared to control rats.

Reduced glutathione

The overall mean of reduced glutathione values of the brain in Group I to IV rats were 18.64 ± 2.62^{a}, 10.11 ± 0.98^{b}, 17.89 ± 0.93^{a} and 16.22 ± 0.84^{a} (µmoles GSH/g of tissue) respectively, and are given in Table 1 and Fig. 1b. There was a significant ($P<0.05$) decrease in GSH values of fipronil treated rats (Group II) when compared to the control group. No significant difference was noticed in

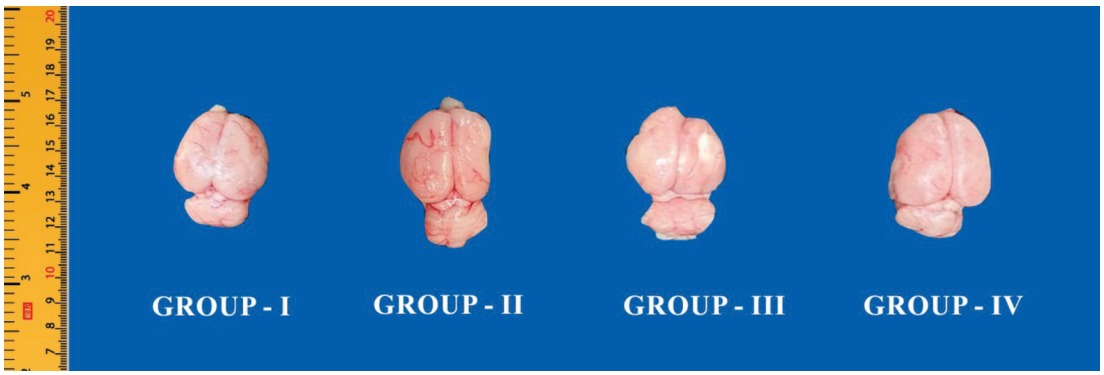

Fig. 2. Brain: Group II 6th week; note congestion of cerebral blood vessels.

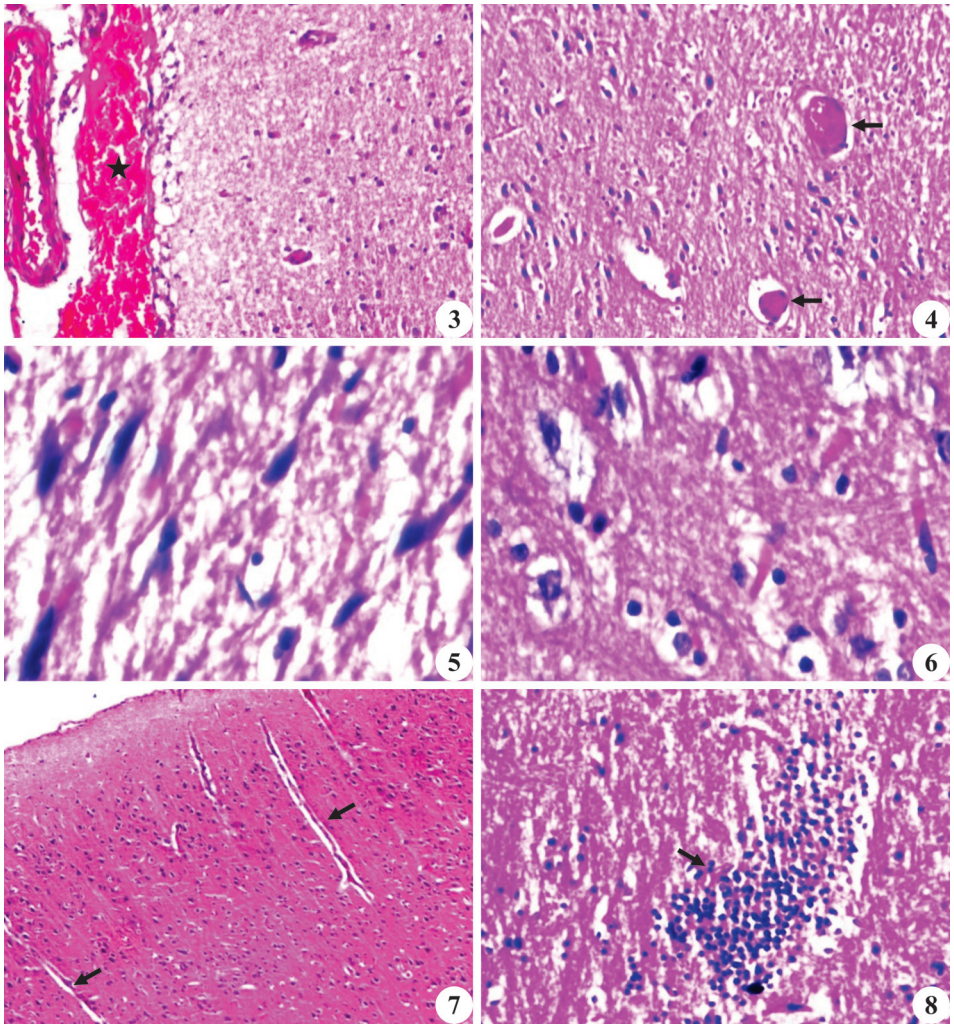

Fig. 3. Brain: Group II: Section showing sub meningeal haemorrhages (asterisk) and congested blood vessels (H&E x100); **Fig. 4.** Brain: Group II: Section showing cerebral blood vessel congestion (arrowed) (H&E x100); **Fig. 5.** Brain: Cerebral cortex: Group II: Section showing shrinkage of neurons with hyperchromatic nuclei (H&E x400); **Fig. 6.** Brain: Cerebral cortex: Group II: Section showing satellitosis and neuronophagia (H&E x400); **Fig. 7.** Brain: Cerebral cortex: Group II: Note moderate capillary proliferation in cerebrum (arrowed) (H&E x40); **Fig. 8.** Brain: Cerebral cortex: Group II: Section showing gliosis (arrowed) (H&E x100).

the reduced glutathione among *Punica granatum* treated (Group III) and control rats.

Glutathione peroxidase (GPx)

The mean GPx values were 24.89±1.41^{a}, 16.35±0.75^{c}, 22.36±0.71^{ab} and 20.09±0.47^{b} (U/mg of protein) in Group I to IV rats respectively, and are given in Table 1 and Fig. 1b. There was a significant ($P<0.05$) decrease in the mean brain GPx values of fipronil treated (Group II) rats compared to ameliorated rats (Group IV) and control rats (Group I). At the same time, the brain GPx values in Group I and Group III rats were statistically insignificant.

Pathology

After the end of the experimental period (6th week), both control (group I) and *Punica granatum* (group III) treated rats showed no gross and microscopic lesions of pathological significance. The brain of fipronil-treated rats (group II) showed cerebral blood vessel congestion (Fig. 2), whereas *Punica granatum* treated rats (group IV) did not show any prominent gross changes.

Histopathological examination of brain of fipronil treated rats revealed submeningeal haemorrhages (Fig. 3), congested cerebral blood vessels (Fig. 4) and choroid plexus. Most of the rats showed various neuronal degenerative changes like shrinkage and hyperchromatic neuronal cells (Fig. 5), central chromatolysis, satellitosis and neuronophagia (Fig. 6) in the cerebral cortex. Moderate to severe proliferation of capillaries (Fig. 7), gliosis (Fig. 8), spongiosis and demyelinating changes were conspicuous findings in the cerebral cortex of all treated rats. Majority of fipronil treated rats showed cerebellar degenerative changes like rounding, shrinkage (Fig. 9) and even complete loss of Purkinje cells in the Purkinje cell layer (Fig. 10). Hyperplasia of Purkinje cells was observed in the Purkinje cell layer of the cerebellum,

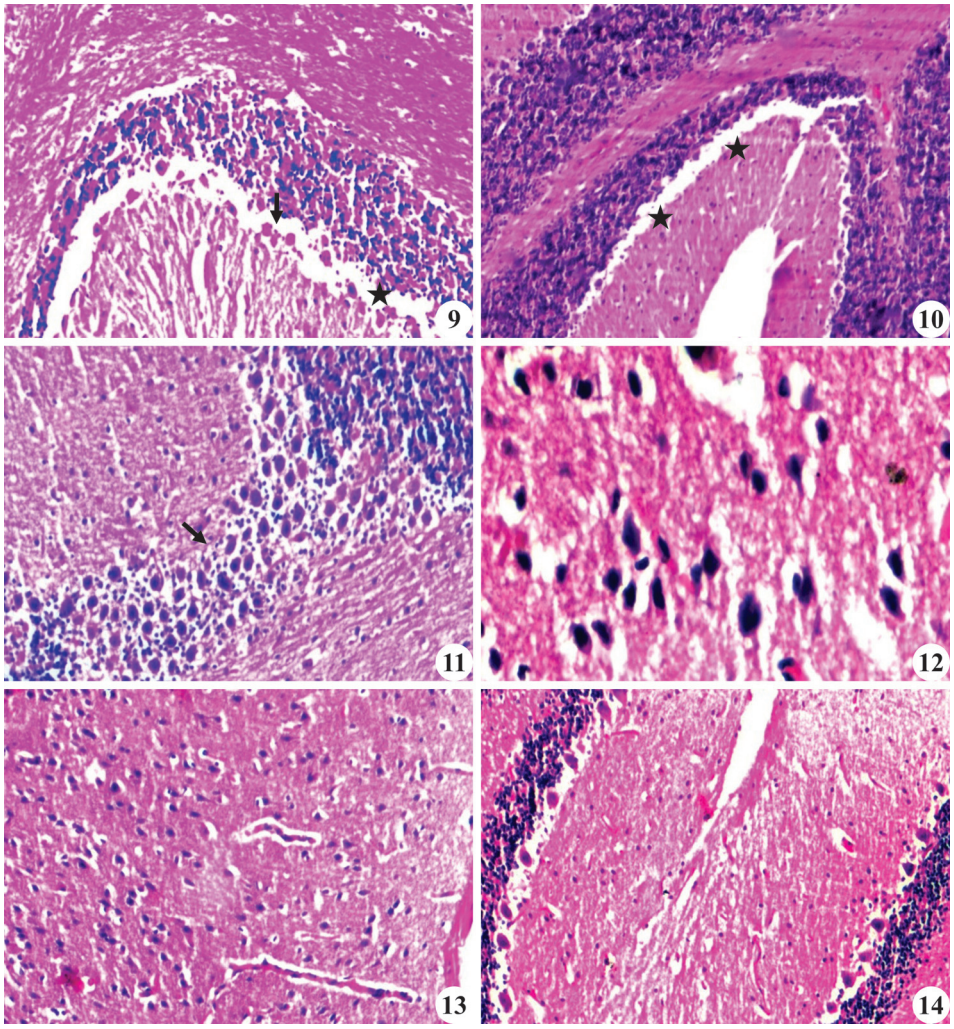

Fig. 9. Section showing rounding (arrowed), shrinkage (asterisk) and loss of Purkinje cells in Purkinje cell layer (H&E x100); **Fig. 10.** Brain: Cerebellum: Group II: Section showing complete loss of Purkinje cell layer (asterisk) (H&E x100); **Fig. 11.** Brain: Cerebellum: Group II: Note hyperplasia of Purkinje cells of Purkinje cell layer (arrowed) (H&E x100); **Fig. 12.** Brain: Cerebral cortex: Group IV: Section normal appearance of neurons (H&E x400); **Fig. 13.** Brain: Cerebral cortex: Group IV: Section showing mild proliferations of capillary blood vessels (H&E x100); **Fig. 14.** Brain: Cerebellum: Group IV: Note normal appearance of Purkinje cell layer (H&E x100).

with these hyperplastic cells extending into the granular layer and forming multiple layers (Fig. 11) in some rats. Neuronal degeneration, spongiosis and demyelinating changes in the molecular layer were more evident in the cerebellum by the end of 6th week of experimental period.

In Group IV rats, similar lesions like group II were observed in ameliorated rats by the end of 6th week of the experimental period with reduced intensity like mild neuronal degenerative changes, reduced spongiosis (Fig. 12) in both cerebrum and cerebellum, reduced capillary blood vessel proliferation (Fig. 13), mild congestion and normal appearance of Purkinje cell layer (Fig. 14).

The brain of *Punica granatum* treated rats (Group III) appeared normal and closely resembled those of the control group.

DISCUSSION

In the present study, a significant (P<0.05) increase in LPO (lipid peroxidation) value was noticed in the brain of fipronil treated rats (group II). When compared to corresponding control rats (group I). The present findings were in accordance with the results reported by earlier authors[28]. The increased LPO value might be due to alterations of lipid bilayer configuration in the cell membrane by ROS generated by fipronil, which in turn produces an excess of lipid peroxides (malondialdehyde) from the damaged membrane[29]. Hence, malondialdehyde which is a secondary product of lipid peroxidation, is used as a marker of cell membrane damage.

In the present research, a significant (P<0.05) decrease in superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione was observed in the brain of fipronil treated animals when compared to corresponding

controls. These findings were in agreement with[30]; who observed decreased levels antioxidant enzymes in liver of mice teated with fipronil. The decreased antioxidant enzymes activities in different organs in the present study might be due to fipronil induced excessive generation of ROS and reduced cell defence mechanism.

Significant reduction in LPO values along with increased levels of SOD, CAT, GPx, GST and GSH was observed in the *Punica granatum* ameliorated group (Group IV). The restoration of these values near to normal range in *Punica granatum* ameliorated rats might be due to anti-lipid peroxidation property of *Punica granatum*[31] and its antioxidant activity[32].

In the present study, brain of fipronil treated rats showed cerebral blood vessel congestion grossly. Microscopical examination of cerebral cortex revealed submeningeal hemorrhages, neuronal degenerative changes, moderate to severe proliferation of capillaries, gliosis, spongiosis and demyelinating changes. In cerebellum, rounding, shrinkage, loss of Purkinje cells in Purkinje cell layer, neuronal degeneration, spongiosis of molecular layer and hyperplasia of Purkinje cell layer were observed. Similar findings like shrinkage and loss of Purkinje cells, depletion of granular cell layers were reported by[33] in his work. These lesions might be caused by fipronil-induced oxidative damage in the brain. Because fipronil and fipronil sulfone are lipophilic, they cross the blood-brain barrier and cause brain damage and they also inhibit GABA-gated chloride ion channels and mitochondrial function, activating apoptotic proteins (caspases-3), ROS and the release of anti-inflammatory cytokines leads to neuronal necrosis and other neurobehavioral and histological changes in the brain[34].

In *Punica granatum* ameliorated rats (group IV), similar changes were noticed but with reduced intensity when compared to fipronil treated rats (group II). These improved brain changes might be due to the neuroprotective action[35] and antioxidant properties of *Punica granatum*[31].

CONCLUSION

The observations made in this study indicates that fipronil @ 10 mg/kg b.wt. / day orally for a period of 6 weeks induces toxic effects on brain in rats due to accumulation of fipronil and associated oxidative damage in brain tissue. Treatment with *Punica granatum* peel extract @ 200 mg/kg b.wt. / day concurrently with the fipronil was shown to have ameliorating effect on different pathological alterations induced in brain which suggest the neuroprotective effect.

ACKNOWLEDGEMENTS

The authors are thankful to the Sri Venkateswara Veterinary University, Tirupati for providing various facilities to carry out the present research work.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 49(3) : 232-237, 2025: DOI: https://doi.org/10.56093/7y77va05

RESEARCH ARTICLE

Pathomorphological alterations associated with gastrointestinal system of chickens in Patna district

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Received: 3.5.2025; Accepted: 31.5.2025

ABSTRACT

Genetical and managemental transformation of commercial poultry to meet the nutritional demands of increasing population can negatively affect the delicate balance among the components of the chicken gut leading to disturbances in the absorption and assimilation of nutrients, decreased feed conversion ratio and growth rate and production losses. The present study was conducted to study the occurrence of different pathomorphological affections of GIS in poultry with reference to age and season. A total of 500 dead birds in farms located at Patna district were necropsied and the lesions were critically recorded. Out of all these birds, 310 birds were found to be having affections related to GIS. Highest incidence of GIS affections was noticed in the monsoon season and least in spring season. Similarly, age-wise analysis revealed maximum incidence in the birds of 0-2 weeks age, while minimum incidence in the birds of more than 20 weeks of age. Among different components of GIS, Intestine was most affected organ while oesophagus was least affected. Histopathological lesions that range from degenerative to inflammatory changes were noticed in different organs of GIS.

Keywords: Chickens, gastro-intestinal system, gross lesions, histopathological lesions, incidence

INTRODUCTION

To meet the increasing demand with increase in population, these days birds are being genetically modified to achieve maximum growth and production in minimum times. Also, methods of poultry husbandry have changed considerably predisposing the birds to various infectious and non-infectious diseases leading to production loss and mortality. Poultry must have a healthy and functional Gastro-intestinal tract to maintain the optimal feed efficiency and productivity. The gastro-intestinal tract (GIT) also acts as a selective barrier between the tissues of the bird and its luminal environment. In addition, gut-associated lymphoid tissues (GALT) comprise of Payer'spatches, caecal tonsils, bursa of Fabricius and other lymphoid aggregates in the urodeum and proctodeum are a part of both primary and secondary lymphoid organs[1]. Thus, any abnormality in functioning of GIT leads to disturbances in the absorption and assimilation of nutrients resulting in decreased feed conversion ratio, decreased growth rate and production losses[2] along with subsequent bacterial translocation, product (meat and egg) contamination, morbidity and death. Such affections of GIT accounts for more than 30% mortality[3].

Since, the GIT has the most extensive exposed surface in the body, it is constantly exposed to a wide variety of potentially harmful substances any physical, chemical or biological disturbances can result in enteric disease[4]. Among infectious diseases, the conditions like Colibacillosis, Salmonellosis, Coccidiosis, Ranikhet disease, necrotic enteritis etc. affecting GIT are quite common. Stress is another factor that could indirectly predispose poultry to enteric diseases including leaky gut and enteritis.

Thus, the present study was undertaken to explore the role of GIT disorders in poultry mortality and to determine the age-wise and seasonal incidence of various gastrointestinal disorders in poultry.

How to cite this article : Vatsa, A., Kumar, S., Kumar, K. and Kumar, S. 2025. Pathomorphological alterations associated with gastrointestinal system of chickens in Patna district. Indian J. Vet. Pathol., 49(3) : 232-237.

MATERIALS AND METHODS

Samples and Sampling

The study was conducted for a period from October 2021 to September 2022. A total of 500 dead birds from different Government and private farms located in and around Patna district were necropsied and those birds showing the lesions of digestive system were included for the current study. The birds were further categorized into five different age groups. The birds were further categorized into five different age groups *viz.* 0-2 weeks, 3-5 weeks, 6-10 weeks, 11-20 weeks and above 20 weeks to determine the effect of age on the Incidence of GIT affections.

Pathomorphological & Histo-

pathological study

The organs were critically and systematically examined during necropsy for presence of any gross lesions in the digestive system. Upon critical systemic evaluation, gross lesions were recorded based on their type and distribution. Representative tissue samples revealing gross lesions were collected in 10% Neutral Buffer Formalin for histopathological tissue processing, sectioning and staining.

RESULTS

Out of 500 dead birds examined, gross pathological lesions were noticed in the digestive system of 310 birds (60.2). Among different age groups, 0-2 weeks age group revealed highest occurrence (76.53%) of GIT affections followed by 3-5 weeks age group. However, minimum GI tract affections were seen in age group of above 20 weeks age (Fig. 1A). Among different seasons, highest occurrence of lesions was noticed in the monsoon season (74.52%) followed by winter (68.06%) season, with lowest occurrence in spring (43.18%) season (Fig. 1B).

Gross lesions

Among different components of GIT, intestine revealed highest occurrence of lesions (38.38%) followed by liver with intestines (19.03%), while the least occurrence was noticed in oesophagus (0.32%). The details of occurrence of lesions in various components of GIT were showed in Table 1.

Lesions in the oral cavity included extensive erosions on the epithelial surface of tongue (Fig. 2A) with yellowish sticky substance covering the entire oral cavity. Oesophageal lesions were characterised by the congestion of mucosal surface with homogeneous, translucent material (Fig. 2B). Crop revealed inflammatory changes in the mucosa characterized by thickening, congestion, hemorrhages with minute ulcers. Dilatation of crop was also noticed with the blockage of feed passage (Fig. 2C). Proventricular lesions were characterized by profuse dilatation (Fig. 2D), serosal haemorrhages, haemorrhage in between the proventricular glands along with necrosis and erosions in the mucosa. Haemorrhages at the junction of proventriculus and gizzard were also noticed in few birds.

Gizzard revealed lesions in both keratinized layer

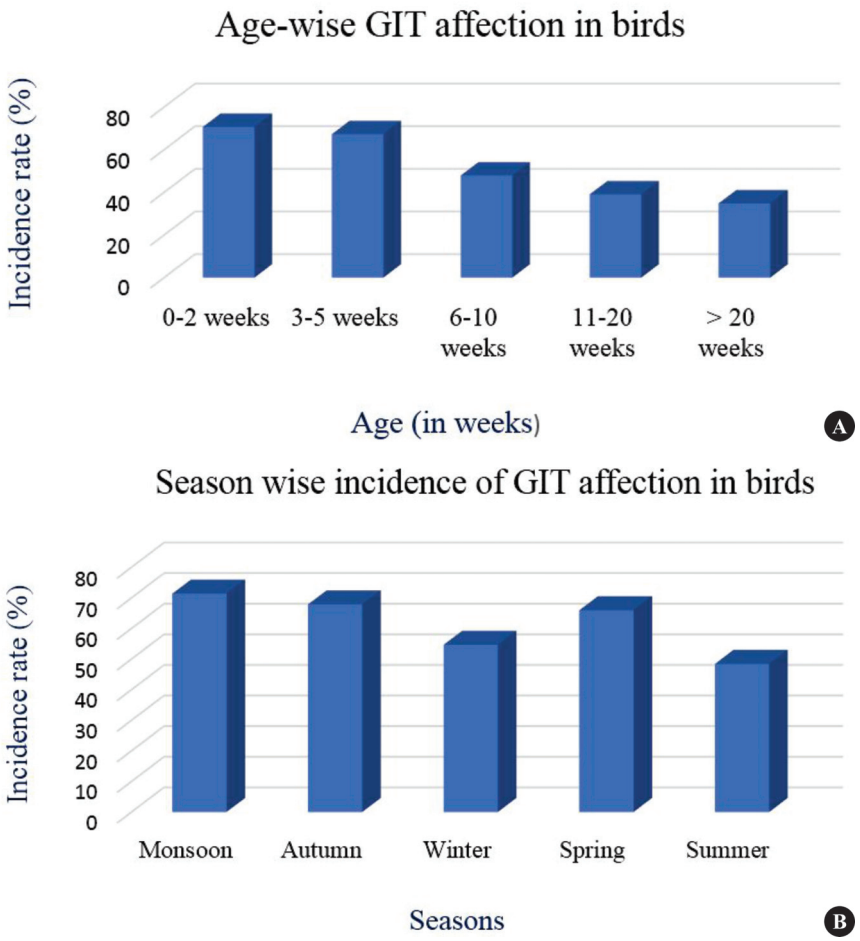

Fig. 1. Occurrence of gastro-intestinal affections in Chickens. **A.** Age-wise occurrence of GIT affections in Chickens. **B.** Seasonal incidence of GIT affections in Chickens.

and mucosal surface, which included erosive changes, ulceration and whitish discoloration of mucosa. Intestine was the mostly affected organ with the significant gross lesions of variable degree of hemorrhages, enteritis (necrotic and catarrhal) (Fig. 3A), thickening of intestine with corrugations (Fig. 3B). Caecal lesions included ballooning with bloody contents with necrosis and ulceration of caecal tonsils. Presence of Ascaris round worm was also evident in some birds. The findings have been depicted in Table 2. Gross lesion of liver

Table 1. Occurrence of gross GIT lesions in different organs.

S.No.	Organs Affected	Number & Percent of Birds Showing Lesions
1.	Oral cavity	3 (0.96%)
2.	Oesophagus	1 (0.32%)
3.	Crop	7 (2.25%)
4.	Proventriculus	25 (8.06%)
5.	Gizzard	7 (2.25%)
6.	Intestine	119 (38.38%)
7.	Liver	55 (17.74%)
8.	Liver and Intestine	59 (19.03%)
9.	Liver, Proventriculus & Intestine	34 (10.96%)

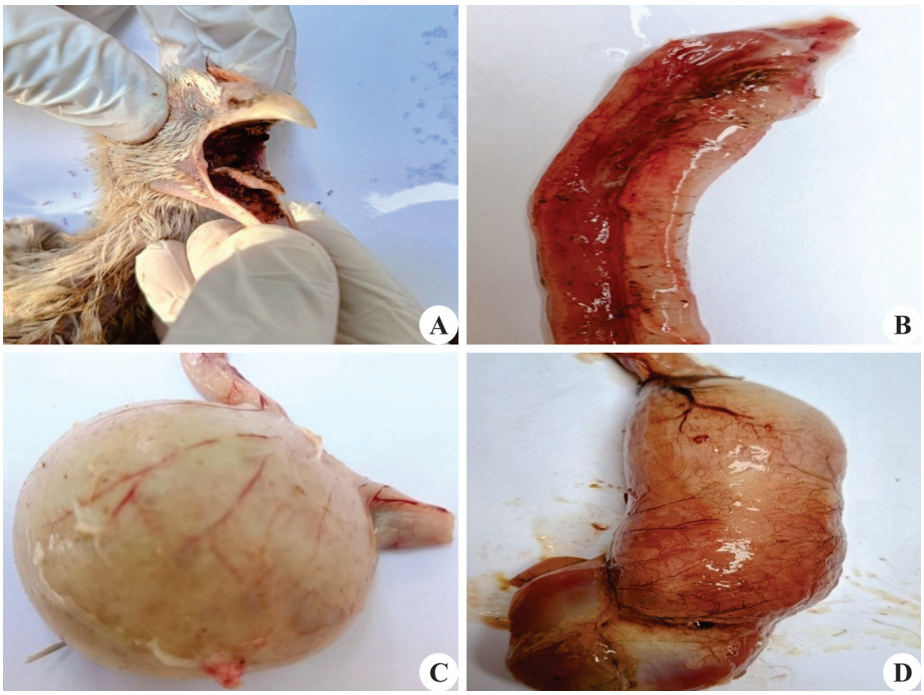

Fig. 2. Gross lesions in GIT of affected Chickens. **A.** Ulcerated oral lesions with extensive epithelial erosions. **B.** Congestion of oesophageal mucosa with homogeneous translucent material. **C.** Crop impaction with complete blockage of feed passage. **D.** Proventricular dilatation.

Table 2. Gross lesions observed in the Intestine.

S.No.	Lesions in intestine	No. of birds affected
1.	Haemorrhagic enteritis	84
2.	Catarrhal enteritis	38
3.	Necrotic enteritis	29
4.	Enlarged Caeca with blood	32
5.	Ascaris affection	14
6.	Corrugation with thickening	06
7.	Ulcerative Caecal tonsil	09
	Total No. of Birds affected	212

noticed in the current study are depicted in Table 3 and they were characterized by swelling and enlargement, fatty change, hemorrhages (Fig. 3C), multiple necrotic foci (Fig. 3D), fibrin deposition over the surface and mottling with deposition of urate crystals.

Histopathological Lesions

Histopathological lesions in different components of GIT were depicted in Fig. 4. Oesophagal lesions were characterized by hyalinization of tissues (Fig. 4A) with increased numbers of spindle cells, focal edema, necrosis and fibrosis without significant inflammatory cell infiltration. Proventricular lesions included congestion, haemorrhages, degeneration and necrosis of glandular epithelium accompanied by marked lymphocytic infiltration. Cellular debris of desquamated cells (Fig. 4B), hyperplasia of ductualar epithelial cells, degeneration and necrosis of proventricular glands and inter-glandular connective tissue proliferation were the other lesions of proventriculus. Histopathological lesions of gizzard included variable degrees of keratinized layer depletion and necrosis of the lining epithelium with infiltration of lymphocytes and few heterophils.

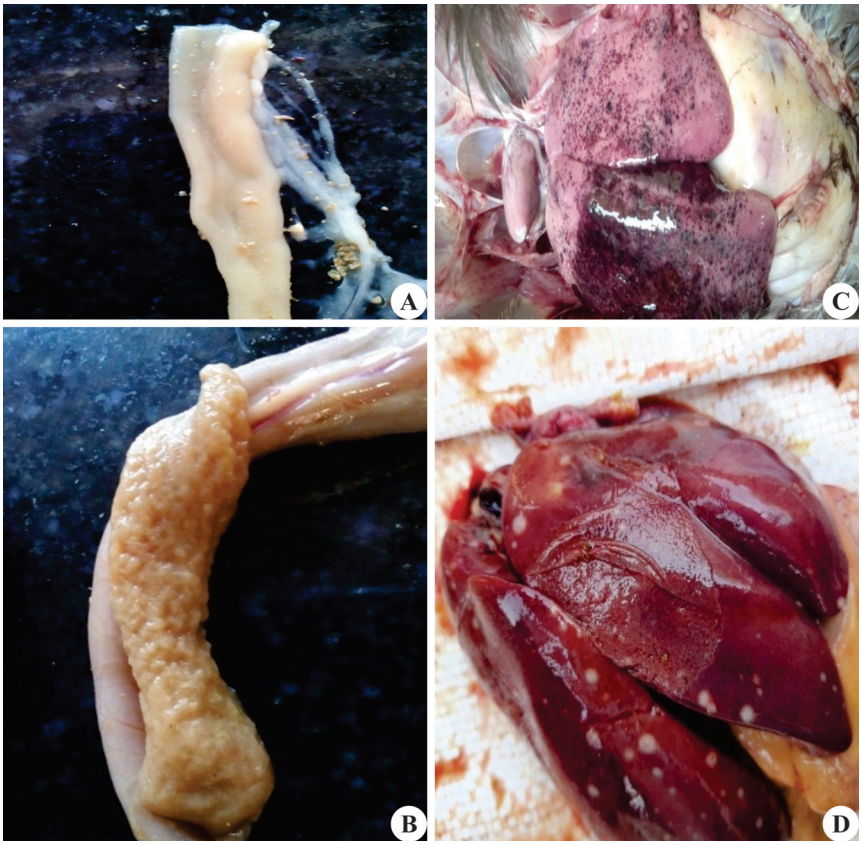

Fig. 3. Gross lesions in different organs of affected chickens. **A.** Catarrhal enteritis with thick layer of mucous. **B.** Thickened and corrugated intestines. **C.** Multiple hemorrhages on the liver surface. **D.** Multiple necrotic foci over the liver surface.

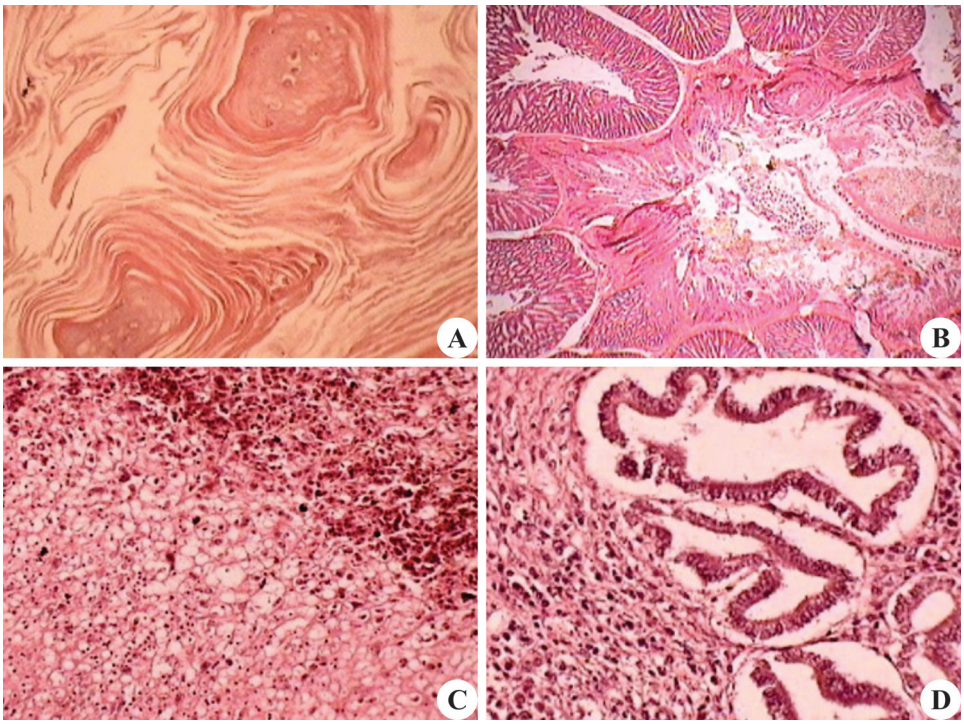

Fig. 4. Histopathological lesions in different organs of affected chickens. **A.** Marked hyalinization of oesophageal mucosa (H&E x100). **B.** Proventriculus with degeneration and desquamation of lining epithelium forming cellular debris in the lumen (H&E x40). **C.** Hepatocytes with cytoplasmic vacuolization and inflammatory changes (H&E x100). **D.** Liver with hyperplasia of bile ducts along with infiltration of heterophils, macrophages and few lymphocytes in the portal area (H&E x100).

Microscopic examination of affected intestines revealed predominant lesions of enteritis. Catarrhal enteritis was characterised by congestion, necrosis of epithelium, hyperplasia of goblet cells and cellular infiltration mainly with heterophils. Necrotic enteritis revealed severe denudation and necrosis of intestinal epithelium extending upto the crypts along severe inflammatory exudates comprising of an admixture of necrotic epithelial cells, fibrin and inflammatory cells mainly heterophils. In some cases, epithelium was found to be sloughed into the lumen along with congestion and edema of the lamina propria with vacuolation and subsequent lifting up of epithelial cells. Clubbing of intestinal villi, Gram positive bacilli lining necrosed villi were other intestinal findings.

Few cases revealed infection of coccidiosis with lesions of congestion, haemorrhages, necrosis of villi along with the presence of developing meronts, schizonts and oocysts in epithelium of villi and crypts. Mucosal scrapings from the affected intestines revealed Eimeria oocysts characterised by four sporocysts with two sporozoites in each sporocyst.

Histopathological lesions in the liver tissues revealed hydropic degeneration (Fig. 4C), fatty change and necrotic changes in centrilobular and periportal areas. Nuclear changes varied from pyknosis to karyorrhexis and karyolysis. Few cases revealed periportal fibrosis, congestion of portal vein, hyperplasia of bile duct epithelium (Fig. 4D) along with deposition of urate crystals within the liver parenchyma.

DISCUSSION

Over the past few decades, poultry husbandry practices have undergone significant transformations, largely driven by the emphasis on intensive rearing systems. While these practices have enhanced productivity, they have also increased the birds' susceptibility to a range of infectious diseases, leading to substantial economic losses for the poultry industry. Among these disorders affecting the GIT are particularly prevalent. In light of this, the present study was designed to investigate the patho-morphological lesions of the gastrointestinal system in poultry from different regions in and around Patna.

A total of 500 birds were examined, and the findings revealed that the highest incidence of gastrointestinal lesions occurred in the 0-2 week age group, while birds older than 20 weeks exhibited the lowest incidence. This observation contrasts with earlier studies, where the highest incidence was reported in birds over 24 weeks of age and the lowest in the 18-24 week age group[6]. Seasonally, the prevalence of lesions was highest during the monsoon and lowest in the spring, although spring recorded the highest mortality rate, with winter showing the least[5].

Among the gastrointestinal organs examined, the intestine was found to be the most frequently affected, followed by the liver. The esophagus was the least affected. Histological examination of the esophagus revealed four distinct layers: mucosa, submucosa, musculosa and adventitia or serosa. Affected birds exhibited glassy appearance and hyalinization of tissues, an abnormal finding in the avian esophagus[7]. Such hyalinization is typically associated with aging or chronic irritation, as also reported earlier, who described similar histopathological changes in multiple organs in broilers naturally infected with colibacillosis.

The proventriculus, continuous with the esophagus at the base of the heart and containing digestive enzymes and goblet cells, showed variable pathological changes. While proventriculitis is commonly linked with infectious bursal disease virus, other etiologies may also be involved[9]. Although multiple factors can cause such lesions, fungal infections are considered a common cause[10]. The ventriculus (gizzard), characterized by its thick muscular walls and protective koilin membrane, demonstrated lesions primarily in the keratinized and mucosal layers[11].

Histologically, the small intestine, composed of the tunica mucosa, submucosa, muscularis and serosa, exhibited a wide range of lesions[12]. These lesions are often associated with various pathogens, including viruses (e.g., astrovirus, avian nephritis virus, rotavirus, reovirus), bacteria (e.g., Clostridium spp.) and protozoa such as *Eimeria acervulina* and *E. tenella*[13,14].

The liver, the largest accessory digestive gland in poultry and encapsulated by Glisson's capsule, also exhibited considerable pathological changes. These may be attributed to infections with hepatitis virus, adenovirus or parasitic migration, such as by *Ascaris*[15,16]. Consistent with the findings of earlier worker[17], this study observed a range of gross liver lesions including congestion (71.27%), hemorrhage (18.05%), necrosis (9.26%), fatty changes (0.78%) and hematomas (0.62%). Histologically, the most common non-inflammatory lesions were congestion (64.52%), hemorrhage (10.20%), fatty liver (0.47%) and hepatosis (3.29%).

These findings were in agreement with those of earlier worker[18], who documented hepatic necrosis (29.41%), congestion (25.88%) and hepatitis (20%) in White broiler breeds. Gross lesions such as multifocal to diffuse necrosis, discoloration, mottling and hepatomegaly as well as histopathological features including sinusoidal and portal vein dilation and infiltration of hepatocytes with heterophils and mononuclear cells were similarly observed in the current study.

In conclusion, the study highlights the significant burden of gastrointestinal lesions in poultry, particularly in younger birds and during the monsoon season. The findings underscore the need for timely diagnosis and effective control measures to mitigate losses in commercial poultry production.

CONCLUSION

Poultry production incurs significant losses due to various conditions affecting the GIT. The findings of the present study highlight that the GIT, being the most directly exposed interface with potential pathogens requires focused attention from poultry farm owners to ensure optimal health, productivity and economic sustainability. The pathology of enteric diseases in poultry is diverse, reflecting the continuous exposure of the GIT to a wide range of harmful agents, including viruses, bacteria and parasites.

Our study revealed that gastrointestinal affections were most prevalent during the monsoon season, with the lowest incidence observed in the spring. Age-wise, young chicks particularly those in the 0-2 week age group were most commonly affected, whereas birds older than 20 weeks exhibited the least susceptibility. Among the various segments of the gastrointestinal system, the intestine emerged as the most frequently affected organ, while the esophagus was comparatively the least involved.

These findings underscore the importance of preventive strategies aimed at protecting the gastrointestinal system to reduce morbidity, enhance productivity and promote economic viability in poultry farming. A comprehensive understanding of GIT lesions can also serve as a valuable foundation for future research and the development of improved management and disease prevention practices in commercial poultry operations.

ACKNOWLEDGEMENT

The authors are extremely thankful to the Dean, Bihar Veterinary College and officers of Bihar Animal Sciences University, Patna for providing necessary guidance and financial support for smooth conduction of this research work.

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Indian J. Vet. Pathol., 49(3) : 238-241, 2025: DOI: https://doi.org/10.56093/675qc250

SHORT COMMUNICATION

Polycystic kidney disease in a Labrador dog

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Received: 28.3.2025; Accepted: 29.5.2025

ABSTRACT

An eleven-month labrador dog was brought to Veterinary clinical complex, Veterinary College and Research Institute, Namakkal with a history of anorexia, frequent vomition and progressive weight loss. Hematology and serum biochemical analysis showed severe anaemia, leukopaenia, azotemia, hyperphosphatemia and hypocalcemia. The case was tentatively diagnosed as Polycystic kidney disease based on clinical signs, haematobiochemical parameters and ultrasonography. In spite of the palliative treatment, the animal died within a week and the same was referred to Department of Veterinary Pathology for necropsy. The carcass was severely emaciated and ulcers were noticed in the oral cavity. Kidneys showed numerous, whitish, irregular and varying-sized fluid-filled cysts studded over the entire renal parenchyma. Histopathological examination of the kidney revealed large amount of fibrous tissue in the interstitium with irregularly dilated cysts and some cysts with homogeneous, acidophilic material in the lumen. On Masson's trichrome staining and Van Gieson's staining, the fibrous stroma appeared blue and bright red in colour respectively. The liver showed portal fibrosis with bile duct hyperplasia. This communication deals with the pathology of congenital polycystic kidney disease in a dog.

Keywords: Anaemia, hyperphosphatemia, Labrador dog, masson's trichrome stain, polycystic kidney, Van Gieson's stain

Polycystic kidney disease (PKD) is a rare genetic disorder that occurs either as an autosomal dominant polycystic kidney disease (ADPKD) in adults or autosomal recessive polycystic kidney disease (ARPKD) in young ones[1]. The PKD is characterized by the formation of multiple cysts in the cortex and medullary region with primary lesions in the renal tubules. These cysts continue to grow and deteriorate the normal renal function leading to chronic kidney disease like symptoms and lesions[2]. The animal often do not develop any clinical signs until the disease has progressed significantly. At the advanced stage, the animals shows symptoms like polydipsia, polyuria, lethargy, poor appetite and progressive weight loss. Microscopically, PKD is characterized by extensive renal fibrosis and dilated cysts throughout the renal parenchyma with limited functional renal tissues. Eventually, these changes associated with PKD compromise the renal function and can progress to chronic kidney disease and end stage renal failure in affected dogs. Histopathological examination of the kidney remains the preferred method for the definitive diagnosis of the condition despite the ultrasonography, which helps in the early diagnosis of the condition. Though there are several literatures on occurrence of PKD in cats, there are only few reports on the occurrence and pathology of PKD in dogs. Understanding the pathology of PKD is essential for the accurate diagnosis of the condition, monitoring the disease progression which provides insights into the breeding programs especially in the predisposing breeds. In this regard the present communication deals with the pathology and various pathological manifestations of renal lesions in a dog diagnosed with polycystic kidney disease.

An eleven-month-old female Labrador dog weighing about 10 kg was presented to the Veterinary Clinical Complex, Veterinary College and Research Institute, Namakkal with a history of anorexia, frequent vomition, polyuria, polydipsia and progressive weight loss. The animal was subjected to routine clinical examination including abdominal ultrasonography. Whole blood was collected for hematology and serum biochemistry. However, the dog died after a week and the carcass was presented to the Department of Veterinary Pathology, VCRI, Namakkal for postmortem examination. A detailed necropsy was carried out and gross lesions were recorded. The organs showing lesions were collected in 10% formalin for histopathological examination. The sections were cut at 4µ thickness and were subjected to routine H&E staining and special staining *viz.*, Masson's trichrome and Van Gieson's stainings.

How to cite this article : Sivaraj, S., Arulmozhi, A., Sasikala, M., Gopal, K., Balachandran, P., Srinivasan, P. and Monisha, D.C. 2025. Polycystic kidney disease in a Labrador dog. Indian J. Vet. Pathol., 49(3) : 238-241.

In the present case, clinical examination of the dog revealed that the animal was lethargic with stunted growth, anaemic, dehydrated with intermittent vomition. Hematology revealed severe anaemia and dehydration (Table 1), while serum biochemistry revealed higher levels of serum creatinine and BUN along with

hyperphosphatemia and hypocalcemia (Table 2). Abdominal ultrasonography revealed the presence of multiple thin-walled anechoic cysts in both the kidneys. Based on the clinical signs, clinical pathology and ultrasonography, the condition was diagnosed as renal disease and the dog died within a week.

On necropsy, the carcass appeared emaciated with pale mucous membranes and ulcers in the oral cavity. The jaw bones were soft and pliable. Internal examination of the carcass revealed pale viscera; rounded up heart with left ventricular hypertrophy; lungs were leathery and revealed atelectatic changes; Liver showed mild hepatomegaly with rounded borders; Gastric and intestinal mucosa were haemorrhagic and showed area of ulcerative changes.

The kidneys were distorted in shape, shrunken and pale with adherent capsule. There were numerous, whitish, irregular and varying-sized fluid-filled cysts studded over the entire renal parenchyma (Fig. 1). The sagittal section of both the kidneys revealed poorly demarcated cortex and medulla (Fig. 2). The urinary bladder exhibited severe thickening of mucosa.

Table 1. Hemogram of the dog affected with Polycystic kidney disease.

Parameters	Results	Units	Reference range
Creatinine	19.5	mg/dl	0.5 - 1.8
BUN	185.6	mg/dl	8 - 28
Total protein	5.6	g/dl	5.4 - 7.1
Albumin	2.5	g/dl	2.3 - 3.3
Calcium	6.6	mg/dl	9.0 - 11.7
Phosphorus	18.9	mg/dl	2.6 - 5.3

Table 2. Serum biochemistry of the dog affected with Polycystic kidney disease.

Parameters	Results	Units	Reference range
Hb	3.6	g/dl	12.0 - 19.0
PCV	11	%	37 - 57
RBC	1.71	$x10^6/\mu L$	5.0 - 9.0
WBC	7.44	$x10^3/\mu L$	5 - 15

Histopathological examination of kidneys showed a large amounts of fibrous connective tissue containing many circular to ovoid irregular cysts with thin walls (Fig. 3). Most of the cysts were empty and few were filled with an eosinophilic homogenous proteinaceaous substance. The cysts were lined with a single layer of flattened squamous to low cuboidal epithelial cells. In addition, there were dilated tubules with atrophied glomeruli, immature tubules and proliferative arteriole in the dysplastic area (Fig. 4). Dilated renal tubules were surrounded by extensive collagenous fibrous tissues which were well demonstrated as blue (Fig. 5) and bright red colour fibers (Fig. 6) in Masson's trichrome and Van Gieson's stain respectively.

Histopathological examination of lungs revealed peribronchiolar fibrosis and desquamation of bronchiolar epithelium into the lumen. Most of the alveoli were

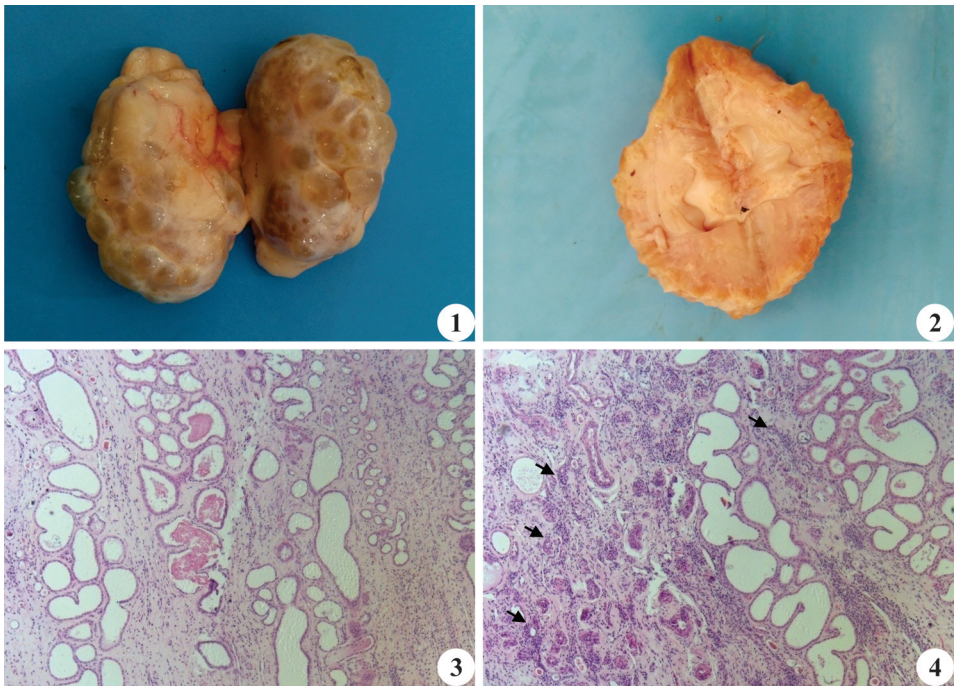

Fig. 1. Kidneys showing numerous, irregular and varying sized fluid-filled cysts; **Fig. 2.** Irregular left kidney showing reduced cortex; **Fig. 3.** Large amounts of fibrous connective tissue in the interstitium surrounding the irregular dilated cysts containing acidophilic material (H&E x40); **Fig. 4.** Dilated tubules with the immature glomeruli (arrows) with inapparent capillary lumina and tubules (H&E x40).

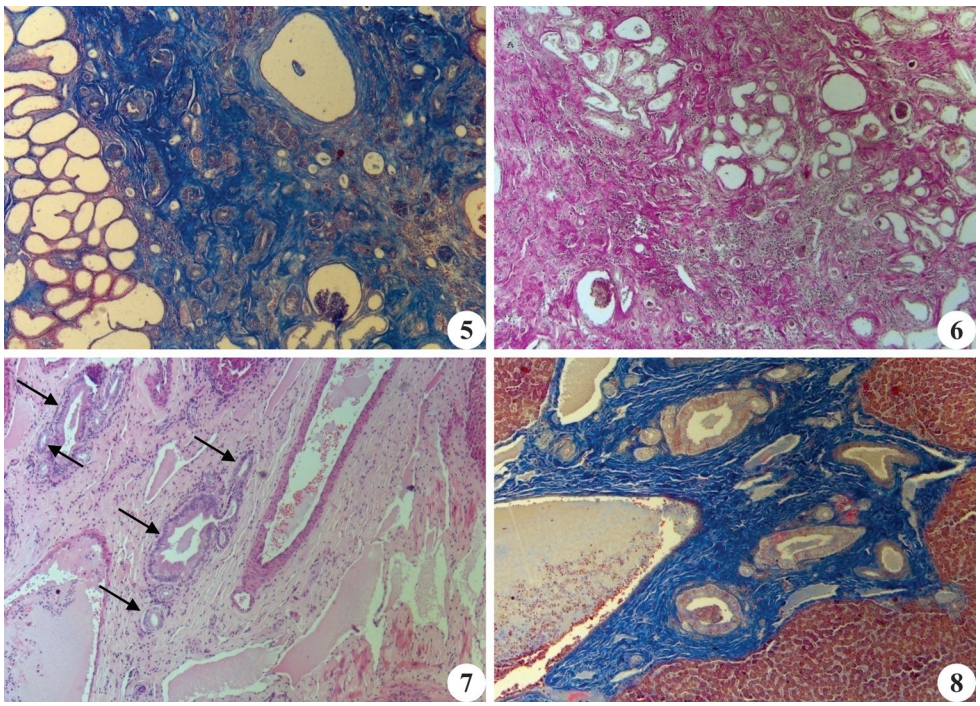

Fig. 5. Kidney showing extensive fibrous tissue proliferation in the interstitium (blue) with dilated renal tubules and atrophied glomeruli (Masson's trichrome stain x40); **Fig. 6.** Kidney showing abundant fibrous stroma (bright red) with dilated tubules replacing the normal renal parenchyma (Van Gieson's stain x40); **Fig. 7.** Liver showing replacement of hepatic tissue with fibrous tissue and bile duct proliferation (arrows) (H&E x40); **Fig. 8.** Liver showing severe periportal fibrosis (blue) and bile duct proliferation (Masson's trichrome stain x40).

collapsed and a few compensatory emphysematous areas were observed. However, there was no significant lesions noticed in the cardiac myofiber.

In liver, replacement of normal hepatic tissue with fibrous tissue proliferation and bile duct hyperplasia (Fig. 7) were noticed. The portal fibrosis was evidenced by blue coloured area in Masson's trichrome staining (Fig. 8). Fibrous tissue proliferation with ulcers were also noticed in the gastric and intestinal mucosa.

Stomach revealed loss of superficial epithelium and thickening of lamina propria with fibrous tissue proliferation. There were destruction of gastric glands and replacement with fibrous tissue noticed. There was severe destruction and desquamation of intestinal villi epithelium as well as crypts of Lieberkühn.

Polycystic kidney disease may occur due to heritable conditions[3] or certain chemicals[4] or defects in the tubular basement membrane[5]. Anaemia and decreased hemogram noticed in the present study was very well correlated with the renal lesions as erythropoietin production is hampered by the polycystic kidneys[6].

Progressively deterioting kidneys fails to excrete the metabolic end products such as urea, creatinine and phosphorus[7] which results in azotemia due to increased BUN and creatinine, hyperphosphatemia and subsequent hypocalcemia. The resultant alteration of the serum calcium and phosphorus level might have attributed to the soft, pliable and rubbery nature of the jaw bones which had been observed in the present case[8]. Similarly the excess accumulation of the nitrogenous waste substance *viz.*, urea and creatinine stimulates the chemoreceptor trigger zone, which irritates the intestinal mucosa resulting in frequent vomition and progressive weight loss noticed in the present case[9].

Microscopically, the large amounts of fibrous tissue with numerous dilated cysts observed can be well correlated with the inflammatory changes within the interstititum and subsequent fibrosis due to prolonged irritation, triggered by the chemical mediators such as cytokines, lymphokines and chemotactic factors[10].

To conclude, in polycystic kidney disease, a large area of the functional renal tissue is replaced by fibrous tissues and dilated tubular cysts resulting in end-stage renal failure mimicking the pathological changes occurring in the chronic renal failure of stage 4 leading to irreversible damage to the kidneys, resulting in anaemia due to the diminished production of erythropoietin and renal osteodystrophy with imbalanced serum calcium and phosphorus levels, finally culminating in the unfavourable prognosis.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that

there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 49(3) : 242-245, 2025: DOI: https://doi.org/10.56093/zk8qdv72

SHORT COMMUNICATION

Therapeutic management of intranasal transmissible venereal tumor in canines

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Received: 17.4.2025; Accepted: 14.5.2025

ABSTRACT

This case study aimed to diagnose and elucidate the therapeutic management of intranasal transmissible venereal tumor (TVT) in four dogs presented with unilateral epistaxis and respiratory distress for several days. The bleeding was characterized by fresh, uncoagulated blood. Cytological, hematological, serum biochemical and radiographic examinations were conducted. Cytological examination of the nasal swab samples showed a neoplastic population of large round cells exhibiting cytoplasmic vacuolation which is characteristic of transmissible venereal tumor, de-differentiation, increased N/C ratio and multiple prominent neucleoli. The animals received intravenous injections of vincristine sulfate as a drug of choice however complications may arise due to its repetitive/excessive use and like development of toxicity and hepatic insufficiency. Additional supportive treatments, administered in three to five weekly doses, intranasal administration of hemocoagulase; botrophase @ 5-6 drops daily for 3-4 days upto cessation of nasal bleeding and injectable broad-spectrum antibiotics were also administered resulting in complete recovery in all the cases examined.

Keywords: Cytology, epistaxis, intranasal, radiography, vincristine

Canines with transmissible venereal tumors (TVTs) have a contagious round cell tumor that is spread through sexual contact. Rarely, TVT spreads to the lips, oral mucosa, peritoneum and other organs such as the muscles and mammary glands. TVT can be malignant growth of mesenchymal origin (sarcoma) and may also occur as veneral granuloma[1]. It can also spread through sniffing or licking of the nasal/oral cavities, skin and rectum[3]. Facial edema, swelling in superficial lymph nodes, oro-nasal fistula, epistaxis and other nasal discharges are symptoms of nasal canine TVT[2]. Tumor cells are transmitted when genital mucous membranes come into contact during coitus. Therefore, the external genitalia often serve as the site of TVT lesions[4,17]. When an animal lick or sniff the preputial or vaginal discharges of an infected dog, extragenital lesions can also be observed in the nasal and/or oral cavities[4,5]. Grossly it appears as cauliflower-like ulceration, hemorrhagic, friable mass that bleed easily[7] and also sometimes manifested by bloody discharge. TVT is diagnosed through history, clinical signs, cytology and histopathology[12]. The treatment of choice for this condition is through chemotherapy[12,13] using Vincristin sulphate as a preffered drug of choice for treatment of TVT[19]. This case study describes the diagnosis of intra-nasal TVT in canines and its treatment.

This case study involves four dogs, irrespective of breed, age or sex, that were registered at the Teaching Veterinary Clinical Complex, College of Veterinary Science, DSVCKV, Anjora, Durg, between 1st January 2023 and 31st August 2024. The clinical presentation included unilateral epistaxis, dyspnea and a few days of fresh, uncoagulated blood discharge, accompanied by painful swelling palpated over the right nasal area. Each case underwent comprehensive hematological and biochemical assessments (Table 1). A ventrodorsal radiographic examination of the skull was performed, followed by a cytological evaluation to confirm the presence of intranasal TVT. Nasal swabs were collected for each case, with samples processed and stained using Giemsa stain. Therapeutic intervention included the administration of intravenous vincristine sulfate at a dose of 0.025 mg/kg body weight, given 3 to 5 times weekly, in addition to supportive care (Table 1).

How to cite this article : Bante, N., Kumar, V., Ratre, H.K., Maiti, S.K., Kalim, M.O., Raj, I.L., Kujur, R.F., A. and Sahu, H. 2025. Therapeutic management of intranasal transmissible venereal tumor in canines. Indian J. Vet. Pathol., 49(3) : 242-245.

The study included three male dogs and one female dog, aged between 3-10 years. Clinical signs included sneezing, epistaxis and painful swelling palpated over the right nasal region. Hematological parameters were assessed (Table 1). There were mild to moderate anemia, relative leukocytosis and neutrophilia in some cases, along with normal

Table 1. Hematological and biochemical analysis.

Parameters	Case 1 Non descriptive, Male, 4 Year	Case 2 Labrador, Male, 3 Year	Case 3 Non descriptive, Female, 9 Year	Case 4 Non descriptive, Female, 5 Year
Total Leukocytes Count (x10^9/L)	32.5	24.6	22.7	18
Neutrophils (%)	85	60	80	68
Haemoglobin (g/dL)	7.9	12.7	11.2	10.9
BUN (mg/dL)	23.14	26.01	32.15	19.01
Creatinine (mg/dL)	0.88	1.78	2.08	1.06
Albumin (g/dL)	2.08	2.96	2.78	1.93
SGPT (U/L)	38.23	27.23	54	42.08
Number of Vincristicine Cycles	3	3	5	4

platelet counts in all dogs. Blood smears were negative for hemoprotozoan infections. Biochemical analyses for kidney and liver function were within normal reference ranges (Table 1). A ventrodorsal radiographic view of the skull revealed no encapsulated tumor or bony abnormalities (Fig. 3). Cytological examination of nasal swab samples showed increased cellularity, neutrophilia, few large round cells (Fig. 4 & 5) with intracytoplasmic vacuolation, characteristic of TVT (Fig. 6), basophilic irregular cytoplasm and increased nucleo-cytoplasmic ratio with anisocytosis, anisokaryosis and multiple prominent nucleoli indicating de-differentiation (anaplasia) leading to neoplasm (Fig. 4 & 5). The definitive diagnosis of intranasal TVT was confirmed based on

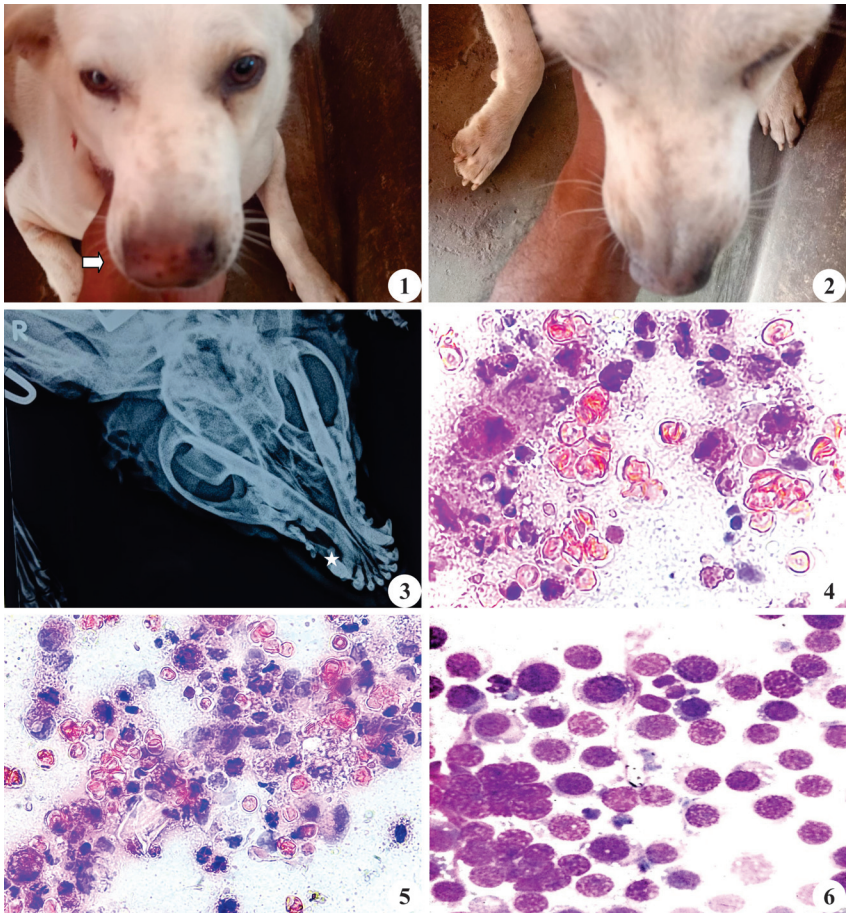

Fig. 1. Swelling of nasal area on right side (arrow); **Fig. 2.** After complete remission of the mass; **Fig. 3.** Confirmation by radiographic examination showing presence of tumorous growth (contor marked with star) on right side of nasal cavity; **Fig. 4.** Cytology revealing numerous large round cells with large neucleus and small cytoplasmic rim with vacuolations (Giemsa 100x); **Fig. 5.** Increased cellularity with neutrophilic infiltration and increased nucleo-cytoplasmic ratio (Giemsa 40x); **Fig. 6.** Presence of intracytoplasmic vacuolation: typical characteristic of TVT (Giemsa 100x).

cytological findings. Each animal received intravenous injections of vincristine sulfate @ 0.025 mg/kg body weight, administered weekly, in addition to supportive care. The treatment regimen consisted of 3 to 5 cycles. Complete remission was achieved in all cases (Fig. 2). At the follow-up, none of the dogs exhibited any adverse effects and all showed an excellent treatment response.

This study is comparable to other studies that have reported that the most common clinical symptoms of primary or secondary oral and nasal TVT are submandibular lymphadenopathy, halitosis, purulent nasal discharge, muscle deformity and the presence of friable cauliflower like lump[2]. There have been reports of secondary involvement at other body locations. The primary extragenital involvement rarely occurs[4]. In contrast to our findings, 19.2% of the dogs were diagnosed with TVT in extragenital locations like: skin, ribs, spleen, liver, nasal cavity, eye orbit, subcutaneous, submandibular, cervical and inguinal lymph nodes[8]. TVT has been found most commonly in vestibule and vagina of female and penile region of male dog and some metastasized in the ovary.

Cytological examination revealed the characteristic intracytoplasmic vacuolation, irregular basophilic cytoplasm, giant tumour cells which were in accordance with the findings of earlier worker[1,7]. TVTs should be distinguished from mast cell tumors, histiocytomas and lymphomas, which are other round cell skin tumors.

Numerous round cells with a noticeable nucleolus and basophilic cytoplasm were seen in the cytology[13]. The tumor's localization is crucial for the diagnosis. Our research indicated that the thickening of the soft tissues in the nostrils causes upper airway blockage and thus affected dogs may exhibit respiratory symptoms. One notable aspect of this dog's past was dyspnea. Oral neoplasms are responsible for blood-tinged saliva, anorexia and difficulty swallowing. Ocular tissue was impacted by the tumorous mass's progressive growth, resulting in conjunctivitis and hyperemia on the same side. According to our description, this benign tumor primarily manifests as a cauliflower-like, friable, hemorrhagic mass[17] although it seems more dispersed in the mouth[18]. In contrast, it was also reported to have an unusual appearance, resembling an ulcerated lesion with profuse granulation tissue. It has been demonstrated that medical treatment using chemotherapeutic drugs such vincristine is successful[16,18]. Vincristine sulfate was injected intravenously once a week at a dose of 0.025 mg/kg body weight to begin treatment. For the treatment of secondary problems, intranasal drops of hemocoagulase; botrophase @ 5-6 drops daily for 3-4 days and injectable broad-spectrum antibiotics were also administered. Every case showed improving signs of overall health and recovered with no problem after three to five weekly cycles of chemotherapy.

CONCLUSION

In canines, intranasal tumors can be cumbersome to diagnose due to the complex structure of the nasal sinus. Advanced imaging methods like CT scans or X ray examinations are frequently needed, coupled with biopsy for histological confirmation. Depending on the tumor's nature and stage as well as the dog's general condition, treatment options may include radiation therapy, surgery and palliative care. Despite the generally bad prognosis for dogs with intranasal tumors, especially when the tumors are malignant, detection and treatment might enhance quality of life and in certain situations, prolong survival. Additionally, palliative care may assist affected dogs retain their comfort and manage their symptoms. Present study concludes that cytological and radiographic examinations can be used to diagnose intranasal TVT and vincristine sulphate chemotherapy assist in the full remission of the neoplasm.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 49(3) : 246-249, 2025: DOI: https://doi.org/10.56093/geztd230

SHORT COMMUNICATION

Prevalence, isolation and antibiogram study of pathogenic *E. coli* from poultry farms in and around Patna

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Received: 17.4.2025; Accepted: 2.6.2025

ABSTRACT

Escherichia coli (E. coli), a member of the *Enterobacteriaceae* family, is a Gram-negative, rod-shaped, facultatively anaerobic bacterium commonly found in the intestines of birds and animals. Although it is part of the normal gut flora, certain enteropathogenic and toxigenic strains can cause a range of enteric and extra-intestinal infections in poultry. While previous studies have investigated the prevalence, isolation and antibiotic susceptibility profiles of *E. coli*, its characteristics are known to vary across regions and time periods. This study was undertaken to assess the prevalence of *E. coli* infections contributing to poultry diseases in and around Patna, with a specific focus on the antimicrobial resistance patterns of isolated strains. A total of 500 birds underwent necropsy, from which 200 suspected cases of colibacillosis on the basis of history and gross lesions were selected for further microbiological analysis and confirmation. *Escherichia coli* was successfully isolated from 120 of these samples, representing 60.0%. Of these, 113 isolates (56.5%) were biochemically confirmed as *E. coli*. Infections were more prevalent among younger chickens during the monsoon season. Antimicrobial susceptibility testing was conducted on all biochemically confirmed isolates using the standard disc diffusion method. The results indicated that tetracycline exhibited the highest resistance, followed by ciprofloxacin. Based on the findings, *E. coli* was identified as a predominant gastrointestinal pathogen in poultry and cephalexin was suggested as an effective treatment option against the prevailing strains.

Keywords: Antibiogram, characterization, *E. coli*, isolation, poultry, prevalence

During the last few decades, systems of poultry husbandry have transformed significantly due to an importance on intensive rearing which has made the birds susceptible for the incidence of various infectious diseases. Poultry business suffers great losses due to these infections. Despite adoption of modern managemental practices, preventive precautions and medications the poultry is plagued with a number of bacterial infections in recent years. Bacterial infections is the most common challenge for the gastro-intestinal tract of bird. In poultry, infections from *Escherichia coli (E. coli)* are most common among all other pathogenic bacteria that are associated with poultry production. *E. coli* are normal inhabitant of the intestines of birds and animals[1], but is of the concern due to the possible presence of enteropathogenic and/or toxigenic strains which lead to wide variety of enteric and extra-intestinal diseases in birds. Avian pathogenic *E. coli* (APEC) causes a syndromic poultry infection known as colibacillosis which can affect birds of all ages and different types of poultry and has zoonotic importance because these organisms are transmitted from raw poultry meat to the human consumers and the workers handling the poultry or poultry products[2,3]. *E. coli* alone accounts for more than 14.0% of the infectious diseases[4]. The above facts have necessitated an in-depth study of prevalence of *E. coli* infection in causation of poultry sufferings in and around Patna. The purpose of this study also aimed to investigate the antimicrobial resistance profile of *E. coli* isolated from poultry samples.

The samples were collected from dead birds of different age groups from different farms of Patna, Bihar and its vicinity. A total of 500 birds were necropsied and based on the gross lesions like fibrinous pericarditis and perihepatitis, opaque air sacs, whitish adherent pericardial sac, congestion and haemorrhagic lesions in the intestinal tract, a total of 200 samples suspected for colibacillosis infection were collected and processed for isolation, identification and their biochemical characterization. Single colony of *E. coli* was picked from agar plate and stained with Gram's stain and their morphological characteristics were examined using microscopy. Isolated colonies were subjected to different biochemical tests like indole test, methyl red test, Voges-Proskauer test, citrate utilization test, TSI etc. using strip tests of standard company. Biochemically confirmed isolates were further tested for antibiotic sensitivity test by disc diffusion method using a set of antibiotics. The antibiotics in study were selected after questionnaire

How to cite this article : Vatsa, A., Kumar, S., Kumar, K. and Kumar, S. 2025. Prevalence, isolation and antibiogram study of pathogenic *E. coli* from poultry farms in and around Patna. Indian J. Vet. Pathol., 49(3) : 246-249.

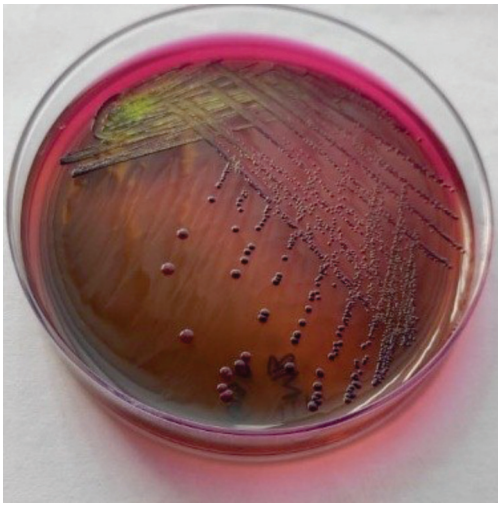

Fig. 1. Showing the characteristic colony of *E. coli* (metallic sheen appearance) in EMB Culture media.

from the poultry owner and practitioners. The zone of inhibition was measured using standard method and results were interpreted as per guidelines and recommendations of CLSI.

All the data collected were statistically analysed using one-way ANOVA and Duncan's multiple range test with the help of software IBM SPSS version 20-bit 32 as per method of[5].

All the 200 samples suspected for colibacillosis were allowed for enrichment in MacConkey broth with an overnight incubation at 37^{O}C followed by streaking on the eosin methylene blue (EMB) agar plates. By conventional methods of enrichment and selective plating on EMB, the characteristic colony of *E. coli* (metallic sheen appearance) was obtained from 120 samples (60.0%), after incubation at 37^{O}C for 24 hours (Fig. 1). In the present study, out of 120 isolates of *E. coli,* only 113 (56.5%) of *E. coli* isolates showed the confirm biochemical characteristic reactions (Fig. 2).

The area wise isolation and cultural study of *E. coli* revealed that out of 200 samples analysed for the presence of *E. coli*, 120 (60%) samples harboured *E. coli* that belongs to maximum occurrence in Masaudhi (76%) followed Patna (70.58%), Naubatpur (63.15%), Danapur (58.97%), Bihta (53.84%), Sonpur (50%), Muzaffarpur (45.45%), Hajipur (37.5%) and minimum occurrence at Jahanabad (22.22%).

Previous studies and observation for the isolation of *E. coli* from poultry samples also detailed the varying percentage of prevalence[6] made an effort for isolation of *E. coli* from poultry samples (chicken meat, n = 228 and eggs, n = 24) in Patna and revealed 27.1% of prevalence. Prevalence of 89.4% *E. coli* by processing different poultry samples in Rajasthan[7]. These findings conclude the variation of prevalence in different areas and the present study also signifies it with a range around 22% to 68% prevalence.

The present study also emphasized on the age-wise study of incidence of colibacillosis. Birds of 0-2 weeks of age were found to suffer most with an incidence of 30.57% followed by 3-5 weeks of birds (18.96%), 6-10 weeks of age (8.45%), 11-20 weeks of age (4.87%) and least in >20 weeks of age (4.34%). These findings indicates that there was decrease in the incidence of colibacillosis with the aging of birds (Fig. 3).

However, a number of studies conducted worldwide have also reported a varying percentage in the incidence of colibacillosis. Highest mortality was found in 11-15 days old chicks (93%) as compared to 6-10 days (83.33%) and 1-5 days old chicks (21.42%) reported[8].

The season-wise incidence of colibacillosis in present study revealed that the highest incidence was seen in the monsoon season (34.39%) followed by autumn (26.43%), winter (17.64%), spring season (11.36%) and least in summer season (10.75%). Few attempts had been done to understand the seasonal prevalence of colibacillosis like reported[9] the total prevalence of colibacillosis in broiler flocks was 12.50%. The season wise prevalence showed the highest in spring season (17.86%), followed by winter 14.47%, summer 9.62% and autumn 7.14%. The

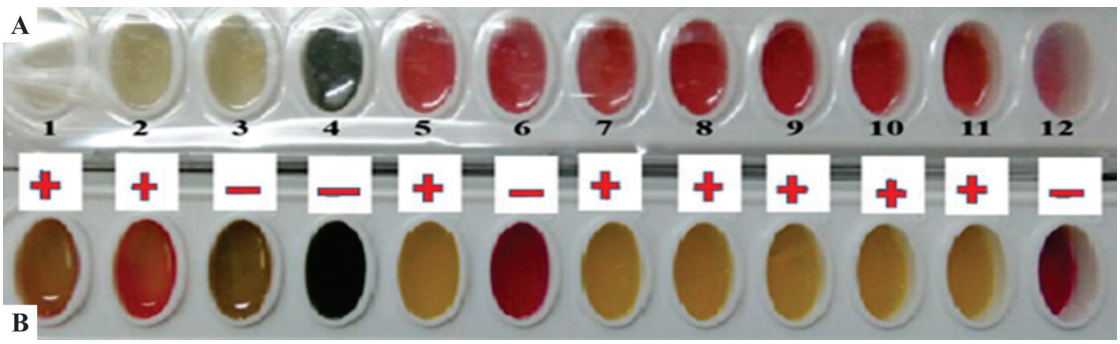

Fig. 2. Showing biochemical characterization of *E. coli* by HiMViC biochemical strip kit. 1: Indole, 2: Methyl red, 3: Voges Proskauer's, 4: Citrate utilization, 5: Glucose, 6: Adonitol, 7: Lactose, 8: Sorbitol, 9: Mannitol, 10: Rhamnose, 11: Sucrose. A: Un-inoculated test kit, B: Test kit inoculated with sample.

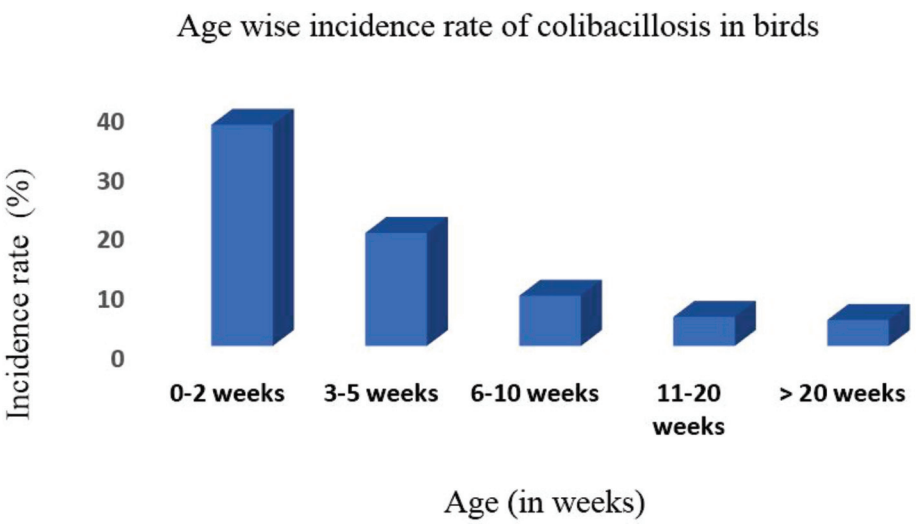

Fig. 3. Bar-diagram showing age-wise incidence of colibacillosis in poultry.

maximum prevalence of colibacillosis in monsoon season and minimum in winter season reported by[10].

Antibiotics are indiscriminately used in poultry rearing as growth stimulants or to treat infectious diseases. The overuse and misuse of antibiotics play a significant role in the emergence and spread of antibiotic-resistant *E. coli*[11]. In this present study, all the biochemically confirmed isolates of *E. coli* were screened for *in-vitro* antibiotic sensitivity profiling by standard disc diffusion assay using the disc of antibiotics *viz.*, tetracycline (30 µg), gentamicin (10 µg), amoxiclav (20/10 µg), cephalexin (30 µg), cefpodoxime (10 µg), ciprofloxacin (5 µg) and ceftriaxone (30 µg) (Fig. 4). The *E. coli* isolates showing zone of inhibition ≤ 11 mm for tetracycline (30 µg), ≤ 12 mm for gentamicin (10 µg), ≤ 13 mm for amoxiclav (20/10 µg), ≤ 18 mm for cephalexin (30 µg), ≤ 17 mm for cefpodoxime (10 µg), ≤ 15 mm for ciprofloxacin (5 µg) and ≤ 13 mm for ceftriaxone (30 µg) were considered to be resistant whereas zone of inhibition ≥ 19 mm, ≥ 15mm, ≥ 18 mm, ≥ 21 mm, ≥ 21 mm, ≥ 21 mm, ≥ 21 mm for respective antibiotics were considered to be susceptible. The range lies in between these were considered as intermediate.

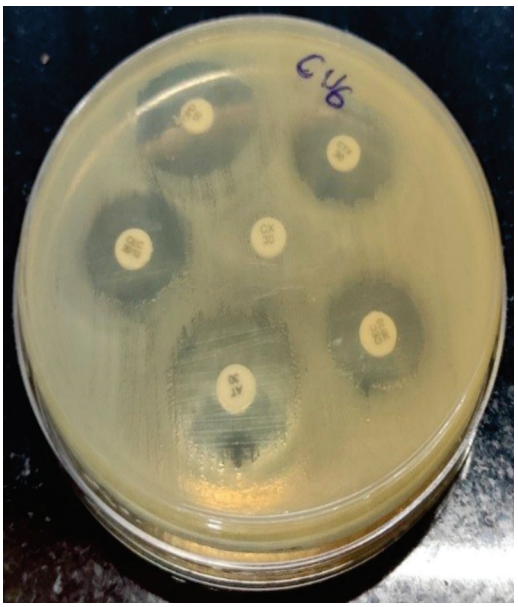

Fig. 4. Antibiotics sensitivity testing of *E. coli* on Mueller Hinton Agar.

The present study revealed that the tetracycline showed the maximum resistant (83.33%) towards *E. coli* followed by ciprofloxacin (76.1%), amoxiclav (52.21%), gentamicin (50.44%), cefpodoxime (43.36%), ceftriaxone (31.85%) and cephalexin (29.02%) showed the least (Fig. 5). However, a number of studies and analysis had reported different resistance of antibiotics against *E. coli*. *E. coli* showed the highest resistance[12] to sulfamethoxazole-trimethoprim (71%), tetracycline (63%), ampicillin (62%), where gentamicin (23%) showed the lowest resistance, followed by ceftriaxone (26%). Reports of[6] showed the maximum resistance of the isolates against cefuroxime (89.1%) and penicillin (89.4%), followed by ampicillin (80.43%), vancomycin (74.1%), co-trimoxazole (73.1%), cephalothin (60.8%), ceftriaxone (28.2%), tetracycline (17.4%), gentamicin (13%), amikacin (13.04%), ofloxacin (13%) and ciprofloxacin (6.5%).

In a study[13] on 30 isolates (94%) showed resistance to more than one antibiotics with percentage resistance were tetracycline 81%, sulphamethoxazole 67%, streptomycin 56%, trimethoprim 47%, ciprofloxacin 42%, ampicillin 36%, spectinomycin 28%, nalidixic acid 25%, chloramphenicol 22%, neomycin 14%, gentamicin 8%, amoxicillin-clavulanate, ceftiofur, cefotaxime, colistin, florfenicol and apramycin 0%.

CONCLUSION

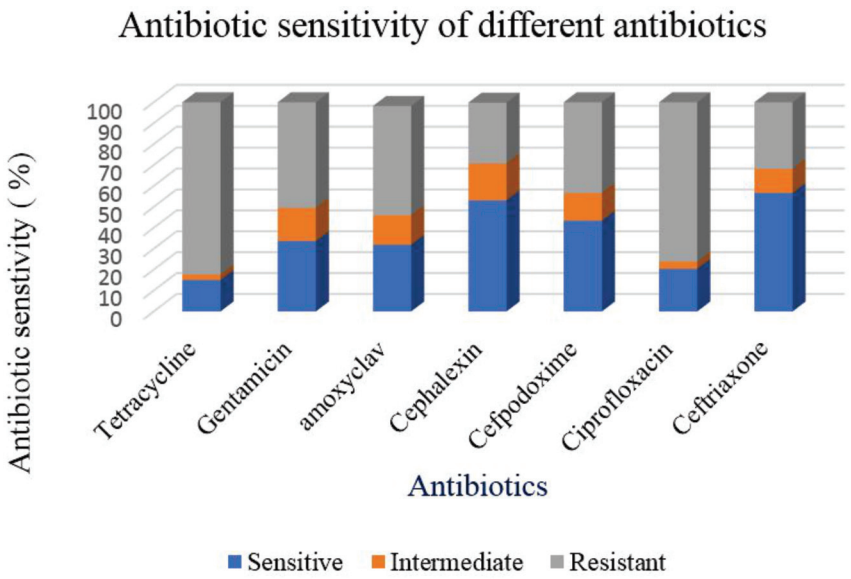

Fig. 5. Showing antibiogram profile of different antibiotics.

From the research findings it could be concluded that the pathology of enteric disease in poultry can be varied as gastro-intestinal tract is being constantly exposed to a wide variety of potentially harmful substances. *E. coli* is the prominent and commonest bacteria affecting GIT with various manifestations and cephalexin can be drug of choice against prevailing strain of *E. coli*. The findings of the present study suggest that being the most exposed surface for the pathogens, gastrointestinal system demands a major emphasis from the poultry farm owners considering for their shake of higher production and economic stabilization. Among one of the major poultry diseases, colibacillosis is of major concern so disease control authority should prioritize the disease burden and plan their efficient control strategies for the poultry farm owners to orchestrate their disease management protocol. As antibiotic resistance potentiates the pathogens to create serious animal as well as human health implications so it is of major concern.

ACKNOWLEDGEMENTS

The authors would like to thank the Dean, Bihar Veterinary College, BASU, Patna, Bihar for providing the necessary facilities for this research work.

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Indian J. Vet. Pathol., 49(3) : 250-253, 2025: DOI: https://doi.org/10.56093/8ky8qx64

SHORT COMMUNICATION

Cytological diagnosis of seminoma in dog

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Received: 7.5.2025; Accepted: 10.6.2025

ABSTRACT

A 10-year-old intact male Spitz dog was presented at Sanchu Animal Hospital, Chennai for diagnosis and treatment with the history of unilateral scrotal swelling since few months. Physical examination of the affected dog showed a marked enlargement of left testis with peeling of skin due to frequent licking. On palpation of affected testis, mild pain was evinced. No enlargement of regional lymphnodes was noticed. Hematobiochemical studies revealed mild anemia, an increase in serum total protein and globulin and a decrease in A/G ratio. Cytological examination of the aspirated smears from the affected testis was suggestive of seminoma which revealed moderate cellularity with cells being discrete, round to ovoid and exhibiting moderate pleomorphism. The cytoplasm was homogenous and slightly basophilic containing prominent large spherical to ovoid nuclei with coarsely reticular chromatin. In many areas, binucleate and multinucleate cells were present. In addition, mitotic figures were also seen in some areas. Based on the cytological examination, the mass was identified as seminoma.

Keywords: Cytology, haematobiochemical studies, seminoma, spitz

Tumours affecting the genital system of dogs include testicular tumors (sertoli cell, interstitial cell, seminoma), vaginal tumors (leiomyoma, fibroleiomyoma, fibroma) and the transmissible venereal tumor (TVT). Of all canine male genital tumours, testicular tumours represent more than 90 per cent and dogs record the highest incidence of all animal species. Among various testicular tumours in dogs, seminoma, which results from neoplastic transformation of germ cells of testes is one of most common tumours encountered in aged male dogs. Although seminoma exhibits malignant evidence like intravascular invasion on histological examination, the biological behavior of this tumour has been found to be mostly benign to less malignant with rare metastases[1,2,3,4].

The dogs with cryptorchid testes have been found be at an increased risk and 15 times more prone to this tumour development[5,6]. The mean age of the affected dogs was observed to be 10 years[7]. Among various breeds affected, Golden Retriever, English Cocker Spaniel, Sheltie, Collie, Boxer, German Shepherd, Fox Terriers, Afghan Hounds and Norwegian Elkhounds have been found to be more susceptible than mixed breed dogs. Exposure to environment contaminated with pesticides and chemicals such as diethylhexyl phthalate (DEHP) and polychlorinated biphenyl 153 (PCB153) could also pose an increased risk to this tumour development[8]. The main clinical finding is enlargement of testicles in affected animals. However this enlargement may not be readily apparent in case of cryptorchid dogs which show no clinical signs[7]. Feminization syndrome, prostatitis, prostrate hyperplasia and perianal adenoma have also been recorded in some dogs affected with seminoma[9]. Although adequate reports are available on incidence of seminoma in dogs, records pertaining to cytological diagnosis are scanty. Hence the present study reports a case of seminoma which was confirmed cytologically.

A 10-year-old intact male Spitz dog was presented at Sanchu animal hospital, Chennai for diagnosis and treatment with the history of unilateral scrotal swelling since few months. A thorough physical examination was carried out on affected animal. Blood samples were collected for hematobiochemical studies. For hematological studies, blood samples were collected in vacutainers containing EDTA as anticoagulant while for biochemical estimations, serum was separated from the clotted blood samples after centrifugation @ 3000 rpm for five minutes. In addition, fine needle aspiration smears prepared from the affected testis were air dried and stained with Leishman and Giemsa cocktail stain for cytological examination[10].

How to cite this article : Ramesh, S., Vijayendran, V., Balakumar, A. and Kumar, V. 2025. Cytological diagnosis of seminoma in dog. Indian J. Vet. Pathol., 49(3) : 250-253.

Physical examination of the affected dog showed a marked enlargement of left testis with peeling of skin covering the testis due to frequent licking. On palpation of the affected testis, mild pain was evinced. No enlargement of regional lymphnodes was noticed. Hematological studies showed no abnormal changes except mild anemia while biochemical estimations revealed an increase in serum total protein and globulin and a decrease in A/G

ratio (Table 1 and 2). Cytological examination of the aspirated smears from the affected testis was suggestive of seminoma which revealed moderate cellularity with cells being discrete, round to ovoid and exhibiting moderate pleomorphism. The cytoplasm was sparse to moderate in amount and showed mild basophilia. The nuclei were prominent, large and spherical to ovoid in shape with coarsely reticular chromatin. In many areas, binucleate and multinucleate cells were present. In addition, mitotic figures were seen in some areas (Fig. 1-6). In few areas, lymphocytic and neutrophilic infiltrations were also observed. Based on the cytological findings, the mass was identified as seminoma.

The clinical findings namely unilateral involvement of testis and non involvement of regional lymphnodes noticed in the present case correlate well with that of previous worker[3] who also observed similar findings in a nine-year-old boxer dog. The involvement of left testis in the present study was also reported in a 10 years old golden retriever affected with seminoma[11]. In contrast to the present findings, an increased incidence of seminoma affecting right testicle has been frequently reported[3,9,12,13]. Testicular enlargement which is the major feature of seminomas is usually unilateral but occasionally bilateral[9]. However, fifty percent of dogs affected with testicular tumours revealed bilateral involvement[14].

The present findings of mild anemia with normal leukocyte and platelet counts were also reported in a nine year old male boxer affected with seminoma[3]. However, they recorded no abnormal findings in serum biochemical parameters.

Table 1. Haematobiochemical parameters of the dog affected with seminoma.

S.No.	Parameters	Values	Reference range[16]
1.	Haemoglobin (g/dL)	11.80	11.90-18.90
2.	PCV (%)	36.10	35-57
3.	RBC (millions/cumm)	5.69	4.95-7.87
4.	MCV (fL)	63.60	66-77
5.	MCHC (g/dL)	32.6	21.0-26.2
6.	MCH (pg)	20.70	32.0-36.3
7.	Total leukocyte count (x10^3/mcL)	12.5	5-14.1
8.	Differential leukocyte count (%)		
	Neutrophil	71	58-85
	Lymphocyte	20	8-21
	Monocyte	5	2-10
	Eosinophil	4	0-9
9.	Thrombocyte count (x10^3/mcL)	250	211-621
10.	BUN (mg/dL)	28.70	8-28
11.	Creatinine (mg/dL)	1.65	0.5-1.7
12.	BUN/Cr ratio	17.40	10-20
13.	Glucose (mg/dL)	78	76-119
14.	Phosphorus (mg/dL)	5.1	2.9-5.3
15.	Total bilirubin (mg/dL)	0.3	0-0.3
16.	Calcium (mg/dL)	9.20	9-11
17.	GGT (IU/L)	7.00	5-14
18.	ALT (IU/L)	30	10-109
19.	ALP (IU/L)	58	1-114
20.	Total protein (g/L)	11	5.4-7.5
21.	Albumin (g/L)	2.60	2.3-3.1
22.	Globulin (mg/dL)	8.4	2.7-4.4
23.	A/G ratio	0.30	
24.	Total cholesterol (mg/dL)	212	135-278

The cytological findings noticed in the present study were also recorded in a nine-year-old seminoma affected boxer dog[3], were in the tumour revealed moderate cellularity with cells of varying sizes and varying amount of bluish cytoplasm. Nuclei were large, round to oval and hyperchromic with fine to coarse chromatin containing prominent multiple nucleoli and mitotic figures as seen in the present study.

Aspiration smears from the mass of seminoma generally contains moderate to large number of neoplastic cells of varying sizes and varying amount of lightly basophilic and homogenous cytoplasm. The nuclei may be multiple and homogenous to finely reticular containing relatively large nucleoli with mitotic figures[9].

Cytological smears of seminoma often reveal large number of lysed cells and free nuclei. The neoplastic cells appear large, round and either discrete or in small aggregates. The nuclei may also appear large and round with reticular to coarse chromatin and prominent nucleoli. In addition moderate anisocytosis, anisokaryosis, binucleation, multinucleation and aberrant mitoses may also be observed. The cytoplasm may exhibit mild to moderate basophilia with rare vacuolations. Nuclear cytoplasmic ratio may be moderate to high. Small lymphocytes are frequently observed. The presence of lacy granular eosinophilic material giving a tigroid appearance may be occasionally seen[15]. In addition mitotic figures are frequently seen. Granular lacy eosinophilic background giving a tigroid appearance along with lymphocytes and aypical mitoses are diagnostic features of seminomas[9].

Financial support & sponsorship: None

Conflicts of Interest: None

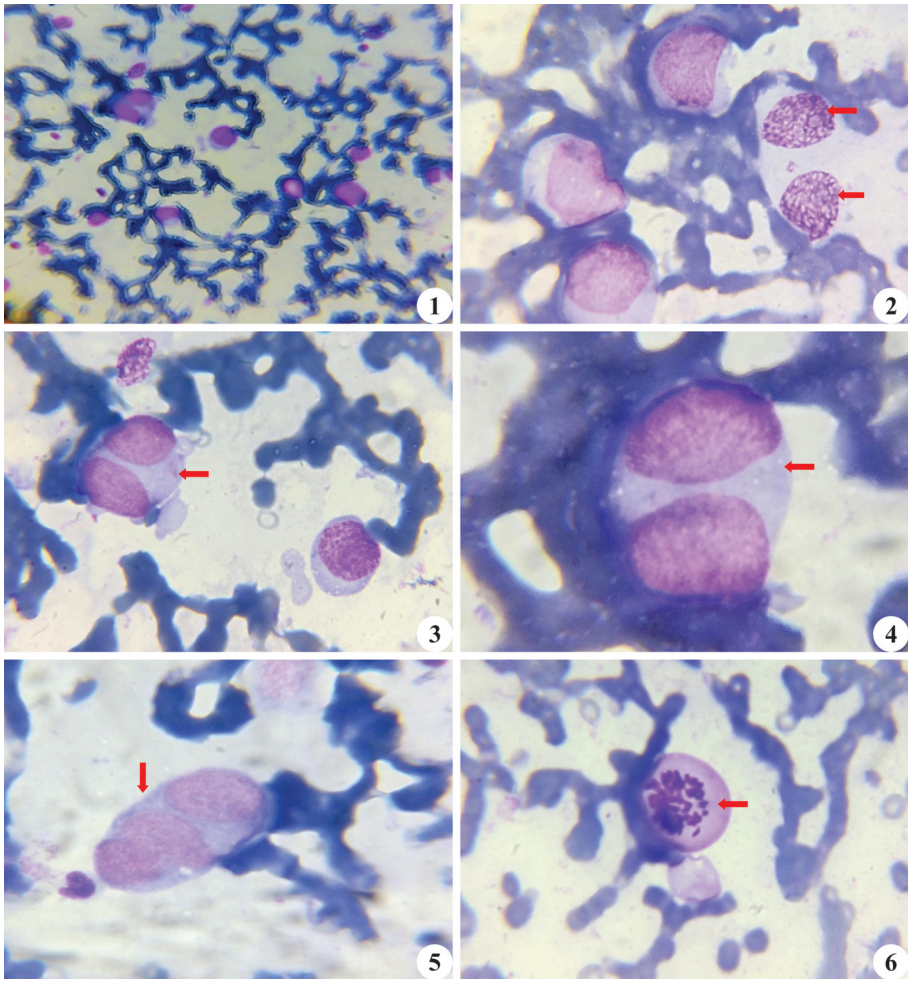

Fig. 1 & 2. Seminoma: Moderate cellularity with discrete, round to ovoid cells exhibiting moderate pleomorphism (FNAC - L&G staining x200); **Fig. 3 & 4.** Binucleate cell with coarse chromatin and varying amount of lightly basophilic homogenous cytoplasm (FNAC - L&G staining x1000); **Fig. 5.** Multinucleate cell with coarse chromatin and prominent nucleoli (FNAC - L&G staining x1000); **Fig. 6.** Seminoma neoplastic cells-mitotic figure (L&G staining x1000).

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 49(3) : 254-257, 2025: DOI: https://doi.org/10.56093/xs22z704

CASE REPORT

Postpartum metritis and septicemia in an adult cross-breed pig: Aetiopathology and antibiotic sensitivity pattern

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Received: 26.3.2025; Accepted: 6.5.2025

ABSTRACT

The adult cross-breed pig had a history of retention of placenta after farrowing and discharge of purulent materials from vagina. At necropsy, epicardial hemorrhage, hemorrhagic lymph nodes, congestion of visceral organs, gastric and caecal mucosa were observed. The uterus was thickened, highly congested and hemorrhagic with presence of necrotic materials in the endometrium. Microscopically, vascular changes in different organs, fibrin thrombi in lymph node, multiple follicular cysts in ovary, severe vascular and inflammatory changes in endometrium and myometrium, meningitis, leucocyte margination and perivascular edemas in brain were observed. *Escherichia coli* was isolated from heart blood. The isolate was sensitive to ofloxacin, ciprofloxacin, chloramphenicol, levofloxacin, meropenem, ceftriaxone, amikacin, oripenem, ceftazidime/clavulanate. Based on pathology and bacteriology, the case was diagnosed as post-partum metritis and septicemia associated with *E. coli*.

Keywords: Antibiotic sensitivity, *Escherichia coli*, pathology, pig, post-partum metritis, septicemia

Infection and inflammation of reproductive organs are leading cause of infertility in domestic animal species[1]. Post-partum and post mating uterine infections occur most commonly in domestic species. Post-partum uterine infection is a significant problem in pig breeding industry causing heavy economic losses by affecting health of sows and by reducing reproductive performance leading to early superannuation from the herd. After parturition, anatomic barriers to infection and reproductive tract compartmentalization is temporarily lost[1]. Uterine contamination occurs due to poor hygiene, prolonged parturition, obstetrical intervention and retained placenta[1,2]. Presence of epithelial tissue debris and fluids during postpartum period creates a suitable environment for bacterial growth. In pigs, uterine infection is caused by many pathogens like virus, bacteria, parasite, fungal toxin and other factors[3]. *E. coli*, *Truperella pyogens*, *Lactobacillus spp.*, *Biofido bacterium*, *Proteus vulgaris* were most common identified pathogens in sow endometritis[4]. *E. coli* is responsible for early puerperal endometritis[5]. Postpartum uterine infection can cause death of animals by causing severe sepsis. Limited literature is available on pathology of swine postpartum metritis. Therefore, the present study is intended to study bacteriology, antibiotic sensitivity and pathology of postpartum metritis and septicemia in an adult crossbreed pig.

One cross-breed adult female pig was submitted to post mortem facility, ICAR-IVRI, Izatnagar with history of farrowing, retention of placenta and discharge of purulent materials from vagina and the animal was under treatment. Detailed necropsy was conducted on the dead animal and gross lesions were recorded. Heart blood samples are collected aseptically in a sterile syringe and stored at -4^{O}C for bacteriological work. Tissue samples were collected from different organs in 10% neutral buffer formalin for routine histopathological processing and Hematoxylin and Eosin staining[6]. For bacteriological examination, heart blood samples stored at -4^{O}C were processed on brain heart infusion agar and MacConkey Agar and incubated at 37^{O}C for 24-48 hours and bacterial smear was stained with Gram staining[7]. The antibiotic sensitivity test (ABST) was carried out in Mueller Hinton agar using disc diffusion method as per standard procedures described earlier[8].

How to cite this article : Das, T., Beena, V., Pathak, M., Sethi, M., Vidhyrani, H.B., John, J.K., Sahoo, M., Dash, B., Chavan, A.S., Das, N.K. and Saikumar, G. 2025. Postpartum metritis and septicemia in an adult cross-breed pig: Aetiopathology and antibiotic sensitivity pattern. Indian J. Vet. Pathol., 49(3) : 254-257.

The nutritional status of the animal was good. The perianal region was soiled with dried bloody discharges (Fig. 1). Petechiae to ecchymosis hemorrhages were present on the epicardium of the ventricles and auricles (Fig. 2). The tonsil, lung, liver and kidneys were congested. Gastric mucosa was highly congested. In the small intestine, watery catarrhal content was present. The caecal mucosa was highly congested with presence of semisolid content in the lumen.

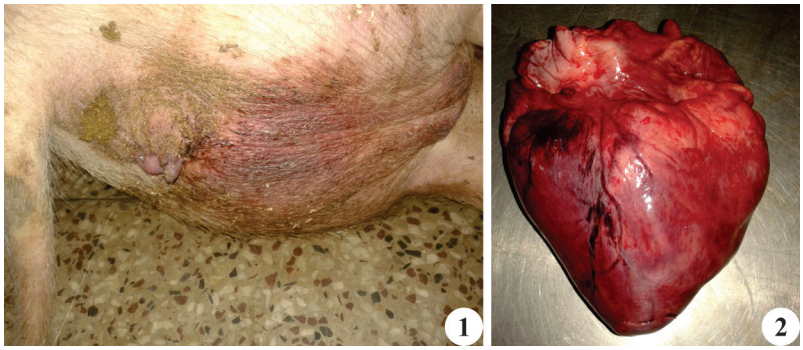

Fig. 1. Soiling of perianal region with dried bloody vaginal discharge; **Fig. 2.** Petechiae to ecchymosis hemorrhages on epicardium.

The lymph nodes were hemorrhagic. The uterus wall was thickened, highly congested and hemorrhagic with presence of necrotic materials in the endometrium (Fig. 3 & 4). The meningeal blood vessels of brain were highly congested.

Histopathology of lungs revealed highly congested blood vessels with presence of red blood cells in the lumen of alveoli and bronchiole and hemosiderin laden macrophages in the parenchyma (Fig. 5). Some alveoli were edematous and filled with fibrin. Bronchiolar lumen was

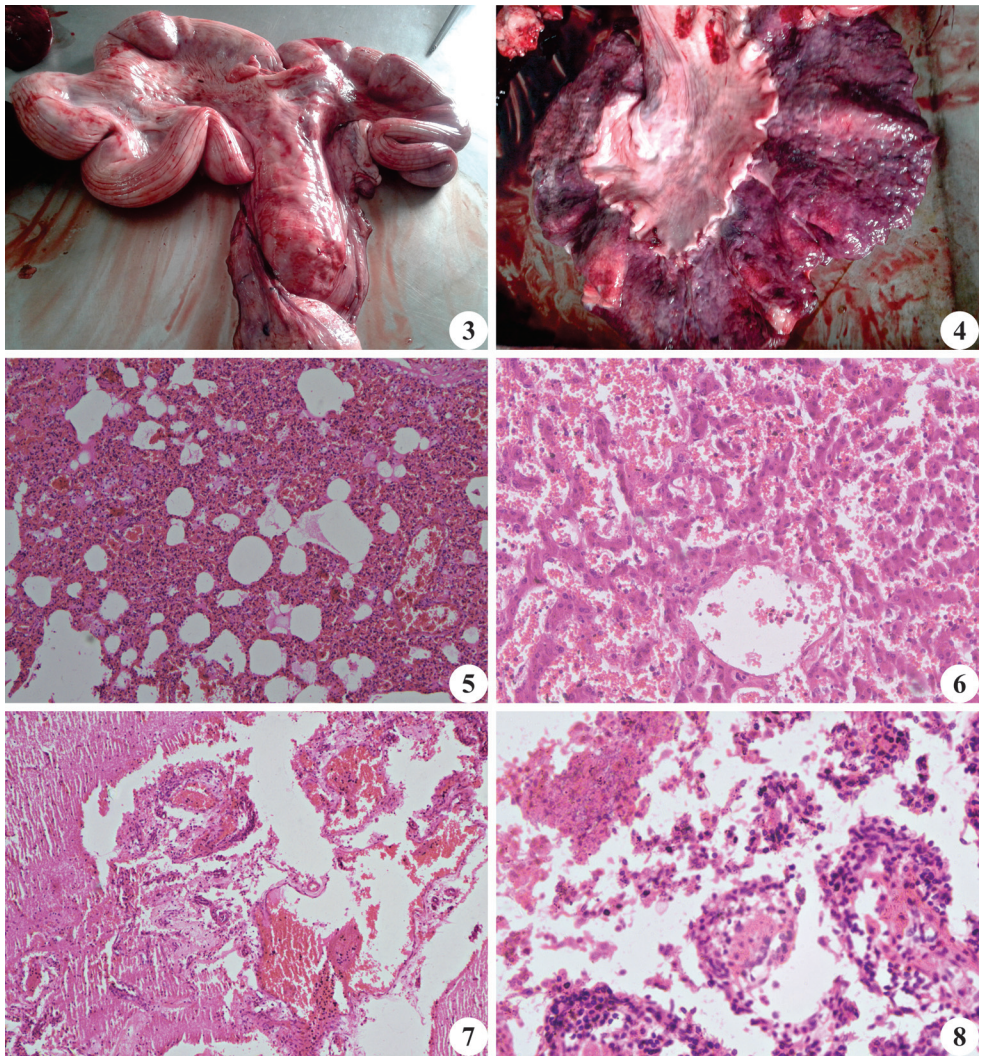

Fig. 3 & 4. Highly congested, thickened and hemorrhagic uterus; **Fig. 5.** Photomicrograph of lung showing hemorrhage, congestion and oedema in lungs (H&E staining x100); **Fig. 6.** Photomicrograph of liver showing highly congested hepatic sinusoidson (H&E staining x200); **Fig. 7.** Photomicrograph of brain showing severe meningitis (H&E staining x100); **Fig. 8.** Photomicrograph of intestine showing necrosis and sloughing off epithelial cells into the lumen, infiltration of inflammatory cells in the lamina propria (H&E staining x400).

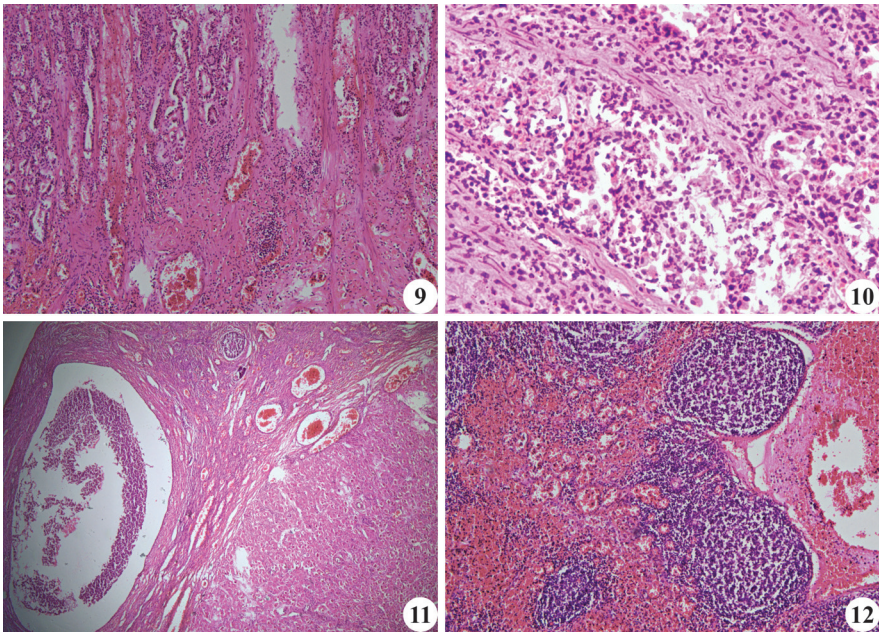

Fig. 9. Photomicrograph of uterus showing severe vascular congestion, necrosis and heavy infiltration of inflammatory cells (H&E staining x100); **Fig. 10.** Photomicrograph of uterus showing necrosis and heavy infiltration of inflammatory cells (H&E staining x200); **Fig. 11.** Photomicrograph of ovary showing cystic follicle (H&E staining x40); **Fig. 12.** Photomicrograph showing hemorrhagic lymph node and fibrin thrombi inside blood vessel (H&E staining x100).

partially blocked by shed epithelial cells. Hemorrhages were present in the myocardium with increased gap among cardiac muscle cells with prominent fibroblasts. The capillaries of the kidney were highly congested with cellular swelling and degeneration of tubular epithelial cells. In liver, severe congestion of hepatic sinusoids and interlobular blood vessels, hemorrhages, disorganization of hepatic cords were noticed with presence of hemosiderin laden Kupffer cells (Fig. 6). In cerebrum, meningitis, capillary congestion, margination of leucocytes, gliosis, perivascular edema with some eosinophilic neurons were observed (Fig. 7). In intestine, severe congestion, necrosis and sloughing off epithelial cells into the lumen and infiltration of inflammatory cells mainly neutrophils in the lamina propria were observed (Fig. 8). In the endometrium of uterus, severe vascular congestion and extensive hemorrhage, necrosis of endometrial gland, heavy infiltration of inflammatory cells mainly neutrophils in the surface epithelium and lamina propria were observed (Fig. 9 & 10). In myometrium, vascular congestion, hemorrhage and accumulation of perivascular mononuclear cells were observed. The ovarian blood vessels were highly congested with presence of multiple cystic follicle lacking ova and diminished granulosa cells thickness, corpus luteum and different developmental stages of follicles (Fig. 11). The retention of CL may be due to impaired transfer of PGF2 alpha from endometrium to ovary due to postpartum metritis. In sows, presence of multiple follicular cysts are common, usually interfering with normal reproductive cycle. In spleen and lymph nodes, severe congestion, hemorrhages, fibrin thrombi in blood vessels and severe lymphoid necrosis with accumulation of neutrophils were observed (Fig. 12).

The bacteria produced lactose fermenting bright pink, moist and circular colonies on MacConkey agar. Microscopic examination of Gram stained slide smear of culture revealed presence of pink colored gram negative bacilli. Based on colony morphology, biochemical tests and Gram's staining, the isolated bacteria was identified as *E. coli*. On ABST, the bacteria were found to be sensitive to ofloxacin, ciprofloxacin, chloramphenicol, levofloxacin, meropenem, ceftriaxone, amikacin, oripenem, ceftazidime/clavulanate; intermediate sensitive to tetracycline, pefloxacin, cefepime, erythromycin, cefoperazone, gentamycin, estapenem, piperacillin/tazobactum and resistant to ampicillin, methicillin, amoxiclav, sulphadiazine, cotrimoxazole and trimethoprim.

Metritis in sow mostly occurs 24-48 hours of post parturition. In the present case, the death of sow occurred after 24-36 hours of farrowing and retention of placenta was the one of the major risk factor associated with postpartum metritis. Prolonged farrowing and impaired placenta expulsion are reported to increase the chances of postpartum metritis[2]. Prolonged farrowing causes energy and electrolyte imbalance and impairs sow immunity. In addition, shortage of oxytocin level hinders expulsion of fetal membrane and other inflammatory material resulting in profound bacterial growth[9]. Poor hygiene, prolonged parturition, obstetrical intervention, large

litter size, number of dead born fetuses per litter and retained placenta are recorded as major cause of uterine contamination in the previous study[1,9]. In the current study, the observed gross lesions were hemorrhages on the epicardium and lymphnodes; moderate to severe congestion of tonsil, lung, liver, kidneys, meningeal blood vessels, gastric and caecal mucosa; thickening, congestion and hemorrhages in the uterus. In a previous report, a case of bacterial metritis and valvular endocarditis caused by *Actinobacillus equuli* was described in a gilt with secondary septicemia in United States[10]. In another case study, post-partum metritis in 4 years old Duroc with vulvar discharge associated with retention of macerated foetus was described[11]. Distention of uterus with purulent fluid mixed with necrotic materials on necropsy and microscopically, edema, bacterial infiltration and purulent exudates in uterine wall were reported in sow metritis[12,13]. In a production sow with nonpuerperal chronic endometritis and pyometra, histological lesions like dilation of endometrial glands, submucosal infiltration of inflammatory cells and vascular congestion in submucosa and myometrium, presence of multiple corpus luteum and albicans in ovaries, gastritis and nephritis were described[13]. In our study bacteriological examination of heart blood collected aseptically revealed presence of *E. coli*. *Truperellapyogens*, *Lactobacillus spp.*, *Biofidobacterium*, *Proteus vulgaris* were reported to bethe most common identified pathogens in sow endometritis[4]. *E. coli* and *Staphylococcus* were also listed as commonly isolated pathogen associated with septicemia and death in animals and birds in Northern India[14]. In other study, bacteria like *E. coli*, *Staphylococcus* and *Proteus* etc. were detected in the vaginal discharges of sow suffering from postpartum metritis and the isolated *E. coli* was sensitive to ofloxacin, streptomycin and gentamicin and were resistant to nitrofurantoin, augmentin, chloramphenicol, ciprofloxacin, rocephin and ampiclox[11]. In the present case, *E. coli* isolate was resistant to ampicillin, amoxiclav, methicillin, vancomycin, sulphadiazine, cotrimoxazole and trimethoprim. ABST assay was used as a guide by the associated pig farm to select suitable antibiotics for similar cases in future and there was no report of further death in postpartum sows in that pig farm. Antimicrobial resistance (AMR) is a major global health threat. Overuse and misuse or unregulated use of antibiotics in veterinary and public health care system and agricultural industry give rise to emergence of antimicrobial resistance. Accurate selection of antibiotics after antibiotic sensitivity test and proper use of antibiotics are crucial to combat AMR. Isolation of pathogens from post mortem cases and determination of their antibiotic sensitivity pattern are helpful in formulating effective treatment regimen for similar cases in future and helpful in proper understanding of antibiotic sensitive and resistance pattern of isolated pathogens.

Postpartum is a very crucial period in animals. Postpartum uterine infection is very common in sows. Postpartum metritis causes delay in uterine involution and can cause sow mortality from severe sepsis. Proper care and treatment of sows during and after farrowing will reduce the incidence of postpartum infection and sow mortality. Identification of pathogens and their antimicrobial sensitivity to different antibiotics are very essential for choosing an effective treatment regimen.

ACKNOWLEDGEMENT

The authors wish to acknowledge the Director, ICAR -IVRI for providing necessary facility to carry out this research work.

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Indian J. Vet. Pathol., 49(3) : 258-261, 2025: DOI: https://doi.org/10.56093/xyjees24

CASE REPORT

Cytological diagnosis of canine cutaneous mast cell tumour with nodal metastasis

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Received: 29.1.2025; Accepted: 21.5.2025

ABSTRACT

A thirteen year old male mongrel dog was presented at Sanchu animal hospital, Palavakkam, Chennai for treatment of two months old wound infected with maggots in the interdigital space of the left forelimb. Physical examination of the animal revealed the presence of a large ulcerated wound infested with maggots and a foul smelling discharge from the interdigital space between 3rd and 4th digit of the left forelimb. In addition, left prescapular lymphnode showed marked enlargement. Radiological examination of the affected limb revealed complete osteolytic lesions in the distal phalanges (P2 and P3) of the 4th digit, while no abnormalities were noticed in thorax and abdomen. After five days of treatment with ivermectin and antibiotics, maggots disappeared completely leaving an ulcerated nodule with soft consistency and measuring 4 cm in diameter. Impression smears from the site showed presence of numerous discrete round cells having moderate amounts of pale basophilic cytoplasm with varying numbers of characteristic metachromatic granules, suggestive of mast cell tumour (MCT). Fine needle aspirates from enlarged lymphnode also showed clusters of neoplastic cells comprised of mast cells along with numerous neutrophils, confirming its metastasis. Based on the laboratory findings, diagnosis was confirmed as cutaneous MCT with nodal metastasis (stage II). Thus, the present paper reports the cytological findings of cutaneous MCT with lymph node metastasis in a mongrel dog.

Keywords: Cytology, mast cell tumour, mongrel dog, node metastasis

Mast cell tumors (MCT) resulting from uncontrolled proliferation of mast cells of hematopoietic origin have been reported in both domestic and wild animals, including dogs, cats, horses, cattle, pigs, ferrets, wild dogs, lion, jaguar, tiger, squirrel, hedgehog and walrus. Among the animal species affected, MCT is more common in dogs followed by cats and is less frequent in other species[1-3]. In dogs, it ranks second among the most frequently diagnosed cutaneous malignant tumours[4]. It is highly invasive and metastatic, comprising 16 to 21 percent of all skin tumors encountered[5] and its biologic behavior is highly variable, ranging from solitary benign to highly malignant multiple masses which are potentially fatal[6]. It can be focal or multicentric affecting the skin and may occasionally spread to the distant sites such as spleen, liver, intestines and bone marrow[1]. The cutaneous forms are mostly located on the hind limbs, abdomen, perineum and scrotum. The tumours affecting preputial, inguinal and sub inguinal regions and other mucocutaneous sites tend to be more aggressive[7]. The tumours which grow slowly and remain localized for a long duration carry a better prognosis. In contrast, rapidly growing tumours with high infiltrating behaviour usually indicate a poor prognosis[8]. Among various breeds affected, Boxers, Boston terriers, Beagles and Labrador retrievers are more prone to MCTs. Malignant transformation of mast cells resulting from mutations in the c-kit tyrosine kinase receptor has been thought to be responsible for the malignant transformation in these breeds. Regarding age affected, middle-aged to older dogs are prone to MCTs. However, younger dogs which are less than three weeks old may also get affected. In dogs, gender predilection has not been reported for MCT[9,10].

Approximately 50 percent of the MCT affected dogs with normal or palpable regional lymphnodes showed either early metastatic or overtly metastatic disease, while the remaining 50 percent revealed lymphnodes with no evidence of metastasis or minimal metastasis[11]. Hence, cytologic evaluation of the regional lymphnodes is advocated for confirming the metastatic involvement and staging of MCTs. Based on the cytological findings, five categories associated with increasing risk of malignancy have been proposed namely normal lymphnodes, hyperplastic lymphnodes, possible, probable and certain metastasis based on the number of mast cells and the number and size of mast cell aggregates[12]. For localized MCT, surgery is advised as the best choice of treatment while for disseminated, non-resectable and high-grade tumours, chemotherapy is recommended[13]. Though numerous reports are available on the incidence in

How to cite this article : Ramesh, S., Kumar, S.D., Harish, L.J., Balakumar, A., Preetha, S. and Kumar, V. 2025. Cytological diagnosis of canine cutaneous mast cell tumour with nodal metastasis. Indian J. Vet. Pathol., 49(3) : 258-261.

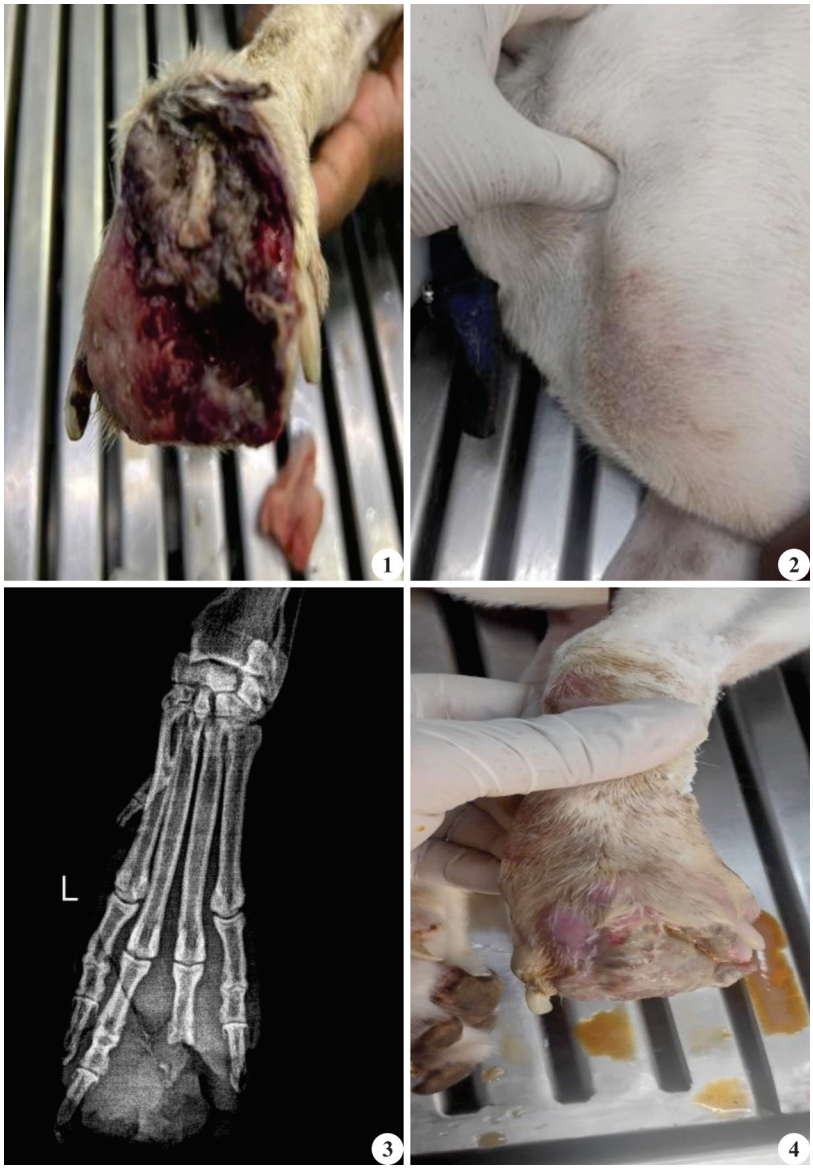

Fig. 1. An ulcerated wound infested with maggots in the interdigital space of the left forelimb; **Fig. 2.** Mongrel dog showing enlarged prescapular lymphnode; **Fig. 3.** Mongrel dog affected with MCT-Radiograph showing complete osteolytic lesions of distal phalanges (P2 and P3) of the 4th digit; **Fig. 4.** Mongrel dog showing growth in the interdigital space of the left forelimb.

dogs, records pertaining to cytology of primary tumour with affected regional lymphnodes are scanty. Hence the present paper reports the cytological diagnosis of cutaneous MCT with nodal metastasis in a mongrel dog, presented at Sanchu animal hospital, Chennai.

A thirteen years old male mongrel dog was presented at Sanchu animal hospital, Palavakkam, Chennai for treatment of wound that was two months old and infested with maggots in the interdigital space of the left forelimb. A thorough physical examination was carried out on the affected dog. In addition, radiological investigations were carried out to assess the extent of tissue damage in the affected limb and to rule out thoracic and abdominal lesions. The wound was cleaned and the animal was treated with Inj. ivermectin and oral cefpodoxime. After five days of treatment, impression smears from the ulcerated lesion and fine needle aspirates from the enlarged prescapular lymphnode were collected for cytological diagnosis. The smears were air dried, stained with Leishman and Giemsa cocktail stain and subjected to microscopic examination[14].

On physical examination of the animal when presented on the first day of examination, the interdigital spaces between the third and fourth digits as well as the respective digital pads of the left forelimb were found to be severely necrotic and ulcerated with presence of numerous maggots. The lesions appeared irregular and multinodular, about half the lesions showed blackish

brown discoloration while the remaining half was pinkish with foul smelling discharges (Fig. 1). In addition, massive enlargement of left prescapular lymphnode was noticed (Fig. 2).

Radiological examination of the affected forelimb revealed complete osteolytic lesions in the distal phalanges (P2 and P3) of the 4th digit (Fig. 3), while thorax and abdomen showed no abnormalities. After five days of treatment, the wound appeared as small, nodular, soft in consistency, measuring 4 cm in diameter with pus filled ulcerated surface (Fig. 4) devoid of maggots.

Cytological examination of impression smear collected from the ulcerated mass showed the presence of discrete round cell population with indistinct cellular borders containing moderate amount of cytoplasm. The cytoplasm which was pale basophilic contained numerous fine to coarse metachromatic granules with centrally to eccentrically placed spherical nucleus, suggestive of mast cells (Fig. 5 & 6). Mild anisocytosis and anisokaryosis of the mast cells were also observed. In addition, extracellular granules released from mast cells were also noticed in many areas along with few non-degenerate neutrophils and eosinophils. Cytological examination of lymphnode aspirates also revealed small clusters of neoplastic cells comprising of mast cells, confirming its metastasis (Fig. 7 & 8). In addition, inflammatory cells, chiefly neutrophils along with few bacterial cocci and bacilli were also observed. Based on the present cytological findings, the ulcerated mass was confirmed as mast cell tumour (MCT) with nodal metastasis. The present case of MCT was classified under the clinical stage II, based on clinical staging system for canine MCTs (Table 1) as per World Health Organisation (WHO) (Table 1) which specifies the presence of single mast cell tumour with involvement of a regional lymph-node.

The present gross findings of an ulcerated lesion measuring 4 cm in diameter were in accordance with that of previous workers[15] who also noticed masses measuring 2 to 10 cm diameter with or without ulcerations in 177 dogs affected with MCT. A dark red ulcerated nodular mass was noticed on the cranial aspect of right forearm with the size measuring 0.5 to 0.7 cm in diameter in a six years old pug affected with MCT[16]. A small elevated nodule, whitish in colour, soft in consistency, measuring approximately 1 cm in diameter was noticed on the perianal region of a senile male captive bush dog housed in a zoological garden diagnosed with MCT histologically[3].

The present radiological findings of thorax and abdomen which revealed no abnormalities were also observed in a six years old dog diagnosed with cutaneous MCT[13].

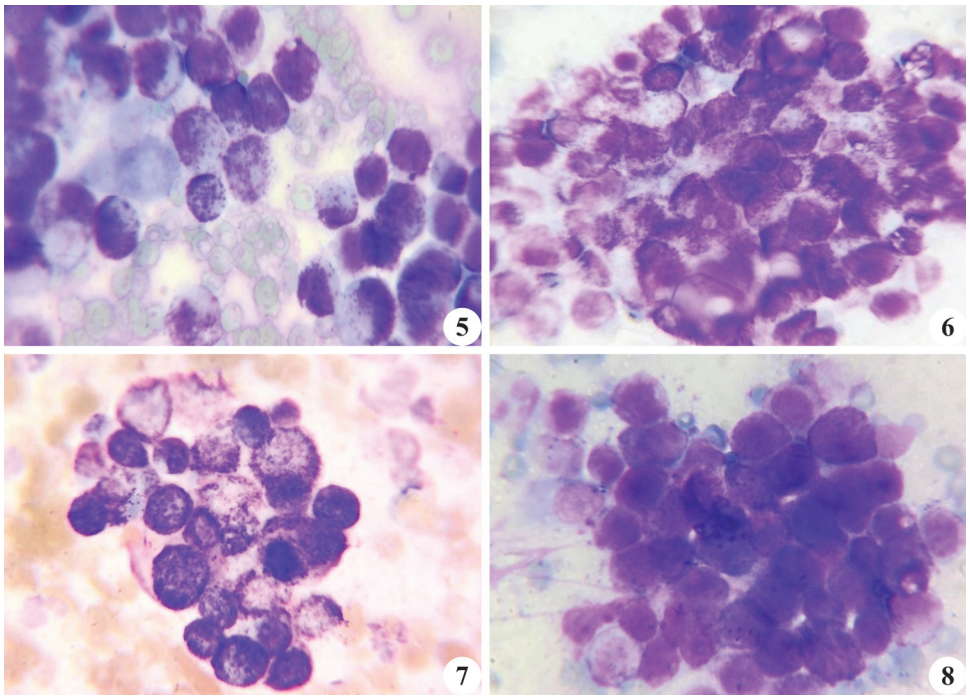

Fig. 5. FNAC from the interdigital mass of affected dog - showing numerous round cells containing spherical nuclei with metachromatic cytoplasmic granules (L&G stain X100); **Fig. 6.** FNAC from the interdigital mass of affected dog - showing numerous round cells containing spherical nuclei with metachromatic cytoplasmic granules (L&G stain X100); **Fig. 7.** FNAC of prescapular LN of MCT affected dog showing a cluster of numerous neoplastic cells comprised of mast cells (L&G stain X100); **Fig. 8.** FNAC of prescapular LN of MCT affected dog - showing a cluster of numerous neoplastic cells comprised of mast cells (L&G stain X100).

Table 1. WHO clinical staging system for canine mast cell tumors.

Stage I	One tumor confined to the dermis, without regional lymph node involvement
Stage II	One tumor confined to the dermis, with regional lymph node involvement
Stage III	Multiple dermal tumors or large infiltrating tumor with or without regional lymph node involvement
Stage IV	Any tumor with distant metastasis or recurrence with metastasis (including blood or bone marrow involvement)

The cytological findings of discrete round neoplastic cells containing metachromatic intracytoplasmic granules with spherical nuclei and presence of extra cellular granules observed in the aspirate collected from the interdigital mass during the present study were in accordance with that of previous workers[15] who also observed similar cytological changes in more than 150 dogs affected with MCT. Similar findings were also reported in an eight years old Pomeranian dog[17] and in a nine years old Dachshund dog affected with MCT[18]. The neoplastic cells were spherical to oval in shape and well differentiated with indistinguishable nuclei and cytoplasm due to the presence of numerous dense purple to pink granules. In addition, some mast cells were stained pale blue due to heavy degree of granulation and lack of stain penetration in the nucleus in the aspirate of cutaneous nodule of a male Frenchbull dog diagnosed with MCT[13].

Cytological findings in the present study of aspirate of enlarged prescapular lymph node confirmed the nodal metastasis and hence present MCT was grouped under stage II. Similar observations have also been documented by earlier researchers as discussed hereunder. Cytological examination of regional lymph node aspirates of 152 dogs affected with mast cell tumour revealed an incidence of 63.8% and 36.2% cases which belonged to stage I and II respectively[12]. However no such nodal metastasis was observed in a six years old dog affected with cutaneous MCT[13]. The presence of nodal metastasis has been considered as a negative prognostic indicator for MCT in dogs[19].

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Indian J. Vet. Pathol., 49(3) : 262-265, 2025: DOI: https://doi.org/10.56093/mw4ym910

CASE REPORT

High-Grade Lymphoblastic Lymphoma Mimicking Burkitt - Like Lymphoma in a Puppy: Case Report

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Received: 25.3.2025; Accepted: 2.5.2025

ABSTRACT

Burkitt-like lymphoma (B-LL) is a rare and aggressive high-grade B-cell malignancy in dogs. Present report describes a case of high-grade lymphoblastic lymphoma in a 2-month-old, non-descriptive male puppy with clinical signs of progressive abdominal distension, emaciation and vomiting. Radiographic, haematological, cytological, histopathological and immunohistochemical evaluations were performed. The neoplastic cells were negative for CD10 and CD20, ruling out classical B-LL. Based on histopathological and immunohistochemical findings, the case was diagnosed as high-grade lymphoblastic lymphoma, likely of T-cell origin. Present case emphasizes the importance of immunephenotyping in diagnosing canine lymphomas and highlights the occurrence of aggressive lymphoid neoplasms in young dogs.

Keywords: Burkitt-like lymphoma, histopathology, immunohistochemistry, Lymphoblastic lymphoma

Neoplasms are a significant health concern in canines, with increasing prevalence due to improved longevity and veterinary diagnostics. Among various tumors, hematopoietic neoplasms are particularly prevalent, accounting for a significant proportion of canine malignancies. Lymphoid neoplasms, especially lymphomas, are among the most frequently diagnosed cancers in dogs, representing approximately 7-24% of all canine neoplasms and 83% of all hematopoietic tumors[1]. Canine lymphomas constitute a heterogeneous group of disorders that differ markedly in their anatomic location, histological grade, immunophenotype (B-cell or T-cell), and clinical behavior, influencing both therapeutic response and prognosis[2].

Lymphoblastic lymphoma (LBL) is a high-grade, aggressive neoplasm arising from immature lymphoid precursors, predominantly affecting young dogs. It is histologically and clinically comparable to Burkitt-like lymphoma in humans, characterized by a diffuse proliferation of medium-sized lymphoblasts with high mitotic index, a "starry-sky" appearance due to interspersed tangible body macrophages, and rapid systemic dissemination[1,3]. In canines, LBL is most commonly of T-cell origin, often presenting with a mediastinal mass, respiratory distress, or signs of thymic involvement. T-cell lymphoblastic lymphomas generally exhibit a more aggressive clinical course and are associated with a poorer prognosis than B-cell variants[1]. Although rare, Burkitt-like lymphomas in dogs are histologically distinct from conventional lymphomas due to their extremely high proliferation rate and potential for extranodal involvement, including bone marrow and central nervous system infiltration[3].

The classification and diagnosis of lymphomas require a multimodal approach, incorporating clinical evaluation, imaging, cytology, histopathology and immunohistochemistry. Immunophenotypic characterization is particularly essential in distinguishing B-lymphoblastic lymphoma (B-LL) from T-cell lymphoblastic lymphoma, as treatment response and prognosis may differ between these subtypes. Burkitt-like lymphoma, is rarely reported in Veterinary Medicine[4]. While lymphoblastic lymphoma predominantly affects young animals, its clinical and histopathological resemblance to B-LL can lead to diagnostic challenges[5].

How to cite this article : Srinivasa, N.H., Jyothi, C., Revathi, P., Yugandhar, C., Charitha, V.G., Sivasudarshan, L., Anjali, K. and David, G. 2025. High-Grade Lymphoblastic Lymphoma Mimicking Burkitt - Like Lymphoma in a Puppy: Case Report. Indian J. Vet. Pathol., 49(3) : 262-265.

In Veterinary oncological diagnosis, the differentiation of lymphomas is often challenging due to their diverse clinical presentations and overlapping cytological features with other diseases. This is particularly true in young dogs, where lymphoblastic lymphoma (LBL) a high-grade, rapidly proliferating neoplasm of immature lymphoid

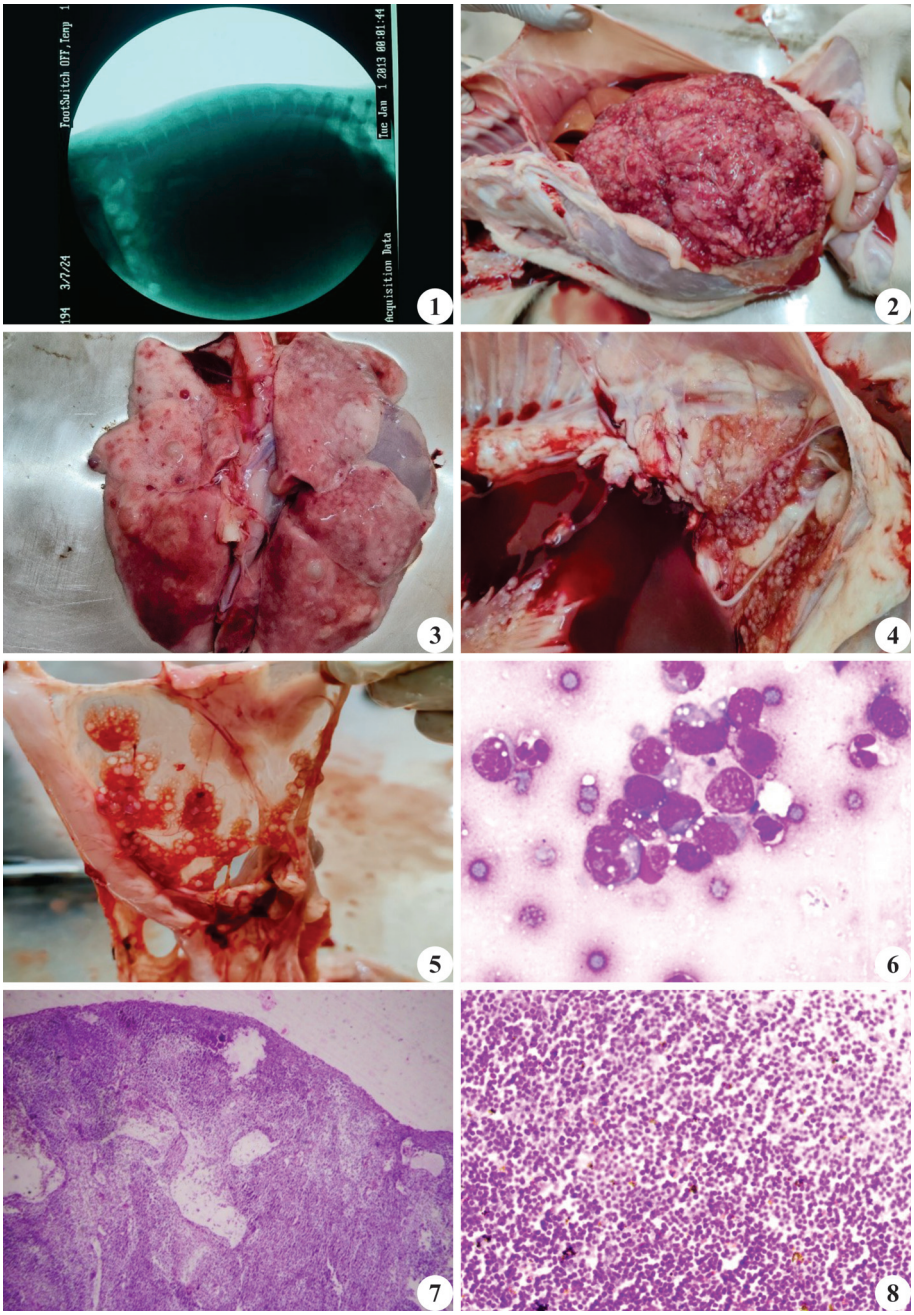

Fig. 1. Lateral abdominal radiograph revealed diffuse soft tissue opacity with poor serosal detail, suggestive of peritoneal effusion or presence of a mass in the right side of the abdominal cavity; **Fig. 2.** Large ball-like soft mass weighing around 2.5 kg with multiple small grey nodules occupied the entire right and ventral abdomen, extended to the left portion of the abdominal cavity; **Fig. 3.** Multiple diffuse raised greyish-white nodules were embedded in all the lobes of lung tissue; **Fig. 4.** Small greyish-white multiple grape-like nodules on the parietal layer of the thoracic cavity; **Fig. 5.** Small greyish-white multiple bunches of grape-like nodules on the mesentery of intestine; **Fig. 6.** Cytological smears revealed malignant neoplastic cells with round to oval nucleoli with a thin rim of basophilic cytoplasm containing lipid vacuoles (Giemsa stain 100X); **Fig. 7.** Section of tumor showing diffuse proliferation of lymphoid cells (H&E 10X); **Fig. 8.** Section of tumor mass showing diffuse proliferation of medium to large sized lymphoid cells (H&E 40x).

precursors can clinically and cytologically mimic a variety of conditions. These include juvenile immune-mediated diseases, reactive or infectious lymphadenitis (e.g., *Ehrlichia* spp., *Leishmania* spp.) and other round cell tumors such as histiocytic sarcoma, acute leukemia, or transmissible venereal tumor[4,6]. LBL often presents with generalized or mediastinal lymphadenopathy, hepatosplenomegaly or leukemic blood profiles, complicating its distinction from acute lymphoid leukemia (ALL) and making immunophenotyping and clonality assays essential for definitive diagnosis[1,3]. Given its aggressive nature and rapid progression, early diagnosis is critical

for implementing therapeutic strategies and determining prognosis.

Present report describes a rare case of lymphoblastic lymphoma in a young puppy, emphasizing the importance of comprehensive diagnostic techniques, including histopathology and immunohistochemistry, in establishing diagnosis. These findings add to the understanding of lymphoid cancers in young dogs and highlight the importance of immunophenotypic markers in identifying different types of lymphoma.

A 2-month-old mixed-breed male puppy, weighing approximately 5 kg, showed progressive abdominal swelling, severe weight loss, emaciation and vomiting. Clinical examination revealed dullness, weakness, pale mucous membranes and a firm mass palpable on the right side of the abdomen. Abdominal radiography showed diffuse soft tissue opacity with poor serosal detail, suggestive of peritoneal effusions or neoplastic infiltration (Fig. 1). While blood picture revealed a decrease in RBC count (5 million/mm^3), Hb (4 g/dL), increase in TLC (> 1 lakh) count and differential leukocyte examination revealed lymphocytosis (92%)[7].

Fine-needle aspiration cytology revealed a population of pleomorphic malignant round cells, comprising of large lymphoblasts with high nuclear-to-cytoplasmic ratios, round to irregular nuclei, coarse chromatin, prominent nucleoli and scant to moderate amounts of deeply basophilic cytoplasm suggestive of lymphoid neoplasm. Due to poor prognosis, on the same day euthanasia was performed with the owner's consent.

A complete necropsy was performed, and gross pathological lesions were systematically recorded. For histopathological evaluation, representative samples from the pulmonary nodule and abdominal tumor mass were collected and fixed in 10% neutral buffered formalin. Tissues were processed using standard paraffin-embedding techniques, and serial sections of 5 µm thickness were stained with hematoxylin and eosin (H&E) as per established protocols[8]. Impression smears were prepared from the cut surfaces of the tumor and lung nodules for cytological assessment. Immunohistochemistry for CD10 and CD20 markers was performed using routine avidin-biotin-peroxidase complex methods to characterize the lymphoid phenotype[3].

Postmortem findings revealed a large, firm abdominal mass (-2.5 kg) occupied the entire right and ventral abdomen, extended to the left portion of the abdominal cavity[7] (Fig. 2). The mass was red to greyish in colour and studded with miliary nodules and soft to firm in consistency. The heart chambers showed a currant jelly clot. Multiple diffuse greyish-white nodules were embedded in all the lobes of lung tissue (Fig. 3) and cut section revealed froth in the bronchi and bronhioles, while the kidneys appeared pale with adherent capsules. Metastatic involvement was evident in multiple organs, including the liver, spleen, kidneys, intestines and mesentery. In addition to metastatic growths, the liver was icteric with a distended gallbladder. Small greyish-white multiple bunches of grape-like nodules were noticed on the parietal layer of the thoracic cavity and peritoneum of the abdominal cavity (Fig. 4 & 5).

Cytological examination of the neoplastic mass revealed round to oval cells with prominent nucleoli and basophilic cytoplasm containing lipid vacuoles, indicative of a high-grade malignant process[7] (Fig. 6).

Histopathological examination of the tumor mass and lung nodule revealed a diffuse proliferation of medium to large-sized lymphoid cells exhibiting moderate to marked anisokaryosis, vesicular nuclei with multiple prominent nucleoli and scant cytoplasm (Fig. 7 & 8). The neoplastic population showed high mitotic activity and scattered apoptotic bodies, consistent with an aggressive high-grade lymphoid malignancy. These features closely resemble those described in canine Burkitt-like lymphoma[7]. To differentiate between B-LL and other lymphoid neoplasms, immunohistochemistry was performed. The neoplastic cells were negative for CD10 and CD20, ruling out classical B-LL (Fig. 9 & 10). Based on histopathological and immunohistochemical

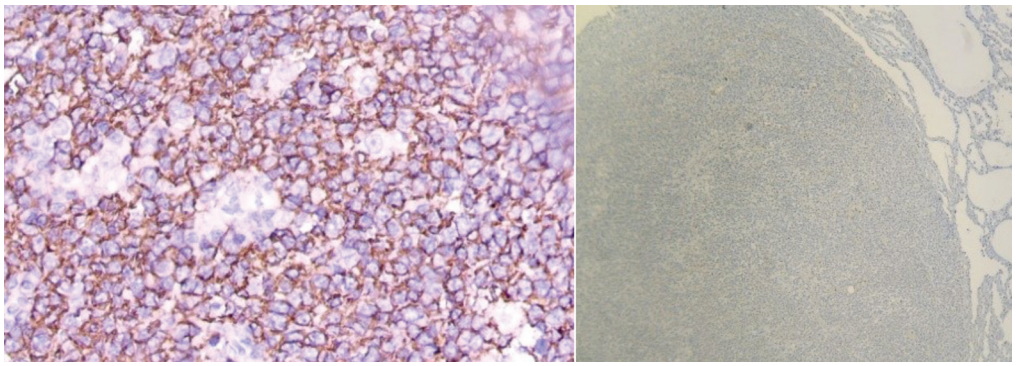

Fig. 9. Immunohistochemical staining of lung tissue revealed negative for CD10, 10X.

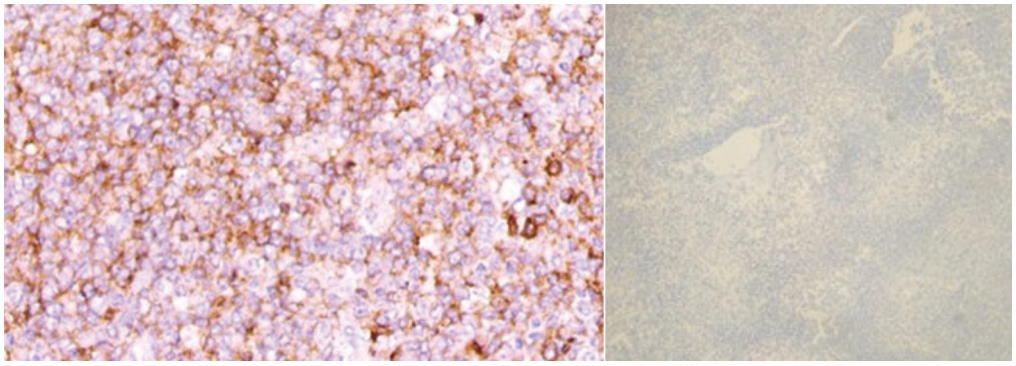

Fig. 10. Immunohistochemical staining of tumor revealed negative for CD20, 10X.

findings, the case was diagnosed as high-grade lymphoblastic lymphoma (lymphosarcoma), likely of T-cell origin. The aggressive nature of T-cell lymphomas correlates with a poorer prognosis in canines, similar to findings in human cases[5].

Lymphoblastic lymphomas in young dogs are rare and may be misdiagnosed as B-LL due to overlapping histological features. Burkitt lymphoma, commonly reported in humans, exhibits a high mitotic rate and a characteristic starry-sky appearance due to tangible body macrophages[9]. Present case report documents a rare case of high-grade lymphoblastic lymphoma mimicking Burkitt-like lymphoma in a puppy. Early diagnosis and classification are crucial for understanding lymphoma progression and optimizing therapeutic application.

ACKNOWLEDGEMENT

The authors' wishes to acknowledge the faculty and staff of the Department of Veterinary Pathology and Veterinary Clinical Complex, College of Veterinary Science, Proddatur for their cooperation. The Facilities of the department were utilized for conducting present study.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 49(3) : 266-270, 2025: DOI: https://doi.org/10.56093/djdznb79

CASE REPORT

A widely metastatic hepatocellular carcinoma and intrahepatic bile duct cystadenocarcinoma in Spitz dog

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Received: 18.4.2025; Accepted: 8.5.2025

ABSTRACT

A ten-year-old male Spitz dog was brought for post mortem examination with a history of emaciation, weakness, difficulty in breathing and death. At necropsy, multiple tumour nodules were noticed on the liver surface. The metastatic tumour growths were also noticed in the lungs, lymph nodes and rectum. Morbid changes were observed in the spleen, heart, kidneys, urinary bladder and stomach. Microscopically, the tumour nodules were characterised by the presence of hepatocellular carcinoma (HCC) and intrahepatic bile duct cystadenocarcinoma (ICC). The pleomorphic neoplastic cells in HCC contained large, hyperchromatic, vesiculated nuclei, prominent nucleoli and multiple mitotic figures. The hepatic tissues around the tumour nodules were invaded with neoplastic cells and infiltration of mononuclear cells. The ICC consists of many cysts lined by single or multiple layers of cuboidal or columnar cells. The cystic lumen contained eosinophilic secretions and exfoliated cells. The metastatic neoplastic cells were noticed in the lungs, lymph nodes, spleen, heart, kidneys, urinary bladder, stomach and rectum. The immunoreactivity of neoplastic cells showed a mild expression of *arginase-1, Hep Par-1, glypican-3, AFP* and *Bcl-2*. The normal hepatic cells showed a marked expression of *arginase-1*, strong expression of *Hep Par-1* and mild expressions of *glypican-3* and *AFP*. Spleen showed marked expression of *PCNA* and *CD3* in the lymphoid cells. Mesenteric lymph node showed a moderate expression of *CD3* in the lymphoid cells.

Keywords: Dog, HCC, histopathology, ICC, immunohistochemistry

Hepatocellular carcinoma (HCC) is rare in dogs and most common in older animals with higher incidence in males. The malignant variant of HCC usually occurs as massive, nodular and diffuse forms[1,2]. The metastasis of HCC occurs mostly within liver by local invasion or extrahepatic organs like lungs, lymph nodes and peritoneum[1]. The other metastatic organs include kidney, heart, urinary bladder, adrenal glands, pancreas and bone marrow in dogs[1-3]. The intrahepatic invasion of neoplastic cells arranged as tubular carcinomas (cholangiocarcinoma) and intrahepatic bile duct cystadenocarcinomas (ICC). The prognosis of mixed variants of HCC and ICC are generally determined by histology and morphology. The pathophysiology, diagnosis and treatments of this tumour is highly challenging in advancing veterinary oncology. Hence, the present report describes the pathological characteristics of a rare variant of HCC and ICC in Spitz dog.

A ten-year-old male Spitz dog was brought for post mortem examination with a history of emaciation, weakness, difficulty in breathing, congested visible mucous membrane and death. After necropsy, representative tissue pieces from liver along with tumour nodules and visceral organs were collected in 10% neutral buffered formalin. Tissue samples were trimmed, processed, embedded, sectioned at 3-5 µm thickness and stained with routine hematoxylin and eosin (H&E) for histopathology[4]. Few sections were taken on positively charged slides for immunohistochemical (IHC) staining. Sections were incubated with primary antibodies (Table 1) followed by Poly Excel HRP / DAB detection system conjugated secondary antibody (PEH002) and counter stained with Mayer's haematoxylin[5,6]. Both primary and secondary antibodies were purchased from M/s PathnSitu Biotechnologies, Hyderabad, India.

How to cite this article : Madheswaran, R., Mahalakshmi, S., Thilagavathi, K. and Sumathi, D. 2025. A widely metastatic hepatocellular carcinoma and intrahepatic bile duct cystadenocarcinoma in Spitz dog. Indian J. Vet. Pathol., 49(3) : 266-270.

Grossly, multiple tumour nodules of varying sizes (8x9x6 cm) were noticed on the surface of liver. Nodules were pinkish-white in colour, well-delineated and had central depressions (Fig. 1). The metastatic tumour nodules were noticed in all lobes of the lungs. Mesenteric and mediastinal lymph nodes were enlarged and grayish-white in colour. Spleen was mildly enlarged and moderately congested. Heart revealed few

Table 1. Immunohistochemical expression of various primary antibodies in tumour tissues and lymphoid organs.

S.No.	Primary antibody	Host	Clone	Dilution	Location of expression	IHC scoring / expressions			
						Normal hepatic cells	Neoplastic hepatic cells	Stromal cells	Lymphoid cells
1.	Arginase-1	Mouse	Monoclonal (ARG1)	1:25	Cytoplasmic	4+	2+	1+	Negative
2.	Hep Par-1	Mouse	Monoclonal (EP265)	1:50	Cytoplasmic	5+	2+	1+	Negative
3.	Glypican-3	Mouse	Monoclonal (GPC3)	1:50	Cytoplasmic	2+	2+	Negative	Negative
4.	Alpha Fetoprotein (AFP)	Rabbit	Monoclonal (EP209)	1:50	Cytoplasmic	2+	2+	Negative	Negative
5.	Bcl-2	Rabbit	Monoclonal (EP36)	1:50	Cytoplasmic	Negative	2+	Negative	Negative
6.	Proliferating cell nuclear antigen (PCNA)	Rabbit	Monoclonal (EP091)	1:50	Nuclear	Negative	Negative	Negative	Spleen - 4+
7.	CD3	Rabbit	Polyclonal (PP160)	1:25	Membranous, cytoplasmic	Negative	Negative	Negative	Spleen - 4+, Lymph node - 3+

1+ minimal; 2+ mild; 3+ moderate; 4+ marked; 5+ strong

whitish raised areas in the cardiac muscles and valves. The kidneys were smaller in size and capsules peeled-off easily. On incision, thinning of cortex and widened medulla were noticed. The urinary bladder contained numerous yellow coloured crystals of various sizes and its mucosa revealed thickening with mild congestion. The mucosal folds of stomach covered with mucous shreds and underlying mucosa revealed congestion. The rectal (8 cm) muscles were thickened due to whitish coloured growth and congestion in the underlying mucosa that causes narrowing of the lumen.

Microscopically, the tumour nodules were mostly encapsulated with few ill-defined borders and infiltration of neoplastic cells. The neoplastic cells were characterized by a mixed variant of HCC and ICC. The HCC characterized by the trabecular pattern of neoplastic cells with eosinophilic and vacuolated cytoplasm intertwined with fibrous tissues (Fig. 2). The pleomorphic neoplastic cells contained large, hyperchromatic and vesiculated nuclei, prominent nucleoli and multiple mitotic figures (25-30 mitoses per 10 high-power fields) (Fig. 3). Angiogenesis, invasion of neoplastic cells in the adjacent hepatic tissues and infiltration of mononuclear cells were noticed. The cystadenocarcinoma developing from ICC consists of many cysts lined by single or multiple layers of cuboidal or columnar cells (Fig. 4). The cystic lumen contained eosinophilic secretions with exfoliated cells and debris. The nuclei were spherical, hyperchromatic and had few mitoses.

The lungs revealed metastatic neoplastic cells in most of the alveoli, atelectasis in the adjacent alveoli and mononuclear cells infiltration in interstitial areas (Fig. 5). The mesenteric lymph node showed a diffuse area of metastatic neoplastic cells replacing the lymphoid cells in the medullary region (Fig. 6). The neoplastic cells infiltration noticed in spleen, heart, kidneys, urinary bladder, stomach and rectum were morphologically similar to the tumour cells found in the liver.

The IHC scoring in tumour tissues and lymphoid organs against various primary antibodies are presented in Table 1. The immunoreactivity of HCC showed mild cytoplasmic expression of *arginase-1* (Fig. 7), *Hep Par-1* (Fig. 8), *glypican-3* (Fig. 9), *AFP* (Fig. 10) and *Bcl-2* (Fig. 11) in the neoplastic cells. The normal hepatic cells showed a marked cytoplasmic expression of arginase-1, strong cytoplasmic expression of *Hep Par-1* and mild cytoplasmic expressions of *glypican-3* and *AFP*. The stromal tissues intertwined with the neoplastic cells showed minimal cytoplasmic expression of *arginase-1* and *Hep Par-1*. The spleen showed marked nuclear expression of proliferating cell nuclear antigen (PCNA) in the lymphoid cells (Fig. 12), and marked nuclear and membranous expressions of CD3 in the lymphoid cells of periarterial lymphoid sheath (Fig. 13). The mesenteric lymph node revealed moderate nuclear and membranous expressions of CD3 in the lymphoid cells (Fig. 14).

A rare variant of HCC and ICC was observed in a ten-year-old male Spitz dog. Available literature stated that the average age of the dogs affected with HCC is more than 10 years and frequency of occurrence is high in males[1]. According to previous workers, the most commonly reported clinical signs in affected dogs were hepatomegaly, anorexia, weakness, emaciation, difficulty in breathing and congested mucous membranes[1]. These unusual signs were attributed to the paraneoplastic syndromes and cause for cancer related death.

Grossly, multiple tumour nodules noticed on the surface of liver is

agreed to the findings of earlier workers[1,2,7]. The nodular variants were most commonly occurs as multifocal in single or all liver lobes. The nodules were coalescing together and efface to hepatic parenchyma[2,8]. The nodules were greyish-white, well-delineated, had central depressions and solid or cystic with increased stromal tissues[9].

Histopathologically, tumour nodules were characterised by the mixed variants of HCC and ICC. The nodules were encapsulated and invaded in few areas of hepatic tissues[1]. The neoplastic cells were organized into trabecular, cystic, adenoid, pleomorphic and mixed patterns indicate malignancy and aggressiveness[1]. The intrahepatic invasion into bile duct commonly manifested as tubular carcinoma[10] and bile duct cystadenocarcinoma[3]. The occurrence of ICC is less common, more malignant and has high frequency of metastasis in dogs[9]. The extra hepatic metastases to distant organs of this study is agreed to the findings of previous workers[1,2,7].

The immunoreactivity of neoplastic cells showed mild expression of *arginase-1*, *Hep Par-1*, *glypican-3*, *AFP* and *Bcl-2* are in agreement with earlier workers who used these markers for the diagnosis of well-differentiated HCC in humans and animals[5,6,11-13]. The normal hepatic cells showed marked expression of *arginase-1*, strong expression of *Hep Par-1* and mild expression of *glypican-3* and *AFP* are similar to the findings of previous workers[5,6,11-13]. The stromal tissues intertwined with neoplastic cells showed minimal expression of *arginase-1* and *Hep Par-1* is similar to the findings of earlier workers[5,6,11-13]. A mild expression in the neoplastic cells, marked expression in the normal hepatic cells and

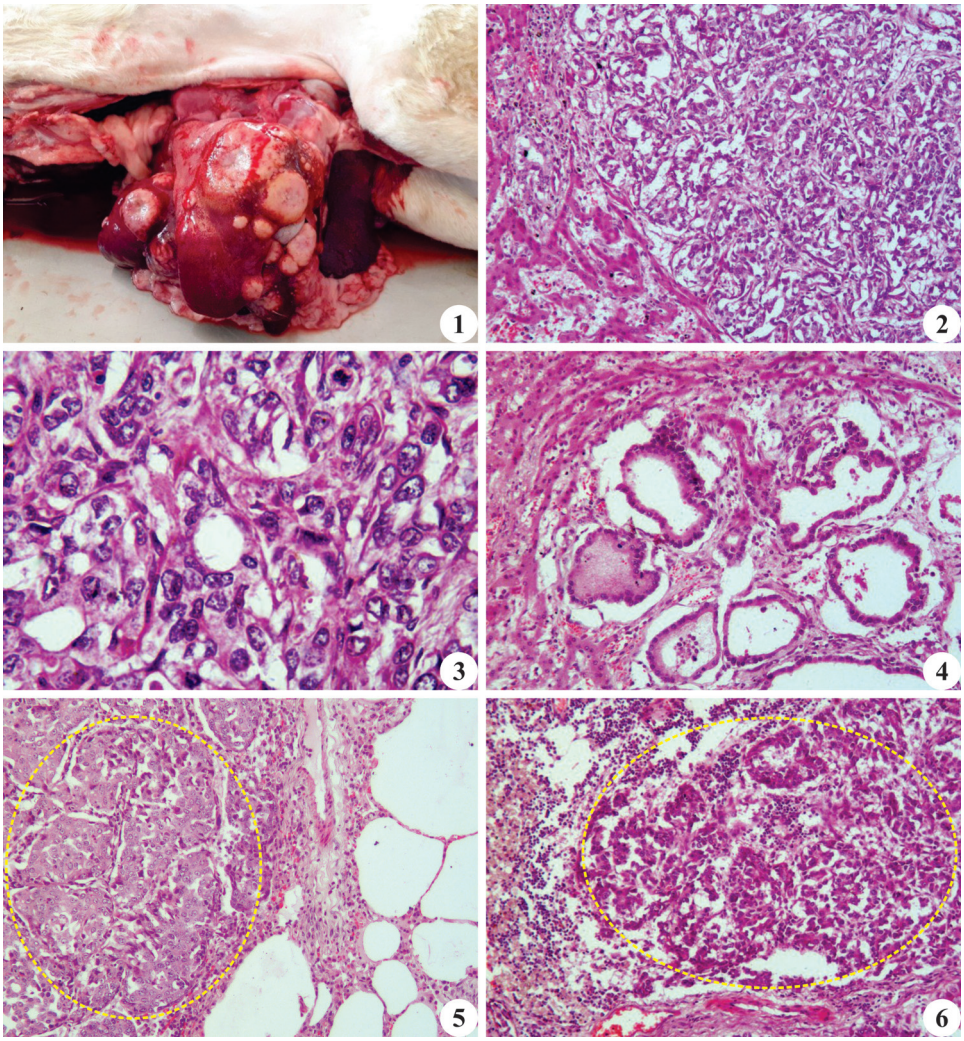

Fig. 1. Spitz: Hepatic tumour nodules showing pinkish-white in colour, well-delineated and central depressions; **Fig. 2.** Hepatocellular carcinoma showing encapsulation, ill-defined areas and invasion of neoplastic cells (H&E x100); **Fig. 3.** Hepatocellular carcinoma showing pleomorphic neoplastic cells with eosinophilic cytoplasm, vesiculated nuclei, prominent nucleoli and mitotic figures (H&E x400); **Fig. 4.** Intrahepatic bile duct cystadenocarcinoma showing many cysts lined by single or multiple layers of cuboidal or columnar cells (H&E x100); **Fig. 5.** Lung showing metastatic neoplastic cells (circle) in most of the alveoli and atelectasis in the adjacent alveoli (H&E x100); **Fig. 6.** Mesenteric lymph node showing metastatic neoplastic cells (circle) replacing the lymphoid cells in the medullary region (H&E x100).

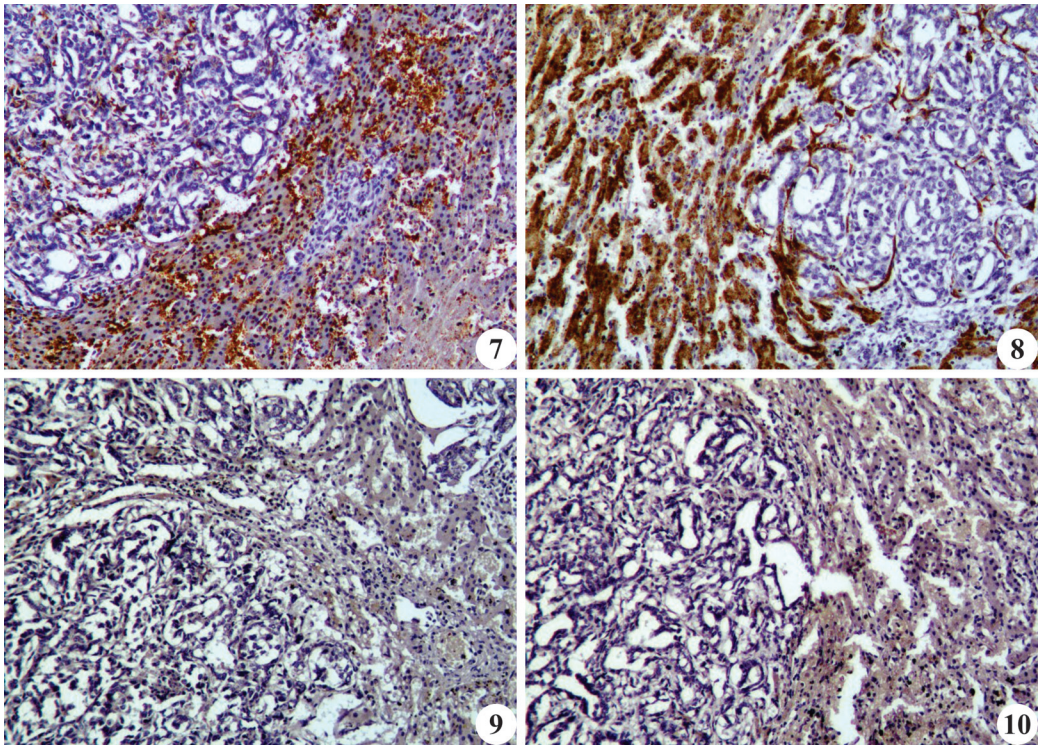

Fig. 7. Tumour section showing marked cytoplasmic expression in normal hepatic cells, mild expression in neoplastic cells and minimal expression in stromal cells to *arginase-1* (IHC, Arginase-1 x100); **Fig. 8.** Tumour section showing strong cytoplasmic expression in normal hepatic cells, mild expression in neoplastic cells and minimal expression in stromal cells to *Hep Par-1* (IHC, Hep par-1 x100); **Fig. 9.** Tumour section showing mild cytoplasmic expression to glypican-3 in the normal hepatic cells and neoplastic cells (IHC, Glypican-3 x100); **Fig. 10.** Tumour section showing mild cytoplasmic expression to AFP in the normal hepatic cells and neoplastic cells (IHC, AFP x100).

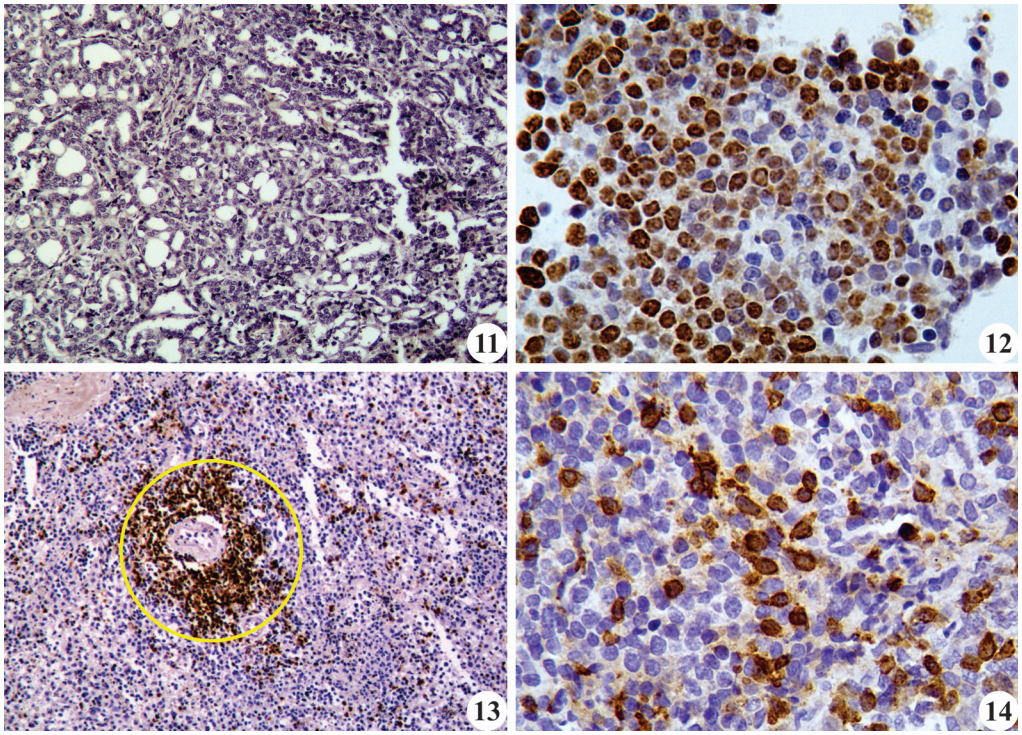

Fig. 11. Tumour section showing mild cytoplasmic expression to Bcl-2 in the neoplastic cells (IHC, Bcl-2 x100); **Fig. 12.** Spleen showing marked nuclear expression to PCNA in the lymphoid cells (IHC, PCNA x400); **Fig. 13.** Spleen showing marked nuclear and membranous expressions to CD3 in the lymphoid cells of periarterial lymphoid sheath (IHC, CD3 x100); **Fig. 14.** Mesenteric lymph node showing moderate nuclear and membranous expressions to CD3 in the lymphoid cells (IHC, CD3 x400).

minimal expression in the stromal cells revealed a mixed variant of HCC and ICC.

Spleen and lymph nodes showed marked expression of PCNA and CD3 in the lymphoid cells of this study are in agreement with earlier reports[14]. PCNA is a nuclear marker usually expressed in proliferating neoplastic and lymphoid cells. CD3 is a nuclear and membranous marker generally expressed in rapidly proliferating lymphoid cells. Expression of both markers in lymphoid organs shows an increased turnover as well as phagocytic activity of lymphoid cells against metastatic neoplastic cells. The intrahepatic invasion and extrahepatic metastases to distant organs shows the malignancy potentials of HCC. The pathological features of HCC and ICC will be useful for the early diagnosis and prognosis, hence the case is reported.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 49(3) : 271-274, 2025: DOI: https://doi.org/10.56093/qy73y297

CASE REPORT

Laboratory diagnosis of Babesiosis in a Rottweiler dog

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Received: 19.4.2025; Accepted: 3.5.2025

ABSTRACT

A three and half years old female Rottweiler dog was brought to the Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli for diagnosis and treatment with a history of anorexia since a week. Physical examination of the ailing animal revealed pyrexia and splenomegaly. Haematological estimation showed anaemia, thrombocytopenia and eosinophilia. Microscopic examination of peripheral blood smears revealed the presence of intra erythrocytic parasites, suggestive of *Babesia gibsoni*. Blood picture showed polychromasia and presence of nucleated red blood cells (RBC) and Howell Jolly bodies. Biochemical studies revealed increased levels of serum alkaline phosphatase (ALP), alanine transaminase (ALT) and blood urea nitrogen (BUN). Polymerase chain reaction (PCR) assay was carried out using the suspected blood sample confirmed the presence of *Babesia gibsoni* infection. Based on the present laboratory findings, diagnosis was confirmed as babesiosis and the affected animal was treated successfully. The animal became active and alert with normal feeding habits after 10 days of post treatment.

Keywords: *Babesia gibsoni*, blood smear examination, PCR, Rotweiller

How to cite this article : Kumar, V., Ponnarasi, S., Thangathurai, R., Ramesh, S., Rajesh, N.V. and Latchumikanthan, A. 2025. Laboratory diagnosis of Babesiosis in a Rottweiler dog. Indian J. Vet. Pathol., 49(3) : 271-274.

Babesiosis is one of the most common vector borne hemoprotozoan diseases, caused by various *Babesia* species affecting both domestic as well as wild animal species including cattle, buffaloes, sheep, goats, dogs etc. Canine babesiosis is caused by two most common species include *Babesia vogeli canis* and *Babesia gibsoni*, of which, the former being the larger one measuring 3-5 µm in diameter, while the latter being smaller, measuring 1-2 µm in diameter[1]. Both the forms are transmitted by Ixodid ticks notably *Rhipicephalus* and *Hemophysalis*. Transmission occurs through transovarian and transtadial route and also by transfusion of blood infected with *Babesia* spp.[2,3,4]. The above two vectors responsible for transmission of disease are widely distributed in Asia, Europe and African countries. The organism proliferates within the erythrocytes and gets released into the blood stream, which in turn eventually produces disease with characteristic clinical signs namely pyrexia, splenomegaly, hemoglobinuria, ascites, azotemia, liver dysfunctions and jaundice[5]. These haemoprotozoan parasites are easily demonstrated in the peripheral blood smears. However, detection of smaller forms of *Babesia* spp. such as *B. gibsoni* in the blood smears is difficult, for which application of molecular techniques such as Polymerase chain reaction (PCR) will be of valuable tool in confirmation of disease[6,7]. Thus, the present study reports a case of *Babesia gibsoni* infection, which was confirmed both by peripheral blood smear examination as well as by PCR assay in a Rotweiller dog.

A three and half years old female Rottweiler dog (Fig. 1) was brought to the Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli for diagnosis and treatment with a history of anorexia since a week. A thorough physical examination was carried out on the ailing animal and blood samples were collected from the saphenous vein for haematobiochemical studies. For haematological studies, blood samples were collected in ethylene diamine tetra acetic acid (EDTA) containing tubes and subjected to detailed analysis of various parameters namely Haemglobin (Hb), Packed cell volume (PCV), total erythrocyte count, total leukocyte count and thrombocyte count using automatic hematology analyser (Mindray - BC-2800). For biochemical estimations, blood samples were collected in clot activator tubes and serum was separated after centrifuging the clotted samples @ 300 rpm for 5 minutes. The separated serum was subjected to various estimations namely urea nitrogen, creatinine, ALP and ALT using automatic biochemical analyser (A15 Biosystems). In addition, blood smears prepared

from the ear vein were stained with Leishman and Giemsa (LG) and subjected to microscopic examination for presence of hemoprotozoan parasites.

For molecular confirmation of hemoprotozoan infections, blood samples were subjected to PCR assay using primers sequence as described[8]. The analysis was performed at Department of Veterinary Parasitology, Veterinary College and Research Institute, Orathanadu, Thanjavur, Tamil Nadu, India. The procedure were as follow, 200 µL of EDTA anticoagulant blood taken and genomic DNA was isolated using (QIAGEN DNeasy® Blood & Tissue Kit, Germany) by following manufacturer's protocol. The PCR cyclic conditions, including initial denaturation at cycling conditions involved initial denaturation at 95^{O}C for 5 minutes, followed by denaturation at 95^{O}C for 30 seconds, annealing at 55^{O}C for 30 seconds and extension at 72^{O}C for 90 seconds for 35 cycles. A final extension step was performed at 72^{O}C for 5 minutes. The 18S rRNA gene *B. gibsoni* target sequences that amplified positively were examined using 1.5% agarose gel electrophoresis, seen under a UV transilluminator and recorded.

Table 1. Haematological profile.

Parameters	Unit	Value	Normal range[14]	Result
Hb	g/dl	5.2	12-18	Anemia
RBC	10^6/µL	2.6	5.5-8.5	Anemia
PCV	%	21.6	37-55	Anemia
WBC	10^3/µL	4.7	6-17	Normal
Platelet	10^5/µL	33,000	2-9 lakhs	Thrombocytopenia
Neutrophil	%	63	60-70	Normal
Eosinophil	%	14	2-10	Eosinophilia
Lymphocyte	%	18	12-30	Normal
Monocyte	%	5	3-10	Normal

Table 2. Biochemical profile.

Parameters	Unit	Value	Normal range[15]	Inference
Blood urea nitrogen	mg/dl	40.4	5-30	Elevated
Creatinine	mg/dl	0.9	0.7-1.8	Normal
Total protein	g/dl	6.1	5-8	Normal
Albumin	g/dl	2.8	2.5-3.7	Normal
Total bilirubin	mg/dl	3.1	0.07-0.61	Normal
Calcium	mg/dl	9.2	9-11	Normal
Phosphorus	mg/dl	3.1	3.0-5.9	Normal
Alkaline phosphatase	IU/dl	312	20-200	Elevated
Alanine transaminase	IU/dl	210	10-109	Elevated

Physical examination of the ailing dog revealed pyrexia (103.5^{O}C), lymph node enlargement, pale mucous membrane and splenomegaly. The results of the hematobiochemical studies are presented in Table 1 and 2. Haematological examination showed anaemia, thrombocytopenia and eosinophilia, while biochemical studies revealed increased levels of serum alkaline phosphatase (ALP), alanine transaminase (ALT) and blood urea nitrogen (BUN). Microscopic examination of peripheral blood smears revealed the presence of numerous intra erythrocytic inclusions of variable sizes and shapes (Fig. 2) comprising both signet ring (Fig. 3 & 4) and comma shaped organisms suggestive of *Babesia gibsoni*. Blood picture showed polychromasia (Fig. 5), presence of nucleated red blood cells (RBC) (Fig. 6) and Howell Jolly bodies. These cells are immature RBCs, produced from bone marrow and appear in response to a high requirement for red blood cells due to increased destruction or loss (hemolytic anemia).

Polymerase chain reaction (PCR) assay (Fig. 7) confirmed the presence of *Babesia gibsoni* infection (671 bp to amplify 18s RNA using forward primer Gibb 599 (5′ CTCGGCTACTTGCCTTGTC 3′) and reverse Primer (5′ GCCGAAACTGAAATAACGGC 3′)[8]. Enlargement of spleen noticed during the present study was in accordance with earlier worker, who also recorded similar findings in a dog infected with *Babesia gibsoni.* Splenomegaly in babesiosis is due to more amounts of RBCs destruction occur. These cells are engulfed and removed by splenic macrophages during phagocytosis, which increases the cellularity of the red pulp and makes it harder to distinguish between the white and red pulp regions. The present haematological findings namely anemia, thrombocytopenia and eosinophila observed due to hemolysis of RBCs in the present case was in agreement with that of[9,10] who also recorded similar findings in *Babesia gibsoni* infected dog. The anemia noticed during the present study could be attributed to the cause of oxidative damage caused by *Babesia* organisms[11]. The present blood picture which revealed polychromasia and presence of nucleated RBC and Howell jolly bodies could be due to intra and extra vascular hemolysis[12].

Biochemical examination which revealed elevated levels of serum ALP and ALT might be attributed to cause of liver damage especially necrosis due to hypoxia and cytokines while increased levels of urea nitrogen (BUN) might be due to renal dysfunction due to slowing done of blood flow caused by refractory hypotension. Similar

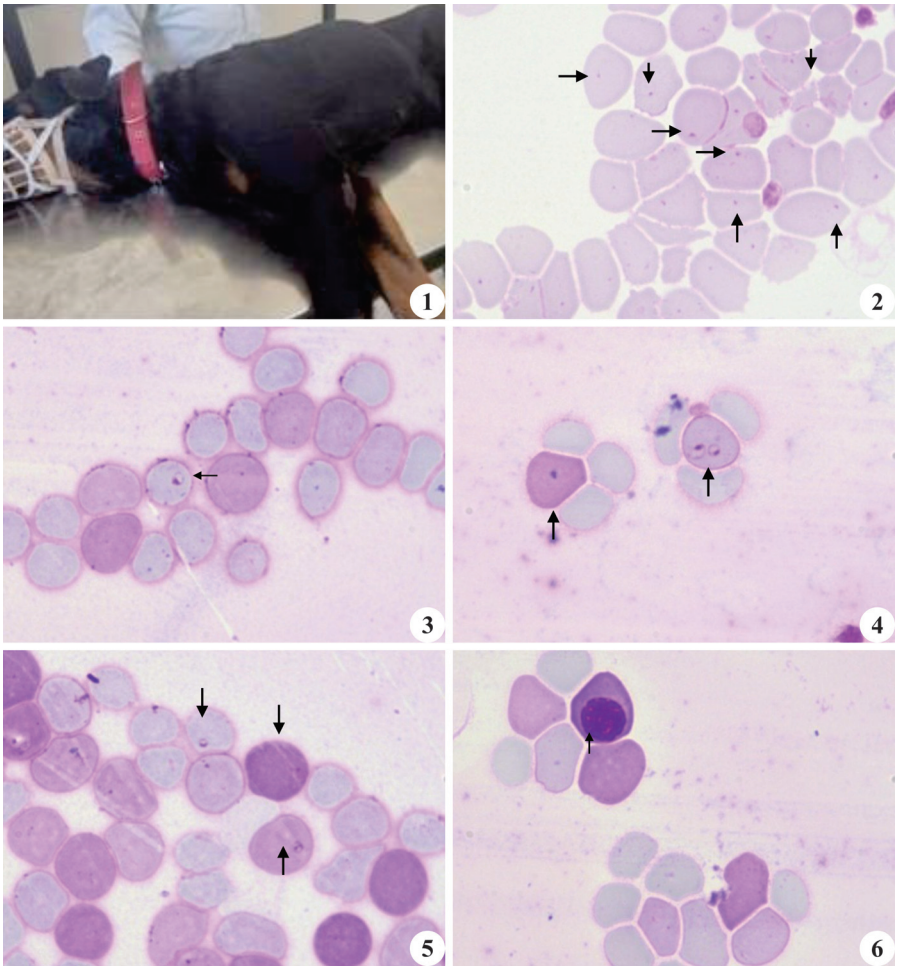

Fig. 1. A female Rottweiler dog affected with Babesiosis; **Fig. 2.** Peripheral blood smears revealed the presence of numerous intra erythrocytic inclusions of variable sizes and shapes (L&G staining x1000); **Fig. 3.** Blood smear of a dog showing the presence of numerous intra erythrocytic signet ring shape merozoite suggestive of *B. gibsoni* (L&G staining x1000); **Fig. 4.** Blood smear of a dog showing the presence of numerous intra erythrocytic double signet ring shaped inclusions suggestive of *B. gibsoni* (L&G staining x1000); **Fig. 5.** Blood smear of a dog infected with *B. gibsoni* showing polychromasia RBC (L&G staining x1000); **Fig. 6.** Blood smear of a dog infected with *B. gibsoni* showing nucleated RBC (L&G staining x1000).

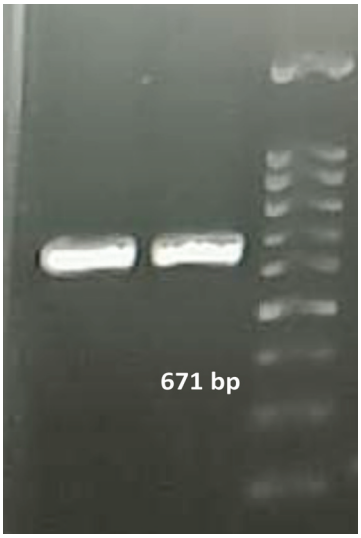

Fig. 7. PCR assay showing positivity of *B. gibsoni*. Lane 1: 100 bp ladder, Lane 2: Positive Control, Lane 3: Sample.

findings were also recorded[11,12,13] in dogs infected with babesiosis. The present microscopic findings of intra erythrocytic inclusions of variable sizes and shapes which were suggestive of *Babesia gibsoni* were in agreement with that of[10,9] who also reported similar morphological appearance in dog infected with *Babesia gibsoni*.

Blood smear examination provide less cost, simple, quick and easy way to identification of the blood protozoan parasite in the infected blood and enabling timely diagnosis and therapy. It can assist in determining the severity of infection. PCR assay which is more sensitive, confirmative and also can assist in distinguishing from plasmodium infection. Other common reliable methods like detecting antibodies against babesia infection in the blood, using procedures like enzyme linked immune sorbent assay (ELISA), indirect immunofluoresent antibody test (IFA) and complement fixation test (CFT), not suited for acute infection as antibodies may not be

present in adequate amounts until later in the infection. Triple therapy was initiated in this case with Clindamycin @ 25 mg/kg b.w., intravenously, BID, Metronidazole @ 15 mg/kg b.w., intravenously, BID and Doxycycline @ 5 mg/kg b.w., intravenously, BID, for 15 days on the date of presentation of this case. Also, the animal had given supportive treatment. Advised the owner with syrup LIV 52 and aRBCe @ 5 mL, Orally, BID and syrup THROMBOFIT @ 10 mL, Orally, BID. For the management of valvular heart dysfunction, Tablet. Envas @ 10 mg, sig. 1 tab, Orally, BID and Tablet. Lasilactone @ 50 mg, sig. 2 tab, Orally, BID was prescribed for 15 days and for supportive liver function, Tablet. Lisybin large, sig. 2 tab, Orally, SID for 10 days and Tablet. Ursodeoxycholic acid @ 300 mg, sig 1 tab, Orally, SID for 10 days were prescribed.

On day 15 following treatment, a peripheral blood smear stained with leishman and Giemsa stain showed the absence of *Babesia gibsoni*. Significant improvements were made to the haematobiochemical parameters. With a remarkable response and a smooth recovery, the dog made a full clinical recovery. In conclusion, microscopic examination of peripheral blood smear of a dog suspected for hemoprotozoan infection revealed intra eryhtrocytic inclusions suggestive of *B. gibsoni* which was further confirmed by molecular technique namely PCR assay.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 49(3) : 275-277, 2025: DOI: https://doi.org/10.56093/nmzjb864

CASE REPORT

Squamous cell carcinoma in a dog - A case report

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Received: 2.5.2025; Accepted: 10.6.2025

ABSTRACT

A 12-year-old male Labrador was presented at a local multispecialty hospital in Chennai with a history of having a multinodular, ulcerated, greyish-white mass on the right forelimb near the digits for surgical removal. Various samples, including blood samples, peripheral blood smears, fine needle aspiration smears of the mass, and the excised mass were collected and sent to the Centralized Clinical Laboratory at Madras Veterinary College in Chennai for laboratory diagnosis. Hematological studies revealed anemia and leukocytosis with left shift neutrophilia, while no changes were observed in serum biochemical parameters. Cytological findings showed clusters of large neoplastic squamous epithelial cells with faintly basophilic cytoplasm, containing prominent vesicular single to multiple nuclei and nucleoli. Additionally, anisocytosis, anisokaryosis, mitotic figures and tadpole cells were suggested, indicative of squamous cell carcinoma. Histopathological examination revealed neoplastic cells that were polygonal in shape, containing prominent vesicular nuclei, mitotic figures and variable-sized keratin pearls. Moreover, immunohistochemical studies showed increased expression of vascular endothelial growth factor (VEGF) in the cytoplasm of the neoplastic cells, confirming the tumor as squamous cell carcinoma. Based on the current laboratory findings, the tumor was identified as well-differentiated squamous cell carcinoma.

Keywords: Cytology, dog, haematobiochemical, histopathology, squamous cell carcinoma

Squamous cell carcinoma (SCC), the most prevalent malignant neoplasm affecting dogs accounts for 5% of all cutaneous tumours. Although the dogs that are aged more than eight years are commonly affected, dogs that are four months age are also affected[1]. The common sites of this tumour include nail bed, scrotum, nasal planum, limbs and anus. Squamous cell carcinoma affecting digits has been commonly recorded in large breeds, namely Standard Poodles, Labrador Retrievers, Giant Schnauzers, Gordon Setters, Rottweilers, Beaucerons and Briards. The etiology of squamous cell carcinoma is multifactorial. Exposure to solar ultraviolet light, ionizing radiation and chemical carcinogens, long-standing dermatoses, scars and other chronic lesions predispose the animals to this malignancy[1]. The papilloma viruses causing immunosuppression and inducing malignant transformation have been found to be a potential risk factor for the development of this malignant neoplasm[2]. Though the tumour can be extremely invasive and destructive, metastases are rarely recorded and occur only during the later stages of the disease. The common sites for metastasis include regional lymph nodes and lungs[3]. Various signs, namely scaly red spots, open sores and raised growths have been noticed in patients affected with squamous cell carcinoma[4].

Based on differentiation, squamous cell carcinoma can be classified into well-differentiated, moderately differentiated and poorly differentiated. The well-differentiated subtype, which has been commonly recorded in canines, is characterized by masses or cords of neoplastic epithelial cells which proliferate and invade the dermis and subcutis. The hallmark of this tumour is the presence of keratin pearls in varying numbers and sizes, composed by concentric layers of squamous cells with a gradual increase of keratinization toward the center[3].

A 12-year-old male Labrador dog was presented at a local multispeciality hospital, Chennai with the history of having a multinodular, ulcerated, greyish white mass on the forelimb near the digits for eight months, for surgical removal.

How to cite this article : Ramesh, S., Nithya, P., Senthilkumar, K. and Kumar, V. 2025. Squamous cell carcinoma in a dog - A case report. Indian J. Vet. Pathol., 49(3) : 275-277.

Samples, namely EDTA added blood samples, clotted blood and peripheral blood smears, were collected and sent to the Centralised Clinical Laboratory, Madras Veterinary College, Chennai for haematobiochemical studies. In addition, fine needle aspiration smears from the mass were also collected and referred for cytological diagnosis. Based on the cytological diagnosis, surgery was performed a week later, and the excised mass was fixed in 10% formalin for histological studies.

Haematogical and biochemical estimations were carried out using the Hemo auto analyzer namely Mindray BC-2800 and A15 Biosystems, respectively. The Fine Needle Aspiration Cytology (FNAC) smears as well as blood

smears were air dried, stained with Leishman and Giemsa cocktail stain and subjected to microscopical examination. Formalin-fixed tissues were processed by the routine paraffin embedding method, and the cut paraffin sections were stained by haematoxylin and eosin for histological studies. Immunohistochemical staining was performed on surgically excised tumour samples following the manufacturer protocol (M/s Biogenex, USA) using super-sensitive labeled poly-HRP polymer method.

Haemogram showed anemia and leucocytosis with left-shift neutrophilia while serum biochemical parameters revealed no abnormal changes. The haemato-biochemical profiles of the case are presented in Table 1.

Cytological findings revealed the presence of clusters of large neoplastic squamous epithelial cells with faintly basophilic cytoplasm containing prominent vesicular single to multiple nuclei and nucleoli. In addition, anisocytosis, anisokaryosis, mitotic figures and tadpole cells were also observed, which were suggestive of squamous cell carcinoma (Fig. 1 & 2).

Histopathological examination revealed neoplastic cells that were polyhedral in shape containing a moderate amount of cytoplasm with distinct cell borders. The nuclei were round to oval and vesiculate, containing 1-3 prominent nucleoli with moderate anisokaryosis. In addition, mitotic figures and variable-sized keratin pearls, which were composed of concentric layers of squamous cells were noticed, confirming the mass as well-differentiated squamous cell carcinoma (Fig. 3). Immunohistochemical studies showed an increased expression of VEGF in the cytoplasm of the neoplastic

Table 1. Haematobiochemical parameters of the dog affected with Squamous cell carcinoma.

S.No.	Parameters	Values	Reference range
1.	Haemoglobin (g/dl)	7.00	12-18
2.	PCV (%)	24	35-55
3.	RBC (millions/cmm)	3.6	5.7-8.5
4.	WBC (x10^3/cmm)	18	5-10
5.	Platelets (x10^5/cmm)	2.6	1.5-3.5
6.	Differential count		
	i. Neutrophils	84%	60-70%
	ii. Lymphocytes	12%	12-28%
	iii. Eosinophils	4%	5-10%
7.	BUN (mg/dl)	12	10-28
8.	Creatinine (mg/dl)	1.02	0.5 to 1.5
9.	SGPT (ALT) (IU/L)	38	21-102
10.	ALP (IU/L)	106	20-156
11.	Total protein (g/L)	6.4	5.4-7.1
12.	Albumin (g/L)	3.2	2.3-3.8

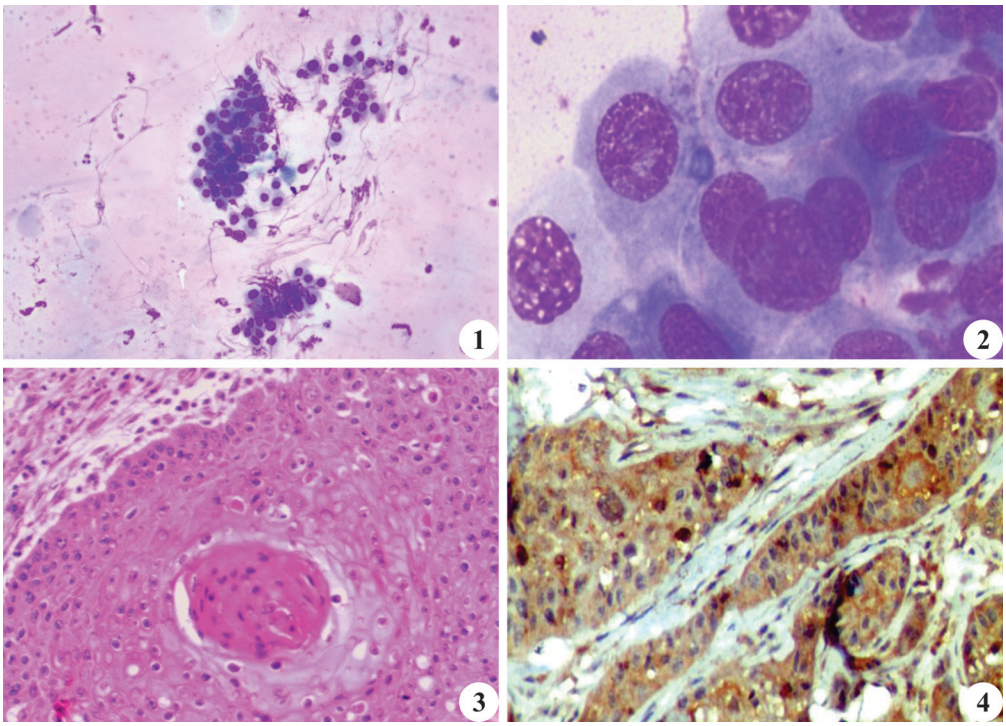

Fig. 1. Dog-Cytology: Squamous cell carcinoma - Clusters of neoplastic squamous epithelial cells showing anisocytosis and anisokaryosis (LG stain X100); **Fig. 2.** Dog-Cytology: Squamous cell carcinoma showing large neoplastic squamous epithelial cells showing prominent vesicular multiple nuclei and nucleoli with faintly basophilic cytoplasm (LG stain X100); **Fig. 3.** Dog-HP: Squamous cell carcinoma showing keratin pearls, composed of concentric layers of squamous cells (H&E X100); **Fig. 4.** IHC: Increased expression of VEGF in the cytoplasm of neoplastic squamous cells (X200).

cells which were suggestive of squamous cell carcinoma (Fig. 4). Based on the present laboratory findings, the tumour was identified as diagnosed as squamous cell carcinoma.

The present haematological findings were in accordance with those of earlier workers[1] who also reported leukocytosis and mild anemia in a 14-year-old dog affected with keratinized squamous cell carcinoma with extensive cranial bone invasion. An increased leukocyte count with normal biochemical findings was noticed in a 16-year-old female Maltese dog affected with squamous cell carcinoma[5] as observed in the present study.

The cytological observations made in the present study correlate well with those of previous workers[6] who also noticed similar cytological changes in dogs affected with squamous cell carcinoma. They observed a large number of malignant squamous cells occurring either individually or in clusters. The cells were pleomorphic, round to caudate in shape, exhibiting prominent anisokaryosis and anisocytosis. Anisokaryosis is characterised by nuclei, varying from pyknotic to large type, variable nuclear to cytoplasmic ratio, binucleation and multinucleation and perinuclear vacuolation. Similar cytological findings were also reported in domestic animals, including dogs, cats, horses and cows affected with squamous cell carcinoma[7]. The tadpole cells observed in the present study were also reported by earlier workers[7] who opined that the presence of tadpole cells is not a diagnostic feature of squamous cell carcinoma but it actually indicates epithelial dysplasia, although it is suggestive of squamous cell carcinoma[7].

The present histological findings agreed with that of previous workers[8,9,10,12] who also recorded similar findings in domestic animals affected with squamous cell carcinoma. They found that neoplastic cells were polyhedral in shape containing moderate amounts of cytoplasm with prominent intercellular bridges. Nuclei were found to be round to oval and finely stippled to vesiculated, containing multiple prominent nucleoli. In addition, moderate to marked anisokaryosis, numerous mitotic figures and keratin pearls were noticed. The positive expression of cytokeratin in the cytoplasm of neoplastic cells recorded during the present study was also reported by previous workers[10] who noticed similar expression of cytokeratin AE1AE3 in the cytoplasm of the neoplastic cells of a six-year-old male Pit Bull dog affected with squamous cell carcinoma.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 49(3) : 278-280, 2025: DOI: https://doi.org/10.56093/4g7xdx96

CASE REPORT

Ascites Syndrome in broiler birds - A case report

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Received: 17.3.2025; Accepted: 2.7.2025

ABSTRACT

Five Vencobb broiler birds approximately eight weeks old were submitted for post-mortem examination to the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Ranchi, Jharkhand, from a nearby private broiler farm. External examination revealed poor body condition and abnormal abdominal distension in all birds. Upon necropsy, approximately 50-60 ml of yellow, watery fluid containing thick, mucus-like yellow clots was found in the abdominal cavity. The liver appeared mottled and friable, the intestines were severely congested, the kidneys showed lesions of nephrosis and the lungs were pneumonic. Histopathological analysis revealed vacuolar degeneration of hepatocytes, congestion of sinusoids and the presence of haemosiderin pigment. The mucosal surface of the proventriculus exhibited degenerative, necrotic and congestive changes. The intestinal villi showed necrosis, epithelial sloughing and karyorrhexis. The heart tissue displayed significant mononuclear cell infiltration - primarily macrophages and epithelioid cells - within the subepicardial region. Based on the clinical history, gross lesions and histopathological findings, the condition was diagnosed as Ascites Syndrome.

Keywords: Ascites Syndrome, broiler, postmortem, Vencobb

How to cite this article : Lakra, P.P., Gupta, M.K., Kumar, V., Sharma, S. and Mahto, A.K. 2025. Ascites Syndrome in broiler birds - A case report. Indian J. Vet. Pathol., 49(3) : 278-280.

Ascites Syndrome (AS), also known as Pulmonary Hypertension Syndrome (PHS), is characterized by the accumulation of fluid within the coelomic cavity. It is a leading cause of morbidity and mortality in the modern broiler industry. In most cases, ascites is diagnosed between 4 and 5 weeks of age[1]. Ascites is most commonly seen in fast growing chickens particularly during the winter[2,3]. In recent years, intensive genetic selection for rapid growth has contributed to the emergence of metabolic disorders, including ascites. Many factors interact to cause ascites, including management practices, environmental conditions and genetic makeup.

Five broiler birds (Vencobb) of about 8 weeks of age were submitted for post-mortem examination in January 2025 from a local poultry farm with a history of mortality that had reached upto 10% in a total strength of 500 birds and was progressively increasing each day. It was also noticed that in the flock about 20% of the birds were smaller in size and weighed lesser than expected at 8 weeks of age. The deaths were sudden and the birds showed signs of poor growth, lethargy, anorexia and distended abdomen.

External examination of the carcasses revealed poor body condition and stunted growth. The abdomen was abnormally distended. Upon opening the carcass, approximately 50-60 ml of yellow colored watery fluid with thick yellow, jelly-like clots were observed within the abdominal cavity (Fig. 1). The liver appeared pale, mottled and friable in consistency. The intestines were severely congested, both kidneys were enlarged and mottled and indicated nephrosis and the lungs were pneumonic. After examining all the organs grossly, samples were collected in 10% Neutral Buffered formalin for histopathology. The tissue were routinely processed, sectioned and stained with Haematoxylin and Eosin (H&E) stain[4].

Histopathological examination of the intestinal tissue revealed necrosis and sloughing of the villous epithelium, accompanied by nuclear karyorrhexis (Fig. 2). Similar necrotic and karyorrhectic changes were also noted in the crypts, though infiltration of inflammatory cells was minimal. Blood vessels within the intestinal tissue were congested. Degenerative and necrotic changes with widespread karyorrhexis was observed in mucosal surface of the proventriculus (Fig. 3). Blood vessels were dialated and highly congested. The deeper glandular part showed milder lesions with intact glandular tissue. Lungs revealed severe congestion of both alveolar and larger inter lobular blood vessels with significant dilatation (Fig. 4). No significant inflammatory cell infiltration was observed. Kidney revealed severe congestion of the interstitial blood vessels along with marked degenerative changes in the tubular epithelium suggestive of nephrosis (Fig. 5). The glomeruli exhibited hypoplastic changes, with no notable infiltration of inflammatory cell. Liver exhibited marked vacuolar degeneration

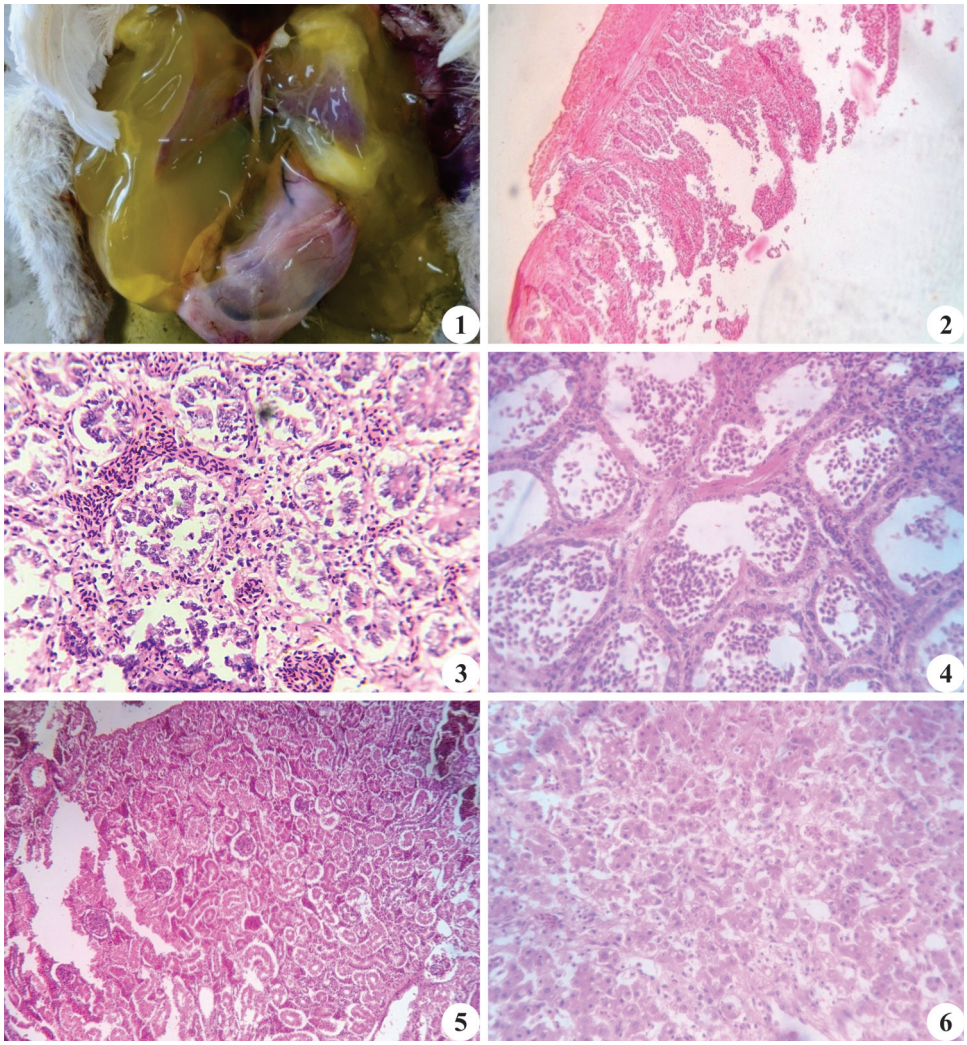

Fig. 1. Yellow colored watery fluid with thick yellow, jelly-like clots in the abdominal cavity; **Fig. 2.** Intestinal epithelium showed necrosis and sloughing of villi with karyorrhectic changes in nuclei (H&E 100X); **Fig. 3.** Section of Proventriculus showing degenerative and necrotic changes in the cells along with dilatation of blood vessels (H&E 400X); **Fig. 4.** Lungs revealed severe congestion of both alveolar and larger inter lobular blood vessels with significant dilatation (H&E 400X); **Fig. 5.** Kidney revealed severe congestion of the interstitial blood vessels along with marked degenerative changes in the tubular epithelium suggestive of nephrosis (H&E 100X); **Fig. 6.** Section of liver showing coagulation necrosis characterized by cytoplasmic acidophilia and pyknosis (H&E 100X).

of hepatocytes along with congested sinusoids and the presence of haemosiderin pigment. In extensive areas hepatocytes revealed coagulation necrosis characterized by cytoplasmic acidophilia and pyknosis (Fig. 6 & 7). Significant fibrosis was observed in the portal tract suggesting initiation of portal cirrhosis were seen in the liver. Heart showed marked infiltration of mononuclear cells, primarily macrophages and epithelioid cells in the subepicardium suggestive of pericarditis. The blood vessels were highly congested (Fig. 8). Cardiac muscle fibres also revealed infiltration of mononuclear cells, predominantly lymphocytes. Cardiocytes showed degenerative changes with loss of cross striations (Fig. 9).

The pathogenesis of ascites syndrome may begin with an elevated basal metabolic rate, triggered by various factors such as cold stress, mild heat exposure, increased physical activity, hyperthyroidism, excessive muscle mass and overeating.

In backyard flocks, cold is regarded to be a major factor in ascites epidemics[5], due to an increased blood flux out of the bird's lungs to provide the body with internal warmth. Ascites is exacerbated by cold weather by raising pulmonary hypertension and metabolic oxygen demands[6,7]. Since in the current case the mortality was occurring in the month of January, it can be correlated with the cold factor.

The occurrence of ascites can also be attributed to the diet's nutritional makeup and/or the way that feed is distributed. Ascites in broiler chickens can be caused by significant dietary parameters, such as high feed

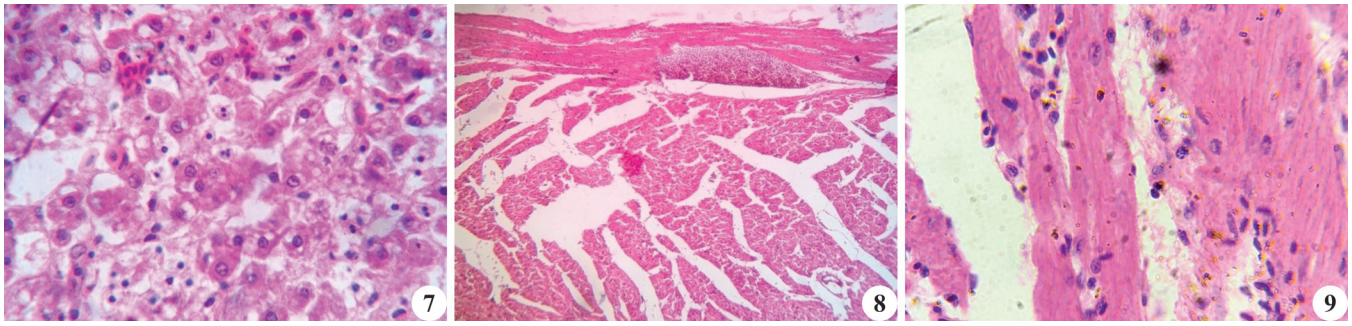

Fig. 7. Liver - Hepatocytes revealed coagulation necrosis characterized by cytoplasmic acidophilia and Pyknosis (H&E 400X); **Fig. 8.** Heart revealed marked infiltration of mononuclear cells, predominantly macrophages and epithelioid cells in the subepicardium suggestive of pericarditis (H&E 100X); **Fig. 9.** Cardiocytes showing loss of cross striations (H&E 1000X).

concentration and an increase in feed consumption, in addition, to feed structure (shape). Diets low in calories have been suggested to lower the prevalence of PHS[8-10]. Apart from temperature and feed, the broilers' genetic makeup can make them susceptible to PHS due to the moderate to high heritability of the genes linked to the disease.

According to published research research, a limited number of genes have a major role in the heritability of ascites[5]. In an investigation mutant genes were shown to increase pulmonary artery reconstruction and be related to ascites incidence[11,12].

In conclusion, the diagnosis in this case was supported by a comprehensive gross and histopathological examination, along with the clinical history. The clinical signs were in accordance with the previously reported earlier worker[13]. Feed restriction and nutrient concentration reduction in the diet can limit growth and prevent ascites-related death[14]. Ascites is a complex condition resulting from the interaction of physiological, environmental and management factors. However, its incidence can be minimized through proper management, medical intervention and nutritional strategies.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Title of Thesis	:	Pathoepidemiology of Elephant Endotheliotropic Herpesvirus in Asian Elephants with Special Reference to Vascular Endothelial Injury
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Name of the Advisor	:	Dr M. Karikalan
Degree/Year	:	MVSc/2023
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The aim of the present work was to study the pathoepidemiology of elephant endotheliotropic herpes virus in Asian elephants with special reference to the vascular endothelial injury. In this study, 150 Asian elephants from different parts of the country were clinically examined and samples such as blood, rectal swab/dung and trunk wash fluids were collected. A total of 87 elephants (58%) were found positive for EEHV1 genome by specific semi-nested PCR. The viral load in the clinical samples determined by SYBR green based qPCR assay using MDBP gene and comes in a range of 101-103 viral copies/μl of the sample. Sequencing and phylogenetic analysis of the POL1 (U38) gene from the representative positive clinical samples with desired amplicon size revealed 99% sequence identity with EEHV1A subtype. Among the 51 dead elephant tissue samples screened, six elephant calves were confirmed to have died due to EEHV1-HD and 14 elephants were found to be subclinically or latently infected as supported by histopathological examinations, semi-nested PCR and qPCR. The six fatal EEHV1-HD cases showed acute onset with specific clinical signs like swelling of head and trunk, cyanosis of mucous membranes and ocular discharge. Grossly, severe vascular changes like congestion, haemorrhages and oedema were observed in most internal organs. Cyanotic tongue, epicardial and endocardial haemorrhages, congested and oedematous liver, lungs and kidneys, haemorrhagic and congested blood vessels of brain were the major gross lesions observed. Histologically, all the organs exhibited severe vascular changes like engorged blood vessels, haemorrhage, swollen endothelial cells and oedema with mild to moderate degenerative changes. Amphophilic to basophilic intranuclear inclusion bodies were detected in heart and liver and was absent in all other organs as well as endothelial apoptotic bodies. While microthrombi were evident in lungs and kidneys. Immunohistochemistry using anti-EEHV DNA polhyperimmune sera revealed positive staining of endothelial cells and aggregates of erythrocytes in blood vessels of heart, liver, lung and kidney. The viral load ranged from 2.8 × 103 - 6.1 × 105/μg of EEHV1-HD tissue samples. The PCR amplicons of the desired size for EEHV1 specific genes POL1 (U38), TER, HEL and vGPCR were partially sequenced and all the sequences showed 99% similarity with previously reported EEHV1A subtype sequences in India as well as throughout the world. The immunostaining of endothelial markers such as PECAM and vWF was significantly ($p<0.05$) higher in EEHV1-HD cases compared to negative cases. The expression of PECAM was noticed more in endothelial cells of blood vessels, whereas vWF revealed immunolabeling in endothelial cells as well as microthrombi or aggregated RBCs. Relative quantification of endothelial markers (PECAM and vWF) and pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) exhibited significant ($p<0.05$) higher in EEHV1-HD cases then negative cases by TUNEL assay. In conclusion, apparently healthy Asian elephants are asymptomatic carriers of EEHV1 caused sporadic haemorrhagic diseases in calves with elevated endothelial markers and endothelial apoptosis contributing to the understanding of EEHV-HD pathogenesis in Asian elephants.

Title of Thesis : Pathomorphological cytological and molecular characterization of canine mammary tumours

Name of the Student : **Gavali Khushboo Raju**

Name of the Advisor : R. Thangathurai

Degree/Year : MVSc/2024

Name of the University : Veterinary College and Research Institute, Tirunelveli-627 358, TANUVAS

The present study was undertaken to assess the incidence and pathology of canine mammary tumours and also to use immunomarkers in the diagnosis and evaluation of canine mammary tumours. During the study period (January 2023 to November, 2023), 30 canine mammary tumour cases were recorded in dogs. Population incidence (n = 24,479) of canine tumour was 0.91 percent and mammary tumour was 13.45 percent. The average age of mammary tumour affected dog was 8.5 years and the highest abundance was recorded in 6-8 years of age group and the lowest was in 14-16 years. Twenty-eight mammary tumours were recorded in female and two were in male dogs. Also, tumours were recorded in both pure breeds and non-descript breeds of dogs. The incidence of canine mammary tumours was highest in pure breeds (n=21, 70 percent) than in mongrels (n=9, 30 percent). Among the 51 mammary tumour lumps, 30 (58.82 percent) palpable masses were seen on the right-side mammary gland and 21 (41.17 percent) on the right chain. Mammary tumours ulceration was noticed in 9 (17.64 percent) dogs.

At the time of presentation 30 dogs with mammary tumours were analysed, of which 10 dogs showed different metastatic patterns in lung parenchyma. Cytologically, carcinoma showed round to oval neoplastic cells with basophilic cytoplasm and acinar pattern in tubular carcinoma. A cluster of neoplastic cells was arranged in tubular or/and papillary like projection in tubulo papillary carcinoma. Neoplastic cells with basophilic cytoplasm, small to medium vacuoles and the nucleus at periphery were in lipid rich carcinoma. In malignant mixed type, the cluster of neoplastic epithelial cells and isolated fusiform mesenchymal cells were observed.

Grossly, the tumour appeared round to oval, with irregular borders some with glandular patterns. Some of the tumours were freely movable and others were fixed to the skin. Cut surface showed a white-greyish area, cystic fluid, multiple necrosis and haemorrhages.

Histopathologically, tumours were classified into different categories like tubular carcinoma, tubule-papillary carcinoma, comedocarcinoma, chondrosarcoma, malignant mixed mammary tumour and benign tumours. Among them malignant mixed types were recorded highest (n=7) followed by tubulopapillary (n=6) and least recorded was, adenoma (n=1) and benign mixed tumours (n=2). Malignant mixed types showed both carcinomatous and sarcomatous components surrounded by fibrous stroma. The cytoplasm of neoplastic cells was sparse with increased nuclear/cytoplasmic ratio. Sarcomatous component revealed cartilage and bony tissues adjacent to the carcinomatous areas. Tubulopapillary carcinoma showed neoplastic cells forming papillae within tubular lumen which were supported by fibrovascular stroma. Tubules were lined by flattened or cuboidal to short columnar neoplastic cells.

Mean mitotic count recorded were 11.20 ± 2.74 in Grade I, 24.00 ± 0.61 in Grade II and 36.71 ± 2.42 in Grade III tumours. Highly significant ($P \leq 0.01$) correlation was observed between the MI and Grade of mammary tumours. Based onthe grades, tumours were grouped into Grade I (n = 5), Grade II (n = 18) and Grade III (n = 7). Mean survival days recorded was 211 days in Grade I tumour, 141 days in grade II and 85.44 days in Grade III tumours.

Expression with Ki-67 mouse monoclonal antibody in all 30 tumour samples showed weak, moderate to intense staining. Grade I showed low positivity and no dead case was recorded. Grade II, comedocarcinoma showed poor prognosis with intense Ki-67 expression with the survival period of 178 days. Grade III, malignant myoepithelioma showed less chances of survival i.e. 12 days. There was a highly significant ($P \leq 0.01$) correlation was found between Ki-67 expression and overall survival period.

p53 mouse monoclonal antibody was used to detect mutant protein expression. All tumours expressed varying degrees of immunoreactivity from mild, moderate to intense staining. Two types of staining patterns were recorded i.e., nucleus and cytoplasmic staining. There was a highly significant ($P \leq 0.001$) correlation found between p53 and overall survival period, but no significant correlation was found between grades of tumours and p53 immunoreactivity.

Anti-VEGF antibodies were used to assess the angiogenesis in the samples. It took cytoplasmic staining. No significant correlation was found between vascular growth factor (VEGF) expression and survival interval period.

In conclusion, it was known that the overall incidence of canine tumours is 0.91 percent (223/24, 479) and same with mammary tumour is 13.45 percent (30/223). The average age of mammary tumour occurrence is 8.5 years with a range of 2-15 years. More mammary tumours were in female dogs than in male. Highest incidence of tumour was found in Labrador and non-descript breeds. Caudal thoracic, cranial and abdominal glands were predominantly affected followed by inguinal glands. Left mammary chains (58.82 percent) had more tumours and most of the canine mammary tumours were malignant than benign. Malignant mixed mammary tumours were recorded the most followed by tubulopapillary carcinoma. Cytology technique was useful for determining the origin, pattern of tumour and to assess the malignancy. Grading of tumours is useful to assess the prognosis of mammary tumours bearers. Majority of tumours were found in Grade II category. Tumours in Grade III had shorter survival period than Grade I and II. Mitotic index was found to be independent prognostic indicator in Grade III mammary tumours. The information presented in this study can help veterinarians and dog breeders to understand the occurrence of canine mammary tumours better and take appropriate measures to prevent or manage this condition in dogs.

Title of Thesis : Epidemiology, pathogenesis and ultra-pathobiology of parvoviral enteritis of canines and felines in and around Indore, Madhya Pradesh

Name of the Student : **Kuldeep Singh**

Name of the Advisor : Dr Supriya Shukla

Degree/Year : PhD/2022

Name of the University : College of Veterinary Science & AH, Mhow, Nanaji Deshmukh Veterinary Science University, Jabalpur, MP

In the present study, 150 dogs and 50 cats were examined irrespective of age, sex and breed showing tentatively symptoms of hemorrhagic gastro-enteritis were screen grossly, histopathological and ultrastructral studies. Out of these 150 dogs and 50 cats, samples of intestine, liver, spleen and heart from 55 dogs and 5 cats tissue samples were collected. The confirmation of incidence was done by using sandwich lateral flow immunechromatography assay as per the protocol provided along with PARVO kit. The overall incidence of CPV infection in dogs and cats was reported as 36.67% and 10%.

In most canine breeds, haemoglobin content, PCV and TEC values decreased initially from 3 months of age with leucopenia. Beagle and bull mastiff recorded higher blood higher blood biochemical values. Decreased sodium and potassium concentrations were observed in all breeds of dogs. In comparison, Persian female cats were more affected from first month onwards, showing decreased haematobiochemical values and higher ALT values indicative of liver damage, along with leucopenia. The values of total protein and potassium levels were less than normal in both Persian and non-descript cat breeds.

In canine on histopathology intestines revealed hemorrhages in the mucosal villi and intervillus crypts, congestion in sub mucosa and serous surfaces, liver had fatty changes with mononuclear periportal infiltration, hyperplasia of splenic trabeculae with infiltration and degeneration and necrosis of myocytes with hemorrhages and infiltration was noticed in heart TEM revealed presence of VLPs outside in crypts and inside intestinal epithelial cells, hepatocytes, splenic pulp and large group of virions in degenerated cardiac myocytes in a single pup. Other changes noticed were alaptisis of entericyte, severe margination of nuclear chromatin, fat bodies disrupted RER and infiltration by lymphocytes.

In felines histopathology revealed similar changes with demarcating fibrinous exudates and infiltration. TEM revealed more changes in mitochondria and cytoplasmic organelles with myeline figures and vesicles formation.

Title of Thesis : Incidence and pathology of bovine and caprine cutaneous lesions in and around Indore district of Madhya Pradesh

Name of the Student : **Rashmi Choudhary**

Name of the Advisor : Dr Supriya Shukla

Degree/Year : PhD/2022

Name of the University : College of Veterinary Science & AH, Mhow, Nanaji Deshmukh Veterinary Science University, Jabalpur, MP

A study was carried out to determine the incidence and pathology of various cutaneous lesions in cattle, buffaloes and goats in and around Indore district of Madhya Pradesh during the period between October, 2020 to March, 2022. A total of 3000 ruminant animals (1000 cattle, 1000 buffaloes and 1000 goats) were screened in the study irrespective of sex, age and breed from organized and unorganized livestock farms. Overall incidence of cutaneous lesions recorded were 15.17%. Out of this 191 (19.1%) cattle, 166 (16.6%) buffaloes and 98 (9.8%) goats were affected. Skin scrapings, culture, preliminary stainings, gross and histopathological examination along with special stainings were the basis for identification of cutaneous lesions. The major skin diseases encountered in and around Indore district were superficial and deep bacterial dermatitis, lumpy skin disease, contagious ecthyma, cutaneous warts, PPR and FMD like skin lesions, dermatophytosis and ectoparasitic infestation of ticks, mites, lice and fleas. The difference in the incidence of skin diseases among the three host species was statistically significant (p< 0.05).

Blood samples were analyzed for haematobiochemical changes by kits. Mean values of Haemoglobin concentration, PCV and TEC were found significantly lower (P< 0.05) while TLC and eosinophil count were higher in parasitic cases. Differential leucocyte count (%) revealed significantly increased lymphocyte count for fungal and parasitic causes of skin diseases. A significant increase in neutrophil count (%) were observed for bacterial as well as fungal causes of skin diseases whereas significantly increased monocyte count (%) was observed for only fungal etiology. Thrombocytopaenia was observed for parasitic cause of skin diseases in both small and large ruminants. There was significant difference observed in the mean values of ALT, AST, GGT, TP and BUN values whereas non significant differences in creatinine concentrations were observed among skin diseases of varying etiology in ruminants. Immunohistochemical staining of tumours tissues using P16 biomarker was done for papilloma virus with negative results, indicates absence of cross reactivity between human and bovine papilloma virus. The skin samples from LSD infected animals were subjected to conventional PCR, targeting ORF103 gene showed positive amplification of expected size. Skin samples of LSD and CE infected animals were subjected to electron microscopy which confirmed the presence of capripox and parapox virus by ultrasectioning of tissues.

Title of Thesis	:	Prognostic significance of canine mammary tumour biomarkers vis-à-vis human breast cancer as a trans model
Name of the Student	:	**Ankur Narad**
Name of the Advisor	:	Dr Supriya Shukla
Degree/Year	:	PhD/2024
Name of the University	:	College of Veterinary Science & AH, Mhow, Nanaji Deshmukh Veterinary Science University, Jabalpur, MP

The present study was carried out to establish aspects of comparison between canine and human mammary gland tumours. In order to achieve that 30 specimens of canine mammary tumours and 30 specimens of human breast tumours were examined in order to state points of similarity in morphology, cells of origin, behavior and existence of tissue markers.

Malignant and benign tumours were mostly observed in senior aged dogs ranging between 6-11 years, all cases of canine mammary tumours were found to be intact females. Total 13 breeds were affected among these highest incidence was recorded in Labrador Retriever. Canines with malignant tumours revealed increased total leucocyte count significantly as compared to benign counter parts. Cytology revealed characters like anisocytosis, anisokaryosis, hypercellularity, multinucleation and mitotic figures in malignant tumours.

Out of 30 CMTs, malignant neoplasms were 83.33% followed by benign 16.66%. The incidence of malignant epithelial neoplasms were 53.33%, malignant mixed tumour 13.33%, malignant mesenchymal 10% and malignant epithelial neoplasm special types 6.66%. Benign neoplasms constituted of simple adenoma 10% and fibroadenoma 6.66%. Canine mammary carcinomas were highest in grade I 56%, grade II 32% and 12% in grade III. Luminal B subtype was maximum 36% followed by HER-2 over expression 28%, Luminal A 20% and Basal like 16%.

Among the 30 HBTs, 66.67% were malignant and 33.33% benign. Invasive ductal carcinoma was the most frequent malignant type (43.34%), followed by ductal carcinoma in situ (23.33%). Benign lesions were primarily fibroadenomas (30%), with a single case of phyllodes tumour (3.33%). Tumour grading showed a predominance of grade II (55%), followed by grade III (35%) and grade I (10%). The molecular subtypes included Luminal A (35%), HER-2 overexpression (25%), Basal-like (25%), Luminal B (10%) and Normal-like (5%).

The study highlights significant morphological and molecular similarities between CMTs and HBTs, supporting the potential of canine mammary tumours as a spontaneous model for human breast cancer research.

SUPERANNUATION

Dr Bakorbhai Joitaram Patel

Dr Bakorbhai Joitaram Patel born on June 1, 1963 in Satlasana, Gujarat. His academic journey in veterinary sciences began with a BVSc & AH degree from Gujarat Agricultural University, Sardarkrushinagar in 1986. He further pursued his passion by completing his MVSc in Veterinary Pathology in 1989 from the same institution. In 1996, he earned his PhD in Veterinary Pathology from the prestigious Veterinary College, Govind Ballabh Pant University of Agriculture and Technology (GBPUAT), Pantnagar, Uttarakhand. Dr Patel's career path reflects a steady progression of academic excellence. He commenced his journey as a Junior Lecturer in September 1986, a position he held for three years and three months till December 1989. Subsequently, he served as an Assistant Professor from December 1989 to December 1998. His dedication and expertise were recognized through his promotion to Associate Professor in December 1998, a role he held till December 2006. Dr Patel's unwavering commitment to leadership and academic advancement culminated in his well-deserved promotion to Professor and Head of the Department of Pathology on January 1, 2016. He continues to lead the department with expertise and dedication. Beyond his professorship, Dr Patel actively contributed to research administration by serving as Associate Director of Research for three years. Since 1st April, 2021, he has further expanded his leadership by holding the role of I/C Principal at GN Patel College of Dairy Science, Kamdhenu University, Sardarkrushinagar.

Dr Patel's scholarly contributions are truly impressive. He has authored or co-authored a remarkable 82 research papers, 1 book, 1 booklet and 12 popular articles. His passion for knowledge dissemination is further evident in his presentation of over 152 research paper abstracts and delivery of 6 lead papers at national symposia. Additionally, he has significantly enriched academic resources by authoring 8 laboratory and training manuals. Dr Patel's dedication extends beyond research and administration. He has served as a guiding light for students, actively mentoring 30 MVSc candidates and 4 PhD scholars. His commitment to academic excellence and scientific advancement has been recognized by 32 prestigious awards and fellowships, including 2 medals for academic achievements and 30 for his outstanding scientific contributions.

His commitment to improving veterinary diagnostics is commendable. Dr Patel played a pivotal role in establishing a modern veterinary clinical pathology laboratory and pathology museum. Additionally, he organized refresher courses on laboratory techniques in animal disease diagnosis for state veterinary officers and dairy veterinarians. He has also actively participated in research, serving as the principal investigator of two plan schemes and coleading an ICAR scheme on fluorosis. Dr Patel's research spans a broad and impactful range, including the toxicopathology of heavy metals and pesticides in laboratory animals, as well as investigations into experimental diabetes in rats.

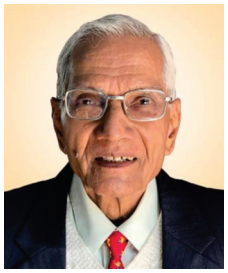
Dr Patel's commitment to life long learning is evident in his active participation in professional development opportunities. He has attended a total of 45 seminars, symposiums and workshops, encompassing both international (4) and national (41) events. Furthermore, he has participated in 5 summer schools and short training courses, further broadening his knowledge base. Dr Patel has demonstrated his organizational skills by successfully organizing the Indian Association of Veterinary Pathologists (IAVP) Congress in Sardarkrushinagar twice. He served as a member of the organizing committee for the 2001 congress and took on the lead role of organizing the 2018 event.

His scholarly contributions, mentorship and commitment to the advancement of veterinary education and research are truly commendable. Dr Patel continues to be a valuable asset to the field of veterinary pathology, shaping future generations of veterinarians and contributing significantly to animal health. He was also Fellow IAVP.

The Staff and Students are proud to honour Dr BJ Patel on his superannuation on 30.6.2025 for his single minded dedication, diligence and outstanding contributions for the veterinary profession. The Indian Association of Veterinary Pathologists wish him a healthy, happy and peacefull family life.

OBITUARY

Dr Jawaharlal Vegad Passes Away



Dr Jawaharlal Vegad was born on August 30, 1936 in Jabalpur, Madhya Pradesh. He secured his Bachelors in Veterinary Science & Animal Husbandry from Veterinary College Jabalpur and thereafter did Masters in Veterinary Pathology from Indian Veterinary Research Institute Izatnagar. He was a recipient of University Gold Medal in BVSc degree program. After securing the commonwealth scholarship in the year 1968, Dr Vegad obtained his PhD from Massey University, New Zealand.

Dr Vegad was Professor and Head Department of Veterinary Pathology, College of Veterinary Science & AH, Jabalpur for over 25 years and was subsequently elevated to the post of Dean, Veterinary College, Jabalpur. Dr Vegad superannuated from the government services in the year 1996. He was a visiting Professor at University of California, Davis, USA during 1988-1989 and Professor Emeritus of Indian Council of Agricultural Research from 1996-1998. Recognizing his intellect and sincerity Dr Vegad was offered consultancy at an evolving Poultry breeding and diagnostic centre, Phoenix, where he worked for more than two decades.

His dynamic and imaginative leadership brought national and international recognition to the Department of Veterinary Pathology. In Jabalpur, a small city with few resources and facilities, Dr Vegad, carried out ground breaking research on inflammation that was recognized globally. In 1987, Dr Vegad received the esteemed Rafi Ahmed Kidwai Award in appreciation of his dominance. As evidenced by the fact that three of his students went on to win the prestigious Jawaharlal Nehru award for best PhD thesis, Dr Vegad was a distinguished educator and excellent mentor, 180 research publications that were published in reputable journals with a high impact factor highlight his contributions to the field. He has published Atlas of Poultry Diseases, written six books in Veterinary Pathology and Poultry Science and written one book chapter.

Dr Vegad was honored to receive numerous accolades from different societies, including the Best Teacher Award from the University, the Dr CM Singh Samman and the Dr Nemi Chand Jain Lifetime Achievement Award from the IAVP. He was a fellow of the National Academy of Veterinary Sciences, the Society for Immunology and Immunopathology and the IAVP. In addition to being elected to the NAVS governing council and serving on the ICAR research advisory group for the Central Avian Research Institute and Project Directorate for Poultry, Dr Vegad served as President of the Indian Association of Veterinary Pathologists from 1997 to 2003. He was on the editorial board of the International journal "Comparative Haematology International" published from England and Indian Journal of Animal Sciences published by ICAR, New Delhi. Looking into the exemplary contributions made by Dr Vegad to Veterinary Pathology, the Indian Association of Veterinary Pathologits unanimously formed "Dr JL Vegad Foundation" in his honour.

As a consultant to the Phoenix Group of Poultry, Dr Vegad made a significant contribution to the organization's expansion. He traveled around numerous Asian nations, giving presentations at various venues on a range of topics related to poultry management and diseases.

He stayed engrossed in research until the very end, turning into an encyclopedia on the theoretical facets of molecular pathology, especially the process of inflammation.

On August 29, 2025, Dr Vegad departed for his celestial home, leaving behind his extensive studies, deep values, and most importantly, his simplicity and pray and hope that many people would recognize and adhere to his values. IAVP family extends their condolences to the departed soul.