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Clinico-pathological and Ultrasonographical Studies in *Babesia gibsoni* Infection in Dogs

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Abstract

The present study was carried out to investigate the prevalence, clinical signs, haematological, biochemical and ultrasonographic alterations in dogs naturally infected with *Babesia gibsoni* at the Medicine unit, Veterinary Clinical Complex, College of Veterinary and Animal Science, Bikaner, Rajasthan from July 2025 to December 2025. Out of 1628 dogs examined in the Medicine OPD, 23 were confirmed positive for *B. gibsoni* by PCR (18S rRNA gene), yielding an overall hospital prevalence of 1.41%. The highest occurrence was recorded in German Shepherds (30.4%), male dogs (56.5%) and the >1–5-year age group (34.8%). Maximum positivity was observed during July (39.1%). The predominant clinical signs included pyrexia (73.9%), anorexia (69.6%) and tick infestation (65.2%). Ultrasonography revealed splenomegaly (52.3%), hepatomegaly (39.1%) and renal cortical changes (17.4%). Haematological examination disclosed highly significant ($p < 0.01$) reductions in haemoglobin (8.56 ± 0.79 g/dl), total erythrocyte count ($4.45 \pm 0.28 \times 10^6/\mu\text{l}$), PCV ($28.09 \pm 1.98\%$) and platelets ($82.52 \pm 11.64 \times 10^3/\mu\text{l}$). Biochemical analysis revealed significant elevations in AST, ALT and total bilirubin, along with significant decreases in total protein and albumin.

Keywords: *Babesia gibsoni*, Prevalence, PCR, Haemato-biochemical, Ultrasonography, Dogs

Introduction

Babesiosis attributable to *B. gibsoni* follows a comparatively chronic and less fulminant course than disease caused by *B. canis*, although severe multi-organ involvement is well documented (Irwin, 2010). Despite increasing reports from other Indian states, epidemiological and clinico-pathological data specific to the Bikaner region of Rajasthan remain scarce (Bhati, 2025). The present study was done to determine the hospital-based prevalence of *B. gibsoni* and to characterise the associated clinical, haemato-biochemical and ultrasonographic alterations in dogs with Babesiosis.

Materials and Methods

The investigation was conducted at the Medicine unit, Veterinary Clinical Complex, College of Veterinary and Animal Science, Bikaner from July 2025 to December 2025. A total of 1628 dogs of varied breeds, ages and both sexes presented to the Medicine OPD underwent thorough physical and clinical examination. Animals exhibiting pyrexia, history or presence of tick infestation, anaemia, icterus or other signs suggestive of haemoprotozoan infection were subjected to detailed clinical, haemato-biochemical and

ultrasonographic examination. Signet-ring, pyriform and comma-shaped organisms appearing singly or in pairs within erythrocytes were recorded as positive findings. The Ubio Quickvet immunochromatographic rapid test kit (Ag-Ab based) was employed for all 110 suspected cases as per Eljadar (2010). Genomic DNA was extracted from 200 μl of EDTA-anticoagulated blood using a standard commercial DNA extraction kit. PCR amplification targeting a 670 bp fragment of the 18S rRNA gene of *B. gibsoni* was performed with species-specific primers and the protocol described by Mahalingaiah *et al.* (2017) with minor modifications to annealing temperature. Amplicons were visualised under UV light after electrophoresis on 1.5% agarose gel stained with ethidium bromide.

Results and Discussion

Of 1628 dogs examined in the Medicine OPD during the study period, 110 were identified as suspects for *B. gibsoni* infection. PCR confirmed 23 positive cases, yielding an overall hospital-based prevalence of 1.41% (23/1628). Among the 110 suspected cases, microscopy detected 9 (8.18%), the rapid test kit detected 7 (6.36%) positives, while PCR identified 23 (20.9%) cases, confirming its superiority as a diagnostic modality. The PCR prevalence of 1.41% closely agreed with Gonmei *et al.* (2020), who recorded 1.25% in Mizoram, and with Kushwaha *et al.* (2018), who reported 1.82% hospital-

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based PCR positivity. Variation in prevalence across different studies may be attributed to differences in agro-climatic conditions, diagnostic techniques employed, tick vector density and host immunity as reported by Baneth (2018). In contrast, significantly higher prevalences of 34.39% and 47.05% have been reported from Tamil Nadu by Sahithi *et al.* (2024) and Soundarya *et al.* (2023), respectively, likely reflecting differences in tick pressure and climate.

Age-wise, the highest positivity was recorded in the >1–5 year group (34.8%), followed by the >5–10 year group (30.4%), while dogs up to 1 year and those above 10 years each contributed 17.4%. This distribution is comparable to Aarthi (2017), who noted peak infections in the 1–5 year cohort (71.68%), and to Chaurasia *et al.* (2022), who documented highest prevalence in dogs aged 1–3 years (41.18%), reflecting greater outdoor activity and tick exposure in younger, more active dogs. Breed-wise, German Shepherds predominated (30.4%), followed by Labradors (26.1%). This aligns with findings of Chaurasia *et al.* (2022), who attributed genetic susceptibility and carrier status in German Shepherds to sustained seropositivity. Gender-wise, males comprised 56.5% of positive cases compared to 43.5% in females, which is consistent with Mahalingaiah *et al.* (2017), and Soundarya *et al.* (2023), who attributed higher male susceptibility to roaming behaviour and greater tick vector exposure.

Seasonally, the rainy months of July–September accounted for 73.8% of all cases, with July alone contributing 39.1%. This pattern is concordant with Bhati (2025) and Chaurasia *et al.* (2022), attributing peak incidence to increased activity of *Rhipicephalus sanguineus* under warm, humid conditions.

Pyrexia (>102.5°F) was the most frequent systemic finding, recorded in 73.9% (17/23) of cases, with a mean body temperature of $103.7 \pm 0.4^\circ\text{F}$. Anorexia was documented in 69.6% (16/23), followed by tick infestation or tick history in 65.2% (15/23) and lethargy in 47.8% (11/23). Pale mucous membranes indicative of anaemia was present in 34.8% of cases. These findings are in close agreement with Hajare (2023) and Bhati (2025), who reported similar predominance of fever, inappetence and tick infestation. Gastrointestinal manifestations such as vomiting (26.1%), melena (30.4%) and diarrhoea (4.3%) were recorded. Gonde *et al.* (2014) similarly documented vomiting in 27.02% and melena in 2.70% of naturally occurring babesiosis cases. Petechial haemorrhages and limb oedema

were each observed in 17.4% of dogs, consistent with thrombocytopenic manifestations. Jaundice, corneal opacity and emaciation were less frequently encountered (8.7% each), while haemoglobinuria, epistaxis, seizures and ascites were noted in solitary cases (4.3%). Chan *et al.* (2025) also recorded jaundice in only 7% of cases and attributed haemoglobinuria to the rapid intravascular haemolysis more characteristic of *B. rossi* than *B. gibsoni*. Atypical signs such as seizures and corneal opacity, observed previously by Shinde *et al.* (2021) in 3.22% and 6.45% of cases respectively, were attributed to cerebral babesiosis arising from sequestration of parasitised erythrocytes in CNS microvasculature.

Splenomegaly was the most common finding, present in 52.3% (12/23) of cases, observed as diffuse hypoechoic parenchymal pattern with enlargement, consistent with observations of Yogeshpriya *et al.* (2018). Moth-eaten echotexture indicative of multifocal splenic necrosis was detected in 8.7% of animals, suggesting vascular compromise with venous outflow obstruction (Raut, 2014). Hepatomegaly was identified in 39.1% (9/23) of dogs, with hyperechoic parenchyma noted in 4 animals correlating with elevated ALT and ALP values, consistent with the findings of Fraga *et al.* (2011). Gallbladder distension was recorded in 21.7% (5/23) of cases and gallbladder sludge or wall oedema in 13%, with Sarma *et al.* (2014) suggesting that distension beyond normal range is a reliable indicator of anorexia in companion animals. Renal ultrasonographic abnormalities included loss of corticomedullary distinction (17.4%), increased cortical echogenicity (13%) and renal pelvis dilation (pyelectasia) in 8.7% of dogs. These findings are consistent with increased renal echogenicity and altered corticomedullary ratio characteristic of *B. gibsoni*-associated renal injury, as previously described by Eduardo *et al.* (2011) and Yogeshpriya *et al.* (2019). Ascitic free fluid was identified in one dog (4.3%), consistent with observations of Kaur (2019) who reported ascites in 14.3% of affected animals.

Haematobiochemical values are summarized in Table 2. The mean $\pm$ SE values of haemoglobin (8.56 ± 0.79 g/dl), TEC ($4.45 \pm 0.28 \times 10^6/\mu\text{l}$) and PCV ($28.09 \pm 1.98\%$) in infected dogs were highly significantly ($p < 0.01$) reduced compared to healthy controls (15.16 ± 0.61 g/dl; $6.98 \pm 0.35 \times 10^6/\mu\text{l}$; $44.56 \pm 1.94\%$), indicative of severe regenerative anaemia. Similar findings were documented by Yogeshpriya *et al.* (2018), Bhati (2025) and Chan *et al.* (2025). Platelet count was highly

significantly ($p < 0.01$) decreased in infected animals ($82.52 \pm 11.64 \times 10^3/\mu\text{l}$) compared to controls ($346.13 \pm 31.92 \times 10^3/\mu\text{l}$), indicative of severe thrombocytopenia, in agreement with Chan *et al.* (2025).

AST (82.89 ± 11.77 IU/L) and ALT (105.37 ± 13.16 IU/L) were highly significantly ($p < 0.01$) elevated compared to healthy controls (30.39 ± 3.16 and 36.72 ± 6.86 IU/L, respectively). Total protein (5.52 ± 0.12 g/

dl) and albumin (2.40 ± 0.21 g/dl) were significantly ($p < 0.05$) reduced in infected dogs, comparable to healthy values of 6.11 ± 0.22 and 2.99 ± 0.16 g/dl respectively, indicating hypoproteinaemia and hypoalbuminaemia. Blood urea nitrogen (35.63 ± 16.98 mg/dl) and creatinine (2.22 ± 0.75 mg/dl) were non-significantly increased relative to healthy controls (15.79 ± 1.38 and 1.02 ± 0.09 mg/dl).

Table 1: Haemato-biochemical values of *Babesia gibsoni* infected dogs (Mean $\pm$ SE)

Parameters	Healthy Dogs (n=8)	<i>B. gibsoni</i> Infected (n=23)
Hb (g/dl)	15.16 ± 0.61	$8.56^{**} \pm 0.79$
TEC ($\times 10^6/\mu\text{l}$)	6.98 ± 0.35	$4.45^{**} \pm 0.28$
PCV (%)	44.56 ± 1.94	$28.09^{**} \pm 1.98$
Platelets ($\times 10^3/\mu\text{l}$)	346.13 ± 31.92	$82.52^{**} \pm 11.64$
WBC ($\times 10^3/\mu\text{l}$)	10.39 ± 0.92	18.01 ± 4.56
MCHC (g/dl)	33.45 ± 0.53	$29.99^* \pm 1.53$
Neutrophils (%)	71.90 ± 2.25	76.27 ± 2.32
Lymphocytes (%)	21.88 ± 2.04	19.15 ± 2.09
AST (IU/L)	30.39 ± 3.16	$82.89^{**} \pm 11.77$
ALT (IU/L)	36.72 ± 6.86	$105.37^{**} \pm 13.16$
ALP (IU/L)	124.69 ± 16.33	173.20 ± 39.87
Total Protein (g/dl)	6.11 ± 0.22	$5.52^* \pm 0.12$
Albumin (g/dl)	2.99 ± 0.16	$2.40^* \pm 0.21$
Globulin (g/dl)	3.0 ± 0.23	3.12 ± 0.21
Total Bilirubin (mg/dl)	0.32 ± 0.06	$1.19^{**} \pm 0.17$
BUN (mg/dl)	15.79 ± 1.38	35.63 ± 16.98
Creatinine (mg/dl)	1.02 ± 0.09	2.22 ± 0.75

* $p < 0.05$ – significant; ** $p < 0.01$ – highly significant; no superscript – non-significant

Acknowledgement

The authors express gratitude to the Dean, College of Veterinary and Animal Science, Bikaner, Rajasthan for facilitating the infrastructure and resources required to carry out this study.

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Received : 08.04.2026

Accepted : 05.06.2026

Published : 30.06.2026

Estimation of Thyroid profile in cats through Radio Immunoassay

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Abstract

Radioimmunoassay (RIA) is a highly sensitive and specific *in vitro* technique for quantifying hormones and other small antigens in biological fluids. The present study was undertaken to establish reference values for total Triiodothyronine (TT3), total Thyroxine (TT4), and free Thyroxine (fT4) in healthy cats. A total of thirty-one (31) healthy cats were ethically enrolled for the establishment of the thyroid hormone reference range. Serum concentrations of TT3, TT4, and fT4 were measured using RIA. The estimation of TT3 was carried out using the procedure recommended by the manufacturers. However, TT4 and fT4 were estimated by doubling the serum and standard volumes and halving the final results. The results of the present study recorded mean serum TT3, TT4, and fT4 values in healthy cats as 0.69 ± 0.13 nmol/l, 28.29 ± 3.78 nmol/l and 31.41 ± 2.88 pmol/l respectively. The reference interval (25th to 75th percentile) for TT3, TT4, and fT4 was found to be 0.31 – 0.84 nmol/l, 13.84 – 34.75 nmol/l and 19.31 – 37.32 pmol/l respectively. The median for TT3, TT4, and fT4 was found to be 0.38 nmol/l, 19.95 nmol/l and 33.46 pmol/l respectively.

Keywords: Feline thyroid function, Radioimmunoassay (RIA); Triiodothyronine (TT3); Thyroxine (TT4) and Free Thyroxine (fT4).

Introduction

Feline hypothyroidism is rare, whereas hyperthyroidism is the most common endocrine disorder of older cats (Crowe, 2004; Peterson *et al.*, 1983; Mooney, 2010). There is increase in the recognition of hyperthyroidism and other thyroid-responsive conditions in cats. The present study was designed to establish reference ranges for TT3, TT4, and fT4 in clinically healthy cats using Radio Immunoassay (RIA).

Materials and Methods

In the present study, thirty-one (n=31) healthy cats (average age in years: 3.35 ± 0.46 ; average body wt. in kg: 4.55 ± 0.31 ; Sex: 14 males & 17 females; recorded: since year 2016) were enrolled ethically with written consent from the pet owner. The cats included in the study were referral cases (presented in year 2016) to the Department of Medicine, Outpatient Department, and Teaching Veterinary Clinical Complex (Goregaon and Parel, Mumbai Veterinary College, Mumbai), private practitioners, and pets of voluntarily participating owners. Inclusion criteria: comprised of Adult cats (male or female) aged 1 year or older, including purebred, mixed-breed, and non-descript

cats, showing no clinical signs of illness, systemic disease, or endocrine dysfunction. Exclusion criteria were Cats receiving treatment for any endocrinopathy, including thyroid disorders; pregnant or lactating cats; cats treated with drugs known to affect thyroid function (e.g., steroids, methimazole, L-thyroxine); and cats with non-thyroidal illnesses such as viral infections, neoplasia of non-thyroidal origin, hepatic dysfunction, or severe renal disease that could secondarily affect thyroid function. The present study was initiated after permission from the Institutional Ethics Committee for Veterinary Clinical Research and the Institutional Biosafety Committee of Mumbai Veterinary College, Maharashtra Animal and Fishery Sciences University-Nagpur, Mumbai-India. Five ml of blood was collected from each cat. Two millilitres were placed in EDTA vials for haematological analysis, and the remaining 3 ml was used to harvest serum for biochemical and thyroid hormone estimation. Serum samples for thyroid profile analysis were stored at -20°C until assayed. Thyroid hormone estimation (TT3, TT4 and fT4) was performed using RIA at the Radio Isotope Laboratory and Veterinary Nuclear Medicine facility of Mumbai Veterinary College, Mumbai. Commercial human RIA kits, manufactured by the Board of Radiation and Isotope Technology (BRIT), Department of Atomic

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Energy, Mumbai, were used for the measurement of feline thyroid hormones. The RIA kits used for thyroid hormone estimation included MAG-3B, a magnetisable particle-based kit for TT3; MAG-4B, a magnetisable particle-based kit for TT4; and BRIACT-1, a coated tube-based kit for fT4. In the present study, total thyroxine (TT4) and free thyroxine (fT4) were initially estimated using the standard procedures provided by the kit manufacturers. However, these assays yielded undetectable or subnormal concentrations. Therefore, the procedures were modified by doubling the volume of serum and standards and halving the final calculated values, as recommended by Anderson and Brown (1979), to obtain accurate TT4 and fT4 measurements. Thyroid hormones were assayed for each sample, providing paired/ duplicate observations. Quality control parameters, including the levels of control samples and recovery percentages, were assessed to validate each assay.

Results and Discussion

Assay passed all recommended quality control parameters, viz., magnitude of control samples provided with kits and percent recovery. The standard curve of the assay was plotted, and the hormonal magnitude of unknown samples was interpolated from the standard curve. Mean, interquartile range (i.e., 25th to 75th percentile), and median of TT3, TT4, and fT4 in healthy cats (n=31) enrolled in the study, and levels of thyroid hormones reported by other authors are presented in Table -1. The mean value of TT3 in healthy cats was found to be 0.69 ± 0.13 nmol/l. Figure -1 depicts a box plot of TT3 concentration in healthy (n=31) cats. For each box plot, 'T bars' represent the data, which is equal to the range (0.15 – 1.31 nmol/l). The box represents the middle half of the data (Interquartile range; 25th to 75th percentile, as 0.31 – 0.84 nmol/l). The horizontal bar in the box is the median of the data (0.38 nmol/l). The mean concentration and interquartile range (25th to 75th percentile) of TT3 recorded in the present study falls within the previously reported normal ranges of 0.60–1.73 nmol/l (Reap *et al.*, 1978), 0.23–1.23 nmol/l (Reimers *et al.*, 1982), 0.13–1.24 nmol/l (Thoday *et al.*, 1984), and 0.4–1.8 nmol/l (Peterson *et al.*, 2001), respectively. The mean value of TT4 of healthy cats was recorded as 28.29 ± 3.78 nmol/l. Figure -2 depicts a box plot of TT4 concentration in healthy cats (n=31). For each box plot, 'T bars' represent the data, which is equal to the range (8.37 – 54.70 nmol/l). The box represents the middle half of the data (Interquartile range; 25th to

75th percentile, as 13.84 – 34.75 nmol/l). The horizontal bar in the box is the median of the data (19.95 nmol/l). The mean concentration and interquartile range (25th to 75th percentile) of TT4 recorded in the present study falls within the previously reported normal ranges 18–83.7 nmol/l (Anderson and Brown, 1979), 9.7–56.3 nmol/l (Reimers *et al.*, 1982), 5–54.1 nmol/l (Thoday *et al.*, 1984) and of 12.9–38.7 nmol/l (Peterson *et al.*, 1981).

The mean value of fT4 of healthy cats was found to be 31.41 ± 2.88 pmol/l. Figure 3 depicts a box plot of fT4 concentration in healthy cats (n = 31). For each box plot, 'T bars' represent the data, which is equal to the range (10.94 – 55.34 pmol/l). The box represents the middle half of the data (Interquartile range; 25th to 75th percentile, as 19.31 – 37.32 pmol/l), the horizontal bar in the box is the median of the data (33.46 pmol/l). The mean concentration and interquartile range (25th to 75th percentile) of fT4 recorded in the present study fall within the previously reported normal ranges 15–50 pmol/l (Peterson *et al.*, 2001) and 10–51 pmol/l (Peterson *et al.*, 2015).

Measurements of circulating thyroid hormones using RIA are now widely employed in the investigation of thyroid disease in humans. These techniques can also be applied in feline practice, provided the methods are modified to accommodate the much lower circulating concentrations of total T4 and T3 found in cats compared with humans. Reap *et al.*, (1978) and Anderson and Brown (1979) have reported details of the RIA methods they used to determine plasma total T4 and T3 levels in normal cats. Reap *et al.*, (1978) measured total TT4 and TT3 in only 10 animals, whereas Anderson and Brown (1979) evaluated TT4 but not TT3 in 92 cats. Both groups used commercially produced RIA kits designed for human serum and recognized the challenges of assaying the low thyroid hormone levels in cats. To address this, they doubled the serum volume used in the assays and halved the measured values to obtain the correct hormone concentrations.

In the present study, commercial RIA kits intended for human serum was used to assess the thyroid profile of cats. Using the manufacturer's recommended protocol, only TT3 results showed clinical correlation in healthy cats, whereas TT4 and fT4 values were low and poorly correlated clinically. Following the approach described by Anderson and Brown (1979), we adapted our RIA procedures for TT4 and fT4 by doubling the

volumes of both the standards supplied with the kits and the serum samples, and then halving the resulting values to obtain the precise hormone concentrations. In our study, the adjusted total volumes of standards and serum samples were 50 μ L for TT4 and 100 μ L for fT4, respectively. This modification produced acceptable concentrations of both TT4 and fT4, with improved clinical correlation in healthy cats.

Acknowledgements

The authors are thankful to the Associate Dean, Mumbai Veterinary College, Parel-Mumbai, India, for providing the facility. The authors also gratefully acknowledge the support of the Board of Radiation and Isotope Technology (BRIT), Department of Atomic Energy (DAE), Government of India, for supplying the RIA kits.

Table 1: TT3, TT4 and fT4 concentration in healthy cats (n=31) using RIA

Parameters	Mean and range recorded in the present study	International published data about thyroid profile			
TT3 (nmol/l)	0.69 $\pm$ 0.13	0.60-1.73 (Reap <i>et al</i> , 1978)	0.23-1.23 (Reimers <i>et al</i> , 1982)	0.13-1.24 (Thoday <i>et al</i> , 1984)	0.4-1.8 (Peterson <i>et al</i> 2001)
TT4 (nmol/l)	28.29 $\pm$ 3.78	18-83.7 (Anderson & Brown, 1979)	9.7-56.3 (Reimers <i>et al</i> , 1982)	5-54.1 (Thoday <i>et al</i> , 1984)	12.9-38.7 (Peterson <i>et al</i> , 1981)
fT4 (pmol/l)	31.41 $\pm$ 2.88	15-50 (Peterson <i>et al</i> 2001)	10-51 (Peterson <i>et al</i> 2015)	--	--

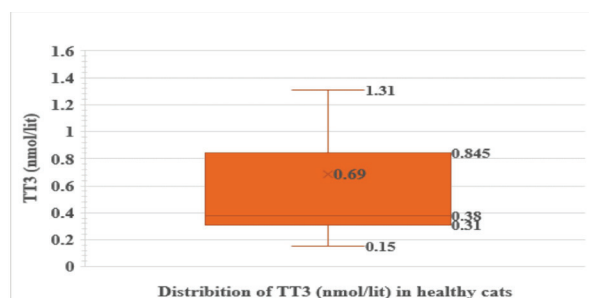


Figure 1: Box Plot of TT3 in healthy cats (n=31)

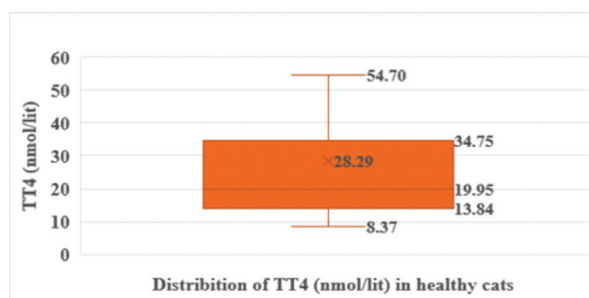


Figure 2: Box Plot of TT4 in healthy cats (n=31)

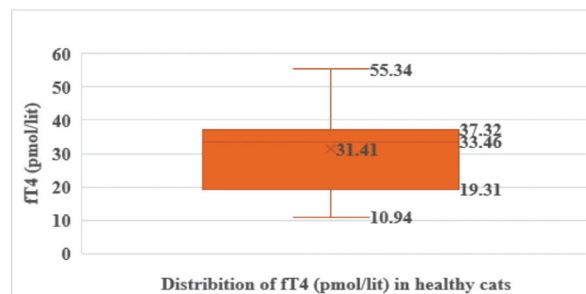


Figure 3: Box Plot of fT4 in healthy cats (n=31)

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Demographic study of Canine Renal Failure in a Tertiary Veterinary Hospital of Bihar, India

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Abstract

The present study evaluated the incidence and demographic distribution of canine renal failure cases presented at the Veterinary Clinical Complex (VCC), Bihar Veterinary College (BVC), Patna, over a six-month period (July–December 2024). Out of 2,975 dogs examined, 700 showed clinical signs suggestive of renal dysfunction, of which 149 cases were confirmed as renal failure through haematological and biochemical investigations, yielding an overall incidence of 5%. The highest prevalence was observed in dogs older than 8 years (36.2%), followed by those aged 5–8 years (30.2%). Breed-wise, Pomeranians (33.5%) were most commonly affected, followed by Labrador Retrievers (28.1%) and German Shepherds (22.1%). Male dogs (52.4%) were slightly more affected than females (47.6%). A clear seasonal trend was noted, with the highest number of cases recorded in September (23.4%).

Keywords: Canine renal failure, breed, clinical signs, incidence, seasonal variation,

Introduction

Renal failure is a life-threatening condition that severely compromises the kidney's essential roles in electrolyte balance, excretion of nitrogenous wastes, and endocrine regulation, ultimately resulting in uremia and systemic complications (Ogobuiro and Tuma, 2023). In dogs, renal failure is among the leading causes of mortality, contributing to approximately 2–5% of clinical presentations worldwide (Lund *et al.*, 1999). In India, the reported incidence of canine renal failure varies from 0.93% (Thade *et al.*, 2019) to 4.84% (Chawla *et al.*, 2020), with regional differences attributed to environmental influences such as endemic leptospirosis, variations in diagnostic facilities, and differing husbandry practices. The present study was undertaken to evaluate the incidence and demographic patterns, age, sex, breed, and seasonal variation of canine renal failure in Patna, with the objective of facilitating early diagnosis and improving clinical management.

Materials and Methods

Dogs diagnosed with renal failure having varying ages, sex, and breed were selected for the study. dogs showing the clinical signs viz. vomiting, anorexia, depression, fatigue, weight loss, weakness, dehydration,

melena, halitosis, diarrhea, oral ulcers, polyuria, polydipsia, lethargy and anemia were initially screened for the diagnosis of renal failure. These were subjected to detailed clinical and laboratory investigations for confirmatory diagnosis. The incidence of renal failure in age group, sex, breed and season was recorded for each dog.

Results

The overall incidence of renal failure among all canine cases presented was 5% (149 out of 2,975 dogs), indicating that renal failure represents a relatively common clinical condition in the canine population.

Sex-based incidence analysis revealed a slight predominance of renal failure in male dogs, with 78 cases (52.4%), compared to 71 cases (47.6%) in females. **Age-wise distribution** showed that dogs older than 8 years exhibited the highest incidence of renal failure, accounting for 54 cases (36.2%), followed by those aged 5–8 years with 45 cases (30.2%). Dogs aged 2–5 years contributed 30 cases (20.1%), while lower incidences were observed in the 1–2-year age group with 15 cases (10.0%), and in dogs younger than one year with only 5 cases (3.3%). Pomeranians was the most affected breed (50 cases; 33.5%), followed by Labrador Retrievers (42 cases; 28.1%) and German Shepherds (33 cases;

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22.1%). **Seasonal distribution** of renal failure cases (n = 149) revealed marked monthly variation, with the highest incidence recorded in September, accounting for 23.4% (35/149) of cases. October followed with 18.1% (27/149) of cases, indicating a sustained high risk during the early post-monsoon period.

Table 1: Sex, Age, Breed wise incidence of renal failure.

Parameter		Number of positive cases (n=149)	Incidence (%)
Sex	Male	78	52.4
	Female	71	47.6
Age	≥1 year	5	3.3
	1-2 year	15	10.0
	2-5 year	30	20.1
	5-8 year	45	30.2
	> 8 years	54	36.2
Breed	Pomeranian	50	33.5
	Labrador retriever	42	28.1
	German Shephard	33	22.1
	Non-descript	7	4.6
	Golden retriever	6	4.0
	St. Bernard	3	2.0
	Beagle	2	1.3
	Bull mastiff	2	1.3
	Pug	2	1.3
	Shih-Tzu	2	1.3

Table 2: Month wise incidence of renal failure

Season	Number of positive cases (n=149)	Incidence (%)
July	23	15.4
August	26	17.4
September	35	23.4
October	27	18.1
November	19	12.7
December	19	12.7

Anorexia and vomiting were the most frequently observed clinical signs; each recorded in 16 out of 18 dogs (88.88%). Diarrhea was noted in 13 dogs (72.22%), whereas halitosis (bad breath) was the least common clinical sign, occurring in 5 out of 18 dogs (27.77%).

Discussion

The present study documented an overall incidence of canine renal failure of 5%, which is comparable with several reports from different regions of India. Chawla *et al.* (2020) reported an incidence of 4.84% in northern India, while Tufani *et al.* (2015) observed a prevalence ranging from 4–6%,

closely aligning with the current findings. Similarly, Karunanithy *et al.* (2019) recorded an incidence of 5.8% in dogs from Bareilly, indicating comparable disease occurrence across geographically distinct regions, including Patna. Age-wise analysis revealed a markedly higher prevalence of renal failure in dogs older than 8 years (36.2%), supporting the established association between advancing age and increased risk of chronic kidney disease (Polzin, 2011). Comparable age-related trends have been reported by Thade *et al.* (2019), and Chawla *et al.* (2020). The progressive and irreversible nature of chronic kidney disease, along with age-associated physiological changes such as declining glomerular filtration rate and tubular function, likely contributes to the higher disease burden in geriatric dogs (Lees *et al.*, 2005). Breed-wise evaluation showed that Pomeranians were the most affected breed, accounting for 33.5% of cases. This finding corroborates earlier reports by Chaitanya *et al.* (2020), and Pathak *et al.* (2023), all of whom reported a higher incidence of renal disorders in this breed. Sex-wise analysis indicated a slight male predominance (52.4%) among dogs affected with renal failure. While Tufani *et al.* (2015) reported a higher prevalence in females, the present findings are consistent with reports by Thade *et al.* (2019), and Chawla *et al.* (2020), who also observed higher incidence in male dogs.

Seasonal analysis revealed a peak incidence during September (23.4%), followed by October (18.1%) and August (17.4%), a similar pattern to that reported by Karunanithy *et al.* (2019). Anorexia and vomiting were the most frequently observed clinical signs, each recorded in 88.8% of affected dogs. These findings reflect the systemic impact of uremic toxicity, wherein accumulation of nitrogenous waste products leads to gastrointestinal irritation and central nervous system involvement (Polzin, 2011). Polyuria and polydipsia, observed in 44.4% of cases, indicated impaired renal concentrating ability, consistent with tubular dysfunction commonly associated with chronic kidney disease.

Acknowledgement

The authors are thankful to Dean, Bihar Veterinary College, Bihar Animal Sciences University, Patna for proving all facilities for this work.

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Platelet-Rich Plasma (PRP) Therapy in Canine Alopecia

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Abstract

The present study was conducted to evaluate the therapeutic efficacy of platelet-rich plasma (PRP) as an adjunct to conventional management in dogs affected with alopecia and to assess the utility of trichogram analysis as a diagnostic and therapeutic monitoring tool. A total of 20 client owned dogs presented with alopecia were divided into two treatment groups and compared with ten healthy controls. Dogs in group I received conventional therapy, while Group II received conventional therapy along with PRP administration once weekly for two consecutive weeks. Haematology, serum biochemistry, and trichogram analysis were performed before and after therapy. Pre-treatment trichogram analysis revealed predominance of telogen hairs along with follicular casts, trichoptilosis, distorted hair shafts, and pigmentary abnormalities, indicating follicular inactivity and impaired hair growth. Post-therapeutic evaluation demonstrated significant clinical improvement in PRP-treated dogs, characterized by earlier hair regrowth, improved coat quality, increased hair density, and a higher proportion of anagen hairs compared to dogs receiving conventional therapy alone. The average recovery time and percentage recovery were superior in the PRP-treated group (9 days and 97%, respectively) compared to the conventional therapy group (17 days and 74%, respectively).

Keywords: PRP, Canine alopecia, Trichogram

Introduction

Alopecia is a common dermatological disorder in dogs characterized by partial or complete loss of hair that occurs due to inflammatory or non-inflammatory aetiologies. Non inflammatory alopecia primarily results from hair cycle arrest and also inadequate production of new hair with diverse causes, making accurate diagnosis essential for successful management. Among the various diagnostic modalities used for dermatological examination, trichography stands out as a valuable, noninvasive diagnostic tool for assessing hair shaft abnormalities, follicular activity and hair growth cycles in the diagnosis of canine alopecia. In recent years, Platelet Rich Plasma (PRP) therapy is emerging as a promising regenerative therapy in human as well as Veterinary practice. The present study documents the efficacy of PRP in alopecic dogs and to assess trichogram findings as indicators of therapeutic response.

Materials and Methods

The present study was conducted on client owned dogs presented to Teaching Veterinary Clinical Complex, NTR College of Veterinary Science, Gannavaram with signs of alopecia were screened for inclusion in the study. A detailed clinical evaluation, skin

scrapings examination, flea comb test, skin cytology, diet exclusion trials were carried out to determine the etiology. The selected cases were subjected to trichographic examination for assessment of hair shaft morphology, follicular activity, and hair growth cycle abnormalities. Hematological and biochemical investigations were performed to rule out underlying systemic disorders associated with alopecia. A total of 20 dogs were selected for the study and were randomly divided into two groups. Group I (n = 10) received conventional therapy comprising antiparasitic treatment, nutritional support and supportive care. Group II (n = 10) was treated with conventional therapy with platelet-rich plasma (PRP) therapy, administered once weekly for two consecutive weeks. Group III i.e., healthy control group (n = 10) consisted of clinically normal dogs used for establishment of reference values. Platelet rich plasma was prepared using standardized double centrifugation technique under aseptic conditions. Five to ten millilitres of venous blood was collected aseptically into sodium citrate anticoagulant (9:1 ratio). The sample was subjected to two-step centrifugation process: a soft spin @1000 rpm for 10 minutes (Fig. 1) followed by a hard spin @1600 rpm for 10 minutes (Fig 2) which yields platelet poor plasma (PPP) and platelet rich plasma (PRP). Trichogram analysis was performed before and after therapy to determine the percentage of anagen, catagen, and telogen hairs along with hair shaft

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abnormalities. Therapeutic response was assessed based on clinical improvement, extent of hair regrowth, and

changes observed in trichogram findings during the post-treatment period.



Figure 1: First centrifugation

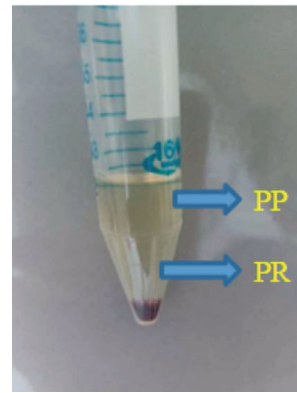


Figure 2: Second centrifugation

Results and Discussion

Haematological and serum biochemical parameters showed no significant alterations in either of the therapeutic groups when compared with the control group dogs, indicating the absence of major systemic metabolic disturbances associated with the condition. Trichogram analysis of dogs affected with non-inflammatory alopecia prior to initiation of therapy revealed a predominance of telogen phase hairs, suggestive of hair cycle arrest and reduced follicular activity. In addition, several microscopic abnormalities including follicular casts, trichoptilosis, distorted hair shafts, and irregular hair pigmentation were observed. These findings were indicative of impaired follicular health and possible nutritional or metabolic deficiencies. In certain cases, trichographic examination also revealed features suggestive of demodicosis, characterized by perifollicular debris and structural damage to hair shafts (Fig 3 and 4).

Post-therapeutic evaluation demonstrated marked improvement in trichographic parameters,

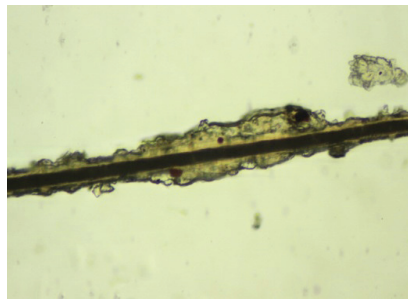
particularly in dogs treated with Platelet-Rich Plasma (PRP) therapy showing higher percentage of anagen phase compared to conventional treatment. A progressive increase in the proportion of anagen phase hairs along with a corresponding reduction in telogen hairs was recorded during the follow-up period in animals treated with PRP (Fig.5). The average time taken and percentage of dogs recovered in group I was 17 days and 74 % respectively while in group II the same was recorded as 9 days and 97 per cent reflecting a superior response with PRP. The reappearance of healthy anagen hairs reflected reactivation of dormant hair follicles and restoration of normal follicular cycling. Furthermore, reduction in follicular casts, improvement in hair shaft integrity, and increased hair density were observed in successfully treated cases. Clinically, these changes were associated with visible hair regrowth, improved coat quality, and reduction in the extent of alopecic lesions. The observed shift in hair cycle dynamics suggested a favourable therapeutic response and highlighted the regenerative potential of PRP in the management of canine non-inflammatory alopecia.



Trichoptilosis



Hair shaft abnormality



Follicular casts



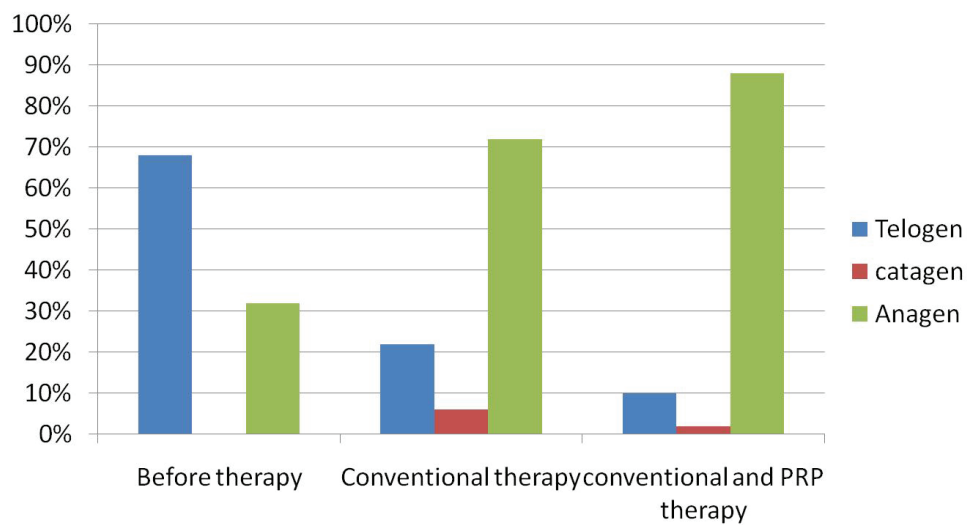
Telogen phase

Fig. 3 Pretherapy trichogram analysis in dogs with non-inflammatory alopecia

Pretreatment



Post treatment

Fig. 4. Pretherapy and Post therapeutic comparison of skin lesions in dogs treated with PRP**Fig. 5. Trichogram analysis**

PRP-derived growth factors may enhance dermal papilla cell activity, increase perifollicular vascular supply, reduce follicular apoptosis, and prolong the anagen phase (Manole et al., 2023). The present findings are in agreement with the regenerative principles described by **Foster et al. (2009)**, who emphasized the clinical potential of PRP in tissue healing. Similarly, **Marx (2004)** and **Anitua et al. (2004)** documented the reparative and regenerative potential of PRP in medical applications. In veterinary medicine, **Jee et al. (2016)** demonstrated beneficial effects of autologous PRP on cutaneous wound healing in dogs, supporting the broader dermatological applications of PRP. Future studies should therefore include larger cohorts, standardized clinical scoring systems, objective trichographic quantification, histopathological correlation, and longer follow-up periods to better evaluate the durability of hair regrowth and recurrence rates.

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Quadriplex PCR assay for diagnosis of haemoprotozoan diseases in bovines

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Abstract

The present study was conducted to standardize a quadriplex PCR assay for rapid, sensitive, specific and simultaneous diagnosis of major haemoprotozoan diseases of bovines. Primers targeting *cytochrome b* gene, repetitive nucleotide sequences, gene encoding carbomyl phosphate synthetase II and small subunit rRNA were used for detection of *Trypanosoma evansi*, *Theileria annulata*, *Babesia bovis* and *Babesia bigemina* generating amplicon of 257bp, 312bp, 446bp and 689bp, respectively. Multiplex PCR assay was optimized in a final volume of 25 µl containing 12.5 µl dream taq green master mix (2X), 0.5 µl of each cyto b1 primer, 0.4 µl of each TR3/TR4 primer, 0.4 µl of Bb1/Bb2 primer and 0.6 µl of Bg3/Bg4 primer, 4µl of template DNA and rest NFW. Twenty DNA samples extracted from field blood samples were screened by quadriplex PCR assay. Primers were found specific as did not produce amplicon with non-target DNA. Limit of detection was determined using tenfold serial dilution of parasitic DNA. Quadriplex PCR assay could able to amplify DNA of *Theileria annulata* and *Babesia bigemina* at copy number 1000 whereas of *Babesia bovis* and *Trypanosoma evansi* at copy number 100, respectively. Out of 20 samples, *T. annulata* was seen in 15% sample, *B. bigemina* and *B. bovis* in 5 % sample and *B. bigemina* + *T. annulata* in 10% samples, rest sample were negative. Assay was found to be highly specific and can be used for simultaneous diagnosis of major haemoprotozoan diseases.

Keywords: Babesia, haemoprotozoan disease, quadriplex PCR assay, Theileria, trypanosoma

Introduction

In India, economic losses due to ticks and tick borne diseases have been projected to be Rs 3873.06 crores annually (Narladkar *et al.*, 2018). Both Giemsa staining and serological assay methods have limitation that they cannot diagnose disease in sample having low parasitemia and carrier state. Serological assay failure to differentiate between past and present infections and can result in false positive result further leading to economic loss to farmer (Sumbria *et al.*, 2015).. PCR assays can detect the parasite within 6 hours after blood collection with higher sensitivity and specificity (Charaya *et al.*, 2016). The present study describes the standardization of a novel multiplex PCR assay for simultaneous detection of *T. annulata*, *B. bigemina*, *B. bovis* and *T. evansi* in bovine blood samples and evaluates its use in detecting parasites from field samples.

Materials and Methods

Five microlitre blood samples positive for respective haemoprotozoans by conventional microscopic technique were collected from jugular

vein in EDTA vials and used as positive controls. DNA from these samples were extracted using pure link DNA extraction kit (Invitrogen, USA) as follows. To 200µl blood sample, 200µl alkaline lysis buffer is added alongwith 20µl proteinase K, mix and incubated at 55°C for 30 minutes. To the mixture 200µl alcohol was added and after thorough mixing the content is transferred to spin column. Spin column was centrifuged at 12000rpm for minutes and column is transferred to new collection tube and 500 µl of wash buffer 1 was added and again centrifuged at same rate and time. Two washing with 500 µl of wash buffer 2 were done and empty column was again centrifuged at same rate and time. Finally DNA was eluted in 30 µl elution buffer. DNA obtained was stored at -20 °C till further used.

Details of oligonucleotide primers used in standardization of multiplex PCR assay has been mentioned in table 1. Primer melting temperatures (T_m) and the potential for self-annealing, i.e. hair-pin loop and primer-dimer formation, were bioinformatically tested using oligonucleotide analyser software and finally primers were procured from Sigma Company. Multiplex PCR assay was standardized using variable conditions of annealing temperature (gradient PCR

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assay), primer concentrations, number of PCR cycles and amount of DNA as template. Reaction conditions comprised an initial denaturation step of 95 °C for 2 min followed by 35 cycles of denaturation (95 °C for 30 s), primer annealing (gradient for 30 s) and extension (72 °C for 1min). A final extension at 72 °C for 10min was performed. For each reaction, 10 µl of PCR product was analysed by gel electrophoresis on a 2% agarose gel, as described above.

To determine the detection limit of the simplex PCR, copy number was calculated as follows. Average mass of nucleotide was taken as 650 daltons meaning one mole of nucleotide weighs 650g. This means 650g contains Avogadro number of nucleotide or 1g of nucleotide contains Avogadro number /650 nucleotide (Factor A). Amount of DNA was estimated using nanodrop and multiplied with factor A to get number of nucleotide in sample (Factor B). Finally Factor B is divided by length of product to get the number of copy number. For determination of sensitivity of Multiplex PCR assay equal amount (10^{10}) of copy number of *B. bovis*, *B. bigemina*, *T. annulata* and *T. evansi* was added in a mixture and ten fold serial dilution was prepared. Multiplex PCR assay was run using optimised conditions. Twenty samples were collected from a cross bred dairy herd for determining the utility of quadruplex in detecting the parasites. DNA was extracted and subjected to multiplex PCR assay. Simplex PCR assay for detection of individual parasites was employed on all the samples to compare the sensitivity of simplex PCR assay with that of quadruplex PCR assay. Microscopic examination of stained blood smear prepared from all the animals were examined and results were compared with both simplex and quadruplex assay.

Results and Discussion

Multiplex PCR assay standardized using published primer set in present study can simultaneously detect these diseases within 6 hour after receiving the samples. Assay was standardized using gradient PCR assay for simultaneous detection of *Trypanosoma evansi*, *Theileria annulata*, *B. bigemina* and *Babesia bovis* could produce amplicons of 257 bp, 312 bp, 689bp and 446 bp, respectively at 55 °C annealing temperature as shown in in Fig. 1 without any non specific amplification. The optimised multiplex PCR was performed in a final volume of 25 µl containing 12.5 µl dream taq green master mix (2X), 0.5 µl of each cyto1 primer, 0.4 µl of each TR3/TR4 primer, 0.4 µl

of Bb1/Bb2 primer and 0.6 µl of Bg3/Bg4 primer, 4µl of template DNA and rest NFW. Gradient PCR assay result showed clear distinguished bands of all four parasite from 52 to 58°C and best at 55.8°C (Fig 1). Reaction conditions comprised an initial denaturation step of 95 °C for 2 min followed by 35 cycles of denaturation (95 °C for 30 s), primer annealing (55 °C for 30 s) and extension (72 °C for 1min). Bands were easily differentiated on gel electrophoresis. Primers were found specific as did not produce amplicon with non target DNA. Fragments of expected size were generated from template DNA representing *T. annulata*, *T. evansi*, *B. bovis* and *B. bigemina* isolates using each of the species-specific primer sets, while non-target species DNA displayed no evidence of fragment amplification as shown in fig. 2. This indicated that each primer set is capable of specifically amplifying the target locus in a single PCR reaction. Sensitivity of quadruplex PCR assay was checked by ten fold serial dilution of DNA mix containing equal copy number of all the parasites. Quadruplex PCR assay could able to amplify DNA of *Theileria annulata* and *Babesia bigemina* at copy number 1000 whereas of *Babesia bovis* and *Trypanosoma evansi* at copy number 100, respectively as shown in Fig 3. Quite similar to our study, Ashuma *et al.*, 2014 developed and evaluated multiplex PCR assay for diagnosis of three parasites viz. *Theileria annulata*, *Trypanosoma evansi* and *Babesia bigemina* whereas in contrast we have standardized multiplex PCR assay with added parasitic identification *Babesia bovis*. Kundave *et al.*, 2018 developed and evaluated multiplex PCR assay for simultaneous detection of *Theileria annulata*, *Babesia bigemina* and *Anaplasma marginale* infections in bovines using different sets of primers and different species of parasites. On analyzing twenty DNA samples by quadruplex PCR assay, it was found it could detect mixed infection in 3 samples. Out of 20 DNA samples, *T. annulata* was seen in 15% sample, *B. bigemina* and *B. bovis* in 5 % sample and *B. bigemina* + *T. annulata* in 10% samples, rest sample were negative. Microscopic analysis revealed 4 samples positive for *Theileria annulata* and 3 samples positive for *Babesia* sp. None of the samples were found positive for *Trypanosoma evansi*. *Theileria annulata* was detected in 7 samples (35%) by simplex PCR assay whereas only 6 samples by multiplex PCR assay. The sample found positive for *B. bigemina* + *T. annulata* was also found positive microscopically for both parasites and by PCR assay. No disparity in results of simplex PCR assay and quadruplex PCR assay was observed. None of the samples were found

positive for *Trypanosoma evansi* by either simplex or multiplex PCR assay. The combination of parasites detected and primer combination used in the present study is novel for development of multiplex PCR assay.

Multiplex PCR assay was found to be highly specific and can be used for easy, early, sensitive, specific and simultaneous diagnosis of haemoprotozoan diseases in epidemiological survey as a robust tool.

Table 1: Details of Primer used for detection of parasites DNA

Parasite	Targeted gene/ sequences	Sequence	Product size	Reference
T. annulata	Cytochrome b	F:5'ACTTTGGCCGTAATGTAAAC 3' R:5'CTCTGGACCAACTGTTTGG 3'	312bp	1
T. evansi	Repetitive nucleotide sequences	F:5' GCGCGGATTCTTTGCAGACGA 3' R:5'TGCAGACACTGGAATGTTACT 3'	257bp	2
B. bovis	Gene encoding enzyme carbamoyl phosphate synthetase II	F: 5' TTTGGTATTTGTCTTGGTCAT 3' R: 5' ACCACTGTAGTCAAACCTCACC 3'	446bp	2
B. bigemina	SSU r RNA	F: 5' TAGTTGTATTTTCAGCCTCGCG 3' F: 5' AACATCCAAGCAGCTAHTTAG 3'	689bp	2

1 Bilgiç *et al.* (2013); 2; Ananyutthawongese *et al.* (1999)

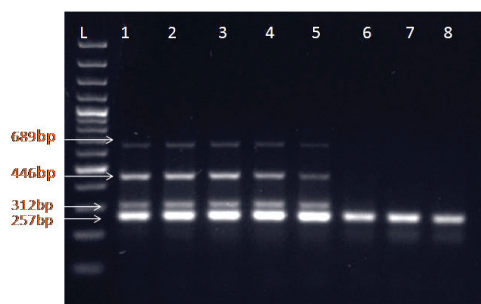


Fig. 1. Agarose gel electrophoresis results of gradient quadriplex PCR assay for simultaneous detection of *Theileria annulata*, *Babesia bovis*, *Babesia bigemina* and *Trypanosoma evansi*. L: 100bp ladder (New England biolab, USA); Lane 1: 52°C; 2: 52.7°C ; 3: 53.9°C; 4: 55.8°C; 5: 58.1°C; 6: 60°C; 7: 61.2°C; 8: 62°C

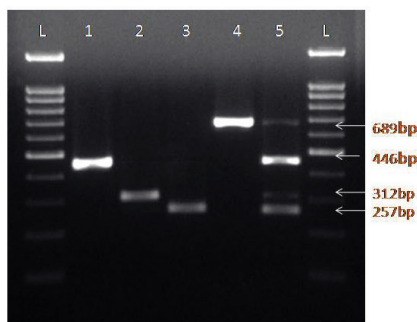


Fig. 2. Agarose gel electrophoresis results showing specificity of Quadriplex PCR assay with known positive DNA samples. L: 100bp ladder (New England biolab, USA) ; Lane 1: positive for *B. bovis*; Lane 2: Positive for *T. annulata*; Lane 3: Positive for *T. evansi*; Lane 4: Positive for *B. bigemina*; Lane 5: 106 dilution of stock solution prepared for checking multiplex sensitivity

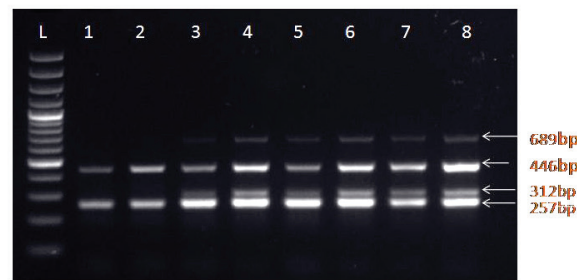


Fig. 3. Agarose gel electrophoresis results for determination of sensitivity of quadruplexPCR assay for simultaneous detection of *Theileria annulata*, *Babesia bovis*, *Babesia bigemina* and *Trypanosoma evansi* at 55 °C annealing temperature. L: 100bp ladder (New England biolab, USA) ; Lane 1 to 8 : Ten fold serial dilution from 10^1 to 10^8

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Studies on thyroid dysfunction in dogs using radioimmunoassay

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Abstract

Fifty-nine clinically healthy dogs, 35 dogs with non-thyroidal illness and 13 suspected clinically hypothyroid dogs were included in the study. Reference ranges were established for thyroid hormone concentrations (TT4, TT3 and fT4) from 59 clinically healthy dogs by RIA. Blood samples were collected from 107 dogs (59 Healthy, 35 Non-thyroidal illness and 13 Hypothyroid) and assessed for thyroid hormone concentration using RIA. Mean (range) values established for TT4, TT3 and fT4 in the present study as 29.67±1.43 nmol/l (11.71-49.26 nmol/l), 1.03±0.02 nmol/l (0.68-1.40 nmol/l) and 9.07±0.52 pmol/l (4.06-18.72 pmol/l) respectively. In geriatric age group thyroid hormone concentration decreased compared to younger dogs. There was non-significant effect of sex on thyroid hormone concentration. TT4 concentration of dogs on homemade diet was lower compared to dogs on commercial and mixed type of diet. Dogs clinically suspected with hypothyroidism had low concentration of thyroid hormone (TT4-18.46±3.20, TT3-0.56±0.07, fT4- 5.68±1.24) as compared to dogs with non-thyroidal illness (TT4-21.78±1.85, TT3-0.85±0.05, fT4-7.80±0.69) and clinically healthy dogs (TT4-29.67±1.43, TT3-1.03±0.02, fT4-9.07±0.52).

Keywords: Thyroid, Radioimmunoassay, TT4, TT3 and fT4

Introduction

Primary hypothyroidism occurs due to either lymphocytic thyroiditis, idiopathic thyroid atrophy, neoplasia or immune destruction of thyroid follicular cells. RIA is considered as the best assay to estimate hormones as compared to Enzyme-linked immunosorbent assay (ELISA) and CLIA. Analysis of animal samples by CLIA method yielded lower concentration of T₄ compared to RIA (Singh et al., 1997). The specificity, reproducibility and sensitivity of thyroid testing is more in RIA and has ability to estimate small molecule in biological samples (Goldsmith, 1975). The present study was planned with an objective to establish data of TT₄, TT₃ and fT₄ in clinically healthy dogs (n=59) using RIA.

Material and Methods

Present study was approved from Institutional Ethics Committee for clinical research (Project Approval

No: IEC-VCR/2018/10 Dated: 26.02.2018) and Institutional Bio-safety Committee (Project Approval No: IBSC, Resolution No: 2.2; Dated:15.12.2017) of Mumbai Veterinary College, Mumbai, Maharashtra Animal and Fishery Sciences University, Nagpur. Maharashtra, India. Total 59 healthy dogs referred to Bai Sakrabai Dinshaw Petit Hospital, Mumbai, Parel-12 (M.S, India) and Teaching Veterinary Clinical Complex (Goregaon and Parel) Mumbai (M.S, India) were selected. Healthy dogs were assessed on the basis of anamnesis, routine clinical examination, and assessment of hemato-biochemical profile. Total 59 healthy dogs, 35 dogs with non-thyroidal illness and 13 suspected hypothyroid dogs were included in the study after due consent from their owners. Age of 59 healthy dogs ranged from 1-12 years (Median=2 years) and Weight from 1.6-45 Kg (Median=24 kg). Among 59 apparently healthy dogs, 35 were males and 24 were females. To appraise hormonal status in non-thyroidal illness and hypothyroidism, thyroid hormone profile was also studied in 35 non-thyroidal illness dogs (altered complete

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blood count, liver function test, kidney function test and lipid profile) and 13 clinically hypothyroid dogs (symptoms of lethargy, bilateral alopecia, rat-tail and obesity). For estimation of hormonal status, ready to use RIA kits supplied by Board of Radiation and Isotope Technology (BRIT, Government of India) was used. For TT_4 and TT_3 methods described by manufacturer were followed. However, for fT_4 partial modification was undertaken (One extra wash before addition of tracer to remove traces of proteins) by keeping rest of procedure as per methods described by manufacturer.

Results and Discussion

Mean concentration of TT_4 , TT_3 and fT_4 of healthy dogs were 29.67 ± 1.43 , 1.03 ± 0.02 nmol/l and 9.07 ± 0.52 pmol/l respectively. Established reference range for TT_4 , TT_3 and fT_4 concentration was 11.71-49.26 nmol/l, 0.68-1.40 nmol/l and 4.06-18.72 pmol/l respectively. Mean and range concentrations of TT_4 , TT_3 and fT_4 hormone in study was found to be in accordance with internationally published reference ranges depicted in Table 1, Table 2 and Table 3 respectively. Age and sex wise changes in thyroid profile in healthy dogs is shown in Table 4 and Table 5. Non-significant ($p < 0.05$) difference was recorded in concentration of TT_4 , TT_3 and fT_4 in all age groups. A trend of gradual increase in thyroid hormone concentration was recorded from age group 1-3 years to 6-9 years. Moreover, after 9 years, there was non-significant decrease in concentration of thyroid hormone. This suggests that in older age there is decrease in thyroid hormone concentration. A non-significant ($p < 0.05$) difference was recorded in concentration of thyroid hormone in males (TT_4 - 29.70 ± 1.90 ; TT_3 - 1.02 ± 0.04 and fT_4 - 8.61 ± 0.61) and females (TT_4 - 29.65 ± 2.23 ; TT_3 - 1.06 ± 0.03 and fT_4 - 9.74 ± 0.94). This suggests that thyroid hormone concentration does not changes as sex differs.

Diet wise thyroid profile is presented in Table 6. The concentration of TT_4 for dogs fed on homemade diet, commercial diet and mixed diet were 21.88 ± 2.08 nmol/l, 31.15 ± 2.57 nmol/l and 34.23 ± 1.94 respectively. As far as diet wise status of thyroid profile is concerned, a significant ($p < 0.05$) difference was recorded in TT_4 concentration, however TT_3 and fT_4 concentration differed non-significantly ($p < 0.05$). Median baseline serum TT_4 concentration of hypothyroid dogs (19.31 nmol/l) was lower than value of clinically healthy dogs (28.09 nmol/l) and dogs with non-thyroidal illness (21.88 nmol/l). Mean (Range) TT_4 concentration of non-thyroidal illness and hypothyroid

dogs was found to be 21.78 ± 1.85 nmol/l (2.28-40.86) and 18.46 ± 3.20 nmol/l (6.89-44.91). TT_4 concentration of healthy dogs (29.67 ± 1.43) was significantly higher ($p < 0.05$) than hypothyroid dogs (18.46 ± 3.20) however nonsignificant difference ($p < 0.05$) was recorded in dogs with non-thyroidal illness (21.78 ± 1.85) and healthy dogs (Table 7). There was overlap in total thyroxine hormone concentration in healthy dogs, dogs with non-thyroidal illness and hypothyroid dogs. (Fig 1). Median baseline serum TT_3 concentration of hypothyroid dogs (0.58 nmol/l) was lower than value of clinically healthy dogs (1.04 nmol/l) and dogs with non-thyroidal illness (0.86 nmol/l). Mean TT_3 (Range) concentration of non-thyroidal illness and hypothyroid dogs was found to be 0.85 ± 0.05 nmol/l (0.31-1.30) and 0.56 ± 0.07 nmol/l (0.19-0.96). TT_3 concentration of healthy dogs (1.03 ± 0.02) was significantly ($p < 0.05$) higher than hypothyroid dogs (0.56 ± 0.07) and significant ($p < 0.05$) difference was recorded in dogs with non-thyroidal illness (0.85 ± 0.05) and healthy dogs (Table 7). Box plot showed difference in thyroid hormone concentration in healthy dogs and hypothyroid dogs. (Fig 2). Median baseline serum fT_4 concentration of hypothyroid dogs (4.12 pmol/l) was lower than value of clinically healthy dogs (7.97 pmol/l) and dogs with non-thyroidal illness (6.95 pmol/l). Mean fT_4 (Range) concentration of dogs with non-thyroidal illness and that in hypothyroid dogs was found to be 7.80 ± 0.69 pmol/l (3.73-14.79) and 5.68 ± 1.24 pmol/l (0.49-13.77). fT_4 concentration of healthy dogs (9.07 ± 0.52) was significantly higher than hypothyroid dogs (5.68 ± 1.24) however non-significant difference ($p < 0.05$) was recorded in dogs with non-thyroidal illness (7.80 ± 0.69) and healthy dogs (Table 7). There was overlap in free thyroxine hormone concentration in healthy dogs, dogs with non-thyroidal illness and hypothyroid dogs. (Fig 3)

Study recorded age wise changes in thyroid hormones. TT_4 , TT_3 and fT_4 concentration increased upto 9 years of age whereas it decreased in group of 9-12 years of age. Percent decrease in old age for TT_4 , TT_3 and fT_4 was recorded to be 12.96, 4.00 and 7.94 respectively. This might be because of relationship between increase in glucocorticoids and decrease in serum TT_4 during aging. Possibility is suggested that increase in glucocorticoid may suppress thyroid hormone secretion (Gonzalez & Quadri, 1988). A non-significant ($p < 0.05$) difference was recorded in concentration of thyroid hormone in males and females. The above findings were in accordance to published work which suggests that concentration of thyroid hormones when compared

on the basis of sex without considering specific stage of reproductive cycle has no apparent effect on serum thyroid hormone concentrations (Reimers *et al.*, 1990). Changes in sex can occur with respect to reproductive status as estrogen concentration has effect on Thyroid Binding Globulin (TBG) synthesis (Franklyn, Ramsden, & Sheppard, 1985). Diet wise comparison of thyroid hormone revealed that dogs fed on homemade diet had lower TT_4 concentration than dogs on commercial and mixed diet. Author feels that lower concentration of thyroid concentration in homemade diet could be because of relatively low concentration of total protein, iodine and minerals in homemade diet as compared to commercial diet. Mean TT_4 concentration of non-thyroidal illness and hypothyroid dogs decreased from healthy dogs by 36.23% and 60.73% respectively. Mean TT_3 concentration of non-thyroidal illness and hypothyroid dogs decreased from healthy dogs by 17.47% and 45.63%. Mean fT_4 concentration of non-thyroidal illness and hypothyroid dogs decreased from healthy dogs by 14.00% and 37.37%. This suggests marginal decrease in thyroid hormonal profile in dogs suffering from non-thyroidal illness and maximum decrease in dogs with hypothyroidism as compared to healthy dogs.

Acknowledgement

Funding for this project was provided by central assistance grants available at Mumbai Veterinary College. Authors are thankful to Associate Dean, Mumbai Veterinary College for providing needful facilities.

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Evaluation of Serum Lactate Dehydrogenase in Dogs with Hepatic and Splenic Tumours

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Abstract

The present study was undertaken to evaluate the diagnostic utility of serum lactate dehydrogenase (LDH) in dogs affected with hepatic and splenic tumours. Dogs presented to Madras Veterinary College Hospital, Chennai, with clinical signs suggestive of intra-abdominal neoplasia were subjected to detailed clinical examination, radiography, and ultrasonography. Cytological and histopathological examinations were performed for definitive diagnosis. Serum lactate dehydrogenase (LDH) activity was evaluated in 15 dogs with hepatic tumours, 15 dogs with splenic tumours, and 12 clinically healthy dogs used as controls for comparison. Serum LDH levels were significantly elevated in tumour-bearing dogs compared to healthy controls. However, no significant difference was observed between hepatic and splenic tumour groups. Among the hepatic and splenic tumour groups, dogs diagnosed with cholangiocellular carcinoma and splenic lymphoma, respectively, exhibited comparatively higher serum LDH concentrations than dogs affected with other tumour types. The findings suggest that serum LDH may serve as a useful biomarker for detecting intra-abdominal neoplasia in dogs, although its utility in differentiating tumour location appears limited.

Keywords: Lactate dehydrogenase, Hepatic tumours, Splenic tumours, Dog

Introduction

Lactate dehydrogenase (LDH) is an intracellular enzyme involved in anaerobic glycolysis and is released into circulation following cellular injury, tissue destruction, and neoplastic proliferation. Increased serum LDH activity has been reported in several human and animal malignancies and is considered an indicator of tumour metabolism and burden. Considering the limited information available regarding LDH concentrations in canine hepatic and splenic tumours, the present study was undertaken to evaluate serum LDH activity in dogs with hepatic and splenic neoplasms.

Materials and Methods

The study was conducted on 30 dogs diagnosed with intra-abdominal tumours, comprising 15 hepatic tumour cases and 15 splenic tumour cases presented to the Small Animal Medicine Outpatient Unit of Madras Veterinary College Teaching Hospital, Chennai. Twelve clinically healthy dogs were included as controls. All dogs were subjected to detailed clinical examination, hematobiochemical evaluation, radiography, and abdominal ultrasonography for tumour identification and

localization (Fig 1- 4). Fine needle aspiration cytology (FNAC) was performed under ultrasonographic guidance using a 24-gauge needle attached to a disposable syringe. Aspirated material was smeared onto clean glass slides, air-dried, and stained with Giemsa stain for microscopic examination. For histopathological examination, tissue samples obtained by biopsy or surgical excision were fixed in 10 per cent neutral buffered formalin, processed routinely, sectioned at 4–5 μ m thickness, and stained with haematoxylin and eosin (Fig 5- 6).

Results and Discussion

The mean $\pm$ standard error (SE) of serum lactate dehydrogenase (LDH) concentration in healthy control dogs was 99.67 ± 4.33 U/L, whereas dogs with hepatic tumours and splenic tumours exhibited significantly higher concentrations of 514.67 ± 24.14 U/L and 473.60 ± 32.58 U/L, respectively. Statistical analysis revealed a highly significant difference among the groups at the one per cent level ($P < 0.01$). The ultrasonographic findings in the present study revealed hepatomegaly, splenomegaly, heterogeneous masses, irregular margins, reticulated splenic parenchyma, and metastatic involvement in dogs with hepatic and splenic tumours. These observations are consistent with the findings of Kambala *et al.* (2026), who reported ultrasonography as a sensitive

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imaging modality for the detection and preliminary characterization of splenic neoplasms in dogs. The significant elevation of serum LDH activity observed in tumour-bearing dogs in the present study is consistent with the findings of Marconato *et al.* (2009), who reported significantly higher serum LDH concentrations in dogs with various malignancies compared to healthy controls. Similarly, Goodwin *et al.* (2019) highlighted the central role of lactate metabolism in tumour progression and reported that increased LDH activity is associated with tumour aggressiveness, angiogenesis, metastasis and poor prognosis. The elevated LDH concentrations recorded in dogs with hepatic and splenic tumours may be attributed to increased cellular proliferation, tissue hypoxia, tumour necrosis and membrane disruption

resulting in leakage of intracellular enzymes into circulation. Likewise, Faloppi *et al.* (2014) reported that higher serum LDH concentrations were associated with poorer clinical outcomes in patients with hepatocellular carcinoma and suggested that LDH serves as a marker of tumour burden and biological aggressiveness. Although the mean LDH concentration was numerically higher in dogs with hepatic tumours than in dogs with splenic tumours, the difference was not statistically significant. This finding suggests that serum LDH reflects the presence and metabolic activity of neoplastic tissue rather than the anatomical location of the tumour. Similar observations were reported by Marconato *et al.* (2009), who noted considerable overlap in LDH values among different tumour types, thereby limiting its utility as a tumour-specific diagnostic marker.



Fig. 1. Dog showing distended abdomen



Fig. 2. Dog showing enlarged submandibular lymph nodes



Fig. 3. Radiography revealed radiodense mass in the hepatic /spleen region

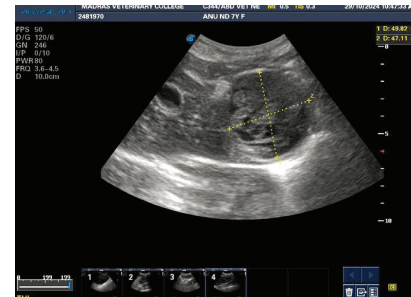


Fig. 4. Ultrasonography revealed heterogenous parenchymal mass in the left lobe of hepatic region.

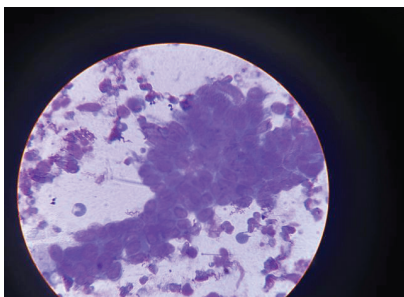


Fig. 5. Cytology of hepatic mass revealed cohesive cluster of epithelial cells with moderate to abundant cytoplasm. Some degree of anisocytosis and anisokaryosis also noticed

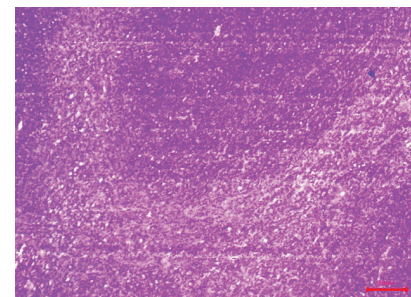


Fig. 6. Histopathology of splenic mass revealed revealed diffuse infiltration and replacement of the normal parenchyma by a dense population of neoplastic lymphoid cells.

Within the hepatic tumour group, dogs diagnosed with cholangiocellular carcinoma exhibited higher serum LDH concentrations than those affected with hepatocellular carcinoma. Similarly, within the splenic tumour group, dogs with splenic lymphoma showed comparatively higher serum LDH concentrations than dogs diagnosed with splenic haemangiosarcoma, histiocytic sarcoma, and haemangioma. This observation is in agreement with the findings of Zanatta *et al.* (2003), who reported significant elevations of LDH isoenzymes in canine lymphoma and observed shorter survival times in dogs with higher LDH concentrations. Furthermore, Bitencourt *et al.* (2026) documented significantly higher LDH levels in dogs with multicentric lymphoma and demonstrated that LDH concentrations decreased substantially following successful chemotherapy, indicating its close association with tumour burden and treatment response. Overall, the significant increase in serum LDH activity observed in dogs with hepatic and splenic tumours supports its potential utility as a marker of tumour-associated metabolic alterations and disease burden, although its value in differentiating tumour type or location appears limited.

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Ultrasonography of coelomic organs in rescued black kites (*Milvus migrans*)

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Abstract

Fourteen rescued kites were examined at centers in Chennai and Ahmedabad between February and September 2024. Each bird underwent general examination and ultrasonographic evaluation using various probes and approaches including ventromedian, ventrocaudal, parasternal, and lateral views. Liver, gallbladder, gizzard, intestines, kidneys, and heart were assessed using ultrasound. Abnormalities such as hepatomegaly, nephromegaly, proventricular dilation, enteritis, and pneumocoelom were identified. The liver, gizzard, and intestines were consistently visualized, while spleen, kidneys, and reproductive organs were less commonly visualized.

Keywords: Avian, black kite, coelomic organs, ultrasonography

Introduction

Black kites or small Indian kites (*Milvus migrans*) (Christie and Ferguson-Lees, 2010) are known to be urban raptors which live in a close proximity with humans. During Uttarayan festival where many Indians fly kites in large numbers, there have been many reports of these black kites getting injured and being presented to veterinarians for their treatment. This study describes the ultrasonographic findings of coelomic organs in rescued black kites.

Materials and Methods

The study was conducted at Madras Veterinary College, Chennai Tamil Nadu, Besant Nagar animal dispensary, Chennai, Tamil Nadu, and Jivdaya Charitable Trust, Ahmedabad, Gujarat during the months of February to September 2024. Permission

was obtained from the Principal Chief Conservator of Forests and Chief Wildlife Warden, Government of Tamil Nadu (Proceeding No: WL5(A)/3066/2024 and Permission No: 35/2024 dated 26.06.2024). Fourteen black kites (*Milvus migrans*) (identified as BK1, BK2 etc.) rescued and brought to the centre, were subjected to general examination followed by the ultrasonography examination. AEROSCAN-250 ultrasound machine at Madras Veterinary College, Chennai, U50 VET ultrasound machine at BMAD, Chennai and SonoSite M-Turbo at JCT, Ahmedabad, Gujarat were used for ultrasonographic examination. The probes used were linear array (8-12MHz), microconvex (7-10MHz) and phased array probe (2-5MHz) and microlinear probe (7-15MHz). The birds were presented either on dorsal recumbency or held in upright position. Some feathers on ventral region were parted or either plucked a bit before applying isopropanol or ultrasound gel for placing of the probe. The ultrasonography examination of the avian coelomic cavity was done as per Garret (2016).

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#part of research submitted by the first author for M.V.Sc in Wildlife Science to Tamil Nadu Veterinary and Animal Sciences University, Chennai – 600 051.



Fig. 2. a) Ventromedian, (b) Ventrocaudal, (c) Parasternal approaches, d) Left lateral and (e) Right lateral approaches.

Results and Discussion

The liver was visualized in all the birds examined and the details are depicted in Fig. 3-8. The liver parenchyma was of average echogenicity compared to the subcutaneous fat which was in agreement with Echols, (2006) and Varshney (2022). The appearance of the liver parenchyma was similar to the mammalian one (Koenhems 2019). Two lobes of the liver were visualized clearly (Da Silva *et al.*, 2024). The gallbladder in association with right lobe of the liver was easy to identify as it was hypoechoic in nature and was in agreement with Krautwald-Junghanns *et al.* (2001) and Da Silva *et al.* (2024). The portal vein in the left lobe was clearly visible due to thick hyperechoic wall and a hypoechoic lumen. Falciform fat appeared to be hyperechoic compared to the echogenicity of the liver but less than that of subcutaneous fats echogenicity. Colour doppler enabled visualization of blood vessels in liver and was in agreement with Pees *et al.* (2006). The liver of BKA1 was enlarged and appeared round and thicker. The echogenicity of the liver was homogenous which was in agreement with Knafo *et al.* (2011) and Koenhems (2019). An engorged hepatic vessel was also visualized in the liver parenchyma as described by previous authors (Fig. 9) (Pees *et al.*, 2006, Cusack *et al.*, 2016 and Dutton *et al.*, 2016). The post mortem examination of this individual confirmed the enlargement of the liver with rounded and thickened borders (Fig 10 and 11). In another black kite BKA8, a congested blood vessel on its long axis with hypoechoic lumen and hyperechoic wall (Figure 12 and 13) were visualized.

Healthy spleen was not visualized in any of the these birds which was in agreement with Da Silva *et al.* (2024). The gizzard was easily identifiable in all black kites and appeared hypoechoic (Fig 14-17) with hyperechoic contents and was in agreement with reports of Dietz *et al.* (1999) and Krautwald-Junghanns *et al.* (2010). Proventriculus was visualized only in two black kites and both were abnormally distended

(Figure 18 -21) Abnormally distended proventriculus could be due to foreign body, neurogenic proventricular dilatation disease (PDD) where massive dilatation of the proventriculus is seen, infection by *Macrorhabdus ornithogaster*, *Streptococcus* spp. or *Escherichia coli* and vitamin A deficiency (Samour, 2015). Mejia-Fava *et al.* (2013) visualized a proventriculus of an umbrella cockatoo (*Cacatua alba*) suffering from proventricular nematodiasis. Intestines with peristalsis with hyperechoic intestinal content (Fig 22 -23) were visualized which was in agreement with Krautwald-Junghanns *et al.* (2010). The empty cloaca was difficult to visualize under ventrocaudal approach. When the cloaca is filled with liquid content the internals could be visualized as oval or roundish with thin regular hypoechoic fluid (Fig 24 and 25).

The normal urogenital tract was not visualized. (Hofbauer *et al.*, 1999) and (Krautwald-Junghanns *et al.*, 2010). The long axis view of the kidneys was unachievable, which may be due to interference by the feathers. No ovaries or egg like structures were visible on ultrasound.

Liver was used as the acoustic window to visualize the heart with the ventromedian and parasternal approach for the black kites as described by Beaufreire *et al.* (2012). The avian heart was similar to that of mammalian heart (Chitty and Lierz, 2008). The visualization of the hypoechoic left ventricle, right ventricle, aorta, left atrium and hyperechoic interventricular septum, was in agreement with Samour (2015). In the three chambered view of heart the two ventricles (the left being larger than the right) along with aorta above the right ventricle was visualized and the findings were in agreement with Krautwald-Junghanns *et al.* (2010). The interventricular septum was hyperechoic in nature whereas the ventricles and the atrium and the aorta were hypoechoic which was in agreement with Rembert *et al.* (2008) and Samour (2015).

Acknowledgments

The authors are thankful to authorities of Principal Chief Conservator of Forests and Chief Wildlife Warden, Government of Tamil Nadu, Besant Memorial Animal Dispensary (BMAD), Chennai, Tamil

Nadu, Jivdaya Charitable Trust, Ahmedabad, Gujarat for their cooperation, permission to use their birds of prey and their veterinarian's assistance for performing this ultrasonography study. The authors are thankful to the Department of Wildlife Science, Madras Veterinary College, TANUVAS for their help.



Fig.3. Visualization of black kite (BKC1) liver and heart and other parts in dorsal positioning with a linear probe (8-12MHz) on the ventromedian approach.

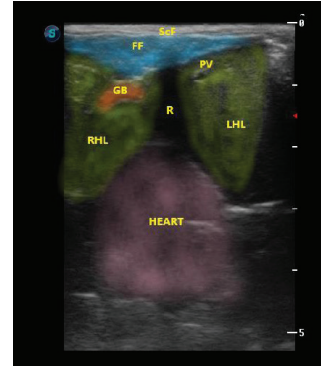


Fig.4. Demarcations highlighting, RHL-Right hepatic lobe, LH-Left hepatic lobe, GB-Gallbladder, PV-Portal vein, R-Ridge, FF-Falciform fat, ScF-Subcutaneous fat and heart.

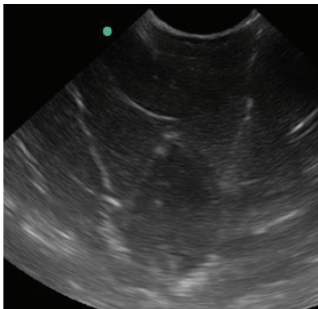


Fig.5. Visualization of black kite (BKA2) liver and heart and other parts in dorsal positioning with a microconvex probe (7-10MHz) on ventromedian approach.

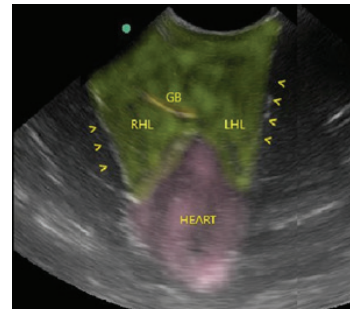


Fig.6. Demarcations highlighting, Normal liver border with (<) sharp edges, RHL-Right hepatic lobe, LHL-Left hepatic lobe, GB-Gallbladder and heart.

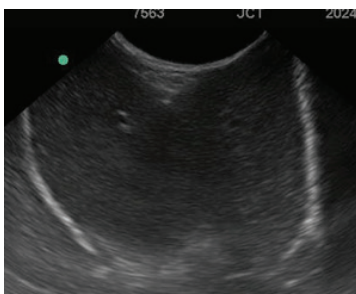


Fig.7. Ultrasound showing hepatomegaly of (BKA1), rounded and thickened liver borders away from each other in dorsal positioning with a microconvex probe (7-10MHz) placed on ventromedian approach.

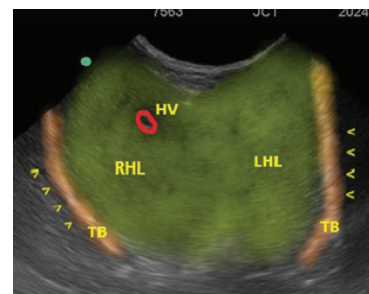


Fig.8. Demarcations highlighting, RHL-Right hepatic lobe, LHL-Left hepatic lobe where both are homogenous, with TB-Thickened borders away from each other marked with (>) and HV-Hepatic vein.

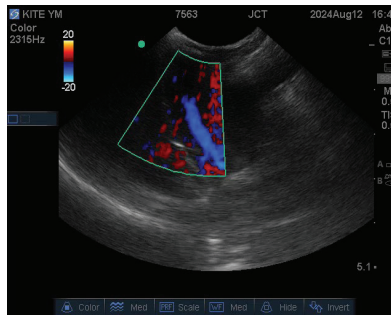


Fig.9. Visualization of engorged blood vessel by colour doppler in the hepatic parenchyma of (BKA1) in dorsal positioning by a microconvex probe (7-10 MHz) with a ventromedian approach

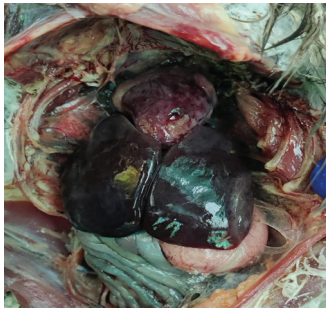


Fig.10. Hepatomegaly confirmed on necropsy of BKA1 as it is almost covering majority of the gizzard and the borders appearing rounded.



Fig.11. Enlarged liver with thickened, rounded border of BKA1

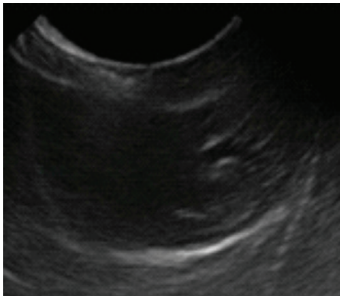


Fig.12. Congested hepatic vessel of BKA8 on longitudinal axis and a slightly enlarged liver visualized in dorsal positioning by a microconvex probe (7-10 MHz) on a ventromedian approach.

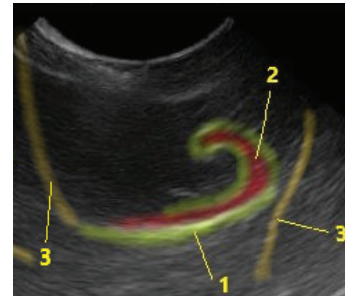


Fig.13. Demarcations highlighting, 1) Hyperechoic wall of the congested vessel, 2) Hypoechoic lumen of the congested vessel and 3) Borders of the enlarged liver away from each other.

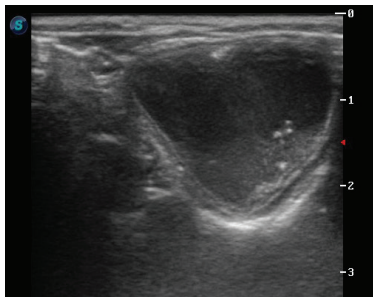


Fig.14. Visualization of gizzard and contents of BKC1 parts in dorsal positioning with a linear probe (8-12MHz) on ventromedian approach.

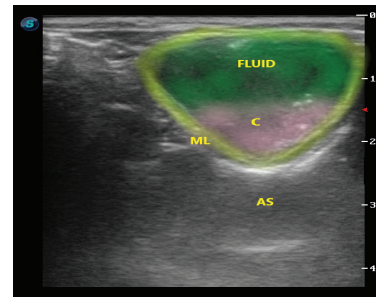


Fig.15. Demarcations highlighting, ML-Muscular layer of gizzard with fluid, C-Contents and AS-Acoustic shadow

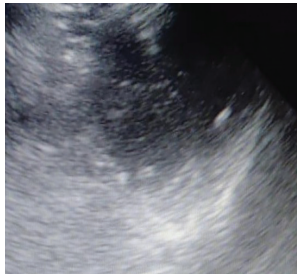


Fig.16. Visualization of gizzard and contents of BKA10 in dorsal positioning with a microconvex probe (7-10MHz) on ventromedian approach.

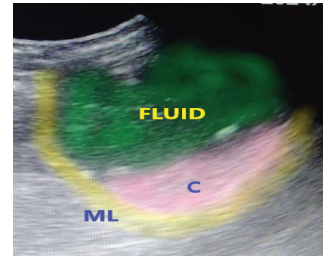


Fig.17. Demarcations highlighting, ML-Muscular layer of gizzard with fluid and C-Contents.

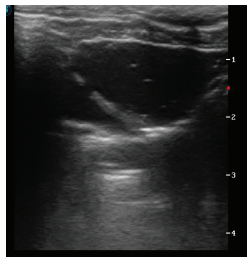


Fig.18. Visualization of proventricular-gizzard junction and contents of BKC1 in dorsal positioning with a linear probe (8-12MHz) on left lateral approach

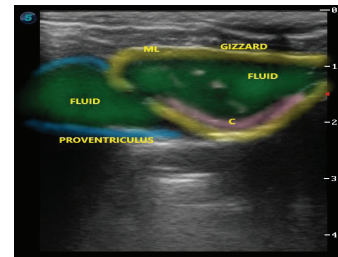


Fig.19. Demarcations highlighting, where proventriculus filled with fluid is indicated in blue and gizzard in yellow, ML-Muscular layer of gizzard with fluid and C-Content

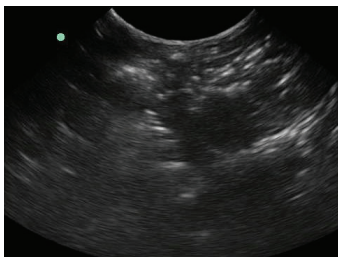


Fig.20. Visualization of the impacted proventriculus and gizzard BKA2 in dorsal positioning with a microconvex probe (7-10MHz) placed laterally on left side

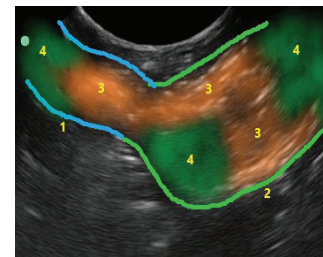


Fig.21. Demarcations highlighting, Proventriculus (1) and gizzard (2) filled with hyperechoic contents (3) and fluid (4)



Fig.22. Visualization of normal intestine of BKA6 on a longitudinal section in dorsal positioning with a microconvex probe (7-10MHz) on ventrocaudal approach

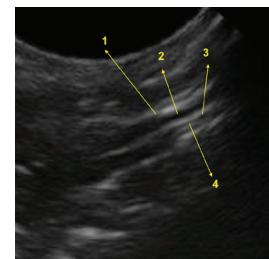


Fig.23. Demarcations highlighting, 1) Serosa, 2) Muscularis, 3) Mucosa and 4) Contents

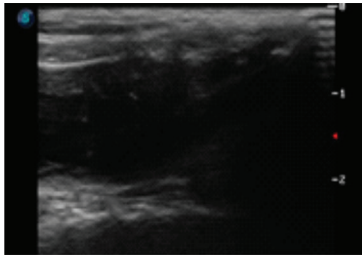


Fig.24. Visualization of normally distended cloaca of BKC1 with fluid in it by dorsal positioning with a linear probe (8-12MHz) directly over it in dorsal presentation

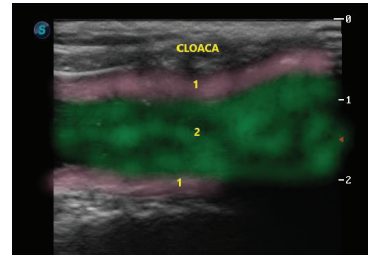


Fig.25. Demarcations highlighting, 1) Mucosa and 2) Fluid

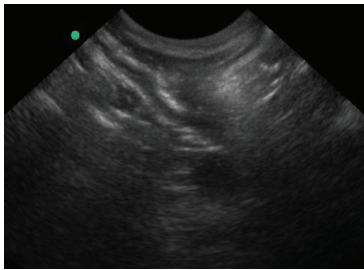


Fig.26. Visualization of inflamed intestinal segment of BKA6 in dorsal positioning with a microconvex probe (7-10MHz) on a ventrocaudal approach

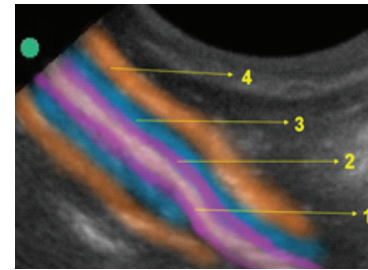


Fig.27. Demarcations highlighting, Arrangement of the intestinal layers as follows 1) Lumen with contents, 2) Mucosa, 3) Muscularis and 4) Serosa (Thickened)

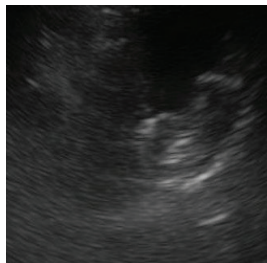


Fig.28. Visualization of enlarged kidneys (both) of BKA9 in cross section in association with renal fossa in dorsal positioning with a microconvex probe (7-10MHz) on ventrocaudal approach

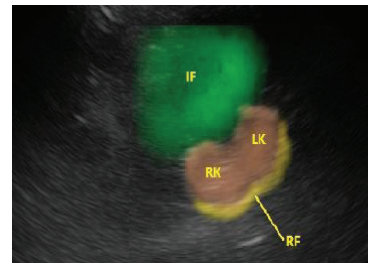


Fig.29. Demarcations highlighting, RK-Right kidney, LK-Left kidney both of them lying in the renal fossa (RF) with hypoechoic Intestinal fluid (IF)



Fig.30. Visualization of horizontal apical four chamber view of BKC1 heart in association with liver in upright position with a microconvex probe (3.6-12.8 MHz) on ventromedian approach

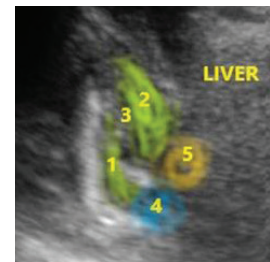


Fig.31. Demarcations highlighting, 1) Right ventricle, 2) Left ventricle, 3) Inter ventricular septum, 4) Aorta and 5) Left atrium and liver

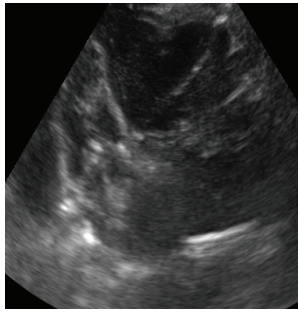


Fig.32. Visualization of parasternal apical three chamber OF BKC1 heart view in upright position by placing the microconvex probe (3.6-12.8MHz) parasternally behind the last rib



Fig.34. Poor visualization of heart of BKA1 in association with liver in dorsal positioning with a microconvex probe (7-10MHz) on ventromedian approach

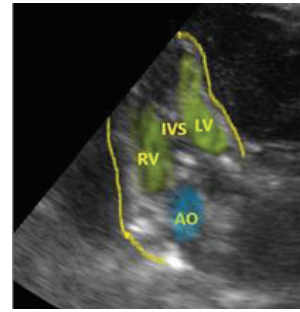


Fig.33. Demarcations highlighting, RV-Right ventricle, LV-Left ventricle, IVS-Inter ventricular septum and AO-Aorta

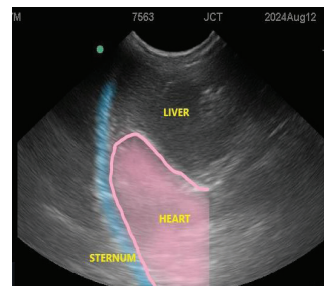


Fig.35. Demarcations highlighting, Heart lying on sternum in association with liver

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Myxomatous Mitral Valve Disease in a German Shepherd Dog

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Abstract

A four-year-old male German Shepherd dog was presented to the Small Animal Clinics, GADVASU, with the primary complaint of exercise intolerance for the past 3-4 days and chronic weight loss. Physical examination revealed poor body condition and dull mentation, fever and tachycardia. Conjunctival mucous membranes and palpation of lymph nodes were normal. The animal was subjected to blood sampling, thoracic radiography, electrocardiography and echocardiography. Haemoglobin was 14.7g% and TLC was 13,900 cu mm, showing absolute neutrophilia. Thoracic radiography showed heart occupying 83% of chest in ventral-dorsal view, suggestive of cardiac enlargement. Electrocardiography revealed an extremely rapid rhythm with a heart rate of 300 beats per minute, indicating emergency condition. Echocardiography revealed left atrium and ventricle enlargement, along with mild flattening of mitral valve and mitral regurgitation. Values of Ao, LA and their ratio (LA/Ao) were 1.80, 4.99 and 2.72 respectively. Based on the findings, the case was diagnosed for Myxomatous Mitral Valve Disease. The dog was treated with digoxin 250mcg and diltiazem 45 mg twice a day for first two days to control arrhythmia. Further treatment was continued with tab pimobendan and enalapril at the dose of 5mg twice a day. The dog was telephonically reported to be stable after 14 days.

Keywords: MMVD; German Shepherd; echocardiography; arrhythmia; mitral regurgitation

Myxomatous mitral valve disease (MMVD) is a chronic degenerative disorder affecting the atrioventricular valves, most commonly the mitral valve complex (Olsen *et al.*, 2010). It is one of the most common acquired heart diseases in dogs. The disease may affect any breed but is often observed in small-breed dogs particularly Cavalier King Charles Spaniels (Keene *et al.*, 2019). The exact etiology of MMVD remains unknown; however, the marked breed predisposition suggests a genetic basis for the disease (Burchell *et al.*, 2014). This article present Myxomatous mitral valve disease in a German Shepherd dog.

A four-year intact male German shepherd dog was presented to Small Animal Clinics, GADVASU with a history of exercise intolerance and inappetence for the past 3-4 days. Progressive weight loss from past one month was also noted. The general physical examination revealed poor body condition and dull mentation. Conjunctival mucous membranes were pale pink. Rectal temperature was noted as 104°F. The patient had arrhythmia and tachycardia on heart auscultation. Peripheral lymph nodes were normal on palpation. The animal was subjected to thoracic radiography in right lateral view and ventrodorsal view. Radiography

showed a globoid heart with cardiothoracic ratio > 83% along with VHS as 11 and sternal contact as 2.5 ICS (Fig 1.1 & 1.2)

Haematological findings revealed haemoglobin of 14.7g% and absolute neutrophilia. Electrocardiography showed heart rate of 300bpm, P waves were not associated with QRS complexes, and wide, bizarre QRS complexes were present. All the observations were suggestive of Ventricular Tachycardia (Fig 2).

2D echocardiography revealed enlargement of the left atrium and left ventricle, and mild flattening of mitral valves. Mitral valve regurgitation was also observed on colour doppler imaging. M-mode echocardiography revealed LA/Ao (Fig 3.2) was 2.72. The ejection fraction was 55.5%, and fractional shortening was 20.34%.

The dog was managed for ventricular tachycardia by digoxin (250 mcg bid) and diltiazem (45 mg bid) for 3 days initially. Further, treatment was continued with pimobendan (5mg bid) and enalapril (5 mg bid) for 15 days. Improvement in exercise intolerance and lethargy was reported telephonically after 15 days of treatment.

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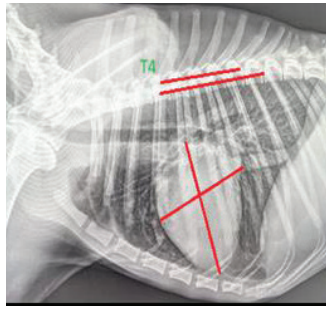


Fig 1.1

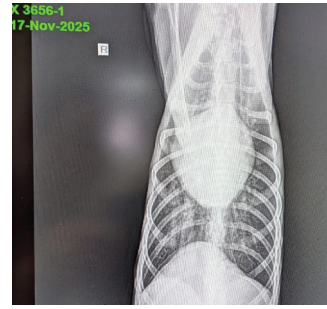


Fig 1.2

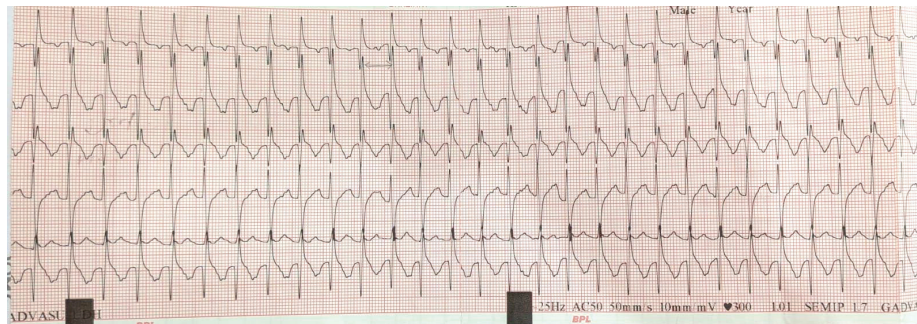


Fig 2

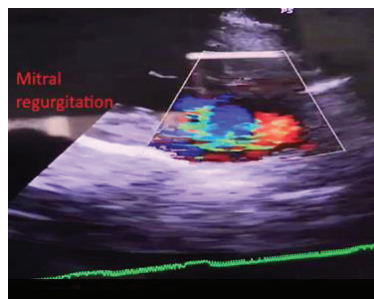


Fig 3.1

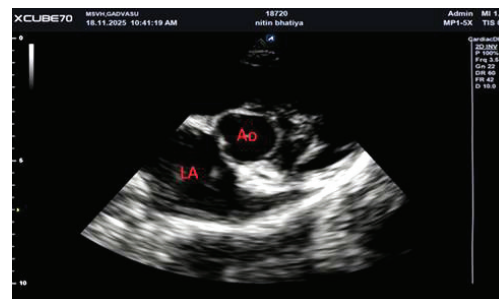


Fig 3.2

Degenerative mitral valve disease represents the predominant acquired cardiac disorder affecting elderly small-breed dogs. (Menciotti & Borgarelli, 2017) however, unlike typical cases, a 4-year-old large-breed German Shepherd dog was diagnosed with the condition highlighting that MMVD can also occur in young to middle age large breed dogs. Although arrhythmias are not a routine feature of myxomatous mitral valve disease (MMVD), their presence in large-breed dogs is of particular clinical significance. They may indicate more advanced myocardial involvement and are often associated with accelerated disease progression, which is consistent with the generally poorer prognosis observed in large breeds (Atkins *et al.*, 2009) While MMVD in large-breed dogs is typically associated with faster progression and a higher risk of developing

decompensated congestive heart failure, prognosis is not determined by breed alone. Factors such as the stage of disease at diagnosis, the extent of cardiac remodeling, and the response to medical therapy play crucial roles in determining outcome. The favourable recovery observed in this case may therefore be attributed to early diagnosis and timely initiation of appropriate therapeutic management prior to the onset of advanced congestive heart failure

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Received : 12.05.2026

Accepted : 21.06.2026

Published : 30.06.2026

Therapeutic Management of *Anaplasma bovis* Infection and Hypophosphatemia in a Cross Breed Cattle

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Abstract

An eight-year-old Holstein Friesian cow in eight months of pregnancy was presented with the history of inappetence to anorexia and decreased rumination from last seven days with clinical signs such as anorexia, tachycardia, tachypnea, fever, swollen lymph nodes, pale icteric mucous membranes and severe tick infestation. Haematological analysis revealed marked anemia, lymphocytosis, granulocytosis, monocytosis and thrombocytopenia. The serum biochemical analysis showed increased levels of alkaline phosphatase, aspartate aminotransferase, total bilirubin, globulin, blood urea nitrogen and creatinine while the levels of total protein, albumin and phosphorus found decreased. The Giemsa-stained blood smears revealed presence of numerous intracytoplasmic morulae of *Anaplasma bovis* in the monocytes. Clinical signs and haemato-biochemical and blood smear findings confirmed the case diagnosis for concurrent *Anaplasma bovis* infection and hypophosphatemia. The treatment of cow with oxytetracycline for 5 days along with buffered phosphorus preparation for 3 days revealed gradual improvement leading to complete clinical recovery in 5 days.

Keywords: *Anaplasma bovis*, Haematobiochemical changes, Hypophosphatemia

Bovine Ehrlichiosis is a tick-borne disease caused by small, pleomorphic, Gram negative, obligate intracellular bacteria belonging to the family Rickettsiaceae, order Rickettsiales and genus *Anaplasma*. *A. bovis* infection in cattle is considered to be asymptomatic or subclinical in most cases but the disease is more serious in crossbred and newly imported cattle in tropical areas (Aguiar *et al.*, 2014; Prasath *et al.*, 2016). Nutritional hemoglobinuria is an economically important disease of buffaloes observed during advanced pregnancy or early lactation in high yielding animals maintained exclusively on dry roughages, or fed on diets containing cruciferous plants, and has been attributed to hypophosphatemia. The present article describes clinical signs, hematobiochemical alterations and therapeutic management of concurrent *Anaplasma bovis* infection and hypophosphatemia in Holstein Friesian cow.

An eight-year-old Holstein Friesian cow in 8th month of pregnancy was presented with the history of inappetence to anorexia and reduced rumination from seven days with clinical signs such as anorexia, fever (103.4 °F), tachycardia (88 beats/minute), increased respiration rate (44/minute), swollen lymph nodes, pale icteric mucous membranes and severe tick infestation.

The haematological analysis revealed marked anemia, leukocytosis (lymphocytosis, granulocytosis, and monocytosis), and thrombocytopenia (Table 1). The microscopic examination of Giemsa-stained blood smears revealed the presence of intracytoplasmic inclusions (morulae) within monocytes or mononuclear cells with variable morphology. The serum biochemical analysis revealed increased levels of alkaline phosphatase, aspartate aminotransferase, total bilirubin, conjugated and unconjugated bilirubin, blood urea nitrogen and creatinine, while the levels of total protein, albumin, and phosphorus were found to be decreased (Table 2).

The cow was treated with oxytetracycline (@ 20 mg/kg IV once daily), dextrose 5% (@ 500 ml IV), vitamin B1, B6 and B12 (@ 10 ml IM) for 5 days. Hypophosphatemia was treated with buffered phosphorous preparation (@ 50 ml IV once daily) for 3 days. With 24 hours of first dose of the treatment, cow showed positive clinical response with resumption of feed intake and rumination on second day of the treatment. Complete recovery was appreciated following therapy.

The clinical signs such as fever, pale icteric mucous membranes, swollen lymph nodes, anorexia,

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and dark yellow urine (bilirubinuria) recorded in the affected eight-month-pregnant crossbred cow of the present study can be attributed as outward manifestations of the above-cited various pathological mechanisms in response to concurrent *Anaplasma bovis* infection and hypophosphatemia (Prasath *et al.*, 2016; Constable *et al.*, 2017). The same therapeutic regimens were found effective to treat *A. bovis* infection and hypophosphatemia separately in earlier studies (Chhabra *et al.*, 2015; Constable *et al.*, 2017).

Table 1: Hematological Changes Associated with Concurrent *Anaplasma bovis* Infection and Hypophosphatemia in Holstein Friesian Cow.

Parameters	Result	Reference Interval (Constable <i>et al.</i> , 2017)
TEC ($\times 10^{12}/L$)	2.57	5.10-7.60
Hb (g/dL)	5.20	8.50-12.20
PCV (%)	15.90	22-33
MCV (fl)	62.20	38-50
MCH (pg)	20.20	14-18
MCHC (g/dl)	32.50	36-39
RDW (%)	15.50	15.50-19.70
TLC ($\times 10^9/L$)	28.42	4.90-12
Lymphocytes ($\times 10^9/L$)	11.99	1.60-5.60
Granulocytes ($\times 10^9/L$)	8.50	1.80-6.30
Monocytes ($\times 10^9/L$)	7.93	0-0.80
Platelets ($\times 10^9/L$)	95.00	200-650

Table 2: Biochemical Changes Associated with Concurrent *Anaplasma bovis* Infection and Hypophosphatemia in Holstein Friesian Cow.

Sr.	Parameter	Result	Reference Interval (Constable <i>et al.</i> , 2017)
1.	Alkaline Phosphatase (U/L)	474.90	0-200
2.	SGPT/ALT (U/L)	36.65	11-40
3.	SGOT/AST (U/L)	140.06	78-132
4.	Bilirubin (Total) (mg/dL)	4.39	0.01-0.50
5.	Bilirubin (Direct) (mg/dL)	1.71	0.04-0.44
6.	Indirect Bilirubin (Direct) (mg/dL)	2.68	0-0.30
7.	Total Protein (g/dL)	5.39	5.70-8.10
8.	Albumin (g/dL)	1.72	2.10-3.60
9.	Globulin (g/dL)	3.67	3.0-3.48
10.	Blood Urea Nitrogen (mg/dL)	37.38	20-30
11.	Creatinine (mg/dL)	2.21	1.0-2.0
12.	Calcium (mg/dL)	12.37	9.70-12.40
13.	Phosphorus (mg/dL)	3.94	5.60-6.50
14.	Glucose (mg/dL)	70.00	45-75

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Received : 29.05.2025**Accepted : 20.06.2026****Published : 30.06.2026**

Cerebrospinal Fluid Analysis in Dogs with Canine Distemper

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Abstract

The aim of this study was to evaluate the cerebrospinal fluid examination findings in neurological form of canine distemper in naturally infected dogs. The study consisted 10 healthy dogs as control group and 10 dogs with neurological form of canine distemper virus. Confirmation of neurological form of canine distemper was done by assessing the neurological abnormalities, lack of vaccination history and further confirmed by commercially available rapid antigen diagnostic CDV test kit. Cerebrospinal fluid was collected from the cerebello-medullary cistern for physical, haemato-biochemical parameters. Cerebro spinal fluid examination in these animals showed the variations in specific gravity, higher red blood cells, leucocyte count, protein and glucose levels.

Keywords: Dogs, Canine distemper, Cerebro spinal fluid analysis

Dogs and other carnivore species are susceptible to a deadly and highly contagious disease caused by the canine distemper virus (Buragohain et al., 2017). Most often spread by oronasal aerosols, canine distemper can cause a variety of multisystemic clinical signs in affected dogs. This study describes cerebro spinal fluid analysis in dogs with canine distemper.

Table 1. Cerebrospinal fluid analysis in dogs with canine distemper with neurological signs

S. No	Parameters		Apparently healthy dogs (10)	Dogs with canine distemper with neurological signs (10)	t-test
1.	Appearance	Colourless	(10/10)	(40 %) 4/10	
		Straw colour		(60 %) 6/10	
2.	Consistency	Clear	(10/10)	(40 %) 4/10	
		Cloudy		(60 %) 6/10	
3.	Specific gravity	<1.005		(20 %) 2/10	
		1.005-1.010		(20 %) 2/10	
		>1.010		(60 %) 6/10	
4.	Total erythrocyte count / μ L		0.11 ^a ±0.01 (0.33-1.46)	1.03 ^b ±0.01 (0.70-1.82)	3.88*
5.	Total nucleated cell count / μ L		2.23 ^a ±0.78 (1.42-3.42)	5.08 ^b ±0.42 (2.81-8.94)	5.23*
6.	Total Protein (mg/dL)		21.01 ^a ±3.81 (19.54 -24.35)	35.44 ^b ±4.08 (24.23-66.08)	4.89*
7.	Albumin (mg/dL)		9.84 ^a ±0.72 (7.61 -12.72)	13.44 ^b ±2.05 (8.55-22.32)	2.07*
8.	Glucose (mg/dL)		60.81 ^a ±7.01 (54.23-79.03)	71.02 ^b ±5.08 (55.63-86.64)	1.82*

*Significant (P<0.05) Columns bearing different superscripts differ significantly.

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The study was conducted from 2023 to 2024 at the College of Veterinary Science, Proddatur, at the Department of Veterinary Medicine. Ten dogs with neurological form canine distemper were diagnosed during the one-year study period, and cerebrospinal fluid was obtained from each dog. The antigen rapid CDV Ag Test Kit was used to confirm the neurological form of canine distemper. Cerebello-medullary cistern was used to collect cerebrospinal fluid (Di Terlizzi and Platt, 2009) and color, consistency, and specific gravity were assessed. Additional haematological and biochemical parameters were evaluated, including total erythrocyte count and total leukocyte count; total protein, albumin and glucose (Kim et al., 2008).

Cerebrospinal fluid from ten dogs with nervous form of canine distemper was analysed, and the results are shown in Table-1. The physical characteristics of the cerebrospinal fluid included the following appearances: colourless in 40 percent (4/10) of cases, and straw-coloured in 60 (6/10). The consistency was clear in 40 percent (4/10) and cloudy in 60 percent (6/10). Specific gravity values were <1.005 in 20 percent (2/10), between 1.005 and 1.010 in 20 percent (2/10), and >1.010 in 60 percent (6/10). The laboratory parameters of cerebrospinal fluid in the dogs were total erythrocyte count with a mean of $1.03 \pm 0.01/\mu\text{L}$ (range: 0.70 to 1.82)

total nucleated count with a mean of $5.08 \pm 0.42/\mu\text{L}$ (range: 2.81 to 8.94), the total protein level was $35.44 \pm 4.08 \text{ mg/dL}$ (range: 24.33 to 66.08), albumin $13.44 \pm 2.05 \text{ mg/dL}$ (range: 8.55 to 22.32) and glucose level $71.02 \pm 5.08 \text{ mg/dL}$ (range: 55.63 to 86.64). Significant increases ($P < 0.05$) were found total erythrocyte count, total nucleated cell count, total protein, albumin and glucose in these dogs. The total protein content of healthy CSF is much lower than that of plasma or serum due to the intact blood-brain barrier, which prevents large molecules from entering the subarachnoid space (Di Terlizzi and Platt, 2009).

Acknowledgement

Authors are thankful to Sri Venkateswara Veterinary University (SVVU) for providing facilities required to conduct the research.

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Endoscopic Evaluation of Spirocercosis in Four Dogs

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Abstract

Spirocerca lupi induces fibroproliferative oesophageal nodules, which may progress to obstruction or aneurysm. Four dogs were presented with anorexia, chronic vomiting, pyrexia, persistent vocalisation, and poor response to symptomatic therapy. Laboratory evaluation revealed mild leucocytosis with unremarkable serum biochemistry. Endoscopic examination identified nodular lesions in the distal thoracic oesophagus, with live worms visualised in two cases. Barium contrast radiography demonstrated intraluminal mass effects. Treatment with doramectin administered at 14 day intervals for three cycles, combined with antiemetics and pantoprazole, resulted in progressive clinical improvement and regression of nodules on follow up imaging.

Keywords: Canine spirocercosis, Endoscopy, Oesophageal nodules, Doramectin

Spirocercosis in dogs can result in a spectrum of clinical manifestations, including esophageal nodules, regurgitation, vomiting, chronic coughing, and in severe cases, life-threatening complications such as aortic

aneurysm or rupture (Traversa, 2012; Mazaki-Tovi *et al.*, 2025). This article describes endoscopic diagnosis of Spirocercosis in our dogs.

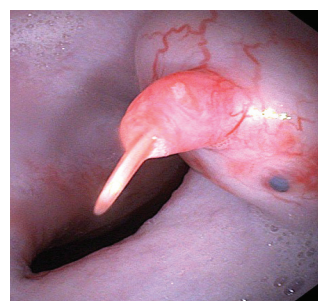
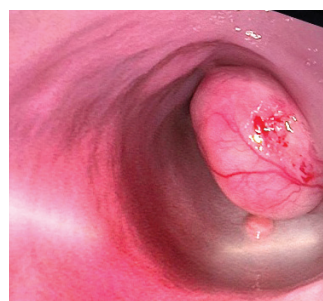


Fig. 1 and 2: Endoscopic images of oesophageal nodules- *Spirocerca lupi*.

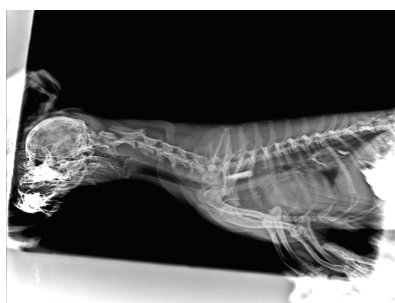


Fig. 3: Barium radiograph showing stasis of contrast

Four dogs were presented to Paw Universe Veterinary Hospital, Ahmedabad, Gujarat with a several-day history of marked anorexia, chronic vomiting, intermittent pyrexia, and persistent vocalisation.

Despite symptomatic management, none of the dogs exhibited clinical improvement, prompting further diagnostic investigation. Mild leucocytosis without any change in the serum biochemical parameters was observed. Endoscopic examination of the oesophagus revealed multiple space-occupying nodular lesions in

the thoracic oesophagus (Fig 1). Live *Spirocerca lupi* worms were visualised in two dogs (Fig. 2). To further characterise the lesions, endoscopic biopsy samples were obtained from the nodules. Barium contrast radiography demonstrated a well-defined intraluminal mass effect in the distal oesophagus (Fig. 3).

Doramectin was administered at 14-day intervals for a total of three treatment cycles. Supportive medical management included ondansetron and pantoprazole. Follow-up endoscopic and radiographic evaluations revealed a marked decrease in the size and prominence of the oesophageal nodules. Therapy with doramectin at 14-day intervals resulted in clinical improvement and reduction in nodule size, reinforcing macrocyclic lactones as the cornerstone of treatment (Lavy *et al.*, 2018).

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Warburg Effect and Tumour-Associated Hyperlactatemia in a Non-Descript Dog

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Abstract

An eight-year-old male non-descriptive dog was presented to Madras Veterinary College Hospital, Chennai, with a history of severe respiratory distress and open-mouth breathing. Clinical examination revealed hyperthermia, SpO₂ 98%, tachypnea, and small multiple skin masses, and a large ulcerated mass on the left medial thigh region. Haematological evaluation showed leukocytosis with neutrophilia, while biochemical parameters were within reference limits. Radiography revealed normal lung parenchyma with no effusions. Arterial blood gas analysis revealed a normal pH with reduced bicarbonate concentration, decreased partial pressure of carbon dioxide, and markedly elevated lactate levels, indicating metabolic acidosis with a compensatory respiratory component. Fine needle aspiration cytology was collected from the small skin masses and from the left thigh mass which revealed a mast cell tumour and fibrosarcoma, respectively. The dog was stabilized with fluid therapy, antipyretics, antibiotics and supportive medications.

Keywords: Warburg effect, Tumour, Lactate levels, Blood gas analyser

Respiratory distress in dogs is commonly attributed to primary cardiac or pulmonary disorders; however, metabolic causes are often overlooked (DiBartola, 2012). Tumour-associated hyperlactatemia may result in metabolic acidosis and secondary respiratory compromise (de Groot et al., 2011). The Warburg effect is one of the most important metabolic characteristics of cancer cells (Hanahan and Weinberg, 2011). This article presents a case of tumour associated hyperlactatemia in a non-descript dog.

An eight-year-old male non-descriptive dog was presented to Madras Veterinary College, Chennai, with a history of severe respiratory distress, open-mouth breathing, and drooling of saliva. On clinical examination, the patient's rectal temperature was 105.4°F, oxygen saturation was 98%, open-mouth breathing, tachypnea, enlarged lymph nodes, normal mentation, and multiple small cutaneous masses and a large ulcerated mass in the left medial thigh region were noted (Fig. 1, 2). Haematology and serum biochemistry revealed leukocytosis, with other parameters within the normal range (Table 1). Thoracic and abdominal radiography revealed normal lung parenchyma with no effusions, and a severely distended stomach with gas

(Fig. 3, 4). No abnormality could be detected through AFAST and TFAST. Arterial blood gas analysis revealed metabolic acidosis with a compensatory respiratory component & hyperlactatemia (Table 2). Fine needle aspiration cytology was collected from the small skin masses and from the left thigh mass which revealed a mast cell tumour and fibrosarcoma, respectively.

The dog presented with marked respiratory distress in the absence of cyanosis, suggesting that the dyspnea was not primarily caused by pulmonary or cardiac insufficiency. DiBartola, (2012) reported, in which compensatory hyperventilation develops secondary to systemic acid-base imbalance rather than an intrinsic respiratory disease in animals and humans with metabolic acidosis. The peripheral oxygen saturation (SpO₂) of 98% further supported adequate oxygenation and reduced the likelihood of hypoxemic respiratory failure. Nelson and Couto (2020) reported that aerophagia and gastric dilatation have been associated with excessive respiratory effort and compensatory hyperventilation in animals with metabolic acidosis and systemic stress. A marked increase in blood lactate concentration was also observed, indicating severe hyperlactatemia. Malignant tumours are known to induce aerobic glycolysis, commonly referred to as the

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Warburg effect, in which neoplastic cells preferentially convert glucose into lactate despite adequate oxygen availability (Liberti and Locasale, 2016). This excessive lactate production contributes to metabolic acidosis and may result in secondary respiratory compensation, manifesting clinically as tachypnea or respiratory distress. The pet was managed symptomatically with fluid therapy, mild sedation, and antibiotic administration. However, despite supportive treatment, the patient died during therapy, indicating the severity

of the systemic metabolic derangement and advanced neoplastic disease. Previous studies have shown that severe lactic acidosis associated with malignancy often carries a grave prognosis due to rapid progression of metabolic failure and underlying tumour burden (de Groot *et al.*, 2011). Arterial blood gas and lactate analysis were crucial in identifying the metabolic derangement and should be considered valuable diagnostic tools in critically ill oncology patients for accurate diagnosis and prognostic evaluation



Fig.1: Open mouth breathing, abdominal distension



Fig. 2: Multiple small masses over the skin, large ulcerated mass at thigh region



Fig. 3: Normal lung praenchyma with no effusions



Fig. 4: Distended stomach with gas due to aerophagia

Table 2: Arterial Blood Gas Analysis

GASES		CHEMICAL		METABOLIC	
Ph	7.4	Na	145	Glucose	121 mg/dl
PCO ₂	31.2	K	3.7	Lactate	10.37mmol/l
P0 ₂	73.8mmHg	Cl	117	BUN	17 mg/dl
CHCO ₃	17.8mmol/l	Ca	1.22	Creatinine	0.9mg/dl
BE (ecf)	-5.6mmol/l	Tco ₂	18.7	Urea	6.0 mmol/l
CSO ₂	81.6%	A gap	14	Bun/Crea	18.6

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Received : 15.05.2025**Accepted : 21.06.2026****Published : 30.06.2026**

Mixed Intestinal Coccidiosis in *Columba livia* and Its Therapeutic Management

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Abstract

Coccidiosis is an important protozoan disease of pigeons causing considerable morbidity and mortality, particularly in young birds. Three young domestic pigeons from a flock with high mortality were presented with severe diarrhoea, inappetence, weight loss and weakness. Parasitological examination of faecal samples revealed mixed infection with *Eimeria columbae* and *Eimeria tropicalis* confirmed based on oocyst morphology and sporulation characteristics. The affected pigeons and the flock were treated with toltrazuril 2.5% administered through drinking water along with supportive therapy and improved hygienic measures. Marked clinical improvement was observed within a few days with complete cessation of mortality and a significant reduction in oocyst shedding following treatment.

Keywords: Pigeon coccidiosis, *Eimeria columbae*, *Eimeria tropicalis*, Toltrazuril, anticoccidial

Coccidiosis is an important enteric parasitic disease affecting pigeons (*Columba livia*), caused primarily by protozoan parasites of the genus *Eimeria*, particularly *Eimeria columbarum*, *Eimeria tropicalis* and *Eimeria labbeana* (Soulsby, 1982). These parasites invade and multiply within the intestinal epithelium leading to mucosal damage and clinical manifestations ranging from subclinical infections to severe diarrhoea, weight loss, emaciation and increased mortality, especially in young squabs and susceptible flocks (Aboelhadid *et al.*, 2021). The present report describes mixed infection with *Eimeria columbae* and *Eimeria tropicalis* in domestic pigeons and its successful therapeutic management.

Three young domestic pigeons (*Columba livia*) were presented with a history of dullness, severe diarrhoea, complete inappetence, weight loss and progressive weakness. The owner reported that more than 12 pigeons from a flock of seventy had died previously showing similar clinical signs. On clinical examination, the affected birds showed ruffled feathers, dehydration, soiling of vent feathers with watery to greenish diarrhoeic droppings and marked emaciation. The birds were lethargic with reduced responsiveness. No neurological signs were observed.

Blood samples were collected aseptically from the wing vein into heparinized vacutainers for haematological analysis (Table. 1). Fresh faecal samples were collected directly from the cloaca for parasitological examination and the samples were subjected to sporulation using 2.5% potassium dichromate solution. Microscopic examination revealed the presence of numerous unsporulated oocysts of *Eimeria* species. Oocysts of *Eimeria columbae* were ovoid measuring 16.4 µm × 14.3 µm with a thin, smooth wall (Fig. 1) and sporulated within four to five days at room temperature (Fig. 2), whereas *Eimeria tropicalis* oocysts were subspherical, measuring 19 -24 µm × 18 -23 µm and sporulated within 40–48 hours under ambient conditions (Fig. 3,4).

Based on the clinical observations and laboratory findings, the affected pigeons were diagnosed with a mixed infection caused by *Eimeria columbae* and *Eimeria tropicalis*. The affected pigeons, along with the remaining birds in the flock, were treated with toltrazuril 2.5% administered through drinking water at a dose rate equivalent to 7 mg/kg body weight once daily for five consecutive days. In birds showing severe infection, the same treatment regimen was repeated after an interval of five days. Supportive therapy included oral multivitamin and electrolyte supplementation for seven days to correct dehydration and facilitate recovery. Following treatment, a marked improvement in appetite and activity levels

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was observed within 3–5 days. Diarrhoea gradually subsided and no further mortality was recorded in the flock. Post-treatment faecal examination revealed a marked reduction in oocyst shedding.

Table. 1. Haematological values (n=3) of domestic pigeons affected with coccidiosis

Parameter	Values (n=3)	Reference Range*
RBC ($\times 10^6/\mu\text{L}$)	1.65 ± 0.07	2.5–4.5
Hemoglobin (g/dL)	9.57 ± 0.41	12–18
PCV (%)	32 ± 2	35–55
WBC ($\times 10^3/\mu\text{L}$)	5.20 ± 0.24	5–10
–Nuterophils	48 ± 2	20–45
– Lymphocytes	42 ± 2	50–75
– Monocytes	5.3 ± 0.6	0–5
– Eosinophils	2.7 ± 0.6	0–5
– Basophils	2 ± 0	0–1

* Orakpoghenor *et al.* (2021)

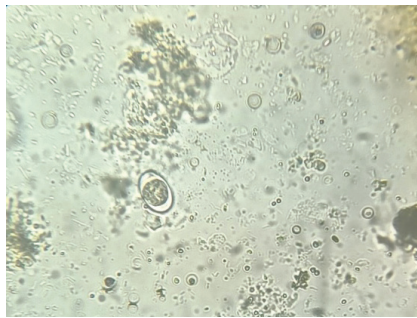


Fig. 1. Unsporulated oocyst of *Eimeria columbae* showing Ovoid shape thin wall (x40)

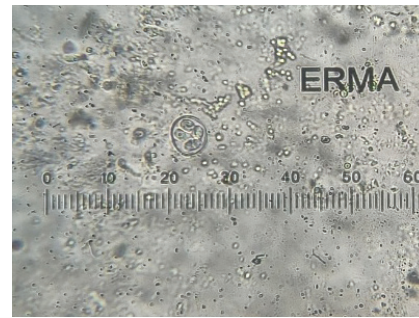


Fig. 2. Sporulated oocyst of *Eimeria columbae* showing 4 distinct sporocysts with refractile sporozoites (x40)

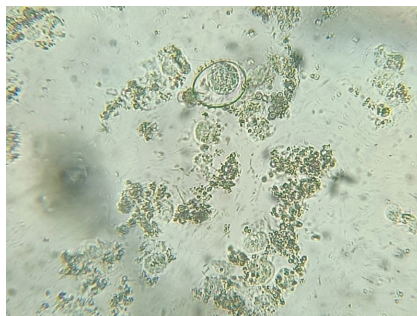


Fig. 3. Unsporulated oocyst of *Eimeria tropicalis* - sub-spherical, thin smooth wall (x40)



Fig. 4. Sporulated oocyst of *Eimeria tropicalis* showing 4 sporocysts with refractile sporozoites.

Coccidiosis remains one of the most common parasitic diseases affecting domestic pigeons worldwide, with reported prevalence ranging from approximately 5.1% to more than 70% in young birds, depending on management conditions and geographical

location. Young pigeons are particularly susceptible, and mortality rates have been documented between 5% and 70%, with most deaths occurring during the third and fourth months of life (Krautwald-Junghanns *et al.*, 2009). Mixed infections involving *Eimeria*

columbae, *Eimeria labbeana* and other related species are commonly reported, reflecting high environmental contamination and efficient faeco-oral transmission among flock members (Gurung and Subedi, 2018). Clinically, *Eimeria* infections are characterized by enteritis, diarrhoea, dehydration and poor growth, resulting from the invasion and multiplication of sporozoites within the intestinal epithelium (Soulsby, 1982). Therapeutically, toltrazuril has been shown in several studies to be highly effective in controlling avian coccidiosis. Its broad anticoccidial spectrum includes activity against both asexual (schizogony) and sexual (gametogony) intracellular developmental stages of *Eimeria*, which significantly reduces oocyst shedding and intestinal lesion severity compared with certain other anticoccidial agents (Lakkundi et al., 2014). Compared with older anticoccidial drugs such as sulfonamides and amprolium, the broader spectrum and prolonged efficacy of toltrazuril make it a preferred therapeutic option in clinical cases of pigeon coccidiosis.

Acknowledgement

The authors express their sincere gratitude to The Dean, Veterinary College and Research Institute, Orathanadu, TANUVAS, for their invaluable support and the provision of research facilities. We wish to express our deep gratitude to The Professor and Head, Veterinary Clinical Complex and The Associate Professor and Head, Department of Veterinary Parasitology, VCRI, Orathanadu, for the consistent support.

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Biomarkers and Acute Phase Proteins in Dogs with Gastro Intestinal Emergencies

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Abstract

Acute abdomen is a sudden onset of abdominal pain which develop due to multiple etiologies. Among those etiologies gastrointestinal emergencies are considered to be common cause of acute abdomen in dogs. Seventeen dogs had gastrointestinal emergencies which included gastric dilatation and volvulus, foreign body, intussusception and acute pancreatitis. Parameters like haematology, biochemistry, blood gas analysis and acute phase proteins and biomarkers were collected and analysed statistically. Biomarkers were analysed in both survivor and non survivor dogs. The findings highlighted the significance of acute phase proteins and biomarker in predicting the outcome of survivor in acute abdomen dogs with gastrointestinal emergencies.

Keywords: Acute abdomen, GI emergencies, Biomarkers, Predictor

Gastrointestinal emergencies constitute a large and clinically important among acute abdominal syndrome in dogs. Common GI emergencies include gastric-dilatation and volvulus, intussusception, gastrointestinal perforation and acute pancreatitis (Mazzaferro, 2003). Blood lactate concentration is widely used as a marker of tissue hypoperfusion and hence serves as a predictor for outcome in dogs with shock, GDV, sepsis and other severe abdominal emergencies, serial evaluation of lactate help to assess treatment response and outcome of acute abdomen dogs (Mooney *et al.*, 2014). This study reports the usefulness of acute phase protein and biomarkers in predicting the outcome of the patient.

The study was conducted in the clinical cases presented to the critical care unit of Department of Veterinary Clinical medicine of Madras Veterinary College for a period of one year. The total of 6 apparently healthy dogs were included under Group I (control group) and 17 dogs with acute gastrointestinal emergencies were included under Group II. Dogs in both groups were subjected to detailed clinical, physical examination, haematology, serum biochemistry, blood gas analysis, radiography and ultrasonography. General and detailed physical examination were done as guided by Heeren *et al.* (2004). All the vital parameters like temperature, heart rate, respiratory rate, CMM, CRT, STT were recorded. SpO₂ and ECG were recorded in acute abdomen dogs. Blood gas analysis was analysed in epoc® blood analysis system and acute phase proteins like

CRP, procalcitonin, Interleukin-6 and biomarkers like D-dimer were analysed using i-chroma III analyser with the principle of Fluorescence immune assay. Abdomen lateral radiography and abdominal ultrasonography using basic ultrasound scanner (Aeroscan CD 25) were performed as per standard protocol. All the patients were initially stabilised and were treated based on aetiology.

Seventeen dogs with gastrointestinal emergencies were grouped into GDV (35.29 %, 6 cases) acute pancreatitis (23.52 %, 4 cases), foreign body (17.65 %, 3 cases), intussusception (17.67%, 3 cases) and haemorrhagic enteritis (5.88 %, 1 case) (Fig.1). Deep chested breed such as Great Dane, Saint Bernard, Dobermann and German Shepherd (one case each) were presented with GDV as reported by Brockman *et al.* (1995). The predominant clinical signs noticed were respiratory distress (77.78 %, 28 cases) and pain on abdominal palpation (72.22 %, 26 cases). Distended abdomen and abnormal posture were recorded in (each 55.55%, 20 cases). Other signs observed were vomiting (69.44 %, 25 cases), diarrhoea (41.66 %, 15 cases), and melena (38.89 %, 14 cases). Less common signs observed were haematemesis (13.88 %, 5 cases), haematochezia (8.33 %, 3 cases) and oliguria (8.33%, 3 cases). There was significant increase in plasma lactate concentration in dogs with acute abdomen and was in agreement with report of Manosalva *et al.*, (2022) who observed similar changes. Mooney *et al.* (2014) found that high serum lactate concentration have been identified as a strong predictor for survival of patients with acute abdomen. There was highly significant increase in serum CRP concentration in dogs with acute

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abdomen in the present study. According to Manosalva *et al.* (2010), CRP concentration increased in cases like GI perforation, peritonitis, pancreatitis or splenic torsion.. There was significant increase in level of procalcitonin in acute abdomen dogs with gastrointestinal disease when compared to other causes of acute abdomen and was in agreement with the reports of Giunti *et al.* (2010). The ultrasonographic changes observed in acute

pancreatitis were hypoechoic pancreatic parenchyma and hyperechoic peripancreatic fat. Xenoulis (2015) also recorded similar ultrasonographic finding in acute pancreatitis dogs. In summary, in the present study the acute phase proteins and biomarkers such as CRP, procalcitonin, lactate, interleukin-6 and D-dimer concentration were remarkably higher in non-survivors than survivor.

Table. 1 Biomarkers (Mean $\pm$ S.E value) in dogs with acute abdomen

Parameters	Group I Control group n = 6	Group II Dogs with GI emergencies n= 17	t value	F value
Lactate (mmol/L)	0.65 $\pm$ 0.11	4.83 $\pm$ 0.73	-3.31	0.00*
C- Reactive Protein (mg/L)	4.78 $\pm$ 0.44	58.45 $\pm$ 6.31	-4.96	0.00*
D- dimer (μ g/dl)	0.15 $\pm$ 0.02	0.50 $\pm$ 0.06	-3.21	0.00*
Interleukin – 6 (pg/mL)	5.65 $\pm$ 0.50	101.07 $\pm$ 11.15	-5.00	0.00*
Procalcitonin (pg/mL)	19.20 $\pm$ 0.94	70.02 $\pm$ 6.80	-4.36	0.00*

Acknowledgement

Authors are highly thankful to the Dean, Madras Veterinary College, TANUVAS for providing necessary facilities for conducting the research work.

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Therapeutic Management of Oviposition Disorder in a Domestic Hen

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Abstract

Two year-old domestic hen was presented with lethargy, straining and failed to lay eggs for 2 days. Clinical examination and palpation suggested egg retention in the coelomic cavity. Ultrasonography and radiography confirmed a retained shell-less egg within a mildly distended oviduct. Biochemical analysis revealed hypocalcaemia. The case was diagnosed as egg retention associated with hypocalcaemia. Treatment with subcutaneous 10% calcium gluconate, oral calcium supplementation and supportive care resulted in oviposition of a shell-less egg followed by a normally shelled egg within 3 days after treatment. No recurrence was observed.

Keywords: Egg retention, dystocia, hypocalcemia, shell-less egg

Introduction

Egg binding/retention is defined as the failure of a fully or partially formed egg to pass through the oviduct within a normal timeframe, resulting in prolonged retention in the shell gland or vagina and potentially life-threatening sequelae if not relieved timely. Disruption of this process can result from a variety of factors. Among metabolic causes, hypocalcaemia is a frequently implicated condition due to its central role in regulating smooth muscle contractility and eggshell calcium deposition (Rosen, 2012). The current paper describes the successful medical management of egg retention in a domestic chicken.

Case History and Observation

Two year-old domestic hen was presented to Veterinary College and Research Institute, Orathanadu, TANUVAS with a history of lethargy, repeated straining and failure to lay egg for a period of 2 days. On clinical examination, the bird was depressed with intermittent cloacal straining and tail pumping. Gentle palpation revealed a soft, ovoid mass within the reproductive tract. Transabdominal B-mode ultrasonography using a 7.5 MHz linear probe revealed a retained ovoid structure

within the oviduct, characterized by an anechoic lumen and absence of acoustic shadowing consistent with a shell-less egg. The oviduct was mildly distended without appreciable coelomic effusion. Radiography revealed a radiolucent ovoid structure consistent with a shell-less egg in the reproductive tract. Blood was collected from the wing vein and subjected to biochemical analysis, which revealed reduced serum calcium concentration (7 mg/dL) and comparatively low-normal serum phosphorus levels (2.3 mg/dL). Based on clinical findings, imaging, and biochemical evidence of hypocalcaemia, the case was diagnosed as egg retention associated with hypocalcaemia.

Treatment and Discussion

Calcium gluconate 10% (10 mL) was administered subcutaneously. Oral calcium and multivitamin supplementation were advised to address ongoing mineral and micronutrient deficiency. The hen was maintained in a thermoneutral environment (28–30 °C) with mild humidity to reduce stress and support oviductal motility. Clinical improvement was observed over the subsequent 48–72 hr post therapy. The bird successfully ovipositioned on day 3 of treatment. The first egg passed was shell-less, followed by a normally shelled egg, indicating restoration of oviductal contractility with gradual recovery of shell gland

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mineralization. Immediate recurrence of dystocia was not observed following short-term follow-up.

Calcium is a critical component of eggshell calcification and of smooth muscle contraction in the oviduct (An *et al.*, 2016). Research on laying hens shows that dietary calcium and its metabolism influence eggshell quality, bone retention and plasma calcium status and that deficits in calcium availability are associated with compromised reproductive performance and eggshell formation, particularly in low-calcium diets or when physiological demands are high (Sinclair-Black *et al.*, 2023). General reviews of avian reproductive

disease highlight calcium-associated oviductal inertia as a key factor in egg impaction syndromes in laying hens (Greenacre, 2015). Egg binding in laying hens represents a multifactorial reproductive disorder in which calcium homeostasis plays a pivotal role. Hypocalcaemia compromises oviductal smooth muscle contractility, predisposing to impaired egg transport and dystocia, while simultaneously disrupting shell gland mineralization, resulting in soft- or shell-less eggs. Early recognition of calcium imbalance and targeted medical management are therefore essential for effective treatment and prevention of dystocia and other oviposition disorders in domestic hens.



Fig. 1. Domestic hen presented with lethargy, repeated straining, and failure to lay eggs

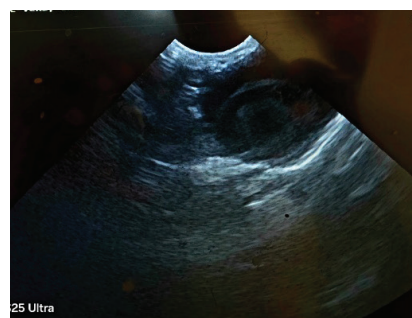


Fig. 2. Ultrasonographic image showing a distended oviduct with an anechoic lumen and absence of acoustic shadowing



Fig. 3. Radiographic view of the coelomic cavity revealing a radiolucent ovoid structure within the reproductive tract



Fig. 4. Sequential egg passage following treatment: initial shell-less egg followed by a normally shelled egg

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Therapeutic Management of Theileriosis in a Calf: A Case Report

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Abstract

Two month-old male Cross-bred Jersey (CBJ) calf was presented with the history of cloudiness in the right eye, tick infestation and anorexia for a period 10 days. Clinical examination revealed pyrexia, enlarged prescapular lymph node and manifestation of unilateral corneal opacity in the right eye. anaemia, neutropenia, lymphopenia and thrombocytopenia were observed. Blood smear was found to be positive for *Theileria annulata*. Calf was treated with buparvaquone @ 2.5 mg/kg BW deep intramuscularly along with supportive treatment. Animal showed uneventful recovery following treatment.

Keywords: Corneal opacity, Theileriosis, Buparvaquone, calf

Introduction

Bovine tropical theileriosis is an endemic protozoal disease primarily affecting cattle, buffalo, sheep, goats and horses. Clinical manifestations include high fever, lymphadenopathy, anemia, icterus, anorexia, lacrimation and occurrence of occasional ocular lesions like conjunctivitis (Constable *et al.*, 2017). The present report describes the successful therapeutic management of theileriosis in a calf.

Case History and Obseeration

Two month-old male Crossbred Jersey (CBJ) was presented with the complaint of anorexia, heavy tick infestation, lacrimation, unilateral cloudiness in the right eye (Fig. 1 and 2) for 15 days. Clinical examination showed pale mucous membrane, enlarged prescapular lymph node, tachycardia and pyrexia (103.6 °F).

Hematological examination revealed anemia, leukopenia and thrombocytopenia. Blood smear revealed signet ring shaped piroplasm's of *T. annulata* in erythrocytes (Fig 3) and was confirmed through Polymerase chain reaction (PCR) assay.

Treatment and Discussion

Calf was administered with two doses of Buparvaquone (@ 2.5 mg/kg b.wt deep IM at 48 hours interval), meloxicam (@ 0.5 mg/kg b.wt IM SID), dextrose normal saline (@ 10 ml/kg b.wt IV SID), Ringers Lactate (@ 10 ml/kg b.wt IV, SID), and B complex vitamin (@ 10 mg/kg IV SID for 5 days). For the tick infestation, ivermectin (@ 0.2 mg/kg b.wt SC) was administered. After 15 days of treatment, the corneal opacity reduced markedly (Fig.5) and uneventful recovery was observed.



Pre and Post treatment image of corneal opacity in a CBJ calf

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Bovine tropical theileriosis is an endemic protozoal disease affecting ruminants like cattle, buffalo, sheep and goats. Ocular involvement in theileriosis is uncommon but may occur due to infiltration of infected leukocytes into ocular tissues, immune-mediated inflammatory reactions, microvascular damage and hypoxic injury. A few reports of ophthalmological conditions were observed in the case of Bovine tropical theileriosis such as exophthalmos, conjunctival hyperaemia, oedema of eyelid and chemosis as reported by Taylor *et al.*, (2007).

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Received : 04.06.2025

Accepted : 20.06.2026

Published : 30.06.2026

Acute on Chronic Kidney Disease in a Non-Descript Bitch

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Abstract

A nine-year-old female mongrel dog was presented to Veterinary Clinical Complex, College of Veterinary Science, Proddatur with history of acute onset of anorexia, vomiting and tarry colored diarrhea. Upon clinical examination, subnormal rectal temperature, blanched conjunctival mucous membranes and moderate dehydration were observed. Auscultation of thorax revealed the presence of grade III systolic murmurs. Anemia, leukocytosis, azotemia, hypoproteinemia and hypoalbuminemia were also observed. Renal vascular doppler showed an increased renal resistive index (RRI) and pulsatility index (PI). Transthoracic echocardiography revealed hyperkinetic left ventricle with concentric hypertrophy. Based on the above findings, the case was diagnosed as Acute on Chronic Kidney Disease (ACKD) with secondary Hypertrophic Cardiomyopathy (HCM). The patient was treated with antibiotic, ACE inhibitor and supplements.

Keywords: ACKD, anemia, HCM, resistive index

Introduction

Acute on chronic kidney disease (ACKD) is often reported in dogs with CKD, resulting from a sudden decrease of glomerular filtration rate (Cowgill and Francey, 2012; IRIS, 2023). In dogs with stable CKD additional renal insult like ischemia, toxin injury, NSAID therapy, or infections precipitate this sudden decline in GFR, resulting in severe metabolic and systemic derangements leading to ACKD. This report describes clinical, haemato-biochemical, ultrasonographic, echocardiographic changes in a nine-year-old mongrel diagnosed with Acute on chronic kidney disease (ACKD) and secondary hypertrophic cardiomyopathy (HCM).

Case History and Observation

A nine-year-old female mongrel was presented to College of Veterinary Science, Proddatur with history of acute onset of anorexia, vomiting and, foul smelling tarry colored diarrhea. On physical examination, the dog was obtunded and recumbent. Clinical examination revealed subnormal temperature (96.9°F) with prolonged skin tenting time and pale mucous membranes. Cardiac auscultation revealed grade III systolic murmur on left apical region while lung auscultation was unremarkable. Haematological parameters revealed severe anemia (Hb – 3.4 g/dl, PCV 11.4%, RBC-1.5 mill/cmm)

along with neutrophilic leukocytosis (TLC- 24,700/cmm). Serum biochemical analysis showed a marked elevation in serum creatinine (24.3 mg/dl) and BUN (159.1 mg/dl). Concurrent hypoproteinemia (4.67 g/dL) and hypoalbuminemia (1.72 g/dL) were also observed, collectively indicating the presence of primary renal disease. Abdominal ultrasound revealed increased cortical echogenicity of both kidneys with an indistinct corticomedullary junction in right kidney (Fig.1). Reduced kidney-aorta ratios (LK:Ao – 5.81 and RK:Ao – 5.4), increased renal resistive index (0.83 in left kidney and 0.88 in right kidney) (Fig.2) were recorded. Echocardiographic examination recorded a hyperkinetic left ventricle with thickened left ventricular free wall (LVFWs- 24mm, LVFWd- 13mm) (Fig.3) and stage III diastolic dysfunction with E/A ratio of 2.20 and E/Elat – 17.10 (Fig.4).

Treatment and Discussion

Based on the above findings, the case was diagnosed as ACKD with secondary HCM. The patient was treated with amoxicillin sulbactam combination (@ 10mg/kg b.wt I/M BID), benazeperil (@0.25mg/kg p/o BID), syrup UTKID (@5-10ml p/o bid), syrup aRBC pet (@5-10ml p/o bid) with weekly darbopoeitin (@0.8mcg/kg SQ). Acute on chronic kidney disease is characterized by acute deterioration of renal function superimposed on a chronic kidney disease. The clinical presentation and laboratory abnormalities of ACKD

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may resemble those of AKI, which occasionally makes differentiation between AKI and ACKD challenging (Dunaevich *et al.*, 2020). The exhibited clinical signs like anorexia, vomiting, melena, lethargy, dehydration, and recumbency, are commonly associated with advanced renal dysfunction and uremic syndrome. Similar nonspecific gastrointestinal and systemic manifestations have been previously described in dogs and cats affected with ACKD (Dunaevich *et al.*, 2020; Ross, 2011). In addition to decline in erythropoietin levels, uremic toxins and G.I hemorrhage secondary to uremic gastritis contributed to severe anemia recorded in this case. Similar mechanisms contributing to anemia in advanced renal disease have been reported previously by Polzin (2013). Concurrent hypoproteinemia and hypoalbuminemia observed in the present case are attributed to protein-losing nephropathy associated with glomerular injury, reduced nutritional intake, chronic inflammation, and possible gastrointestinal protein loss. Ultrasonographic findings including increased renal

cortical echogenicity, reduced kidney-to-aorta ratio, and poor corticomedullary distinction were suggestive of chronic renal parenchymal degeneration and fibrosis. Increased RRI is considered indicative of elevated intrarenal vascular resistance and reduced renal perfusion associated with chronic renal parenchymal disease ((Rivers *et al.*, 1997; Pouchelon *et al.*, 2015). Another important finding is the secondary HCM and stage III diastolic dysfunction evidence through increased E/A ratio and E/E_{at} which emphasizes the relation between renal disease and cardiovascular changes (Brown *et al.*, 2007; Pouchelon *et al.*, 2015). Chronic activation of RAAS and blood pressure contributes to increased left ventricular afterload leading to concentric left ventricular hypertrophy and diastolic dysfunction. Early recognition of this alteration in cardiac function is vital in management of renal disease as a bidirectional relation exists where renal disease contributes to cardiac disease which inturn compromise renal perfusion and function.



Figure 1 : Transabdominal ultrasound of right kidney



Figure 2: Elevated Renal resistive index (RRI) of 0.83 increased cortical echogenicity

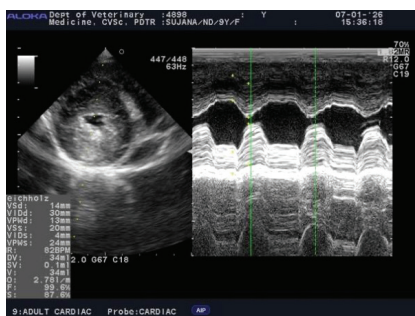


Figure 3 : Rt.Parasternal short axis view: Thickened LV free wall

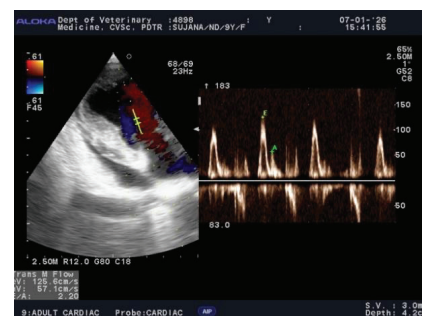


Figure 4: Trans mitral flow velocities measured in Left parasternal apical 4C view narrowed LV lumen

Acknowledgement

The authors thank the authorities of Sri Venkateswara Veterinary University, Tirupati, Andhra

Pradesh and the College of Veterinary Science, Proddatur for the facilities provided.

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Indian Journal of Veterinary Medicine

Vol. 46 No. 1 (June 2026)

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Meter	m	Centimeter	cm	Microlitre	μl
Counts per minute	cpm	Milligram	mg	Cubic centimeter	cm ³
Millilitre	ml	Degree centigrade	°C	Minute(s)	min
Degree Fahrenheit	°F	Once a day	od	Decilitre	dl
Parts per million	ppm	Gram	g	Percent	%
Hour(s)	hr	Picogram	pg	Inch	in
Revolution per min	rpm	Intramuscular	im	Seconds(s)	sec
Intraperitoneal	ip	Square centimeter	cm ²	Intravenous	iv
Subcutaneous	sc	Kilo calories	kcal	Thrice a day	tid
Kilogram	Kg	Year(s)	yr	Twice a day	bid
Volts	V				

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INDIAN JOURNAL OF VETERINARY MEDICINE

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1. Place of publication : Department of Clinical Veterinary Medicine
Madras Veterinary College
Tamil Nadu Veterinary and Animal Sciences University
Chennai - 600007, Tamil Nadu
2. Periodicity of its publication : Bi-annual
3. Printer's Name : Shri Vignesh Prints
- Nationality : Indian
- Address : Old No. 33 (New No.65), 2nd Street, Pudur,
Ashok Nagar, Chennai - 600 083
5. Editor's Name : Dr. G. Vijayakumar
- Nationality : Indian
- Address : Department of Veterinary Clinical Medicine
Madras Veterinary College
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Dated: 30th June, 2026

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