RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2023.54.2.477-485

Aetiopathology of canine hepatitis in Kerala#

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Citation: Joseph, A., Vinodkumar, K., Tresamol, P.V., Vijayakumar, K. and Sajitha, I.S. 2023. Aetiopathology of canine hepatitis in Kerala. *J. Vet. Anim. Sci.* **54**(2):477-485
DOI: https://doi.org/10.51966/jvas.2023.54.2.477-485

Received: 14.11.2022 *Accepted: 13.01.2023* *Published: 30.06.2023*

Abstract

Hepatitis in dogs could be due to infectious, toxic or metabolic causes. Among infectious causes, leptospira, canine adenovirus (CAV 1) and Babesia are responsible at varying levels, depending on the local geo-climatic conditions. This study, conducted on 40 dogs with clinical signs of hepatitis in Thrissur, Kerala, revealed occurrence of 2.5 per cent (1/40) for CAV 1, 20 per cent (8/40) for leptospirosis and 22.5 per cent (9/40) for babesiosis. The aetiologies were confirmed by polymerase chain reaction (PCR) targeting the E3 gene for CAV 1, microscopic agglutination test (MAT) for leptospirosis and peripheral blood smear examination for babesiosis. Haematological profile of CAV 1 infected animals revealed thrombocytopaenia, lymphopaenia, monocytopaenia and granulocytosis. Serum biochemical analysis divulged elevated alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total bilirubin and creatinine, with decreased total protein and albumin. Significant decrease in volume of packed red cells (VPRC), haemoglobin (Hb) and total erythrocyte count (TEC), with significant increase in total leukocyte count (TLC) were evident in dogs positive for leptospirosis. Serum biochemical analysis revealed significant increase in ALP, AST and blood urea nitrogen (BUN) and significant decrease in total protein. Severe anaemia and thrombocytopenia with mild leucocytosis and significant increase in ALP and total bilirubin, with hypoproteinemia were noticed in animals with babesiosis.

Keywords: *Infectious canine hepatitis, Canine adenovirus 1, leptospirosis, babesiosis*

Hepatitis is one of the most frequent liver disorders in dogs and affects approximately one per cent of the referred population of companion animals in the clinics (Boomkens *et al*., 2004). Many dogs might not exhibit any clinical signs of hepatitis until late in the course of the disease as liver has considerable reserve capacity (Bexfield and Watson, 2006). The clinical manifestations of

#Part of MVSc thesis submitted to the Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala.

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canine hepatitis are frequently non-specific and subclinical, making it difficult to diagnose, and the dogs often remain undiagnosed for a very long time (Eman *et al*., 2018). Most common causes of canine hepatitis are infectious, toxic and metabolic, with leptospirosis, infectious canine hepatitis and babesiosis contributing to the major part of infectious causes. Canine adenovirus 1 is the only virus with primary tropism for the liver. Infection with CAV 1 can cause a multisystemic disease involving the liver, kidney, brain and other organs (Decaro *et al*., 2008). Hepatic dysfunction occurs in dogs with leptospirosis without any significant histologic changes because of subcellular damage produced by the bacterial toxins (Adamus *et al*., 1997). Babesiosis could be presented in a complicated manner, including coagulopathies, immune-mediated haemolytic anaemia (IMHA), acute renal failure, hepatopathy, jaundice, pancreatitis and multiorgan failure (Karasova *et al*., 2022). Identification of the infectious aetiology and the prominent haemato-biochemical alterations in the host are of paramount importance for formulating effective therapeutic protocols. Hence, this study was conducted to determine the presence of these three infectious agents among dogs with canine hepatitis in Thrissur district of Kerala, and assess the haemato-biochemical changes associated with their infections.

Materials and methods

The study was performed from September, 2021 to September, 2022, and the study population included 40 dogs that were presented to University Veterinary Hospital, Kokkalai and Teaching Veterinary Clinical Complex, Mannuthy with clinical signs of progressive anorexia, gastroenteritis, respiratory involvement and ocular or neurological signs.

Microscopic examination of peripheral blood smear was done using Field's stain (Himedia laboratories Pvt. Ltd.) for the diagnosis of babesiosis. Serum samples were collected from animals suspected for leptospirosis, and subjected to microscopic agglutination test (MAT).

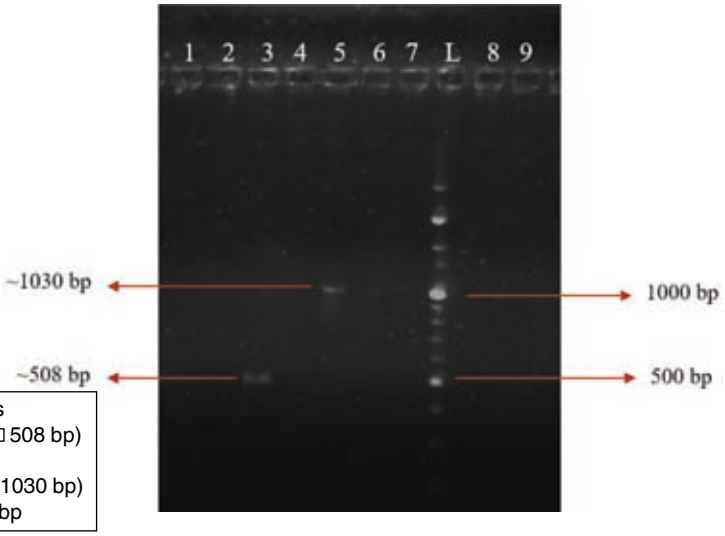
Ocular, nasal, throat and faecal swab samples were collected from 40 dogs and deoxyribonucleic acid (DNA) was extracted from all the collected samples using DNeasy® Blood and Tissue Kit and QIAamp® DNA Stool Mini Kit (QIAGEN, Germany). The extracted DNA was subjected to genus-specific PCR for identifying the presence of CAV using a pair of common primers targeting E3 gene of CAV as per Hu *et al.* (2001). The following primers were used to amplify 508 bp region of E3 gene of CAV 1: forward primer, HA 1: 5'-CGC GCT GAA CAT TAC TAC CTT GTC-3'and reverse primer HA 2: 5'-CCT AGA GCA CTT CGT GTC CGC TT-3'. The polymerase chain reaction was carried out using the S1000™ Thermal cycler (Bio-Rad, USA). The commercial vaccine Biocan® DHPPiL (Bioveta, Czech Republic) was used as positive control for CAV. The PCR protocol for amplification of sequences of the E3 gene for CAV 1 and CAV 2 included an initial denaturation at 95°C for 1 min followed by 31 cycles of denaturation at 95°C for 45s, annealing at 58°C for 1 min and extension at 72°C for 1 min and a final extension at 72°C for 5 min

Two ml of blood, collected in EDTA treated vials from all dogs were subjected to complete blood count, using an automatic haematology analyzer (Orphee, Mythic Vet 18, UK). Four millilitre of blood, collected in blood collection vial coated with clot activator was subjected to serum biochemistry analysis using a semi-automatic analyzer (Erba Manheim, Chem-5 Plus v2, USA) according to manufacturer's instruction. Haematobiochemical values obtained from all the dogs were compared with normal reference values standardised at the clinical laboratory in the College of Veterinary and Animal Sciences, Mannuthy, and analysed by one sample t-test, using SPSS version 24.0.

Results and discussion

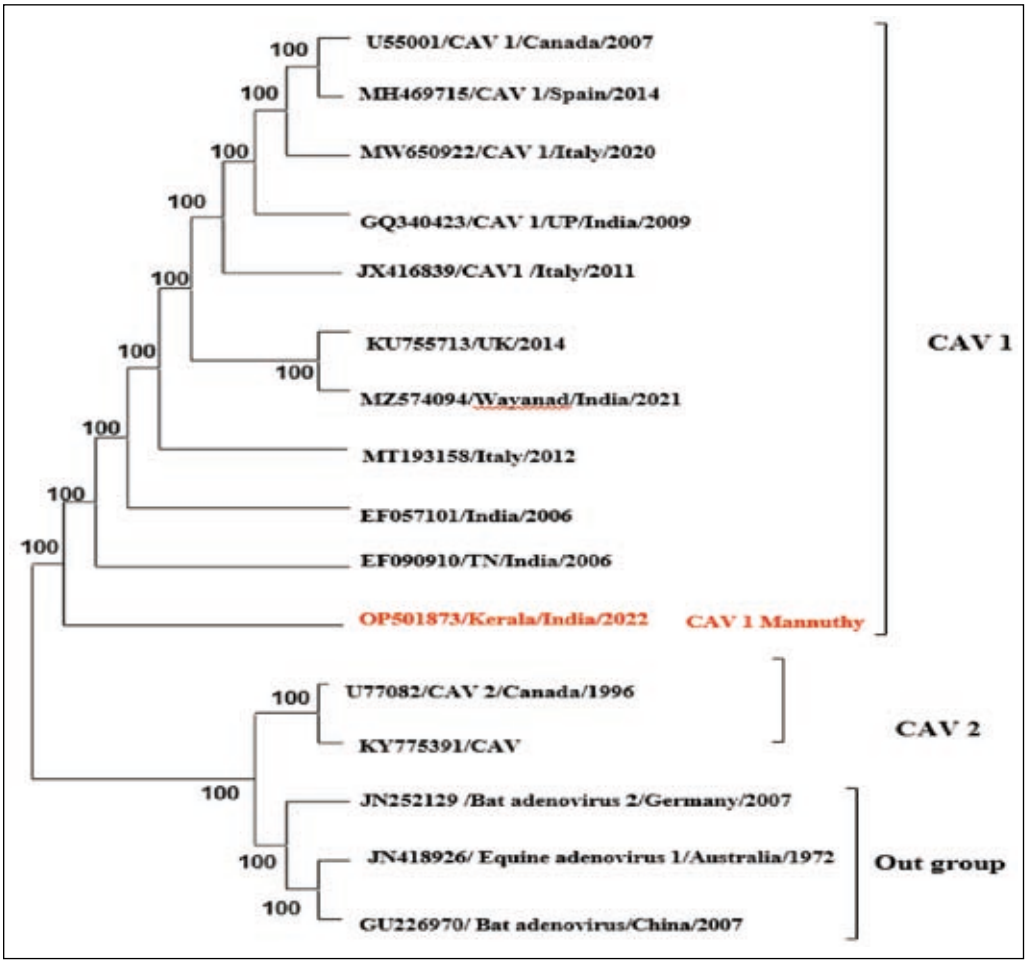
Infectious canine hepatitis caused by canine adenovirus 1

Specific amplicon of 508 bp size for CAV 1 was yielded by PCR of the DNA extracted from the ocular sample of only one among the 40 dogs (2.5 per cent) under study (Fig. 1). Relatively lower occurrence of CAV 1



Lane 1 & 2	: Negative samples
Lane 3	: Positive sample (□ 508 bp)
Lane 4	: Negative control
Lane 5	: Positive control (□1030 bp)
Lane L	: DNA marker 100 bp

Fig. 1. Agarose gel electrophoresis of PCR products for CAV 1



OP501873 */ CAV 1/Mannuthy – CAV 1 obtained in the present study

Fig. 2. Phylogenetic tree constructed using E3 gene of CAV 1.
The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model.

in the present study might be either due to less incidence and low endemicity of the diseases or a lower rate of virus excretion during sample collection (Greene, 2006). Canine adenovirus 1 specific positive PCR product was sequenced and the sequences were analysed using Basic Logarithmic Alignment Tool (BLAST) under the NCBI database to determine the genotype homology and deposited in GENBANK under accession number OP501873. The result showed 100 per cent similarity towards CAV 1 complete genome sequences in database. The phylogenetic relationship was constructed using E3 gene of CAV 1 involving 16 nucleotide sequences (CAV 1, CAV 2, equine adenovirus 1 and bat adenovirus (Fig. 2). From the phylogenetic tree it was concluded that the CAV 1 isolate in the present study (OP501873/ Kerala/India/2022) had close homology with the genotypes of other Indian isolates, thus indicating absence of any new genotypic variant.

Even though the occurrence for CAV 1 in the present study was low, it still represented re-emerging status for India. Only a few studies had been conducted on the prevalence of this virus in India. Chethan *et al.* (2021) found the prevalence of CAV 1 as 5.55 per cent by PCR in a study conducted at IVRI, Izatnagar. However, Raja *et al*. (2021) reported the prevalence of CAV 2 as 3.84 per cent in 14 nasal and 12 faecal swab samples collected from dogs with clinical signs of gastroenteritis and respiratory tract infections by PCR in Chennai, India during 2019-20, with no amplification for CAV 1. Again, Ramidi *et al*. (2019) who screened 302 faecal samples from dogs in Hyderabad, India by real-time PCR obtained positive results for CAV 2 only (15 Nos). But CAV 2 was not detected from any of the samples in the present study.

Only one pair of primers were involved in the PCR procedure in the present study which were advocated to be faster and easier to perform by Hu *et al*. (2001). The results were quicker than any of the assays used for detecting CAV and needed only one step centrifugation for the preparation of samples. This advantage enables application of the method in rapid confirmation and differentiation of CAV by PCR.

Clinical signs of CAV 1 positive animal

The single CAV 1 positive animal in the present study had clinical signs of ocular discharge, icteric mucous membrane, normal temperature, anorexia, vomiting, melena, dark yellow urine and seizures with a frequency of four times on the day before presentation. The dog succumbed to death next day. Similar clinical symptoms of ICH were also reported by Cabasso, (1981). But signs of abdominal pain, blue eye, haemorrhages on the oral mucosa and fever, which are reported as common clinical signs of ICH (Piacesi *et al*., 2010), were not present. Episodes of seizures were noticed in the CAV 1 positive animal. Neurological manifestation of CAV 1 is typically the main clinical sign seen in wild animals, and rare among dogs, as reported by Williams and Barker (2001). The affected dog in this study was revaccinated 14 months back with a multicomponent vaccine containing live attenuated CAV 2 virus.

Haematological and biochemical studies of CAV 1 positive animal

The haematobiochemical values of CAV 1 positive animal are shown in table 1. Kahilo *et al. (*2012) reported a significant decrease in the mean values of haemoglobin (Hb), erythrocytes, platelets and haematocrit, moderate leukocytosis and significant lymphocytosis in ICH. But the haematological profile of CAV 1 infected animal in the present study revealed thrombocytopenia, lymphopenia and granulocytosis only. In spite of severe hepatic change and thrombocytopenia, this animal did not exhibit any spontaneous bleeding. According to Boone (2008), lymphopenia was noticed in dogs with acute viral infections like infectious canine hepatitis, canine parvovirus, coronavirus, canine distemper, gastrointestinal diseases, acute severe stress and inflammatory conditions.

Neutrophilia was present in the animal in spite of normal total leukocyte count. As per Davis *et al*. (1991), the homeostatic stress placed on the body secondary to the hepatopathy resulted in increased activation of the hypothalamic-pituitary-adrenal axis and increased circulating cortisol had the effect of

Table 1. Haematobiochemical values of CAV 1 positive animal

Sl. No.	Parameters	CAV 1 positive animal	Reference range
1.	Volume of packed red cells (VPRC) (%)	43.3	37-55
2.	Haemoglobin (Hb) (g/ dL)	15.9	12-18
3.	Total erythrocyte count (TEC) ($\times 10^6$/ µL)	7.05	5.5-8.5
4.	Total leukocyte count (TLC) ($\times 10^3$/ µL)	15.3	6-17
5.	Thrombocyte count (PLT) ($\times 10^3$/ µL)	74	160-525
6.	Lymphocyte (%)	8.50	12-30
7.	Granulocyte (%)	89.10	60-74
8.	ALT (IU/ L)	112.2	17-95
9.	ALP (IU/ L)	2303.4	7-115
10.	AST (IU/ L)	80.6	18-56
11.	Total bilirubin (mg/ dL)	8.01	0.1-0.3
12.	Total protein (g/ dL)	3.71	5.4-7.5
13.	Albumin (g/ dL)	2.19	2.3-3.1
14.	BUN (mg/ dL)	16.5	8-28
15.	Creatinine (mg/ dL)	4.29	0.5-1.7

inducing neutrophil production and release into the circulation. Breheny *et al*. (2020) reported that the reduction in Kupffer cells seen with hepatic disease resulted in a lack of removal of apoptotic and dysfunctional neutrophils, with the effect that ineffectual neutrophils spend a longer time in circulation, artefactually presenting as neutrophilia.

Kahilo *et al. (*2012) reported elevation of alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and a reduction in the serum total protein, albumin and globulin in pups experimentally infected with CAV 1, by four weeks post-infection. Similar findings were noted in the present study except for elevated total bilirubin and creatinine. Lakshmi *et al*. (2018) suggested that significant increase in the mean values of ALT and AST in hepatitis might be due to altered hepatocellular membrane permeability, hepatocellular necrosis and inflammation with a degree proportional to the extent of injury to the hepatocytes. Chapman and Hostutler (2013) reported that high ALP could indicate a primary hepatic disease, however in dogs, ALP was not liver-specific and its elevation might also be due to extrahepatic sources.

The creatinine values were elevated in CAV 1 positive animal and this was in accordance with the findings of Sampaio *et al*. (2014). According to Tantary *et al*. (2014), the disturbed balance between the rate of production, metabolism and excretion of bilirubin explained hyperbilirubinaemia in hepatic disorders. Hypoproteinemia was the most common finding in canine hepatitis because liver was the main site for synthesis and degradation of proteins.

Haematological and serum biochemical parameters in canine leptospirosis and babesiosis

Eight (20 per cent) animals were detected as positive with MAT for leptospirosis and nine (22.5 per cent) for babesiosis (*Babesia gibsoni*) infection. Results of haematological and biochemical parameters of leptospirosis and babesiosis were analysed and represented in table 2 and 3 respectively.

Analysis of the haematological parameters of the leptospirosis positive dogs revealed a significant decrease in VPRC, Hb, TEC and significant increase in TLC. Lappin (1997) reported decreased RBC count, PCV and Hb concentrations characterised by normocytic, normochromic anaemia associated with signs of haemorrhage and blood loss in leptospirosis. All eight animals exhibited profound leucocytosis which might be due to stimulation of neutrophil adherence and activation involved in inflammatory and coagulatory abnormalities (Chandrasekaran *et al*., 2011). The decrease observed in thrombocyte count was non-significant.

Table 2. Statistical analysis of haematological values of dogs with leptospirosis and babesiosis

Haematological parameters	Test value	Leptospirosis (n=8)			Babesiosis (n=9)		
		Mean ± SE	t value	p value	Mean ± SE	t value	p value
Volume of packed red cells (VPRC) (%)	46	23.975 ± 5.355	4.113**	0.005	17.156 ± 2.890	9.979**	< 0.001
Haemoglobin (Hb) (g/ dL)	15	8.463 ± 2.088	3.130*	0.017	5.467 ± 0.985	9.677**	< 0.001
Total erythrocyte count (TEC) ($\times 10^6$/ µL)	7	3.703 ± 0.950	3.470**	0.010	2.546 ± 0.455	9.798**	< 0.001
Total leukocyte count (TLC) ($\times 10^3$/ µL)	11.5	38.556 ± 9.285	2.914*	0.023	24.304 ± 4.404	2.908*	0.020
Thrombocyte count (PLT) ($\times 10^3$/ µL)	342.5	300.38 ± 67.432	0.625	0.552	155.560 ± 47.430	3.938**	0.004

** - Mean is significantly different at 1 per cent level

* - Mean is significantly different at 5 per cent level

Table 3. Statistical analysis of serum biochemical values of dogs with leptospirosis and babesiosis

Serum biochemical parameters	Test value	Leptospirosis (n=8)			Babesiosis (n=9)		
		Mean ± SE	t value	p value	Mean ± SE	t value	p value
ALT (IU/ L)	56	281.974 ± 146.904	1.538	0.168	76.238 ± 23.509	0.861	0.414
ALP (IU/ L)	61	877.308 ± 228.154	3.578**	0.009	552.419 ±112.346	4.374**	0.002
AST (IU/ L)	37	122.899 ± 31.405	2.735*	0.029	66.833 ± 15.11	1.974	0.084
Total bilirubin (mg/ dL)	0.2	9.429 ± 5.059	1.824	0.111	7.253 ± 2.26	3.118*	0.014
Total protein (g/ dL)	6.45	5.295 ± 0.233	4.961**	0.002	5.090 ± 0.583	2.331*	0.048
Albumin (g/ dL)	2.7	2.898 ± 0.154	1.282	0.241	2.176 ± 0.289	1.810	0.108
Blood urea nitrogen (mg/ dL)	18	63.786 ± 17.790	2.573*	0.037	29.78 ± 8.232	1.431	0.190
Creatinine (mg/ dL)	1.1	4.695 ± 1.754	2.049	0.080	1.174 ± 0.209	0.356	0.731

** - Mean is significantly different at 1 per cent level

* - Mean is significantly different at 5 per cent level

Analysis of the haematological parameters of babesiosis confirmed dogs revealed a significant decrease in VPRC, Hb, TEC and thrombocyte count suggestive of severe anaemia and thrombocytopenia. This was in agreement with the findings of Anju *et al.* (2022). Anaemia in babesiosis resulted from an increased osmotic fragility of erythrocytes, increased erythrophagocytic activity of macrophages and immune-mediated cleavage. Additionally oxidative stress in babesiosis might cause damage to erythrocytes that result in their increased susceptibility to phagocytosis (Murase *et al.,* 1996). Thrombocytopenia might be due to platelet sequestration in the spleen or immune mediated platelet destruction and development of disseminated intravascular coagulation (Boozer and Macintire, 2003). Significant increase in TLC was noted in dogs which might be due to leukemoid response in immune-mediated haemolytic anaemia (Guitian *et al*., 2003). This was contrary to the findings of Evers *et al*. (2003) who reported that babesiosis was often accompanied by leucopenia.

Analysis of the serum biochemical parameters of leptospirosis positive dogs revealed a significant increase in ALP, AST and BUN and significant decrease in total protein. Higher levels of AST are due to changes in liver attributable to leptospira toxins and lipase production by the organism which help in release of fatty-acids inducing haemolytic and cytotoxic reactions. The haemotoxin from pathogen also causes breakdown of erythrocytes resulting in intravascular haemolyis, as evident by the clinical signs of haemoglobinuria and jaundice (Tangeman and Littman, 2013). Significant increase in mean value of BUN in dogs in spite of non-significant increase in creatinine levels, could be explained by the impaired kidney functions associated with liver disorders due to decreased capacity of the liver to detoxify harmful products (Sultana *et al*., 2022).

Analysis of the serum biochemical parameters of dogs with babesiosis revealed a significant increase in ALP and total bilirubin and significant decrease in total proteins. Hepatocyte damage is reportedly less common in babesiosis, thus explaining the variations in ALT being non-significant in the present study. Hyperbilirubinemia observed in the study population might be due to the increased destruction of RBCs leading to increase in unconjugated as well as conjugated bilirubin. Hepatopathy also results in imbalance in production, metabolism and excretion of bilirubin (Saravanan *et al*., 2014). However, ALP is not liver-specific in dogs and its elevation in two dogs might be due to extra hepatic sources like bone marrow and intestinal mucosa (Nelson and Couto, 2019).

Hypoproteinemia noticed in leptospirosis and babesiosis, could be due to the disruption in the hepatic protein metabolism, marked decline in diet intake, malabsorption and ongoing protein-losing enteropathies (Deepika *et al*., 2022)..

Histopathologic examination of visceral organs in a dog with leptospirosis revealed centrilobular necrosis with sinusoidal dilatation and congestion and subsequent thinning and atrophy of hepatocytes in liver. Haemosiderin laden macrophages could also be observed. Congestion, haemorrhage and accumulation of pink stained oedema fluid in alveoli of lung, depletion of white pulp in spleen and congested glomerular capillaries, peritubular congestion, degeneration of tubular epithelial cells in kidney were the other lesions observed.

Conclusion

Infectious aetiology of canine hepatitis in the study area was multifactorial with varying pathogenesis as evident from the haematobiochemical alterations associated with each organism. Presence of CAV 1 after two decades indicates the necessity for considering this virus as probable aetiology for canine hepatitis in the area. Leptospirosis and babesiosis were also the major infectious agents causing canine hepatitis in the study area, as in most other parts of the country.

Acknowledgement

The authors are thankful to the Kerala Veterinary and Animal Sciences University for providing the facilities needed for carrying out the research.

Conflicts of interest

There were no conflicts of interest reported by the author(s).

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