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Prevalence of Ehrlichiosis in Dogs with Gastrointestinal Bleeding Signs and Meta-analysis of Causal Association

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ABSTRACT

Background: Most of the canine monocytic ehrlichiosis (CME) cases are left undetected until they reach the chronic stage which is non-responsive to the treatment resulting in death associated with panleucopenia. Hence, diagnosis of clinical or subclinical CME is of paramount importance to prevent entering into the fatal chronic stage.

Methods: Dogs (n=50) presented with haemorrhagic signs were subjected to microscopic examination and immunochromatography test. Positive cases were subjected to haemato-biochemical analysis, radiography, ultrasonography and computerized tomography. Meta-analysis of clinical variables was done to determine the causal association.

Result: CME was observed in 22.0 per cent of cases by lateral flow assay (LFA) and 2.0 per cent by microscopic examination. Pathological changes were noticed in the liver, spleen, kidney, lungs, urinary bladder and intestine. A decrease ($P<0.05$) in haemoglobin level (8.66±1.59), increase ($p<0.05$) in white blood cell (21.87±5.2), monocyte count (8.5±1.06) and serum alkaline phosphatase level (151.33±48.12) and increase ($p<0.01$) in blood urea nitrogen (57.64±12.82) was observed. Melena (91.6%) was the predominant sign among positive cases. The sign- specific prevalence rate was high for epistaxis, circling/ joint pain (100.0%), emaciation (100.0%) and monocytosis (75.0%). The relative risk for the prevalence of CME was 5 to 6 times more in dogs with emaciation (95% CI, 2.26 to 12.18) and melena (95% CI, 0.73 to 6.71). High prevalence was observed in females (28.5%, $p>0.05$), 4-6-year-old (45.0%, $p>0.05$), pure breeds (100.0%, $p<0.01$) and recent tick infestation (31.2%, $p>0.05$). Antibody-based test, imaging techniques, haemato-biochemical analysis and the causal association could be useful tools in identifying the persistent ehrlichial infection.

Key words: Canine ehrlichiosis, Diagnostic imaging, Haemato-biochemical analysis, Immuno chromatography test, Meta-analysis.

INTRODUCTION

Canine monocytic ehrlichiosis (CME) is a significant vector borne disease caused by the obligate intracellular bacteria, *Ehrlichia canis*, transmitted by the tick, *Rhipicephalus sanguineus* and dogs act as reservoir (Aziz *et al.,* 2022). The pathogen attacks macrophages and leucocytes in dogs (Mylonakis and Theodorou, 2017) and is reported to be zoonotic (Andric, 2014).

The CME occurs as acute, subacute and chronic, with the clinical findings such as fever, lethargy, anaemia, lymphadenomegaly, hepatomegaly, petechiae or ecchymoses, vasculitis and extended bleeding period during estrus (Aziz *et al.,* 2022). Some dogs due to ineffective treatment may become subclinical carriers for months or years by evasion of immune response (Greene *at al*., 2012). Most of dogs do not show any visible signs except mild thrombocytopenia (Maggi *et al.,* 2014) and may enter into the most fatal chronic stage (myelosuppressive form). In chronic form, bleeding disorders such as ecchymoses, epistaxis, haematuria, melena and prolonged bleeding from venipuncture sites are more common (Mylonakis *et al.,* 2019).

The diagnosis of the ehrlichiosis becomes challenging owing to the several overlapping and non-specific clinical signs (Bai *et al.,* 2017). Further, in recovered cases, the low ehrlichaemia is undetectable by molecular methods such as polymerase chain reaction (PCR) (Theodorou *et al.,* 2013). An early diagnosis would help in prompt treatment of cases of ehrlichiosis before entering into the fatal chronic stage (Aziz *et al.,* 2022). Hence, the study is undertaken to report the early diagnosis of CME by lateral flow assay, to

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identify changes in the organs and to determine the causal association in the occurrence of CME.

MATERIALS AND METHODS

A cross-sectional study in the dogs (n=50) presented with the signs of gastrointestinal bleeding in 2023 to the Veterinary Clinical Complex, Veterinary College and Research Institute, Namakkal was conducted. Serum samples were subjected to lateral flow immuno assay based immunochromatography test (Pet X®, J and B Biotech Ltd, UK) for detection of *E. canis* antibodies. The positive result was indicated by the red lines both in control (C) and test (T) zones, whereas, negative result was indicated by the red line only in C zone. Giemsa stained peripheral blood smears were subjected to microscopic examination.

The prevalence rate was statistically analysed between groups by Chi-square test. Blood were collected for haemato-biochemical analysis by auto Veterinary hematology Analyzer (Rayto®, Version 2.4e, RT7600Vet, Rayto Life and Analytical Sciences Co., Ltd., China) and auto Biochemistry analyzer (A15 Biosystems SA, Spain and the values were compared with healthy control group by Student t-test (using SPSS software). Radiography, ultrasonography (USG) (Esoate®, Italy) and computerized tomography (CT) (Toshiba Alexion 16 Slice CT scanner, Japan) were performed in positive cases. The causal association for the clinical variables was determined by logistic regression analysis using Med calc Statistical software and a forest tree analysis of RR (CI:95%) was plotted on a logarithmic scale.

RESULTS AND DISCUSSION

Microscopic examination (× 100) could detect morulae of ehrlichia within monocytes (Fig 1) in one case (2.0 per cent), whereas sandwich lateral flow assay could detect positivity (Fig 1) in 11 cases (22.0 per cent) and an overall prevalence of 24.0 per cent was observed for CME in dogs. Kappa analysis revealed only a slight agreement (Kappa value=0.18) between the tests (Landis and Koch, 1977). Ehrlichia could not be detected in most of the cases owing to the poor sensitivity of microscopic examination (Singh *et al.,* 2021; Angkanaporn *et al.,* 2022), as detection of morulae could be possible only in 4 to 6% of cases (Aziz *et al.,* 2022; Sarawade *et al.*, 2023; Gamit *et al*., 2024 and Checa *et al*., 2024). Previously, Senthil *et al.* (2023) recorded a highest prevalence of 56.37 per cent of *E. canis* in dogs from Chennai city. In this study, the immunochromatography test could detect more ehrlichia infections before entering into chronic form. Serological tests could detect both current and past ehrlichia infections and molecular test is recommended to distinguish the active infection (Sainz *et al.,* 2015). Immuno chromatography test (ICT) is reported to be a quick and useful diagnostic tool with 87% sensitivity and 95% specificity (Geromichalou and Faixova, 2017). Previously, Checa *et al.* (2024) also reported a high prevalence of CME by LFA.

The clinical manifestations of positive cases are presented in Table 1 and Fig 2. From Table 1, most of the cases (84.4%) were found to be sub-acute as they showed clinical signs without fever (Waner and Harrus, 2013). Melena, anaemia, lymphadenopathy and thrombocytopenia were the most common signs observed in the positive cases which concurred to the findings of Singh *et al.* (2021). The bleeding signs could be associated with anti-platelet antibodies or bone marrow suppression or vasculitis (Asawapattanakul *et al.,* 2021; Aziz *et al.,* 2022). A very few were chronic cases (16.6%) showing panleucopenia, as chronic CME is indicated by severe pancytopenia due to myelosuppression caused by the ehrlichiae (Sainz *et al.,* 2015; Singh *et al.* 2021).

Radiography (Fig 3), USG (Fig 4) and CT scan (Fig 5) of abdomen revealed hepatic, splenic and pulmonary changes**.** Splenic congestion was observed as hypoechoic area than liver, myelolipoma as hyper echoic mass with homogenous echo texture in spleen, distended renal pelvis and mineralization seen (hyper echogenic), kidney with indistinct cortico-medullary junction and small intestinal mucosa thickening (hyper echogenic). The CT scan in one chronic case was suggestive of lymphoma of the liver with an increased attenuation scale of 145 Hounsfield Units (HU), enlargement of spleen with an attenuation of 56 HU

Table 1: Distribution of signs in the positive cases of CME with gastrointestinal bleeding signs.

Clinical signs in cases (n=50) with GI bleeding signs	Test positives (n=12)	Per cent distribution of signs
Melena	11	91.6
Lymphadenopathy	10	83.3
Anaemia	9	75.5
Thrombocytopenia	7	58.3
Emaciation	6	50.0
Leucocytosis	5	41.6
Renal changes	5	41.6
Lymphocytosis	4	33.3
Hepatic changes (USG/Radiography/CT)	3	25.0
Hyperglobulinemia	4	33.3
Monocytosis	3	25.0
Neutrophilia	3	25.0
Pulmonary signs (Radiography)	3	25.0
Splenic changes (USG/Radiography/CT)	3	25.0
Epistaxis	2	16.6
Leucopenia	2	16.6
Pancytopenia (Myelosuppressive)	2	16.6
Petechiae /Ecchymoses	2	16.6
Neutropenia	2	16.6
Hypoalbuminemia	2	16.6
Vomiting, Joint pain	2	16.6
Circling	2	8.3
Haematochezia	1	8.3

and inflammation of urinary bladder with an attenuation of 50 HU. Ultrasonographic study for the lymphoproliferative changes in liver, spleen and kidney in positive cases is reported to be more useful in supporting other diagnostic procedures especially during the subclinical phase (Singh *et al.,* 2021a). Splenomegaly was observed in 25.0% of the CME cases (Table 1), whereas positivity was observed in 37.5% of the cases with splenomegaly (Table 2). These splenic changes were in accordance with Singh *et al.* (2021). Previously, Sarma *et al.* (2016) recorded splenomegaly in 8.33% of CME cases and in contrast, Angkanaporn *et al.* (2022) recorded in 76.9% of CME cases. Splenic enlargement could be associated with sequestration of ehrlichiae in infected macrophages (Waner and Harrus, 2013). Similar to this study, Cassaro *et al.* (2021) also reported splenic myelolipoma in an adult dog recovered from ehrlichiosis, though the neoplasm is reported to be rare in dogs. Renal, hepatic and pulmonary

Fig 1: (a) Microscopic examination of Giemsa stained blood smears showing morulae of *E. canis* within monocyte (× 100); (b)Lateral flow assay based immunochromatography test showing positivity for CME and (c) LFA showing negativity for CME.

Fig 2: Clinical signs in CME cases showing (a) Emaciation in an young rottweiler breed; (b) epistaxis; (c) Blanched mucosa; (d) Mucopurulent nasal discharge; (e) ecchymoses in the chest and axilla and (f) ecchymoses on the ventral abdomen of a young great dane breed.

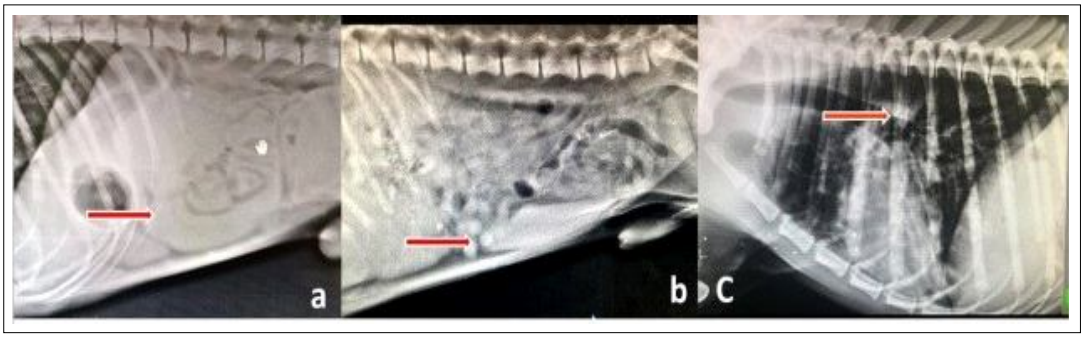

Fig 3: Radiography showing (a) Severely enlarged liver and spleen; (b) moderately enlarged spleen and (c) pulmonary infiltration in dogs affected with CME.

changes were observed in 41.6, 25.0 and 25.0 per cent, respectively of the CME cases (Table 1), whereas positivity was observed in 26.3, 21.4 and 25.0 per cent of the cases presented with renal, hepatic and pulmonary changes, respectively (Table 2). The hepato-renal changes observed in this study were in accordance with that of Singh *et al.* (2021a). Hepatic lymphoma in one of the positive cases could be associated with chronic inflammation and plasma cell proliferation caused by ehrlichiae resulting in development of B-cell lymphoma (Brunker and Hoove, 2007). These changes in liver, spleen and kidney could be due to immunological complications associated with immune dysregulation resulting in tissue injury (Singh *et al.,* 2021a), since ehrlichia affects whole lymphatic system resulting in hyperplasia (Aziz *et al.,* 2022). Inflammatory changes observed in other organs such as lungs, urinary bladder and intestinal mucosa might be due to the systemic inflammatory response (SIRS) caused by cytokines, acute phase protein and nitric oxide elicited by ehrlichiae (Asawapattanakul *et al.,* 2021).

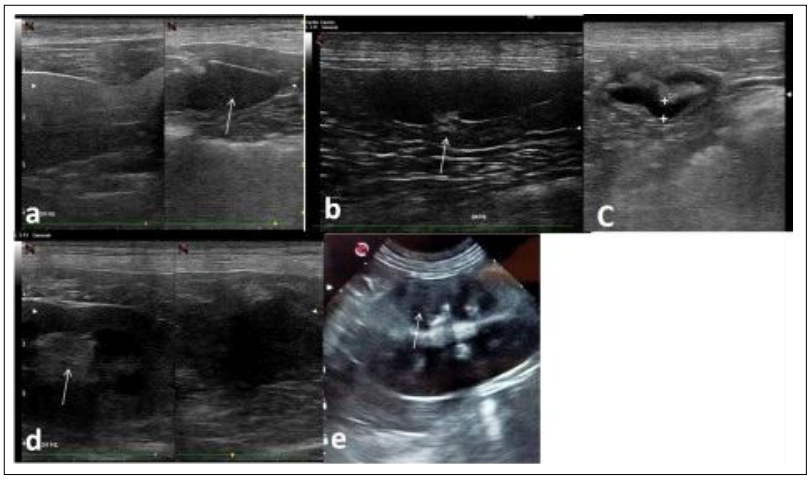

Fig 4: Ultrasonography showing (a) splenic congestion (hypoechoic area); (b) myelolipoma (hyper echoic area) of spleen; (c) mucosal thickening of small intestine; (d) distended renal pelvis and mineralization (hyper echogenic area) and (e) indistinct cortico-medullary junction of kidney in CME positive cases.

Table 2: Comparison of Haemato-biochemical values (Mean ± SE) of CME cases with control group.

Values	Control group (Mean ± SE)	Positive cases (Mean ± SE)	P value
Haemoglobin (g/dl)	16.95±0.41	8.66±1.59	0.022*
Packed cell volume (%)	46.3±3.38	25.65±4.57	0.109^{NS}
Red blood cells (× 10^6/µl)	6.78±0.55	4.05±0.71	0.088^{NS}
White blood cells (× 10^3/µl)	10.95±1.29	21.87±5.2	0.028*
Neutrophils (%)	73.17±1.37	69.91±3.24	0.020*
Lymphocytes (%)	19.67±1.74	22.41±2.5	0.101^{NS}
Monocytes (%)	5.5±0.56	8.5±1.06	0.029*
Platelets (× 10^3)	288.17±46.18	218.58±60.99	0.063*
Total protein (g/dl)	6.8±0.19	6.775±0.37	0.294^{NS}
Albumin (g/dl)	3.17±0.12	2.85±0.21	0.110^{NS}
Globulin (g/dl)	3.63±0.26	3.99±0.32	0.169^{NS}
Alanine transferase (U/L)	75.66±10.28	79.79±19.81	0.087^{NS}
Serum alkaline phosphatase (U/L)	62.6±7.33	151.33±48.12	0.038*
Blood urea nitrogen (mg/dl)	30.17±2.8	57.64±12.82	0.007**
Creatinine (mg/dl)	1.18±0.24	3.89±1.73	0.083^{NS}
Calcium (mg/dl)	10.92±0.52	8.77±0.79	0.191^{NS}
Phosphorus (mg/dl)	4.13±0.48	6.39±1.07	0.088^{NS}
Glucose (mg/dl)	113.33±8.40	80.5±6.86	0.828^{NS}
Sodium (mmol/L)	139.41±6.28	123.08±8.81	0.410^{NS}
Potassium (mmol/L)	3.510±0.22	2.93±0.26	0.089^{NS}
Chloride (mmol/L)	119.67±3.47	95.6±2.9	0.518^{NS}

^{NS}Non-significant (P>0.05); *significant (P<0.05); **Highly significant (P<0.01.

Haematological values (Mean ± SE) in positive cases (Table 2) revealed a significant decrease ($p<0.05$) in haemoglobin (Hb) level and increase ($p<0.05$) in white blood cell (WBC) and monocyte count than control group. Serum biochemical values (Table 2) revealed an increase ($p<0.05$) in the blood urea nitrogen (BUN) level ($p<0.01$) and serum alkaline phosphatase (SAP) ($p<0.05$) than control group. Haematological changes (Table 1 and 2) such as anaemia, thrombocytopenia and neutropenia could be associated with the bone marrow hypoplasia caused by ehrlichiae in chronic form. Lymphocytosis, monocytosis and the resultant leucocytosis could be associated with the pathogen targeting mononuclear cells and lymphocytes (Asawapattanakul *et al.,* 2021). However, lymphocytosis was reported to be the most common finding in the subclinical canine ehrlichiosis (Lorente *et al.,* 2008). Serum biochemical changes (Table 2) such as increased levels of BUN, creatinine and phosphorus and decreased calcium level indicated chronic kidney disease (CKD). The CKD could be associated with immune mediated glomerulonephritis (IMGN) (Crivellenti *et al.,* 2021) or SIRS specific to ehrlichiae resulting in increased C-reactive protein (CRP) and interleukin-6 (IL-6) levels (Asawapattanakul *et al.,* 2021). Hypoalbuminemia might be due to vasculitis resulting in loss of proteins or hepatopathy (Singh *et al.,* 2021; Gamit *et al.,* 2024) and hyperglobulinemia as a sequel of strong humoral response associated with persistent ehrlichiae (Crivellenti *et al.,* 2021).

The prevalence rate of ehrlichiosis with logistic regression analysis for various clinical variables was presented in Table 3. The prevalence rate was high in dogs with signs such as epistaxis, circling/ joint pain, emaciation, monocytosis, leucopenia and ecchymoses. The risk for the prevalence of CME was 5 to 6 times more in dogs with emaciation and melena, 3 to 4 times more with monocytosis, leucopenia, epistaxis and circling/ joint pain, 2 to 3 times with anaemia, thrombocytopenia and lymphadenopathy, than in dogs not associated with these clinical signs. A negative causal association was noticed for hepatic changes, haematochezia, vomiting, neutrophilia and no causal association was found in dogs with haemorrhagic diarrhoea, haematemesis and haematuria.

The specific prevalence rate (Table 3) was higher in dogs in the age group of 4-6 year ($p>0.05$) with two times

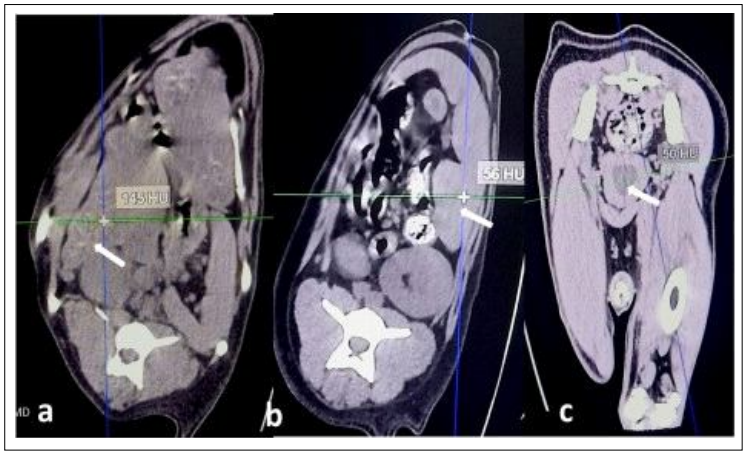

Fig 5: Computerized tomography showing (a) lymphoma of the liver (scale of 145 HU); (b) enlargement of spleen (scale of 56 HU) and (c) inflammation of urinary bladder (a scale of 50 HU) in a CME positive case.

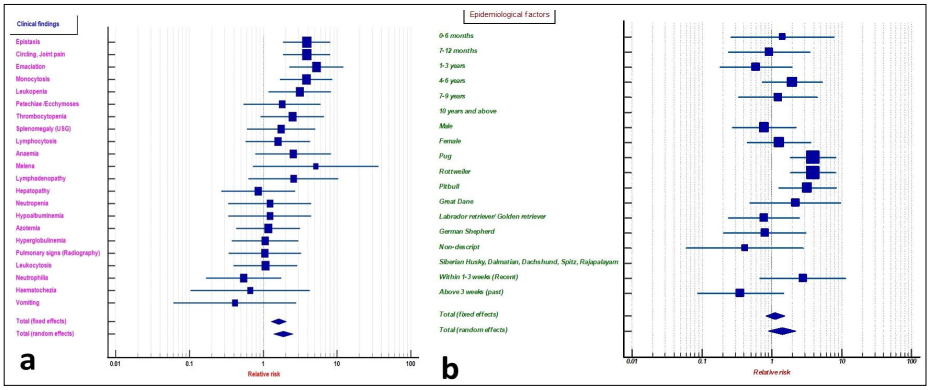

Fig 6: Forest tree analysis of relative risk (CI: 95%) for (a) clinical findings and (b) host determinants drawn on a logarithmic scale in the prevalence of CME.

Table 3: Prevalence rate of ehrlichiosis with logistic regression analysis specific to various clinical signs (95% CI=28.2 to 76.22; df-15; P=0.0016) and host determinants (95% CI=7.07 to 66.07; df =21, P=0.015).

Variables		Number of cases(n=50)	Test positives (n=12)	Specific prevalence of CME (%)	RR	95% CI
Clinical signs						
Epistaxis		2	2	100.0	3.889	1.861 to 8.129
Circling, Joint pain		2	2	100.0	3.889	1.861 to 8.129
Emaciation		8	6	75.0	5.250	2.262 to 2.185
Monocytosis		4	3	75.0	3.833	1.698 to 8.656
Leucopenia		3	2	66.6	3.133	1.187 to 8.273
Petechiae/Ecchymoses		5	2	40.0	1.800	0.540 to 6.004
Thrombocytopenia		18	7	38.8	2.489	0.923 to 6.710
Splenic changes		8	3	37.5	1.750	0.603 to 5.080
Lymphocytosis		12	4	33.3	1.583	0.577 to 4.346
Anaemia (Non-haemolytic)		27	9	33.3	2.556	0.783 to 8.337
Melena		34	11	32.3	5.176	0.730 to 6.713
Lymphadenopathy		33	10	30.0	2.576	0.635 to 0.455
Neutropenia		7	2	28.6	1.229	0.338 to 4.468
Hypoalbuminemia		7	2	28.5	1.229	0.338 to 4.468
Renal changes		19	5	26.3	1.165	0.431 to 3.154
Hyperglobulinemia		16	4	25.0	1.062	0.374 to 3.015
Pulmonary infiltration		12	3	25.0	1.056	0.340 to 3.281
Leucocytosis		20	5	25.0	1.071	0.395 to 2.908
Hepatic changes		14	3	21.4	0.857	0.271 to 2.711
Neutrophilia		19	3	15.8	0.544	0.168 to 1.762
Haematochezia		6	1	16.6	0.667	0.104 to 4.287
Vomiting		9	1	11.1	0.414	0.061 to 2.813
Haemorrhagic diarrhoea (10), Haematuria (5) and Haematemesis (3)		18	-	-		
Pyrexia		7	-	-		
Determinants						
Age	0-6 months	03	01	33.3	1.424	0.265 to 7.656
	7-12 months	09	02	22.2	0.911	0.240 to 3.465
	1-3 years	18	03	16.7	0.593	0.184 to 1.913
	4-6 years	10	04	40.0	2.000	0.751 to 5.329
	7-9 years	07	02	28.6	1.229	0.338 to 4.468
	10 years and above	03	-	-	-	-
	Chi square value		2.03^{NS}			
Sex	Male	36	08	22.2	0.778	0.278 to 2.177
	Female	14	04	28.5	1.286	0.459 to 3.599
	Chi square value		0.22^{NS}			
Breed	Pug	02	02	100.0	3.889	1.861 to 8.129
	Rottweiler	02	02	100.0	3.889	1.861 to 8.129
	Pitbull	01	01	100.0	3.261	1.264 to 8.409
	Great dane	02	01	50.0	2.182	0.497 to 9.583
	Labrador retriever/ Golden retriever	15	03	20.0	0.778	0.244 to 2.477
	German shepherd	10	02	20.0	0.800	0.207 to 3.089
	Non-descript	09	01	11.1	0.414	0.0610 to 2.813
	Siberian Husky, Dalmatian, Dachshund, Spitz, Rajapalayam	09	-	-	-	-

Table 3: Continue....

Table 3: Continue....

	Chi square value		13.9**			
Tick exposure	Within 1-3 weeks (Recent)	32	10	31.2	2.813	0.691 to 11.452
	Above 3 weeks (past)	18	2	11.1	0.356	0.0873 to 1.448
	Chi square value		2.56^{NS}			

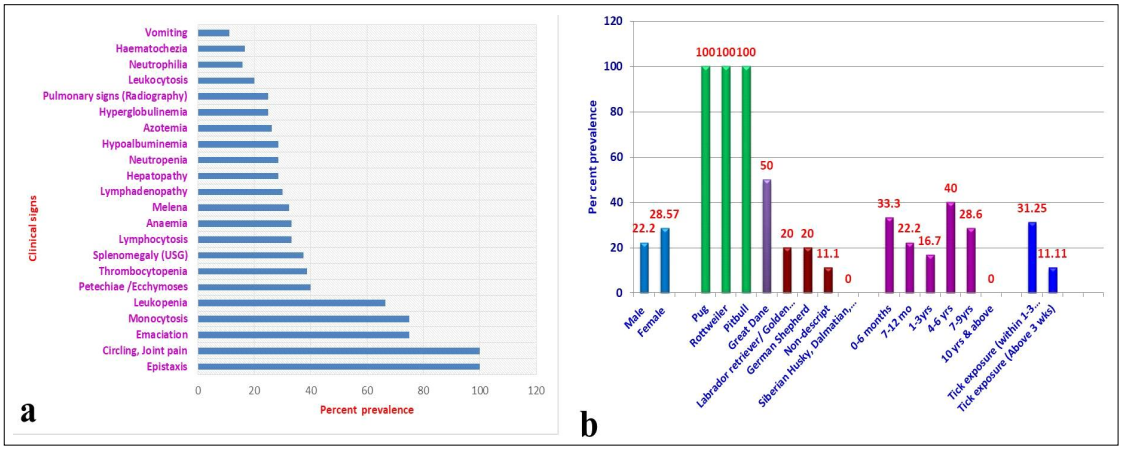

Fig 7: Graphical representation of prevalence of CME associated with the (a) spectrum of signs and (b) host determinants in dogs with GI bleeding signs.

the risk, females (p>0.05) with less than 2 times the risk, pure breeds (p<0.01) with 3 to 4 times the risk and recent exposure to ticks (p>0.05) with 2 to 3 times the risk, than in dogs of other groups. The increased risk in older dogs could be due to the increased exposure to the Ehrlichiae (Singh *et al.,* 2021 and Aziz *et al.,* 2022). In contrast, a high prevalence in dogs less than one year old (Kalaivanan *et al.,* 2020), males (Asawapattanakul *et al.,* 2021), both sex (Costa *et al.,* 2007) and absence of sex preference (Asawapattanakul *et al.,* 2021) was also reported. The high susceptibility in certain pure breeds could be associated with increased preference to Rottweiler, Pitbull, Pug and Great Dane breeds by pet owners. In contrast, a high prevalence was recorded in German Shepherd (Kalaivanan *et al.,* 2020 and Singh *et al.,* 2021a), associated with their low cell mediated immune response (Dhankar *et al.,* 2011) and in Labrador retriever (Pothiappan *et al.,* 2024). More positivity in dogs with recent exposure to tick bites could be due to the seroconversion associated with an active ehrlichial infection.

In the forest tree analysis (Fig 6), the boxes represented the point estimates of RR and the whiskers represented the confidence interval for the variables. Since, the 95% CI from the pooled estimates (diamond) was entirely on the right side of no-effect line (corresponding to 1), the difference in the RR between the clinical variables was statistically significant. In contrast, 95% CI from the pooled estimates crossed the no-effect line indicating that there existed no significant difference. The graphical representation of prevalence of CME is shown in Fig 7.

CONCLUSION

Nearly one fourth of the dogs presented with gastro intestinal bleeding signs were found to be infected with *E. canis*, where majority of the cases were sub-acute and a few cases were chronic. Lateral flow assay as a point- of-care assay with on-spot diagnosis was useful in detecting the ehrlichia cases which were missed by conventional microscopy. Melena, monocytosis, lymphocytosis and leucocytosis were identified as early ehrlichia predictors in initiating the specific treatment to prevent the cases entering into the fatal chronic (myelosuppressive) form. Imaging techniques such as ultra sonography and computerized tomography could be useful in detecting the lymphoproliferative changes caused by ehrlichia in liver, spleen, kidney, lungs, urinary bladder and intestine.

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Conflict of interest

This study does not require approval of ethical committee. The authors have no relevant financial or non-financial interests to disclose.

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