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Study of *Ehrlichia canis* in febrile dogs of Kathmandu valley of Nepal

Chet Narayan Gyawali, Mohan Prasad Gupta, Subir Singh and Ram Prasad Poudyal

Abstract
Canine ehrlichiosis is a tick-borne disease caused by an obligate intracellular alpha- proteobacterium, *Ehrlichia canis* transmitted by *Rhipicephalus sanguineus* characterized by thrombocytopenia, variable leucopenia, anaemia, fever, anorexia, lymphadenopathy, haemorrhages in mucous membrane, peripheral oedema, emaciation and hypotensive shock leading to death.
Methods: Among the febrile dogs presented to 8 different veterinary hospitals and clinics of Kathmandu valley, 270 samples were randomly selected and tested by SensPERT *E. canis* Antibody Test Kit. Positive samples were tested for morula detection by blood smear microscopy and measurement of hematological and biochemistry parameters. Questionnaire was done for risk factor analysis.
Results: Overall seroprevalence by Rapid antibody test was 11.85% (32/270) and prevalence of 4.07% (11/270 by blood smear microscopy. Variation in prevalence of *E. canis* with housing, history of anorexia, hemorrhage in mucous membrane and edema were statistically significant (p<0.05) and that with sex, location and age groups were found statistically insignificant (p>0.05) in dogs seropositive by rapid test. In dogs positive to blood smear similarity was found except housing was found statistically not significant (p>0.05). In hematological and biochemical parameters variation in monocyte, hemoglobin, packed cell volume, platelets, albumin and glucose with reference value were found statistically significant by z test (p<0.05) and WBC, Neutrophil, Lymphocyte, Eosinophil, Basophil, erythrocyte sedimentation rate, blood urea nitrogen, serum creatinine, total/direct bilirubin, SGPT (ALT), SGOT (AST), ALP and total protein were not statistically significant (p> 0.05) in febrile dogs positive by rapid test. Moreover, in dogs positive by smear microscopy significant relation was seen also in Eosinophil (p< 0.05). Febrile dogs with hemorrhage in mucous membrane, edema anorexia are most prone to get infection with *E canis* (p<0.05). Similarly, febrile dogs with hematological and biochemical parameters like thrombocytopenia, monocytopenia, low packed cell volume, low hemoglobin, decreased albumin and glucose level strongly suggest an infection with *Ehrlichia* infection.

Keywords: *Ehrlichia*, hematology, biochemistry, blood smear, antibody test

Introduction
Parasitic diseases are a major health concern in dogs as they are among the most common pets and their population has increased tremendously (Mcbride *et al*., 1996) [25]. Several blood protozoan parasites like Trypanosomes, Leishmania and Babesia cause infection and death in dogs and man in the tropical regions (Urquhart, 1996) [42]. Brown dog tick is the major carrier of Tick-borne infectious disease of dogs, Ehrlichiosis. Etiological agent of Canine monocytic ehrlichiosis is an obligate intracellular alpha-proteobacterium, *Ehrlichia canis* (*E. canis*) which replicates within mononuclear cells in the host (Harrus & Waner, 2011) [20]. The most common rickettsial species causing CME is *Ehrlichia canis* although other strains of the organism may also affect dogs.
It has also been called tracker dog disease and tropical canine pancytopenia because of its origin in military dogs in Vietnam. It has been reported as very common disease from subtropical and tropical areas of the world (Abd Rani *et al*., 2011) [1]. *Rhipicephalus sanguineus* sensu lato, is commonly available in tropical and subtropical areas of the world in the rural and urban areas, which is the major reasons for the common occurrence of *E. canis* (Aktas *et al*., 2015) [3].

Disease is divided into acute, subclinical, and chronic phases (Harrus *et al*., 1999) [21] with the incubation period that ranges from 8 to 20 days (Greene, 2006) [19]. The rapid acute phase is of 2 to 4 weeks (Greene, 2006) [19] with symptomps of fever, weight loss, anorexia, depression, limphadenomegaly, spleenomegaly, vasculites, and ocular and musculoescheletic signs (de Castro *et al.*, 2004) [14]. Thrombocytopenia, decrease in number of platelets, in naturally or experimentally infected dogs is the most common abnormality in this phase of the disease (Waner *et al.*, 1995) [43].

Traditionally the disease was diagnosed using techniques: hematology, cytology, serology and isolation which were valuable diagnostic tools for CME, however a confirmatory diagnosis of Ehrlichiosis requires molecular techniques (Harrus & Waner, 2011) [20]. Other diagnostic tools include blood smears microscopy where identification of inclusion bodies or morulae of *E. canis* in leucocytes (Elias & Menon, 1991) [17], or buffy coat smears and lymph node aspiration (Mylonakis *et al*., 2003) [28] is performed. Some other diagnostic methods include specific antibodies detection by the Immuno-fluorescent Antibody Test (IFAT) and dot-ELISA and by molecular techniques such as nPCR (Nakaghi *et al*., 2010) [32].

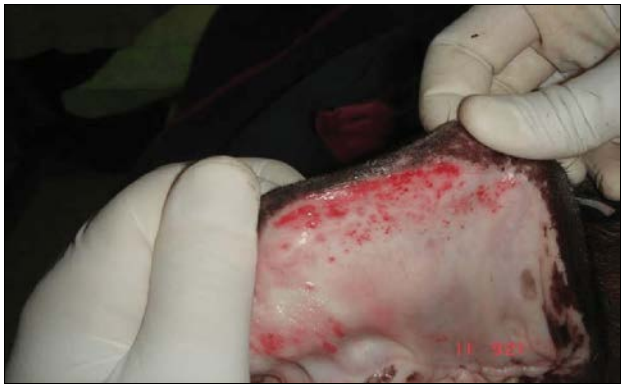


Fig 1: Numerous petechiae and ecchymoses on the upper lip mucosa from a dog with acute CME (Mylonakis & Theodorou, 2017) [29]

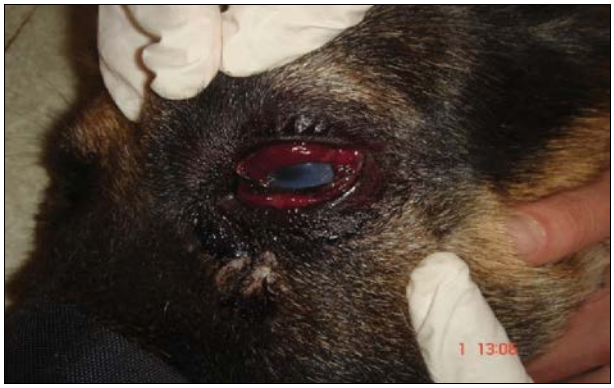


Fig 2: Conjunctival hemorrhage and anterior uveitis in a dog with CME (Mylonakis & Theodorou, 2017) [29]

Fig 3: Penile mucosal pallor petechiae and ecchymoses in a dog with CME associated aplastic (Mylonakis & Theodorou, 2017) [29]

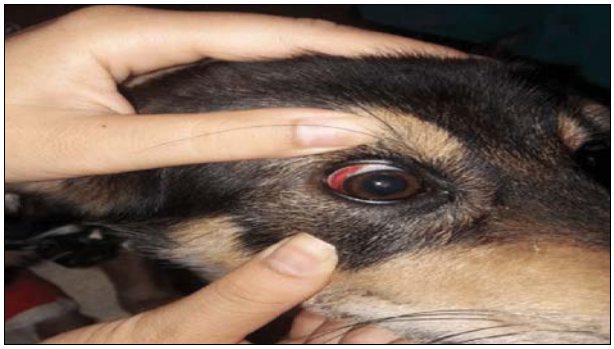


Fig 4: Scleral hemorrhage in a dog with CME (Mylonakis & Theodorou, 2017) [29]

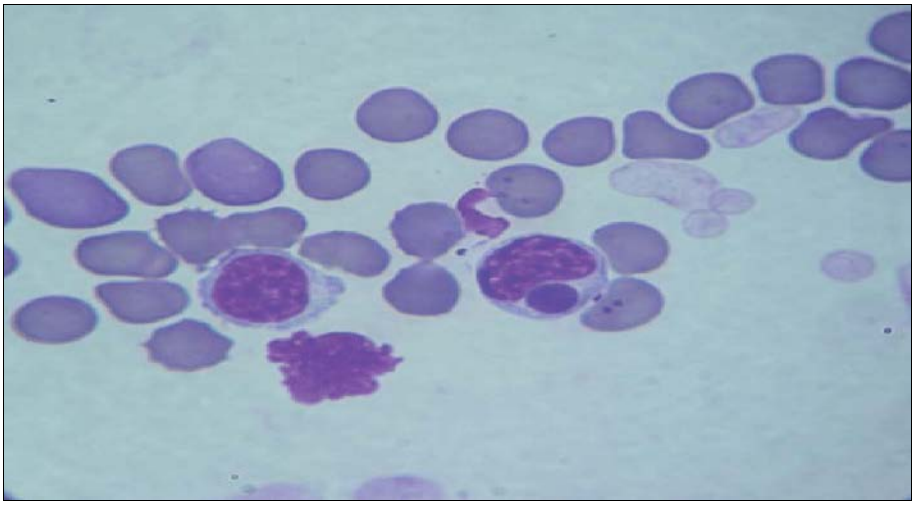


Fig 5: Buffy coat smear from a dog experimental – CME – Diff Qyuik objective 100x (Mylonakis & Theodorou, 2017) [29]

Materials and Methods

Survey design

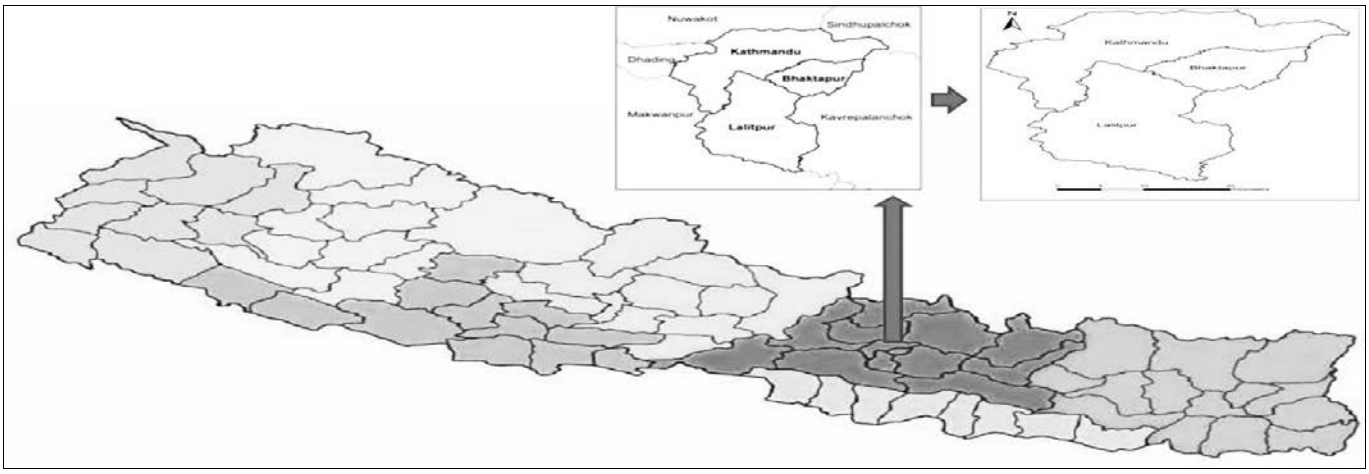


Fig 6: Study Site of Kathmandu Valley with Kathmandu, Bhaktapur and Lalitpur district of Nepal

Kathmandu Valley is the capital of Nepal with three large cities, Kathmandu, Lalitpur and Bhaktapur whose geographical location is 27'27" East to 27' 49" East and 85'10" North to 85'32" North (Bhatta, Acharya, Acharya & Thapa, 2018) [8]. Population of housed pet and stray dogs has been increasing in Kathmandu and there is no any exact information on population distribution and density of pet dogs and stray dogs. So, for this study, infinite population is taken and Daniel and Cross (2018) [13] formula is used to calculate the sample size. Due to lack of information on distribution and density of dogs in different locations, cases presented to the different hospitals will be taken as the sample. Among the random cases of dogs from various random locations attended in those hospitals and clinics of Kathmandu Valley, samples were selected on random basis (irrespective of age, sex and breed).

$$n = \frac{z^2 P (1-P)}{d^2}$$

Where,

n = required sample size;

z = reliability coefficient at 95% level of confidence (= 1.96)

P = estimated prevalence based on previous study

d= level of precision (5%)

According to Díaz-Regañón *et al*., (2012) [15], the prevalence (P) was 27.14% (19/70), Phuyal, Jha & Subedi, (2017) [35] was 8% (4/50) and Bhatta, Acharya, Acharya & Thapa, (2018) [8] was 10.66% (16/150) in different studies done at Kathmandu Valley. The average prevalence from the previous literature equals to 15.27%. Thus required sample size from the above formula was calculated to be 198.81 (~ 199). During sampling and questionnaire survey, there is high chance of occurrence of error or in some samples some data might be missing. To overcome this error 10% more of calculated sample size has been suggested to be collected. Thus, total sample size for the study equals to 219.

But, 270 samples were collected from different hospitals from December 2019 to September 2020. The conduction of questionnaire survey about information regarding age, breed, sex, presence of clinical signs and management factors was done by owner's interview.

Table 1: Number of cases attended from different hospitals and clinics

S. N.	Name of hospital	Number of cases
1.	Central Veterinary Hospital	39
2.	Advance Pet hospital and research center	55
3.	Valley Animal Clinic	82
4.	Vet for your Pet -Kathmandu Clinic	19
5.	Nepal Police Canine Division	22
6.	Animal Nepal	17
7.	Kathmandu Animal Treatment Centre	17
8.	Animal Medical Centre	19
	Total	270

Laboratory test

In all, 270 blood samples were collected on random basis from dogs with febrile cases presented to different 8 veterinary hospitals and private veterinary clinics of Kathmandu valley. Blood samples were taken from dogs with hyperthermia (temperature > 103-degree Fahrenheit). Blood samples were collected by jugular vein puncture in sterile 5 ml EDTA vial and fresh blood for blood smear preparation, hematology and biochemistry. The tube for further investigation was kept under refrigeration at -4 ˚C until further laboratory works were performed. Serum was extracted and then tested by using SensPERT Ehrlichia Anitbody Test Kit (Sensitivity: 97.7% vs IFAT, Specificity: 100% vs IFAT). Blood and serum samples were further tested using blood smear with Giemsa stain (Qualigens) under microscope for morula detection and also sent to laboratory for hematology (HumaCount 5D) and biochemistry profile (Erba Chem 7 chemistry analyzer).

Table 2: Normal reference value of hematological and biochemical tests

Hematological Tests (Dog)			
Test		**Result**	**Normal Value**
Total Leukocyte Count (/cumm)			4000-11000
Differential Leukocyte Count-DLC (%)	Neutrophils		40-65
	Lymphocyte		12-30
	Monocyte		3-10
	Eosinophil		0-9
	Basophil		0-1
Hemoglobin (g/dL)			14-20
Packed Cell Volume -PCV (%)			37-55
Erythrocyte Sedimentation Rate –ESR (mm/hr)			5-8
Platelets (*1000/cumm)			150-400
Biochemical Tests (Dog)			
Blood Urea Nitrogen- BUN (mg/dl)			7-27
Serum Creatinine (mg/dl)			0.5-1.6
Total Bilirubin (mg/dl)			0.1-0.3
Direct Bilirubin (mg/dl)			0-0.1
SGPT/ALT (U/L)			10-34
SGOT/AST (U/L)			10-62
ALP (U/L)			10-150
Total Protein			5.1-7.8

Data analysis

The collected data were entered into Microsoft Excel Worksheet and analyzed with statistical software; Open Source Epidemiologic Statistics for Public Health, Version 3.01 (https://www.openepi.com/Menu/OE_Menu.htm) and R version 3.0.3 (R Core Team, 2014). Similarly, z test was used for hematological and biochemistry parameters for calculation of p-value to check statistical significance (p<0.05) in Microsoft Excel Worksheet.

Result

Prevalence of *E. canis* in febrile dogs of Kathmandu

Overall sero-prevalence of *E. canis* by rapid antibody test was found to be 11.85% (32 positives of 270 samples) and prevalence of 4.07% (11 positive of 270 samples) by blood smear microscopy in febrile dogs presented at different hospitals of Kathmandu valley during 2019-2020.

Prevalence of *E. canis* on the basis of sex in febrile dogs of Kathmandu

Table 3: Prevalence of *E. canis* on the basis of Sex in febrile dogs of Kathmandu

Rapid antibody test kit					
Sex	**Total**	**Positive sample**	**Sero/prevalence (%)**	**Odd Ratio**	**Chi-squared P value**
Female	97	9	9.28	0.667 (0.2955-1.506)	0.3273
Male	173	23	13.30		
Blood smear microscopy					
Female	97	3	3.09	0.6582 (0.1705-2.541)	0.7942 (Fisher exact Test)
Male	173	8	4.62		

Note: The figures in bracket indicate confidence interval at 95%.

Prevalence of *E. canis* on the basis of housing in febrile dogs of Kathmandu

Table 4: Prevalence of *E. canis* on the basis of housing in febrile dogs of Kathmandu

Rapid antibody test					
Housing	**Total**	**Positive sample**	**Sero/prevalence (%)**	**Odd Ratio**	**Fisher Exact Test P value**
Housed	231	17	7.36	0.1271 (0.05641-0.2864)	0.000003722
Street Dog	39	15	38.36		
Blood smear microscopy					
Housed	231	7	3.03	0.2734 (0.0761-0.9825)	0.1157
Street Dog	39	4	10.26		

Note: The figures in bracket indicate confidence interval at 95%.

Prevalence of *E. canis* on the basis of Location in febrile dogs of Kathmandu

Table 5: Prevalence of *E. canis* on the basis of location in febrile dogs of Kathmandu

Rapid antibody test					
Location	**Total**	**Positive sample**	**Sero/prevalence (%)**	**Odd Ratio**	**Chi-squared P value**
Bhaktapur	65	7	10.77	N/A	0.9011
Kathmandu	125	16	12.80		
Lalitpur	80	9	11.25		
Blood smear microscopy					

Bhaktapur	65	1	1.538	N/A	0.3994
Kathmandu	125	7	5.6		
Lalitpur	80	3	3.75		

Prevalence of *E. canis* on the basis of age groups in febrile dogs of Kathmandu

Table 6: Prevalence of *E. canis* on the basis of age group in febrile dogs of Kathmandu

Rapid Antibody Test					
Age groups	**Total**	**Positive sample**	**Sero/ prevalence (%)**	**Odd Ratio**	**Chi-squared P value**
Upto 1 year	47	7	14.89	1.2 (0.4507-3.195)	0.6121
1-5 years	110	14	12.73	1.352 (0.5853-3.124)	
Above 5 yrs.	113	11	9.74	1.623 (0.5877-4.481)	
Blood smear microscopy					
Upto 1 year	47	2	4.26	0.7704 (0.1497-3.963)	0.5704
1-5 years	110	6	5.46	2.115(0.5157-8.678)	
>5 years	113	3	2.66	1.63(0.2634-10.08)	

Note: The figures in bracket indicate confidence interval at 95%.

Prevalence of *E. canis* in relation to anorexia in febrile dogs of Kathmandu

Table 7: Prevalence of *E. canis* in relation to anorexia in febrile dogs of Kathmandu

Rapid antibody test					
	Total	**Positive sample**	**Sero/prevalence (%)**	**Odd Ratio**	**Chi-squared P value**
Anorexia	167	25	14.97	2.414 (1.004-5.805)	0.04354
No anorexia	103	7	6.80		
Blood Smear Microscopy					
Anorexia	112	10	8.93	6.497 (0.8193-51.51)	0.001671 (Fisher Exact Test)
No anorexia	158	1	0.63		

Note: The figures in bracket indicate confidence interval at 95%.

Prevalence of *E. canis* in relation to hemorrhage in mucous membrane in febrile dogs of Kathmandu

Table 8: Prevalence of *E. canis* in relation to hemorrhage in mucous membrane in febrile dogs of Kathmandu

Rapid antibody test					
Mucous membrane	**Total**	**Positive samples**	**Sero/prevalence (%)**	**Odd Ratio**	**Chi-squared P value**
Hemorrhage	59	23	38.98	14.34 (6.14-33.49)	<0.0000001
No	211	9	4.27		
Blood Smear Microscopy					
Hemorrhage	59	8	13.56	10.88(2.787-42.45)	0.0007(Fisher Exact Test)
No	211	3	1.42		

Note: The figures in bracket indicate confidence interval at 95%.

Prevalence of *E. canis* in relation to edema in febrile dogs of Kathmandu

Table 9: Prevalence of *E. canis* in relation to edema in febrile dogs of Kathmandu

Rapid antibody test kit					
Edema	**Total**	**Positive sample**	**Sero/prevalence (%)**	**Odd Ratio**	**Fisher Exact Test P value**
Yes	26	9	34.62	5.087 (2.038-12.7)	0.002273
No	244	23	9.43		
Blood smear microscopy					
Yes	26	4	15.39	6.156(1.671-22.67)	0.0282
No	244	7	2.87		

Note: The figures in bracket indicate confidence interval at 95%.

Hematology and Biochemistry Result of *E. canis* positive febrile dogs of Kathmandu

Table 10: Statistical analysis of hematological and biochemistry parameters of *E. canis* rapid antibody test positive febrile dogs in Kathmandu

S.N.	**Parameter**	**Reference value**	**Population Mean**	**Sample Mean**	**Sample SD**	**z test p value**
1	WBC	4000-11000	7500	13393.75	6700.069	0.999999676
2	Neutrophil	40-65	52.5	79.6875	10.01752	1
3	Lymphocyte	20-30	21	18.45313	9.762799	0.070007575
4	Monocyte	3-10	6.5	1.265625	0.933121	2.7855E-221
5	Eosinophil	0-9	4.5	0.21875	0.420013	0
6	Basophil	0-1	0.5	0	0	N/A
7	Hemoglobin	14-20	17	7.95	2.264452	1.8113E-113

8	PCV	37-55	46	24.30313	6.548454	1.10921E-78
9	ESR	5-8	6.5	26.84375	23.1072	0.999999683
10	Platelets	150-400	275	120.4063	64.49149	3.44971E-42
11	BUN	7-27	17	72.87813	77.15807	0.999979047
12	S. Creatinine	0.5-1.6	1.05	2.7	3.796688	0.993022282
13	T. Bilirubin	0.1-0.3	0.2	0.471875	0.709491	0.984908935
14	D. Bilirubin	0-0.1	0.05	0.134375	0.070066	1
15	SGPT (ALT)	10-34	22	60.54375	41.8335	0.999999907
16	SGOT (AST)	10-62	36	60.71875	46.77002	0.998603942
17	ALP	10-150	80	213.25	109.5907	1
18	T. Protein	5.1-7.8	6.45	6.6125	0.764853	0.885289258
19	Albumin	2.7-4.4	3.55	3.190625	0.749348	0.003334509
20	Glucose	60-117	88.5	76.65625	40.66512	0.04972115

Note: 1E-n means 1*10^(-n) where n= 1,2,3,……,∞

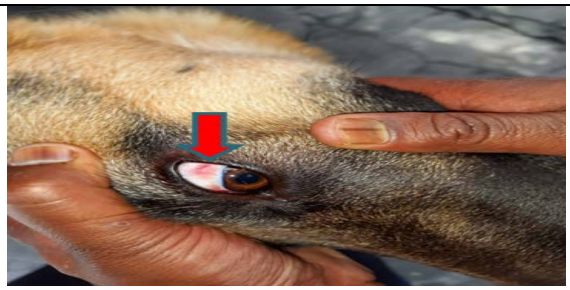
Table 11: Statistical analysis of Hematological and Biochemistry parameters of *E. canis* positive in blood smear microscopy in febrile dogs in Kathmandu

S.N.	Parameter	Reference value	Population Mean	Sample Mean	Sample SD	z test p value
1	WBC	4000-11000	7500	15763.64	10063.53	0.996769677
2	Neutrophil	40-65	52.5	78.18182	10.86111	1
3	Lymphocyte	20-30	21	18.5	10.74011	0.220051817
4	Monocyte	3-10	6.5	1.954545	0.960587	8.29632E-56
5	Eosinophil	0-9	4.5	0.272727	0.467099	3.0689E-198
6	Basophil	0-1	0.5	0	0	N/A
7	Hemoglobin	14-20	17	7.790909	1.856585	4.10959E-61
8	PCV	37-55	46	23.88182	3.480465	6.4682E-99
9	ESR	5-8	6.5	34.09091	28.79741	0.999257684
10	Platelets	150-400	275	127.5455	70.27711	1.7147E-12
11	BUN	7-27	17	62.88182	50.69806	0.998656956
12	S. Creatinine	0.5-1.6	1.05	2.3	3.531006	0.879824067
13	T. Bilirubin	0.1-0.3	0.2	0.690909	1.172565	0.917514651
14	D. Bilirubin	0-0.1	0.05	0.118182	0.040452	0.999999989
15	SGPT (ALT)	10-34	22	67.09091	55.16422	0.996645865
16	SGOT (AST)	10-62	36	67.72727	74.75439	0.92038154
17	ALP	10-150	80	214.7273	103.6205	0.999991921
18	T. Protein	5.1-7.8	6.45	6.872727	0.634178	0.986474439
19	Albumin	2.7-4.4	3.55	3.172727	0.593449	0.017494997
20	Glucose	60-117	88.5	69.72727	13.58007	2.27194E-06

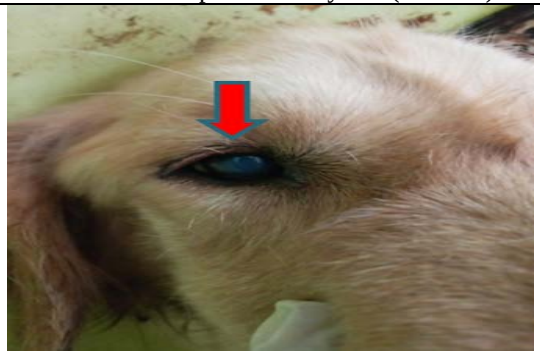
Note: 1E-n means 1*10^(-n) where n= 1,2,3,……,∞

Research findings

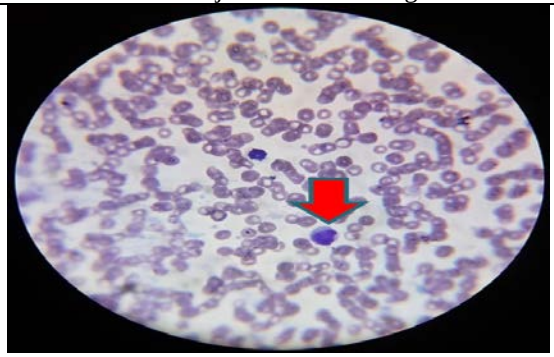
SensPERT Rapid Antibody test (Positive)



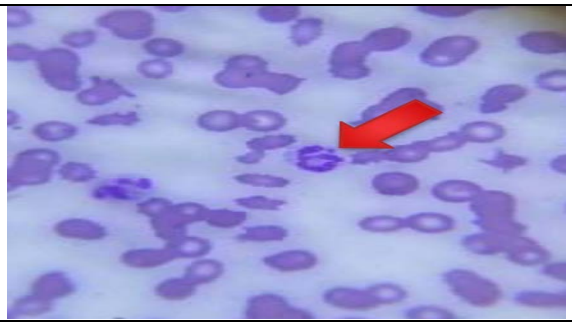
Sub-conjuctival hemorrhage



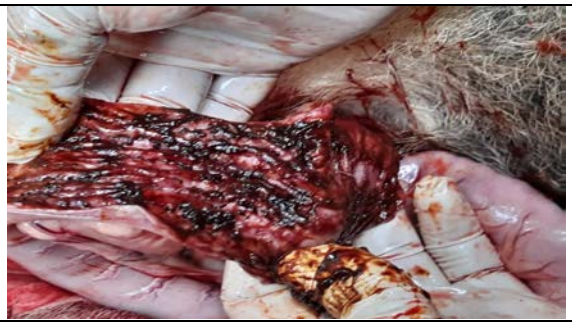
Corneal clouding



E. canis in blood smear (40X)



E. canis in blood smear oil immersion (100X)



Intestinal hemorrhage (Necropsy finding)

Discussion

Overall seroprevalence of *E. canis* in febrile dogs presented at different veterinary hospitals by rapid antibody test kit (SensPERT *E. canis* antibody test) was 11.85% (32 positives of 270 samples) with the prevalence of 10.77% (7/65) in dogs of Bhaktapur, 12.8% (16/125) in Kathmandu and 11.25% (9/80) in Lalitpur. Overall sero-prevalence was in congruence with the study of 11.43% Manandhar and Rajawar (2008) [24], 11.06% (16/150) by Bogicevic *et al*., (2017) [10], 10.41% (40/384) in domestic dogs from Paraguay (Pérez-Macchi, Pedrozo, Bittencourt & Müller, 2019) [34] and 12.5% using the SNAP® 4Dx® Plus kit from IDEXX Laboratories (Angelou *et al*., 2019) [4].

Higher to our study, than the study of Díaz-Regañón *et al*., (2012) [15] obtained the prevalence of 27.14% (19/70). Very high sero-prevalence 80% (48/60) was found by Singla *et al*., (2011) [40] using Immunocomb(®) Dot-ELISA and study of Kukreti *et al*., (2018) [22] has a finding of 57.5% (293/510) by ELISA, 50% (49/98) in Chennai (Lakshmanan *et al*.,2007) [23] and 20.6% from four different regions of India (Abd Rani *et al*. 2011) [1].

Lower prevalence (1.5%) in comparison to our study was reported in the findings by SNAP 4Dx ® test (IDEXX Laboratories) and negative by PCR (Dzięgiel *et al*., 2016) [16], 7.6% (Piantedosi *et al*., 2017) [36], 0.7% by canine point-of-care ELISA kit in dogs in Washington, Oregon, and California (Carrade, Foley, Sullivan, Foley & Sykes, 2011) [11] and 0.8% in dogs in North America (Beall *et al*., 2012) [5]. Working dogs in organized kennel in India, overall prevalence of ehrlichiosis was estimated to be 1.3% (lower to our finding) by microscopic examination, 19.1% (higher to our finding) by commercial dot-ELISA kit and 5.8% by nested PCR assay (Mittal *et al*., 2017) [27].

Overall prevalence of *E. canis* found was 4.07% (11/270) by blood smear microscopy for the detection morula by Giemsa stain (Qualigens) which was 1.538% (1/65) in Bhaktapur, 5.6% (7/125) in Kathmandu and 3.75% (3/80) in Lalitpur. Overall prevalence was similar to the finding of 2.34% (5/214) and 2.12% in the study of Bhattacharjee & Sarmah, (2013) [9] and 2.2% in dogs in Switzerland (Pusterla *et al*., 1998) [37]. Our finding was lower in comparison to the finding of 8% (4/50) by Phuyal, Jha & Subedi, (2017) [35] and 10.66% of Bhatta, Acharya, Acharya & Thapa, (2018) [8] by Giemsa Stain (Himedia) blood smear examination.

When comparing the overall prevalence of the findings from blood smear microscopy and serological examination, false positives chance was low whereas of false-negative results were high with the blood smear result. However, for false positives, morulae of *Ehrlichia* spp can be confused to other similar structures (Dagnone, Souza, A, André & Machado, 2009) [12]. The sensitivity of blood smear microscopy varies at the time of sample taken from host with the stage of infection. During higher parasitic load, high occurrences of infected leukocytes are seen in the blood smear; whereas in the subclinical and chronic stage, infected leukocytes are scarce, that may result false-negative. The chances increase to find specific antibodies rises approximately after 15 days of infection due to the time body takes to develop immunoglobulins (Nakaghi, Machado, Costa, André & Baldani, 2008) [31]. In addition, due to the fact that serological testing is based only on detection of antibodies, serology positive result may give negative result in blood smear.

Male dogs were found higher prevalence 13.30% (23/173) in comparison to female 9.28% (9/97) similar to the finding of (male% > female %) Bhatta *et al*., (2018) [8]. No significance difference (p>0.05) for age groups was similar to the finding of Bhatta *et al*., (2018) [8]. There was no significant difference between the prevalence of infection and the host age or gender similar to the finding of Bogićević *et al*., (2017) [10]. Age <1 years showed higher prevalence which coincides to the finding of Milanjeet *et al*., (2014) [26]. Similar to our finding, no statistically significance was found in relation to sex (Singh, Haque, Singh & Rath, 2012; Silva *et al*., 2012) [39, 38] and prevalence in females was found greater to male (Milanjeet *et al*., 2014) [26] which differ to our finding.

Due to the varied clinical, haematological and biochemical variations, the laboratory diagnosis of CME is difficult (Waner 2008) [44]. The prevalence of *E. canis* was correlated significantly with study's findings of hematological profile with decreased TLC, PCV, and Hemoglobin. In dogs affected by *Ehrlichia* spp, the low count of Total Leucocyte Count was significant statistically (p<0.05) (Bhatta *et al*., 2018) [8] which differ from our finding.

In case of CME, drastic changes in the count for hematological parameters are observed in the acute, subclinical and chronic phases (Waner, 2008) [44]. A very important finding of restricted or absent bone marrow response is observed in affected dogs leading to normocytic, normochromic and non-regenerative anemia (Gaunt *et al*., 2010; Silva *et al*., 2012) [18, 38]. To support CME as an important cause of anemia in canines, similar study statistically verifies significant drop (p<0.05) in haemoglobin levels (Milanjeet *et al*., 2014) [26] similar to our finding.

The test positive samples by blood smear microscopy were statistically significant (p<0.05) for decrease in platelet count that indicates thrombocytopaenia (Milanjeet *et al*., 2014) [26] similar to our findings. In dogs with CME and dogs experimentally infected, thrombocytopaenia is most common and prominent blood parameter finding. Mild to severe low platelets count in Ehrlichia affected dogs has been found (Niwetpathomwat *et al*., 2006; Silva *et al*. 2012) [33, 38] similar to our finding. Low level of platelets count obtained in data can be due to immune-mediated breakdown, increased use of platelets by sequestration or by low production, vasculitis and abnormal functioning of platelet (Milanjeet *et al*., 2014) [26].

There was increase in serum ALT, AST and alkaline

phosphatase values in affected dogs, suggesting hepato-biliary dysfunction. These findings were in agreement with other workers (Srikala, Satish Kumar, Amruth Kumar & Tirumala Rao, 2012; Bhardwaj, 2013; Agnihotri, Khurana, Jain & Singh, 2012) [41, 7, 2] similar to our findings. Correlation to our study, due to infiltration of perivascular mononuclear cells in the hepatic cells of the liver leading to histopathological changes and high values of AST and ALT (Nair *et al*. 2016) [30]. Low levels of serum albumin and globulin in serum protein profile in dogs affected (Agnihotri *et al*., 2012; Srikala *et al*. 2012; Bhadesiya & Raval, 2015) [2, 41, 6] coincides to our finding.

Summary and Conclusion

Overall seroprevalence by SensPERT *E. canis* Antibody test was 32 positives of 270 samples (11.85%) and 11 positive of 270 samples (4.07%) by blood smear microscopy. Among the risk factor considered, variation in prevalence of *E. canis* with housing, history of anorexia, hemorrhage in mucous membrane and edema were statistically significant and that with sex, location and age groups were found statistically insignificant in dogs seropositive by rapid test. In dogs positive to blood smear similarity was found except housing was found statistically not significant. In hematological and biochemical parameters variation in monocyte, hemoglobin, PCV, Platelets, Albumin and Glucose with reference value were found statistically significant and WBC, Neutrophil, Lymphocyte, Eosinophil, Basophil, erythrocyte sedimentation rate, blood urea nitrogen, serum cretinine, Total/Direct Bilirubin, SGPT (ALT), SGOT (AST), ALP and total protein were not statistically significant in febrile dogs positive by rapid test. Moreover, in dogs positive by smear microscopy significant relation was seen also in Eosinophil. Febrile dogs with hemorrhage in mucous membrane, edema anorexia are most prone to get infection with *E canis* ($p<0.05$). Similarly, dogs with hematological and biochemical parameters like thrombocytopenia, monocytopenia, low packed cell volume, low hemoglobin, decreased albumin and glucose level strongly suggest an infection with *Ehrlichia* in febrile dogs.

This study finding of *Ehrlichia canis* prevalence in febrile dogs presented to different hospitals of Kathmandu valley suggest the importance of further study of canine ehrlichiosis in Kathmandu as well as in other parts of the country. The prevalence and statistical significance in street dogs over housed dogs shows the disease occurrence and transmission cycle between the street dog and housed dog. This disease should be regarded as an important disease by pet owners and veterinarians should be monitored closely by veterinary authorities and further study with gold standard test and molecular diagnosis is suggested.

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