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Prevalence of Canine *Ehrlichiosis* in the Vindhya Region of Madhya Pradesh (Rewa)

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ABSTRACT: Canine ehrlichiosis also known tropical "canine pancytopenia and Nairobi bleeding disorder is an earmarked rickettesial disease caused by an intracytoplasmic parasite *Ehrlichia canis,* seen in circulating monocytes and lymphocytes. It is an important disease in dogs and humans worldwide with higher frequencies reported from tropical and subtropical regions due to presence of the *Rhipicephalus sanguineus*. Epidemiological studies in canine ehrlichiosis have not been conducted in Rewa district of Madhya Pradesh, hence it was done for the first time. Therefore, the present study was undertaken to study the prevalence of canine ehrlichiosis in and around Rewa (M.P.) in affected dogs. The present study was carried out in the Department of Veterinary Medicine, COVS & AH, Rewa. The epidemiological survey was carried out in and around Rewa and the study revealed an overall prevalence of 19.5% with maximum prevalence in 1-3 years age group (30.76%), breed wise - German Shepherd (35.89%), sex wise - males (53.84%), and then in area wise – rural areas (19.81%). PCR assay proved to be a sensitive and rapid diagnostic tool. Canine ehrlichiosisi characterised by fever, neurological, ocular signs and bleeding through natural orifices in the form of epistaxis, hematemesis and dermal petechiae, lymphadenomegaly, splenomegaly.

Keywords: *Ehrlichia canis*, *Rhipicephalus sanguineus*, Prevalence, Rickettsial disease.

INTRODUCTION

Canine *ehrlichiosis* has been observed as an emerging and earmarked rickettesial diseases, caused by rickettsial microorganism, *Ehrlichia canis*. It is currently reported throughout the world but at higher frequencies in tropical and subtropical regions due to presence of their vectors (Unver *et al*., 2003). The disease affects dogs, other domestic and wild animal species as well as humans (Perez *et al*., 2006).

Rhipicephalus sanguineus, the brown dog tick, is the most widespread tick in the world and is a well-recognized vector of *Ehrlichia canis* and occasionally humans. The prevalence of *E. canis* is dependent on the distribution of the tick, which occurs mainly in tropical and subtropical regions. *Ehrlichia canis* is transmitted transtadially and intrastadially by *Rhipicephalus sanguineus* ticks (Bremer *et al.,* 2005). It is a multisystemic disease with clinical symptoms like fever, leukopenia, thrombocytopenia, depression, anorexia, diarrhoea, depression, lethargy, neurological, ocular signs and bleeding through natural orifices in the form of epistaxis, hematemesis, hematuria and melena have been reported (Kumar and Varshney 2006) which vary considerably in severity and frequency of occurrence in the initial and terminal phases of infection. The study has been designed with the objective of estimation of Canine *ehrlichiosis* in and around Rewa (M.P.).

MATERIAL AND METHODS

200 dogs irrespective of age, sex and breed, with a history of anorexia and showing clinical signs of pale mucous membranes and dermal petechiae and with a history of recent tick infestation were screened for presence *ehrlichia* organism by blood smear examination and PCR assay.

Thin blood smears were prepared after puncturing the ear tips with 24-gauge needle, fixed in methanol and stained with Giemsa and Leishman's stains following standard procedures (Benjamin, 1982), which were observed under microscope for presence of ehrlichia organism and identified on the basis of characteristic morphology (Soulsby, 1982). One ml blood samples were also obtained from suspected dogs from cephalic/saphenous vein for isolation of DNA for PCR and those showing band of 412 bp on PCR assay were included in the study.

Molecular detection of *Ehrlichia canis* by PCR:

Genomic DNA extraction:

The blood samples collected in vaccutainer containing sodium EDTA were brought at room temperature and genomic DNA was extracted as follows:

1. Four hundred microliters of the sample was taken and centrifuged at 4,000 rpm for 3 min.
2. The cell pellets were resuspended in 1 ml of erythrocyte lysis solution, mixed, and centrifuged as described below:

Erythrocyte lysis solution:

155 mM NH_4Cl,

M $NaHCO_3$,

disodium EDTA [pH 7.4]

3. Treatment with erythrocyte lysis solution was repeated until the leukocyte pellets lost all reddish colour. Template DNA was obtained as follows:

(a) Four hundred microliters of lysis solution (2% Triton X-100, 1% sodium dodecyl sulfate, 100 mMNaCl, 10 mMTris-HCl [pH 8.0]) and 10 µl of proteinase K (10 mg/ml) were added to the samples, and the contents were mixed thoroughly and incubated for 30 min at 50°C.

(b) Four hundred microliters of saturated phenol was added, and the contents were mixed thoroughly and centrifuged at 10,000 rpm for 10 min.

(c) The aqueous layer was transferred to a fresh eppendorf tube, and an equal volume of chloroform-isoamyl alcohol (24:1) was added; the contents of tubes were mixed thoroughly and centrifuged at 10,000 rpm for 10 min.

(d) The upper layer was again transferred to a fresh eppendorf tube, and 200 µl of 7.5 M ammonium acetate was added and mixed thoroughly. Two volumes of absolute ethanol were added, the contents were mixed, and the tubes were stored at -20°C.

(e) DNA was recovered by centrifuging the samples at 10,000 rpm for 10 min.

(f) The pellets were rinsed with 1 ml of 70% ethanol, dried, and resuspended in 30 µl of TE buffer [10 mM Tris-HCl (pH 8.0), 1 mM disodium EDTA] and stored at -20°C until they were processed.

Polymerase Chain Reaction: The PCR reaction was performed in 25 µl reaction volume containing 12.5 µl of 2X GoTaq® Green master mix (Promega, USA) (reaction buffer (pH 8.5), 400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP and 3mM $MgCl_2$) (Table 1), 1 µl of each forward and reverse primer (10 p-mole of each) (Integrated DNA Technology), 5 ul of DNA template and 5.5 µl of nucleic acid free distilled water to maintain the volume. The reaction was performed in thermal cycler (Applied Biosystem Veriti™, ThermoFisher) with the lid temperature of 105°C with cycling condition as follows:

- Initial denaturation at 96°C followed by 35 cycles of denaturation at 96°C for 45 sec, annealing at 59°C for 45 sec, extension at 72°C for 45 sec and final extension at 72°C for 7 min.
- The amplified PCR products of 412 bp were subjected to electrophoresis in 1.5 % agarose gel (Bengaluru, Genei, India) with ethidium bromide at final concentration of 0.5µg/L and visualised in Gel documentation system (E-Gel Imager, Life Technologies, Invitrogen, USA) along with molecular marker (100 bp DNA ladder, Thermofisher, USA).

Table 1: Oligonucleotide sequence used in PCR.

Sr. No.	Gene Target	Primer	Primer sequence	Amplicon Size (bp)	Reference
1.	16S rRNA	ECA	5′-AAC ACA TGC AAG TCG AAC GGA-3′	412 (bp)	Wen et al. 1997
2.		HE3R	5′- TAT AGG TAC CGT CAT TAT CTT CCC TAT-3′		

RESULTS AND DISCUSSION

Overall Prevalence: The overall prevalence of canine ehrlichiosis is shown in the Table 2. Out of the 200 dogs surveyed, 39 dogs were found positive showing an overall prevalence of canine ehrlichiosis as 19.5%.

Our findings in the present study are in agreement with those of Katyal (2000); Dagnone *et al.* (2003) who reported a prevalence of 17.58% and 21.70% respectively. However, Bhadesiya and Raval (2015) reported the overall incidence of ehrlichiosis as 62.07%

and Guedes (2015) reported an overall prevalence of 46.9%.

Prevalence on the basis of blood smear examination and PCR Assay: Thin blood smears stained with Geimsa's stain and PCR assay were used for the diagnosis of the canine ehrlichiosis. The results are shown in Table 3.

Conventional microscopic parasitological diagnosis of the stained blood smear is the most commonly used techniques for the canine monocytic ehrlichiosis. But this is a challenging task, as sometimes the organism may not be detectedon peripheral thin blood smear, particularly seen in case of low parasitaemia or in chronic or inapparent infections. PCR based assays are utilized for the early diagnosis of the disease, especially in epidemiological surveys, owing to their higher sensitivity and specificity.

In the present study, out of 200 dogs screened for ehrlichiosis, 14 dogs were found positive by blood smear method (7%) and 25 found positive by PCR method (12.5%). Higher prevalence of canine ehrlichiosis as detected by PCR based assays as compared to microscopy indicates the higher sensitivity levels of PCR. These findings are in agreement with the observations of Mittal *et al*. (2007); Parmar *et al*. (2013)

Area-wise - Prevalence of *E. canis.* In the present study, a higher prevalence was recorded in rural area (19.81%) in comparison with urban area (19.14%) (Table 4). Higher prevalence in rural area may be due to unhygienic conditions and more tick population in these areas.

Table 2: Overall prevalence of canine ehrlichiosis.

Time period	Dog surveyed	Positive dogs	Prevalence (%)
Six months	200	39	19.5%

Table 3: Prevalence on the basis of blood smear examination and PCR Assay.

Total Positive dogs	39	Prevalence (%)
Dogs Positive for *E. canis* on blood smear	14	7 %
Dogs negative for *E. canis* on blood smear but positive in PCR	25	12.5 %

Table 4: Area-wise Prevalence of *E. canis.*

Sector	No. of dogs examined	Positive dogs	Prevalence (%)
Rural	106	21	19.81%
Urban	94	18	19.14%

Age-wise prevalence of *E. canis.* In the present study, maximum prevalence of canine ehrlichiosis was recorded in 1-3 years age group (30.76%), followed by less than 1 year age group (23.07%), 3-6 year age group (20.51%), 6-9 year age group (17.94%) and least prevalence was found in dogs more than 9 year age group (7.69%) (Table 5).

Our findings corroborate with those of Karthika *et al.* (2014); Choudhary *et al.* (2012), who reported highest prevalence in dogs in the age group of 1-3 years. However, Rahman *et al*. (2010) reported no significant difference between age groups. Higher prevalence of canine ehrlichiosis was found in the age group of 1-3 years (30.76%) and lowest in dogs of more than 9 years. The reason for this is that the organism parasitizes monocytes. High bone marrow activity with active precursor cells i.e., monoblasts in young age gives an opportunity for the organism to parasitize more cells and multiply rapidly. The immune system in young animals is also in the developmental stage. However, many authors reported *E. canis* in different age group of dogs. Therefore, it can be opined that the age is not the criteria for *Ehrlichia* infection and it depends largely on the transmitting vector and the immune status of the host.

Breed-wise prevalence of *E. canis.* The present study reported maximum prevalence in German Shepherd dogs (35.89%) followed by 30.76% in Non-Descript, 17.39% in Labrador, 7.69% in Pomeranian, 5.64% in Saint Bernard, and 2.56 % in Rottweiler (Table 6).

These findings are in agreement with the earlier observations of Guedes *et al.* (2015); Singh *et al*. (2014), who reported higher prevalence in GSD. However, Tresamol *et al.* (1998) reported higher occurrence of canine ehrlichiosis in Doberman pinschers while and Costa *et al.*, (2007) found it to be more prevalent in non-descript dogs. Higher susceptibility of GSD to the disease may be due to unnoticed tick infestation as the hair coat is comparatively thick with long hair.

Sex-wise prevalence of *E. canis.* In the present study, higher prevalence was recorded in males (53.84%) as compared to females (48.71%) (Table 7).

Similar findings were reported by Choudhary *et al*. (2012) and Kitaa *et al.* (2014). However, Tanikawa *et al.* (2013) reported no considerable differences between male and female dogs for *E. canis* infection. No sex wise predilection for the disease seems to be present as, it depends purely on the preference of the owners for keeping pets and generally males are more preferred over females as pets.

Frequency of predominant signs of Canine Ehrlichiosis. Among the 18 positive dogs with canine ehrlichiosis, the characteristic clinical features observed

were pyrexia (83.33%), lymphadenopathy (77.7%). anorexia (66.6%), depression and lethargy (66.6%), presence of ticks (61.1%), pale mucous membrane (55.5%), congested mucous membrane 26 (44.4%), melena (11.1%), epistaxis (33.3%), petechial hemorrhages (44.4%) (Table 8).

Table 5: Age-wise prevalence of *E. canis.*

Age group	Positive dogs	Prevalence%
Less than 1 year	9	23.07%
1-3 years	12	30.76%
3-6 years	8	20.51%
6-9years	7	17.94%
More than 9 years	3	7.69%

Table 6: Breed-wise prevalence of *E. canis.*

Breed of dogs	Positive dogs	Prevalence%
Non-descript	12	30.76%
German shepherd	14	35.89%
Labrador	7	17.39%
Pomeranian	3	7.69%
Saint Bernard	2	5.64%
Rottweiler	1	2.56%

Table 7: Sex-wise prevalence of *E. canis.*

Sex	Positive dogs	Prevalence
Male	21	53.84%
Female	19	48.71%

Table 8: Frequency of predominant signs of Canine Ehrlichiosis.

Sr. No.	Clincal Symptoms	No. of Animals (N=18)	Percentage%
1.	Pyrexia <101.5°F	15	83.33%
2.	Lymphadenopathy	14	77.7%
3.	Congested Mucosal Layers	8	44.4%
4.	Pale Mucosal Layers	10	55.5
5.	Epistaxis	6	33.3
6.	Petechial Hemorrhages	8	44.4
7.	Anorexia	12	66.6
8.	Dehydration	8	44.4
9.	Vomiting	6	33.3
10.	Presence of Ticks	11	61.1
11.	Melena	2	11.1
12.	Depression and Lethargy	12	66.6

These findings are in agreement with the earlier reports of Choudhary *et al*. (2015) and Sosa-Gutierrez *et al.* (2013).

The clinical manifestations of ehrlichiosis in dogs varies between and within different geographical locations due to many factors like strain variations, breed of dogs infected, immunological status of the dog, concurrent infection with other tick borne infections.

Elevated temperature, tachycardia and polypnoea were also reported by Choudhary *et al.* (2015). The clinical signs observed may be the result of inflammation which causes marked release of cytokine resulting in pyrexia and associated symptoms.

The palpable lymph nodes were found to be enlarged in most of the dogs under study. Similar findings were recorded by Moreira *et al*. (2003). Intracytoplasmic leucocytic invasion by the rickettsia and due to generalized infection, lymphadenitis is observed. Replication of the organisms in the reticulo-endothelial system along with proliferation of medullary and para-cortical lymphocytes and aggregation of reactive histiocytes in the lymph nodes results in generalized lymphadenopathy. Pallor mucosae (55.5%) as observed in the present investigation in canine monocytic ehrlichiosis has been attributed to the loss of blood due to thrombocytopaenia, suppression of bone marrow and probably due to immune mediated red cell destruction. Whereas, bleeding tendencies (epistaxis, malena, haematemesis, petechial haemorrhages on oral gums and ventral abdomen) is mainly due to thrombocytopaenia and damage to vascular endothelium due to deposition of immune complexes on the vascular wall. Severe antibody mediated cytotoxic destruction of erythrocytes, release of pro-inflammatory cytokines in response to canine ehrlichiosis inhibiting the secretion of erythropoietin results in decreased RBC production together with immune mediated RBC destruction which are

responsible for low RBC count and pale mucous membrane.

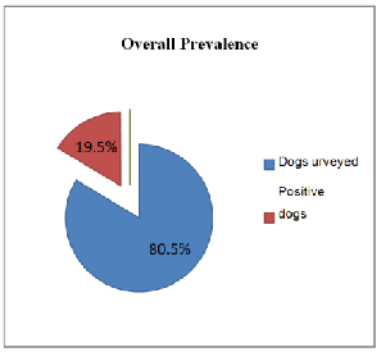

Fig. 1. Showing the overall prevalence of *E. canis* of in Rewa (M.P.)

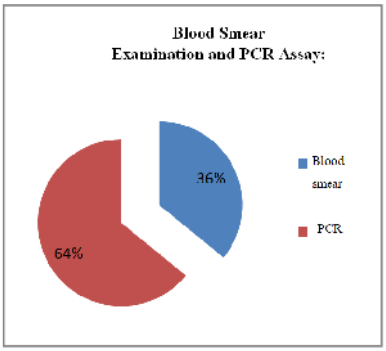

Fig. 2. Showing the prevalence *E. canis* on the basis diagnosis techniques.

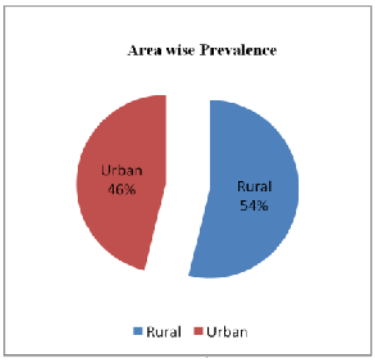

Fig. 3. Showing the areawise prevalence of *E. canis.*

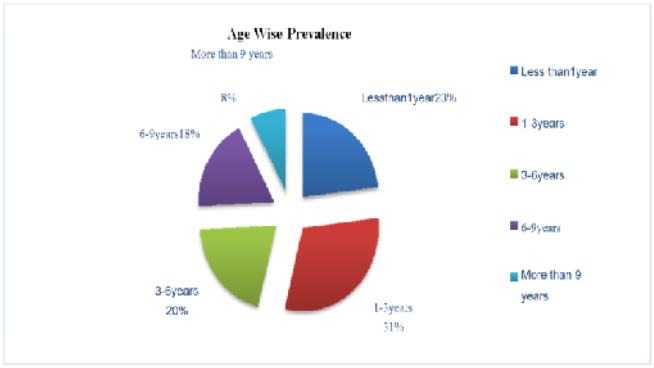

Fig. 4. Showing the agewise prevalence of *E. canis.*

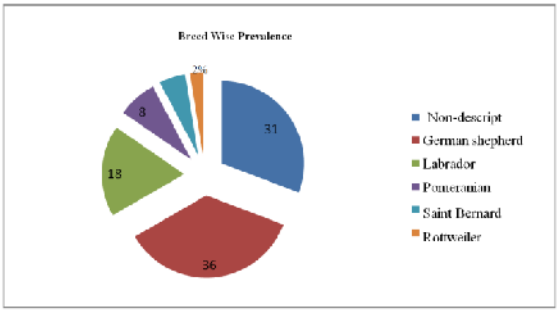

Fig. 5. Showing the Breedwise prevalence of *E. canis.*

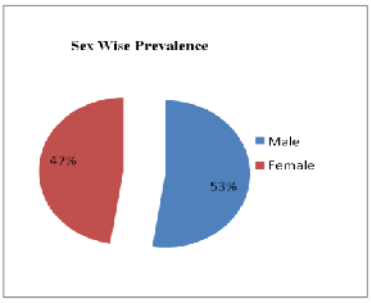

Fig. 6. Showing the sexwise prevalence of *E. canis.*

Plate 1: Showing Tick infestation.

Plate 2: Showing Heavy Tick infestation.

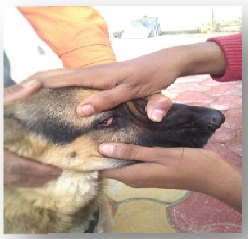

Plate 3: Congested Mucous Membrane.

Plate 4: Epistasis.

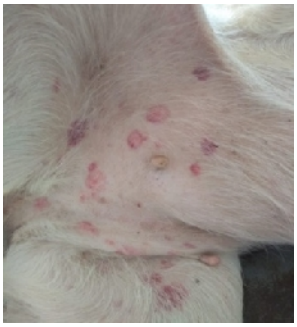

Plate 5: Dermal Petechiae.

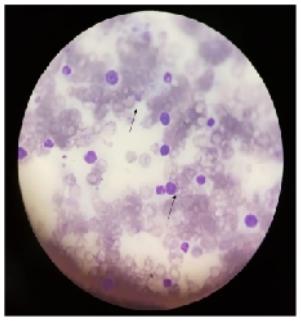

Plate 6: Monocytes are marked by the arrows.

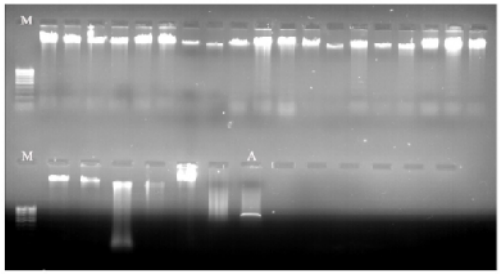

Plate 7: Agarose gel showing genomic DNA of DOG blood samples.
M: 100bp DNA Ladder A: Amplification product

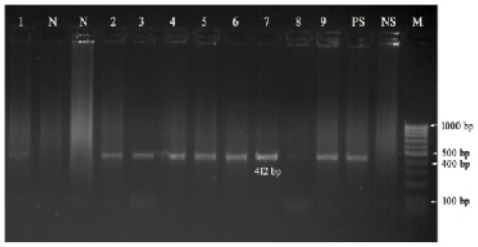

Plate 8: Agarose gel showing the amplification product (412 bp) of 18S rRNA gene of *Ehrlichia canis*.
1-9: Positive sample N: Negative sample
PS: Positive standard NS: Negative standard:
M: 100 bp DNA Ladder.

CONCLUSION

From the above results it was concluded that epidemiological survey carried out in and around Rewa revealed an overall prevalence of 19.5% with maximum prevalence in 1-3 years age group (30.76%), German Shepherd (35.89%), males (53.84%), and then in rural area (19.81). PCR assay proved to be a sensitive and rapid diagnostic tool. Prominent clinical features observed were pyrexia (83.33%), lymphadenopathy (77.7%) & epistaxis (33.3%).

FUTURE SCOPE

Further work on prevalence can be done on other Haemoprotozoan and Rickettesial diseases of dogs.

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Conflict of interests. None.

REFERENCES

Benjamin, M. M. (1982). Outline of Veterinary Clinical Pathology, 3rd Edn., Kalyani Publishers, New Delhi, pp 86.

Bhadesiya, C. M. and Raval, S. K. (2015). Hematobiochemical changes in ehrlichiosis in dogs of Anand region. *Gujarat Veterinary World*, *8*(6): 713-717.

Bremer, W. G., Schaefer, J. J., Wagner, E. R., Ewing, S.A., Rikihisa, Y., Needham, G. R., Jittapalapong, S., Moore, D. L. and Stich, R.W. (2005). Transstadial and intrastadial experimental transmission *Ehrlichiacanis* by male *Rhipicephalus sanguineus*. *Veterinary Parasitology*, *131*: 95–105.

Choudhary, S., Muralidhara, A., Yathiraj, S., Placid, E. D., Suryanarayana, T. and Sengupta, P. P. (2012). Epidemiological study on *Ehrlichiacanis* in Bangalore. *Indian Journal of Veterinary Medicine*, *32*(1): 54-55.

Costa, L. M. J. R., Rembeck, K., Ribeiro, M. F. B., Beelitz, P., Pfister, K. and Passos, L. M. F. (2007). Sero-Prevalence and Risk Indicators for Canine Ehrlichiosis In Three Rural Areas Of Brazil. *Veterinary Journal*, *174*: 673-676.

Dagnone, A. S., Autran de Morais, H. S., Vidotto, M. C., Jojima, F. S. and Vidotto, O. (2003). Ehrlichiosis in anemic, thrombocytopenic or tick-infested dogs from a hospital population in South Brazil. *Veterinary Parasitology*, *117*: 285-290.

Guedes, P. E. B., Oliveira, T. N. A., Carvalho, F. S., Carlos, R. S. A., Albuquerque, G. R., Munhoz, A. D., Wenceslau, A. A. and Silva, F. L. (2015). Canine ehrlichiosis: prevalence and epidemiology in northeast Brazil. *The Brazilian Journal Veterinary Parasitology*, *24*(2): 115-121.

Karthika, K., Vijaylakshmi, P., Thanislass, J., Sreekrishnan, R., Antony, P. X. and Selvi, D. (2014). Incidence of ehrlichiosis in dogs of puducherry region. *Indian Veterinary Journal*, *9*: 96-97.

Katyal, D. P. (2000). Studies on the prevalence of canine blood parasitic infections with special reference to the diagnosis and chemotherapy of *Ehrlichia canis*. M.V.Sc. Thesis (Veterinary Medicine), Konkan Krishi Vidyapeeth, Dapoli.

Kitaa, J. M. A., Mulei, C. M., Mande, J. D. and Wabacha. J. (2014). A retrospective study of canine ehrlichiosis in Kenya. *International Journal Veterinary Science*, *3*(3): 122-124.

Kumar, A. and Varshney, J. P. (2006). Studies on clinical variants in naturally occurring cases of canine ehrlichiosis. *Intas Polivet*, *7*: 94-101.

Mittal, M., Kundu, K., Chakravarti, S., Mohapatra, J. K., Nehra, K., Sinha, V. K. and Kumar, A. (2017). Canine Monocytic Ehrlichiosis among working dogs of organized kennels in India: A comprehensive analyses of clinicopathological, serological and molecular epidemiological approach. *Preventive Veterinary Medicine*, *147*: 26–33.

Moreira, S. M., Bastos, C. V., Araujo, R. B., Santos, M. and Passes, L. M. F. (2003). Retrospective study (1998-2001) on canine ehrlichiosis in Belo Horizonte. MG, *The Journal Arquivo Brasilerirode Medicina Veterinary Zootecnia*, *55*(2): 55-60.

Parmar, C., Pednekar, R., Jayraw, A. and Gatne, M. (2013). Comparative diagnostic methods for canine ehrlichiosis. *Turk Journal Veterinary Animal Science*, *37*: 282-290.

Perez, M., Bodor, M., Zhang, C., Xiong, Q., and Rikihisa, Y. (2006). Human infection with *Ehrlichia canis* accompanied by clinical signs in Venezuela. *Annals of The New York Academy of Sciences*, *1078*(1): 110–117.

Rahman, W. A., Ning, C. H. and Chandrawathani, P. (2010). Prevalence of canine ehrlichiosis in Perak State, Penisular Malaysia. *Topical Biomedicine*, *27*(1): 13-18.

Singh, M., Singh, N. K., Singh, N. D., Singh, C. and Rath S. S. (2014). Molecular prevalence and risk factors for the occurrence of canine monocytic ehrlichiosis. *Veterinarni Medicina*, *59*(3): 129-136.

Sosa-Gutierrez, C. G., Quintero Martinez, M. T., Gaxiola Camacho, S. M., Cota Guajardo, S., Esteve-Gassent, M. D. and Gordillo-Pérez, M. G. (2013). Frequency and clinical epidemiology of canine monocytic ehrlichiosis in dogs infested with ticks from Sinaloa, Mexico. *Journal of Veterinary medicine*, *10*: 1-3.

Soulsby, E. J. L. (1982). Helminths, Arthropods and Protozoa of domesticated animals. 7th Edn., ELBS Bailliere and Tindall, London, pp 809.

Tanikawa, A., Labruna, M. B., Costa, A., Aguiar, D. M., Justiniano, S. V., Mendes, R. S., Melo, A. L. T., Alves, C. J. and Azevedo, S. S. (2013). *Ehrlichia canis* in dogs in a semiarid region of Northeastern Brazil: Serology, molecular detection and associated factors. *Research in Veterinary Science*, *94*: 474–477.

Tresamol, P. V., Dinakaran, M. and Suresh, S. (1998). Serological diagnosis of *Ehrlichia canis* infection in dogs. *Cheiron*, *27*: 3-4.

Unver, A., Rikihisa, Y., Kawahara, M., Yamamoto, S., (2003). Analysis of 16S rRNA gene sequences of *Ehrlichia canis*, *Anaplasma platys*, and *Wolbachia* species from canine blood in Japan. *Annals of the New York Academy Sciences*, *990*: 692–698.

Wen, B., Rikihisa, Y., Mott, M. J., Greene, R., Kim, H. Y., Zhi, N., Couto, G. C., Unver, A., and Bartsch, R (1997). Comparison of Nested PCR with Immunofluorescent-Antibody Assay for Detection of *Ehrlichia canis* Infection in Dogs Treated with Doxycycline. *Journal of Clinical Microbiology*, *35:* 1852–1855.

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