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Clinico-pathological and haemato-biochemical alterations in Canine Ehrlichiosis

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ABSTRACT

Ehrlichiosis, a vector-borne disease of dogs, has gained importance worldwide because of its growing prevalence and zoonotic importance. An investigation was conducted in the Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar to record the clinical, haematological, biochemical and ultrasonographic alterations in 35 dogs with canine ehrlichiosis that was confirmed through polymerase chain reaction (PCR) producing amplicon at 843 bp. The predominant clinical signs were pyrexia, weight loss, epistaxis, conjunctivitis, vomition and pale mucous membrane. Other clinical signs were weakness, limping and lymphadenomegaly. Majority of the affected dogs (32/35) had tick infestation. Anaemia and thrombocytopenia were two important pathognomonic haematological alterations noticed. Ultrasonography revealed hepatomegaly, gall bladder distention, splenomegally and ascites, in descending order of intensity. Elevated level of alkaline phosphatase (ALP), blood urea and creatinine were considered specific biochemical alterations in ehrlichiosis.

Key words: Ehrlichia, vector-borne, clinical signs, haematological, biochemical, ultrasonography

INTRODUCTION

Ehrlichiosis, a hemoparasitic disease, is caused by a special type of rickettsia that belongs to the genus *Ehrlichia* of family Anaplasmataceae. Among canine vector-borne diseases, ehrlichiosis is most prevalent across the globe especially in tropical and subtropical countries including India with risk to both canine and human population (Perez et al., 1996). Canine monocytic ehrlichiosis was first described in 1935 in Algeria (Pyle, 1980) and subsequently found in different parts of the world i.e., India (Lakshmanan et al., 2007), USA (Bowman et al., 2009), Brazil (Bulla et al.,2004), Turkey (Tuna et al.,2009), Thailand (Laummaunwai et al.,2014) etc. There are at least 9 *Ehrlichia* species that may infect dogs (Kelly, 2000) among which *Ehrlichia canis* is mostly seen in India (Bhatacharjee et al., 2014 and Singh et al., 2014). The prevalence rate varied from 3.1 to 88.0% (Murphy et al., 1998; Bulla et al. 2004; Alexandre et al., 2009; Silva et al., 2012). As regards the Indian scenario, the prevalence of *E. canis* was reported to be 50% (49/98) in Chennai (Lakshmanan et al., 2007) and 20.6% from four different regions of India (Abd Rani et al., 2011). *E. canis* is transmitted transstadially but not transovarially in *Rhipicephalus sanguineus* ticks (Groves et al., 1975). Affected dogs suffer from pyrexia, pale mucus membrane, loss of body weight, epistaxis, lymphadenomegaly, conjunctivitis etc. (Harrus et al., 1997; Waner et al., 1999; Behera et al., 2015). In human, *Ehrlichia* infection causes fever, skin rashes, renal failure, neurologic syndrome and sometimes death in immunosuppressed people. Further studies are in progress to confirm the zoonotic importance of this vector-borne diseases in this geographic region of India.

MATERIALS AND METHODS

The study was undertaken in pet dogs presented in the College of Veterinary Science, Orissa University of Agriculture and Technology, Bhubaneswar from nine districts of Odisha viz. Khurda, Puri, Cuttack, Jagatsinghpur, Mayurbhanj, Ganjam, Malkangiri, Sambalpur and Dhenkanal during June 2015 to May 2016.

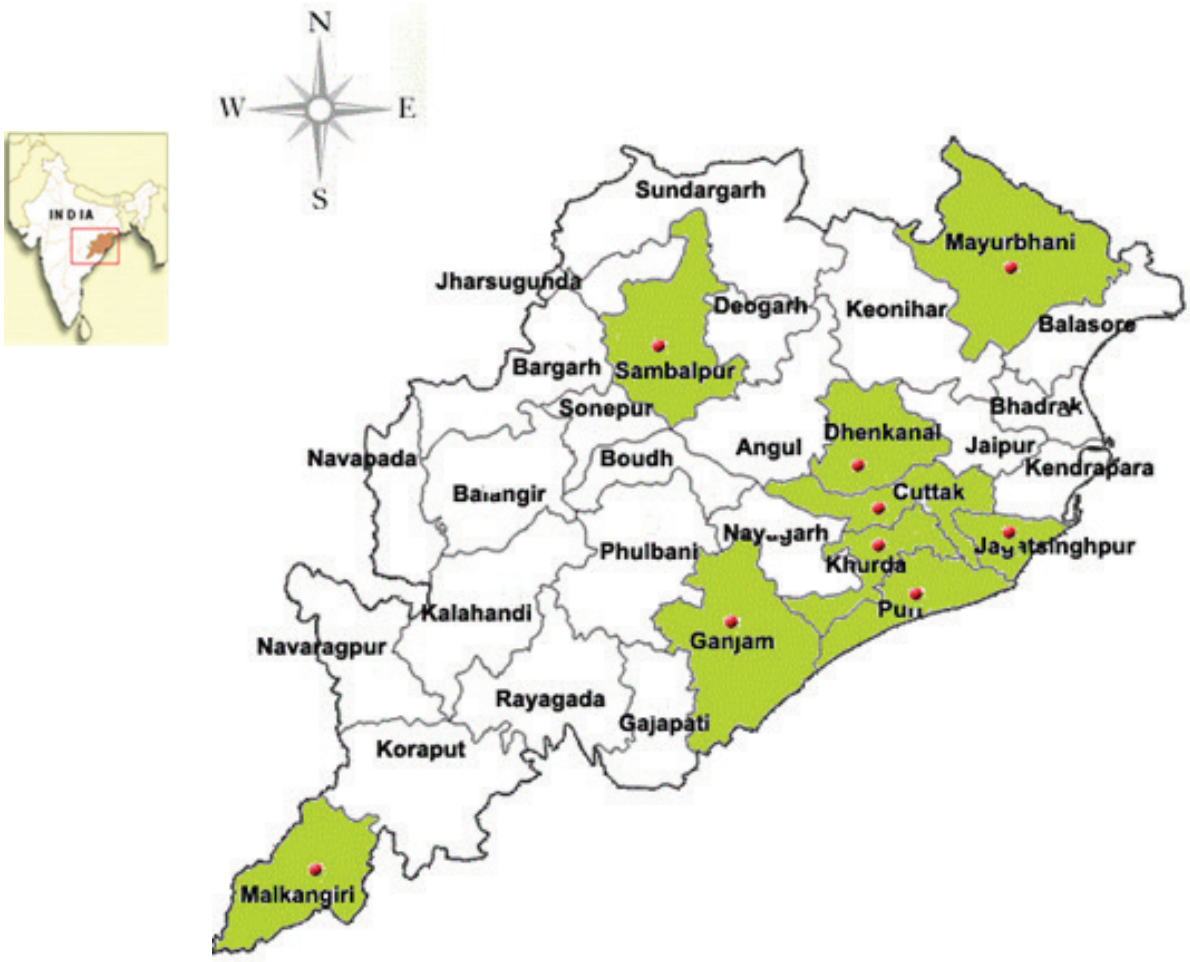

Fig. 1. Shaded area show the districts covered under the study

A data sheet was prepared to take into account the important clinical signs those were earlier documented by various workers in canine ehrlichiosis that include pyrexia, loss of body weight, pale mucus membrane, limping, epistaxis, conjunctivitis, lymphadenomegaly, vomition, diarrhoea, seizure, convulsion and duration of illness. Clinical signs persisting beyond seven days were considered as chronic illness. The data sheet was prepared gathering information from pet owners and the suspected cases were subjected to further laboratory investigation for confirmation. Two milliliter of blood samples were collected in EDTA vial (0.5ml) and clot activator vial (1.5 ml) from each suspected dog by vein puncture for hematological tests and PCR whereas the serum samples collected in clot activator vial was used for biochemical analysis. The genomic DNA was extracted from a blood sample by using QIA amp^{R} DNA blood mini kit according to the recommended procedure and stored at -20^{0}C for subsequent use. These DNA samples were subjected to polymerase chain reaction (PCR).

Haematological tests were conducted as per the procedures described by Benjamin (1978). Serum concentrations for alkaline phosphatase (ALP), urea and creatinine were performed in Auto-analyser (TURBO CHEM100 © 2013 CPC Diagnostics, model 4611) using commercially available kits (Fig 2).

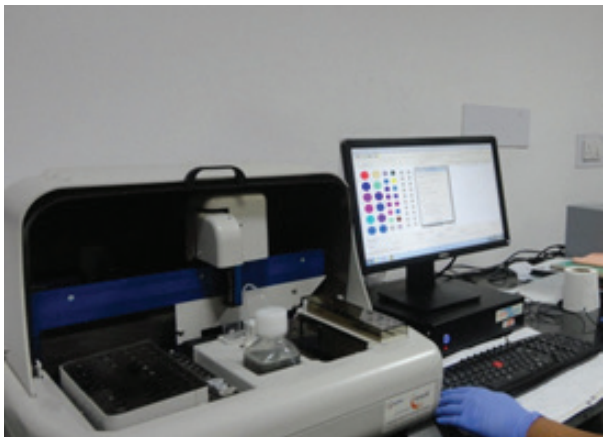

Fig. 2. Biochemical estimation through auto analyzer

The dogs found positive for *Ehrlichia* infection by PCR were subjected to ultrasonography using 5.0 MHz transducer (Hitachi Aloka Medical, Ltd. Model-UST9137) with focused attention to the abdomen.

RESULTS AND DISCUSSION

A total of 35 dogs were found positive for *Ehrlichia* infection during the PCR assay. Weakness/ anorexia, weight loss, pyrexia, vomiting, pallor mucus membrane, limping, conjunctivitis, epistaxis, lymphadenomegaly, coughing, seizure/paresis, polymyositis/ peripheral edema were recorded in 31(88.57%), 29(82.86%), 27(77.14%), 20(57.14%), 17(48.57%), 12(34.29%), 8(22.85%), 7(20.0%), 5 (14.28%), 2(5.71%) and 1(2.85%) dogs with *Ehrlchiosia* infection, respectively (Table 1). Such observation is in accordance with the findings of Glaus et al*.,* 1992; Nakaghi et al., 2008 and Dhankar et al., 2011. All the dogs were either infested or had the history of tick infestation which potentiated its mode of transmission i.e., through ticks. Based on the duration of illness, the clinical signs are divided into three phases i.e., acute, subclinical and chronic (Waner et al., 1999; Unver et al., 2001b). Dogs can harbor *E. canis* for years without developing the clinical form of illness and these dogs can eliminate the parasite and recover from canine monocytic ehrlichiosis (CME) without medical treatment (Harrus et al., 1998b). Herein, under the study both the acute (14/32) and chronic (18/32) forms of the disease were recorded.

Table 1. Clinical manifestations observed in dogs suffering from ehrlichiosis (N=35)

Clinical signs	Number of dogs (%)
Tick infestation	32(91.43)
Weakness	31(88.57)
Anorexia	31(88.57)
Weight loss	29(82.86)
Pyrexia	27(77.14)
Vomition	20(57.14)
Pallor mucus membrane	17(48.57)
Limping	12(34.29)
Conjuctivitis	8(22.85)
Epistaxis	7(20.00)
Lymphadenomegaly	7(20.00)
Coughing	5(14.28)
Seizure	2(5.71)
Paresis	2(5.71)
Polymyostis	1(2.85)
Peripheral edema(hind limbs and scrotum)	1(2.85)

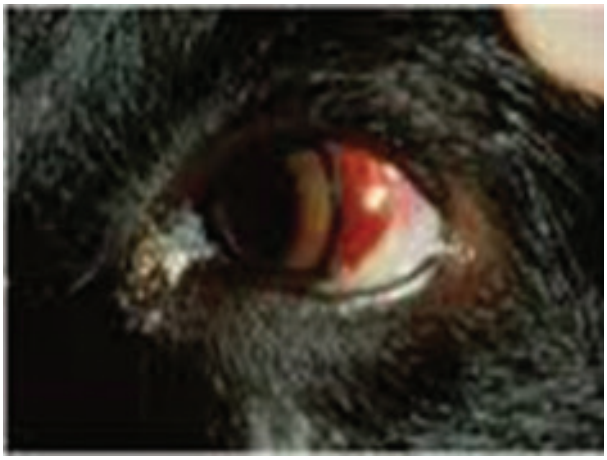

Fig. 3. Conjunctivitis, a marked symptom

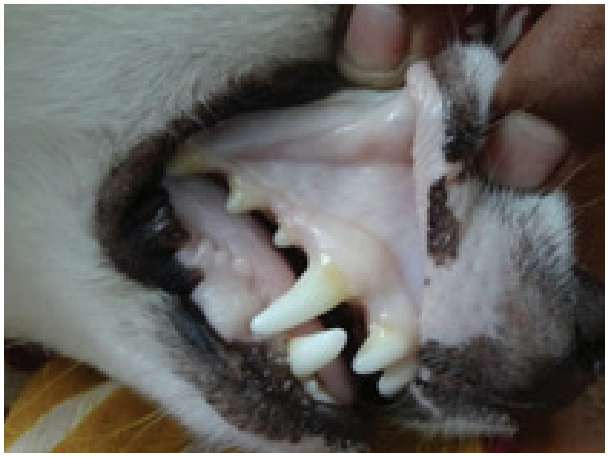

Fig. 4. Pale mucous membrane in ehrlichiosis

Fig. 5. Epistaxis recorded in ehrlichiosis

Fig. 6. Clinical signs of Lymhadenomegaly

The ultrasonographic imaging of the dogs with Ehrlichiosis revealed hepatomegaly, gallbladder distention, splenomegaly and ascites. Pathological alterations were found in either single or multiple organs. Hepatomegaly with gallbladder distention was found in majority dogs i.e., 11(31%) followed by hepatomegaly with ascites in 7(20%), hepatomegaly alone in 6(17%), ascites in 4(11%), gallbladder distention in 3(9%), splenomegaly in 2(6%) and hepatomegaly, gall bladder distention and ascites in 2(6%) dogs (Fig. 4). Such observations were in agreement with Kumar (2004) and Sarma et al. (2013). The main target organs of tick-borne intracellular diseases were bone marrow, spleen and lymph node including other internal organs such as liver, kidney and lungs (Jackobson and Clark, 1994). Multi-organ dysfunction with liver and spleen involvement is common in clinical cases of canine monocytic ehrlichiosis (Ganguly and Mukhopadhayay, 2008). The study by Harrus et al. (1998b) suggested that the spleen is the organ most likely to harbor *E. canis* parasites during the subclinical phase and the last organ to accommodate the parasite before elimination. Hence, splenomegaly is one of the ultrasonographic alterations of dogs with ehrlichiosis.

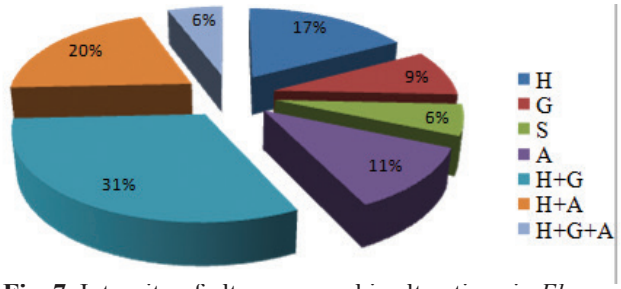

Fig. 7. Intensity of ultrasonographic alterations in *Ehrlichia* infected dogs (N=35)

H: Hepatomegaly
G: Gall bladder distention
S: Splenomegaly
A: Ascites
H+G: Hepatomegaly + Gall bladder distention
H+A: Hepatomegaly + Ascites
H+G+A: Hepatomegaly+ Gall bladder distention+ Ascites

Haemato-biochemical tests were performed in 35 dogs, positive for *Ehrlichia* infection where haemoglobin concentration was found in the range from 4.2 to 13.5 g % with a mean of 8.26±0.42 g %. The minimum and maximum platelet count were 0.02 × 10^6/cu mm and 0.195 × 10^6/cu mm with a mean of 0.077±0.007 × 10^6 /cu mm. The mean value of alkaline phosphatase(ALP) concentration was 175.76±16.43 IU L^{-1} which was within the range of 57.9 to 398.5 IU L^{-1}. Blood urea concentration was found to be 42.67±2.13 mg dl^{-1} with a range from 22.9 to 69.7 mg dl^{-1}. Creatinine concentration was found to be in a range from 0.48 to 15.38 mg dl^{-1} with a mean of 2.9±0.53 mg dl^{-1}. The *Ehrlichia* infected dogs presented with Hb < 3.0 g % and creatinine concentration > 10.0 mg dl^{-1} were succumbed during our investigation. Anaemia and thrombocytopenia were two significant haematological alterations which were also recorded in the present study. This is in agreement of the findings of Bhadesiya and Raval (2015), Singh et al. (2014) and Harrus et al. (1999). Anemia might be due to bone marrow hypoplasia by the parasites leading to impaired production of cellular components of blood (Neer et al., 2002). The variation in the type of anemia can be attributed to several influential factors such as nutritional status, iron reserves in the body, concurrent infection and age of the infected dogs. The development of thrombocytopenia may be due to large-scale destruction of the cells in the spleen that begins a few days after the infection (Smith et al., 1975) or due to bone marrow hypoplasia leading to impairment of normal functions (Waner,2008). The development of thrombocytopenia has also been attributed to an immune-pathological mechanism described by Waner et al., 1999, who demonstrated significant levels of serum anti-platelet IgG, 17 days after experimental *E. canis* infection that resulted in the removal of antibody adsorbed thrombocytes by the mononuclear phagocyte system in the liver and spleen. Elevated level of alkaline phosphatase (ALP), blood urea and creatinine recorded in the study were in accordance with the reports by Behera et al. (2015) and Morar et al. (2015). *E. canis* resides and replicates in the cytoplasm of circulating monocytes and macrophages affecting other internal organs like liver and kidney, hence elevating the ALP, blood urea and creatinine level. Harrus et al.(1996) documented biochemical alterations like hypoalbuminemia, hyperglobulinemia, increased serum enzyme activities of AST and ALT. Hypoalbuminemia is seen in all stages of canine ehrlichiosis and maybe a consequence of anorexia and associated with decrease in protein uptake, blood loss and peripheral loss to oedematous inflammatory fluids as a consequence of vasculitis (Woody and Hoskin, 1991), decreased protein production due to concurrent liver disease (Reardon and Pierce, 1981) or due to proteinuria. Studies have indicated that proteinuria might occur independently or concurrently with glomerulonephritis (Frank and Breitschwerdt, 1999; Codner and Maslin, 1992; Waddle and Littman, 1988).

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REFERENCES

Abd Rani, P. A.M., Irwin, P.J., Coleman, G.T., Gatne, M. and Traub, R.J. 2011. A survey of canine tick borne diseases in India. *Parasites and Vectors* **4**: 141–148.

Alexandre, N., Santos, A.S., Nuncio, M.S., Sousa, R., Boinas, F. and Bacellar, F. 2009. Detection of Ehrlichia canis by polymerase chain reaction in dogs from Portugal. *Veterinary Journal* **181**: 343–344.

Behera, S.K., Dimri, U., Banerjee, P., Garg, R., Dandpat, S. and Sharma, B. 2015. Molecular detection and assessment of hemato-biochemistry, oxidant / antioxidant status in natural canine monocytic Ehrlichiosis cases from Northern India. *The National Academy of Sciences, India* **40011**(015): 0605.

Benjamin, M.H. 1978. Outline of Veterinary Clinical Pathology. 3rd Ed, Lowa State University Press, Ames, U.S.A. pp. 38-40.

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

Bhattacharjee, K., Sarmah, P.C. and Barman, N.N. 2014. Seroprevalence of vector borne parasites and other infections in naturally exposed dogs of Assam. *Indian Veterinary world* **7**: 87.

Bowman, D., Little, S.E., Lorentzen, L., Shields, J., Sullivan, M.P. and Carlin, E.P. 2009. Prevalence and geographic distribution of Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia canis and Anaplasma phagocytophilum in dogs in the United States: Results of a national clinic-based serologic survey. *Veterinary Parasitology* **160**(1-2): 138-148.

Bulla, C., Kiomi Takahira, R., Pessoa A.J.J., Aparecida T.L., Souza L.R. and Wiedmeyer, C.E. 2004. The relationship between the degree of thrombocytopenia and infection with Ehrlichia canis in an endemic area**.** *Veterinary Research* **35**: 141–146.

Codner, E.C. and Maslin, W.R. 1992. Investigation of renal protein loss in dogs with acute experimentally induced *Ehrlichia canis* infection. *American Journal of Veterinary research*. **53**: 294-299.

Dhankar, S., Sharma, R.D. and Jindal, N. 2011. Some epidemiological observations on canine ehrlichiosis in Haryana and Delhi states. *Haryana Vet* **50**: 9-14.

Frank, J.R. and Breitschwerdt, E.B. 1999. A retrospective study of ehrlichiosis in 62 dogs from North Carolina and Virginia. *Journal of Veterinary Internal Medicine* **13**: 194-201.

Gangly, S. and Mukhopadhayay, S.K. 2008. Tick-borne ehrlichiosis infection in human beings. *Jornal of Vector Borne Diseases* **45**: 273-280.

Glaus, T. and Jaggy, A. 1992. Ehrlichiosis in dogs: literature review and case description. *Schweiz Arch Tierheilkd*. **134**(7): 319-323.

Groves, M.G., Dennis, G.L., Amyx, H.L. and Huxsoll, D.L. 1975. Transmission of *Ehrlichia canis* to dogs by ticks (*Rhipicephalus sanguineus*). *American Journal of Veterinary Research* **36**(7): 937-940.

Harrus, S., Waner, T. and Bark, H. 1997. Canine monocytic ehrlichiosis update. *Compendium for Continuing Education for the Practicing Veterinarian* **19**: 431–444.

Harrus, S., Waner, T., Aizenberg, I., Foley, J.E., Poland, A.M. and Bark, H. 1998b. Amplification of ehrlichial DNA from dogs 34 months after infection with *Ehrlichia canis*. *Journal of Clinical Microbiology* **36**: 73–76.

Harrus, S., Waner, T., Bark, T., Jongeja, F. and Cornelisen, A.W.C.A. 1999. Recently advances in determining the pathogenesis of canine monocytic ehrlichiosis. *Journal of Clinical Microbiology* **37**: 2745-2749.

Harrus, S.,Waner, T., Avidar, Y., Bogin, E., Peh, H. and Bark, H.1996. Serum protein alterations in canine ehrlichiosis. *Veterinary Parasitology* **66**: 241–249.

Jacobson, L.S. and Clark, I.A. 1994. The patho-physiology of canine babesiosis: new approaches to an old puzzle. *Journal of South African Veterinary Association* **65**: 134-145.

Kelly, P.J. 2000. Canine ehrlichiosis: an update. *Journal of the South African Veterinary Association* **71**(2): 77–86.

Kumar, A. 2004. Clinico-therapeutic aspect of canine ehrlichiosis. M.V.Sc.Thesis, Indian Veternary Research Institute, Izatnagar, India.

Lakshmanan, B., John, L., Gomathinayagam, S. and Dhinakarraj, G. 2007. Moleclar detection of *Ehrlichia canis* from blood of naturally infected dogs in India. *Veterinary archieve* **77**: 307-312.

Laummaunwai, P., Sriraj, P., Aukkanimart, R., Boonmars, T., Boonjaraspinyo, S., Sangmaneedet, S., Potchimplee, P., Khainman, P. and Maleewong, W. 2014. Molecular detection and treatment of tick borne pathogens in domestic dogs in Khon Kaen, northeastern Thailand. *Southeast Asian Journal of Tropical Medical Public Health*. **45**(5): 1157-1166.

Morar, D., Darabs, G., Imre, M., Ilie, M.S. and Imre, K. 2015. First record of autochthonous canine ehrlchiosis caused by *Ehrlichia canis* in Romania. *Veterinary Clinical Pathology* **44** (2): 200-204.

Murphy, G.L., Ewing, S.A., Whitworth, L.C., Fox, J.C. and Kocan, A.A. 1998. A molecular and serologic survey of *Ehrlichia canis*, *E. chaifeensis* and *E. ewingii* in dogs and ticks from Oklahoma. *Veterinary Parasitology* **79**: 325–339.

Nakaghi, A.C.H., Machado, R.Z., Costa, M.T.,Andre, M.R. and Baldan, C.D. 2008. Canine ehrlichiosis: clinical, hematological, serological and molecular aspects. *Ciência Rural* **38**(3): 766-770.

Neer, T.M., Breitschwerdt, E.B., Greene, R.T. and Lappin, M.R. 2002. Consensus statement on ehrlichial disease of small animals from the infectious disease study group of the ACVIM. *Journal of Veterinary Internal Medicine* **16**: 309-315.

Perez, M., Rikihisa, Y and Wen, B. 1996. *Ehrlichia canis*-like agent isolated from a man in Venezuela: antigenic and genetic characterization. *Journal of Clinical Microbiology* **34**(9): 2133-2139.

Pyle, R.L. 1980. Canine ehrlichiosis. *Journal of American Veterinary Medicne Association* **177**: 1197-1199.

Reardon, M.J. and Pierce, K.R. 1981. Acute experimental canine ehrlichiosis. Sequential reaction of the hemic and lymphoreticular systems. *Veterinary Pathology* **18**: 48-61.

Sarma, K., Mondal, D.B. and Saravanan, M. 2014. Ultrasonographic changes in dogs naturally infected with tick borne intracellular diseases. *Jornal of Parasitic Dis*ease **12639**(014): 0485-0488.

Silva, G.C.F., Benitez, A.N., Girotto, A., Taroda, A., Vidotto, M.C., Garcia, J.L., Freitas, J.C., Headley, S.A. and Vidotto, O. 2012. Occurrence of Ehrlichia canis and Anaplasma platys in household dogs from northern Parana. Revista Bra-sileira de Parasitologia Veterinaria **21** : 379–385.

Singh, M.H., 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. *Veterinary Medicina* **59**(3): 129-136.

Smith, R.D., Ristic, M., Huxsoll, D.L. and Baylor, R.A. 1975. Platelet kinetics in canine ehrlichiosis: evidence for increased platelet destruction as the cause of thrombocytopenia. *Infection Immunology* **11**: 1216-1221.

Tuna, G.E. and Ulutas, B. 2009. Prevalence of *Ehrlichia canis* infecton in thrombocytopenic dogs. *Lucrari stintifice medicina veternara* XLII: 160-164.

Unver, A., Perez, M., Orellana, N., Huang, H. and Rikihisa, Y. 2001b. Molecular and antigenic comparison of *Ehrlichia canis* isolates from dogs, ticks and a human in Venezuela. *Journal of Clinical Microbiology* **39**: 2788-2793.

Waddle, J.R. and Littman, M.P. 1988. A retrospective study of 27 cases of naturally occurring canine ehrlichiosis. *Journal of American Animal Hospital Association* **24**: 612-620.

Waner, T. 2008. Haematopathological changes in dogs infected with *Ehrlchia canis*. *Israel Veterinay Medicine Journal* **63**: 1-8.

Waner, T., Keysary, A.,Bark, H.,Sharabani, E. and Harrus, S. 1999.Canine Monocytic Ehrlichiosis – an overview. *Israel Journal of Veterinary Medicine* **54**(4): 103-107.

Woody, B.J. and Hoskins, J.D. 1991. Ehrlichial diseases of dogs. *Veterinary Clinics of North America: Small Animal Practice* **21**: 75–98.