

SOME EPIDEMIOLOGICAL OBSERVATIONS ON CANINE EHRLICHIOSIS IN HARYANA AND DELHI STATES

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ABSTRACT

In the present study, 203 dogs of different breeds brought to veterinary hospitals and private veterinary clinics of Haryana and Delhi states during the period from September 2002 to September 2005 were screened for ehrlichiosis. Based on blood and buffy coat smear examinations, 23 dogs were found positive for canine ehrlichiosis. Of these, 9 (39.13%) dogs were found positive by peripheral blood smear examination whereas showed 100% positivity for ehrlichial inclusions or morulae was observed by examination of buffy coat smears. The dogs affected with canine ehrlichiosis showed main clinical symptoms of depression, anorexia, pyrexia, anaemia, epistaxis, skin lesions, petechiae/ecchymoses, lymphadenopathy, respiratory dyspnea and neurological disorders. Of the 23 *Ehrlichia*-affected dogs, 13 (56.52%) suffered from ehrlichiosis alone while 10 cases had concurrent infection of either *Babesia* spp. (9 cases) or *Hepatozoon canis* (1 case). The occurrence of the disease was higher in pure bred dogs than the non-descript ones, however, there was no major effect of age and sex on disease occurrence.

Key words: Canine ehrlichiosis, Haryana, Delhi

In India, canine ehrlichiosis has been reported from different parts of the country (Murali Manohar and Ramakrishnan, 1982; Thilagar *et al.*, 1990; Thirunavukkarasu *et al.*, 1993; Tresamol *et al.*, 1998; Manigandan *et al.*, 2003; Harikrishnan *et al.*, 2009; Megat Abd Rani *et al.*, 2010). The disease exhibits itself mainly in acute, subclinical and chronic forms (Van Heerden, 1982). The diagnosis of canine ehrlichiosis poses challenge to the clinicians owing to vague and non-specific clinical symptoms, variations in haematological and biochemical findings and resemblance with a number of diseases which mimic ehrlichiosis in clinical presentation. Though the identification of morulae in monocytes in peripheral blood smears is diagnostic, yet their low number in blood smears and transient nature makes the diagnostic efforts unrewarding. Indirect fluorescent antibody test, ELISA and western blotting have been developed for confirmatory diagnosis but are not being widely practiced in small animal clinics because of the requirement of sophisticated facilities and specialized trained personnel. Examination of buffy coat smears appears to be rewarding under field conditions (Greene and Harvey, 1990).

Systematic large scale clinical studies on canine ehrlichiosis are lacking in north India. Therefore, the present study was undertaken to determine the prevalence and epidemiology of canine ehrlichiosis in Haryana and Delhi states.

MATERIALS AND METHODS

Clinical Cases: The present study was conducted in the Department of Veterinary Epidemiology and Preventive Medicine, CCS HAU, Hisar (now the Department of Veterinary Public Health and Epidemiology, LLRUVAS, Hisar) in association with various veterinary hospitals and private veterinary clinics in Haryana and Delhi states. Two hundred and three dogs reported in these hospitals during the period from September, 2002 to September, 2005 were included in this study. These dogs had tick-infestation and showed depression, pyrexia, anaemia, vomition, skin lesions or bleeding tendencies such as ecchymoses, petechiae or epistaxis. Particulars regarding such as breed, age, sex, diet, vaccination status, deworming status, complete past and present medical history, previous treatment given, if any, and details of current

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problem of each dog were recorded. These dogs were subjected to further clinical examination as per the standard procedure (Jones, 1994).

Rectal temperature of all these dogs was recorded daily. Ocular examination was carried out in those cases which showed eye involvement on clinical examination. Detailed neurological examination was conducted in dogs showing neurological signs as per procedure described by Taylor (1998). Mental state, posture, gait, spinal and cranial reflexes were recorded as per the requirement of each case.

Cytological Examination: Blood and buffy coat smears prepared from 203 dogs were examined for the detection of ehrlichial inclusion bodies or morulae. At least 200 leukocytes in each smear were screened for the presence of ehrlichial inclusions and/or morulae. Buffy coat collected from the microhaematocrit capillary tube, after centrifugation, was used for smear preparation. The smears were examined under oil immersion and upto 100 oil immersion fields were examined for the detection of ehrlichial inclusions.

RESULTS AND DISCUSSION

Of the 203 dogs examined, 23 (11.35%) were found positive for canine ehrlichiosis either through blood smear examination or by buffy coat smear examination in Haryana and Delhi States during this period. This observation may not represent true prevalence of this disease as the study did not include all cases of dogs that were brought to the veterinary hospitals or private veterinary clinics as well as those hospitals/clinics that were not included in this study. Hence the true prevalence in both states over a period of time may differ from 11.33%. Various reports have estimated varying prevalence rate of ehrlichiosis in dogs in India. Thirunavukkarasu *et al.* (1993) and Kumar *et al.* (2010) on the basis of blood and buffy coat smears examination reported 1.12% and 2.94% prevalence of canine ehrlichiosis in Chennai and Ranchi, respectively. In contrast, Tresamol *et al.* (1998) reported a very high seroprevalence (68.60%) on the basis of IFAT in Chennai. In a study, Samradhni *et al.* (2003), on the basis of examination of blood

smears, reported 18.90% prevalence of *E. canis* in dogs in Nagpur.

Clinical Manifestations: The dogs affected with canine ehrlichiosis showed wide variability in clinical presentations (Table 1). The main clinical signs were depression (73.91%), anorexia (65.21%), pyrexia (65.21%), anaemia (56.52%), epistaxis (26.08%), skin lesions (26.08%), bleeding tendencies i.e petechiae/ecchymoses (26.08%), lymphadenopathy (21.74%) and respiratory dyspnea (13.04%) (Table 1). More or less similar types of clinical manifestations in dogs with canine ehrlichiosis have been reported by other workers (Troy *et al.*, 1980; Van Heerden, 1982; Price *et al.*, 1987; Thirunavukkarasu *et al.*, 1994; Kumar *et al.*, 2010). The variations in clinical signs seem to be due to a number of factors including differences in pathogenicity between strains of *Ehrlichia*, breeds of dogs, concurrent infections with other diseases, immune status of the dog etc. The clinical findings which generated the highest index of suspicion for ehrlichiosis included pyrexia, depression, pale mucosa, bleeding

Table 1
Clinical manifestations observed in dogs suffering from ehrlichiosis*

Clinical signs	Number of dogs with manifestations (%)
Depression	17 (73.91)
Anorexia	15 (65.21)
Pyrexia	15 (65.21)
Anaemia	13 (56.52)
Bleeding tendencies i.e.	
Ecchymoses/Petechiae	6 (26.08)
Epistaxis	6 (26.08)
Skin lesions	6 (26.08)
Lymphadenopathy	5 (21.74)
Respiratory dyspnea	3 (13.04)
Coughing	3 (13.04)
Diarrhoea	3 (13.04)
Haematuria	3 (13.04)
Eye involvement	3 (13.04)
Polydypsia	2 (8.69)
Lameness	2 (8.69)
Vomition/Haematemesis	2 (8.69)
Malena	2 (8.69)
Neurological disorders	1 (4.34)

*A total of 23 dogs were diagnosed as suffering from ehrlichiosis

tendencies and peripheral lymphadenopathy singly or in combination. Haemorrhages were manifested as epistaxis, cutaneous petechiae and ecchymoses, haematuria, haematemesis and malena either alone or in combination in ehrlichiosis with or without concurrent infections. Of these, epistaxis was the most common single finding observed during the present study as also observed by Troy *et al.* (1980). Present observation of haemorrhages in 47.83% cases of canine ehrlichiosis with or without concurrent infections were higher than the earlier reports (Kuehn and Gaunt, 1985; Thirunavukkarasu *et al.*, 1994) and may be attributed to thrombocytopenia and the deposition of immune complexes on vascular wall. Corneal opacity was observed only in two cases.

The clinical signs of dogs with single infection of *Ehrlichia* have been well characterized (Troy *et al.*, 1980; Waddle and Littman, 1988) but the manifestations remain ambiguous in concurrent infections. The clinical findings of dogs with concurrent infections of *Ehrlichia* spp. and *Babesia* spp. did not differ significantly from that of single infection of *Ehrlichia* spp. except frequency of the signs and severity of the disease which were more in mixed infections. More or less similar observations have been reported by Chou (1995).

Comparative efficacy of blood and buffy coat smears: Out of 23 dogs diagnosed with ehrlichiosis, peripheral blood smears were found positive only in 9 (39.13%) dogs whereas buffy coat smears showed 100% positivity for ehrlichial inclusions or morulae. The present findings are in agreement with the observations of Elias (1991), Thirunavukarasu *et al.* (1994) and Mylonakis *et al.* (2003) who also observed that cytological examination of buffy coat appears to be more diagnostic for ehrlichiosis than peripheral blood particularly in dogs with leukopenia.

Morphological Features of *E. canis*: *E. canis* were observed as intracytoplasmic bodies of varying sizes and shapes in monocytes or lymphocytes or neutrophils. The most commonly encountered form was the large spherical morulae (Fig. 1) whereas another form viz., inclusion bodies were also recorded (Fig. 2). Many of the morulae were dark with closely knit small units. The morulae were observed in 17 cases (73.91%) whereas

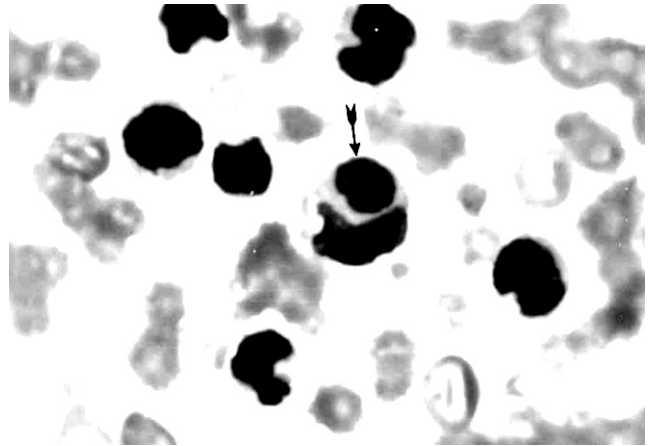


Fig 1. Blood smear of a dog showing morula of *Ehrlichia* in the cytoplasm of a monocyte (arrow). (Giemsa stain x 1000)

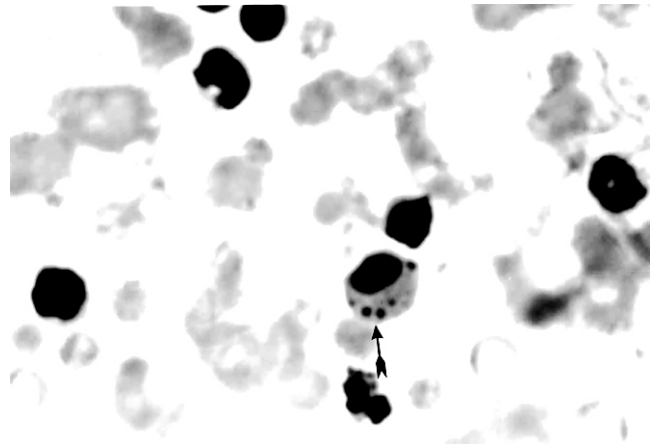


Fig 2. Blood smear of a dog showing inclusion bodies of *Ehrlichia* in the cytoplasm of a lymphocyte (arrow). (Giemsa stain x 1000)

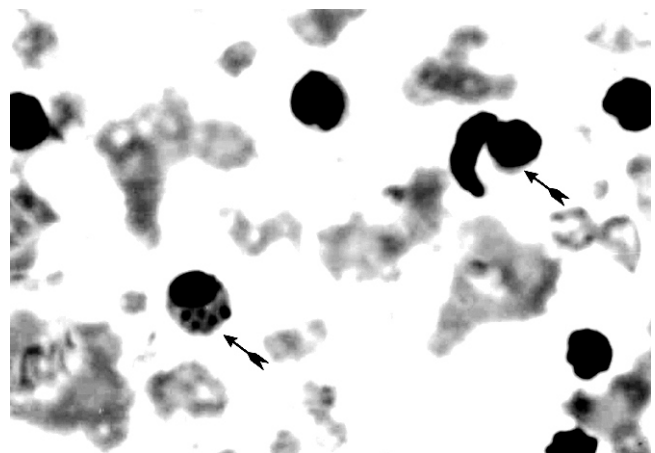


Fig 3. Blood smear of a dog showing morula and inclusion bodies of *Ehrlichia* in the cytoplasm of leucocytes (arrow). (Giemsa stain x 1000)

inclusion bodies were observed in 6 cases (26.09%). The majority of morulae were basophilic, homogenous and dense inclusions. Granulocytic *Ehrlichia* were detected as morulae or inclusion bodies in granulocytes mainly neutrophils (Fig. 3). In the present study, morulae of *E. canis* were detected more in monocytes than lymphocytes as also reported by Mylonakis *et al.* (2003). However, Harrus *et al.* (1997) observed more morulae in lymphocytes than monocytes. In the present study, morulae of granulocytic *Ehrlichia* were also detected in neutrophils. This finding is in conformity with the observations of Ewing *et al.* (1971). Thus more emphasis should be laid to detect either morulae or inclusion bodies in blood or buffy coat smears from suspected cases of ehrlichiosis so that the chances of false negative results can be minimized.

Prevalence of Canine Ehrlichiosis Either Alone or as Mixed Infections: Of the 23 *Ehrlichia*-affected dogs, 13 (56.52%) suffered from ehrlichiosis alone while in 10 cases concurrent infection of *Babesia* spp. (9 cases) or *Hepatozoon canis* (1 case) was also recorded. Concurrence of *E. canis* with *Babesia* spp. has also been reported by other workers (Matthewman *et al.*, 1993; Chou, 1995;

Varshney and Dey, 1998), while concurrent infection of *E. canis* with *H. canis* was in agreement with the findings of Juyal *et al.* (1992) and Smitha *et al.* (2003). Thus under field conditions, a holistic approach is required particularly for diagnosis of tick-borne diseases. Concurrent infections with *Hepatozoon canis* or *Babesia* spp. and strain or species variations of *Ehrlichia* may lead to variation in clinical signs between different geographic regions, making the diagnosis confusing and treatment unrewarding.

Breed-wise Prevalence: Out of 203 dogs, 133 were pure-bred while the remaining 70 were non-descript (Table 2). Of the 133 pure-bred dogs, 19 (14.28%) were positive for the disease. Of the 70 non-descript, 4 (5.71%) were positive. The results indicated higher prevalence of the disease in pure-bred dogs as compared to non-descript dogs. This finding is in agreement with that of Seamer and Snape (1970) who also reported that more susceptibility of pure bred dogs to canine ehrlichiosis. In this study, highest prevalence was recorded in German Shepherd breed (20.58%) followed by Spitz (17.39%), Cocker Spanial (16.67%), Saint Bernard (14.28%) and so on (Table 2). This finding is in accordance with other workers (Murali Manohar and Ramakrishnan, 1982; Thirunavukkarasu *et al.*, 1993; Chandrasekar *et al.*, 2002; Manigandan *et al.*, 2003) who also reported different prevalence rates in different breeds. German Shepherd breed has been reported to be more susceptible to this disease and is more severely affected (Nyindo *et al.*, 1980; Harrus *et al.*, 1997).

Age-wise Prevalence: Prevalence of canine ehrlichiosis in different age groups is presented in Table 3. It is evident from this table that the disease

Table 2
Breed-wise prevalence of clinical cases of canine ehrlichiosis

Breed	Number of dogs examined	Number positive (%)
German Shepherd	34	7 (20.58)
Spitz	23	4 (17.39)
Cocker Spanial	6	1 (16.67)
Saint Bernard	7	1 (14.28)
Boxer	7	1 (14.28)
Dalmation	8	1 (12.50)
Great Dane	8	1 (12.50)
Doberman Pinscher	9	1 (11.11)
Labrador	23	2 (8.69)
Mongrels	42	3 (7.14)
Cross bred	28	1 (3.57)
Gaddi	2	Nil
Golden Retriever	4	Nil
Lhasa Apso	2	Nil
Pure Breed	133	19 (14.28)
Non descript	70	4 (5.71)
Overall	203	23 (11.33)

Table 3
Age-wise prevalence of clinical cases of canine ehrlichiosis

Age (Months)	Number examined	Number positive (%)
< 6	17	2 (11.76)
6 - 12	33	4 (12.12)
12 - 24	27	3 (11.11)
24 - 36	34	5 (14.70)
36 - 48	39	3 (7.69)
48 - 60	31	3 (9.60)
> 60	22	3 (13.64)

affected dogs of all age groups. However, there was a difference in percent prevalence in dogs of different age groups. For example, the dogs of 24-36 months of age revealed the highest prevalence of ehrlichiosis among the *Ehrlichia*-affected dogs (Table 3). Murali Manohar and Ramakrishnan (1982) and Harikrishnan *et al.* (2009) also reported disease in dogs of different age groups. However, Thirunavukkarasu *et al.* (1993) and Chandrasekar *et al.* (2002) reported that younger dogs (below 1 year) were more susceptible to the disease.

Sex-wise Prevalence: Out of 138 males and 65 females, 16 (11.59%) and 7 (10.76%) were positive for canine ehrlichiosis, respectively; the difference in prevalence being statistically non-significant. Manigandan *et al.* (2003) also reported that there was no major difference in the occurrence of disease in males and females. However, Thirunavukkarasu *et al.* (1993) recorded higher prevalence in males, which may be due to over representation of males in the study population.

In conclusion, this study indicates the presence of canine ehrlichiosis in dog population in Haryana and Delhi states and buffy coat smear examination may be a better diagnostic approach for proper diagnosis of this disease.

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