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SHORT COMMUNICATION

Prevalence and Molecular Characterization of *Ehrlichia canis* in Dogs of Jammu Region

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ABSTRACT

The present study was conducted on dogs presented to Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu, between March 2015 and December 2016. A total of 5711 dogs were presented for treatment in small animal medicine OPD of Referral Veterinary Hospital of the Faculty of Veterinary Science and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu. Out of which a total of 200 dogs suspected to be suffering from various Tick borne diseases (TBD's) were screened and 100 were found positive for different TBD's, of which 36 were found positive for *Ehrlichia canis (E. canis)*. The prevalence of *Ehrlichia canis* was found to be 36 percent (based on PCR) with maximum occurrence in summer. 13 cases were found positive in giemsa stained thin blood smear. PCR was performed using standard protocol. In SNAP4Dx plus kit 30 out of 60 cases (50 %) were found positive. Males (63.88 %) were more affected than female with highest prevalence in Labrador breed. Dogs in the age group of (1 - 5 year) (72.23%) were found most susceptible to *E. canis*. Canine ehrlichiosis causes acute febrile illness in dogs but subclinical stage, lasting months to years, is not associated with clinical signs of illness and therefore may go unnoticed by pet owners and undiagnosed by veterinarians unless antibodies are detected during annual screening with in-clinical kits. Therefore, it is not possible to rely on a single serological result for diagnosis of *E. canis*, it may be concluded that PCR is most reliable method, useful in the clinical laboratory for specific and early diagnosis of ehrlichiosis in dogs.

Keywords: *E. canis*, Prevalence, PCR, TBD's, SNAP4Dx plus kit, Dogs

Ticks are notorious vectors of various pathogenic protozoa, rickettsiae, bacteria, and viruses that cause serious and life threatening illnesses in humans and animals worldwide (Alekseev *et al*., 2001). Tick transmitted infections are an emerging problem in dogs. In addition to causing serious disease in traditional tropical and semi-tropical regions, they are now increasingly recognized as a cause of disease in dogs in temperate climates and urban environments (Shaw *et al*., 2012). Ticks transmit a greater variety of pathogenic micro-organisms than any other arthropod vector group, and are among the most important vectors of diseases affecting animals (Jongejan, 2007). Twelve species of ticks are known to occur. The brown dog tick, *Rhipicephalus sanguineus*, is the only species that can become established as a pest in homes and kennels. Canine ehrlichiosis are a group of tick-borne malady caused by lipopolysaccharide deficient, obligatory, intracellular gram-negative bacteria of the genus *Ehrlichia* and family *Anaplasmataceae*. This *Ehrlichia* spp. are of either monocytotropic (*E. canis*), granulocytotropic (*Anaplasma phagocytophilum*, *E. ewingii* and human granulocytic ehrlichiosis), or thrombocytotropic (*E. platys*) (Skotarczak, 2003; Greene, 2006) in nature. Among all the ehrlichia spp. the most well studied and pathogenic organism is *E. canis*, the causative organism of canine monocyteic ehrlichiosis (CME), also called tropical canine pancytopenia and has got worldwide prevalence (Skotarczak, 2003). The distribution of CME is related to the distribution of the vector and has been reported to occur in Asia, Africa, Europe and America (Baneth *et al*., 1996). In India there are sporadic reports of *E. canis* infection in dogs from time to time.

Keeping in view the scarcity of information as no systematic effort through conducting a planned study of dog population in the region has been done till date and importance of tick borne diseases in dogs, present work was undertaken to determine the prevalence of *E. canis* and associated risk factors in dogs.

The present study was conducted on dogs presented to Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu, between March 2015 and December 2016. A total of 5711 dogs were presented for treatment in small animal medicine OPD of Referral Veterinary Hospital of the Faculty of Veterinary Science and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu. Out of which a total of 200 dogs suspected to be suffering from various TBD's were screened and 100 were found positive for different TBD's and were found positive for *Ehrlichia canis*.

A comprehensive history comprised of description of patient with respect to age, sex, breed, vaccination, deworming status, owner's chief complaint about dog and main symptoms observed, time of onset of symptoms, previous treatment if any and response thereof were recorded. Details regarding environment contact with other pets or stray dogs and migration of dog from distant place were also recorded.

Patient examination included present status of appetite, water intake, urination, type of feed given, defaecation, vomition, behaviour, conformation, posture or gait, fever, cyanosis, hind limb weakness, oedema, ascitis and exercise intolerance, epistaxis and cyanosis. Animals showing these signs were suspected for tick borne diseases and investigated thoroughly. Observation for the presence or absence of ticks was also made. Clinical examination involved observation of the rectal temperature, heart rate, respiration rate and pulse rate. Conjunctival or gingival mucous membrane was examined and dehydration status was ascertained by state of muzzle, nostrils and skin tenting time. Body weight of the animal was also recorded. On the basis of history, clinical symptoms, blood smear examination and PCR, diagnosis of disease was done. Blood smear were fixed in methanol and standby stained by Giemsa method of staining (Jain, 1986). Prevalence was recorded as age wise and classified as juvenile (upto 1 year of age), adult (1-5 years of age) and old dogs (>5 years). Prevalence data was recorded on the season basis.

Each dog was subjected to detailed clinical examination as per standard procedure (Jones, 1994). Presence of symptoms/signs/manifestation of involvement of different body systems and systemic states were recorded. A clinical score of each ailing dog was worked out based on 17-points scale (Jones, 1994) (Table 1).

Table 1: 17 points scale clinical score of dogs with TBD's

Signs	Weightage Presence	Absence
Temperature > 102.4 °F	1	
Anorexia/Inappetance	1	0
Vomiting	1	0
Diarrhoea	1	0
Dehydration	1	0
Melena	1	0
Respiratory signs	1	0
Haemorrhage	1	0
Staggering gait	1	0
Lymphadenopathy	1	0
Ocular signs	1	0
Nervous signs	1	0
Ascites/edema/abdominal distention	1	0
Presence of ticks	1	0
Musculoskeletal signs	1	0
Total	**17**	**0**

PCR was performed using standard protocol given by Murphy *et al.* 1998 and 389 base pair (bp) product specific for *E. canis* was visualized in 1% Agarose gel electrophoresis (Table 2).

Table 2: Details of specific primer used for molecular diagnosis and PCR conditions for amplification of *E. canis*

Parasite	Primers	Product size	Reference
E. canis	ECAN5: 5'-CAATTATT-TATAGCCTCTGGCTCTG GCTATAGGA-3' HE3:5'-TATAGGTA CCGT-CATTATCTTCCCTAT-3'	389 bp	Murphy *et al.* 1998

Parasite	PCR conditions (steps of reaction)	
E. canis	Initial denaturation at 94 ºC for 3 min	
	Denaturation at 94 ºC for 1 min	
	Annealing at 55 ºC for 2 min	37 cycles
	Extension at 72 ºC for 1.5 min	
	Final extension at 72 ºC for 8 min	

SNAP 4 Dx plus kit canine ehrlichia antibody test kit is multivalent (enzyme linked immunosorbent assay) based test uses synthetic peptide reagents for in vitro diagnosis of *Dirofilaria immitis* antigen*, A. platys/ phagocytophilum* antibodies, *B. burgdorferi* antibodies*, E. canis/ ewingii* antibodies.Any development of colour in sample spots indicates presence of *Dirofilaria immitis antigen, A. platys/ phagocytophilum antibodies, B. burgdorferi antibodies, E. canis/ ewingii antibodies* (Fig. 1).

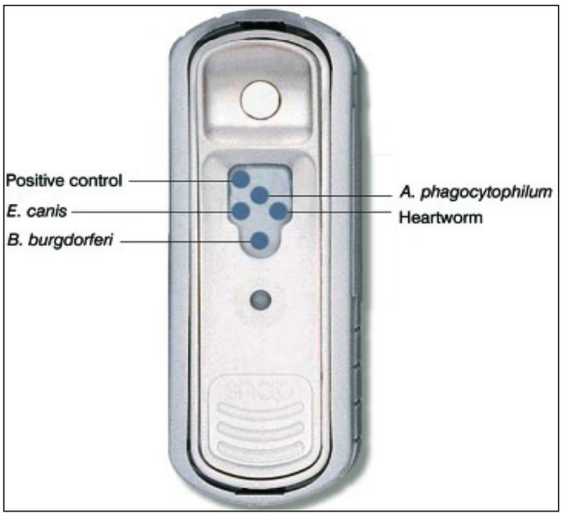

Fig. 1: CANINE SNAP 4 Dx plus test

A total of 5711 presented for treatment of various ailments and in health examination were screened over a period of 22 months. Out of 5711, 200 dogs were suspected to be suffering from TBD's based on history and clinical examination, 100 were found positive for different TBD's, of which 36 were found positive for *E. canis* based on PCR. PCR was performed as per standard protocol given by Murphy *et al.* 1998 and 389 base pair (bp) product specific for *E. canis* was visualized in 1% Agarose gel electrophoresis (Fig. 2). 13 cases were found positive in giemsa stained thin blood smear (Fig. 3). Out of 60 cases 30 were found positive in SNAP 4Dx plus kit (Fig. 4). The overall prevalence of *E. canis* was found to be 36 per cent with maximum (4 cases; 11.11 percent) during May, (3 cases; 8.33 percent) in June July 2015 March, June, July 2016 and minimum (1 case; 2.77 percent) in August September, October 2015 and January, February August and December 2016.

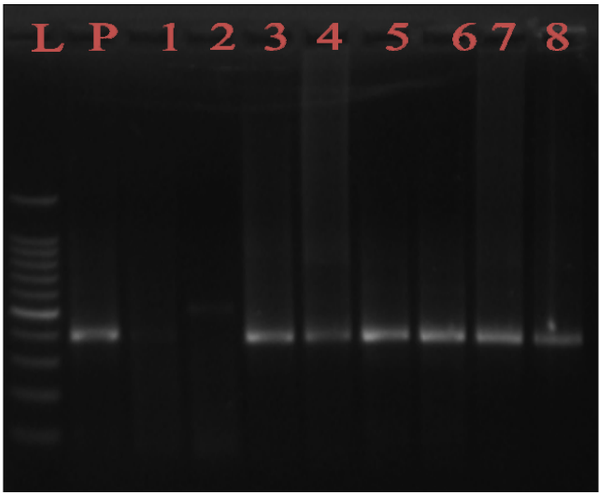

Fig. 2: Agarose Gel (1%) electrophoresis of PCR amplicon (389bp) *E. canis*. Lane L: 100 bp ladder; Lane P: Positive control; Lane: 1 to 8 samples

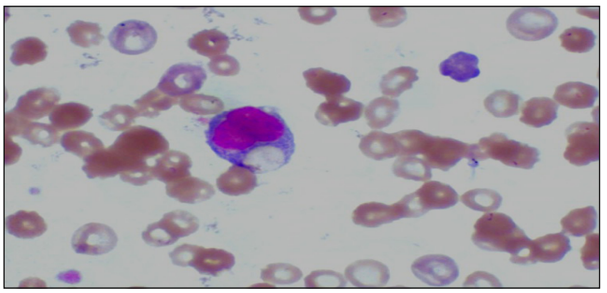

Fig. 3: Morulae stage of *E. canis* in monocyte as morulae from peripheral blood smear of dog

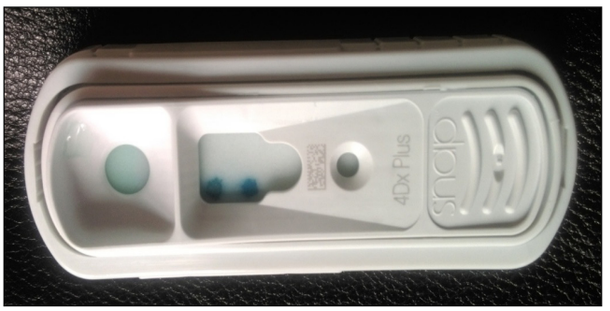

Fig. 4: SNAP4Dx plus test positive for *E. canis*

The prevalence of *E. canis* was recorded to be 36 percent in present study which is in agreement with work done by Singh *et al.* (2011) who carried out a prevalence

study on canine parasitic infections in Ludhiana district of Punjab and reported 1.43 per cent prevalence rate of *Ehrlichia canis*. Similar type of work done by Dhanakar *et al*. (2011) found 21.35% dogs positive for ehrlichiosis in Haryana and Delhi States. From South-Western regions the republic of Korea (South Korea) morula of *Ehrlichia canis* was found in 7.8% of the screened canine blood smears by Lim *et al*. (2007). Lakshmanan *et al.* (2006) had observed 5.66 per cent of dogs positive when screened for the presence of inclusion bodies of *E. canis* by blood smear examination in the Small Animal Clinic of Madras Veterinary College, Chennai.

The breed wise prevalence in the present study of *Ehrlichia canis* was found to be higher in Labrador Retriever (38.8 percent) German Shephard (25 %) followed by Rottweiler (5.55 %), Bully (5.55 %t), Bakerwali (5.55 %) and lowest in Saint Bernard (2.77 %), Pitbull (2.77 %), Great Dane (2.77 %), Beagle (2.77 %), Golden Reteriever (2.77 %) and Whippet (2.77 %) (Table 3), which is in comparable with sporadic study at Bareilly (Behera, 2011). The variation in the occurrence of *E. canis* among various breeds of dogs may be due to difference in the population size of different breeds in and around Jammu district. Hornok *et al.* (2006) attributed higher incidence of Ehrlichiosis in Labrador breed due to increased risk to them of unnoticed ticks attached under their heavy hair coat rather than a genetic or breed predisposition.

Table 3: Breedwise prevalence of *Ehrlichia canis*

Sl. No.	Breed	Positive animals
1	Labrador	14
2	German Shephard	9
3	Bakerwali	2
4	Bully	2
5	Rottweiler	2
6	English pointer	1
7	Whippet	1
8	Saint Bernard	1
9	Beagle	1
10	Pitbull	1
11	Great dane	1
12	**Golden retriever**	**1**

The higher susceptibility of German shepherd to *E. canis* is well established (Bhardwaj *et al.,* 2013; Nyindo *et al*., 1980). The age wise prevalence in the present study was found higher (72.23%) in 1 – 5 years dogs as compared to > 5 years (25%) and <1 year (2.77%). Age wise analysis of data to study the distribution of *Ehrlichia canis* revealed presence of infection in almost in adult and older dogs which was in agreement with Harrus *et al*. (1997b) and Harikrishnan *et al*. (2001). The higher incidence was observed in the adult group (1- 5 years) which corroborates with findings of Mundim *et al*. (1994), Gavazza *et al*. (2003) and Chaudhuri, (2006). This prevalence may be ascribed to the low immunity of adult animals. The month wise prevalence in the present study revealed highest prevalence of *Ehrlichia canis* in the month of May, June, July and April and least number of cases in August, September, October and December. These observation are in agreement with the findings of other workers (Harrus *et al*., 1997 a,b; Greene, 2006). The low incidence during November and December can be explained on the basis of reduced vector activity whereas high incidence in summer might be due to high ambient temperature conducive for breeding and hence increased activity of the vector *Riphicephalus sanguineus* tick (Soulsby, 2006). The sex wise prevalence in the present study was higher among males as compared to female counterpart. It was found in agreement with Okubanjo *et al.* 2014 and Gavazza *et al.* 2003.The higher occurrence of males in this study can be attributed to higher population male dogs, or it may be related with their higher exposure to ticks, the vector of the disease or due to behavioral habits (Okubanjo *et al.,* 2014).

CONCLUSION

It may be concluded that canine ehrlichiosis causes acute febrile illness in dogs but subclinical stage, lasting months to years, is not associated with clinical signs of illness and therefore may go unnoticed by pet owners and undiagnosed by veterinarians unless antibodies are detected during annual screening with in-clinical kits. Therefore, it is not possible to rely on a single serological result for diagnosis of *E. canis*, it may be concluded that PCR is most reliable method, useful in the clinical laboratory for specific and early diagnosis of ehrlichiosis in dogs.

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