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Prevalence and *In vitro* Detection of *Anaplasma platys/ phagocytophilum* Infection 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 number of 5711 dogs were presented for treatment of various ailments and in health examination at 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 200 dogs were suspected to be suffering from TBD's were screened and 100 dogs were found positive for different TBD's and 4 were found positive for *Anaplasma platys/ phagocyophilum* infection. The prevalence of *Anaplasma platys/ phagocytophilum* was found to be 6.6 percent (6/60) (based on SNAP4Dx plus test) with maximum occurance in monsoon season. No case was found positive in giemsa stained thin blood smear. Dogs in the age group of (1 - 5 year) were found most susceptible (50%) to *Anaplasma platys/ phagocytophilum*. German Shephard breed was most suseptible. SNAP4Dx plus test was performed using standard protocol. Little information is available about Anaplasmosis in this region due to lack of planned work so present study was undertaken to determine prevalence of *Anaplasma platys/ phagocytophilum* infection. It was concluded Anaplasmosis is present in this region and SNAP4Dx plus test is reliable and sensitive tool for early detection.

HIGHLIGHTS

- Study focuses on *in vitro* detection of *Anaplasma platys/ phagocyophilum* infection.
- SNAP4Dx plus test is reliable and sensitive tool for early detection.
- Clinical signs anaplasmosis vary but the disease most commonly presents as an acute febrile syndrome.

Keywords: *Anaplasma platys/ phagocytophilum*, Prevalence, SNAP4Dx plus, Dogs

Tick transmits a greater variety of pathogenic microorganisms, protozoa, rickettsiae, spirochaetes and viruses (Lee *et al*., 2010) and are the second most vectors after mosquitoes (Dennis and Piesman, 2012) affecting livestock, humans and companion animals. Tick-borne canine infectious diseases constitute an emerging problem in veterinary medicine in recent years. Ticks are notorious vectors of various pathogenic protozoa, rickettsiae, bacteria, and viruses that cause serious and life threatening illnesses in animals as well as in humans worldwide (Alekseev *et al*., 2011; Jongejan, 2007). *Anaplasma phagocytophilum* is the causative agent of granulocytic anaplasmosis in dogs. This is a tick-transmitted disorder that was previously known as canine granulocytic ehrlichiosis (Greig and Armstrong, 2013). In 2001, molecular analysis prompted the reclassification of several *Ehrlichia* species to the genus *Anaplasma.* The species *Ehrlichia equi, Ehrlichia phagocytophila,* and the unnamed causative agent of human granulocytic ehrlichiosis (HGE) were merged into a single species and renamed, *Anaplasma phagocytophilum* (Greig and Armstrong, 2013). These are gram-negative,

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obligate intracellular bacteria that parasitize neutrophils in dogs, people, cats, cattle, sheep, and goats and horses worldwide. *Anaplasma phagocytophilum* is an obligate, intracytoplasmic coccus that belongs to the family *Anaplasmataceae*. The agent affects mostly neutrophils and rarely eosinophils; it is present within intracytoplasmatic vacuoles (morulae) (Dumler *et al*., 2011). Canine cyclic thrombocytopenia is caused by *Anaplasma platys*, a gram-negative, obligate intracellular bacterium that infects platelets. *A. phagocytophilum* is transmitted by ticks, which include *Ixodes pacificus* in the western United States, *Ixodes scapularis* in the upper Midwestern and northeastern United States, *Ixodes ricinus* in Europe, and *Ixodes persulcatus* and *Dermacentor silvarum* in Asia and Russia (Cao *et al*., 2012). Dogs were first identified with *A. phagocytophilum* infection in California in 1982. Risk factors for *A. phagocytophilum* infection in dogs include season of the year, co infection with other tick-borne pathogens, and signalment.

Due to paucity of information, lack of planned work on tick borne diseases of dogs of Jammu region present study was undertaken to determine prevalence of *Anaplasma platys/ phagocytophilum* infection and associated risk factors.

MATERIALS AND METHODS

The present study was conducted on clinical cases of dogs of different age groups and breeds presented to Referral Veterinary Hospital Sher-e-Kashmir University of Agricultural Sciences and Technology. A total number of 5711 dogs were presented for treatment of various ailments and in health examination at Small Animal Medicine OPD of Referral Veterinary Hospital during the period from March 2015 to December 2016. Out of which total of 200 dogs were suspected to be suffering from TBD's were screened and 100 dogs were found positive for different TBDs and 4 were found positive for *Anaplasma platys/ phagocytophilum.*

A detailed history regarding the 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, haemato-biochemical observations, radiographic findings, oxidative stress indices and PCR, diagnosis of disease was done. Blood smear were fixed in methanol and standby Stained by Giemsa method of staining). Prevalence was recorded age wise and differentiated 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 ailing dog was worked out based on 17-points scale (Jones, 1994) (Table 1).

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

Signs	Weightage Presence	Absence
Temperature > 102.4 ºF	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**

Serological detection by SNAP 4 Dx plus kit canine antibody test kit (IDEXX Labs. Inc., Westbrook, ME). This is multivalent (enzyme linked immunosorbent assay) based test uses synthetic peptide reagents independent in vitro diagnosis of *Dirofilaria immitis antigen, A. platys/ phagocytophilum antibodies, B. burgdorferi antibodies, E. canis/ ewingii antibodies.*

CANINE SNAP 4 Dx plus test kit protocol

All components were equilibrated at room temperature (18 - 25ºC) for 30 min before use. 3 drops of serum sample dispensed into sample tube then 4 drops of conjugate added to sample tube. Contents mixed thoroughly by inverting 3 – 5 times. Kit placed on horizontal surface and entire contents of sample tube were added to sample well. Sample crosses the result window, reaching the activation circle in 30 – 60 seconds. With first appearance of colour in activation circle, push the activator firmly until flushed with the device body

Results

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).

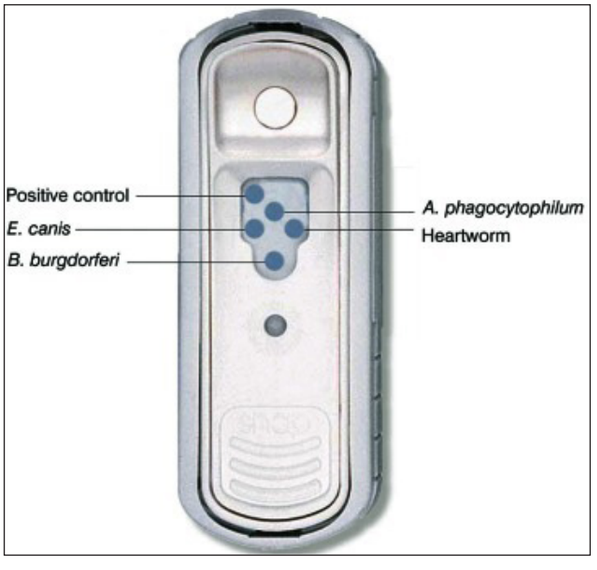

Fig. 1: CANINE SNAP 4 Dx plus test

RESULTS AND DISCUSSION

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 4 were found positive for *A. platys/ phagocytophilum* based on SNAP 4 Dx plus test (Fig. 2). No case was found positive in giemsa stained thin blood smear. The overall prevalence of *A. platys/ phagocytophilum* was found to be 6.6 per cent with maximum cases in monsoon.

Fig. 2: SNAP4Dx plus test positive for *Anaplasma platys/ phagocytophilum*

The prevalence of *Anaplasma platys/ phagocytophilum* was found to be 6.6 percent which is in agreement with different studies which indicated 1.6 – 4.5% prevalence rate of *A. phagocytophilum* in Germany (Leonhard, 2005; Pichon *et al*., 2006; Silaghi *et al*., 2008), but lesser reports are available from any part of India. In other study at Punjab

A. phagocytophilum has been reported most frequently as a subclinical infection in the north-west region of India, with a prevalence range of 3 to 9% in Punjab based on SNAP4D$_x$ plus test (Pawar and Gatne, 2008). The breed wise prevalence of *A. platys/ phagocytophilum* was found to be higher in German Shephard (4 percent) followed by Labrador Retriever (2 percent) and lowest in Pomeranian (1 percent) (Table 2), which in agreement with work done by coworkers who observed that tick borne diseases occurred most frequently in German shepherd, but the dogs of other breeds were also affected.

Table 2: Breedwise prevalence of *A. platys/ phagocytophilum*

Sl. No.	Breed	Positive animals
1	German Shepherd	2
2	Labrador	1
3	Pomeranian	1

The age wise prevalence in present study showed higher prevalence (50%) infection in adult dogs which is validated by Swedish study who postulated that proportion of seropositive dogs increased with the age of the dog population reflecting an increased likelihood of exposure over time (Egenvall *et al*., 2006).

The season wise prevalence in the present study revealed highest prevalence of *A. platys/ phagocytophilum* in monsoon season. These observation corroborate with the findings of (Beall *et al*., 2010), a bimodal seasonal distribution of illness has been reported that reflects the activity of the *I. scapularis* tick, in late spring (May, June) and fall in October and November. In Berlin, 17 of 18 cases were reported between the months of April and September, with the remaining case occurring in November (Kohn *et al*., 2008). The seasonal distribution of disease most likely reflects periods of peak nymph and adult tick activities, as well as periods when humans and their dogs are engaged in increased outdoor activities.

The sex wise prevalence in the present study was higher among males as compared to female counterpart. It was found in agreement with (Gavazza *et al.* 2013). 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 clinical signs of anaplasmosis vary but the disease most commonly presents as an acute febrile syndrome. Lower prevalence recorded by blood smear examination is due to the fact that it lacks sensitivity, time consuming and chances of successfully finding the organism in stained smears are very low, particularly in subclinical stage of disease SNAP 4 Dx plus is multivalent (enzyme linked immunosorbent assay) based test uses synthetic peptide reagents independent in vitro diagnosis The major advantage of this test is the capability of generating accurate serologic results in a short time. a high sensitivity (99. 4%) and specificity (100%).

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