SERO-CLINICAL PROFILE OF CANINES AFFECTED WITH VECTOR BORNE DISEASE IN JAMMU DIVISION

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ABSTRACT

In the present sero-clinical study on canine vector borne diseases (CVBD). Out of 2203 canine cases presented to different clinics for various ailments/ routine health check-up, 172 cases (7.81%) cases suspected for suffering from vector borne diseases, exhibiting clinical signs of pyrexia and tick infestation formed study population. Examination of peripheral blood smears (PBS), buffy coat (BCS) besides Canine SNAP 4Dx test (indirect ELISA based) showed *Ehrlichia canis* positivity as 13.37 (23), 22.09 (38), and 90 (27) per cent, respectively in 172 canines. Clinico-haematological profile of 38 Ehrlichia positive cases revealed statistically significant in haemoglobin, indices, packed cell volume, total erythrocytic count, platelet count compared to the healthy control besides monocytopaenia. Identification of collected tick from affected cases revealed *Rhipicephalus sangiuneus* as major vector for transmission of disease.

Key words: Canine vector borne diseases, *Ehrlichia canis*, indirect ELISA, monocytopaenia, *Rhipicephalus sangiuneus*

Introduction

The Indian climatic diversity plays an important role in the spread of diseases which are of medical and veterinary importance. The confounding between emergence and re-emergence of arthropod borne disease and change in the climatic conditions due to global warming and the increasing inclination of population of dogs has augmented the chance of disease transmission due to easy accessible hosts (Patz *et al*., 2005). Canine vector borne diseases (CVBDs) are caused by a diverse range of pathogens, transmitted to dogs by different arthropod vectors (ticks, fleas, lice, mosquitoes, tabanids and phlebotomine sand flies) which includes *Ehrlichia canis*, *Dirofilaria immitis*, *Borrelia burgdorferi, Anaplasma phagocytophilum, Anaplasma platys, Leishmania infantum and Babesia gibsoni* as the major vector borne agents infecting dogs. These constitute an important group of illness affecting the dogs around the world, thus recognised as emerging problem worldwide due to their frequency, morbidity and zoonotic potential (Day, 2011).

Evidence about the canine vector borne disease (CVBD) in India is still scarce and available information points out that filariasis, babesiosis, anaplasmosis, ehrlichiosis are endemic at different times in different parts of India (Irwin and Traub, 2006). Therefore, a widespread investigation of these disease is the need of the day enhancing the understanding of diseases encompassing the veterinary clinical aspects and thereby prevention of potential threat to the canine population. Thus the present investigation was aimed at studying the clinic-haematological profile of dogs suffering from vector borne diseases in Jammu division.

Materials and Methods

Model

The present study was a retrospective study carried out on 172 dogs of different breeds (German Shepherd, Labrador, Pomerian, Doberman, Bakerwali and Non-Descriptive) brought for treatment to Teaching Veterinary Clinical Complex (TVCC), Faculty of Veterinary Sciences and Animal Husbandry, R.S. Pura and private pet clinics in and around Jammu division (Table 1) with a history of pyrexia of un-known origin, non-responsiveness to treatment along with history of tick infestation during the course of disease. Apparently healthy dogs (n=10) visiting TVCC, R.S. Pura for vaccination and routine check-up were used as control.

Proceedings followed

Case history of each dog was ascertained admitted for cure before undertaking clinical examination which included age, sex, breed, vaccination status, thermal reaction, feed intake, vomition, diarrhoea, epistaxis, nervous signs and weight loss (if any). Further the dogs were examined clinically for temperature, tick infestation (if any), anaemia (status of mucous membrane), general body condition, dehydration status, heart rate, respiratory rate, lymphandenopathy and haemoglobinuria.

Blood smear examination and haemato-serological studies

Blood smear was prepared by taking one drop of blood following ear vein puncture, which was stained with Geimsa stain (1:10 dilution). The smear was examined under oil immersion (100X) for the presence or absence of vector borne pathogens.

Table 1: Sample collection

Source of Samples	No. of dogs
Small Animal OPD, Teaching Veterinary Clinical complex, R.S. Pura	85
Private Pet Clinics in and around Jammu city	87
Total	172

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Blood samples of animals under study were collected aseptically by venepuncture of either cephalic or saphenous vein. In severely dehydrated and young ones, venepuncture of jugular vein was done since blood collection was a difficult proposition in such cases. Blood was collected in K_2EDTA vacutainers and serum vacutainers for haematological and serological studies, respectively.

Preparation of buffy coat smear and its examination

Microhaematocrit tubes were filled 2/3rd with blood treated with di-potassium salt of Ethylene diamine tetra acetic acid (K_2EDTA) and the other end was sealed. The tubes were centrifuged at 3000 rpm for 5 min and then broken 1 mm below buffy layer. The contents of tube containing buffy coat were tapped out onto a glass slide and a smear was made, which was further stained with Giemsa stain and observed under microscope.

Serological test

Cases which were positive for any of the vector borne diseases after blood smear/buffy coat examination were serologically confirmed using Canine SNAP 4DxTest kit, 2006 (IDEXX Laboratories Inc., Westbrook, ME) which is a multivalent ELISA (enzyme-linked immunosorbent assay) based test uses synthetic peptide reagents in independent *in-vitro* diagnosis of *Diroflaria immitis* antigen, antibodies to *Anaplasma phagocytophilum*, *Anaplasma platys*, *Borrelia burgdorferi*, *Ehrlichia canis* and *Ehrlichia ewingii* in canine serum.

Haematological study

Haemoglobin (Hb) using Drabkin's method, packed cell volume (PCV) using micro-haematocrit method, total erythrocyte count (TEC) and total leucocyte count (TLC) by improved Haemocytometer and differential leucocyte count (DLC) as per Jain (1993) were determined within 5-10 hrs of blood collection.

Erythrocytic indices *viz.* mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were also calculated. Platelets were counted per 100 WBCs in the Giemsa stained blood smears and following formula was used for calculation.

$$\text{Platelet/µl} = \frac{\text{Number of platelets}}{\text{100 WBC}} \times \text{Total leucocytic count}$$

Vector collection and its identification

Ticks were rifled by fleeting hands through the animal's coat and different stages of ticks (larva, nymph and adult) were collected with the aid of forceps and brushes without damaging their mouthparts. Identification of ticks was done by following identification keys and check lists authored by Soulsby (1982) and Walker (1994).

Statistical analysis

The data collected was analysed using SPSS (Statistical Package for Social Sciences) software (Version 14.0-SPSS Inc.).

Results and Discussion

The history of dogs reporting for the treatment including age, sex, breed, vaccination status, thermal reaction and previous tick infestation was collected. Most of the dogs underwent with hyperthermia from last two to three days and showed anorexia.

Clinical considerations

All the dogs were hyperthermic (100%) and 92.10% were infested with ticks. Severe anorexia and dehydration was observed in 92.10% cases. Poor body condition was seen in 81.57%, severe depression in 84.21%, tachycardia in 76.31%, dyspnoea in 65.78%, epistaxis in 55.26% and pale mucous membrane in 52.63%. Moreover, lymphadenopathy was seen in 42.10% and haemoglobinuria in 21.05% cases which are in close covenant with the findings of Bissett *et al*. (2007), Gianfranchesco *et al.* (2019), Qurollo *et al.* (2019) and Yadav *et al*. (2018).

Confirmation of vector borne disease

Primary screening of the suspected cases of vector borne diseases based on anamnesis and clinical examination was done initially using peripheral blood smear (PBS) examination followed by buffy coat smear examination and subsequently serologically by Canine SNAP 4Dx kit. Blood samples from 172 dogs suspected for vector borne infections based on history and clinical findings were examined by PBS and buffy coat smear examination using Giemsa staining. Only 23 blood smears (13.37%) and 38 (22.09%) buffy coat smear were found positive for *Ehrlichia canis* infection (Fig.1 and Fig. 2).

Only 30 buffy coat positive animal sera were subjected to Canine SNAP 4Dx test for detection of *Dirofilaria immitis* antigen, antibodies to *Anaplasma phagocytophilum*, *Anaplasma platys, Borrelia burgdorferi*, *Ehrlichia canis*, and *Ehrlichia ewingii* in canine serum. The results are presented in Table 2.

The ticks collected from all the Ehrlichiosis cases were identified as *Rhipicephalus sanguineus* as the ticks had short mouth parts and hexagonal basis captuli (Fig. 3).

Table 2: Canine SNAP 4Dx test for validation of vector borne infection

Samples tested	Test	Sample positive			
30	Canine SNAP 4Dx test	*E. canis***, *E. ewingii*	*Dirofilaria immitis*	*Anaplasma phagocytophilum*, *Anaplasma platys*	*Borrelia burgdorferi*
		27 (90)	Nil	Nil	Nil

*Figures in parenthesis denote percentage; **All samples were positive for *E. canis*

Table 3: Comparative haematological alterations

Parameter	Healthy Group	*E. canis* infected
Haemoglobin (g/dl)	12.92 ±0.33	8.39 ±0.68*
PCV (%)	39.10 ±0.67	26.50 ±1.88*
TEC (x 10^6/µl)	5.97 ±0.20	3.74 ±0.43
MCV (fl)	59.73 ±0.08	61.37 ±0.29
MCHC (g/dl)	33.49 ±0.09	32.04 ±0.23
MCH (pg)	20.01±0.05	19.60±0.15
TLC (10^3/µl)	9.62 ±1.12	9.06 ±1.70
Neutrophil (%)	69.80 ±1.08	72.12 ±2.82
Lymphocyte (%)	16.90±1.20	18.63±2.88
Eosinophil (%)	1.80±0.47	2.00±0.37
Basophil (%)	-	0.44±0.06
Monocyte (%)	11.40±0.81	6.81±1.12
Platelet count (10^5/µl)	2.04 ±0.17	0.38±0.55*

*denote significant changes

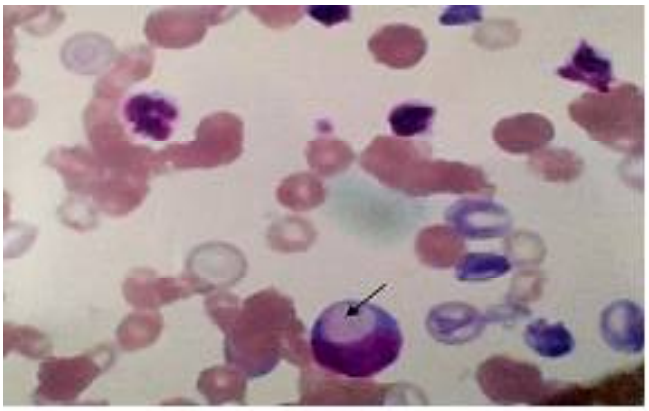

Fig. 1: Giemsa Stained (100X) blood smear of a dog showing *E.canis* morula in a monocyte (arrow marked

Fig. 2: Giemsa Stained (100X) buffy coat smear showing *E.canis* morula in a monocyte (arrow marked)

Fig. 3: Morphological feature of *Rhipicephalus sanguineus*

All the 38 cases of canine Ehrlichia infection were in acute stage of infection. Ten dogs reporting for routine examination were taken as healthy control. The comparative haematological alterations in 38 positive cases of *E. canis* infection at the time of admittance (day 0) were investigated as illustrated in Table 3.

The mean Haemoglobin (Hb) values of healthy control group and *E. canis* infected groups were 12.92 ±0.33 and 8.39 ±0.68 g/dl, respectively. There was significant decrease (P<0.01) in the Hb level between dogs infected with Ehrlichia and control group which is in agreement with the findings of Matthewman *et al.* (1993) and Yadav *et al*. (2017). This reduction could be attributed to severe haemorrhagic tendencies and bleeding disorders and consequent blood loss.The mean PCV of healthy group dogs was 39.10 ±0.67 per cent and infected dogs had highly significant lower value of 26.50 ±1.88 per cent (P<0.01). The mean total erythrocytic count (TEC) in the infected group was 3.74 ±0.43 x 10^6/µl which was significantly decreased (P<0.01) compared to healthy control group (5.97 ±0.20 x 10^6/µl). Drop in PCV in affected dogs could be possibly due to intracellular and intercellular dehydration associated with movement of fluid to the vascular system and our study is in consonance with the findings of Chou (1995) and Davoust *et al.* (1996) and Sharma *et al.* (2016).

The mean corpuscular volume (MCV) of infected group was 61.37 ±0.29 fl which was non-significantly higher as compared to healthy group dogs with mean values of 59.73±0.08 fl. The mean corpuscular haemoglobin concentration (MCHC) of infected group and healthy group dogs were 32.04 ±0.23 g/dl and 33.49 ±0.09 g/dl, respectively.

Similarly, the mean corpuscular haemoglobin (MCH) value was statistically non-significant in infected group (19.60±0.15 pg) as compared to healthy group (20.01±0.05 pg). The total leucocytic count (TLC) of infected group dogs was 9.06 ±1.70 x 10^3/µl and that of healthy group was 9.62 ±1.12 x 10^3/µl. No marked change in mean leucocytic concentrations of infected as compared to the healthy ones was seen. The changes in the erythrocytic indices is attributed to the regenerative anaemia due to haemorrhages like epistaxis.

The mean neutrophil percentage of healthy group dogs and infected group dogs was 69.80 ±1.08 and 72.12 ±2.82 per cent, respectively. The recorded lymphocyte, eosinophil and basophil counts for healthy group dogs in percentage were 16.90±1.20, 1.80±0.47, nil and for infected group were 18.63±2.88, 2.00±0.37, 0.44±0.06, respectively. The mean monocytic percentage of healthy group dogs and infected group dogs were 11.40±0.81 and 6.81±1.12, respectively and statistically the decrease in values was non-significant paralleled to the healthy ones. The findings obtained are in agreement with the conclusions of Kuhen and Gaunt (1985), Davaoust *et al*. (1996) and Tremasol *et al.* (1998).

There was highly significant decrease (P<0.01) in the platelet count of infected group as compared to healthy ones with mean values 0.38±0.55 x 10^5/µl and 2.04 ±0.17 x 10^5/µl, respectively which is probably due to decreased circulating half-life of platelets and increased platelets destruction during the course of the disease and the results are in close conformity to the findings of Smith *et al*. (1974), Collet *et al*. (1987), Abeygunwardena *et al*. (1990), Waner *et al.* (1995) and Gupta *et al*. (2017).

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