Applied Biological Research 14 (2):207-210, 2012 **Short Communication**

MORPHOMETRIC VARIATIONS IN PIROPLASMS OF *Babesia canis* FROM NATURALLY INFECTED DOGS FROM PUNJAB (INDIA)

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ABSTRACT

This study presents the morphometeric characteristics of canine haemo-protozoan, *Babesia canis*, by using software DP2-BSW (Olympus). Most of the *B. canis* in erythrocytes were pyriform bodies at an acute angle whereas a few were at obtuse angle. The other erythrocytic stage of *B. canis* was commonly tetrad with some polymorphic forms including ovoid, amoeboid, elongated, umbrella-like, etc. The micrometric measurements of *B. canis* piroplasms varied from 2.93 - 5.80 x 2.10 - 3.30 µm and a quartered parasite of 5.1 x 5.4 µm size was also seen. The only species of tick, *Rhipicephalus sanguineus*, a known vector of *B. canis*, was recovered from infected dogs.

Keywords: *Babesia canis*, dog, piroplasms, morphometery

INTRODUCTION

Blood protozoan and rickettsial pathogens are transmitted by ticks in dogs and cause diseases resulting in considerable morbidity and mortality. The prevalence of tick-borne haemoprotozoan diseases in India is quite high because of favourable climatic conditions, characterized by high temperature and humidity, in many parts of India including Punjab and other northwestern states (Harikrishnan *et al.*, 2005; Kumar *et al.*, 2006). Scanty information is available on the occurrence of these vector-borne haemoprotozoan pathogens in India (Kalra and Singh, 1984; Juyal *et al.*, 1992; Kaur *et al.*, 2011). *Babesia* species are tick-transmitted apicomplexan parasites infecting a wide range of wild and domestic animals (Kuttler, 1988). In canines, the species of this genus have been divided into two main groups, large (*Babesia canis*) and small (*Babesia gibsoni*) forms on the basis of their morphlogical appearance in the erythrocytes. *Babesia canis* was first described by Piana and Galli-Valerio (1895) and was historically recognized as the only species of large piroplasm known to infect dogs. Since then noticeable differences in vector specificity and infection pathology between isolates led to the proposal of three separate subspecies of *Babesia canis* (Uilenberg *et al.*, 1989). *Babesia canis rossi* is transmitted by *Haemophysalis leachi* and is reported to have the most severe pathogenic manifestations. *Babesia canis canis*, transmitted by *Dermacentor* spp., gives rise to moderate clinical disease and *B. canis vogeli*, transmitted by *Rhipicephalus sanguineus*, produces least severe clinical disease. The above three sub-species of *B. canis* have been classified according to geographic area, epidemiological features, tick species involved as vector, clinical signs and molecular characteristics (Taylor *et al.*, 2007). Since the information on morphometric characteristics of *B. canis* in naturally infected from India is scanty, therefore, the present work was carried out by using software DP2-BSW (Olympus).

MATERIALS AND METHODS

Blood samples were collected from the cephalic vein or the recurrent tarsal vein in EDTA coated vials from dogs naturally infected with *B. canis* presented to small animal clinics of GADVASU Ludhiana

(India). Thin blood smears were prepared and stained by the standard Wright Giemsa staining method (Jain, 1986). The blood smears from infected dogs (10) were randomly selected and examined under oil immersion (100X) lens. Measurement of morphological characteristics of *B. canis*, was done by software DPZ-BSW (Olympus) and analyzed.

RESULTS AND DISCUSSION

Babesiosis was suspected in dogs based on clinical signs including fever, anorexia, pale mucous membranes, anaemia, haemoglobinuria and tick infestation (Fig. 1). Confirmatory diagnosis was made by identifying *B. canis* piroplasms within erythrocytes in stained blood smears. Majority of *B. canis* in erythrocytes were pyriform bodies at an acute angle (Fig. 2) and in some cases these bodies were at obtuse angle (Fig. 3). Tetrads were common (Fig. 4) and polymorphic forms of *B. canis* were ovoid, elongated, amoeboid, umbrella-like, etc. (Fig. 5-7). The micrometric measurements of *B. canis* piroplasms varied from 2.93 - 5.80 x 2.10 - 3.30 µm. A quartered parasite was also seen with a size of 5.1 x 5.4 µm (Fig. 8). Some piroplasms were seen outside the erythrocytes in a few blood smears (Fig. 9).

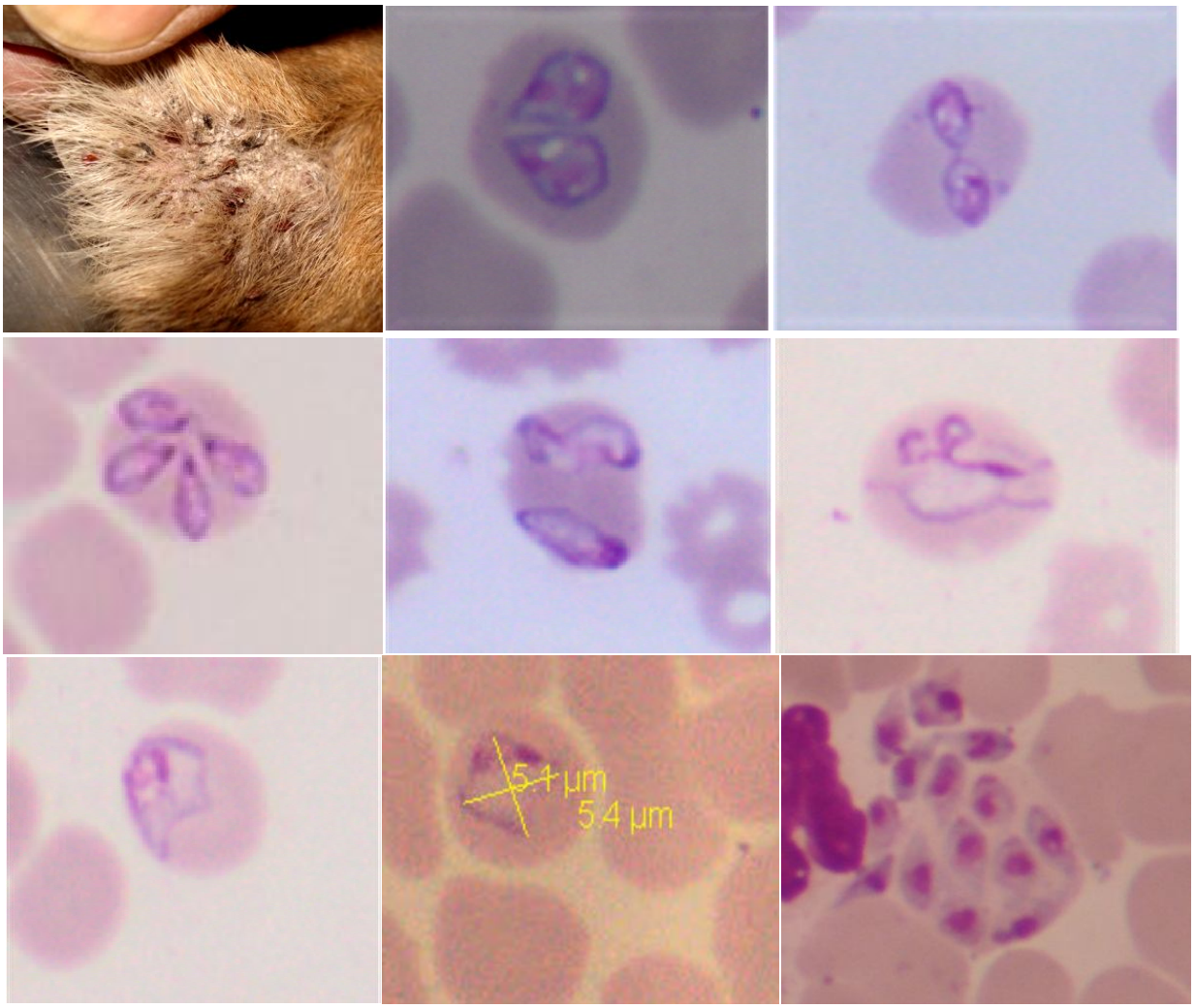

Fig. 1-9 (1st row, left to right) 1) Tick infested in dog; 2) *B. canis* at acute angle (pyriform); 3) *B. canis* at obtuse angle; (2nd row, left to right) 4) Piroplasms in tetrads; 5) Ovoid and elongated piroplasms; 6) Amoeboid piroplasms; (3rd row, left to right) 7) **Umbrella shaped piroplasms; 8) Large size, square shaped piroplasms; 9) Extracellular piroplasms**

Microscopic examination of stained peripheral blood smears was carried out to confirm the infection of *B. canis* in dogs (Guimara˜es *et al.*, 2002). This method has many advantages including simplicity, high specificity and low cost. During the parasitaemic phase, intraerythrocytic inclusions of *Babesia* were observed in stained peripheral blood smears, especially in febrile dogs (Guimara˜es *et al.*, 2004). *Babesia canis* is presumed to be the only large *Babesia* species to infect dogs throughout the world. Though, microscopic identification of large sized (3 - 5 µm) piroplasms in canine erythrocytes was sufficient for the diagnosis of *B. canis* infection, geographic location, differences in pathogenicity, antigenic variation and differences in vector specificity have all been used to support the existence of three subspecies of *B. canis: B. c. canis, B. c. rossi* and *B. c. vogeli* (Uilenberg *et al*., 1989; Hauschild *et al.*, 1995; Hauschild and Schein, 1996; Lewis *et al*., 1996).

Majority of *B. canis* were pyriform bodies at an acute angle inside the erythrocytes and in some cases these bodies were at obtuse angle. As the parasites grow, the nucleus and chromatin begins to separate and fine dividing forms are seen as pyriform bodies inside the erythrocytes (Bosman *et al.*, 2010). Polymorphgic forms including ovoid, amoeboid, elongated and umbrella-like of *B. canis* in erythrocytes observed in present study have also been reported by Levine (1985). Pleomorphic forms observed may be due to the presence of developmental stages of piroplasms inside the erythrocytes (Bosman *et al.*, 2010). The morphological transformation of merozoites into gamonts most likely starts inside the erythrocyte of vertebrate host (Mehlhorn and Schein, 1998). Occasionally, piroplasms become large and oddly shaped. Round piroplasms noticed sometimes are precursors of gamonts in the gut of tick. It is believed that these changed merozoites are gametocytes that can only differentiate further into gamonts within the tick gut upon ingestion (Mehlhorn and Schein, 1984). Tetrad and multiple forms are multiplying forms. The piroplasm size (2.93 - 5.80 x 2.10 - 3.30 µm) of *B. canis* parasite was also comparable to the earlier reports (Lewis and Huxsoll, 1977; 4.5 - 5.0 x 2.5 - 3.0 µm), (Yonamine *et al*., 1984; 3.3 - 4.2 x 2.2 - 3.3 µm) and (Ishibashi *et al.*, 1994; 1.24 - 7.15 x 0.88 - 4.40 µm). A large *Babesia* species sharing a high genetic identity with *Babesia bigemina* from cattle has also been reported in a dog from North Carolina (Birkenheuer *et al.*, 2004). The morphological measures observed in the present study correlated with *B. canis vogeli*. These observations were further substantiated with the recovery of only species of tick, *R. sanguineus*, which is a known vector of *B. canis vogeli*.

The study provides useful insights about morphometeric measurement of *B. canis***,** which can be regarded accurate and definitive in the identification of the agent.

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REFERENCES

Birkenheuer, A.J., Neel, J., Ruslander, D., Levy, M.G. and Breitschwerdt, E.B. 2004. Detection and molecular characterization of a novel large *Babesia* species in a dog. *Veterinary Parasitology*, **124:** 151-160.

Bosman,A.M., Oosthuizen, M.C., Peirce M.A., Venter E.H. and Penzhorn, B.L. 2010. *Babesia lengau* sp. nov., a novel *Babesia spcies* in cheetah (*Acinonyx jubatus*, Schreber, 1775) populations in South Africa. *Journal of Clinical Microbiology,* **48**: 2703-2708.

Guimara˜es, A.M., Oliveira, T.M.F.S. and Santa-Rosa, I.C.A. 2002. Babesiose cania: uma visa˜o dos clı´nicos veterina´rios de Minas Gerais. *Clinica Veterinaria,* **41**: 60-68.

Guimara˜es, J.C., Albernaz, A.P., Machado, J.A., Junior, O.A.M. and Garcia, L.N.N. 2004. Aspectos clı´nico-laboratoriais da babesiose canina na cidade de Campos do Goytacazes, RJ. *Revista Brasileira de Parasitologia Veterinária,* **13** (Suppl. 1): 229.

Harikrishnan, T.J., Pazhanivel, N. and Chellappa, J.D. 2005. Concomitant *Babesia gibsoni* and *Ehrlichia canis* infection in a dog. *Veterinarski Arhiv,* **75:** 513-520.

Hauschild, S. and Schein, E. 1996. The subspecies specificity of *Babesia canis*. Berl. Munch. *Tierarztl. Wochenschr*, **109**: 216-219.

Hauschild, S., Shayan, P. and Schein, E. 1995. Characterization and comparison of merozoite antigens of different *Babesia canis* isolates by serological and immunological investigations. *Parasitology Research*, **81**: 638-642.

Ishibashi, T., Sunaga, F., Namikawa, K., Kishikawa, S. and Kanno, Y. 1994. Isolation of *Babesia canis* from a dog on Okinawa Island. *Journal of the Japan Veterinary Medical Association*, **47:** 202-204.

Jain, N.C. 1986. *Schalm's Veterinary Hematology* (4th edn.). Lea and Febiger, Philadelphia, USA.

Juyal, P.D., Sandhu, B.S., Kalra, I.S. and Sood, N. 1992. *Ehrlichia canis* and *Hepatozoon cains* in naturally infected dogs in Punjab. *Journal of Veterinary Parasitology*, **6:** 21-26.

Kalra, I.S. and Singh, K.B. 1984. Observations of naturally occurring *Babesia canis* infection in dog. *Indian Veterinary Journal*, **61**: 98-100.

Kaur, P., Deshmukh, S., Singh Rajsukhbir, Bansal, B.K., Randhawa, C.S. and Singla, L.D**.** 2011. Para-clinico-pathological observations of insidious incidence of canine hepatozoonosis from a mongrel dog: A case report. *Journal of Parasitic Diseases*, **36**: 135-138.

Kumar, A., Varshney, J.P. and Patra, R.C. 2006. A comparative study on oxidative stress in dogs infected with *Ehrlichia canis* with or without concurrent infection with *Babesia gibsoni*. *Veterinary Research Communication*, **30**: 917-920.

Kuttler, K.L. 1988. Chemotherapy of Babesiosis. **In**: *Babesiosis of Domestic Animals and Man* (ed. M. Ristic), CRC Press, Florida, USA.

Levine, N.D. 1985. *Veterinary Protozoology*. Iowa State University Press, Ames, IA, USA.

Lewis, B.D., Penzhorn, B.L., Lopez-Rebollar, L.M., De Waal, D.T. 1996. Isolation of a South African vector specific strain of *Babesia canis*. *Veterinary Parasitology*, **63**: 9-16.

Lewis, J.E. Jr. and Huxsoll, D.L. 1977. Canine babesiosis. *Current Veterinary Therapy*, **6**:1330-1333.

Mehlhorn, H. and Schein, E. 1984. The piroplasms: Life cycle and sexual stages. *Advances in Parasitology*, **23**: 37-103.

Mehlhorn, H. and Schein, E. 1998. Redescription of *Babesia equi* (Laveran, 1901) as *Theileria equi* (Mehlhorn, Schein 1998). *Parasitology Research*, **84**: 467-475.

Piana, G.P. and Galli-Valerio, B. 1895. Su di un' infezione del cane con parassiti endoglobulari nel sangue. *Mod Zooiatr*, **6**: 163-169.

Taylor, M.A., Coop, R.L. and Wall, R.L. (eds.). 2007. *Veterinary Parasitology* (3rd edn.). Blackwell Publishers, Oxford, UK.

Uilenberg, G., Franssen, F.F., Perie, N.M. and Spanjer, A.A. 1989. Three groups of *Babesia canis* distinguished and a proposal for nomenclature. *Veterinary Quarterly*, **11:** 33-40.

Yonamine, H., Ichiki, H., Hamakawa, M., Shimabukuro, T., Sugiyama, M. and Isoda, M. 1984. *Japanese Journal of Veterinary Science*, **46:** 511-518.