Morphometric variations in gametocytes of *Hepatozoon canis* from naturally infected dogs

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Abstract This study presents the morphometric characteristic of canine haemoprotozoan, *Hepatozoon canis*, using software DP2-BSW (OLYMPUS). The gametocytes of *H. canis* found inside the neutrophils were characteristic in shape and size and varied from 9.50 to 11.80 $\mu\text{m} \times 5.10$ –6.00 μm . Parasitaemia ranged from 1.00 to 39.00 %. Few gametocytes without nuclei and of abnormal shapes were also observed. The results were compared with the measurements done by using ocular micrometer.

Keywords Dogs · Gametocytes · *Hepatozoon canis* · Morphometry · Parasitaemia

Introduction

Hepatozoon canis is a haemoprotozoan that is transmitted to dogs by the ingestion of the ixodid tick *Rhipicephalus sanguineus*, containing mature oocysts. This haemogregarine was first described in dogs by James in 1905 (Laird 1959). Infections of *H. canis* are present in southern Europe, Asia,

Africa and Latin America (Gondim et al. 1998; O'Dwyer et al. 2001; Criado-Fornelio et al. 2003; Kaur et al. 2011). In infected dogs, gametocytes are present within peripheral blood leukocytes. *H. canis* infections range from an asymptomatic type to a lethal type in which symptoms such as fever, anaemia, emaciation and debility are displayed. Concurrent infections with pathogens such as *Babesia canis* and *Ehrlichia* spp. may aggravate the clinical condition of dogs infected with *H. canis* (Karagenc et al. 2006). The first report of the size of the gametocyte of *H. canis* was made by Perak in 1906 in Malaya and he reported its dimensions as 11–12 μm by 4.2–5.2 μm (Laird 1959). Porter (1919) reported that the gametocytes were in the range 8–12 μm by 4.5–6 μm . These measurements were made microscopically using micrometer and measuring a representative number of parasites but, this technique is not regarded as accurate. Warner et al. (1994) used image analysis technology for this purpose and reported its dimensions as 10.66–12.99 μm by 4.74–8.22 μm and proposed that this technique is standard and accurate method as an aid in classification of organisms like *H. canis*. The present study documents some of the physical size characteristics of *H. canis*, from a number of naturally infected dogs and is the first report from India about the morphometric measurements of this canine hemoprotozoan.

Materials and methods

Blood samples were collected from the cephalic vein or the recurrent tarsal vein in EDTA coated vials from cases of dogs naturally infected with *H. canis* presented to small animal clinics of GADVASU Ludhiana (India). Thin blood smears of each blood sample were prepared. The blood smears were stained by the standard Wright Giemsa

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Fig. 1 *H. canis* gametocyte with nucleus in cytoplasm of the neutrophils

Fig. 2 *H. canis* gametocyte without nucleus in cytoplasm of the neutrophils

staining method (Jain 1986). Ten positive cases from the total collection were randomly selected and examined under oil immersion (100×) lens and level of parasitaemia was determined by counting 100 leucocytes (neutrophils) in stained blood smears by the method of Rosenfeld (1947). Comparison of the measurement of the physical size characteristics of *H. canis* was done by ocular micrometry and software DPZ-BSW (OLYMPUS).

Results

Anorexia, pale mucous membranes, weight loss, pain, diarrhoea, vomiting, gait abnormalities, fever, polyuria, polydipsia and tick infestation were the most common

Fig. 3 *H. canis* gametocyte outside neutrophil (arrow)

signs observed clinically. Diagnosis of canine hepatozoonosis was made by identifying *H. canis* gametocytes within leucocytes in stained blood smears. *H. canis* gametocytes were found as capsular structures in cytoplasm of the neutrophils (Figs. 1, 2). 1–39 % (7.70 ± 3.77 %) neutrophils were found infected in different cases. Some parasites were seen without nucleus (Fig. 2) even gametocytes outside the neutrophils were also observed inside the smears (Fig. 3). The average parasitized neutrophils were $813.10 \pm 468.97 \mu\text{l}^{-1}$ with a variation from 14 to $4,913 \mu\text{l}^{-1}$ in different cases. The measurements of gametocyte of *H. canis* inside neutrophils varied from 10.26 to $11.21 \mu\text{m} \times 5.23$ – $5.80 \mu\text{m}$ when done by ocular micrometer (Table 1). Micrometry done by using software DP2-BSW (OLYMPUS) (Fig. 4) showed almost similar values regarding size of *H. canis* as shown by ocular micrometry (Fig. 5) and measurements varied from 9.50 to $11.80 \mu\text{m} \times 5.10$ – $6.00 \mu\text{m}$ (Table 2). Even abnormal shapes of gametocytes were observed (Fig. 6).

Discussion

Though *H. canis* in a dog in Punjab was reported by Kalra et al. (1989), no previous studies from India have reported the morphometric characteristics till date. In blood smears, levels of parasitaemia ranged from 1 to 39 %. Levels of *H. canis* parasitaemia observed by previous researchers ranged from as low as 0.5 % (Gondim et al. 1998) and as high as 90 % (Baneth et al. 1995). Elias and Homans (1988) found 6–43 % of the circulating neutrophils to be infected. Vincent-Johnson et al. (1997) reported that the number of infected leukocytes in American hepatozoonosis did not exceed 0.1 %. The disease severity was greater in dogs with high parasitaemia which is in concurrence with

Table 1 Ocular micrometric measurements and parasitaemia of *Hepatozoon canis* infection in neutrophils of infected dogs

	1	2	3	4	5	6	7	8	9	10
Length, mean $\pm$ SE (range)	10.93 $\pm$ 0.16 (10–12)	10.81 $\pm$ 0.17 (10–12)	10.77 $\pm$ 0.32 (9–12)	11.02 $\pm$ 0.16 (11–12)	11.08 $\pm$ 0.32 (11–12)	11.21 $\pm$ 0.19 (10–12)	10.93 $\pm$ 0.27 (10–12)	10.26 $\pm$ 0.36 (10–11)	10.69 $\pm$ 0.24 (10–12)	10.83 $\pm$ 0.23 (10–12)
Breadth, mean $\pm$ SE (range)	5.32 $\pm$ 0.16 (5–6)	5.46 $\pm$ 0.16 (5–6)	5.38 $\pm$ 0.32 (5–6)	5.80 $\pm$ 0.22 (5–7)	5.70 $\pm$ 0.00 (6)	5.66 $\pm$ 0.27 (5–6)	5.70 $\pm$ 0.00 (6)	5.32 $\pm$ 0.23 (5–6)	5.23 $\pm$ 0.27 (5–6)	5.46 $\pm$ 0.24 (5–6)

Fig. 4 Gametocytic measurement by software DP2-BSW—OLYMPUS **Fig. 5** Gametocytic measurement by ocular micrometer

the findings of Baneth and Weigler (1997). The gametocytic measurements have been done by various researchers using simple microscopic techniques (Laird 1959; Soulsby 1968). Porter reported in 1919 that the gametocytes were in the range of 8–12 μm by 4.5–6 μm (Laird 1959). Soulsby (1968) states that gametocytes measure 6 μm $\times$ 3 μm . In the present case measurements of *H. canis* using software varied from 9.50 to 11.80 μm $\times$ 5.10–6.00 μm , which was compared well with the measurements done by using ocular micrometer (10.26–11.21 μm $\times$ 5.23–5.80). The gametocytic measurement of *H. canis* can be regarded as accurate and definitive owing to the software used (DP2-BSW—OLYMPUS), further more the chances of error are minimized to a greater extent, when compared with ocular micrometry. This software used requires less time to execute the work and does not involve the requirement of a

Table 2 Morphometric observations of *Hepatozoon canis* in dogs as confirmed by software DP2-BSW (OLYMPUS)

	1	2	3	4	5	6	7	8	9	10
Length, mean $\pm$ SE	11.60 $\pm$ 0.08	10.10 $\pm$ 0.25	10.90 $\pm$ 0.84	11.40 $\pm$ 0.17	11.70 $\pm$ 0.11	11.80 $\pm$ 0.42	11.80 $\pm$ 0.09	9.50 $\pm$ 0.08	11.60 $\pm$ 0.08	11.40 $\pm$ 0.19
Breadth, mean $\pm$ SE	5.50 $\pm$ 0.09	5.10 $\pm$ 0.28	6.00 $\pm$ 0.17	5.30 $\pm$ 0.28	5.20 $\pm$ 0.09	5.30 $\pm$ 0.19	5.20 $\pm$ 0.05	5.50 $\pm$ 0.18	5.50 $\pm$ 0.09	5.30 $\pm$ 0.20

Fig. 6 Abnormal shaped gametocytes (Laogenze shaped)

bird's eye when compared with ocular micrometry, which is cumbersome and more error prone.

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