Para-clinico-pathological observations of insidious incidence of canine hepatozoonosis from a mongrel dog: a case report

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Received: 15 September 2011 / Accepted: 27 December 2011 / Published online: 21 January 2012
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Abstract A rare case of canine hepatozoonosis in a mongrel dog with para-clinico-pathological observations has been reported. The study included detailed haemato-biochemical changes at two stages, i.e. before treatment and after treatment with adopted therapy. Before therapy, blood picture revealed normocytic hypochromic anaemia and neutrophilic leucocytosis with variable counts of platelets. Thirty-seven percent of neutrophils were found infected with gametocytes of *Hepatozoon canis*. Following treatment, further decrease in haemoglobin value with a relative increase in lymphocyte count was seen. Biochemically, increase in alkaline phosphatase, blood urea nitrogen and creatinine levels along with hyperproteinemia was seen. The 14 days chemotherapy did not bring a respite for the dog and the level of parasitaemia was 33% after the treatment. The alkaline phosphatase and creatinine level further rose up following therapy with sulphadiazine and clindamycin. Continual study is required to explain the best possible therapeutic combination to deal *H. canis*.

Keywords *Hepatozoon canis* ·
Haemato-biochemical parameters · Mongrel dog ·
Treatment

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Introduction

Canine hepatozoonosis is a tick borne disease of dogs caused by *Hepatozoon canis*, an apicomplexan protozoan parasite and is being transmitted by ingestion of an ixodid tick, *Rhipicephalus sanguineus* containing mature oocysts. In India, the first authenticated incidence of the disease was reported from the blood samples of dogs by James (1905). The perusal of literature revealed that there are occasional number of singly affected natural cases of canine hepatozoonosis in dogs (Priya et al. 2004), and with rate of incidence varying between 3 and 9% in India (Sharma et al. 1997). Changes in pattern and incidence(s) of hepatozoonosis with increased cases of mixed infection(s) have also been reported (Harikrishnan et al. 2001; Banerjee et al. 2008). Studies on tick biology revealed that a small percentage of ticks are responsible for harbouring multiple pathogen(s) and successfully transmitting all pathogens to the host (Levin and Fish 2000; Adelson et al. 2004) or at some times, multiple ticks simultaneously feeding on a host are efficiently producing co-infection(s), resulting in either co-expression of more than one organism clinically in the host (Mordecai et al. 2006). Amazingly, one of the experimental study also described pathological appearance of one particular protozoan or bacterial organism within the hosts, following mixed or co-infections (Thomas et al. 2001), thus accounting for mixed insidious infections (Zeidner et al. 2000). The possible extant of co-infection could be due to myeloperoxidase deficiency in *H. canis* infected neutrophils or due to immunologic alterations as a result of mixed infections (Ibrahim et al. 1989; Pappalardo et al. 1997; Zeidner et al. 2000). The uncertainty prevailing over specific treatment regimen towards *H. canis* infection may be due to the possibility of mixed infection, which at times goes unnoticed clinically (Thomas et al. 2001;

Banerjee et al. 2008). There are reports on few therapeutic agents and regimens found effective in clearing the organism (*H. canis*) from the affected animal (Baneth et al. 1995; Voyvoda et al. 2006). At the same time, incidental improvement or insidious relapse of the *H. canis* infection was also observed with drugs like trimethoprim, sulphadiazine and clindamycin, suggesting the deficiency of myeloperoxidase enzyme to be responsible for the poor response of *H. canis* to chemotherapeutic agents (Ibrahim et al. 1989). It is also believed that there are no specific drug therapies for *H. canis* infection, while treatment with corticosteroids, aspirin and anti-microbial agent proved effective in clinical improvement (Craig et al. 1978). We document present communication to be a rare report on para-clinicopathologic observation(s) of insidious form of canine hepatozoonosis from a mongrel dog may be due to mixed infections. This report explains various haemato-biochemical parameters along with parasitological context of information at two stages, i.e. before and after treatment with adopted drug therapy.

Materials and methods

A male-mongrel dog aged between 6 and 7 years was presented to small animal clinics, GADVASU, Ludhiana, Punjab, India with a history of anorexia along with emaciation for the last 2 months, just prior to admission. The dog had a normal defecation interval with no changes in colour and consistency of faeces. On physical examination, the animal revealed a temperature of 100°F with thick discharge from both the eyes. There was a congestion of mucous membrane of eyes and the body coat appeared to be dull, dry and dehydrated. For parasitological and haematological observations, blood was collected in potassium salt of ethylene diamine tetra acetic acid (K^+EDTA) at 2 mg/ml and for biochemical analysis, blood was collected in a clean and sterile tube, and serum was harvested following slanting of tubes. The serum was kept at $-20^{\circ}C$, till further use. Following microscopy, the neutrophils were found to be parasitized with either non-nucleated or stained nucleated forms of *H. canis*. The relative parasitaemia was evaluated by manual counting of the number of parasitized neutrophils out of relative percentage of neutrophils counted. The dog was immediately put on Septran® (sulphatrimethoprim at 15 mg/kg BID) and Dalcin® (clindamycin at 10 mg/kg TID) for 14 days besides supportive therapy which included Ringer's lactate, dextrose normal saline, amoxirum forte and polybion injection. The various haematological parameters viz. haemoglobin (Hb), total leucocytes count (TLC) and differential leucocytes count (DLC) were estimated as per the procedure suggested by Benjamin (1985). The biochemical parameters viz. alkaline

phosphatase (ALKP), total proteins (TP), albumin, blood urea nitrogen (BUN) and creatinine were estimated as per the manufacturer's instruction provided in the kit (Merck).

Results

Interestingly, around 37% of neutrophils were found having capsule shaped *H. canis* gamonts in their cytoplasm either entangling the segmented nuclear lobe within or pressing aside to the periphery, before treatment (Fig. 1). The complete blood count revealed normocytic hypochromic anaemia and neutrophilic leucocytosis with variable counts of platelets. Table 1 explains various haemato-biochemical parameters before and after treatment with sulphatrimethoprim at 15 mg/kg BID and clindamycin at 10 mg/kg TID.

Following treatment, the haemogram of the animal revealed variable results, characterised by further decrease in Hb value with a relative increase in lymphocyte count. However, the TLC came to within normal range following therapy. The biochemical analysis showed an increase in ALKP, BUN and creatinine levels along with hyperproteinemia. The 14 days chemotherapy did not bring a respite for the dog in terms of level of parasitaemia indicating the persistence of infection in the body, which further evident by the number of affected neutrophils (33%) (Fig. 2). The ALKP and creatinine level further rose up to 287 IU/l and 8.4 mg/dl, respectively following post therapy. While slight decrease in TP level was observed after treatment with sulphadiazine and clindamycin, indicating a gradual lowering down of inflammatory process in the body following prescribed therapy.

Fig. 1 Blood smear of dog exhibiting the gamonts (arrows) of *H. canis* in neutrophils before treatment

Table 1 Haemato-biochemical parameters

Parameters (units)	Before treatment	14 days post treatment
Haemoglobin (g)	5.0	4.6
Total leukocyte count ($10^3/\mu\text{l}$)	22.2	8.7
Neutrophils (%)	78	62
Lymphocytes (%)	18	33
Eosinophils (%)	4.0	3.0
Monocytes (%)	0.0	2.0
Platelet count ($10^3/\mu\text{l}$)	280	321
Alkaline phosphatase (IU/l)	180	287
Total protein (g/dl)	10.6	8.5
Albumin (g/dl)	2.9	2.4
Blood urea nitrogen (mg/dl)	110.4	89.3
Creatinine (mg/dl)	4.0	8.4

Fig. 2 Blood smear of dog exhibiting the gamonts of *H. canis* in neutrophils following 14 days post treatment

Discussion

The study on pathogenicity of *H. canis* infection is scanty and obscure. Recently, one report explains the experimental development of *H. canis* infection in dogs (Harikrishnan et al. 2008), while Pawar and Gatne (2005) explained haematological and biochemical profiles during canine hepatozoonosis. In case of infection, the infected dogs generally do not reveal any specific clinical symptoms, while some non-specific clinical symptoms are evident like weakness, myalgia, fever and generalised wasting or weight loss (Baneth et al. 1995; Panciera et al. 1997; Voyvoda et al. 2006). In our study, almost similar symptoms like continual state of anorexia with change in condition of body coat along with progressive weakness were noted. On haematological analysis, marked decrease in Hb value was appreciated, which was evident by hypochromasia of every erythrocyte. The low Hb can be

insinuated towards either deficiency of iron or reduced synthesis of proteins due to progressive damages of hepatocytes following *H. canis* infection. The parasite, *H. canis* is believed to affect liver and spleen in a wide scale manner (Klopfer et al. 1973; Hervas et al. 1997). Further decrease in Hb value despite therapy, suggests extensive damage to liver, probably as a result of concomitant therapy of sulphatrimethoprim and clindamycin, which may have acted con-jointly to cause over functioning and simultaneous dysfunctioning of hepatocytes due to the damage caused by *H. canis*. In addition, there is also a possibility that the therapeutic regimen adopted in present case was not effective to treat the ensuing infection in the body to its fullest potency. However, decrease in TLC was observed following therapy. This decrease indicates the lowering down of inflammatory changes, which were otherwise inflicted by either *H. canis* infection or any other secondarily associated pathogen. The pathogenicity of *H. canis* in this case is questionable, because there is a chance of mixed infection, as the animal belonged to native mongrel breed. It has been observed that dogs generally acquire concurrent infection of *Ehrlichia* spp., *Anaplasma* spp., *Babesia canis*, *Bartonella vinsonii* and *Rickettsia rickettsii* following cohabitation with ticks, mice and rodents in the vicinity of the house and during frequent travel to agricultural land or bushes (Kordick et al. 1999). Unfortunately, we did not approach to delve with isolation of secondary pathogen(s) or any other associated pathogen in this case. The relative counting of parasitised neutrophils exhibited a decrease in appearance of *H. canis* following treatment; however, virtual decrease in parasitaemia was minimal. The platelet count showed variation, i.e. after treatment, thrombocytosis was evident. The variability in thrombocyte count was also observed by Pawar and Gatne (2005). The serum ALKP level was elevated following infection, which might be due to progression of schizogony (merogony) within the bone marrow and hepatocytes, besides spleen (Pawar and Gatne 2005; Harikrishnan et al. 2008). After treatment, almost 1.5 times increase in the value of ALKP was observed which is assumed to be associated with further exasperation of hepatic damage either because of combined antibiotic therapy or concurrent mixed infection. With regards to TP estimation, marked increase in the value(s) was observed, which is attributed to the setting up of inflammatory conditions, as the body intends to go for preferential use of amino acids for the synthesis of globulin, despite hepatic damage against infection. The resilient hepatic tissue has a unique potential to regenerate to support the functions, thus helping the body to recover from further damage. Following therapy, some level of decrease in TP value was observed, which supports the concept of lowering down of inflammatory conditions in the body. Even after the treatment, hypoalbuminaemia remained a consistent finding; however, a slight improvement in terms of lowered value was noted possibly due to fast regeneration of hepatocytes. The hypoalbuminaemia associated with

infection may be due to liver damage, as it is the principal site for albumin synthesis, which gets affected mostly (Pawar and Gatne 2005). The increase in BUN level in the present case also cues towards possible damage to kidney, particularly to glomeruli, which is responsible for filtration process. *H. canis* has been incriminated in causation of glomerulonephritis, as schizogony also progresses in kidney (Baneth and Weigler 1997; Harikrishnan et al. 2008), thus possibly leading to impairment of renal functions. Creatinine level also increased following infections, however, marked increase in value was observed following therapy which probably suggests a further damage to kidney due to prolonged therapy of sulpha drugs in combination. Prolonged sulpha drug therapy is responsible for kidney damage owing to its detrimental effect on tubular epithelial cells. Till date, no clear cut treatment regimen is available for *H. canis* infection or mixed infections. In the present case, after treatment, the clinical signs did not improve much and also persistent parasitaemia prevailed, possibly suggesting co-infection with some other secondary pathogen. It has been observed that during co-infection, the animal generally exhibit aberrant clinical signs and does not respond to therapy directed at a single pathogen or show delayed response with minimal recovery (Tuttle et al. 2003; Neer 2006). The present findings are in consonance with the reports of earlier worker(s) suggesting a possible case of canine hepatozoonosis with concomitant mixed infection. The observation in the study on parasitised neutrophils with *H. canis* organism revealed no deviation in ranges from the earlier reported percentages of affected circulating neutrophils (Craig et al. 1978; Elias and Homans 2008) and hence, it seems that in occasional cases, the infection can go up to 60–90% of neutrophils (Baneth et al. 1995).

To conclude present study reports haematological and biochemical changes during *H. canis* at two stages, i.e. before and after treatment and warrant further continual study to explain the best possible therapeutic combination to deal with either singly infected cases of *H. canis* or cases of mixed infection. Mixed infections are on high rise and are to be taken care primarily, while dealing with suspected natural cases of *H. canis* infection particularly in dogs belongs to mongrel category or to a pastoral owner.

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