Clinical *Hepatozoon canis* infection in a dog in Turkey

A five-year-old female dog was presented with a four-week history of inappetence, weight loss, and skin and gait abnormalities. Physical examination revealed weakness, depression, incoordination of the posterior limbs, emaciation, skin and hair coat alterations, peripheral lymphadenopathy, pale mucous membranes and fever. Laboratory analysis of samples revealed abnormalities which included anaemia, neutrophilic leucocytosis, thrombocytopenia, low serum glucose and albumin concentrations, and increased serum alkaline phosphatase activity. The diagnosis was confirmed microscopically, by demonstrating the presence of *Hepatozoon canis* gametocytes within neutrophils in Giemsa-stained peripheral blood smears. Treatment consisting of toltrazuril and a trimethoprim-sulfamethoxazole combination was effective in relieving the clinical signs and clearing the blood of *H canis* gametocytes. To the authors' knowledge, this is the first detailed clinical description of *H canis* infection in a dog in Turkey.

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INTRODUCTION

Hepatozoon canis is a tickborne protozoan parasite of leucocytes and parenchymal tissues, which has been reported in dogs and other carnivores from many regions in the world (Craig 1990, Baneth 2001). The brown dog tick (*Rhipicephalus sanguineus*) has been described as the main vector for *H canis* (Craig 1990, Baneth 2001, Baneth and others 2003), although other species may also transmit the parasite (Murata and others 1993b). In addition, vertical transmission has been documented in puppies born to infected dams (Murata and others 1993a).

H canis infection (HCI), often referred to as Old World canine hepatozoonosis, varies from being subclinical and identified incidentally in apparently healthy dogs (Murata and others 1993b, Baneth 2001) to being a severe and life-threatening clinical disease (Baneth and Weigler 1997, Macintire and others 1997, Shaw and others 2001). The most common presentation of the infection in dogs is asymptomatic to mild disease, and it is usually associated with a low level of *H canis* parasitaemia in which 1 to 5 per cent of the animal's neutrophils are infected (Baneth and Weigler 1997, Baneth and others 2003). Some dogs exhibit high parasitaemia, often approaching 100 per cent of the peripheral blood neutrophils, and severe illness characterised by fever, anorexia, weight loss, anaemia, ocular discharge, weakness of the hindlimbs and signs of chronic debilitating disease (Ezeokoli and others 1983, Barton and others 1985, Elias and Homans 1988, Craig 1990, Baneth and others 1995, Baneth and Weigler 1997, Macintire and others 1997, Gondim and others 1998, Baneth 2001). Common abnormalities found through laboratory analysis include anaemia, neutrophilic leucocytosis, hypoglycaemia, hypoalbuminaemia and increased serum alkaline phosphatase activity (Barton and others 1985, Craig 1990, Baneth and others 1995, Macintire and others 1997, Baneth 2001).

HCI is diagnosed mainly by finding *H canis* gametocytes within neutrophils and monocytes in stained blood smears, and/or by identifying a cyst-like structure containing *H canis* organisms in biopsy specimens (Elias and Homans 1988, Craig 1990, Macintire and others 1997, Baneth 2001, Baneth and others 2003).

Several antiprotozoal drugs including imidocarb, diminazene, primaquine, toltrazuril tetracyclines, trimethoprim-sulfonamide and clindamycin, either alone or in various combinations, have been used in an effort to treat HCI. However, the response has been variable (Craig 1990, Baneth and others 1995, Beaufils and others 1996, Parra and Arraga-Alvarado 1996, Baneth and Weigler 1997, Macintire and others 1997, Baneth 2001). Non-steroidal anti-inflammatory drugs, such as aspirin, phenylbutazone or flunixin meglumine, have been recommended as supportive treatment to relieve fever and signs of pain in affected dogs (Barton and others 1985, Craig 1990).

To the authors' knowledge, HCI in a domestic dog was first described in Turkey

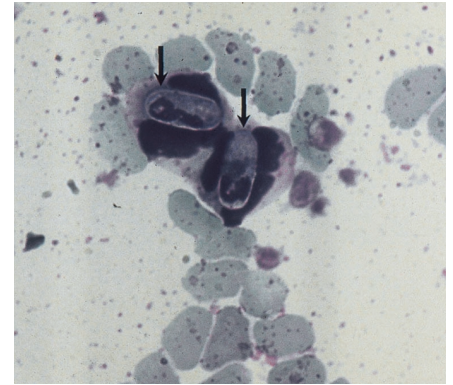FIG 1. General appearance of the dog at presentation

FIG 2. *Hepatozoon canis* gametocytes (arrows) in peripheral blood neutrophils. Giemsa stain. ×1200

in the 1930s (Nevzat 1933), but there was no discussion of clinicopathological characteristics and medical management and no further cases have been reported since. The purpose of this report is to describe the clinical and laboratory findings, and the therapy employed, in a dog with naturally occurring HCI in Turkey.

CASE HISTORY

In September 2002, a five-year-old, female mixed-breed dog weighing 10·2 kg was presented to the Adnan Menderes University Veterinary Teaching Hospital. The dog, from Aydin in western Turkey, had a four-week history of anorexia, weight loss, depression, gait abnormalities and fever after parturition. Vaccinations for canine distemper, infectious canine hepatitis, canine parvovirus, kennel cough and rabies had been performed regularly, but *Leptospira* and canine coronavirus vaccinations were incomplete. According to the owner's information, the dog had been infested with ticks during pregnancy and after parturition. One month prior to presentation to the Veterinary Teaching Hospital, the dog had been seen by a local veterinarian who diagnosed it as having scabies and tick infestation. The dog had been given 200 µg/kg avermectin (Ivomec; Topkim) administered subcutaneously and an acaricide dip (Sebacil; Bayer). There was no response to this therapy.

On the day of presentation, the dog appeared depressed and emaciated, and skin and hair coat alterations were visible (Fig 1). Physical examination also revealed weakness, incoordination of the posterior limbs, a mildly painful hindlimb, peripheral lymphadenopathy, pale mucous membranes and fever (39·6°C). An *R sanguineus* nymph and a larval tick were found on the dog. Findings on radiographic examination of the limbs were normal.

Haematological and serum biochemical evaluations were performed on the day of admission. A haemogram revealed a moderate anaemia, neutrophilic leucocytosis and thrombocytopenia (Table 1). Giemsa-stained peripheral blood smears revealed ellipsoid, elongated, pale-staining cytoplasmic bodies (mean length 11·1 µm, mean width 4·9 µm) inside neutrophils (Fig 2). These bodies were identified as

H canis gametocytes based on their morphological characteristics.

The number of neutrophils parasitised by gametocytes per µl of blood was calculated by multiplying the percentage of parasitised neutrophils counted in the blood smear by the total number of neutrophils. On the day of presentation, the dog had a parasitaemia of 21 per cent of the neutrophils with 3284 parasitised neutrophils/µl blood (Table 1). On the peripheral blood smears, there were no cells infected with other agents. Results of serum biochemical analyses indicated low glucose and albumin concentrations, and high alkaline phosphatase activity (Table 2).

Skin scrapings for scabies mites and a faecal sample for parasite ova were negative. Based on the clinical findings and laboratory results, *H canis* was diagnosed to be the primary cause.

Table 1. Results of haematological analyses performed

| Variables | Pretreatment | Days after treatment | | | Normal range* |
		10	20	30	
Haemoglobin (g/litre)	62†	106†	109†	128	120-180
Haematocrit (litre/litre)	0·20†	0·33	0·35†	0·41	0·37-0·55
Platelets (×10⁹/litre)	139†	322	299	213	150-500
Total white cells (×10⁹/litre)	18·4‡	10·2	11·6	8·0	6·0-17·0
Band neutrophils (×10⁹/litre)	0·368	0·102	0·116	0·040	0·0-0·3
Neutrophils (×10⁹/litre)	15·272‡	7·956	8·004	5·560	3·0-11·5
Lymphocytes (×10⁹/litre)	1·472	1·632	2·204	2·000	1·0-4·8
Monocytes (×10⁹/litre)	0·736	0·408	0·464	0·001	0·1-1·35
Eosinophils (×10⁹/litre)	0·552	0·102	0·812	0·400	0·1-1·25
Parasitaemia of neutrophils (%)	21	26	9	0	0
Parasitised neutrophils (µl)	3284	2095	731	0	0

*Meinkoth and Clinkenbeard (2000), †Below normal range, ‡Above normal range

Table 2. Serum biochemistry results

Variables	Pretreatment	Days after treatment			Normal range*
		10	20	30	
Urea (mmol/litre)	8·1	4·7	5·0	5·7	3·3-8·3
Creatinine (µmol/litre)	53·0	46·8	53·0	85·7	35-106
Glucose (mmol/litre)	2·3†	6·1	4·3	5·9	3·1-6·7
Total protein (g/litre)	56	69	61	78	54-75
Albumin (g/litre)	21†	31	32	32	25-45
Alkaline phosphatase (U/litre)	423‡	230‡	116‡	92	to 108
Alanine aminotransferase (U/litre)	36	36	33	39	to 55
Aspartate aminotransferase (U/litre)	14	23	19	21	to 25
Creatinine kinase (IU/litre)	70	175‡	95‡	80	to 90

*Kraft and Durr (1995), †Below normal range, ‡Above normal range

FIG 3. General appearance of the dog after treatment

The dog was hospitalised and treatment was initiated with a combination of toltrazuril (Baycox; Bayer) and trimethoprim-sulfamethoxazole (Co-Trimoxazole; Phenix). Toltrazuril was given at a dose rate of 10 mg/kg, orally once daily for five days, along with trimethoprim-sulfamethoxazole at a dose of 15 mg/kg, intravenously twice daily. B complex vitamins (Berovit B$_{12}$; Sanofi) were also administered intramuscularly for the first five days of treatment. Trimethoprim-sulfamethoxazole (Bactrim; Roche) was continued orally at the initial dose for 25 days. Toltrazuril was added orally at a dosage of 5 mg/kg for 10 days from days 20 to 30 due to incomplete clearance of *H canis* gametocytes. The dog was monitored daily by physical examination and at 10-day intervals by laboratory examination for a total of 30 days. The animal was re-examined one, two and four months after being discharged from the hospital.

The dog responded to treatment within 72 hours. Improvement in attitude and appetite was noted, and rectal temperature returned to within the normal range. Recovery continued, and on day 10, the dog was bright, more alert and eating. The animal had a haematocrit value of 0·33 litre/litre, but the percentage of parasitised neutrophils had increased to 26 per cent (Table 1). Alopecia, crusting and scaling had diminished. Cutaneous erosion and excoriation, and signs of pain and stiffness had resolved. The dog's condition continued to improve and, on day 20, the animal showed an improved tolerance to exercise and an increased appetite, but had mild anaemia and *H canis* gametocytes were still present (Table 1). Bodyweight had increased to 12·9 kg. By day 30, the dog had gained normal activity and appetite, with a bodyweight of 13·8 kg. Blood samples showed no *H canis* gametocytes, and

haematological and serum biochemical analysis indicated a return to normal ranges (Tables 1 and 2). The dog was discharged (Fig 3) and over the following four months remained clinically normal, with no *H canis* gametocytes being observed.

DISCUSSION

Canine hepatozoonosis caused by *H canis* has been reported from many geographic areas, including Africa, southern Europe, Asia and the USA (Craig 1990, Panciera and others 1997, Baneth 2001, Shaw and others 2001). Although diseases transmitted by *R sanguineus*, such as babesiosis and ehrlichiosis, occur commonly in dogs in Turkey, only one case of HCI, without clinicopathological characteristics and medical management, has been reported (Nevzat 1933). In the case reported here, clinical and laboratory findings and treatment results support the presence of HCI in the dog. This provides further documentation that *H canis* can cause primary clinical disease in dogs.

There is no agreement in the literature on the degree of pathogenicity of the organism, as *H canis* has been detected in leucocytes of clinically normal dogs in Africa (Ezeokoli and others 1983), Japan (Murata and others 1993b) and Israel (Baneth 2001). Therefore, some investigators consider *H canis* as incidental or non-pathogenic, unless the dog is affected by a concurrent disease (Ezeokoli and others 1983, Elias and Homans 1988). In contrast, Baneth and Weigler (1997) reported that parasitaemia with this agent is not a benign finding and should be addressed clinically because of the significant difference in haematological parameters and body temperature between *H canis*-positive dogs and healthy dogs. *H canis* induces a mild or non-apparent illness associated with low parasitaemia, whereas high parasitaemia results in a severe life-threatening disease (Baneth 2001, Baneth and others 2003). High parasitaemia is defined by numbers that exceed 800 gametocytes/µl

of blood, and in Israel (Elias and Homans 1988, Baneth and others 1995) and Africa (Ezeokoli and others 1988) affected dogs may have parasitaemias ranging from 20 to 90 per cent.

The majority of dogs with high *H canis* parasitaemia show emaciation, lethargy and decreased appetite or anorexia, and are febrile and anaemic (Baneth and others 1995, Baneth and Weigler 1997). On the day of presentation, the dog described here had a parasitaemia of 21 per cent of the neutrophils with 3284 parasitised neutrophils/µl of blood, and clinical signs were similar to those reported for dogs with HCI by other investigators (Craig 1990, Baneth and Weigler 1997, Macintire and others 1997, Panciera and others 1997, Gondim and others 1998), except for a bloody diarrhoea. Bloody diarrhoea was reported to have affected half of the dogs examined by Craig (1990), but was not detected in the case reported here.

Skin and hair coat abnormalities were observed in this case. To the authors' knowledge, skin and hair coat alterations have not been reported to be associated with HCI. The skin and hair coat abnormalities described in this report may have resulted from anorexia and cachexia. The high level of parasitaemia in HCI reflects the large number of tissue meronts, and takes its toll on the host by demanding nutrients and causing direct injury to affected tissues, leading to extreme weight loss and cachexia (Baneth and others 2003). As previously described (Hoskins 1991), the fever and pain found in this case may be a consequence of inflammatory cell infiltration of muscles that occurs when merozoites are released from schizonts.

Laboratory abnormalities in the dog reported here include anaemia, neutrophilic leucocytosis, thrombocytopenia, low serum glucose concentration, hypoalbuminaemia and increased serum alkaline phosphatase activity. These findings are consistent with those previously reported (Barton and others 1985, Elias and Homans 1988, Craig 1990, Baneth and Weigler 1997, Macintire and others

1997, Panciera and others 1997, Gondim and others 1998). The neutrophilia and anaemia observed in the case reported here are presumably secondary to necrosis and inflammation in the spleen, lymph nodes, liver and lungs (Gaunt 2000). The mechanism responsible for thrombocytopenia associated with HCI is not understood. A mild thrombocytopenia in the case discussed here may be a result of general causes of thrombocytopenia, either in combination or alone (Rich and Coles 1995). The decreased glucose concentration in serum may be explained by the effect of neutrophilia on glucose metabolism from the time of collection until the time of serum separation (Craig 1990, Macintire and others 1997). Hypoalbuminaemia in combination with normal globulin concentrations occurs secondarily to selective loss of albumin, sequestration into extravascular spaces or decreased production (Kraft and Durr 1995, Rich and Coles 1995). The hypoalbuminaemia detected at presentation of this dog probably reflects decreased production or chronic loss of protein. Increased serum alkaline phosphatase activity may reflect increased osteoblastic activity or hepatic lesions (Kraft and Durr 1995, Rich and Coles 1995). The size, shape and staining characteristics of the gametocytes found inside the neutrophils of the dog are in agreement with reports by other investigators (Craig 1990, Baneth and others 2003).

Dogs with naturally occurring HCI often have concomitant infections of bacterial or viral origin, that potentially weaken their immune defences, including canine monocytic ehrlichiosis, canine distemper and canine parvovirus infection (Elias and Homans 1988, Baneth and others 1995, Baneth and Weigler 1997, Baneth 2001). Although the initial condition described in the current case is similar to that seen in some cases of canine monocytic ehrlichiosis, co-infection with *Ehrlichia canis* can be ruled out since no *E canis* morulae were found on examination of blood smears. Furthermore, drugs with

known efficacy against *E canis* do not include thrimethoprim-sulfamethoxazole and toltrazuril, which were used with apparent success in this case. The initial clinical signs were most likely not associated with canine distemper or canine parvovirus infection because the dog had been regularly vaccinated against these infections. It is possible that leptospirosis or canine coronavirus infection may have played a role in the initial condition of the dog because of its incomplete vaccination status. However, initial clinical signs did not resemble the clinical presentation of these infections. For these reasons, the current case was diagnosed as hepatozoonosis with apparently no interference from bacterial or viral pathogenic agents.

The response to treatment of dogs infected with *H canis* is inconsistent. Some dogs respond to treatment while others develop fatal complications (Craig 1990, Baneth and others 1995, Beaufils and others 1996, Parra and Arraga-Alvarado 1996). Imidocarb seems to be the drug of choice for dogs with HCI (Baneth and Weigler 1997, Baneth 2001, Shaw and others 2001), but is currently not available for clinical use in Turkey. Therefore, other treatment options must be considered. The dog in this report was treated with a combination of toltrazuril and trimethoprim-sulfamethoxazole. The clinical recovery and disappearance of gametocytes from peripheral blood may have been directly attributable to treatment with these drugs because the dog had not apparently been affected by any concurrent disease.

Toltrazuril is an anticoccidial agent efficacious against nearly all coccidia of domestic and laboratory animals. It has been reported that toltrazuril prevented death in voles with experimentally induced hepatozoonosis, but did not completely eliminate the parasite (Krampitz and Haberkorn 1988). Regression of clinical and biological profiles (Beaufils and others 1996) and remission of clinical signs for several months (Macintire and others 1997) in dogs with HCI has also been achieved using toltrazuril. Treatment with

trimethoprim-sulfamethoxazole also was found to be effective in dogs with HCI (Parra and Arraga-Alvarado 1996). Interestingly, the percentage of parasitised neutrophils increased to 26 per cent at day 10 of treatment despite there being an observed improvement in clinical condition. This finding was also described by Beaufils and others (1996).

Conclusions

Incidental detection of the parasite in blood smears in subclinical infections and concurrent diseases in clinical cases may have confused the pathogenic importance of *H canis*. The naturally acquired case of HCI described in this report demonstrates that *H canis* can induce primary clinical disease in dogs. *H canis* should therefore be considered as a potential pathogen in dogs with typical clinical signs. Treatment consisting of a combination of toltrazuril and a trimethoprim-sulfamethoxazole should be considered effective in relieving the clinical signs and clearing the blood of *H canis* gametocytes.

References

BANETH, G. (2001) Canine hepatozoonosis. In: Encyclopedia of Arthropod-transmitted Infections of Man and Domesticated Animals. Ed M. W. Service. CABI Publishing, Wallingford. pp 215-220

BANETH, G., HARMELIN, A. & PRESENTEZ, B. Z. (1995) *Hepatozoon canis* in two dogs. *Journal of the American Veterinary Medical Association* **206**, 1891-1894

BANETH, G., MATHEW, J. S., SHKAP, V., MACINTIRE, D. K., BARTA, J. R. & EWING, S. A. (2003) Canine hepatozoonosis: two disease syndromes caused by separate *Hepatozoon* species. *Trends in Parasitology* **19**, 27-31

BANETH, G. & WEIGLER, B. (1997) Retrospective case-control study of hepatozoonosis in dogs in Israel. *Journal of Veterinary Internal Medicine* **11**, 365-370

BARTON, C. L., RUSSO, E. A., CRAIG, T. M. & GREEN, R. W. (1985) Canine hepatozoonosis: a retrospective study of 15 naturally occurring cases. *Journal of the American Animal Hospital Association* **21**, 125-134

BEAUFILS, J. P., MARTIN-GRANEL, J. & JUMELLE, P. (1996) Hepatozoonosis in dogs and foxes: epidemiology, clinical findings and treatment. *Pratique Medicale and Chirurgicale de l'Animal de Compagnie* **31**, 243-253

CRAIG, T. M. (1990) Hepatozoonosis. In: Infectious Diseases of the Dog and Cat. 2nd edn. Ed C. Greene. W. B. Saunders, Philadelphia. pp 778-785

ELIAS, E. & HOMANS, P. A. (1988) *Hepatozoon canis* infection in dogs: clinical and haematological findings; treatment. *Journal of Small Animal Practice* **29**, 55-62

EZEOKOLI, C. D., OGUNKOYA, A. B., ABDULLAHI, R., TEKDEK, L. B., SANNUSI, A. & ILEMOBADE, A. A. (1983) Clinical and epidemiological studies on canine hepatozoonosis in Zaire, Nigeria. *Journal of Small Animal Practice* **24**, 445-460

GAUNT, S. D. (2000) Extreme neutrophilic leukocytosis. In: Schalm's Veterinary Hematology. 5th edn. Eds B. F. Feldman, J. G. Zinkel and N. C. Jain. Lippincott, Williams & Wilkins, Baltimore. pp 347-349

GONDIM, L. F. P., KONAYAGAWA, A., ALENCAR, N. X., BIONDO, A. W., TAKAHIRA, R. F. & FRANCO, S. R. V. (1998) Canine hepatozoonosis in Brazil: description of eight naturally occurring cases. *Veterinary Parasitology* **74**, 319-323

HOSKINS, J. D. (1991) Canine haemobartonellosis, canine hepatozoonosis, and feline cytauxzoonosis. *Veterinary Clinics of North America: Small Animal Practice* **21**, 129-140

KRAFT, W. & DURR, U. M. (1995) Klinische Labordiagnostik in der Tiermedizin. 3rd edn. Eds. W. Kraft and U. M. Durr. Schattauer, Stuttgart

KRAMPITZ, H. E. & HABERKORN, A. (1988) Experimental treatment of *Hepatozoon* infections with the anticoccidial agent toltrazuril. *Journal of Veterinary Medicine Series B* **35**, 131-137

MACINTIRE, D. K., VINCENT JOHNSON, N., DILLON, A. R., BLAGBURN, B., LINDSAY, D., WHITLEY, E. M. & BANFIELD, C. (1997) Hepatozoonosis in dogs: 22 cases (1989-1994). *Journal of the American Veterinary Medical Association* **210**, 916-922

MEINKOTH, J. H. & CLINKENBEARD, K. D. (2000) Normal haematology of the dog. In: Schalm's Veterinary Haematology. 5th edn. Eds B. F. Feldman, J. G. Zinkel and N. C. Jain. Lippincott, Williams & Wilkins, Baltimore. pp 1057-1063

MURATA, T., INOUE, M., TATEYAMA, S., TAURA, Y. & NAKAMA, S. (1993a) Vertical transmission of *Hepatozoon canis* in dogs. *Journal of Veterinary Medical Science* **55**, 867-868

MURATA, T., SHIMODA, K., INOUE, M., SHIRAMIZU, K., KANOE, M., TAURA, Y. & NAKAMA, S. (1993b) Seasonal periodical appearance of *Hepatozoon canis* gamont in the peripheral blood. *Journal of Veterinary Medical Science* **55**, 877-879

NEVZAT, A. (1933) Bizde ilk defa görülen bik (*Hepatozoon canis*) vak'ası. *Türk Baytarlar Cemiyeti Mecmuası* **3**, 5

PANCIERA, R. J., GATTO, N. T., CRYSTAL, M. A., HELMAN, R. G. & ELY, R. W. (1997) Canine hepatozoonosis in Oklahoma. *Journal of the American Animal Hospital Association* **33**, 221-225

PARRA, M. O. C. & ARRAGA-ALVARADO, C. M. (1996) Canine hepatozoonosis in Venezuela. Clinical and laboratory findings. Revista Cientifica, Facultad de Ciencias Veterinarias Universidad del Zulia **6**, 125-133

RICH, L. J. & COLES, E. H. (1995) Tables of abnormal values as a guide to disease syndromes. In: Textbook of Veterinary Internal Medicine. 4th edn. Eds S. J. Ettinger and E. C. Feldman. W. B. Saunders, Philadelphia. p 14

SHAW, S. E., DAY, M. J., BIRTLES, R. J. & BREITSCHWERDT, E. B. (2001) Tick-borne infectious diseases of dogs. *Trends in Parasitology* **17**, 74-80