Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 03, pp.14052-14056, March, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

SEQUENTIAL BABESIOSIS AND EHRLICHIOSIS IN A DOG WITH CANINE DISTEMPER- A RETROSPECTIVE ANALYSIS

***,[1]Kanta Bhattacharjee, [1]Prabhat Chandra Sarmah, [1]Dipak Kumar Sarma, [1]Parikshit Kakati, [1]Debesh Chandra Pathak and [2]Ramgopal Laha**

[1]Department of Parasitology, College of Veterinary Science, Assam Agricultural University, Guwahati-781022, Assam, India
[2]Division of Animal Health, ICAR Research Complex for NEH Region, Umiam-793103, Meghalaya, India

ARTICLE INFO

Article History:
Received 04th December, 2014
Received in revised form
06th January, 2015
Accepted 18th February, 2015
Published online 31st March, 2015

Key words:

Babesia gibsoni,
Babesia canis,
Ehrlichia canis,
Anaplasma platys,
Dog.

ABSTRACT

A female adult Labrador dog adequately vaccinated and trained in explosive detection suddenly developed nervine symptoms characterized by foreleg paresis and lateral recumbency in about 20 days of sickness which started with fever, inappetence and depression. Examination of giemsa stained blood smear revealed presence of *Babesia gibsoni* and PCR analysis showed presence of *B. canis* in addition to *B. gibsoni*. Antibabesial treatment first with Clindamycin hydrochloride, Quinine sulphate upon microscopic detection of *B. gibsoni* followed by diminazene diaceturate and imidocarb dipropionate against *B. canis* detected in Polymerase Chain Reaction resulted clinical, parasitological and haematological improvement with gradual correction of the gait due to knuckling and impaired motor function in the left foreleg. Ten months later, oxytetracycline and doxycycline treatment consequent upon detection of inclusions indistinguishable from *Ehrlichia canis* and *Anaplasma platys* during microscopic examination of blood of the animal with symptoms of anorexia, hemiplegia and absence of pedal reflex also resulted in clinical and haematological improvement as before. However, in another 3 months, the animal developed incoordination, circling movement, convulsion, chorea and coma leading to death. Post mortem examination revealed haemorrhages in many organs. Histopathological examination showed intranuclear and intracytoplasmic acidophilic inclusions of canine distemper in the epithelial cells of the urinary bladder, bronchioles and stomach. The present analysis discusses vaccine failure or infections with haemoparasites precipitating vaccine induced canine distemper as the cause of death.

INTRODUCTION

Dogs are known to suffer from infections due to bacterial, viral, rickettsial, fungal pathogens and parasites which can cause diseases, some of them being serious and life threatening. The commonly associated serious diseases include leptospirosis, rabies, parvo, canine distemper, canine hepatitis, babesiosis and ehrlichiosis. Many of them alone or in combination produce common manifestations which can mimic the other making the clinical diagnosis difficult. There have been considerable literature describing several case reports in dog with multiple blood parasitic infections due to *Babesia, Ehrlichia, Hepatozoon, Trypanosoma* and *Dirofilaria* (Alan *et al*., 1991; Harikrishnan *et al*., 2005; Banerjee *et al*., 2008). However, information on parasites causing disease in association with viral pathogen is scanty. The present communication reports the retrospective analysis of a cerebral form of babesiosis followed by ehrlichiosis and their successful treatments in a dog during about 14 months of day care/ domiciliary observation which ended with fatal canine distemper.

Corresponding author: Kanta Bhattacharjee
Department of Parasitology, College of Veterinary Science, Assam Agricultural University, Guwahati-781022, Assam, India.

MATERIALS AND METHODS

(a)Case history: A 21-months old female Labrador having trained in explosive detection and attached to a paramilitary force posted at Itanagar, Arunachal Pradesh in north east region of India was referred to the Teaching Veterinary Clinical Complex of the College of Veterinary Science, Guwahati, Assam with neurological manifestations. The dog received regular vaccinations against canine distemper, canine hepatitis, parvo and rabies virus and had no history of serious illness since birth. Soon after 3 months of stay with normal day to day outdoor activities in and around the place of posting, the animal showed illness with fever, anorexia and depression for which treatment with antipyretic and antibiotics was provided locally for 20 days without any fruitful result. The condition of the animal worsened with sudden development of central nervous system derangement over the 24 hours prior to

emergency shifting to the clinics. The case of present analysis was one of the 88 working dogs owned by security agencies examined for haemoparasite prevalence study (Bhattacharjee, 2011).

(b) Clinico-pathological study and treatment

First attack: Physical examination of the dog at its first presentation (Fig 1) revealed lateral recumbency, inability to stand due to forelimb paresis, paddling with hind legs, elevated body temperature (103ºF), tachypnoea, tachycardia and dehydration. Laboratory investigations included examination of giemsa stained blood smear for the presence of haemoparasites and cytological abnormality, estimation of haemoglobin (Hb), packed cell volume (PCV), total leucocyte count (TLC), differential leucocyte count (DLC) in EDTA anticoagulated blood by standard laboratory techniques (Schalm *et al*., 1975). Polymerase Chain Reaction (PCR) was conducted as an additional confirmative method for the presence of *Babesia* organisms. Enzyme linked immunosorbant assay (ELISA) based commercial kit "SNAP 3Dx" (IDEXX, USA) was employed for serological detection of *Ehrlichia canis* antibody and heartworm (*Dirofilaria immitis*) antigen. Following detection of *Babesia gibsoni* in blood smear (Fig 2), the animal was treated with Clindamycin hydrochloride (@ 10 mg/kg b.wt i/v 12 hrly for 15 days) and Quinine sulphate (@ 10 mg/kg b.wt i/v 12 hrly for 7 days) along with intravenous fluid and supportive therapy. The animal was also treated with Diminazene diaceturate (@ 3.5mg/kg i/m) and imidocarb dipropionate (@ 6.6 mg/kg b.wt s/c in 2 doses at 15 days interval) following detection of *B. canis* in addition to *B. gibsoni* in PCR.

Fig 1. Dog showing lateral recumbency

Second attack: Amidst periodic checkup and 10 months after getting fully recovered from *Babesia* infection, the dog at 31 months age suffered a second attack characterized by hemiplegia and left sided recumbency, off fed, high fever, hard foot pad, dry muzzle and hyperkeratinization of eyelid. Parasitological, haematological and serological tests were conducted as before. Following detection of intracytoplasmic inclusions indistinguishable from the morula of *Ehrlichia canis* in lymphocytes and monocytes (Fig 3) and *Anaplasma platys* in the platelets (Fig 4), the animal was treated with oxytetracycline (@ 20 mg/kg b.wt i/v 12 hrly for 5 days) followed by Doxycycline (@ 10 mg/kg b.wt orally twice daily for 6 weeks) along with supportive fluid therapy for a week.

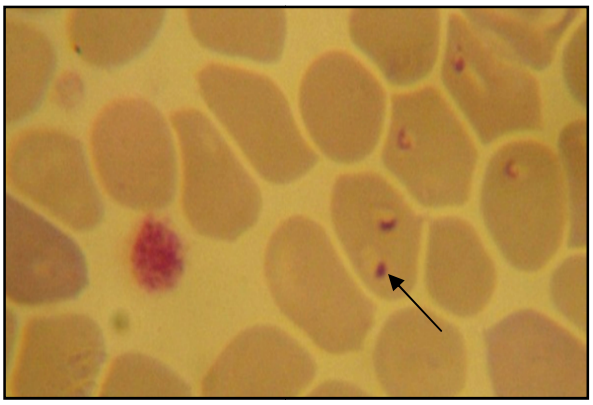
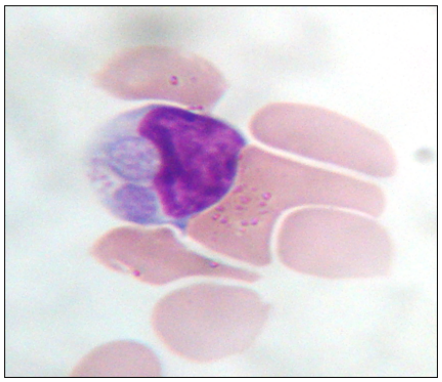


Fig. 2. *Babesia gibsoni* inside erythrocytes



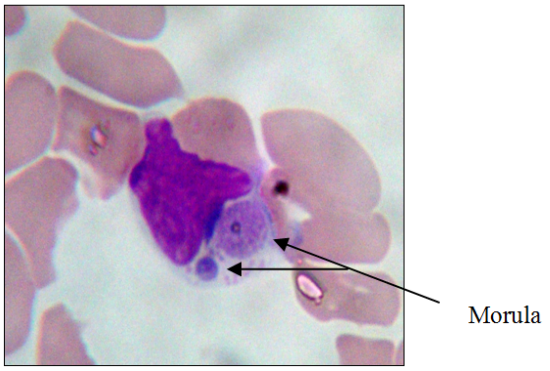


Fig. 3. *Ehrlichia canis* morula (2 nos) inside monocytes

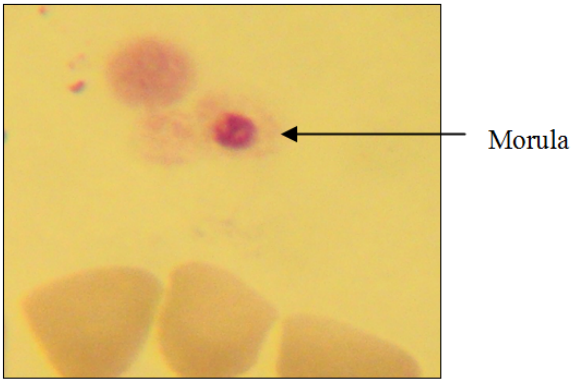


Fig. 4. *Anaplasma* (*Ehrlichia*) *platys* inside platelet

Third attack: Three months after the second attack the animal was again presented with additional symptoms like frequent vomiting, anorexia, inability to swallow food, facial paralysis, biphagic body temperature variable between 99°F and 106°F during morning and evening, hemiplegia with right sided recumbency, hard footpad, dry muzzle, champing of jaws and chorea of head and neck extending posteriorly. After laboratory investigations the animal was treated with antibiotics, antiemetic, H2 blocker in addition to intravenous fluid therapy. The condition of the animal gradually worsened and proved fatal within 15 days of third attack. Gross lesions were studied at post mortem and tissues were processed for routine histopathological study.

(c) Response to therapy: Therapeutic response was assessed by comparing the parasitological, video recorded clinical conditions and haematological observations made before and after institution of treatments.

RESULTS AND DISCUSSION

Results of parasitological, haematological and serological investigations conducted during the whole period of observation are summarized in Table-1.

Table 1. Laboratory findings before and after treatment of a dog with babesiosis followed by ehrlichiosis and canine distemper during 14 months of observation

Parameter	Observation period							
	1st attack				2nd attack			3rd attack*
	Day 1 (26.8.2010)	Day 45 (9.10.10)	Day 57 (21.10.10)	Day 246 (28.4.11)	Day 315 (6.7.11)	Day 360 (20.8.11)	Day 399 (28.9.11)	Day 408 (7.10.11)
A. Parasitological								
i) Blood smear	*Babesia* (F)	*Babesia* (R)	*Babesia* (R)	-ve	*E.canis,E.platys*	-ve	-ve	-ve
ii) PCR for *B. canis* & *B.gibsoni*	+ve	N.D	+ve	N.D	-ve	-ve	N.D	N.D
iii) Snap 3Dx for *Ehrlichia* Ab & *Dirofilaria* Ag	-ve	N.D	N.D	N.D	-ve	N.D	N.D	-ve
B. Haematological (Ref range)								
i) Hb (12-15g%)								
ii) PCV (37-55%)	9.0	10.2	7.0	12.0	8.0	14.0	14.0	14.0
iii) TLC (6-17x10^3/mm^3)	33.0	33.5	23.0	38.0	27.0	45.0	44.0	44.5
iv) DLC: N (62-80%)	14.0	10.0	12.0	10.6	5.0	8.0	7.0	4.5
v) L (10-28%)	78	52	58	62	46	72	68	72
vi) M (3-9%)	18	38	35	33	48	22	24	18
vii) E (2-9%)	1	7	6	4	2	1	4	10
viii) Nucleated RBC	3	3	1	1	4	5	4	-
ix) Howell jolly body	+	+	+	-	-	-	-	-
x) Polychromasia	+	+	+	-	-	-	-	-
	+	+	+	-	-	-	-	-
C. Specific treatments	Clindamycin hydrochloride, Quinine sulphate		Diminazene diaceturate, Imidocarb dipropionate		Oxytetracycline, Doxycycline			

F- frequent parasitaemia with small *Babesia*; R- detectable but rare parasitaemia N.D- Not done; Clindamycin hydrochloride (Clincin) from 27.8.10, Quinine sulphate (Qinarsol) from 2.9.10, Diminazene diaceturate (Nilbery) from 26.10.10, Imidocarb dipropionate (Imizol) from 18.11.10.
* Started on 30.9.11 and the animal died within 15 days, i.e. on 415th day (14.10.11) of observation.

Detection of multiple intraerythrocytic dot with vacuole, ring and oval shaped small forms of *Babesia* indistinguishable from *B. gibsoni* commensurated with the haematological abnormalities which included low Hb and PCV values and neutrophilic leucocytosis. Cytological evaluation in blood smear also showed presence of nucleated red blood cells, howell jolly bodies and polychromasia which indicated regenerative type of anaemia. Present findings are in conformity with the reports made by Varshney *et al*. (2003) and Ayoob *et al*. (2010). Clindamycin and quinine sulphate treatment resulted progressive improvement in physical condition within 48 hours. Body temperature declined towards normal and lateral recumbency improved to sternal recumbency with regaining of sensation in the forelegs. The animal started showing interest in food and water and attempted to bear weight on its forelegs within 96 hours of treatment, could stand and sit on its own and follow the commands of the owner within 20 days. After 45 days of treatment the animal was seen playful but with a persistent complain of knuckling in the left foreleg. Inspite of clinical recovery with 2.5 kg body weight gain, the Hb value was found increased marginally by only 1.2 g% from the pretreatment value. Further treatment at this stage with diminazene diaceturate and imidocarb dipropionate on the basis of detection of *B. canis* in addition to *B. gibsoni* in PCR yielded increase of Hb level to 12g%.

Cerebral babesiosis, an unusual but one of the major complications of *Babesia* infection associated with high mortality has been reported by several workers (Jacobson and Clark, 1994; Vande Maele *et al*., 2008). Neurological condition of this animal associated with presence of parasites was suggestive of babesiosis in which immediate treatment contributed clinical improvement within 48 hours similar to earlier reports (Boozer and Macintire, 2003).

Adoption of combined treatment yielded excellent result in clinical and haematological recovery which are in accordance with the reports of Vial and Gorenflot (2006), Van de Maele *et al*. (2008), Ayoob *et al*. (2010) and relapse of *Babesia* infection was not seen thereafter. However during second attack 10 months after, examination of blood revealed non-regenerative anaemia with neutropenic leucopenia and relative lymphocytosis. These findings accompanied by detection of ehrlichial inclusions and seronegative result in SNAP 3Dx test were suggestive of acute ehrlichiosis which are in agreement with Mylonakis *et al.* (2003) and Iqbal *et al.* (2010). The

animal also responded well to oxytetracycline and doxycycline therapy as reported by earlier workers (Greene and Harvey, 1984; Alan *et al*., 1991). Dog's mentation due to CNS derangement which are not uncommon in ehrlichiosis (Ewing and Buckner, 1965, Alan *et al*, 1991) returned to normal enabling to take food and water within 7 days of treatment and the animal fully regained its health with Hb and PCV values reaching 14 g% and 45% respectively within 45 days of treatment. In another 3 months, the animal presented more severe neurological symptoms and showed leucopenia (lymphopenia) although Hb and PCV values remained unchanged. Inspite of intravenous fluid, antibiotic and symptomatic supportive therapy, the condition finally proved fatal within 15 days. Post mortem examination revealed haemorrhage and congestion in visceral organs including brain and ulcerative lesions in the stomach and small intestine. Presence of intranuclear and intracytoplasmic inclusion bodies in the epithelial cells of the urinary bladder, bronchioles and stomach in histopathological examination confirmed canine distemper as the underlying cause of death. The present findings are in agreement with Machida *et al*. (1993) and Beineke *et al*. (2009) who also reported among others demyelinating encephalitis resulting muscle incoordination, muscle spasm, partial to complete paralysis, deterioration of mental status, lymphoid depletion causing immunosuppression and necrotizing gastroenteritis as the striking pathological features of canine distemper. Although the animal under report was vaccinated against canine distemper initially with Nobivac puppy DP (Intervet) as per standard protocol and thereafter with Megavac-6 (Indian Immunologicals Ltd) at 1 year age, the virus ultimately proved fatal which might be due to vaccine failure as reported earlier by Carpenter *et al*. (1976) or the intercurrent infections like *Babesia* and *Ehrlichia* precipitating vaccine induced distemper (Povey, 1986). Nervine symptoms observed by other workers in canine babesiosis (Varshney *et al*., 2003; Bourdoiseau, 2006; Jacobson, 2006; Choudhuri *et al*., 2009), ehrlichiosis (Kumar and Varshney, 2007) and canine distemper (Beineke *et al*, 2009) were of similar nature. Habus *et al*. (2010) reported a case of cerebral babesiosis due to *B. canis* with concurrent infection of rabies virus that proved fatal in a dog with unknown vaccinal status. We report cerebral babesiosis followed by ehrlichiosis and canine distemper with presentation of overlapping nervine symptoms which also made the clinical diagnosis extremely difficult. Although laboratory diagnosis followed by specific treatments saved the animal from the earlier two infections the latter ultimately proved fatal.

Acknowledgement

The authors are thankful to the Dean and Director of Clinics, Faculty of Veterinary Science, AAU, Guwahati-781022 for facilities provided.

REFERENCES

Alan, R.K., Laurie, E.D. and Christine, A.G. 1991. Concurrent ehrlichiosis and babesiosis in a dog. *Can. Vet. J.,* 32: 305-307.

Ayoob, A.L., Hackner, S.G. and Prattle, J. 2010. Clinical management of canine babesiosis. *J. Vet. Emerg Crit Care.,* 20(1): 77–89

Banerjee, P.S., Mylonakis, M.E., Garg, R., Vatsya, S. and Yadav, C.L. 2008. Concurrent hepatozoonosis, monocytic and granulocytic ehrlichiosis in a dog. *J. Vet. Parasitol*., 22: 9-11

Beineke, A., Puff, C., Seehusen, F. and Baumqartner, W. 2009. Pathogenesis and immunopathology of systemic and nervine canine distemper. *Vet. Immunol. Immunopathol.,* 127: 1-18

Bhattacharjee, K. 2011. Studies on haemoparasitic infections of dog with special reference to *Dirofilaria immitis*. PhD thesis submitted to Assam Agricultural University, 165 pp.

Boozer, A. L. and Macintire, D.K. 2003. Canine babesiosis. Vet. Clin. North Am. *Small Anim. Pract.,* 33: 885-904

Bourdoiseau, G. 2006. Canine babesiosis in France. *Vet. Parasitol.,* 138: 118-125

Carpenter, J.W., Appel, M.J., Erickson, R.C. and Novilla, M.N. 1976. Fatal vaccine induced canine distemper virus infection in black-footed ferrets. *J. Am. Vet. Med. Assoc.,* 169: 961-964

Choudhuri, S., Changkija, B. and Varshney, J.P. 2009. Cerecral babesiosis in dogs- a report. Indian J. Vet. Med., 29: 60-61

Ewing, S.A. and Buckner, R.G. 1965. Manifestations of Babesiosis, ehrlichiosis and combined infections in the dog. *An. J. Vet. Res.,* 26: 815-828

Greene, C.E. and Harvey, J.W. 1984. Canine ehrlichiosis. In: Clinical Microbiology and infectious disease of dog and cat. W.B. Saunders Co., *Philadelphia.* pp 545-561

Habus, J., Stritof, Z., Stevanovic, V., Martinkovic, K., Milas, Z., Staresina, V. and Turk, N. 2010. Dual infection of rabies virus and *Babesia canis* in a dog: a case report. *Vet. Med.,* 55: 294-296

Harikrishnan, J.T., Pazhanivel, N. and Chellapa, J. 2005. Concomitant *Babesia gibsoni* and *Ehrlichia canis* infection in a dog. *Vet. Archiv.,* 75: 513-520

Iqbal, A., Tripathi, A.K. and Agarwal, R. 2010. Canine ehrlichiosis: An overview. Pet India., 5:20-23

Jacobson, L.S. 2006. The South African form of severe and complicated canine babesiosis: clinical advances 1994-2004. *Vet. Parasitol.,* 138: 126-139

Jacobson, L.S. and Clark, I.A. 1994. The pathophysiology of canine babesiosis: new approaches to an old puzzle. *J. S. Afr. Vet. Assoc.,* 65: 134-145

Kumar, A. and Varshney, J.P. 2007. Clinico-haematological and biochemical investigations in dogs concurrently infected with *Anaplasma platys* and other blood parasites. *J. Vet. Parasitol.,* 21: 43-45

Machida, N., Kiryu, K.O., Kanda, E., Izumisawa, N. and Nakamura, T. 1993. Pathology and epidemiology of canine distemper in raccoon dogs (*Nyctereutes procyonoides*). *J. Comp. Pathol.,* 108: 383-392

Mylonakis, M.E., Koutinas', A.F., Billinis, C., Leontides, L.S., Kontos, V., Papadopoulos, O., Rallis, T. and Fytianou, A. 2003. Evaluation of cytology in the diagnosis of acute canine monocytic ehrlichiosis (*Ehrlichia canis*): a comparison between five methods. *Vet. Microbiol.,* 91:197-204

Povey, R.C. 1986. Distemper vaccination of dogs: factors which could cause vaccine failure. *Can. Vet. J.,* 27: 321-323

Schalm, O.W., Jain, N.C. and Carrol, E.J. 1975. Veterinary haematology, 3rd Edition, Lea and Febiger, Philadelphia, USA. 807 pp

Van de Maele, I., Savary-Bataille, K., Gielen, I. and Daminet, S. 2008. An unusual form of canine babesiosis. *Can. Vet. J.,* 49: 283-286.

Varshney, J.P., Varshney, V.P. and Hoque, M. 2003. Clinico-haematological, Biochemical, endocrinological and ultrasonographic findings in canine babesiosis. *Indian J. Anim. Sci.,* 73: 1099-1101

Vial, H.J. and Gorenflot, A. 2006. Chemotherapy against babesiosis. *Vet. Parasitol.,* 138 (1-2): 147-160

****