CEREBRAL BABESIOSIS IN A RIVERINE BUFFALO (*Bubalus bubalis*) AND ITS SUCCESSFUL THERAPEUTIC MANAGEMENT

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ABSTRACT

Babesiosis has long been recognized as persistent major constraint restricting the cross border movement and export of highyielding buffalo breeds from their native homelands to the advanced countries. The disease accounts for high mortality, poor growth and lower productivity of the bubaline host. A she Murrah buffalo, aged 2 years and weighing approximately 300 kg, owned by a person from a weaker section of the society, suffered from critically high temperature (110^{o}F), ruminal hypotonocity (1/3 minutes), anorexia, aggressiveness, grinding of teeth, corneal opacity, cessation of defaecation, abducted hind limbs, icterus, and mild anaemia. She was observed repeatedly making to and fro movements. Clinical and haematological findings coupled with the presence of *Babesia bovis* piroplasms in the circulating erythrocytes made us infer that the animal was suffering from the cerebral form of babesiosis. The buffalo promptly responded the specific therapy and successfully restored normal haematological indices and erythrocytes free from the piroplasms. Differential diagnosis of "cerebral babesiosis" *vis-à-vis* bovine diseases exhibiting analogous neurological signs has been discussed. This seems to be the pioneer documentation of "cerebral babesiois" in a buffalo from the semi-arid enzootic areas of the Indian subcontinent.

Keywords: Murrah buffaloes, cerebral babesiosis, *Babesia bovis*, nervous signs, diminazine aceturate

INTRODUCTION

The buffalo -the incredible Asian dairy animal, popularly known as the "Black Diamond" -plays a versatile role in the socio-economic uplift of its owners from the rural agricultural communities. It is the largest highenergy milk and lean meat producer in India (Gupta and Singh, 2002). Amongst a few parasitic diseases affecting growth, development and productivity of the dairy animal, bovine babesiosis caused by six species of the pathogen, has since long being recognized as an economically important disease of the wild as well as the domesticated buffalo population in the

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tropics and subtropics (Ristic, 1988; Roberts *et al.,* 1998; Aiello and Mays, 1998 and Lefevre *et al.,* 2010). The disease is characterized by exceptionally high pyrexia, extensive erythrolysis leading to fast-developing anaemia, icterus, haemoglobinurea and eventual death of the host (Wright, 1972; Urquhart *et al.,* 2003; Soulsby, 2005; Taylor *et al.,* 2007 and Lefevre *et al.,* 2010). The prevailing epizootiological determinants on the Indian subcontinent offer the most favored and optimum opportunity for faster propagation of the acarine intermediate host (*Boophilus microplus*) and *in situ* development of the pathogen- an apicomplexan parasite restricting the cross border movement of buffaloes and export of high yielding buffalo breeds from native home land to the advanced countries. The authors have come across an interesting and unusual observation about the pathogen associated with the cerebral form of the disease involving central nervous system (CNS), "cerebral babesiosis", in buffalo. This is described herein.

MATERIALS AND METHODS

A she Murrah buffalo, aged 2 years and weighing approximately 300 kg, was presented on the 8th of July, 2011, before the clinicians at the Teaching Veterinary Clinical Complex, Apollo College of Veterinary Medicine, Jaipur. The cow was reported having suffered from high temperature and subsequent anorexia for the previous 10 days. Further enquiry revealed that the animal had been administered systemic antibiotics, antipyretics and appetizers by a field veterinarian but to no eventual success for previous 7 days. The owner belonged to a socio-economically weaker section of the Indian society, with meager family income from sale of milk and agricultural labour. The management of the buffalo shed, housing, sanitation, etc. were not in absolute conformity with scientific standards. These were reported to be of typical traditional type, suited for small animal holdings of three to five animals.

A closer look at the animal revealed severe dyspnoea, frothy nasal discharge, lacrymation, grinding of teeth, and abducting hind limbs, and it was standing in a typical posture with arched back and head extended in the forward direction. There was no haemoglobinurea. The eyes were slightly opaque suggestive of progressively developing corneal opacity. Ophthalmic examinations of the eyes in respect of symmetry, confirmation were carried out from 2-3 feet distance with the least head restraint. In absence of tonometric and ophthalmic equipmental facilities, ocular pressure was roughly assessed by application of gentle pressure on the orbits. The ruminal motility of the animal was assessed by pressing the fist in the left paralumbar fossa. Peripheral blood (5 ml aliquot in EDTA) was aseptically collected and the body hair coat was carefully searched for acarine parasites. Rectal coprological samples were also collected. The samples were brought to the laboratory for identification of the pathogen(s) and its vectors using standard keys/ techniques (Bowmann *et al.,* 2003; Urquhart *et al.,* 2003; Soulsby, 2005; Hendrix and Robinson, 2006 and Taylor *et al.,* 2007).

Overall clinical assessment of the patient was suggestive of grave prognosis as the temperature was critically high. The owner was suitably informed. In order to overcome serious neurological plight, the animal was forthwith managed by symptomatic fluid and supportive therapy, comprising of intravenous drip of 4 liters of normal saline solution (NSS), intramuscular injection of meloxicam 0.5 mg/ kg body weight, Based on the identification of the pathogen, the

animal was given specific therapy consisting of diminazine aceturate 5.0 mg/kg body weight. The animal was re-examined on Day-21 post therapy as evidenced by progressive restoration of body temperature to normal by Day-3 post therapy. The blood of animal was reexamined on Day-21 post therapy.

RESULTS

Clinical examination of the buffalo revealed lusterless dull hair coat infested with the developmental instars, including adults of *Boophilus microplus* over dewlap, axilla, ventral abdomen, udder and the peri-anal region. The animal was weak, uneasy and frequently kicking down. The conjunctival mucous membrane was congested and palid progressively turning to icteric. The muzzle was dry with frothy nasal discharge from both the nostrils. There was excessive drooling salivation, rapid shallow respiration (32 /minutes) besides, accelerated pulse (80 / minutes) and almost dry and hard rectal faeces. The superficial lymph nodes were neither swollen nor oedematous. The rectal temperature was critical, reaching 110.0°F. Signs of respiratory distress were quite evident and the animal was breathing in an arched back position with extended head and abducted limbs. Auscultation sounds revealed harsh sounds from the lungs and audible heart beat from distance. The animal was seen uneasy, frequently exhibiting to and fro movements. Ophthalmic examination of the eyes in respect of symmetry and confirmation did not reveal any deformity and / or blindness. The eyeballs were devoid of intra-ocular lesions suggestive of trauma, insect bite and / or inherited genetic defects. However, the animal was progressively developing corneal opacity and transient blindness (Figure 1). Neither of the eyes was completely blind. Ruminal hypotonocity was quite evident (1 per 3 minutes).

Laboratory investigation of the thin and watery peripheral blood revealed suppressed heamatological indices [Haemoglobin- 11.0 g/dl, PCV-34%, TLC-4.0x10^3/µL] and altered differential cell counts [Neutrophils 18%, Lymphocytes 78%, Monocytes 2% and Eosinophils 2%], these data were suggestive of the animal suffering from a milder form of anaemia, severe leucopenia, lymphocytosis and moderate eosinophilia. Microscopic examination of the Leishman stained thin smear evidenced characteristic intra-erythrocytic piroplasms identified as *Babesia bovis* (Figure 2) whereas, copro samples did not demonstrate parasitic ova and/or cysts. Blood of the animal re-examined on Day-21 post therapy revealed that the altered haematological indices had returned to normal levels and the erythrocytes were completely free from the pathogen.

DISCUSSION

On the Indian subcontinent, in the enzootic semi-arid desert, bubaline babesiosis is mainly caused by *B. bovis and B. bigemina* (buffalo strain). The one host tick *Boophilus microplus* is the vector of the disease in India, transmitting both transstadially and transovarianly bebesiosis in the susceptible exotics and / or their cross-bred progenies in buffaloes (Urquhart *et al.,* 2003; Soulsby, 2005; Taylor *et al.,* 2007 and Lefevre *et al.,* 2010). Though *Babesia* spp. are host and vector specific, yet the pathogens have been documented intertransmissible from buffalo to cattle and vice versa (Callow *et al.,* 1976). The nymph and adult instars of the tick are widely distributed in the

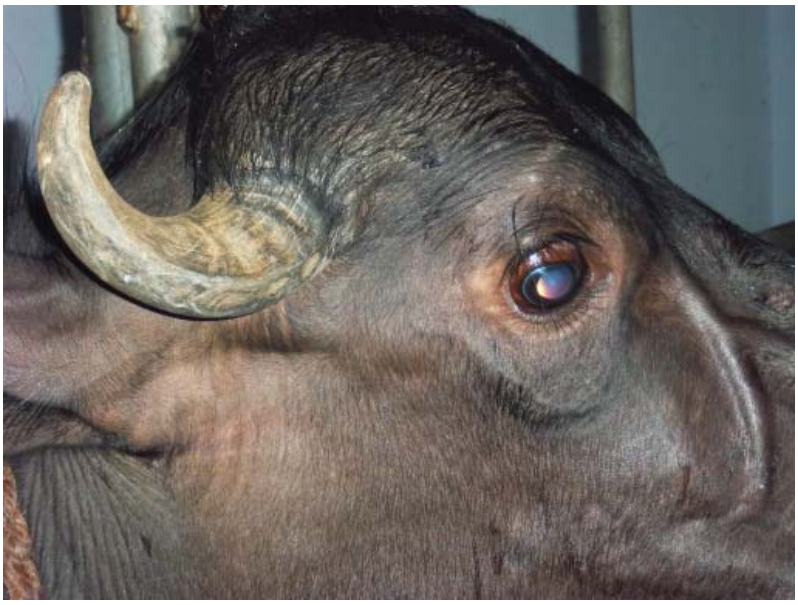

Figure 1. Corneal opacity in the affected animal.

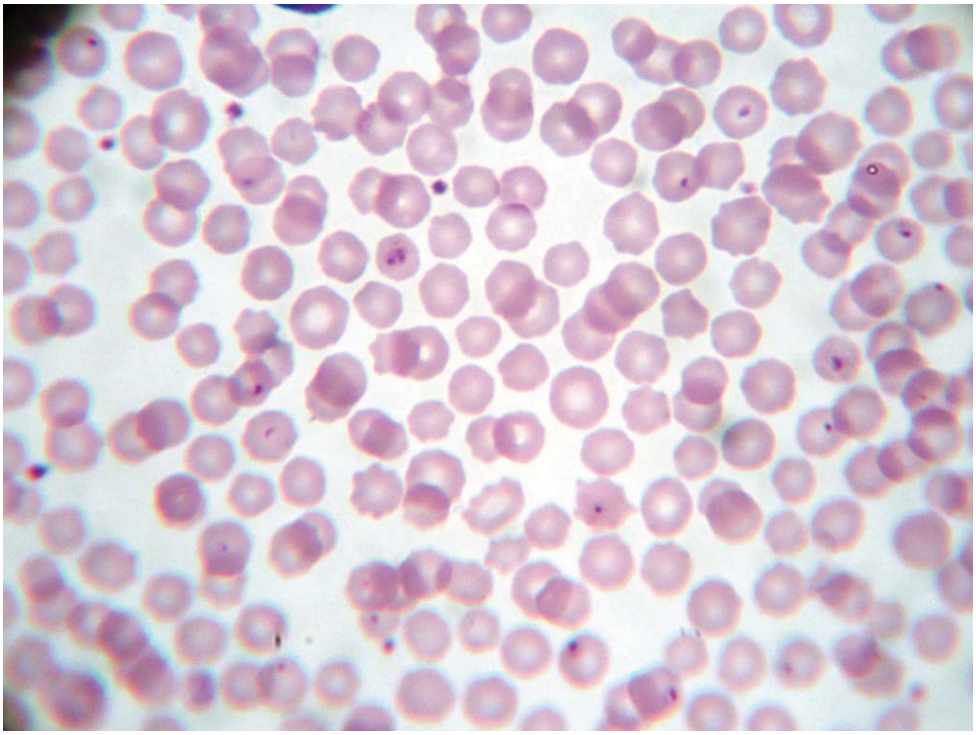

Figure 2. Blood smear showing intra erythrocytic piroplasms.

semi-desert environment, prevailing in a majority of the Middle Eastern and South Asian countries, including India (Radostits *et al*., 1994; Aiello and Mays, 1998 and Taylor *et al.,* 2007). Once the adult female tick is infected, it can transmit the infection for 32 generations (Markov and Abramov, 1966). Epizootilogically, vector borne diseases are regulated by four component systems, wherein susceptible hosts, optimum environments, the parasite and its vector play an integral role and account for the high occurrence of the disease especially during summer months (May to June) in the semi-desert ecosystem of Jaipur. Extremely high temperature, high milk yield and heat stress during summer months are recognized major predisposing factors.

The pathogen is widespread in buffalo native to Africa, Asia, and Australia. Young animals are reasonably resistant to the pathogen and do not cause clinical disease. In older animals, clinical signs can be very severe; however, differences in pathogenicity may occur with various *Babesia* spp. isolates associated with different geographic areas. The anemia may occur very rapidly, with 75% or more of the erythrocytes being destroyed in just a few days. After the onset of hemoglobinuria, the prognosis is guarded. Among fully susceptible older animals, the mortality may reach 70% without treatment. Anaemia, anorexia and / or anoxia are contributory factors to the weakness and loss of condition seen in cattle that survive the acute phase of the disease (Purnell, 1981; Ristic, 1988 and Aryeetey and Jimenez-Lucho, 2002).

Interestingly, to our knowledge, this seems to be the first ever documentation of "cerebral babesiosis" associated with *B. bovis* infection in the buffaloes from the sub-tropical, semi-arid, enzootic region of the Indian subcontinent. The disease occurs sporadically especially in adult bovines and generally terminates fatally. Infections with *B. bovis* have been incriminated as the main causal agent of the "cerebral syndrome", characterized by paddling of limbs, ataxia, and mania followed by death in the majority of cases. Pathogenesis of the syndrome has been debatable. Possibilities of crossing the CNS barriers by the parasite and causing auto immune disorders, characterized by intra-vascular agglutination of the erythrocytes in the cerebral capillaries with consequential embolism / thrombosis could not be ruled out (Soulsby, 2005; Taylor *et al.,* 2007 and Lefevre *et al.,* 2010). The diary animals, especially exotics and / or their cross-bred progenies, have been documented to be more susceptible to the disease than the native breeds of buffaloes in the enzootic areas (Taylor *et al.,* 2007 and Lefevre *et al.,* 2010).

The characteristic clinical signs in buffalo, reported here-in, were consistent with the critically "nervous signs" encountered in cattle [marked fall in general condition, caesation and / or ruminal hypotonocity, elevated body temperature, accelerated pulse and respiratory rate suggestive of dyspnoea and trachycardia, drooling saliva, frothy nasal discharge, constipated rectal faeces, etc.], coupled with sudden development of pathognomic neurological signs (aggressiveness, incoordinated gait and convulsions, paddling of limbs, persistent abduction of hind limbs, ataxia, mania) and demonstration of the pathogen in circulation were suggestive of acute "Cerebral Babesiosis" with grave prognosis. Further, depressed haematological indices confirming anaemia, severe leucopenia, lymphocytosis and moderate eosinophilia and above all, successful response to single specific anti-bebesial therapy and complete elimination of the pathogen from the circulation on Day-21 post therapy, made authors to strongly speculate and believe that the buffalo suffered from cerebral

form of Babesiosis. The nervous signs appeared due to blood stasis incidental to clogging of brain capillaries by accumulation and/ or agglutination of parasitized erythrocytes (Purnell, 1981; Aiello and Mays, 1998; Taylor *et al.,* 2007 and Lefevre *et al.,* 2010). The buffalo, while undergoing critical phase of the disease, exceptionally high pyrexia and high parasitemia, might have adversely affected the *in situ* physiology of pituitary and adrenal glands resulting in poor feed intake, nutrient utilization and rise in body temperature to critical levels and consequently, faster deterioration of the general health of the animal (Wright and Goodger, 1977; Radostits *et al.,* 1994; Soulsby, 2005; Taylor *et al.,* 2007 and Lefevre *et al.,* 2010).

Activation and spontaneous release of kallireins and kininins (vasoactive amines), consequential to host defence-parasite interaction might have played a significant role in the pathobiology and development of "cerebral babesiosis". The cytokines have been incriminated in vasodilatation and increased permeability of the affected fine blood vessels supplying tissues. Synchronously, the activity was coupled with concentration and sequestration of the parasitized erythrocytes, obstructing the free blood flow (Wright and Goodger, 1977; Mahoney and Seal, 1977; Purnell, 1981; Aryeetey and Jimenez-Lucho, 2002; Allred, 2003 and Lefevre *et al.,* 2010). Eventually, during the acute phase of the disease, the buffalo seems to suffer the coagulopathy syndrome of the disease resulting in blood stasis, tissue anoxia and specific neurological signs originating from the brain (Wright, 1972; Wright and Goodger, 1977 and Lefevre *et al.,* 2010).

The differential diagnosis of the cerebral form of the babesiosis *vis-à-vis* other disease conditions, (listeriosis, rabies, polioencephalomalacia, cerebral theileriosis), exhibiting almost analogous neurological signs *viz.* aggressiveness, absence of haemoglobinuria, circling, loss of herding instinct, paresis, muscular tremors, stiffness of hind legs and incoordinated gait seems logical and imperative. In listeric encephalitis, the animal suffers from unilateral facial nerve paralysis causing drooping of the eyelids and ears, dilated nostrils, and drooling of saliva, incidental to pharyngeal nerve partial paralysis. The duration of the disease is invariably longer (2-6 weeks). The lesions are mainly localized in the brain stem and the clinical signs indicate dysfunction of the third to seventh cranial nerve (Radostits *et al.,* 1994 and Lefevre *et al*., 2010).Whereas the rabies affected animal makes unprovocated attacks and is unable to swallow and / or drink. It is characterized by early paralysis of the throat and masseter muscles. The animal often exhibits changed behavior, stops eating and drinking, frequently passes urine, seeks solitude, produces characteristic loud bellowing sounds, and its lower jaw drops (Radostits *et al.,* 1994 and Lefevre *et al*., 2010). Polioencephalomalacia (PEM) is primarily a non-infectious neurological disease of the young and intensively reared cattle and buffaloes. It is incidental to the depressed activity of the tissue thiamine and related enzymes. The disease is characterized by amaurosis "glass eye" and strabismus followed by neurological signs and recumbancy. The disease is not prevalent on the Indian subcontinent (Radostits *et al.,* 1994 and Lefevre *et al*., 2010). The cerebral form "turning sickness" is characterized by a marked fall in general condition and lactation, caesation of rumination and digestive disturbances, pre mortal moderately elevated body temperature, accelerated pulse and respiratory rate, drooling saliva, frothy nasal discharge and respiratory distress, coupled with sudden development of pathognomic neurological signs (occasional circling, head pressing and

persistent abduction of hind limbs, animal falling in lateral recumbancy and finally transient posterior paresis) and demonstration of the *Theileria* piroplasms in erythrocytes and Koch blue bodies in the affected lymphoid tissues (Radostits *et al.,* 1994; Lefevre *et al*., 2010 and Sudan *et al.,* 2012). However, the bubaline cerebral form of babesiosis is devoid of circling signs, haemoglobinuria, paresis, muscular tremors, stiffness of hind legs, gastrointestinal disorders, etc.

It would be interesting to precisely investigate through well-planned experimental studies in buffaloes to elucidate (a) comparative efficacy of anti-babesial drugs ensuring complete elimination of the pathogen from the cerebral circulation; (b) prevalence of the disease, epizootiological determinants and reasons for its underreporting by the 0field veterinarians in the enzootic areas; (c) pathophysiological impact of the disease on endocrine glands especially the pituitary and adrenal glands, feed intake and nutrient utilization, spontaneous marked fall in lactation and deterioration of the general health of the affected animal besides, onset of shock and failure of thermoregulation, reaching critical levels in buffaloes; (d) mechanism of *in vivo* migration and access of the pathogen to cerebral capillaries and / or tissues and multiplication of the pathogen therein and intravascular sequestration of the parasitized erythrocytes; and (e) immunological response of the host, prior to sudden onset of sequential events discussed above and development of specific neurological signs.

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REFERENCES

Aiello, S.E. and A. Mays. 1998. *The Merck Veterinary Manual,* 8th ed. Merck & Co. Inc. New Jursey, USA. 2305p.

Allred, D.R. 2003. Babesiosis: Persistence in the face of adversity. *Trends Parasitol*., **19**: 51-55.

Aryeetey, R. and V. Jimenez-Lucho. 2002. Babesiosis: Current treatment options. *Infect. Dis*. **4**: 319-326.

Bowman, D.D., R.C. Lynn, M.L. Eberhard and A. Alcaraz. 2003. *Georgis Parasitology for Veterinarians*, 8th ed. Saunders-An imprint of Elsevier, USA.): 422p.

Callow, L.L., R.J. Parker, B.J. Rodwell and M.L. Ottley. 1976. Piroplasmosis in buffaloes and its serological diagnosis based on a homology between buffalo and bovine immunoglobulins. *Aust. Vet. J.,* **52**: 40-41.

Gupta, S.C. and B.P. Singh. 2002. Fasciolosis in cattle and buffaloes in India. *J. Vet. Parasitol*., **16**: 139-145.

Hendrix, C.M. and Ed Robinson. 2006. *Diagnostic Tarasitology for Teterinary Technicians*, 3rd ed. Mosby IMC (Elsevier) St Louis Missouri, USA. 304p.

Lefevre, P.S., J. Blancou, R. Chermette and G. Uilenberg. 2010. *Infectious and Parasitic Diseases of Livestock*. Lavoisier Tec & Doc, France. 1985p.

Mahoney, D.F. and J.R. Seal. 1961. Bovine babesiosis; Thick blood films for the detection of parasitaemia. *Aust. Vet. J.,* **37**: 44-47.

Markov, A.A. and LV. Abramov. 1966. Reciprocal

adaptations of certain blood parasites and their hosts, p. 268-269. *In Proceedings of 1st International Congress of Parasitology,* Italy. **I**: 268

Purnell, R.E. 1981. Babesiosis in various hosts, p. 25-63. *In* Ristic, M. and J. Kreier (eds.) *Babasiosis.* Acedemic Press, New York, USA.

Radostits, O.M., D.C. Blood and C.C. Gay. 1994. *Veterinary Medicine: A tTextbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*, 8th ed. Bailliere Tindall, London, UK. 1763p.

Ristic, M. 1988. *Babesiosis of Domestic Animals and Man*. CRC Press, Boca Raton, Florida, USA. 264p.

Soulsby, E.J.L. 2005. *Helminths, Arthropods and Protozoa of Domesticated Animals*, 7th ed. Bailliere Tindal, London, UK.

Sudan, V., R.L. Sharma, R. Yadav and M.K. Borah. 2012. Turning sickness in a cross bred cow naturally infected with *Theileria annulata*. *J. Parasit. Dis.*, **36**(2): 226-229.

Taylor, M.A., R.L. Coop and R.L. Wall. 2007. *Veterinary Parasitology*, 3rd ed. Edition Blackwell Publishing LTD, UK. 874p.

Urquhart, G.M., J. Armour, J.L. Duncan, A.M. Dunn and F.W. Jenningsis. 2003. Babesiosis, pp. 242-246. *In Veterinary Parasitology.* Blackwell Science LTD, UK.

Wright, I.G. 1972. Studies on the pathogenesis of *B. argentina* and *Babesia bigemina* infections in splenomectized calves. *Z. Parasitenkd*., **39**: 85-102.

Wright, I.G. and B.V. Goodger. 1977. Acute *Babesia bigemina* infection: Changes in coagulation and kallikrein parameters. *Z. Parasitenkd*., **53**: 63-73.