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SHORT COMMUNICATION

PIROPLASMOSIS IN A CAPTIVE GRANT'S ZEBRA *EQUUS QUAGGA BOEHMI* (MAMMALIA: PERISSODACTYLA: EQUIDAE) - A CASE STUDY

Sarat Kumar Sahu, Niranjana Sahoo, Bijayendranath Mohanty & Debabrat Mohapatra

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Piroplasmosis in a captive Grant's Zebra *Equus quagga boehmi* (Mammalia: Perissodactyla: Equidae) - a case study

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Abstract: An apparently healthy 2½-year-old male Grant's Zebra weighing approximately 200kg located at Nandankanan Zoological Park, Odisha, India, procured from Zoological Centre, Tel Aviv- Ramat Gan, Israel during September 2015 was noticed in a sitting position making frequent attempts to get up. The zebra was immobilised the same day with a combination of 1.96mg etorphine hydrochloride, 8.0mg of acepromazine and 40.0mg of xylazine hydrochloride to facilitate diagnosis and treatment. Clinical examinations did not reveal any signs suggestive of disease or disorder of the musculoskeletal system. Microscopic examination of blood smears stained in Giemsa's stain revealed the presence of intra-erythrocytic inclusions, either single or pairs, suggestive of haemoprotozoans, i.e., *B. caballi* and/or *T. equi*. The zebra was administered with two divided doses of imidocarb injection @4.0mg/kg b.wt. deep intramuscularly in the neck region with supportive therapy. Progressive improvement in posture, gait, and appetite were noticed following 24h of medication. Three more doses of imidocarb were administered at 72h intervals, each time after immobilisation.

Keywords: *Babesia caballi*, imidocarb, immobilisation, sub-clinical carriers, *Theileria equi*.

'Equine piroplasmosis', a tick borne haemoparasitic disease of Equidae (horses, mules, donkeys, and zebras), is widely distributed across the globe including tropical

and subtropical areas, and some temperate zones (Alhassan et al. 2005; Acici et al. 2008). Piroplasmosis is prevalent amongst Burchell's Zebra *Equus quagga burchellii* and Cape Mountain Zebra *Equus zebra zebra* in southern Africa (Lampen et al. 2009; Bhoora et al. 2010). It is caused by two morphologically distinct intra erythrocytic protozoans, viz., *Theileria equi* (formerly known as *Babesia equi*) and/or *Babesia caballi*. *T. equi* infection having shorter incubation period is more pathogenic than *B. caballi* (de Waal & van Heerden 2004). The disease appears in acute, sub-acute, and chronic forms with signs of fever, anaemia, icterus, hepatomegaly, edema, intravascular haemolysis, and haemoglobinuria. Mortality may reach up to 50% (de Waal 1992). Laminitis is one of the clinical signs of secondary complications (de Waal 1992). Antiprotozoan drugs are quite effective in bringing clinical recovery but fail to make the infected animal sterile. Hence, infected animals may remain life-long carriers of *T. equi* infections while *B. caballi* for up to four years (de Waal

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& van Heerden 2004). Thirty species of ixodid ticks of the genera *Dermacentor*, *Hyalomma*, and *Rhipicephalus* have been identified as vectors (de Waal 1992). It is also not possible to distinguish between *T. equi* and *B. caballi* infections based on clinical signs alone and mixed infections do occur (de Waal 1992). Available literature is silent about documentation of these infections amongst Grant's Zebras in Indian zoos. The present case describes a case report of Piroplasmosis in a Grant's Zebra *Equus quagga boehmi* at Nandankanan Zoological Park.

Case history

On 28 December 2016 (15.30h Indian time), a 2½-year old apparently healthy male Grant's Zebra (approx. body weight 200kg) of Nandankanan Zoological Park (NKZP) was noticed in a sitting position making repeated attempts to get up. But the zebra failed to bear its weight on the hind limbs. When approaching close to the animal, it moved with difficulty and dragged its right hind limb fetlock on the ground. Initial attempt with an intramuscular injection of NSAID (non-steroid anti-inflammatory drug) of 10ml Melonex Power (M/S Intas pharmaceuticals Ltd, Ahmedabad, Gujarat, India) through a blow pipe did not result in any remission even after three hours post-administration.

Four zebras (two males + two females) including the present ailing one were procured from Zoological Center, Tel Aviv- Ramat Gan, Israel. As per health records, the zebras were apparently healthy during the time of procurement as well as on arrival at Nandankanan Zoological Park on 13 September 2015. The zebras were kept in 'pre-export' quarantine at Ramat Gan, Israel for a period of 30 days. During the quarantine period at Israel, they were screened against *Theileria equi* and *Babesia caballi* by complement fixation test (CFT) and test reports were negative for both. At NKZP, these zebras were housed in an open air exhibit enclosure of 3,510m² area.

Of these four zebras, two females died on 20 August 2016 and 29 October 2016 with the predominant signs of limping in one or more limbs that continued for a period of 20–60 days in spite of supportive treatment consisting of nervine stimulants, NSAID, and broad spectrum antibiotics (BSA), all given with the help of a blow pipe. Further investigations could not be initiated due to non-availability of supporting facilities during that concerned period.

Earlier painful experience of casualties in two valuable animals triggered efforts to immobilise the sick zebra in the late evening (20.00h) to extend all possible therapeutic measures.

Clinical investigation

The zebra was darted using a drug mixture of 0.8ml of large animal immobilon (Novartis Animal Health, UK Limited, Frimley, South Africa) containing 1.96mg etorphine hydrochloride & 8.0mg of acepromazine and 0.4ml of Xylazil 100 (Troy Lab Pty Ltd, 35 Glendenning Road, Australia) containing 40.0mg of xylazine hydrochloride. This drug mixture was administered intramuscularly to the thigh muscle through 'Dist-Inject Syringe Projector Mod30N' from a distance of about 10m using a blue cartridge.

Detailed clinical examination was carried out including examination of hooves, joints, and other vulnerable body regions. Blood samples were collected with anticoagulants (EDTA & fluoride) and clot activator in three different sterile vials for further investigation.

Laboratory investigation was performed the same night with respect to haemato-biochemical and parasitological examinations to initiate a specific line of treatment. Blood smears were stained with Giemsa's stain and examined under oil immersion with the objective to detect haemoparasite. It revealed the presence of pear/oval shaped intra-erythrocytic inclusions, either single or pairs, suggestive of *Babesia* organism. Haemato-biochemical parameters with respect to Hb, TLC, DLC, sodium, potassium, ALP, AST, total protein, urea, creatinine, cholesterol, bilirubin, glucose, triglyceride, calcium, magnesium, and phosphorus were carried out following standard procedures (Table 1).

Treatment

The sick zebra was administered Imicarb 8.0ml (M/S Sava Health Care Ltd. Sava House, Pune, India) deep i/m in two equally divided doses at the neck muscle. The dose was calculated @4.0mg imidocarb per kg body weight. Additional treatment included 1L Lactated Ringer's and 1L 5% dextrose, 2.0g Tazar (Piperacillin and Tazobactam from M/S Lupin Limited, Mumbai, India) and 15ml Optineurone (M/S Lupin Ltd, Gujarat, India).

The zebra was reversed after 40min of induction by intravenous injection of 0.8ml of large animal revivon (Novartis Animal Health) that contained 2.6mg of diprenorphine hydrochloride and 0.5ml Reverzine (Bomac Pty Limited, Hornsby, NSW 2077) that contained 5.0mg of yohimbine hydrochloride. All the activities were accomplished under artificial electric flood light (Image 1).

As a supportive therapy, the zebra was provided with mineral mixture (Bestomin Gold, Provimi Animal Nutrition India Pvt Ltd.) @ 30g/day, calcium granules (Orcal-P, TTK Healthcare Limited, Chennai, India) @ 50g/

Table 1. Haemato-biochemical values of a 2½-year-old ailing male Grant's Zebra on different days of illness.

Parameters	Haemato-biochemical values on different days of treatment		Reference values of horse (Radostits et al. 2006)
	28.xii.2016	01.i.2017	
Haemoglobin (g%)	15.5	15.4	11–19
TLC (cu mm)	14,350	11,600	5400–14300
DLC(%)			
Neutrophil	78	70	52–70
Eosinophil	02	01	0–7
Lymphocyte	18	26	21–42
Monocyte	02	03	0–6
Sodium (mEq/L)	134.5	131.6	132–146
Potassium (mEq/L)	4.1	4.0	3.0–5.0
ALP (IU/L)	225.6	210.9	140–4003
ALT (IU/L)	6.0	5.8	3.0–23
Total Protein (g/dl)	6.5	6.4	6.0–7.7
Urea (mg/dl)	59.7	70.2	10–24
Creatinine (mg/dl)	1.81	1.25	0.9–1.9
Cholesterol (mg/dl)	132.9	107.4	46–180
Bilirubin (mg/dl)	0.95	1.01	1.0–2.0
Glucose (mg/dl)	163.0	102.1	75–115
Triglyceride (mg/dl)	67.5	67.5	4.0–44.0
Calcium (mg/dl)	12.1	11.1	11.2–13.6
Magnesium (mg/dl)	2.2	2.2	2.2–2.8
Phosphorous (mg/dl)	3.7	4.1	3.1–5.6



Image 1. Ailing Grant's Zebra after recovery from anaesthesia on 28.xii.2016 night. © Nandankanan Zoological Park.

day and a commercially available herbal antirheumatic preparation (R-Compound from M/S Alarsin Pharmaceuticals, Mumbai, India) @ 10 tablets per day in its concentrate feed for a period of three months.

The sick zebra was segregated from the other male zebra to facilitate treatment and monitoring. Based on the literature (Radostits et al. 2006), three more doses of imidocarb injections were administered with the same dose and route at 72h intervals. Blood samples

were also collected during the post-treatment period to record haemato-biochemical alterations.

RESULTS AND DISCUSSION

The severity of clinical signs shown by the ailing zebra coupled with the earlier tragic end of two other zebras in the same enclosure warranted immediate intervention. Etorphine used in this case is the most recommended drug to immobilise the zebra. The drug combination, i.e., Etorphine, Acepromazine, and Xylazine were also used previously by different workers to immobilise zebras (Walzer 2003; Senthilkumar et al. 2005; Nath et al. 2012). Following tranquilisation the zebra started showing signs of anaesthesia four minutes post-injection period and complete immobilisation was achieved in seven minutes.

Clinical signs of equine piroplasmosis are often nonspecific. It may be confused with a variety of other viral diseases like equine influenza, encephalosis virus infection and equine infectious anaemia. The haemato-biochemical parameters analysed in the present case were found to be within reference range (Table 1). This showed the absence of any of the viral infections described above. Clinical examinations did not reveal any appreciable musculo-skeletal disorders or deformity correlating clinical signs exhibited by the zebra. Body



Image 2. Ailing Grant's Zebra in standing posture on 01.i.2017. © Nandankanan Zoological Park

vitals like rectal temperature, respiration, and heart rate were recorded as 99.3°F, 12 breaths/minute, and 70 beats/minute, respectively. Both rectal temperature and respiration rates were within the normal range. Heart beats were on a higher side as against the reference value of 28–40 bpm. This transient increase could be correlated with the excitement during pre and post tranquillisation procedure.

Anaemia and haemoglobinuria which is marked in case of *T. equi* infection (Soulsby 1982) was not seen here and posterior paralysis found in this case is common to *B. caballi* infection and not found in *T. equi* infection (Soulsby 1982).

Blood smear examination revealed the presence of pear/oval shaped intra-erythrocytic inclusions, either single or pairs (Image 3), suggestive of haemoprotozoans, i.e., *B. caballi* and/or *T. equi*. No tetrads/ maltese cross, specific for *T. equi*, (Soulsby 1982) were noticed here. Clinical signs coupled with parasitological examination confirmed the case to be equine piroplasmosis and more likely to be *B. caballi* infection. This corroborated the earlier report of Zwegarth et al. (2002) who detected both *B. caballi* and *T. equi* in zebras from two national parks in South Africa.

The present report is substantiated by the fact that *Theileria equi* and *Babesia caballi* infections are endemic in Israel (Levi et al. 2018). Most of the infected animals (equids) may remain as sub-clinical carriers of these parasites with no clinical signs and act as a source of infection (Friedhoff & Soulè 1996). Though these zebras were tested negative against *B. caballi* & *T. equi* through complement fixation test during pre-export quarantine period at Israel, the possibility of carrier state can't be ruled out as documented by Radostits et al. (2006) and



Image 3. Blood smear (Giemsa's stain) of an 2½-year-old male Grant's Zebra showing pyriform intra-erythrocytic *Babesia* sp. in pair or single under oil immersion objective. © Department of Veterinary Parasitology.

the zebra is suspected to have carried *B. caballi* and/or *T. equi* from Israel in sub-clinical stage.

Several techniques/tests are employed for the diagnosis of equine piroplasmosis that include clinical signs, direct demonstration of parasites in blood smears, serological assays, cell-culture, and PCR assays, however, the present diagnosis is based on the clinical signs, blood smear examination, and response to treatment. Advanced molecular techniques couldn't be carried out due to lack of facilities at that time. The clinical signs noticed here, i.e., sudden onset of impaired mobility with posterior paralysis were also akin to observations by other authors (Radostits et al. 2006; Kaandorp 2010).

Drugs available for the treatment of equine piroplasmosis are Diminazene for *B. caballi* and Parvaquone for *T. equi* infections (de Waal 1992). Imidocarb, which is considered to be the safest of all drugs available, is effective in treating clinical cases of both the protozoans (Radostits et al. 2006).

Within 24 hours after administration of the first dose of imidocarb injection, significant improvement was observed with respect to gait, movement, and appetite. The zebra could stand and walk with moderate speed (Image 2). The signs of limping subsided completely and appetite was regained within 72h after the first dose of imidocarb. In order to ensure proper administration of the required drug, the zebra was immobilised every time using the same drug and dose schedule.

This favourable response to imidocarb confirmed

our diagnosis of piroplasmosis. Imidocarb is the most trusted drug for the treatment of equine babesiosis (Radostits et al. 2006; Donnellan & Marais 2009). To the best of our knowledge, this seems to be the first report of the piroplasmosis in Grant's Zebra in Indian zoos.

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