Putative clinical piroplasmosis in a Burchell's zebra (*Equus quagga burchelli*)

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ABSTRACT

A 10-year-old tame zebra gelding was presented after suffering from lethargy, nervousness, reported anaemia and icterus as well as a decreased appetite. These symptoms were seen over some months, with changing severity. The animal was immobilised, treated, and blood specimens were submitted for haematology and biochemistry. This report describes molecular characterisation of *Theileria equi* recovered from this animal, as well as the clinical findings, treatment and historical relevance of piroplasmosis in zebra in southern Africa.

Keywords: Burchell's zebra, *Equus quagga burchelli,* piroplasmosis, *Theileria equi,* treatment.

INTRODUCTION

Piroplasms have been reported from various wild ungulate species; frequently they are documentations of organisms seen on blood smears randomly taken from clinically normal animals[25].

Equine piroplasmosis is caused by 2 intra-erythrocytic protozoan parasites, *Babesia caballi* and *Theileria equi* (previously known as *Babesia equi*)[4,11,21,31]. These parasites are tick-transmitted and infect horses, mules, donkeys and zebras. Soon after first being described from horses[20,28], *Theileria equi* was reported from an East African plains zebra (*Equus quagga*) in 1905[18]. Inoculation of blood from this zebra into horses caused high fever and the appearance of piroplasms, leading to the conclusion that zebras are carriers of the parasite[18]. Piroplasms were also reported as an incidental finding from plains zebras from Umfolozi in Kwa-Zulu-Natal, South Africa, in 1931[23]. *Babesia* spp. were reported from both plains and Grévy's zebras (*Equus grevyi*) from East Africa[6]. *Theileria equi* has also been reported from Cape mountain zebras (*Equus zebra zebra*) in South Africa[30,32].

In many cases, identification of piroplasms based on morphology in a blood smear is no longer appropriate; more specific definition requires molecular characterisation[7,13,22]. This is especially true for the parasites which cause equine piroplasmosis. Recently 3 genetically distinct *T. equi* groups have been identified based on 18S rRNA sequence data; similarly 2 *B. caballi* groups have been characterised[4].

The only reported account of clinical piroplasmosis, attributed to *Theileria equi,* was in newly captured Grévy's zebras in Kenya[9]. A zebra owner and a trapper from Uganda stated that they lost 20–25 % of their animals to the disease, usually 2–3 weeks after capture[9].

CASE HISTORY

The behaviour of a 10-year-old tame Burchell's zebra (*Equus quagga burchelli*) gelding, from Cullinan, Gauteng, South Africa, changed markedly over the course of a few months. The animal had recently been separated from a zebra mare and foal due to uncharacteristic and severe aggression, and now grazed with the local horses on a 100-ha farm. Grazing consisted mostly of a mixture of sweet and sour veld grass, but the animal also received additional pelleted horse food. He was regularly treated for ticks and none could be seen on the animal at the time of examination. The owner noticed that he started behaving in an increasingly abnormal and irritable manner and that his appetite gradually decreased. His coat became very dull, and his gingivae were orange-yellow, later turning grey-ish. He showed pronounced nervousness, which was against his nature.

The animal was examined in November 2008. His estimated weight was 350 kg. From a distance, he appeared in good condition, slightly irritable, with a dull coat. He was still eating selected food offered by the owner and could be led around a paddock wearing a harness.

The owner reported a history of lameness, which had been examined years previously. At that stage no treatment was given, other than his hooves being trimmed. The animal managed to cope with the lameness, but chose to generally graze in the vicinity of the farm house. A farrier was standing by to cut the hooves again concurrently with the examination.

To allow closer examination, treatment and blood collection, the animal was immobilised by pole syringe using 5 mg etorphine (M99®, Novartis South Africa; 9.8 mg/mℓ), combined with 60 mg Azaperone (Stresnil®, Janssen Pharmaceutica; 40 mg/mℓ). Induction was very good, with sternal recumbency reached within 3.5 minutes. The heart rate was 60 beats/minute and respiratory rate 9 breaths/minute. Temperature was not taken. The mucous membranes appeared normal and no other noticeable clinical abnormalities were found. Blood was collected from the jugular vein into a 10 mℓ serum (BD Vacutainer® CAT) and 6 mℓ EDTA (BD Vacutainer®, K2E) vacutainer tube and the farrier commenced trimming the hooves.

Treatment, by intramuscular injection, consisted of 20 mℓ long-acting penicillin (Peni LA®, Virbac), 12 mℓ sulpha-diazine/trimethoprim (Norotrim 24®, Norbrook; Sulphadiazine 200 mg/mℓ and Trimethoprim 400 mg/mℓ), 10 mℓ Vit B Co injection (Oberon Pharma) and 5 mℓ non-steroidal anti-inflammatory drug (Ketofen®, Merial; 0.1 g/mℓ). An anthelmintic (5 mℓ Ivermectin, MDB Iver 1 %, CEVA) was also administered.

In spite of the gelding having a normal mucous membrane colour, he was treated for piroplasmosis based on the history of grey and at times 'orange' mucous membranes, lethargy, decreased appetite, a waxing and waning condition and other non-specific symptoms. Eight mℓ imido-carb dipropionate (Forray 65®, Schering-Plough Animal Health), which is a similar

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dose as given to that for a horse of 400 kg, was administered intramuscularly.

The general anaesthetic was reversed by administering 1.5 mℓ diprenorphine hydrochloride (M5050®, Novartis South Africa; 12 mg/mℓ) intravenously. Recovery was prolonged, and the gelding struggled to stand up. Even after half an hour he appeared sedated, with his head hanging. The owner reported that he looked listless for the next 3 days, but then picked up, and recovered well, until he appeared to have returned to his normal demeanour and appetite.

The blood samples were stored in a cooler box after collection and, on completion of the procedure, were taken to the Clinical Pathology Laboratory, Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, for further analysis. The laboratory prepared a blood smear stained with a Diff-Quick Romanowsky stain. Intra-erythrocytic piroplasms were identified under the microscope. A full blood count and biochemistry analysis was also performed (Table 1). The sample was then sent on to the Department of Veterinary Tropical Diseases for molecular characterisation of the piroplasm.

Haematology findings (Table 1) were within the normal range (mean ± 1 standard deviation) given for this species. Among clinical chemistry findings, urea, creatinine and AST levels were reduced, while TSP and chloride levels were elevated (Table 1).

Three months after treatment the zebra again started showing similar clinical signs. The animal was once more treated with 8 mℓ imidocarb dipropionate, this time without requiring general anaesthesia. He made an uneventful recovery, and has since then not shown any further clinical signs.

MOLECULAR DIAGNOSIS

Genomic DNA was extracted from 200 μℓ of EDTA-treated blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Primers RLB-F2 (5'-GAC ACA GGG AGG TAG TGA CAA G-3') and RLB-R2 (Biotin-5'-CTA AGA ATT TCA CCT CTG ACA GT-3') specific for *Theileria* and *Babesia* species, were used to amplify the V4 hypervariable region of the 18S rRNA gene of the parasite/s present in the sample, as described previously[24]. PCR products were subjected to reverse line blot (RLB) hybridisation as previously described[4]. The piroplasm status of the animal was further confirmed using a TaqMan real-time PCR assay developed for the detection of *T. equi* parasites[17], and for the

detection of *B. caballi* parasites, a recently developed TaqMan MGB real-time PCR assay was used[4].

A fragment of the parasite 18S rRNA gene was subsequently amplified using 3 different primer sets. Primers NBabesia1F and 18SRev-TB were used in a primary PCR reaction to amplify a fragment of approximately 1600 bp. Two subsequent nested PCR reactions were performed using primers NBabesia1F and BT18S3R, and primers 18SRev-TB and BT18S3F. All reactions were performed as previously described[4]. Amplicons were purified using the Qiagen PCR purification kit (Qiagen, Hilden, Germany) and samples were sequenced using BigDye chemistry (v.3.1, Applied Biosystems) in a 3130XL sequencer (Applied Biosystems).

Sequences were assembled and edited using gap4 of the Staden software suite[26,27]. Multiple sequence alignments were performed using the MAFFT alignment (Multiple Sequence Alignment) employing the FFT-NS-1 algorithm[15,16]. The alignments

were adjusted manually using BioEdit (version 7.0.5.2)[14]. Searches of databases for homologous sequences were performed using BLASTN[3].

The zebra sample tested negative for the presence of parasite DNA when using the RLB assay. However, the presence of *T. equi* parasite DNA was confirmed using the real-time PCR assay developed for the detection of *T. equi* parasites[17]. Parasite DNA could be detected at a cycle threshold (Ct) value of 30 which indicated the presence of low parasitaemia. The fact that *T. equi* parasite DNA could not be detected using the RLB was probably due to the low parasitaemia in the sample. No *B. caballi* DNA was detected using the TaqMan MGB real-time PCR assay.

A fragment of the 18S rRNA gene (~1170 bp) was sequenced and BLASTN analysis revealed that the sequence showed 98 % identity to the 18S rRNA gene sequence of a *T. equi*-like isolate from a horse from Spain (strain ET1, accession number AY534882)[22] and 99 % identity to

Table 1: **Clinical chemistry and haematology readings for a Burchell's zebra gelding, compared to normal values.**

	Normal	Mean* (SD)**	Unit
Clinical chemistry			
Urea	4.3	6.4261 (1.428)	mmol/ℓ
Creatinine	126	167.96 (35.36)	μmol/ℓ
Total serum protein	75.3	64 (6)	g/ℓ
Albumin	34.7	31 (4)	g/ℓ
Globulin	40.6	35 (3)	g/ℓ
Albumin/globulin	0.85		
Aspartate aminotransferase	226	334 (88)	U/ℓ, 37°C
Gamma-glutamyl transferase	51	53 (23)	U/ℓ, 37°C
Na$^+$	138.2	137 (3)	mmol/ℓ
K$^+$	3.81	3.9 (0.4)	mmol/ℓ
Ca^{++}(total)	2.78	2.65 (0.13)	mmol/ℓ
mg$^+$	0.72	0.66 (0.18)	mmol/ℓ
Cl$^-$	103.1	97 (3)	mmol/ℓ
Haematology			
Haemoglobin	148	150 (12)	g/ℓ
Red cell count	9.81	9.59 (1.15)	10^{12}/ℓ
Haematocrit	0.41	0.428 (0.059)	ℓ/ℓ
Mean corpuscular volume	42.2	44.6 (4.2)	fl
Mean corpuscular haemoglobin	15.1	15.7 (1.7)	g/dℓ cells
Mean corpuscular haemoglobin concentration	35.6	35.2 (3.5)	g/dℓ cells
Red blood cell distribution width	16.9		%
White cell count	9.73	7.8 2(2.6)	x10^9/ℓ
Neutrophils (total)***	7.59		x10^9/ℓ
Neutrophils (mature)***	7.59	5.19 (2.47)	x10^9/ℓ
Neutrophils (immature)***	0	0.16 (0)	x10^9/ℓ
Lymphocytes***	1.95	2.37 (0.94)	x10^9/ℓ
Monocytes***	0.19	0.276 (0.16)	x10^9/ℓ
Eosinophils***	0	0.22 (0.22)	x10^9/ℓ
Basophils***	0	–	x10^9/ℓ
Platelet Count	219	256 (84)	x10^9/ℓ
Anisocytosis	1+		
Acanthocytes	1+		

*International Species Information System Physiological Data *Reference Values 2002:* Common zebra (*Equus burchellii*), male, 3–50 >20 year old, http://www.isis.org/cmsHome/
** Standard deviation.
***Absolute count.

that of a sample from a mountain zebra from the Bontebok National Park (RBE 101, accession number EU642507)[4].

DISCUSSION

In horses, clinical signs of equine piroplasmosis range from acute fever, inappetence and malaise, to anaemia and jaundice, sudden death, or chronic weight loss and poor exercise tolerance[11,31]. The clinical signs seen in this zebra were very non-specific, but similar to those found in horses. The gelding's response following treatment further substantiates that he was showing clinical signs attributable to the parasite. Treatment can suppress clinical signs, but the currently available treatments are ineffective in clearing *T. equi* from carriers[11,29]. Early claims of successful clearing of *T. equi* infections by means of an intensive imidocarb treatment regimen[8,12] (4 treatments of 4 mg/kg at 72 h intervals) have not been substantiated by subsequent research[5,19].

While the RLB assay failed to detect parasite DNA in a blood sample from this zebra, a quantitative real-time PCR assay confirmed the presence of *T. equi* parasite DNA. The parasitaemia was low, however, as indicated both by the negative result obtained using the RLB assay and by the high Ct value obtained in the real-time PCR test. The reasons for such low parasitaemia are unknown. The blood samples were collected before the treatment was given. It can be speculated that parasitaemia levels were low on the day of sampling, when no specific clinical signs were seen, but that they fluctuated over time in conjunction with the reported clinical signs as described by the owner. The first treatment managed to control the infection until a relapse occurred 3 months later, which necessitated further treatment.

At least 3 distinct groups of *T. equi* 18S rRNA genotypes are known to occur in horses and zebra in South Africa[4]. The 18S rRNA gene sequence identified in this study was very similar to that of a *T. equi*-like parasite identified in a horse from Spain (strain ET1, AY534882)[22], and in a sample from a mountain zebra from the Bontebok National Park (RBEQ101, accession number EU642507)[4]. These sequences fall within *T. equi* 18S rRNA group B[4]. Interestingly, in South Africa, this *T. equi*-like genotype has currently only been identified in parasites infecting zebra. Unfortunately, it is not known whether the different *T. equi* genotypes can be associated with different clinical signs.

Endemic stability to babesiosis has been well studied in cattle[10] and horses[1], where constant superinfections induce a stable

immunity[1]. It has been shown that intrauterine infection with *T. equi* in horses may be the rule rather than the exception[2]. In cattle, passive immunity, acquired from the dam, will protect newborn calves. Subsequent to this, any infection before 9 months will stimulate a solid immunity without the animal showing any clinical signs. This stable situation requires a high prevalence of infection in cattle, as well as a large enough tick population to ensure that calves become infected during the critical period. There are indications that in wildlife populations a similar endemically stable situation may exist[25]. It is speculated that any disruption to such stability may then result in the animal not building up the necessary immunity[25], leading to a flare-up of the infection.

During the 2nd consultation, the owner stated that the gelding was treated for ticks on a monthly basis, a protocol also followed with the horses on the property. It was recommended that the owner enforces a less stringent tick control programme to enable the animal to build up a stable immunity to the parasite through constant superinfection[1].

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