Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 154 (2008) 233–241
veterinary parasitology
www.elsevier.com/locate/vetpar

Health management of horses under high challenge from trypanosomes: A case study from Serengeti, Tanzania

Harriet Auty [a,*], Alison Mundy [b], Robert D. Fyumagwa [c], Kim Picozzi [a], Susan Welburn [a], Richard Hoare [c]

[a] *Centre for Tropical Veterinary Medicine, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin, Midlothian EH25 9RG, UK*
[b] *Singita-Grumeti Reserves Ltd., P.O. Box 65, Mugumu, Tanzania*
[c] *Tanzania Wildlife Research Institute—Messerli Foundation Wildlife Veterinary Programme, P.O. Box 707, Arusha, Tanzania*

Received 5 September 2007; received in revised form 23 February 2008; accepted 27 February 2008

Abstract

Horses kept for recreational riding purposes by a wildlife tourism company in a heavily tsetse fly-infested region of north-western Tanzania were systematically monitored to investigate the occurrence, presentation and management of tsetse-transmitted trypanosomosis. During a 23-month period, 18 clinical cases were diagnosed (*Trypanosoma brucei* or *Trypanosoma congolense* were identified) and treated and trypanosomes were implicated of involvement in four deaths. Pyrexia consistently aided early detection (17 cases). Ataxia, weight loss and anaemia were seen in chronic cases and conferred a poor prognosis. Delaying treatment by more than 2 days from the onset of clinical signs led to prolonged disease course and more severe anaemia. Early detection, prompt treatment, thorough post-treatment health monitoring and rigorous prophylactic measures helped keep clinical cases to manageable levels, but re-infection remained a constant, insidious threat.

Keywords: Horse; Tanzania; *Trypanosoma* spp

1. Introduction

Tsetse-transmitted trypanosomes, protozoan blood-borne parasites which cause potentially fatal disease in horses, are a major constraint to horse keeping in Africa, with the more trypanosome tolerant donkeys used where draught power is required (Barrowman, 1990). However, many aspects of trypanosomosis in horses remain poorly understood, and previous studies have only focussed on the management of single outbreaks. With the growth of horse safaris within the expanding wildlife tourism industry, management of horses under trypanosome challenge is likely to become increasingly important.

Horses are susceptible to infection with *Trypanosoma brucei*, *T. congolense* and *T. vivax* (Stephen, 1970). During an outbreak of trypanosomosis in Kenya in 1990 all three trypanosome species were isolated. *T. congolense* was considered the main species responsible for clinical signs. Both *T. congolense* and *T. vivax* were identified as sole pathogens in sick horses. *T. brucei* was only present in mixed infections and was considered incidental (Kihurani et al., 1994). The implication of *T. brucei* as the sole pathogen in an outbreak in Zambia in 1970 (Awan and Johnston, 1979) reveals some uncertainty regarding its role in causing disease. However, with the advent of molecular

* Corresponding author. Tel.: +44 131 650 6269; fax: +44 131 651 3903.
E-mail address: h.k.auty@sms.ed.ac.uk (H. Auty).

0304-4017/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2008.02.034

technologies, tools now exist to differentiate species with a high degree of accuracy compared to morphological examination often previously relied on.

Clinical signs recognised in association with trypanosomosis in horses include ataxia, paralysis and ventral oedema. Pyrexia, anaemia, anorexia, jaundice, tachycardia and keratoconjunctivitis may also be reported (Kihurani et al., 1994; Stephen, 1970; Taylor and Authie, 2004). The frequency of these clinical signs and their value as diagnostic or prognostic indicators has not previously been evaluated, although it has been suggested that differences in disease presentation exist between trypanosome species.

Maintenance of horse populations in tsetse-infested areas relies on chemotherapy for treatment and prophylaxis. Most trypanocidal drugs have been developed for use in cattle or camels. Treatment regimens in horses have been extrapolated from these species, but are associated with uncertain efficacy and frequent side effects.

Diminazene aceturate is widely used in cattle at a dose rate of 3.5–7 mg/kg bodyweight. When used at 3.5 mg/kg bodyweight in horses, side effects are common: of seven horses treated for *T. brucei* infection in Zambia, all became anorexic, and three developed ataxia (Awan and Johnston, 1979), and in treatment of *T. evansi* in Thailand four out of eight horses developed moderate to severe side effects including oedema, hypersalivation, recumbancy, restlessness and dsypneoa, which was fatal in one case (Tuntasuvan et al., 2003). Diminazine is rapidly excreted and considered to have no prophylactic effect.

Isometamidium chloride is used in horses at a recommended dose of 0.5 mg/kg as both treatment and prophylaxis. The period of prophylactic cover is variable but in general lasts for 2–4 months in cattle. Severe local reaction at the injection site is commonly reported (Kinabo and Bogan, 1988).

Quinapyramine is no longer used in cattle but is produced for the treatment of *T. evansi* in camels. In addition to quinapyramine sulphate, Triquin® (Wockhardt Europe Ltd., Ireland) contains quinapyramine chloride which forms a depot at the injection site and provides prophylactic cover in camels for approximately 3 months. Quinapyramine has been used to treat *T. brucei* and *T. evansi* infections in horses. Side effects of hypersalivation, restlessness and colic are described; the proportion of horses affected is not reported (Awan and Johnston, 1979; Leach and Roberts, 1981; Maqbool et al., 1996).

Melarsomine is used for treatment of *T. evansi* infections in camels and horses. Efficacy is also reported against *T. brucei* in horses (Raynaud et al., 1989). Melarsomine has no prophylactic activity.

We describe 2 years of health monitoring in a well-managed stable of horses that are kept for recreational riding purposes by a wildlife tourism company operating in a heavily tsetse-infested region of north-western Tanzania. Documentation of natural infections gave the opportunity for detailed study into the presentation and management of equine trypanosomosis. This case study evaluates (i) the clinical picture seen in natural infections of horses; (ii) a range of diagnostic methods used; and (iii) the success of treatment and prophylactic regimens attempted.

2. Materials and methods

2.1. Study area

The study population of horses is owned by Singita-Grumeti Reserves Ltd., and kept adjacent to Grumeti Game Reserve, a wildlife protected area which borders the north-west of Serengeti National Park in northern Tanzania. The property has an area of about 2500 ha which is dominated by acacia wooded grassland and has very high densities of *Glossina swynnertoni* and *G. pallidipes*. Tsetse populations vary seasonally, increasing after the start of the rainy season and declining during the dry season (Challier, 1982). Sixteen horses (predominately thoroughbred) are kept by the tourist lodge and ridden in the surrounding area for game viewing. Horses are periodically bought and sold to maintain a constant population in the stable. All introductions come from areas outside the tsetse fly belt and are thus naïve to trypanosome infection.

2.2. Health monitoring and diagnostic procedures

Systematic health monitoring was carried out between January 2005 and November 2006, following cases of undiagnosed and recurrent illness. A diagnostic protocol was established for rapid identification of trypanosome infections and medical records were kept for all horses which detailed clinical signs during disease episodes. Any change from normal demeanour or appetite was noted. Rectal temperatures of all horses were routinely taken twice daily and those above 38.5 °C were then taken repeatedly throughout the day. Pyrexia was recorded if rectal temperature exceeded 39 °C. Anaemia or jaundice was initially assessed subjectively by examination of buccal and scleral mucous membranes and the limbs, sheath and ventral areas were examined for oedema. Tachycardia and

cardiac arrhythmia were frequently detected on auscultation carried out once pyrexia was noticed, and as such were often recorded as presenting signs.

Jugular venipuncture was performed on all horses showing pyrexia or other clinical signs consistent with trypanosome infection, and the blood collected in 10 ml heparinized tubes. Fresh blood films were examined microscopically on site. Blood samples were then centrifuged and a Giemsa-stained buffy coat smear examined microscopically. Following a negative result, the above tests were repeated daily, during periods of pyrexia if observed. Anaemia was assessed by measuring packed cell volume (PCV) by centrifugation of blood samples in micro-haematocrit capillary tubes, and carried out both routinely in healthy horses to establish the normal PCV of each horse, and during episodes of disease. Anaemia was recorded if the PCV fell below 30%.

2.3. Molecular analysis

Samples of heparinized blood from microscopy-positive horses were applied to Whatman Classic FTA Cards for characterisation of trypanosome species using polymerase chain reaction (PCR) in the United Kingdom. Cards were sealed in foil pouches with desiccant and maintained at room temperature. Two 2 mm punches per sample were analysed using a PCR which detects differences in length of the internal transcribed spacer (ITS) regions of the trypanosome ribosomal genes, enabling differentiation of the main African trypanosome species (Cox et al., 2005). The wash protocol, primer sequences and PCR conditions were as detailed by Cox et al. (2005).

Samples showing band sizes specific for *T. brucei* sensu lato were screened for the presence of the human-serum-resistance-associated (SRA) gene to differentiate *T. brucei brucei* from *T. b. rhodesiense* (pathogenic to humans), following the protocol described by Picozzi et al. (2005).

2.4. Treatment regimen

Of the four trypanocides described above, two were used in this study. Melarsomine was unavailable in this area during the study period. Diminazine was not used due to concerns over side effects and lack of prophylactic cover. Quinapyramine is regarded as the most effective treatment for *T. brucei* in horses (Fenger, 2004) and was therefore chosen for treatment (Triquin®, Wockhardt Europe Ltd., Ireland). The severe side effects caused by quinapyramine meant that isometamidium (Trypamidium-Samorin®, Merial, France) was preferred for routine prophylaxis. However, isometamidium is more difficult to administer, therefore quinapyramine was used in fractious horses.

When trypanosomes were found on microscopic examination of fresh blood film or buffy coat, parenteral treatment was immediately administered using quinapyramine (0.025 ml of suspension per kg bodyweight subcutaneously, when one bottle of Triquin® (1.5 g quinapyramine sulphate, 1 g quinapyramine chloride) was made up with 15 ml sterile water). Flunixin meglumine (Finadyne®, Norbrook, UK) was used as supporting treatment for alleviation of pyrexia. A vitamin/mineral supplement (Mirablud®, Bayer, South Africa) was given for 10 days to counter anaemia. Recovering horses were rested for at least 10 days, or approximately 1 week per day of pyrexia. Buffy coat samples were examined daily after treatment until parasites disappeared and PCV was monitored regularly during recovery.

2.5. Prevention

Quinapyramine (Triquin®, Wockhardt Europe Ltd., Ireland) (in 8 horses) and isometamidium (Trypamidium-Samorin®, Merial, France) (in 12 horses) were administered as prophylaxis. Horses were only covered by chemoprophylaxis for part of the study period due to unavailability of drugs at the start of the study.

A combination of physical and chemical measures was put in place to reduce tsetse fly density near the stable complex where horses spent the majority of their time. Acetone-baited tsetse control targets (Vale et al., 1988) treated with deltamethrin (Glossinex®, Ecomark Ltd., Zimbabwe) were deployed in the grazing paddocks and around the stables. Commercial equine insect repellents containing a synthetic pyrethroid (Ultrashield®, Absorbine, USA) were applied topically. Nighttime usage of stable lighting was minimised to reduce the attraction of tsetse flies and other insects, and thick vegetation was cut back around the buildings.

2.6. Statistical analysis

A positive case was defined as parasites being found by microscopy, in combination with at least one clinical sign consistent with trypanosomosis. Incidence was calculated as the number of new cases per horse-year at risk and incidence rate ratio used to compare periods when horses were covered by chemoprophylaxis to periods they were not. For further analysis, only the first case recorded for each horse was considered. At the

beginning of the study period, difficulties were experienced obtaining drugs for treatment and treatment was delayed in some cases. Cases treated within 2 days of the first recorded clinical signs were regarded as treated promptly. Cases in which treatment was administered after more than 2 days were regarded as having delayed treatment. After assessment using the Anderson–Darling test for normality, a two-sample *T*-test was used to compare the PCV in cases where treatment was delayed to cases treated promptly. Fishers exact tests were used to identify associations between clinical signs and case outcome, and between treatment delay and resolution of clinical signs. Statistical analysis was conducted in R 2.4.1 (The R Foundation for Statistical Computing, http://www.r-project.org).

3. Results

Records were examined for 24 horses, present for all or part of the 23-month study period. Eighteen cases of trypanosome infection occurred in 14 horses.

3.1. Trypanosome species identification

Blood samples were collected onto FTA card from six of the eighteen cases, and were positive for *T. brucei* s.l. (three cases), *T. congolense* (one case) and *T. brucei* s.l. and *T. congolense* together (one case) by PCR. One sample was negative. Further analysis of the four *T. brucei* s.l. positive samples ruled out the human infective sub-species, *T. b. rhodesiense*.

3.2. Clinical signs and treatment

The clinical signs observed in each case were varied (Fig. 1). Depression, pyrexia and jaundice were the most common clinical signs (Fig. 1). At presentation, pyrexia was the most common clinical sign, either alone or in combination with other signs; only in one case was pyrexia not observed (Fig. 2).

Most horses responded quickly to treatment with quinapyramine, with pyrexia and oedema disappearing within 1 or 2 days (Fig. 3). In cases treated promptly (within 2 days of clinical signs first being observed) clinical signs were statistically significantly more likely to resolve within 5 days after treatment (Fishers Exact test, *P*-value 0.029). The distribution of case outcomes can be seen in Table 1.

Whilst cases that resolved quickly generally showed pyrexia, jaundice, depression and anorexia (Fig. 3), long-standing cases after delayed treatment were characterised by ataxia, anaemia, weight loss and behavioural changes (Fig. 4). Pyrexia and jaundice were not observed in the later phases of chronic cases. Ataxia, weight loss and severe anaemia (PCV $< 20\%$) were all statistically significant risk factors in case fatality (Fishers Exact test, *P*-values <0.01, 0.011, 0.015, respectively) (Table 1).

PCV (normal range 30–46% (Knottenbelt, 2005)) was reduced in every case of trypanosomosis (Fig. 5). Anaemia was severe when treatment was delayed, reaching as low as 12% in two cases. PCV was statistically significantly lower when treatment was delayed by more than 2 days (mean PCV 18.1%,

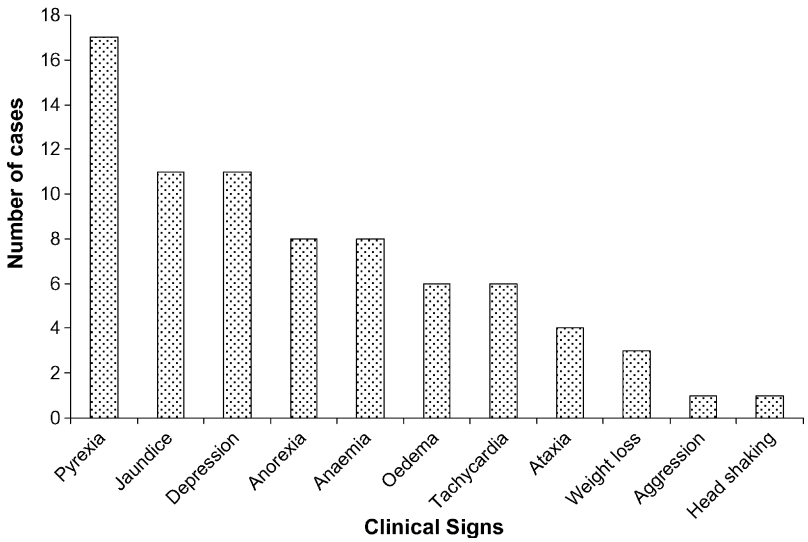

Fig. 1. Relative frequency of clinical signs observed during cases of trypanosomosis in horses ($n = 18$).

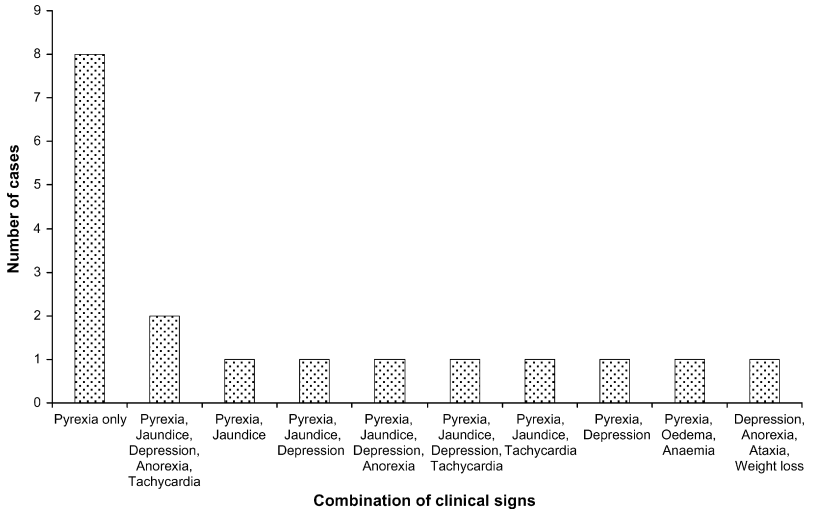

Fig. 2. Combinations of clinical signs seen *at presentation* in cases of trypanosomosis in horses ($n = 18$).

Fig. 5b, calculated from means of lowest PCV observed for each case during a 1-month period post-presentation) compared to cases treated within 2 days of presentation (mean PCV 29.1%, Fig. 5a) (two-sample T-test, d.f. = 11, $T = 3.71$, $P < 0.01$).

Thirteen cases were treated successfully, requiring no further treatment. In one case (case 17 in Fig. 4) the horse exhibited signs of mild head shaking which resolved after a second treatment 2 months after the initial episode. Four horses died despite treatment. These cases occurred at the beginning of the study period when treatment was delayed due to difficulties in obtaining drugs for treatment. One of these horses (case 18 in Fig. 4) is the only case that did not show pyrexia at presentation (Fig. 2) and showed a pattern of clinical signs consistent with chronic infection (anaemia, weight loss and ataxia). This horse is likely to have become infected before the start of the study period.

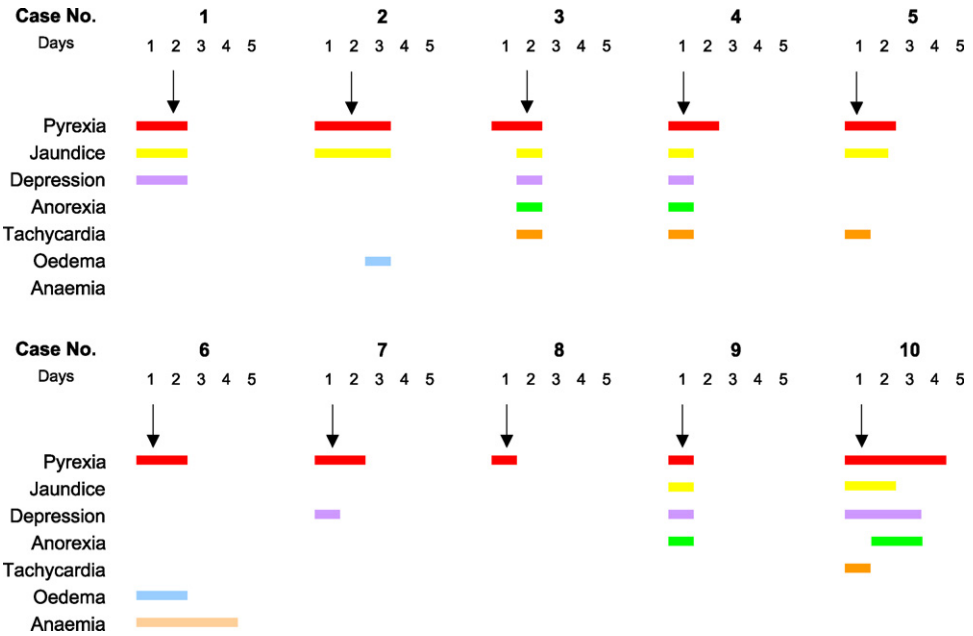

Fig. 3. Pattern of clinical signs seen in cases of trypanosomosis in horses where treatment was administered within 2 days of the onset of clinical signs. Arrow represents time of treatment.

Table 1
Distribution of the explanatory variables and outcomes used in statistical analysis

Resolution of clinical signs after treatment	Resolved in <5 days	Resolved in >5 days
Treatment <2 days after presentation		
Yes	6	1
No	1	5

Case outcome	Recovery	Fatality
Ataxia		
No	10	0
Yes	0	4
Weight loss		
No	10	1
Yes	0	3
Severe anaemia		
No	8	0
Yes	2	4

Post-mortem examination of the horse showed severe lymphoplasmacytic meningo-encephalomyelitis consistent with the presence of trypanosomes in the central nervous system.

Adverse effects were seen in all of the 15 horses where quinapyramine was used for treatment or prophylaxis. These effects were: colic, sweating and restlessness lasting 0.5–3 h, localised non-painful swelling at the injection site persisting for several months in some cases. No adverse effects were seen in the 12 horses where isometamidium was used.

Assuming a period of prophylactic cover of 3 months for both quinapyramine and isometamidium, the incidence rate whilst covered by prophylaxis was 0.39 cases per horse-year at risk, compared to 0.98 when not covered. The incidence rate ratio is 2.50 (95% CI 0.84–7.36).

4. Discussion

4.1. Diagnosis

The systematic diagnostic procedure used in this study helped to identify trypanosome infections as early as possible. Clinical signs previously described for trypanosomosis in horses, namely ventral and limb oedema, ataxia and paralysis (Taylor and Authie, 2004), were only observed late in the course of disease in our study. Kerato-conjunctivitis was not observed during the study. Pyrexia, jaundice and depression were the most prevalent, although less specific, early clinical signs. Pyrexia was a consistent feature of acute cases. Twice daily temperature monitoring is therefore a valuable and easily implemented tool for early case

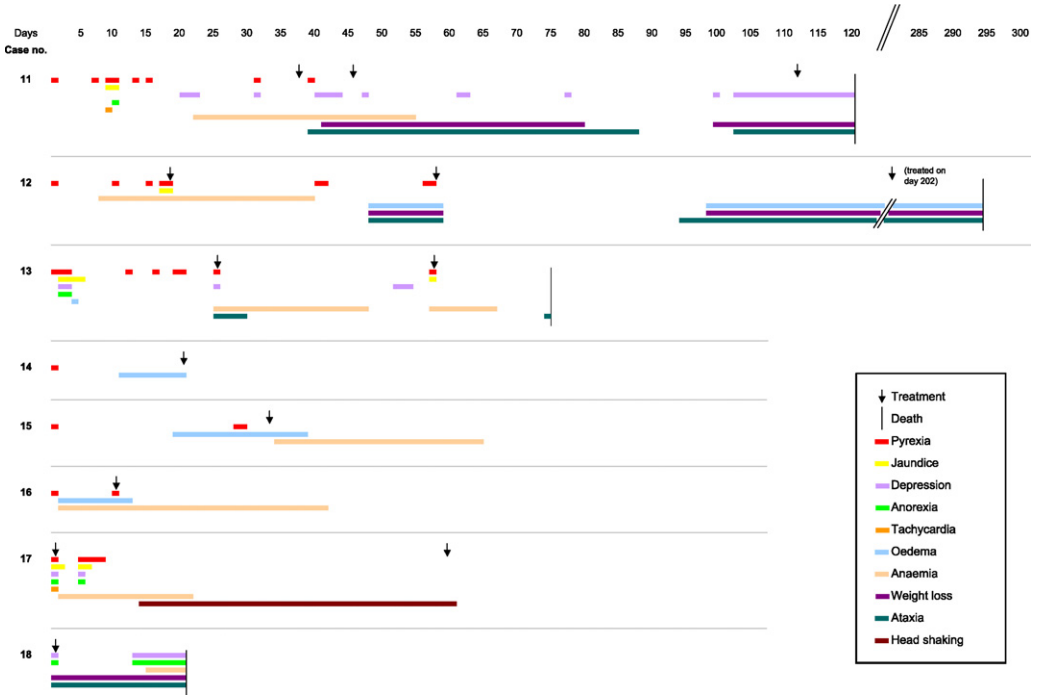

Fig. 4. Pattern of clinical signs seen in each case of trypanosomosis in horses where treatment was delayed for more than 10 days. Two anomalous cases are included (case 17 treated promptly but required second treatment; case 18 likely to have been infected before start of study period).

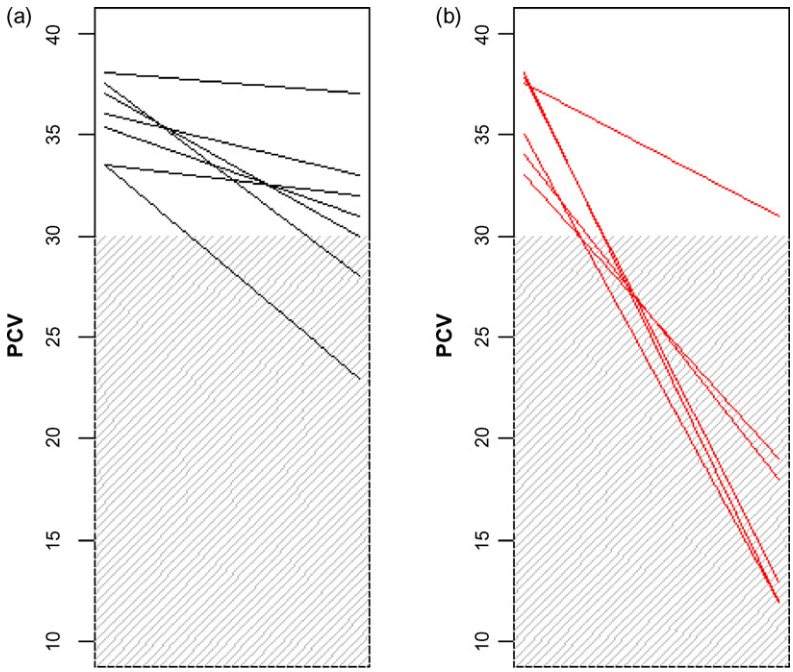

Fig. 5. Change in PCV during cases of trypanosomosis in horses. (a) Where treatment was administered <2 days after clinical signs were first observed ($n = 7$) and (b) where treatment was administered >2 days after clinical signs observed ($n = 6$). Lines join two points (i) initial PCV is the normal level for each individual horse when healthy and (ii) is the lowest PCV reached during a 1-month period post-presentation. Shaded area represents anaemia.

indication. Repeated examination of blood smears is essential in pyrexic cases to confirm trypanosomosis and eliminate other causes of pyrexia such as equine babesiosis (*Babesia caballi*) and theileriosis (*Theileria equi*). Anaemia, a clinical sign frequently utilised for diagnosis of trypanosomosis in cattle (Murray and Dexter, 1988), was uncommon in acute infections. Therefore monitoring PCV to identify cases of trypanosomosis may only be reliable as an aid in detecting more chronic infections. Diagnosis of chronic trypanosomosis becomes more difficult as parasitaemia detectable by microscopy is rarely seen (Van den Bossche et al., 2005), and is therefore more reliant on the clinical signs observed. Identification of a chronic case showing signs that include ataxia, anaemia and weight loss must confer a poor prognosis.

Molecular diagnostics showed that in contrast to previous findings (Kihurani et al., 1994), *T. congolense* and *T. b. brucei* are both able to cause disease as sole pathogens in horses. These trypanosome species are prevalent in the adjacent Serengeti ecosystem in a range of wildlife species (Kaare et al., 2007). Analysis of further case records to determine individual clinical presentations for different trypanosome species would be valuable.

4.2. Treatment

The susceptibility of horses to trypanosome infections makes prompt treatment essential. Delaying treatment increases both the course of disease and the severity of clinical signs. Quinapyramine is an effective treatment in acute infections, with severe but transient side effects. Use of quinapyramine in chronic cases, whilst sometimes leading to a short-term improvement in condition, did not usually prevent progression of disease in the longer term. The neurological signs such as ataxia, head shaking and behavioural changes observed in these cases indicate that trypanosomes have invaded the central nervous system. Quinapyramine, isometamidium and diminazene are unable to cross the blood brain barrier. However, melarsomine is able to penetrate the cerebrospinal fluid (Raynaud et al., 1989) and it is hoped this may provide a future treatment option for cases in which neurological signs are present.

4.3. Prophylaxis

Maintenance of insecticide-treated tsetse control targets and use of pour on or spray formations is costly

and cannot prevent horses from some contact with tsetse. Tsetse challenge varies considerably both spatially and temporally. Maintenance of a rigorous prophylactic protocol is necessary, and particularly important at times of high tsetse challenge. Cases are likely to be minimised by combining physical tsetse control measures with strategic chemoprophylaxis. In this location resistant trypanosome populations are unlikely to arise due to the presence of a large and diverse wildlife population which forms the source of infection for the horses. However quinapyramine has been implicated in the development of multiple drug resistance and the use of sanative pairs of trypanocides may be prudent. Further work is needed to quantify the protective effect of chemoprophylaxis and to determine the relative efficacy of quinapyramine and isometamidium.

5. Conclusion

We conclude that health management of horses under high challenge from trypanosomes requires considerable management skill and resources. The clinical presentation of trypanosomosis in horses differs from that in cattle and early diagnosis and prompt treatment are essential to prevent progression to chronic illness with a poor prognosis. This study has highlighted that with a combination of prompt diagnosis and treatment, strategic use of prophylactics and implementation of measures to reduce fly exposure, cases may be reduced to manageable levels. However, infection remains a constant threat and demands continual vigilance. Being a susceptible exotic species, it appears that domestic horses can only be maintained under conditions of high trypanosome challenge if health and stable management are of the highest standard.

Acknowledgements

We gratefully acknowledge the material and financial contributions given to this study on a pro-rata basis by the partner organisations: Singita-Grumeti Reserves Ltd., the University of Edinburgh and the Messerli Foundation of Switzerland that provides full funding for the Tanzania Wildlife Research Institute (TAWIRI) Wildlife Veterinary Programme. We are grateful to Rob Jackson for his invaluable advice on treatment protocols, Vijay Varma for his commitment to the project and ongoing veterinary care of the horses at Singita-Grumeti Reserves Ltd., and Maulidi Mdaki for sample processing and initial diagnostic work in the TAWIRI Veterinary Laboratory. We thank Sarah Cleaveland, Darren Shaw, Crawford Revie and two anonymous referees for helpful comments on the manuscript.

References

Awan, M.A.Q., Johnston, R.S., 1979. Some observations on an outbreak of trypanosomiasis in horses in the Republic of Zambia. Bull. Anim. Health Prod. Afr. 27, 177–179.

Barrowman, P.R., 1990. Equine trypanosomiasis. Donkeys Mules and Horses in Tropical Agricultural Development. University of Edinburgh, Edinburgh.

Challier, A., 1982. The ecology of tsetse (*Glossina* spp.) (Diptera, Glossinidae): a review (1970–1981). Insect Sci. Appl. 3, 97–143.

Cox, A., Tilley, A., McOdimba, F., Fyfe, J., Eisler, M., Hide, G., Welburn, S., 2005. A PCR based assay for detection and differentiation of African trypanosome species in blood. Exp. Parasitol. 111, 24–29.

Fenger, C.K., 2004. Antiprotozoal drugs. In: Bertone, J.J., Horspool, L.J.I. (Eds.), Equine Clinical Pharmacology. Saunders.

Kaare, M.T., Picozzi, K., Mlengeya, T., Fevre, E.M., Mellau, L.S., Mtambo, M.M., Cleaveland, S., Welburn, S.C., 2007. Sleeping sickness—a re-emerging disease in the Serengeti? Travel Med. Infect. Dis. 5, 117–124.

Kihurani, D.O., Nantulya, V.M., Mbiuki, S.M., Mogoa, E., Nguhiumwangi, J., Mbithi, P.M.F., 1994. *Trypanosoma brucei*, *Trypanosoma congolense* and *T. vivax* Infections in horses on a farm in Kenya. Trop. Anim. Health Prod. 26, 95–101.

Kinabo, L.D.B., Bogan, J.A., 1988. Pharmokinetic and histopathological investigations of isometamidium in cattle. Res. Vet. Sci. 44, 267–269.

Knottenbelt, D.C., 2005. Saunders Equine Formulary, 2nd edition. Saunders.

Leach, T.M., Roberts, C.J., 1981. Present status of chemotherapy and chemoprophylaxis of animal trypanosomiasis in the eastern-hemisphere. Pharmacol. Ther. 13, 91–147.

Maqbool, A., Ahmad, S., Raza, M.A., 1996. Trypanocidal efficacy of different drugs and their effect on the blood picture on equine trypanosomiasis. Indian J. Anim. Sci. 66, 315–317.

Murray, M., Dexter, T.M., 1988. Anemia in bovine African trypanosomiasis. Acta Trop. 45, 389–432.

Picozzi, K., Fevre, E.M., Odiit, M., Carrington, M., Eisler, M.C., Maudlin, I., Welburn, S.C., 2005. Sleeping sickness in Uganda: a thin line between two fatal diseases. Br. Med. J. 331, 1238–1241.

Raynaud, J.P., Sones, K.R., Friedheim, E.A.H., 1989. A review of Cymerlarsan: a new treatment proposed for animal trypanosomiasis due to *T. evansi* and other trypanosomes of the *T. brucei* group. In: Proceedings of the 20th Meeting on International Scientific Council for Trypanosomiasis Research and Control, Mombasa, Kenya, pp. 334–338.

Stephen, L.E., 1970. Clinical manifestations of the trypanosomiases in livestock and other domestic animals. In: Mulligan, H.W. (Ed.), The African Trypanosomiases. George Allen and Unwin, London, pp. 778–789.

Taylor, K., Authie, E.M.-L., 2004. Pathogenesis of animal trypanosomiasis. In: Maudlin, I., Holmes, P.H., Miles, M.A. (Eds.), The Trypanosomiases. CABI Publishing.

Tuntasuvan, D., Jarabrum, W., Viseshakul, N., Mohkaew, K., Borisutsuwan, S., Theeraphan, A., Kongkanjana, N., 2003. Chemotherapy of surra in horses and mules with diminazene aceturate. Vet. Parasitol. 110, 227–233.

Vale, G.A., Lovemore, D.F., Flint, S., Cockbill, G.F., 1988. Odor-baited targets to control tsetse flies, *Glossina* spp. (Diptera, Glossinidae), in Zimbabwe. Bull. Entomol. Res. 78, 31–49.

Van den Bossche, P., Ky-Zerbo, A., Brandt, J., Marcotty, T., Geerts, S., De Deken, R., 2005. Transmissibility of *Trypanosoma brucei* during its development in cattle. Trop. Med. Int. Health 10, 833–839.