



Virulence of *Trypanosoma congolense* strains isolated from cattle and African buffaloes (*Syncerus caffer*) in KwaZulu-Natal, South Africa

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Trypanosoma congolense and *Trypanosoma vivax* are major species that infect cattle in north-eastern KwaZulu-Natal (KZN), South Africa. Of the two genetically distinct types of *T. congolense*, Savannah and Kilifi sub-groups, isolated from cattle and tsetse flies in KZN, the former is more prevalent and thought to be responsible for African animal trypanosomosis outbreaks in cattle. Furthermore, variation in pathogenicity within the Savannah sub-group is ascribed to strain differences and seems to be related to geographical locations. The objective of the present study was to compare the virulence of *T. congolense* strains isolated from African buffaloes (*Syncerus caffer*) inside Hluhluwe-iMfolozi Park, and from cattle on farms near wildlife parks (< 5 km), to isolates from cattle kept away (> 10 km) from parks. To obtain *T. congolense* isolates, blood of known parasitologically positive cattle or cattle symptomatically suspect with trypanosomosis, as well as isolates from buffaloes kept inside Hluhluwe-iMfolozi Park were passaged in inbred BALB/c mice. A total of 26 *T. congolense* isolates were obtained: 5 from buffaloes, 13 from cattle kept near parks and 8 from cattle distant from parks. Molecular characterisation revealed 80% and 20% of isolates to belong to *T. congolense* Savannah and Kilifi, respectively. To compare virulence, each isolate was inoculated into a group of six mice. No statistical differences were observed in the mean pre-patent period, maximum parasitaemia or drop in packed cell volume (PCV). Significant differences were found in days after infection for the drop in PCV, the patent period and the survival time. These differences were used to categorise the isolates as being of high, moderate or low virulence. Based on the virulence, 12 of 26 (46%) isolates were classified as highly virulent and 27% each as either of moderate or of low virulence. Whilst 11 of 12 high virulent strains were from buffaloes or cattle near the park, only 1 of 7 low virulent strains was from these animals. All the Kilifi *T. congolense* types were less virulent than the Savannah types. These results confirmed the higher virulence of *T. congolense* Savannah type compared to Kilifi type and indicated the prevalence of highly virulent strains to be higher in wildlife parks and in cattle near the parks than on farms further away. The geographical location of these strains in relation to the wildlife parks in the area was discussed.

Introduction

African animal trypanosomosis or nagana is a wasting disease of livestock caused by unicellular blood parasites of the genus *Trypanosoma* commonly known as trypanosomes (Minnaar 1989). Pathogenic trypanosomes infecting livestock are primarily transmitted cyclically by various species of *Glossina*, tsetse flies (Diptera: Glossinidae). However, mechanical transmission of certain trypanosome species, such as *Trypanosoma vivax* and *Trypanosoma evansi*, by biting flies of the family Tabanidae does occur (Allsopp & Newton 1985; Gardiner 1989; Hoare 1972). In South Africa, animal trypanosomosis is restricted to the north-eastern parts of KwaZulu-Natal (KZN) Province. Pathogenic trypanosomes infecting cattle in KZN are *Trypanosoma congolense* and *T. vivax* (Gillingwater, Mamabolo & Majiwa 2010; Mamabolo *et al.* 2009; Van den Bossche *et al.* 2006). *Trypanosoma congolense* is considered the most pathogenic trypanosome species infecting livestock with great variation in its virulence (Bengaly *et al.* 2002). Recently, two genetically distinct types of *T. congolense*, Savannah and Kilifi, have been isolated from cattle and tsetse flies in KZN (Mamabolo *et al.* 2009). The main vector for *T. congolense* in this region is *Glossina austeni* (Motloang *et al.* 2012). Variation in pathogenicity of *T. congolense* Savannah sub-group is ascribed to strain differences (Masumu *et al.* 2006; Van den Bossche *et al.* 2011). In South Africa, Savannah sub-group is more prevalent and thought to be responsible for occasional disease outbreaks in north-eastern KZN (Gillingwater *et al.* 2010; Mamabolo *et al.* 2009).

African trypanosomes exhibit a wide range of virulence in vertebrate hosts (Joshua 1990). Nonetheless, the range and severity of pathological effects are influenced by a range of variables

that involves the parasite, geographic regions and the host (Murray & Morrison 1978; Taylor & Authié 2004). Substantial variations in pathogenicity between strains of Savannah subgroup exist, with some strains producing more severe disease outcomes than others (Masumu *et al.* 2006; Van den Bossche *et al.* 2011). This variation has been associated with the type of transmission occurring either inside wildlife reserves or in communal areas where large wild animals are rare or absent. Sylvatic transmission associated with large wild animals is responsible for acute disease in livestock kept at the wildlife–livestock interface. In contrast, infection in cattle kept where large wild animals are rare or absent is mild, as a result of the domestication of trypanosome strains (Van den Bossche 2001). In a recent study, transmissibility of *T. congolense* to tsetse flies was shown to be influenced by its pathogenicity within the cycle type (Chitanga *et al.* 2013). Both sylvatic and domestic strains were significantly better transmitted if they are more pathogenic to mice.

This study was carried out to investigate the virulence of *T. congolense* strains isolated from African buffaloes (*Syncerus caffer*) kept inside Hluhluwe-iMfolozi Park (HiP) and from cattle kept by communal farmers near and farther away from wildlife parks in north-eastern KZN, South Africa. The study not only expands on the findings of Van den Bossche (2001) but is also the first to determine the geographical location of virulent *T. congolense* strains in KZN, South Africa.

Research method and design

Sampling

Blood samples were collected from cattle at eight communal diptanks and one commercial farm in north-eastern KZN. Five of the eight diptanks were located less than 5 km from wildlife parks. None of the blood samples taken at diptank 1, 3, 5 and 6 (Ekuhlehleni, Khume, Ntabayengwe and Ekuphindisweni, respectively), were parasitologically positive. As a result, only four diptanks: 2, 4, 7 and 8 circled in black (Figure 1) are referred to in the present study. Of these, Mvutshini (28.124369 S; 32.134825 E) and Ocilwane (28.412914 S; 32.992797 E) are situated 2.6 km and 3.7 km from HiP, respectively, whilst Ndumo (26.933675 S; 32.281539 E) lies 2.8 km south of Ndumo Game Reserve. The fourth diptank, Nhlanzana (27.312822 S; 32.237039 E) is located 47.9 km south of Ndumo Game Reserve (Figure 1). Additional sampling was undertaken at Boomerang commercial farm (28.23285 S; 32.298717 E) situated about 10 km away from Charters Creek, the closest protected area harbouring large wild animals. Buffalo blood samples originating from HiP (28.219592 S; 31.947886 E) were obtained in the form of stabilate from the reference collection at the Department of Veterinary Tropical Diseases, Faculty of Veterinary Sciences of the University of Pretoria.

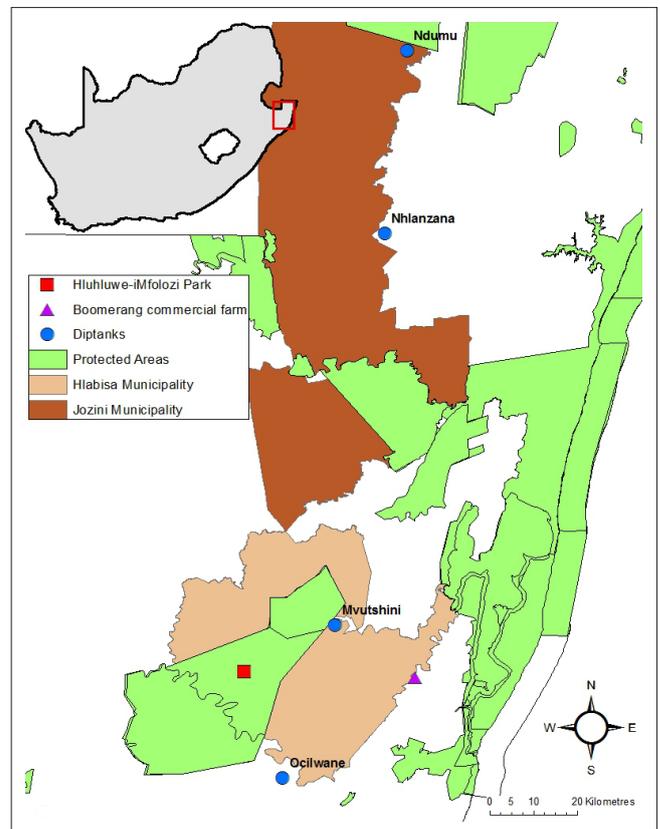
Stabilate preparation

Blood from individual cattle was screened to determine the packed cell volume (PCV) and the presence of trypanosome

parasites using the buffy coat dark ground/phase contrast technique (Murray, Murray & McIntyre 1977). Blood samples from infected cattle or cattle symptomatically suspected to be suffering from nagana as determined by low PCV values (Marcotty *et al.* 2008) were inoculated intraperitoneally in inbred BALB/c mice (Masumu *et al.* 2006). The inoculated mice were monitored three times a week for the development of parasitaemia by examination of wet blood films prepared from tail-derived blood drops (Herbert & Lumsden 1976). Blood of parasitologically positive mice was used to prepare buffy coat on FTA[®] elute micro-cards for DNA analysis. The micro-cards were allowed to air-dry and stored in sealed plastic bags at room temperature until use. The remaining blood was stored as stabilate at -180 °C until required. In total, 26 stabilates were prepared, amongst which 5 originated from buffaloes, 13 from cattle near wildlife parks and 8 from cattle kept away from parks.

Molecular characterisation of trypanosomes

Trypanosoma DNA was characterised using universal primer pairs targeting the segment of the 18S ribosomal RNA gene of all trypanosomes described by Geysen, Delespau and Geerts (2003). Species characterisation was performed with the application of oligonucleotide primers amplifying the satellite DNA monomers of *Trypanosoma brucei* (Sloof *et al.* 1983), *T. congolense* Kilifi type, (Masiga *et al.* 1992), *T. congolense* Savannah type (Majiwa & Otieno 1990) and *T. vivax*



Source: Map drawn by Chantel de Beer

FIGURE 1: Map showing the location of the six sampling sites in KwaZulu-Natal Province: Ndumo, Nhlanzana, Mvutshini, Ocilwane, Hluhluwe-iMfolozi Park and Boomerang commercial farm.



(Masake *et al.* 1997). Known Kilifi (OVIKZNTT/7098/07) and Savannah (IL1180) *T. congolense* strains were used as positive controls. In addition, TVIL 2160, TBA4 and OV11 were used as positive controls for *T. vivax*, *T. brucei* and *Trypanosoma equiperdum*, respectively, whilst pure grade water was added as a negative control.

Following DNA extraction, amplification for both the 18S rRNA gene and species-specific polymerase chain reaction (PCR) was carried out in an automated Eppendorf Mastercycler gradient cycler (Eppendorf, Hamburg, Germany). PCR conditions for the 18S rRNA gene included denaturation for 4 min at 94 °C, followed by 35 cycles of 30 s denaturation at 94 °C, 30 s annealing at 58 °C and 2 min extension at 72 °C, whereas conditions for species-specific amplification included denaturation at 94 °C for 3 min, followed by 30 cycles of 1 min each at 94 °C, 60 °C and 72 °C. Amplified products were resolved by electrophoresis through 1.5% agarose gel containing 0.5 µg/mL of ethidium bromide. Products were run against a 100 bp molecular marker, O'GeneRuler (Fermentas, Waltham, USA). Separated PCR products were analysed using a gel documenting system (Quantity One 4.6.3; BioRad, Berkeley, USA) and species identified according to their molecular band sizes.

Assessment of virulence

The virulence of each *T. congolense* isolate was determined in a group of six BALB/c mice. Each isolate was first resuscitated in two mice to prepare the infective doses. The mice were monitored every second day for the development of parasitaemia as described above. When parasitaemia in infected mice was estimated to be $10^{7.8}$ trypanosomes per mL or higher, a drop of tail blood was diluted in phosphate buffer saline glucose (PSG) to make a final concentration of 10^5 trypanosomes in a total volume of 0.2 mL (Masumu *et al.* 2006). The resultant mix was used immediately as an infection dose (0.2 mL). An additional group of six mice injected with 0.2 mL PSG was used as a negative control.

Parasitaemia was recorded daily for the first 2 weeks and every second day for the remainder of the experiment, up to 90 days post infection, as described by Masumu *et al.* (2006). Mice were considered parasitologically negative when no trypanosomes were detected in at least 50 microscopic fields. Mice were declared uninfected when parasites could not be detected for 30 successive days, that is, for 15 successive samplings. A baseline PCV was recorded for each mouse in all the groups by microhaematocrit centrifugation methods before infection. Subsequent PCV was measured three times a week for the first 2 weeks following infection and once a week for the duration of the experimental period. Death was recorded every day for all experimental and control groups. Virulence categories were determined according to the criteria described by Bengaly *et al.* (2002) and Masumu *et al.* (2006), with some modification. This included days to percentage drop in PCV, whilst the pre-patent period (PPP), the patent period (PP) and the survival time (ST) of each group of mice were retained. Based on these parameters, the field isolates were subsequently classified as high, moderate and low virulence strains (high virulence strain [HVS],

moderate virulence strain [MVS] and low virulence strain [LVS], respectively).

All experimental mice were kept under standard animal housing facilities in an air conditioned room (22 °C – 23 °C) with a relative humidity of 60% – 70%. Mice were fed mouse pellets and supplied with clean water *ad libitum*.

Statistical analysis

For the purpose of this study, a generalised linear model was used firstly to compare strain variability irrespective of origin and secondly with respect to origin. The number of days measured for the PPP, PP, ST and percentage PCV were used as continuous explanatory variables, whilst trypanosome strains were used as categorical explanatory variables. For the first three continuous explanatory variables, no measurement was made for the controls. The data were subjected to a one-way analysis of variance (ANOVA) excluding the controls to test for a significant strain effect. In the case where the strain effect was significant, the Student's t-LSD (least significant difference) was calculated at a 5% level of significance to compare and group strain means. Controls were included for the PCV variable, therefore the first ANOVA was performed to test whether the controls differ significantly followed by the final ANOVA performed to compare the different strains with the pooled controls.

Results

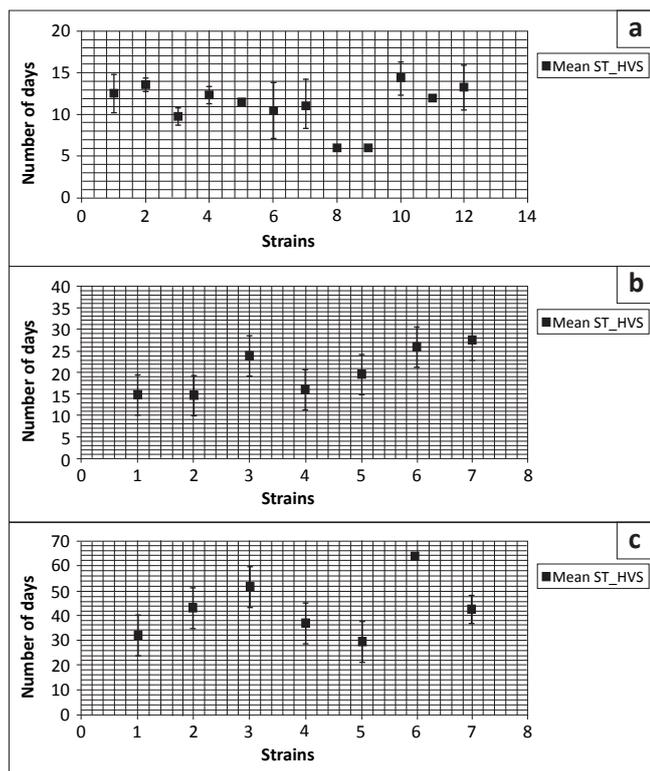
Molecular identification of trypanosomes

Using genotype-specific primers, amplification products for Savannah and Kilifi revealed 26 *T. congolense* isolates, of which 24 (92.3%) belonged to the Savannah and 2 (7.7%) were Kilifi sub-groups. All the strains gave a single molecular band profile following amplification with species-specific primer, confirming that there were no mixed infections. DNA of samples amplified with Savannah-specific primers could not be amplified when Kilifi, *T. brucei* and *T. vivax* specific primers were used. Moreover, when the primer sets for *T. vivax* and *T. brucei* were used, none of the samples were amplified except for the controls. Of the 24 *T. congolense* Savannah isolates, 5 were isolated from buffalo, 13 from cattle at the HiP interface and 6 from cattle kept further away from parks. All of the *T. congolense* Kilifi were obtained from areas away from the parks.

Classification of virulent strains

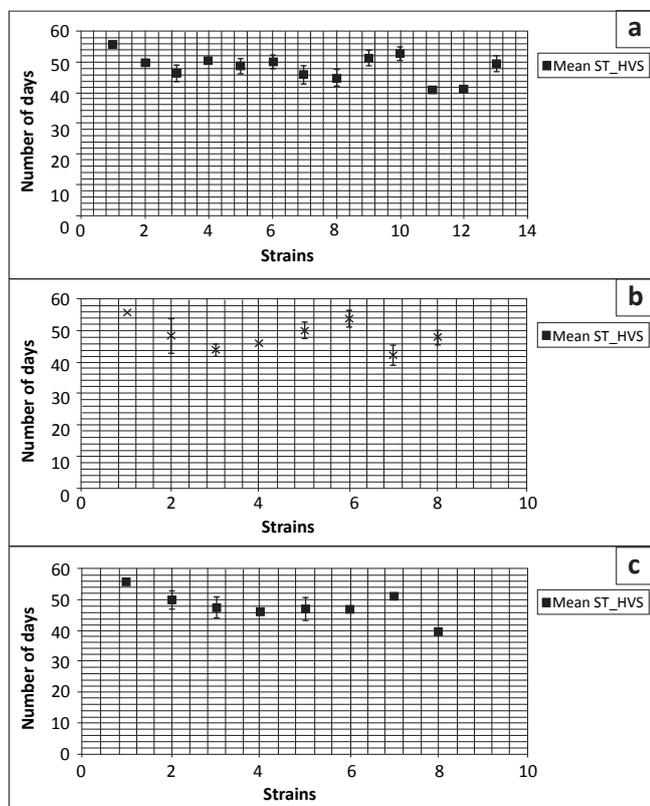
Isolates that killed mice within 2 weeks post infection were classified as HVS ($6 \pm 0.0 - 14 \pm 2.1$). Those that killed mice after 2 weeks up to a month were MVS ($16 \pm 2.1 - 27 \pm 7.3$) and where mice survived for longer than a month, the strains were categorised as LVS ($31 \pm 6.4 - 64 \pm 0.0$). Graphical representation of the results is shown in Figure 2. Mortality in the HVS, MVS and LVS groups was 100%, 100% and 82.0%, respectively. Only 8.3% of mice injected with the Kilifi strain died.

Variability between individual strains was highly significant for all explanatory variables ($p < 0.0001$). Grouping of strains



Source: Authors' own creation
ST, survival time; HVS, high virulence strain; MVS, moderate virulence strain; LVS, low virulence strain.

FIGURE 2: Trends in the mean survival time for the (a) high virulence strain ($n = 12$), (b) moderate virulence strain ($n = 7$) and (c) low virulence strain ($n = 7$).



Source: Authors' own creation
PCV, packed cell volume; HVS, high virulence strain; MVS, moderate virulence strain; LVS, low virulence strain.

FIGURE 3: Development of packed cell volume represented as mean for (a) high virulence strain, (b) moderate virulence strain and (c) low virulence strain. Mean control values are represented as the first point for the three categories.

using the LSD value revealed that 46.2% were HVS, whilst 26.9% were either MVS or LVS (Table 1). The PPP and the PCV mean differences between the HVS, MVS and LVS categories were not significant ($p = 0.2739$ and $p = 0.7738$, respectively). In Figure 3, the first point in the three graphs represents the mean control PCV values.

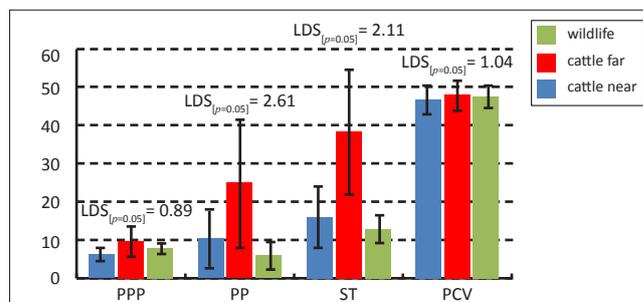
The mean differences for PP and the ST, on the other hand, were highly significant ($p < 0.0001$) between the three categories (Table 2).

Comparison and geographical distribution of virulent strain

A summary of the prevalence and distribution of *T. congolense* strains is presented in Table 3. In all, 26 strains were collected and analysed. Of these, 5 (19.2%), 13 (50.0%) and 8 (30.8%) were collected inside the Park, near to, and away from the parks, respectively. The difference in PPP between strains collected from the buffaloes inside HiP, cattle near the parks and cattle far from the parks was highly significant ($LSD_{[p=0.05]} = 0.89$). The PPP was recorded earlier in strains collected near the parks and strains collected inside HiP (mean 6.2 ± 2.3 and 7.9 ± 2.0 , respectively), compared to strains collected far from the parks (mean 9.7 ± 4.3). Similarly, the PP ($LSD_{[p=0.05]} = 2.67$) and ST ($LSD_{[p=0.05]} = 2.11$), although significant between the three areas, were shorter in strains collected from the buffaloes (mean 5.9 ± 4.1 and 12.8 ± 4.1 , respectively) and from cattle near the parks (mean 10.4 ± 8.2 and 16.0 ± 8.6 , respectively). Longer PP and ST were observed in strains collected far from the parks (mean 25.0 ± 17.3 and 38.3 ± 16.9 , respectively). When the PCV variable was considered, the difference was not significant ($LSD_{[p=0.05]} = 1.04$). The means were 46.8 ± 4.3 , 48.0 ± 4.5 and 47.6 ± 3.5 for strains collected from cattle close to and far from parks and buffaloes inside HiP, respectively (Figure 4).

Ethical considerations

Animal ethics approval for the experiments was obtained from the Animal Ethics Committee of the Agricultural Research Council – Onderstepoort Veterinary Institute (ARC-OVI) (ref. 07/20/C174) and the Animal Use and Care Committee of the University of Pretoria, Faculty of Veterinary Science (ref. VO56-09).



Source: Authors' own creation
PPP, pre-patent period; PP, patent period; ST, survival time; PCV, packed cell volume; LSD, least significant difference.

FIGURE 4: Significance in the pre-patent period, patent period, survival time and packed cell volume between strains collected from Hluhluwe-iMfolozi Park, cattle near (< 5 km) and far (> 10 km) from game parks, with least significant difference calculated at $p = 0.05$.

**TABLE 1:** Means with standard deviations to compare and group strains into high, moderate and low virulence categories using the least significant difference.

N	%	Strain	Origin	Virulence category	Mean					
					PPP ± s.d.	PP ± s.d.	ST ± s.d.	PCV ± s.d.		
12	46.2	Bmrng_A1	Far	High	10 ± 0.0	3 ± 1.3	12 ± 1.0	49 ± 2.4		
		MVUKZN_10	Close	High	6 ± 2.1	8 ± 0.5	12 ± 0.0	41 ± 1.6		
		MVUKZN_5	Close	High	5 ± 0.8	7 ± 2.5	11 ± 2.9	45 ± 2.8		
		MVUKZN_6	Close	High	6 ± 1.6	2 ± 1.6	6 ± 0.0	51 ± 2.3		
		MVUKZN_7	Close	High	3 ± 0.6	4 ± 0.5	6 ± 0.0	53 ± 2.0		
		MVUKZN_9	Close	High	5 ± 2.1	10 ± 3.3	14 ± 2.1	41 ± 1.6		
		MVUZN_2	Close	High	8 ± 0.0	5 ± 0.5	12 ± 0.5	50 ± 2.5		
		OClKZN_1	Close	High	7 ± 0.1	6 ± 2.7	13 ± 2.6	49 ± 2.1		
		TC-UP-KZNB/1/06	Wild	High	9 ± 0.1	5 ± 1.8	13 ± 2.3	50 ± 1.5		
		TC-UP-KZNB/3/06	Wild	High	10 ± 1.6	5 ± 0.9	14 ± 0.8	46 ± 2.7		
		TC-UP-KZNB/4/06	Wild	High	7 ± 0.8	4 ± 1.7	10 ± 1.0	50 ± 0.8		
		ndmT_1	Close	High	5 ± 1.2	7 ± 3.6	11 ± 3.3	46 ± 2.9		
		7	26.9	MVUKZN_1	Close	Moderate	7 ± 0.0	17 ± 2.6	24 ± 3.7	46 ± 1.6
				MVUKZN_3	Close	Moderate	6 ± 0.0	22 ± 7.1	27 ± 7.3	48 ± 2.6
MVUKZN_8	Close			Moderate	10 ± 4.2	7 ± 3.6	16 ± 2.1	50 ± 2.4		
NdmH_2	Close			Moderate	5 ± 0.8	17 ± 9.2	26 ± 3.1	42 ± 3.1		
TC-UP-KZNB/2/06	Wild			Moderate	9 ± 3.0	7 ± 5.2	15 ± 6.4	48 ± 5.5		
TC-UP-KZNB/5/06	Wild			Moderate	6 ± 1.3	9 ± 6.6	15 ± 6.5	44 ± 2.1		
bmrngK_1	Far			Moderate	16 ± 4.0	5 ± 2.0	20 ± 5.4	54 ± 2.3		
7	26.9	BMRNGK_5*	Far	Low	12 ± 5.6	10 ± 11.0	64 ± 0.0	51 ± 1.2		
		BmrngK_2	Far	Low	9 ± 0.0	23 ± 6.4	31 ± 6.4	50 ± 2.8		
		MVUKZN_4	Close	Low	8 ± 2.3	23 ± 10.6	30 ± 8.6	47 ± 1.6		
		bmrngK_3	Far	Low	14 ± 3.8	39 ± 15.0	51 ± 12.8	46 ± 1.8		
		njB_1	Far	Low	8 ± 1.2	38 ± 8.7	43 ± 6.4	48 ± 3.4		
		njT_1	Far	Low	7 ± 0.0	31 ± 7.3	37 ± 6.9	47 ± 3.3		
		njT_2*	Far	Low	4 ± 0.0	46 ± 5.7	43 ± 5.7	40 ± 1.8		
		Control	-	-	-	-	-	-	56 ± 1.1	
LSD ($p = 0.05$)	-	-	-	-	2.43	7.11	5.75	3.07		

*, *Trypanosoma congolense* Kilifi.

TABLE 2: Mean pre-patent period, patent period, survival time and the packed cell volume progression of South African *Trypanosoma congolense* strains belonging to high, moderate and low virulence strains.

Parameters	Virulence categories			p-value
	HVS ± s.d.	MVS ± s.d.	LVS ± s.d.	
PPP (days)	6.8 ± 2.3	7.8 ± 3.8	8.8 ± 4.0	0.2739
PP (days)	5.5 ± 3.0	12.7 ± 8.0	29.9 ± 14.0	0.0001
ST (days)	11.1 ± 3.0	20.5 ± 7.0	42.8 ± 13.0	0.0001
PCV (%)	47.5 ± 4.0	47.4 ± 4.0	46.9 ± 4.0	0.7738

PPP, pre-patent period; PP, patent period; ST, survival time; PCV, packed cell volume; HVS, high virulence strain; MVS, moderate virulence strain; LVS, low virulence strain; s.d., standard deviation.

TABLE 3: Prevalence and distribution of high, moderate and low virulence strains in the study areas.

Category	Hluhluwe-iMfolozi Park		Near game parks (< 5 km)		Far from game parks (> 10 km)	
	N	%	N	%	N	%
HVS	3	60.0	8	61.5	1	12.5
MVS	2	40.0	61.5	30.7	1	12.5
LVS	0	0.0	4	7.6	6	75.0
Total	5	100	13	100	8	100

Discussion

Three pathogenic species of *Trypanosoma* have been identified in South Africa. *Trypanosoma brucei* was identified in the 19th century by Sir David Bruce (Bruce 1895) and *T. congolense* and *T. vivax* have been isolated on several occasions from tsetse flies, buffalo and cattle blood but no isolates of *T. brucei* have been found for a long time (Gillingwater *et al.* 2010; Mamabolo *et al.* 2009; Van den Bossche *et al.* 2006). It is therefore possible that *T. brucei* disappeared from South Africa when *Glossina pallidipes* was eradicated between 1945 and 1952 (Du Toit 1954).

Both *T. congolense* and *T. vivax* and tsetse fly vectors are present in the study area (Gillingwater *et al.* 2010; Mamabolo *et al.* 2009; Motloang *et al.* 2012; Van den Bossche *et al.* 2006).

In the present study, *T. congolense* was selected as the species of interest because of its prevalence in KZN. *Trypanosoma vivax* infections may have been eliminated by the isolation method used which involved sub-inoculation of mice. There is evidence that wild isolates of *T. vivax* fail to develop and establish in mice (Eisler *et al.* 2004), whilst the *Nannomonas* subgenus, to which *T. congolense* belongs, has been shown to grow readily in laboratory rodents (Joshua 1990; Morrison *et al.* 2010). In the present study, molecular analysis has confirmed the identity of 26 isolates belonging to *T. congolense*. This confirmation excludes the possibility of contamination with any other trypanosome species. In addition, genotype-specific PCR unequivocally distinguished between *T. congolense* Savannah and Kilifi sub-groups based on specific band products observed for each.

Although the presence of *T. congolense* virulent strains is reported in other studies (Masumu *et al.* 2006; Van den Bossche *et al.* 2011), this is the first attempt to determine the geographical distribution of virulent strains in KZN. Notwithstanding the limited number of samples used, it is evident from the results obtained that Savannah type *T. congolense* is more prevalent (92.3%) with severe infections than Kilifi (7.7%). Although sampling was random, preference was occasionally given to symptomatically sick animals to increase the chances of obtaining parasitologically positive blood samples and to confirm that trypanosomosis does contribute to disease burden in these animals. The possibility therefore exists that some LVS, including Kilifi infections, may have been missed. The current results indicating low prevalence of Kilifi strains are in agreement with recent reports by Mamabolo *et al.* (2009) and Gillingwater *et al.* (2010). In both studies, 0.8% and 16.0% of mixed infections with Savannah and Kilifi were reported. In endemic areas cattle are continuously challenged with infected tsetse. As a result, a large proportion of cattle harbour



infections with more than one trypanosome species or sub-species (Masumu *et al.* 2009), thus explaining mixed infections in the two former studies. In the present study, however, mixed infections were eliminated by selecting strains that gave a single molecular band profile when amplified with genotype-specific primers to fulfil the objective of this study.

The *T. congolense* Savannah strains characterised in this study suggested a range of clinical outcomes ranging from high, moderate to low virulence. On the other hand, neither of the two Kilifi strains was highly or moderately virulent as they both fell into the low virulence category. These findings confirm the observations made by Bengaly *et al.* (2002) that Kilifi is non-pathogenic. There was no remission or self-cure following the infection in mice infected with the Savannah types, but where infections were caused by the Kilifi type, the course of the disease was brief and the infection cleared within 2 weeks and remained so throughout the experimental observation. This indicates that the strains were very mild or of non-virulent nature.

Long PPPs and the height of parasitaemia peaks observed in the present study did not influence the outcome of the disease. Thus, HVS killed mice rapidly once the parasitaemia was detectable in the blood compared to the MVS and LVS categories. These results are comparable to those of Bengaly *et al.* (2002) and Masumu *et al.* (2006) in that there was a shift in the survival time in the present study as a result of longer PPP. Their short PPP was accountable for shorter survival time, whilst, in our case, long PPP resulted in longer survival times. However, the behaviour was similar in that the rise in parasitaemia in HVS correlated with a steep drop in PCV, as reported earlier by Masumu *et al.* (2006). The difference between the PPP in the former studies and the present study may be attributed to either the mice used or the trypanosome strains themselves. Extensive work undertaken to unravel susceptibility versus resistance in inbred strains of mice shows that the development of infection and the pathological events involved differ between laboratory models (D'Leteren, Authié & Murray 1998). On the other hand, genetic variation in trypanosomes of the same species from distinct geographic origin may influence the outcome of infection (Taylor & Authié 2004).

In animals infected with trypanosomes, anaemia plays a key role in determining severity of the infection (Noyes *et al.* 2009). The change in red blood cell count was apparent in infected individual mice. However, the mean PCV values were not significant when compared to those of the uninfected control groups. In mice infected with the HVS, MVS and, to a lesser extent, LVS Savannah, the drop in PCV was not an indication that death in these groups correlated with anaemia. Similarly, mice infected with the LVS Kilifi were also able to control anaemia and parasitaemia. However, very limited numbers of this strain were used and further research is necessary to confirm these observations.

Of the above 26 strains characterised in mice, HVS and MVS categories were found to be prevalent inside HiP and in

the wildlife–livestock interface (Mvutshini, Ocilwane and Ndumo). All of the LVS, with the exception of one (MVU4), originated from locations considered far from any wildlife reserve, as in Nhlanzana and Boomerang commercial farm. High prevalence of HVS inside HiP and areas in the vicinity of parks has been reported (Masumu *et al.* 2009; Van den Bossche 2001). The results of this study confirm the finding in 2006, when 60.5% of animals at Mvutshini were found to be infected with *T. congolense* Savannah sub-group by PCR restriction fragment length polymorphism (Van den Bossche *et al.* 2006). According to these authors and Ntantiso (2012), high proportions of animals considered to be in poor condition were indeed infected with trypanosomes. The virulence profile obtained in the present study supports these findings and indicates that high disease impact occurring near wildlife areas is indeed caused by extremely virulent *T. congolense* strains. The strain obtained from Ocilwane, located at the southern part of HiP, was also allocated to the HVS category. Although a single strain was used in the present study, evidence exists that nagana was acute in affected cattle at Ocilwane and that 100% of positive cattle were anaemic (Ntantiso 2012). On Boomerang commercial farm, 20.0% of the strains were highly virulent, whilst 80.0% were either of moderate or low virulence. Given the fact that this farm is situated > 10 km away from Charters Creek, the closest wildlife reserve, cattle on this farm may constitute the main host of tsetse, thus limiting the transmission cycle of trypanosomes (Van den Bossche 2001). According to Masumu *et al.* (2006), this is attributed to the fact that strains most likely to survive in susceptible host populations have low virulence, producing mild disease. To support the latter statement, more studies conducted at Boomerang showed that cattle had a very high trypanosome infection rate, although the level of anaemia in the herd did not exceed 27.0%, compared to percentage of anaemic cattle at Mvutshini diptank (Ntantiso 2012). Taking this into account, our findings reveal that the low disease impact in cattle at Boomerang, as in most endemic areas located far away from parks, is possibly indicative of high prevalence of low virulent trypanosome strains in circulation as compared to cattle around parks. Our results further indicate that there is correlation between virulence and geographic distribution.

Conclusion

In the present study, HVS produced shorter PPP, PP and ST, followed by MVS and, lastly, LVS. Comparative observations were made when the same parameters were considered for strains collected from cattle near to and far from parks, and buffaloes inside HiP. Only strains collected from cattle away from parks exhibited longer periods compared to the other two areas. This study confirms that *T. congolense* occurring in South Africa has similar pathogenicity to other strains reported from other countries with severe infections occurring near the wildlife reserves.

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Competing interests

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

Authors' contributions

M.Y.M. (ARC-OVI) was the project leader, performed most of the experiments and wrote the manuscript. J.M. (Institute of Tropical Medicine) was a student mentor who assisted with the laboratory experiments and the edition of the manuscript. B.J.M. (ARC-OVI) was the co-supervisor of the study and contributed to revision and editing of the manuscript. A.A.L. (ARC-OVI) was the project supervisor and also contributed to revision and editing of the manuscript.

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