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Regional report

Corridor disease (buffalo-associated *Theileria parva*) outbreak in cattle introduced onto a game ranch and investigations into their carrier-state

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ABSTRACT

East Coast fever (*Theileria parva* infection in cattle) was eradicated from South Africa in the mid-1900. However, another form named Corridor disease (CD), associated with *T. parva* carrier buffaloes exists and outbreaks have increased in endemic areas. The occurrence of a CD carrier state in cattle under field situations has not been demonstrated but remains a subject of controversy. The current study investigated the *T. parva* carrier state following a severe outbreak in cattle introduced onto a game ranch. Monitoring of the outbreak included clinical signs, mortality, microscopy, serology, real-time PCR and xenodiagnoses. The herd of cattle received block treatment using oxytetracyclines (OTC) by the farmer during the outbreak. Cattle were sampled early during the outbreak and twice within the following 75 days. All buffaloes were tested for a *T. parva* carrier state. Two batches of questing adult *R. appendiculatus* were collected at the time of disease occurrence and a year later. These ticks were fed on susceptible cattle under controlled conditions and monitored for disease transmission. Ticks infected with a buffalo-derived stock of *T. parva* were fed on one bovine under controlled conditions and simultaneously injected with OTC, simulating the infection and treatment method of vaccination and was used as a positive control. Clean *R. appendiculatus* nymphs were fed on four recovered PCR positive cattle from the outbreak and on the positive control animal. The adult ticks were tested for infectivity by xenodiagnoses on susceptible bovines. For the initial outbreak the CD prevalence was 62.3% with a mortality rate of 29.5%. However, the outbreak was contained by block OTC treatment of the herd since only 3.4% cattle subsequently died until the end of the investigations. Adult ticks fed on one field bovine and the laboratory established *T. parva* carrier both transmitted fatal infections to susceptible cattle. Ticks fed on two field cattle transmitted *T. taurotragi* and one failed to transmit any infection. Questing adult *R. appendiculatus* collected during the outbreak transmitted fatal CD to two bovines while ticks collected a year later transmitted *T. taurotragi*. These findings demonstrated the effectiveness of disease control either by cattle treatment using OTC simulating the ITM or by intensive cattle dipping following the outbreak or by both interventions. The potential risk of creating carrier cattle by OTC treatment during CD outbreaks should be considered, supporting the continued control measures of segregation of cattle and buffalo herds.

1. Introduction

Cattle-adapted *Theileria parva*, the agent of East Coast fever (ECF), which causes more than 90% mortality in cattle, was eradicated in the Republic of South Africa by 1955, 53 years after its introduction into the country (Neitz, 1957). Another form of the disease known as Corridor disease (CD), caused by infection of cattle with buffalo-derived *T. parva* (formerly known as *T. parva lawrencei)*, was reported in the following years (Neitz et al., 1955). The African buffalo (*Syncerus caffer*), which is not normally clinically affected by this parasite, plays a major role in the epidemiology of this disease as a natural reservoir host and the source of infection to vector ticks. Corridor disease outbreaks occur at the contact interface between *T. parva* carrier buffalo and cattle in the presence of *Rhipicephalus appendiculatus* and *Rhipicephalus zambeziensis*, the vectors of the parasite in South Africa (Walker et al., 1981). Corridor disease also occur in the rest of Africa where African

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buffalo and the tick vectors occur and may not be easily distinguished from ECF when these diseases occur sympatric (Oura et al., 2011; Sitt et al., 2015). Corridor disease was first reported in South Africa with an outbreak that occurred in the corridor between Hluhluwe and Umfolozi game reserves in northern KwaZulu-Natal (Neitz et al., 1955). Corridor disease was reported earlier in Zimbabwe as buffalo-disease (Lawrence, 1935). Another form of the disease known as Zimbabwean theileriosis or January disease does not require contact with carrier buffalo, but is transmitted from carrier cattle to cattle (Lawrence, 1979). January disease has not been reported in South Africa.

Corridor disease outbreaks in cattle populations can result in a mortality rate of more than 90% (Neitz et al., 1955; Potgieter et al., 1988). It is believed to be self-limiting in cattle populations with no development of a carrier state in recovered cattle. However, in ECF and Zimbabwean theileriosis, the cattle adapted forms are known to produce a long-term carrier state in cattle (Young et al., 1986; Koch et al., 1992; Latif et al., 2001). The pathogenesis and clinical features of CD are different to that of classical ECF and Zimbabwean theileriosis. The buffalo-derived *T. parva* infections in cattle are characterized by a shorter course of the disease compared to ECF, low *Theileria* schizont parasitosis and very low piroplasm parasitaemia, the infective stages to vector ticks (Neitz et al., 1955; Potgieter et al., 1988; Uilenberg, 1999; Lawrence et al., 2004; Thompson et al., 2008). Despite the high severity of the disease, not all cattle that show clinical signs die, but recover, possibly through treatment interventions using antibiotics at the onset of an outbreak (Thompson et al., 2008; Yusufmia et al., 2010; Mbizeni et al., 2013). However, xenodiagnoses studies by Thompson et al. (2008) and Mbizeni et al. (2013) have failed to demonstrate the occurrence of a *T. parva* carrier state in previously infected and recovered cattle obtained from farms that experienced CD outbreaks.

In experimental serial cattle-tick transmission of a buffalo-derived *T. parva* isolate in East Africa, the parasite stock transformed into a form behaviorally indistinguishable from cattle-adapted *T. parva* (Barnett and Brocklesby, 1966; Maritim et al., 1992; Grootenhuis et al., 1987). In South Africa, this transformation into a cattle–adapted type was not achieved (Neitz, 1957; De Vos, 1982; Potgieter et al., 1988). Stoltsz (2011) continued with the earlier work of De Vos (1982) and Potgieter et al. (1988) and transmitted *T. parva* for eight consecutive passages in intact cattle, without the use of chemotherapeutic treatment. No behavioral changes in the *T. parva* buffalo-derived isolate was observed that would suggest transformation. There is a view that parasite population selection rather than parasite transformation occurred in the original transformation studies (Collins et al., 2002). Alternatively, the "*Theileria lawrencei* (Kenya)" isolate originally studied by Barnett and Brocklesby (1966) may have been contaminated by a cattle-derived isolate as was suggested for the Serengeti-transformed isolate from the Muguga cocktail (Norling et al., 2015; Hemmink et al., 2016). It may also be noted, that cattle infection using the buffalo-derived "*Theileria lawrencei* (Kenya)" isolate failed on more occasions than it was successful (Barnett and Brocklesby, 1966), suggesting that establishment of a carrier state in cattle from buffalo-derived *T. parva* stocks is not trivial.

In South Africa, repeated and fatal outbreaks of CD were reported to be on the increase in endemic areas since 2004 (Thompson et al., 2008; Mbizeni et al., 2013). While there is no confirmation of the occurrence of a CD carrier state in cattle or of cattle to cattle transmission, it is still a worry to farmers and the Veterinary Authorities. As such, the disease has only been confirmed based on molecular and serological identification of *T. parva* in cattle after CD outbreaks occurred (Thompson et al., 2008; Yusufmia et al., 2010; Mbizeni et al., 2013). In the present study, the possible development of a *T. parva* carrier state and cattle to cattle transmission of infections was investigated on a game ranch where buffaloes and cattle grazed together, and the farmer had used antibiotic treatment to save his cattle herd.

2. Materials & methods

2.1. Ethics approvals

Animal experiments were approved by the Animal Ethics Committee of ARC-OVR (AEC12.11; AEC11.13; AEC03.14).

2.2. The game ranch

Bedrog Ranch, KwaZulu-Natal Province, has been a registered game ranch since 2009. The game ranch is located in the identified CD free zone with a total of 19 buffaloes on the ranch. The buffaloes were introduced to the game ranch as disease-free according to the Department of Agriculture, Fisheries and Forestry Regulations (Animal Diseases Act 1984, Act No. 35) i.e. animals that tested negative for the four controlled diseases of African buffalo in South Africa that include brucellosis, CD, foot and mouth disease and tuberculosis. The buffaloes had been introduced at different times; the earliest in December 2009, then in January 2010, September 2012 and September 2013. The owner introduced 200 weaner cattle (1–2 years old) on 8th October 2013 for fattening and they were grazed on the buffalo frequented pastures. The cattle were purchased from different locations but allegedly not from CD endemic areas. Cattle started to become sick and clinical and fatality cases were recorded by 20th November 2013; 40 days after their introduction. The owner of the game ranch started treating his cattle using long-acting oxytetracycline (OTC) covering the whole herd. Cattle received treatment more than once during the course of the outbreak. The disease investigations started on the 3rd December, 13 days after the occurrence of the outbreak.

2.3. Herd monitoring

The cattle herd was examined on 3 follow-up visits and 69, 100 and 96 animals were sampled, respectively. Diagnosis of the cause of death included clinical and post mortem examination done on dead and euthanized cattle. Samples obtained included serum for the indirect immunofluorescent antibody test (IFAT) (Burridge and Kimber, 1972), EDTA blood for molecular testing by real-time PCR (Sibeko et al., 2008) and impression smears from lymph nodes, liver, spleen stained by Giemsa's stain for parasite detection using light microscopy. All 19 buffaloes present on the ranch were sampled for blood, serum and ticks. Buffaloes were immobilized by professional wildlife veterinarians following accepted procedures (Grobler et al., 2002; Oosthuizen et al., 2009). Buffaloes were darted individually from the ground ($n = 19$) and immobilized for an average of 15 min. Buffaloes, all adults, were immobilized with a combination of etorphine hydrochloride (M99®; Novartis, South Africa) and azaperone tranquillizer (StresniT; Bayer Pharmaceutical, South Africa). The dose was approximately 8 mg etorphine hydrochloride with 100 mg azaperone adjusted according to estimated body mass. The anesthesia was reversed by intravenous administration of diprenorphine hydrochloride (M5050; Novartis) and all the animals were observed until they were mobile, a process that generally took about 2–5 min.

Clinical reactions were classified as either mild to moderate or progressed to severe reactions. Mild to moderate reactions: Few *Theileria* schizonts parasites detected in Giemsa stained lymph node biopsy smears for 3–4 days, no fever or fever (39.6–40.5 °C) persisted for less than 4 days, not accompanied by clinical reactions and recovery occurred without intervention. Progressed to severe cases: *Theileria* schizonts were detected in superficial lymph nodes and blood stained smears until the animal died or were euthanized, with a fever for 3–4 days (41–42 °C). The animal showed clinical signs; marked enlargement of one or more superficial lymph nodes, rapid loss of condition, red conjunctiva, tears, coughing, nasal discharge, and labored breathing, corneal opacity and copious froth when the animal was recumbent. Animal treatment is prohibited in the country, and such drugs

are not registered in South Africa. Cattle were euthanized using a captive bolt fired through the forehead and death was rapidly brought about by exsanguination.

2.4. Genomic DNA extraction and real-time PCR

Genomic DNA was extracted from 200 µl blood using the MagNa Pure Large Volume Kit and MagNAPure LC and eluted in 100 µl elution buffer as previously described (Pienaar et al., 2011a). Real-time PCR for *T. parva* was performed according to the procedure of Sibeko et al. (2008) using 2.5 µl genomic DNA as template. The cut off for the test was implemented at a crossing point value (Cp) of 37 cycles. Hydrolysis probe PCR for *T. taurotragi* was performed as described (Pienaar et al., 2018).

2.5. Collection of questing ticks off-grass for infectivity testing

During the *R. appendiculatus* seasonal activity that coincide with the summer rainy season, and during the morning and early evening of the day, brown ear ticks migrate to the grass tops, where they are visible and easy to collect by hand (Latif, 2013). *Rhipicephalus appendiculatus* unfed adults were collected from grass for infectivity testing in susceptible cattle under controlled conditions. Tick batches were collected two times: during the onset of the outbreak (15.01.2014) and one year after the outbreak (14.02.2015). A total of three hundred males and female *R. appendiculatus*, respectively, were allowed to feed on each bovine (2 animals per batch). Ticks were fed on the ears contained in cloth ear-bags glued to the base of the ears. Ticks were fed two days after collection and engorged females were counted after dropping. The animals were monitored daily for body temperature, clinical signs and thin blood smears and lymph nodes biopsies were taken when these glands were found swollen and examined as mentioned above.

2.6. Tick pick-up and transmission attempts using recovered cattle from the outbreak

A total of five *T. parva* carrier cattle from the outbreak game ranch, confirmed by PCR and IFAT were identified and brought to ARC-OVR quarantine facility for experimental tick pick-up and transmission attempts. Infected and recovered cattle were brought from the ranch to Onderstepoort 70 days after the last clinical cases were reported. Carrier cattle were kept under tick-free conditions for 5 months before tick pick up was performed. Attempted tick transmission was carried out 40 days later. Two of the five animals (Bedrog 53 and Bedrog 121) were splenectomized on 5 August 2014, approximately nine months after the outbreak. Five susceptible bovines were used to test the infectivity of *R. appendiculatus* adults fed as nymphs on the four carrier cattle, eleven months after the Corridor outbreak and one on a carrier bovine established under controlled conditions (Section below). Tick application and animals monitoring for development of Corridor infection were done as mentioned above.

2.7. Establishment of a corridor carrier state in cattle after infection and treatment

Bovine number 9574/9, splenectomised on 27 March 2012, was infected thirteen months later with three-hundred *R. appendiculatus* adults which were fed as nymphs on a *T. parva* buffalo-derived carrier bovine, animal 8301 infected using ticks fed on animal 9288 which received ticks fed on a buffalo from Welgevonden (Sibeko et al., 2008). The animal was treated simultaneously with short acting OTC 10 ml/kg on Day 0 and the dose was repeated for the following three days. This simulates the infection and treatment method of ECF immunization (Radley et al., 1975). The bovine body temperature was normal (below 39.5 °C) for the total period of tick feeding and a month after ticks had dropped. This animal developed a *T. parva* carrier state for the next eighteen months as tested by PCR and had significant antibodies titres to *T. parva* as detected by the IFAT.

A susceptible bovine (9618), was challenged with adult *R. appendiculatus* which fed as nymphs on 9574/9. The animal was monitored for CD development as mentioned above.

3. Results

3.1. Corridor disease prevalence and mortality rate during the course of the outbreak in cattle introduced onto a buffalo ranch

On arrival to the site of outbreak, one animal was found dead and another one was euthanized to stop its suffering. Impression smears from the organs were found positive for *Theileria* schizonts with high parasitosis. The DNA extracts analyzed by real-time PCR were found positive for *T. parva*. All cattle on the ranch received "block treatment" with OTC (20 mg/kg live body mass) when clinical signs were noticed by the owner. Several animals which showed clinical symptoms of CD received two treatments.

All nineteen buffalo on the game ranch were confirmed *T. parva* carriers with high parasitaemia as exhibited by the Cp values from the real-time PCR that ranged from 19 to 27 (Fig. 1). Clinically sick cattle also showed high schizonts parasitosis. Of the buffalo, nine had IFAT titres of 1/40 and ten had titres of 1/80.

Adult *R. appendiculatus* were found in high numbers feeding on buffalo. Cattle were however, dipped as a measure of disease control after the initial outbreak. Adult ticks identified from one infested cow which missed the previous dipping included *R. appendiculatus* (16 males/15 females), *Rhipicephalus evertsi evertsi* (19 males/21 females), *Rhipicephalus microplus* (0 males/4 females), *Rhipicephalus decoloratus* (1

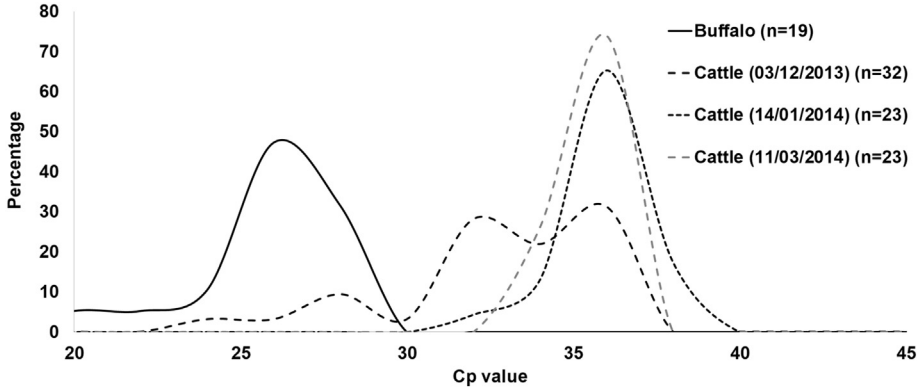

Fig. 1. Distribution of Cp values for *T. parva* positive animals from Bedrog ranch. Indicated are frequency distribution plots of the Cp values and the percentage observed in buffalo and cattle sampled during the outbreak on various dates as indicated.

Table 1
Corridor disease prevalence and mortality rate during the outbreak in cattle introduced into a buffalo ranch.

Animals sampled	Number sampled (%)	q PCR (%)	q PCR + IFAT (%)	IFAT (%)	Total Positive (%)	Number died (%)
Buffalo	19/19 (100%)	0	19/19 (100%)	0	19/19 (100%)	0
Cattle 1st sampling (D13) 3/12/2013	69/165 (41.8%)	14/69 (20.3%)	21/69 (30.4%)	8/69 (11.6%)	43/69 (62.3%)	54/200 (27%)
Cattle 2nd sampling (D55) 14.01.2014	100/146 (68.5%)	0/100 (0%)	24/100 (24%)	35/100 (35%)	59/100 (59%)	5/146 (3.4%)
Cattle 3[d] sampling (D111) 11.03.2014	96/141 (68.1%)	2/96 (2.1%)	22/96 (23%)	31/96 (32.3%)	55/96 (57.3%)	0
Animal Reactions		Stage 1	Stage 2	Stage 3	Prevalence (59.2%)	Mortality 59/200 (29.5%)

Stage 1: Pathogenic stage, clinical signs, new cases.
Stage 2: Infective stage to ticks.
Stage 3: Sterile Immunity.

males/5 females) and *Hyalomma rufipes* (6 males/6 females). The activity season of the adult ticks started early in November or October. All adult ticks collected from grass were *R. appendiculatus* except one male *R. evertsi evertsi*.

Table 1 shows the summary of the results for the follow up investigations of the outbreak. On the 1st sampling, 41.8% (69/165) of cattle were sampled. The morbidity rate was 62.3% (43/69) and mortality rate was 27% (54/200) 42 days after cattle introduction and during the first 13 days of disease occurrence. Cattle that were only positive by PCR (20.3%) were considered in Stage 1 of the disease as clinically sick (detection of schizont DNA). A total of 30.4% (21/69) of cattle survived and remained PCR positive with high antibody (Ab) titres to *T. parva*. These are considered as potential carriers since antibody titres develop after ~30 days consecutively with piroplasms (Stage 2). On the other hand, 11% of cattle became positive by the IFA test only, indicating that they had lost their *T. parva* piroplasm parasitaemia or that the schizonts did not develop into piroplasms (Stage 3). A total of 100/146 (68.5%) of cattle were tested in the 2nd sampling, 55 days after the onset of disease occurrence. None of cattle were allocated to Stage 1 of the disease while 24% and 35% were considered in Stage 2 and Stage 3, respectively. The number of dead cattle increased by 3.4% (5/146). In the 3rd sampling, 96/141 (68.1%) of cattle were sampled 4 months following the first case report. There were only 2/96 bovines (2.1%) which had acquired new infections (Stage 1) and 23% and 32.5% were considered in Stage 2 and Stage 3, respectively. The overall prevalence rate and mortality rate were 59.5% (57.3% - 62.3%) and 29.9% (59/200), respectively. The percentage of cattle in Stage 3 that was serologically positive, but PCR negative for *T. parva* had increased over time from 11.6% (1st sampling) to 32% by day 111 (3rd sampling).

The Cp values for positive cattle during the 1st sampling after the outbreak ranged from 23 to 35 (Fig. 1). The Cp value ranges changed from 32 to 37 during the 2nd sampling and from 33 to 35 during the 3rd sampling (Fig. 1).

3.2. Infectivity of questing ticks in susceptible bovines

Two batches of questing adult *R. appendiculatus* collected at different times from the ranch were applied to feed onto susceptible cattle kept under controlled conditions and were monitored for disease transmission (Table 2). The ticks from Batch 1 collected during the disease outbreak transmitted fatal CD in the two bovines; both died by day 17 and 18 after tick application. Ticks from Batch 2 collected a year later from the same areas, transmitted *T. taurotragi* and the animal reactions were mild.

3.3. Tick pick-up and transmission attempts using recovered cattle from the outbreak

Rhipicephalus appendiculatus nymphs were applied on 5 bovines; 4 from the outbreak game reserve which were confirmed *T. parva* infected by PCR (Cp values from 33.5–35.0) and serology, while the 5th animal was the carrier state established by ITM (Cp value 29.05) (Table 3). The subsequent adult ticks from nymphs fed on bovines Bedrog 164 and ITM 9574/9, transmitted *T. parva* to susceptible bovines. The infections were severe, and both animals died acutely by day 18. Ticks from Bedrog 120 and Bedrog 121 both transmitted *T. taurotragi* to susceptible animals. One animal had mild reactions and the other experienced moderate reactions, and both spontaneously recovered. Ticks fed on bovine Bedrog 24 did not transmit any *Theileria* parasites and remained PCR negative.

4. Discussion

The apparent increase of CD (buffalo-associated *T. parva*) outbreaks since the year 2004 and the development of a potential carrier state in recovered cattle have been a cause of worry to farmers and the veterinary authorities (Mbizeni et al., 2013). In the present study, the possible development of a *T. parva* carrier state and cattle to cattle transmission of *T. parva* was investigated during a CD outbreak on a game ranch where the farmer had used OTC treatment to save his cattle herd. The current reported outbreak is surprising and has not followed the normal known trend observed for *T. parva* in KwaZulu-Natal (Mbizeni et al., 2013), in several ways. The ranch where the outbreak occurred is located outside the Corridor line i.e. not in the known CD endemic area. The buffaloes on the ranch were designated as “disease-free” according to the Veterinary Authorities including CD based on IFAT and real-time PCR assay, performed at the ARC-OVR diagnostic laboratory prior to

Table 2
Theileria transmission attempts using questing ticks collected from buffalo ranch during the Corridor outbreak (Batch 1; collected 15.01.2014) and one year after the outbreak (Batch 2; collected 14.02.2015).

Tick origin	Susceptible recipient bovine	Number female ticks dropped	Days to temp	Duration temp	Days of schizonts	Clinical reactions	*Theileria* species
Batch 1	9613	68	9	7	12–16	S/D (Day 16)	Tp
Batch 1	9616	65	10	7	12–17	S/D Day 17)	Tp
Batch 2	1	148	13	2	14–17	M/R	Tt
Batch 2	2	160	14	3	15–18	M/R	Tt

Batch 1: Ticks collected off grass in January 2014 during the course of disease outbreak.
Batch 2: Ticks collected in February 2015 one year after the disease outbreak.
Clinical reactions: M (mild reaction); R (recovered); S (severe); D (died);
Tp (*Theileria parva*); Tt (*Theileria taurotragi)*.

Table 3
Tick pick up and transmission attempts using recovered cattle from the Corridor outbreak that developed a carrier state (Bedrog 24, 120, 121, 164) together with a carrier bovine after infection & treatment method (ITM).

Cattle number	PCR Cp value	Susceptible recipient	Female ticks dropped	Days to Temp	Duration Temp	Days of schizonts	Theileriosis reactions	PCR
Bedrog 24	33.8	9603	111	0	0	0	No/R	Neg
Bedrog 120	33.7	9604	201	0	0	16–19	M/R	Tt
Bedrog 121	35.0	9617	184	16	5	16–22	Mo/R	Tt
Bedrog 164	33.5	9605	311	13	4	15–18	S/D	Tp
ITM 9574/9	29.05	9618	199	13	4	15–18	S/D	Tp

Clinical reactions: S (severe); D (died); Mo (Moderate); M (mild); No (no reaction), R (recovered).
Tp: *Theileria parva*; Tt: *Theileria taurotragi.*

movement of the buffalo to the game ranch. The outbreak occurred during the month of November which is very early in the reported Corridor outbreak season that normally start in January and last till July/August as reported every year since 2004 (Mbizeni et al., 2013). The activity and occurrence of the adult stage of *R. appendiculatus* earlier in the summer season than reported previously (Rechav, 1981, 1982; Londt and Whitehead, 1972), may be related to early summer rain in this region. However, the *R. appendiculatus* population dynamics in this region has not been studied. We can only speculate about the occurrence of the disease outbreak and the origin of Corridor infection in the ranch. A straying carrier buffalo from the endemic area may have grazed the buffalo/cattle pastures in the game park during *R. appendiculatus* nymphal activity, while subsequent adult ticks infected the clean buffaloes that became *T. parva* carriers. Alternatively, humans (game hunters, game workers) and their vehicles might have carried infected ticks from endemic areas to the game park which were infective to buffaloes. Thus, the clean buffaloes became infected and were the source of infection to ticks and subsequently the introduced susceptible herd of cattle.

The disease outbreak caused high cattle mortality of 27% (54/200) initially, within 13 days after the start of clinical symptoms. However, the outbreak had been contained by the OTC block treatment of the herd since there were only 3.4% (5/146) additional cattle that died up to the end of the four months of investigation. Furthermore, the overall disease prevalence was maintained during the three sampling dates, varying narrowly between 59% - 62.3%. These findings demonstrated the effectiveness of disease control either by cattle treatment using OTC, thereby simulating the ITM, or by intensive cattle dipping following the outbreak, or by both interventions. The removal of either cattle or buffalo from the tick infested pastures and the introduction of stringent tick control measures normally results in the termination of an outbreak (Lawrence et al., 2004).

The parasitemia ranges for the buffalo compared well with previous studies on carrier state parasitemia that showed that buffalo may harbor a range of parasitemia from Cp values of 15–35 (Pienaar et al., 2011a; Pienaar et al., 2011b; Pienaar et al., 2014). The buffalo from Bedrog ranch shows a narrow range of Cp values from 19 to 27. This is at the higher end of the parasitemia scale and corresponds with ranges seen in a National Park such as Hluhluwe-Imfolozi Park (Pienaar et al., 2014). Such a park has a well-established buffalo population within a relatively small range area, i.e. limited buffalo are exposed to constant tick challenge, leading to a high parasite prevalence, i.e. most buffalo are *T. parva* positive, as seen in Bedrog ranch as well. This also correlates with the high level of infectivity observed in the ticks collected from the farm after the CD outbreak. This would suggest that the buffalo on the ranch has been infected a number of years (3–4) prior to the outbreak, with constant re-exposure to infected ticks as well as infection of ticks themselves, since the buffaloes show high parasite prevalence, high parasitemia and high antibody titres. This emphasize the importance of constant disease surveillance on buffalo ranches located outside the CD, but inside the tick vector endemic zones, since such ranches may be at high risk for establishment of *T. parva* in buffalo carriers with high parasitemia and prevalence. Such ranches pose a huge risk to other buffalo and cattle farms in the immediate area.

For cattle, parasitemia values progressively declined over the course of the monitoring period with the expectation that carrier animals would have lost their carrier status by the next nymphal tick cycle (Mbizeni et al., 2013). In contrast, cattle in the current study retained a carrier state after OTC treatment. This correlated with earlier reports that under controlled conditions, cattle which recover from *T. parva* infections, after chemotherapeutic intervention, can become carriers and would infect ticks feeding on them (Neitz, 1958; De Vos, 1982; Potgieter et al., 1985; Potgieter et al., 1988, Stoltsz, 2011). It is known that vaccination by infection with a sporozoite stabilate and simultaneous treatment with a long-acting tetracycline induces a carrier state with most *T. parva* stocks (Maritim et al., 1989; Bishop et al., 1992; Kariuki et al., 1995). *Theileria parva* parasites were found to be sensitive to OTC during development to the schizonts stage, but when mature and established within host cells, schizonts were not demonstrably affected. Parasite mitochondrial protein synthesis was also inhibited by OTC (Spooner, 1990a,b). The infectivity of sporozoites and the binding of sporozoites to lymphocytes were not directly inhibited by OTC. It is suggested that these results may explain the action of OTC when used prophylactically (Spooner, 1990a), or during the time of an ongoing outbreak, as in the present study if cattle received the treatment concurrently with feeding of infected ticks.

Our study had also confirmed previous reports on the state of *T. parva* “sterile immunity”, referred to in this study as Stage 3 (Table 1). Eleven percent of cattle lost their circulating *T. parva* parasites by day 43 reaching 35% within the following 2–3 months. Mbizeni et al. (2013) have also demonstrated that 10% of animals recovered from field outbreaks lose their *T. parva* piroplasms as early as 41 days after infection. Such cattle failed to transmit infection to susceptible bovines under controlled conditions (Thompson et al., 2008; Mbizeni et al., 2013). This highlights the issue that detection of *T. parva* DNA with PCR does not necessarily equate to an infective carrier state. In this case, many of the PCR positive animals may have infected schizonts but no piroplasms, which is the infective stage to ticks.

The xenodiagnoses attempt from the four *T. parva* carriers obtained from the Corridor outbreak produced various transmission results in recipient susceptible cattle. The ticks from one carrier transmitted fatal CD infection to a susceptible control. On the other hand, ticks from two carriers transmitted *T. taurotragi*, while ticks from one bovine failed to transmit any of the two species of *Theileria* to a susceptible host, even though the number of ticks engorged and dropped were comparable to others. The failure of three out four bovines to transmit *T. parva* to susceptible cattle confirmed previous reports by Thompson et al. (2008) and Mbizeni et al. (2013).

At the time of the reported outbreak in this study, questing adult ticks had high infectivity, transmitting fatal infections into susceptible cattle. In contrast, ticks from the succeeding season failed to infect cattle and only *T. taurotragi* was transmitted. In this regard, the buffalo were removed from the ranch 30 days before the last clinical cases (2/96) were reported on 11th March 2014. In addition, buffalo has been showed to be non-significant carrier hosts for *T. taurotragi* (Pienaar et al., 2018). This suggests that infected ticks were cleared from

infection by feeding on other wildlife present on the game ranch. This supports the Animal Health Regulations in the control of CD in South Africa that advocate the removal of cattle from tick infested pastures for 18 months after a disease outbreak to minimize the risk of new outbreaks. Newson et al. (1984) found that the *R. appendiculatus* population died off gradually up to 24 months under 'ideal' rainfall and temperature conditions in the Kenya highlands, and the *T. parva* infection within these ticks declined with age. In South Africa there is only one tick population generation per year (Rechav, 1982), and it is not known whether adult ticks can pass through a second phase of diapause.

In South Africa, vaccination of cattle against *T. parva* and prophylactic treatment is prohibited by law. Vaccination with the Muguga cocktail may introduce exotic vaccine parasite strains into local tick populations (Morrison and McKeever, 2006). Furthermore, there are risks associated with establishment of carrier infections with the foreign parasite strains present in the vaccine and hence their introduction into local tick populations. Specifically, the main concern is that the introduced parasites may be more virulent or differ antigenically from the indigenous parasites (Morrison and McKeever, 2006). In addition, current vaccines are composed of cattle-derived *T. parva* strains that could cause a persistent carrier state in cattle, leading to establishment of an endemic *T. parva* status similar to East Coast fever in the rest of southern and East Africa. The current study also highlighted the dangers of prophylactic treatment that can result in a carrier state in cattle. This may lead to subsequent CD outbreaks in cattle if such animals are moved within the tick vector endemic region.

In conclusion, the carrier state in cattle resulting from buffalo-associated infections with concurrent antibiotic treatment has been demonstrated under controlled conditions and in the field. The movement of such cattle could result in an uncontrolled spread of this disease. Corridor disease is unintentionally a neglected disease due to the limited control and management options available to veterinary authorities, such as dipping and strict separation of buffaloes in the absence of disease treatment and vaccination. The high mortality rate in cattle caused by CD is a cause of significant economic losses such as reported in the current study, making CD a major contributor to poverty of communal farmers in northern KwaZulu-Natal.

Ethics statement

Animal experiments were approved by the Animal Ethics Committee of ARC-OVR (AEC12.11; AEC11.13; AEC03.14). The ethics committee follows the guidelines of the South African National Standard for the care and use of animals for scientific purposes (SANS 10386:2008) that is based on the Australian Standard of Practice for the care and use of animals for scientific purposes, September 1997, drawn up by the National Health and Medical Research Council of Australia (copyright Commonwealth of Australia, reproduced by permission), and on the European Convention for the protection of vertebrate animals used for scientific study and for other scientific purposes.

Declaration of Competing Interest

The authors declare no conflict of interest.

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