Exposure of vaccinated and naive cattle to natural challenge from buffalo-derived *Theileria parva*

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ABSTRACT

Integrative management of wildlife and livestock requires a clear understanding of the diseases transmitted between the two populations. The tick-borne protozoan parasite *Theileria parva* causes two distinct diseases in cattle, East Coast fever and Corridor disease, following infection with parasites derived from cattle or buffalo, respectively. In this study, cattle were immunized with a live sporozoite vaccine containing three *T. parva* isolates (the Muguga cocktail), which has been used extensively and successfully in the field to protect against cattle-derived *T. parva* infection. The cattle were exposed in a natural field challenge site containing buffalo but no other cattle. The vaccine had no effect on the survival outcome in vaccinated animals compared to unvaccinated controls: nine out of the 12 cattle in each group succumbed to *T. parva* infection. The vaccine also had no effect on the clinical course of the disease. A combination of clinical and post mortem observations and laboratory analyses confirmed that the animals died of Corridor disease. The results clearly indicate that the Muguga cocktail vaccine does not provide protection against buffalo-derived *T. parva* at this site and highlight the need to evaluate the impact of the composition of challenge *T. parva* populations on vaccine success in areas where buffalo and cattle are present.

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1. Introduction

For many African countries, maintaining wildlife populations is important as a source of tourism revenue and as an integral part of global biodiversity conservation (Wambwa, 2005; Thomson et al., 2013). However, sustainable increases in livestock productivity are seen as essential to the economic and social development of rural Africa. These two activities are often in conflict and modern conservation efforts are aimed at integrative management of wildlife and livestock. These efforts are constrained by competition for feed, land and water resources, and by the presence of pathogens that can be transmitted between the populations (Grootenhuis and Olubayo, 1993). Although isolation of livestock and wildlife through fences has been used to limit disease transmission, this approach is not practical in extensive pastoral farming systems and has detrimental consequences through the resultant segregation of wildlife sub-populations. The development of more effective control methods for wildlife-derived livestock diseases is required. Essential to this is a better understanding of the interaction between the pathogens, mammalian hosts and the vectors that transmit disease. This will enable the implementation of strategies where wildlife and livestock can cohabit with minimal adverse impact on both.

In eastern, central and southern Africa, a disease of major interest in this context is caused by infection with *Theileria parva*, an apicomplexan protozoan parasite which is transmitted by the brown ear tick (*Rhipicephalus appendiculatus*). *T. parva* infects both cattle and the African buffalo (*Syncerus caffer*), although it is believed that the parasite evolved with the buffalo in eastern Africa long before the introduction of cattle (Uilenberg, 1981; Young, 1981). There are very few, if any, clinical signs in infected buffalo, whereas in cattle the parasite causes a severe, often fatal, lymphoproliferative

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disorder called East Coast fever (ECF). ECF results in considerable economic losses as a consequence of high mortality rates and the inability of livestock owners to introduce the more productive but highly susceptible European breeds of cattle into enzootic areas (Conelly, 1998).

Of particular importance in understanding the interaction between *T. parva*, its tick vector and mammalian hosts is the difference between the buffalo- and cattle-maintained populations of *T. parva*, in their biological properties and genotypic diversity. Infection of cattle with buffalo-derived parasites results in Corridor disease (CD), which is characterized by low levels of parasitized leukocytes in peripheral lymph nodes compared to the high parasitosis seen in ECF (Neitz et al., 1955; Barnett and Brocklesby, 1966). Results based on monoclonal antibody profiles and comparison of the sequences of known *T. parva* antigens have shown that parasites derived from buffalo are much more heterogeneous than those from cattle (Conrad et al., 1987; Grootenhuis and Olubayo, 1993; Pellé et al., 2011). This may be because only a subset of parasite strains from the more heterogeneous buffalo-maintained *T. parva* population have crossed into cattle populations since the relatively recent introduction of cattle into eastern Africa (Hanotte et al., 2002).

The functional significance of the heterogeneity seen in *T. parva* and whether it confers any selective advantage, during either the mammalian or tick stages of the parasite life cycle, are unclear. However, there is evidence that this diversity has an important influence on immunity. The immunity induced by *T. parva* infection is strong and long-lasting and has been extensively studied in the bovine host. The principal mediators of the protective immune response are CD8+ cytotoxic T lymphocytes (CTL) and several antigens recognized by CTL have been identified and characterized (Graham et al., 2006). Extensive polymorphism has been observed in three of the known antigens Tp1, Tp2 (Pellé et al., 2011) and Tp9 (Sitt and Pellé, unpublished observations), and natural variants of known CTL epitopes can escape CTL recognition (MacHugh et al., 2009; Connelley et al., 2011). These findings support the view that strain specificity is due to antigenic differences between the immunizing and challenge parasites.

The strong immunity following *T. parva* infection provides the basis for a commercial vaccination procedure against ECF using live parasites, called the Infection and Treatment Method (ITM) (Radley et al., 1975a). Vaccination involves inoculation of cattle with sporozoites in the form of a ground homogenate of infected ticks and simultaneous administration of a long-acting formulation of oxytetracycline to prevent the infection progressing to fatal disease. Cross-immunity studies conducted in Kenya in the 1970s showed that immunity is strain-specific, as cattle immunized against one strain of the parasite are not necessarily protected against challenge with heterologous parasite strains (Radley et al., 1975a). The studies undertaken during the development of the method showed that immunization with a combination of three parasite isolates provided much broader protection than single isolates (Radley et al., 1975b). This combination of parasite isolates, termed the Muguga cocktail, is now the most widely used formulation for ITM vaccination. It has been shown to reduce the incidence of ECF in field situations where cattle predominate, including parts of Kenya, Uganda, Zambia, Tanzania and Malawi (Uilenberg et al., 1977; Morzaria et al., 2000).

Previous experiments with other versions of the ITM vaccine have shown that they do not always provide complete protection under field conditions (Snodgrass et al., 1972; Cunningham et al., 1974). A number of cross-immunity studies have been conducted using different cattle-derived *T. parva* stabilates, including locally derived parasites, which have shown mixed results in development of protective immunity (Hove et al., 1996; Musisi et al., 1996; Latif et al., 2001a). In particular, studies using the Muguga cocktail have indicated that vaccination provided partial protection against challenge

with buffalo-derived *T. parva* (Radley et al., 1979). However, these studies used tick stabilates delivered by needle inoculation or tick challenge in a paddock artificially seeded with buffalo-derived parasites and on which other groups of cattle had grazed.

Although the Muguga cocktail has been deployed in areas where both buffalo and cattle are present, it has not been tested in natural field situations where challenge arises solely from buffalo-derived parasites. The purpose of this study was to address this question by monitoring groups of vaccinated and control (unvaccinated) calves introduced into a *T. parva*-endemic area grazed only by buffalo and other wildlife. The results were expected to have implications on the use of the vaccine in areas where cattle co-graze with buffalo.

2. Materials and methods

2.1. Animal care and use

The study protocol was approved by ILRI's Institutional Animal Care and Use Committee (References 2011–11 and 2013–03). The humane end-point was defined as 10 days of elevated temperature or if the animal developed severe signs of disease. Sampling of buffalo was approved by the Kenya Wildlife Service (KWS/BRM/5001).

2.2. Study site

The study was undertaken on the Ol Pejeta Conservancy in Kenya, which is revolutionizing efforts in integrative management of wildlife and livestock (OPC, 2015). The field site was located at the southern end of the Ol Pejeta Conservancy, Nanyuki, Kenya (00°03.052′S, 36°52.302′E; altitude 1784 m) and comprised a fenced area of about 500 hectares containing buffalo but not cattle. An attempt at maintaining cattle in the area a number of years previously resulted in extremely high mortality due to suspected CD and any surviving cattle were moved out of the location immediately.

2.3. Study animals

Twenty-four castrated male Boran calves, between the ages of 7 and 12 months, were obtained from the ILRI ranch, Kapiti, Kenya. Prior to selection for the study, the animals were screened by antibody-capture ELISA (Katende et al., 1998) and found to be negative for antibodies against *T. parva*. Animals were also tested by PCR for the presence of *T. parva* using a nested PCR based on the p67 gene as will be described in 2.11, below. The calves were relocated to the ILRI farm, Nairobi, and held in a tick-free environment for 2–4 weeks prior to vaccination. On arrival at ILRI, the calves were treated with acaricide, an anthelminthic (Levamisole with cobalt; Coopers K. Brands) and Imizol (Coopers K. Brands), and vaccinated against FMD (Kenya Veterinary Vaccines Production Institute).

Blood samples were collected from eight African buffalo at different locations on Ol Pejeta, but not within the study site due to the thick vegetation present there. The locations were between 9 and 13 km from the study area. The buffalo were chemically restrained using a combination of etorphine and xylazine mixed in a single dart and delivered using a Danject dart gun, according to standard Kenya Wildlife Service protocols. The animals were placed in sternal recumbency and blood was drawn from the jugular vein into EDTA and plain vacuum tubes (BD), stored in a cool box and transported to the field laboratory for processing. All animals were sampled within 5 minutes of recumbency and revived using appropriate dosages of a combination of atipamazole and diprenorphine.

2.4. Vaccination

Animals were randomly allocated into two groups of 12, vaccinated or control (unvaccinated), using a random number generator in Excel. Animals were vaccinated by ITM with the ILRI 0801 batch (Patel et al., 2011) of the Muguga cocktail vaccine. Each vaccinated animal received 1 ml of a 1:100 dilution of the vaccine and 30 mg/kg long-acting oxytetracycline (Alamycin LA 300, Norbrook Laboratories). Control animals received 1 ml of vaccine diluent and oxytetracycline.

2.5. Clinical reactions to vaccination

Calves were held in a tick-free environment for 40 days after vaccination during which time they were monitored daily for changes in body temperature and observed for adverse reactions. Blood was collected on a weekly basis into plain or EDTA tubes for serology and parasite detection by p67 PCR, respectively. Lymph node (LN) aspirates were obtained from the vaccinated animals from days 8 to 21 from the right parotid lymph node (LN) draining the site of infection for detection of infected or hyperplastic cells.

2.6. Field study

On day 41 after vaccination and 6 days after their previous acaricide treatment, the calves were relocated from ILRI to the study site after receiving a pre-shipment treatment of 15 mg/ml Betamox LA and multivitamins (Norbrook Laboratories). During the study period, the calves were kept in a pen at night and allowed to graze freely during the day. Ticks were allowed to attach naturally onto the calves for the initial 10 days of field challenge, thereafter, the animals were treated with acaricide every 3–4 days. The study was ended 30 days after introduction to the study site (71 days after vaccination), and the surviving animals were incorporated into the Ol Pejeta herd.

Calf health was assessed every morning before the calves were released for grazing. Key clinical parameters such as temperature, respiration and LN swelling, were recorded daily. Field scientists were blinded to the identity of the vaccinated and control groups.

2.7. Field sampling

Blood samples for serology and parasite detection were taken from all calves at twice weekly intervals up to the detection of pyrexia. Thereafter, samples were taken every other day until death or the termination of the field study on day 30. LN aspirates were taken only from swollen parotid or pre-scapular nodes on every second or third day depending on the microscopy results and animal health. For animals remaining after day 24 macroschizont positive LN were aspirated on a daily basis; to decrease LN damage, the same lymph nodes were not necessarily sampled every day. If a LN was negative two samplings in a row, after having been positive macroschizont positive, it was no longer sampled.

2.8. Serology

Serum was stored at –20 °C until use in ELISA. The samples were analyzed in duplicate and the results are presented as percent positivity (PP) as previously described (Katende et al., 1998). Samples with values of 20 PP or greater are considered positive for anti-*T. parva* antibodies.

2.9. DNA extraction

Whole blood from the calves was aliquoted into cryovials and kept at –20 °C for later DNA analysis. DNA extraction was conducted on 200 µl of the frozen blood using High Pure PCR Template Preparation Kit (Roche). Samples were eluted in 50 µl. The eluate was run through the column twice to achieve a higher DNA concentration. DNA concentration was measured using a Nanodrop 1000 Spectrophotometer (Thermo-Scientific) and the DNA was stored at –20 °C. Buffalo DNA was extracted from homogenized blood clots from serum collection. DNA extraction was performed as earlier, except that samples were eluted into 30 µl.

2.10. Microscopy

LN and blood smears were stained with Giemsa and examined for the presence of parasites and hyperplastic cells.

2.11. PCR

The nested p67 PCR was performed as described previously (Nene et al., 1996) with the following modifications. The master mix consisted of 2.5 µl of 10× FastStart PCR buffer (Roche), 2.5 µl 25 mM MgCl$_2$, 0.25 µl 25 mM dNTP, 0.25 µl each primer, 0.125 µl FastStart Taq (Roche), 19.125 µl H$_2$O and 2.5 µl (outer primer reaction) or 1 µl (inner primer reaction) of DNA. The cycling conditions were 94 °C for 5 min, 40 cycles (outer reaction) or 35 cycles (inner reaction) of 95 °C for 1 min, 55 °C for 1 min, 74 °C for 1 min, 72 °C for 10 min and a hold at 10 °C. PCR products were subjected to electrophoresis through a 1.5% agarose gel containing GelRed (Biotium) and visualized under UV light. Both positive controls and negative (no DNA) controls were included in each PCR.

2.12. Cloning and sequencing of p67 gene fragments

Fragments of the p67 gene from the last samples collected prior to death or day 20–23 samples in survivors were generated by nested PCR as follows. The master mix comprised 2.5 µl 10× PCR buffer, 0.16 µl 25 mM dNTPs, 0.25 µl primers, 0.25 µl PFU DNA Polrecombinant (Fermentas) and 21.34 µl H$_2$O. Cycling conditions for outer primers were 95 °C for 3 min, 40 cycles (outer reaction) or 30 cycles (inner reaction) of 95 °C for 45 s, 55 °C for 45 s, 72 °C for 5 min, 72 °C for 10 min and a hold at 10 °C. The PCR products were purified using the HighPure PCR product purification kit (Roche), according to protocol. Ligation was conducted using the CloneJet PCR Cloning kit (Thermo-Scientific), as instructed. Transformation was conducted via standard heat shock protocol using DH5α cells. Six positive clones were selected from each animal, and plasmid DNA was prepared using a GeneJet MiniPrep kit (Thermo-Scientific) as per manufacturer's instructions. The presence of the desired plasmid insert was confirmed by gel electrophoresis. Sanger sequencing was conducted on the cloned PCR products using the GeneAmp® 9700 system (Applied Biosystems). Sequence analysis and alignments were conducted using Geneious version 6.0.6 created by Biomatters (http://www.geneious.com).

2.13. Histology

Tissue samples from spleen, liver, lung and kidney were collected from nine calves (three vaccinated and six control) during post mortem examination, and placed in phosphate-buffered formalin. The tissues were processed by a local pathology laboratory (Aga Khan University Hospital, Nairobi) and hematoxylin-and-eosin-stained histological sections were prepared for microscopic examination.

2.14. Statistics

Statistics were conducted using GenStat (VSN-International, 2013). Survival analysis was conducted using Kaplan–Meier to evaluate the survival function of the two groups over time and a log-rank test used to compare these survival curves. A non-parametric

Fisher's exact test was used to compare final survival between the two groups. To test for differences between vaccinated and control groups in the onset and progress of disease (time to, and duration of, pyrexia and LN parasitosis) a random permutation t-test equivalent was used.

3. Results

3.1. Response to vaccination

As shown in Table 1, all but one of the vaccinated calves had seroconverted by 35 days after vaccination, and all but one of the vaccinated calves were positive by PCR on at least one sampling day post vaccination. Microscopic examination of LN aspirates from the right parotid lymph node between 8 and 21 days after vaccination revealed varying degrees of hyperplasia in all vaccinated animals. Macroschizonts were noted in only one animal (BJ047), between 11 and 13 days after vaccination. The control animals remained negative by ELISA and PCR. The results confirm that the vaccinated cattle were exposed to viable *T. parva* parasites, indicating that the animals were successfully immunized.

3.2. Response to field challenge

Ticks were observed to attach to the animals from day 2 of being placed in the field site. The majority of the ticks were identified as *R. appendiculatus*.

The survival outcome of the challenge is summarized in Table 1. Despite defining clinical endpoints for treating the animals, the very rapid progression of clinical symptoms in the later stages of infection resulted in death of many animals before treatment could be implemented. Seventeen of the 24 animals died during the 30-day field exposure. An additional animal (BJ049), which was found

recumbent, received treatment with a theilericidal compound (Buparvaquone) and survived; for the purposes of analyses of results, this animal was considered to have suffered a fatal infection. Thus, only six animals survived the challenge, of which three were vaccinates and three were controls. Clearly, there was no difference in survival between the two groups (Fishers exact test, p = 1.000). The time to death in the control and vaccinated groups is illustrated by the Kaplan–Meier survival function in Fig. 1. There was no discernible difference in the survival curves in the vaccinated and control groups (N observed = 9 for both, N expected = 9.11, 8.89 for control and vaccinated, respectively; log-rank test statistic = 0.001 on 1 degree of freedom, p = 0.953). The time of death appeared to be biphasic: 15 animals (eight vaccinates and seven controls) died between 20 and 22 days after exposure and three (one vaccinate and two controls) died on day 27 or 28 after exposure. The findings demonstrate that vaccination with the Muguga cocktail provided no protection against the field challenge in this study.

3.3. Clinical signs

Briefly, the clinical findings were typical of severe disease caused by *T. parva*. The key signs observed included pyrexia, swollen parotid and prescapular lymph nodes, labored breathing, nasal discharges, lacrimation and corneal opacity, all of which indicate severe *T. parva* infection.

3.4. Parasitology findings

Macroschizonts were observed in at least one LN of every animal during the course of the study (Table 1). The macroschizonts were first detected between days 14 and 19 after exposure. In all animals that died, macrochizonts remained detectable until death, whereas

Table 1
Clinical, parasitological and serological observations in vaccinated and control calves following immunization and field exposure.[a]

| | Immunization | | Field challenge | | | | | |
	Seroconv.	p67[b]	Pyrexia	LN parasitosis	Piroplasm	p67[b]	Seroconv.	Death
Controls								
2104	–	–	14 (**7**)	14 (7)	–	+	–	20
2150	–	–	17 (**5**)	14 (8)	–	+	–	22
2158	–	–	20 (**5**)	15 (14)	–	+	–	28
2253	–	–	15 (**4**/*2*/**1**)	15 (8)	–	+	–	22
2310	–	–	14 (**5**/*1*/**1**)	14 (7)	–	+	–	21
2325	–	–	15 (**7**/*2*/**4**)	15 (13)	22 (7)	+	20	28
2352	–	–	15 (**5**)	15 (5)	–	+	–	20
2381	–	–	15 (**4**)	14 (7)	–	+	–	20
2384	–	–	16 (**5**)	15 (8)	–	+	–	21
2124 (S)[c]	–	–	15 (**8**)	15 (10)	–	+	22	n/a
2166 (S)	–	–	18 (**8**)	16 (≥14)	–	+	24	n/a
2227 (S)	–	–	21 (**1**)	18 (3)	21 (2)	+	19	n/a
Vaccinated								
2170	28	+	15 (**6**)	15 (7)	–	+	11	21
2174	21	+	15 (**6**)	14 (7)	–	+	–	20
2202	21	+	14 (**7**)	15 (6)	–	+	–	21
2206	21	+	14 (**7**)	14 (7)	–	+	–	21
2223	21	+	17 (**8**)	16 (8)	–	+	–	27
2252	35	+	16 (**1**/*1*/**4**)	16 (5)	–	+	16	21
2265	–	+	15 (**7**)	15 (8)	–	+	–	22
2345	28	–	16 (**3**)	14 (7)	–	+	–	21
2375	21	+	17 (**5**)	16 (5)	–	+	–	22
2164 (S)	21	+	21 (**6**)	18 (≥11)	–	+	–	n/a
2317 (S)	21	+	20 (**2**)	17 (8)	–	+	9	n/a
2320 (S)	35	+	22 (**3**/*1*/**3**)	19 (10)	–	+	23	n/a

[a] All data, except for p67 results, are shown as days to first occurrence following immunization or field exposure. The numbers in brackets refer to the duration of pyrexia and parasitosis. For pyrexia, brackets showing three figures indicate animals in which the pyrexic days (bold) were interrupted by days of normal temperature (italics).

[b] The p67analysis was undertaken on samples taken at day 28 after immunization (detection of parasite post vaccination) or on days 20–23 after field exposure (survivors), or on the final sample before death (non-survivors).
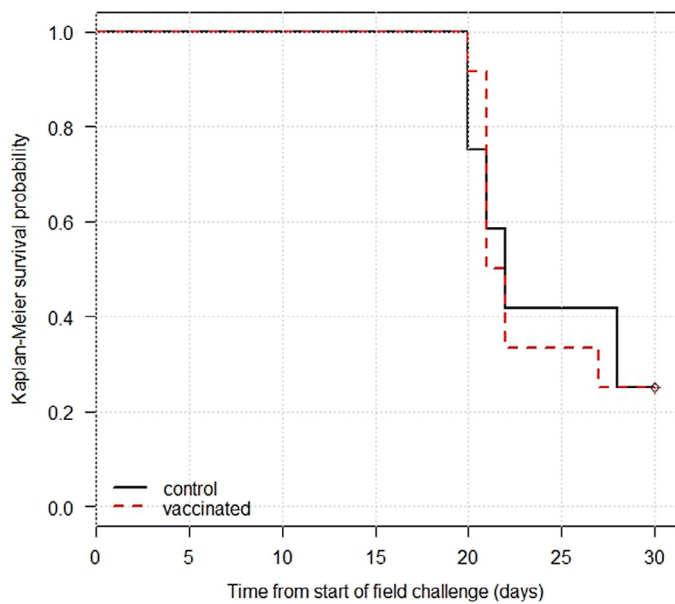
[c] (S) indicates animals which survived.

Fig. 1. Kaplan–Meier survival analysis. Kaplan–Meier survival analysis of vaccinated (dashed line) and control (solid line) calves following field exposure. The black circle and red cross at day 30 represent the animals remaining alive at the end of the experiment (censored). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in four of the six surviving cattle, the number of macroschizonts fell below the level of detection before the end of the experiment.

Microscopic examination of blood smears revealed the presence of piroplasms in only two calves. One surviving control calf had a piroplasm level of <1/1000 on days 21 and 22 post exposure. A second control calf had piroplasms for 7 days leading up its death on day 28, increasing from <1/1000 on day 22 to 7% on day 28.

Blood from the last sample prior to death, or days 20–23 for survivors, from all control calves was analyzed by nested p67 PCR to confirm exposure to *T. parva*. All samples were positive, showing all animals were infected with *T. parva* (Table 1).

Post mortem organ impression smears were made from liver, lung and spleen from 9 calves and also from kidney for three of the same calves. Macroshizonts were seen in all kidney, lung and spleen impression smears and in seven of the liver smears (results not shown).

3.5. Post mortem observations

Of the animals that died, 13 showed a conspicuous frothy white exudate from the nasal cavities (Fig. 2A). Post mortem examinations were performed on 11 calves. In all cases, there was extensive pleural exudate (Fig. 2B) and in all cases that died between 20–22 days, there was also copious frothy exudate in the trachea (Fig. 2C). None of the three calves that died on day 27 or 28 post exposure had frothy exudate. These findings are consistent with previous observations on fatal *T. parva* infections.

3.6. Histology

Histological examination revealed generalized severe pathological changes in the spleen and lungs. There was extensive lymphocytic depletion and disorganization of the splenic white pulp (typical of *T. parva* infection) and also extensive cellular infiltration into the interstitial spaces and to a more variable degree the alveolar septa in the lung, often associated with edematous fluid in the alveolar

spaces. The histological observations are consistent with severe *T. parva* infection.

3.7. Serology

All three control animals that survived and one animal that died showed evidence of an anti-*T. parva* antibody response, with seroconversion occurring between days 19 and 24 after exposure (Table 1). The remaining eight control animals that succumbed showed no evidence of an anti-*T. parva* antibody response. These included the calf (BJ049) that was treated and survived, although this animal subsequently seroconverted after it had been removed from the study. Immunized animals were considered to have experienced a restimulation of the antibody response if the antibody titer value was 10 PP units greater than that immediately prior to field challenge. As shown in Table 1, four of the vaccinated calves were restimulated, including two that survived the field challenge. The median time to seroconversion after field exposure for the controls (21 days) was the same as that following the immunization of the vaccinated group by ITM (21 days). On the other hand, the median time to increased antibody levels for the vaccinated animals after field exposure was only 13.5 days. The results are consistent with exposure of the animals to infection with *T. parva* soon after their introduction into the field. The more rapid response in the vaccinated animals, compared to the controls, is indicative of an anamnestic antibody response in these animals.

The serum taken from the last field sample for each animal was also examined for antibody to other blood pathogens. Three cattle were positive for *T. mutans*, two for *B. bigemina* and none for *A. marginale* (results not shown).

In summary, the results presented earlier support the view that the animals died from infection with *T. parva*. More specifically, the absence of piroplasms in most of the animals is typical of theileriosis caused by buffalo-derived *T. parva* (Neitz et al., 1955).

3.8. p67 sequence analysis

Previous studies of the p67 gene sequence in *T. parva* isolates have identified a single allele in isolates derived from cattle residing in buffalo-free areas, but several additional alleles in buffalo (Nene et al., 1996; Sibeko et al., 2010). To obtain further evidence that the cattle were infected with buffalo-derived parasites, the p67 gene sequences in the control cattle were compared to those obtained from eight buffalo on the Ol Pejeta conservancy (Table 2). The sequences were grouped according to the presence or absence of two indels of 129 bp and 174 bp (Sibeko et al., 2010). Type 1 lacks the 129 bp indel, type 2 has both, while type 3 lacks the 174 bp sequence. The results showed that the occurrence and distribution of the sequence types was similar in the two populations. All three types were found in both the cattle and buffalo populations, with the two types previously found to be associated with buffalo (types 2 and 3) being the most common. Type 1, which is the only type previously identified in cattle-maintained *T. parva*, was found only in a single animal in each group. The results strongly suggest that the cattle were infected with buffalo-derived parasites.

3.9. Effect of vaccination on disease development

Although vaccination did not affect the survival of cattle, it was of interest to determine if vaccination affected the progress of the disease by comparing various parameters associated with *T. parva* infection. There was no statistically significant difference between the vaccinated and control cattle in the time to pyrexia (16.8 and 16.3 days, respectively; $p = 0.602$–0.626), the time to LN parasitosis (15.8 and 15.0 days, respectively; $p = 0.245$–0.261), the time from initial pyrexia to death for non-survivors (6.3 and 6.8 days,
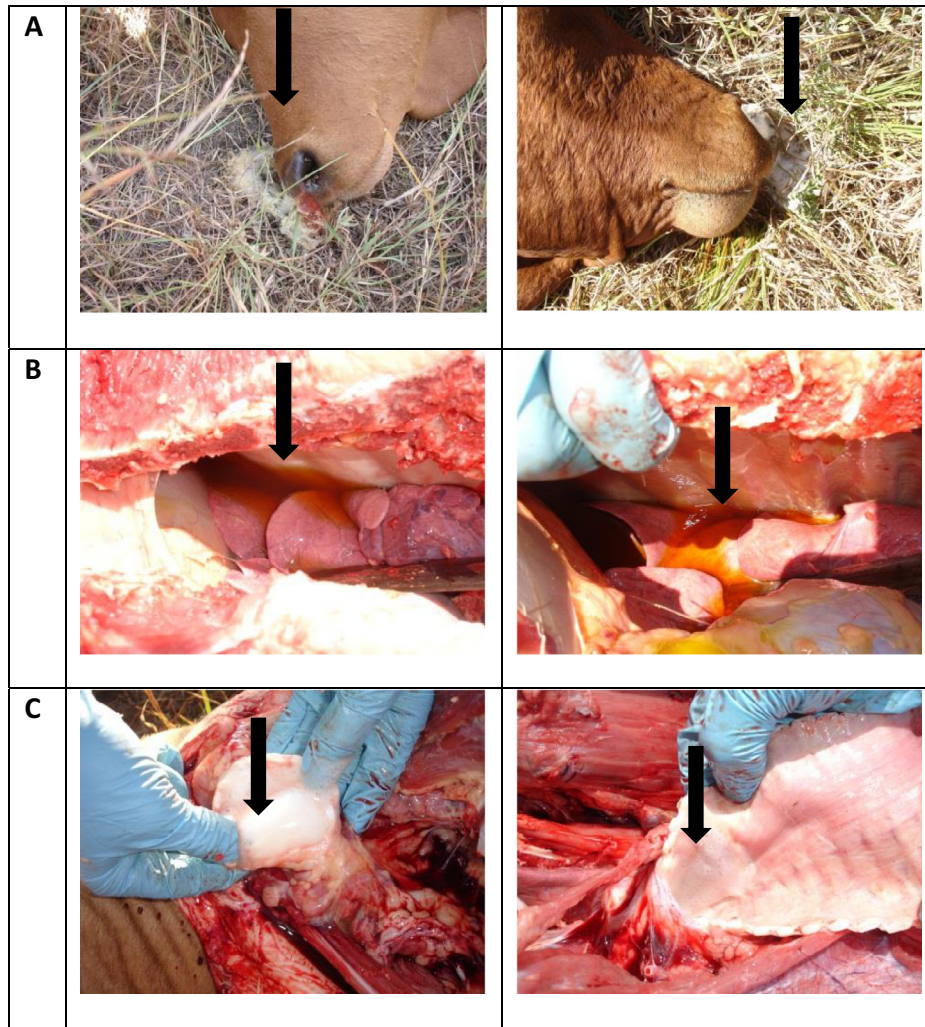
Fig. 2. Post mortem examination. Post mortem examination for typical calves showing signs associated with *Theileria* infection. A: Copious frothy exudate from nasal cavities of BJ031 and BJ037. B: Pleural exudate in thoracic cavities of BJ033 and BJ041. C: Frothing in trachea of BJ026 and BJ033.

respectively; p = 0.721–0.735) or the time from initial LN parasitosis to death for non-survivors (6.8 and 7.9 days, respectively; p = 0.354–0.366). The results show that vaccination did not affect the onset or progress of the disease.

4. Discussion

The results presented here show that the Muguga cocktail ITM vaccine had no discernible protective effect in cattle in a field situation where animals are exposed to ticks infected from buffalo. Further, the vaccine appeared to have no effect on the progression of the disease, with vaccinated animals dying and developing pyrexia and parasitosis as rapidly as the unvaccinated animals.

Previous experimental studies of cattle vaccinated with the Muguga cocktail have indicated that vaccination does not always protect against challenge with *T. parva* isolated from buffalo (Radley et al., 1979). The current study is the first attempt to examine immunity in vaccinated cattle exposed to natural tick challenge in an area where only the buffalo host is present. Intensive monitoring of the animals during both vaccination and exposure, employing parasitological, serological and molecular tools, failed to detect any significant differences between the responses in vaccinated and unvaccinated animals.

Table 2
Occurrence and distribution of p67 sequence types in control cattle and buffalo on Ol Pejeta.

	Type 1	Type 2	Type 3
Cattle			
BJ024	+		
BJ026		+	+
BJ027		+	+
BJ038		+	
BJ041			+
BJ044		+	+
BJ046		+	+
BJ048			+
BJ049			+
BJ025 (S)[a]			+
BJ029 (S)		+	+
BJ036 (S)			+
Buffalo			
301			+
302			+
303		+	+
304		+	+
305			+
306	+		+
307		+	+
308		+	+

[a] (S) indicates animals which survived.

The clinical observations together with post mortem and laboratory results support the contention that calves in this study died from infection with buffalo-derived *T. parva* manifesting in CD. These observations and signs include the lack of detectable piroplasm parasitemia in most animals, the history of the study site (lack of cattle), and the similarities between the p67 alleles found in the cattle and buffalo, including alleles previously only associated with buffalo. Previous studies have provided evidence that buffalo-derived parasites are more antigenically diverse than those maintained in cattle (Pellé et al., 2011). Hence, it is possible that the lack of immunity reflects the greater antigenic heterogeneity in the challenge parasite population. However, further work is required to confirm whether or not this is the case. In contrast to the high levels of schizont parasitosis in peripheral lymph nodes of cattle infected with cattle-maintained *T. parva* (Barnett and Brocklesby, 1966), the levels of schizonts are much lower in animals infected with buffalo-derived parasites (Neitz et al., 1955). Nevertheless the terminal clinical symptoms, characterized by severe pulmonary edema, are very similar and the histopathological observations in the lungs and spleens of cattle that died in the current study exhibited similarities to those seen in ECF. Hence, it is possible that cells infected with buffalo-derived parasites preferentially migrate to internal organs.

The general lack of seroconversion in the control calves that died is consistent with the results of a recently published field study in a buffalo-free area (Kiara et al., 2014) as well as a study assessing cattle exposed to *T. parva* from buffalo (Mbizeni et al., 2013). This is presumably due to the animals dying before an antibody response has been generated, combined with generalized dysfunction of the immune system as a consequence of pathology caused by the infection. A novel observation in this study was that most of the vaccinated animals that died also tended to show a lack of boosting to the extant antibody response, again suggesting disruption of immune function. The seroconversion observed in the animals that survived confirms that these animals were exposed to parasite challenge and that their survival was not due to the absence of infection.

Vaccination with the Muguga cocktail has been deployed successfully in cattle in a number of countries in East Africa, including areas where buffalo are present. The batch of the Muguga cocktail vaccine used in the current study (the ILRI 08 batch) has shown widespread efficacy in the field (unpublished observations). Moreover, a recent field study in northern Tanzania suggested that there is no difference in the efficacy of the vaccine when used in areas containing buffalo compared to those where buffalo were absent (Homewood et al., 2006). However, there is currently no detailed information on the antigenic diversity of the buffalo parasites in this region or the proportion of the ticks that carry buffalo-derived parasites. If ticks carrying cattle-derived parasites predominate, most vaccinated animals will initially be challenged with parasites derived from other cattle in the herd, which may allow the development of a sufficiently broad immunity to protect against subsequent exposure to the buffalo parasites.

Within the Ol Pejeta conservancy, with the exception of the area where the current study was conducted, cattle and buffalo co-graze under an integrated wildlife/livestock management system. Theileriosis caused by *T. parva* is the most important disease in cattle within the conservancy, and has been controlled predominantly by regular application of acaricides to prevent tick infestation. Recent attempts to instigate an ECF immunization regime using the Muguga cocktail have not been successful. Several vaccinated calves died from apparent *T. parva* infection, when the routine 7-day acaricide application interval was extended to 14 days. It is possible that the tick control regime deployed on the conservancy has influenced the nature of the parasite challenge by preferentially reducing the population of cattle-maintained *T. parva*, thus increasing the likelihood that, upon initial exposure, cattle will be challenged with *T. parva* parasites transmitted directly from buffalo.

5. Conclusion

The Muguga cocktail has been successfully implemented in Northern Tanzania to protect against development of East coast fever (cattle-derived *T. parva*) in cattle; at this study site in Kenya, the vaccine does not protect against development of Corridor disease (buffalo-derived *T. parva*) in cattle. Further knowledge of the molecular, antigenic and genetic differences between buffalo- and cattle-derived *T. parva* populations is imperative to gain a more complete understanding of the epidemiology and pathogenesis of the disease and the development of better control tools, including vaccines. Such control should aim to allow cattle to co-graze safely with cattle in *T. parva* endemic areas, and provide an illustrative example of advancing the integrative management of livestock and wildlife.

Conflict of interest

The authors declared that there is no conflict of interest.

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