Ticks and Tick-borne Diseases 6 (2015) 587–591

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Incremental effect of natural tick challenge on the infection and treatment method-induced immunity against *T. parva* in cattle under agro-pastoral systems in Northern Tanzania

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ARTICLE INFO

Article history:
Received 13 September 2014
Received in revised form 28 April 2015
Accepted 29 April 2015
Available online 6 May 2015

Keywords:
Northern Tanzania
Maasai
ECF vaccination
ITM
Theileria parva

ABSTRACT

This study was conducted to assess the incremental effect of natural tick challenge on the infection and treatment method-induced immunity against *T. parva* under agro-pastoral systems in Simanjiro district, Northern Tanzania. *T. parva* specific antibody percent positivity and prevalence of *T. parva* parasites were studied in relation to duration post vaccination and proximity to Tarangire National park. A total of 381 cattle were included in this study, of which 127 were unvaccinated and 254 had been vaccinated at different time points between 2008 and 2014. Antibody percent positivity (PP) determined by the PIM-based *T. parva* ELISA and the prevalence of *T. parva* parasites detected by a nested PCR based on the p104 gene were used to compare vaccinated and unvaccinated cattle. Results showed that seroprevalence was significantly higher in vaccinated than unvaccinated cattle (OR 10.89, $p = 0.0341$). Only 1.6% (6/381) of all cattle were seronegative and 5/6 of these were unvaccinated. Prevalence of *T. parva* parasites was significantly higher in vaccinated (50.39%) than unvaccinated (19.69%) cattle (OR 2.03, $p = 0.0144$). While there was a positive association between PP and duration post vaccination but the latter was inversely associated with *T. parva* parasite prevalence. This study also showed that cattle which were closer to the park had higher antibody PP and *T. parva* prevalence. It is concluded that duration post vaccination as well as proximity from the wildlife in Tarangire National park together may exert an incremental effect on the outcome of ECF vaccination by influencing stronger antibody immunity of cattle and ability to withhold high *T. parva* infection pressure under constant field tick challenge. Further, the high seroprevalence in vaccinated and unvaccinated cattle suggests a likely state of endemic stability to *T. parva* in the study area.

Introduction

Tick-borne diseases constrain the improvement of livestock production efficiency in 11 countries of eastern, central and southern Africa (Mukhebi et al., 1992). The most important of these diseases is East Coast fever (ECF), which is caused by the protozoan parasite *Theileria parva*. The disease is the major cause of deaths, especially among calves in Maasai pastoralist herds in Northern Tanzania, causing calf mortality of about 40–80% (Homewood et al., 1987, 2006; Di Giulio et al., 2003). The predominant control strategy for ECF is based on the use of acaricides to control the tick vector, *Rhipicephalus appendiculatus* (Norval et al., 1992). This method is limited by the high cost of acaricides, development of resistance by vector ticks, disruption of endemic stability, food safety concerns and environmental impacts (deCastro, 1997; George et al., 2004; Ministry of Water and Livestock Development, 2004). Immunization of cattle by the infection and treatment method (ITM) offers a valuable alternative for ECF control (Oura et al., 2004). Immunized animals develop an attenuate infection but also a long-lasting

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http://dx.doi.org/10.1016/j.ttbdis.2015.04.014
1877-959X/

efficient immune response (Di Giulio et al., 2009; McKeever, 2009).

Radley et al. (1975) developed the first *T. parva* vaccine about 35 years ago, which is known as Muguga cocktail. There are several factors limiting the wide adoption of this vaccine, these include; its form of delivery as a potentially lethal dose of cryopreserved *Theileria* parasites, which are inoculated in cattle simultaneously with a long-acting antibiotic. Also, the requirement of cold chain mode of delivery to remote areas makes it relatively expensive (up to US$10 per animal) for small-holder Maasai herders (Di Giulio et al., 2009). Furthermore, ITM does not completely eliminate the need for acaricide application due to the potential existence of other tick-borne diseases. Inadequate studies have been conducted to address scientific questions related to ITM use, such as the potential spread of vaccine parasite strains into the field by ticks and impact of the resulting genetic recombination with local parasite populations (Oura et al., 2007) as well as the risk of introduction of the disease in previously free areas (McKeever, 2007).

The aim of this study was to determine the likely value of incremental effect of natural tick challenge on ITM-induced immunity in agro-pastoral cattle of Northern Tanzania. This study is expected to provide useful information to guide farmers' decisions on ITM adoption especially in agro-pastoral herds in Tanzania, where small-holder livestock keepers usually vaccinate only a proportion of their cattle. Such practice results into intermingling of vaccinated and unvaccinated cattle, what offers a good platform to study the potential spread of Muguga vaccine strains into unvaccinated cattle by ticks as well as the development of carrier state to *T. parva* under constant natural challenge. Here we present results of prevalence of specific antibodies and *T. parva* parasites in vaccinated and unvaccinated cattle kept in same herds in order to advise on efficiency of the ITM in the pastoral herds.

Materials and methods

Study area

The study was conducted in Simanjiro district, which is part of the Maasai steppe in Northern Tanzania and lies south of Arusha city. Eighty five percent of its inhabitants are Maasai agro-pastoralists. The district lies between 3°52′ and 4°24′ south and 36°05′ and 36°39′ east. The five villages included in this study, Emboreet, Loiborsoit, Narakawo, Sukuro and Terrat were chosen according to their proximity to the Tarangire National Park (TNP) on the eastern border of Simanjiro district. The area has a high density of both vaccinated and unvaccinated cattle and is a unique ecosystem with a livestock–wildlife interface (Lamprey, 1964; Kahurananga, 1981; Reid and Kruskal, 1998), where vector-borne diseases including ECF are endemic.

Study design

This was a cross-sectional study done in 20 households keeping local zebu cattle. Selection of the households (kraals) was based on two criteria: (i) presence of both vaccinated and unvaccinated cattle in the same herd and (ii) proximity to TNP, which was in the range of 1–4 km (near park) and 5 km or more (far from park). Vaccinated cattle were easily identified by ear-tag numbers, which indicate year of vaccination what was also confirmed by farmers. Upon receipt of consent from local and village authorities as well as farmers, individual cattle were randomly selected and data on their vaccination status and duration since vaccination were retrospectively collected. Sample size was calculated assuming a 95% confidence level, 50% expected seroprevalence and parasite prevalence and 0.05 tolerable error. Blood was collected from a total of 384 cattle just at the end of the rainy season (April 2014). All samples were properly labeled and stored for further analyses. Three blood samples did not yield good DNA and were removed from further analysis.

Blood samples

Blood samples for serum preparation were taken from each animal by jugular venipuncture using 10-ml vacutainer tubes (Becton Dickson Vacutainer Systems, England). Blood samples were then kept in cool boxes with ice for 2–5 h before refrigeration. The blood samples were centrifuged at 3000 × *g* for 20 min in the laboratory and the serum aliquots were stored in a freezer at −20 °C until ELISA tests were done. Blood samples for DNA extraction were kept frozen at −20 °C until day of analysis.

DNA extraction

Genomic DNA was extracted from 200 μl whole blood using the protocol as described in Thermo Scientific GeneJET Genomic DNA Purification Kit (#0721). Extracted DNA was eluted in 100 μl elution buffer and then stored at −20 °C until further analysis.

p104 nested PCR

The nested p104 PCR was used to screen cattle DNA samples for the presence of *T. parva*. Primers derived from the *T. parva*-specific 104-kDa antigen (p104) gene were used in the PCR amplification as previously described by Odongo et al. (2010) and Iams et al. (1990). The sequences of the forward and reverse primers were 5′ATT TAA GGA ACC TGA CGT GAC TGC 3′ and 5′ TAA GAT GCC GAC TAT TAAT-GACAC C 3′, respectively, for first round and 5′ GGC CAA GGT CTC CTT CAG AAT ACG3′ and 5′TGG GTG TGT TTC CTC GTC ATC TGC 3′, respectively, for the second round. The nested polymerase chain reaction (nPCR) amplifications were performed in a total volume of 20 μl containing 14 μl nuclease-free water, 0.5 μl (10 pmol) of each of forward and reverse primers and 5 μl of genomic DNA (20 ng/μl) template added into the lyophilized pellet (Bioneer PCR Premix – Korea), followed by vortexing and brief spin down to dissolve the pellet. For the second round, the amount of water was 18.5 μl, and 0.5 μl of the primary PCR product was used as a template. Reaction conditions for the primary PCR included initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 60 s, annealing at 60 °C for 60 s and extension at 72 °C for 60 s, and the amplification was done in 30 cycles. The cycling profile condition for the second PCR was the same as the primary amplification, except for the annealing temperature which was 50 °C. The nPCR reactions were carried out in a thermocycler (Veriti™, Applied Biosystems, USA). The nPCR products were separated on 1.5% agarose gel and images visualized and documented on a Gel Doc™ (Bio Rad, USA). Positive nPCR products were identified as 277 bp DNA fragments.

Indirect ELISA for T. parva *antibodies*

The PIM-based enzyme-linked-immunosorbent assay (ELISA) described by Katende et al. (1998) was used to measure specific antibodies to *T. parva* (sensitivity > 99%, specificity 94–98%). Optical density (OD) of each sample was measured at 405 nm using an Erba Lisascan II ELISA reader (ERBA diagnostics, Mannheim GmbH, Germany). The OD readings were used to compute percent positivity (PP) for each sample using the formula: Mean OD (sample or negative control) divided by mean OD of positive control multiplied by 100. PP of 20% or higher was considered positive.