Research article

Tubulin vaccinated Ankole cattle develop less severe lesions than the non-vaccinated cattle when experimentally infected with *Trypanosoma brucei brucei*

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Abstract

Invasion of the Central Nervous System (CNS) by African trypanosomes represents a critical step in the development of human African trypanosomiasis. The study aimed ats assessing the role of tubulin vaccine candidate in protection of cattle against trypanosomiasis using *Trypanosoma brucei brucei* subspecies that is highly related to *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* that cause sleeping sickness in man. The tissue behavior and cerebral fate of *T. b. brucei* in cattle should mimic the situation in humans and since cattle are also natural hosts for trypanosomes, it was envisaged that the cattle system would be a more suitable model for vaccination studies than the rodent model. Experimental infection of tubulin vaccine candidate vaccinated and non-vaccinated Ankole long horn cattle breed calves was done using a *Trypanosoma brucei brucei* parasite strain that had been previously isolated from naturally infected cattle in Uganda. Trypanosomiasis disease progression and associated pathology were assessed by clinical and extensive post mortem examinations. Marked organ abnormalities and severe lesions were observed in the non-vaccinated cattle, however, the findings revealed that tubulin vaccination in cattle lowers tissue parasitiasis and ameliorates the inflammatory pathology and clinical signs of trypanosomiasis by *Trypanosoma brucei brucei*. The trypanosome tissue invasion may be susceptible to immunological attenuation.

Introduction

African trypanosomes cause a disease syndrome of high morbidity and mortality across large areas of sub-Saharan Africa in both humans (sleeping sickness) and domesticated animals (nagana). The parasites (trypanosomes) are mainly spread by blood-feeding tsetse flies (genus: Glossina). Trypanosome species that infect cattle and the disease picture associated with each species was described by Hornby (1921) [1]. The species include *Trypanosoma congolense*, *T. vivax* and *T. brucei brucei*. The latter is closely related to *T. b. rhodesiense* and *T. b. gambiense* that cause sleeping sickness in humans but itself does not establish in humans because it is lysed by a human serum resistance factor ((Holmes, et al., 2004)) [2]. However, the human species do occur in animals which act as reservoirs, but cattle experimentally infected with *T. b rhodesiense* can develop a syndrome similar to that caused

by *T. b. brucei* (Wellde et al., 1989) [3]. The clinical presentation of infected hosts and consequences differ depending on complex factors, including breed, species, strain and nutritional status (Murray, et al. 1984)) [4]. *Trypanosoma congolense* and *T. vivax* are known to cause acute disease conditions in the mammalian hosts, whereas *T. b. brucei* is usually considered a low-grade pathogen (Killick-Kendrick, 1971) [5]. Thus, the generalization is that *T. b. brucei* is not a significant pathogen in cattle but there is no conclusive evidence for this assertion (Ikede and Losos, 1972) [6], the significance of this parasite being obscured by mixed infections with *T. congolense* and/or *T. vivax* ((Killick-Kendrick, 1971) [5]. Indeed, the pathological role of *T. brucei* may be greater in mixed than in single infections (Van den Bossche, et al., 2004) [7]. On the other hand, *T. b. brucei* is very pathogenic for almost all laboratory animals, equine, canine and swine (Uilenberg, 1998) [8]. Another feature that may contribute to obscuring its pathological and economic role is that at some stage *T. b. brucei* disappears into connective tissue so that the organisms, which

could nevertheless be causing a lot of ill-health, are not detected in blood during routine diagnosis of infections.

The more important trypanosome species affecting man and his domestic animals have been subdivided into two groups, the haematinic group (*Trypanosoma congolense*, *T. vivax*) which remains in the plasma and the tissue invading group (*T. brucei, T. evansi, T. gambiense, T. rhodesiense and T. equiperdum*) which is found extravascularly and intravascularly (Losos and Ikede, 1972) [9]. Because of their presence in the blood, they produce numerous changes in the cellular and biochemical constituents of blood. The lesions in tryapanosomiasis also vary considerably depending on species, strain and host. The principal lesion in hematinic infections is severe anaemia. Although anaemia occurs with *T. b. brucei*, inflammatory, degenerative and necrotic changes associated with cellular invasion (mononuclear cell infiltration) and edema of various organs are more dramatic (Ikede and Losos, 1972; Moulton and Sollod, 1976) [10,11]. This study hypothesized that any successful intervention such as immunization could result in the diminishing of such lesions. The present study reports the less severe pathological changes in recombinant tubulin vaccinated Ankole cattle in comparison to the non-vaccinated when challenged with a *T. b. brucei* UTRO 010291B strain that is known to cause high mortality in cattle under experimental conditions.

Trypanosomiasis is classified as an immunological disease (Dargantes et al, 2005; Velthuysen et al., 1994) [12,13] whereby it has been suggested that many of the lesions such as glomerulonephritis is the result of deposition of immune complexes that interfere with organ function. The intense antibody response to the glycoprotein coat kills the trypanosomes and the released antigens provoke further antibody reaction that results in the formation of plenty of antigen-antibody complexes; this would reoccur repetitively as new variants appear repetitively. Confounding to this is the marked immunosuppression that lowers host's resistance to other infections and complicate the clinical and pathological picture. In normal circumstances, any factor that would limit the number or magnitude of trypanosome waves would lower the histopathogical effects qualitatively and/or quantitatively.

This study was conducted with *T. b. brucei* rather than *T. congolense* or *T. vivax* because the vaccine candidate, tubulin, under consideration was previously tested with *T. b. brucei* in mice (Lubega, et al, 2002) [14] and the homology of tubulin among different trypanosome species is such that cross-immunizations are not farfetched (Li, et al., 2007) [15]. The tissue behavior and cerebral fate of *T. b. brucei* in cattle should mimic the situation in humans and since cattle are natural hosts for trypanosomes, it was envisaged that the cattle system would be a more suitable model for vaccination studies than the rodent model. Thus, the *T. b. brucei*-cattle system would be a universal model for both nagana and sleeping sickness. At the moment vaccine candidates against

trypanosomiasis are limited but with advent of bioinformatics many vaccine candidates are likely to be encountered (Gull, 2002) [16]. *Trypanosoma. b. brucei* causes animal African trypanosomiasis (AAT), along with several other *Trypanosoma* species. *Trypanosoma b. brucei* is not human infective due to its susceptibility to lysis by human apolipoprotein L1 (Vanhamme, et al., 2003) [17]. However, as it shares many features with *T. b. gambiense* and *T. b. rhodesiense* (such as antigenic variation) it is used as a model for human infections in laboratory and animal studies. This study reports the mild pathological lesions due to trypanosomiasis in Ankole long horn cattle vaccinated with a recombinant tubulin subunit protein vaccine candidate through experimental challenge with *T. b. brucei* under controlled conditions.

Materials and methods

Ethical statement/considerations

The maintenance and care of experimental animals complied with the Institutional Maintenance and Care Guidelines (College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB) Animal Health Ethical Committee, Makerere University). The study received ethical approval from the School of Biotechnical and Laboratory Sciences (SBLS) (Reference: SBLS.AN. 2010).

Trypanosome parasites

Trypanosomes (*T. b. b* UTRO 010291B strain) from stabilates (cryo-preserved in liquid nitrogen) were revitalized in mice and the parasites propagated (numbers increased) in rats. The mice and rats were kept in cages, with food and water provided ad libitum, at 21-25^{0}C with a 12-hour light: 12-hour dark photoperiodicity. For revitalization, two mice were each infected intramuscularly with 200µl of infected blood from the trypanosome stabilate. Parasitaemia was monitored microscopically daily by wet blood smears of tail blood starting on day 2 post-infection. The trypanosomes observed under wet smears were quantified by matching with a parasitemia scoring scale according to Herbert and Lumsden (1976) [18]. At maximum parasitaemia ($10^{8.2}$-$10^{8.4}$ parasites/ml), the mice were sacrificed and the infected blood harvested by cardiac puncture. The blood was then diluted in 60mM phosphate- buffered saline containing 40mM glucose (PSG) and the parasites counted using the Neubauer haemocytometer. The counted parasites were further diluted in PSG to get a dose of 1x10^4 trypanosomes per 0.2ml inoculums. This parasite dose (200µl per rat) was then inoculated intramuscularly into five rats with the aim of increasing parasite numbers. The rats were also monitored for parasitaemia post-infection as described above. At maximum parasitaemia ($10^{8.2}$ -$10^{8.4}$ parasites/ml), the rats were sacrificed, infected blood harvested by cardiac puncture, diluted in PSG and parasites counted as described above and then used to infect cattle with a known dose at the appropriate time.

Cattle selection and maintenance

Female Ankole calves 1-2 years of age and weighing 120-200kg were used for the study. The animals had been purchased from Western Uganda, an area where trypanosomiasis is least anticipated. The initial selection of animals was based on Enzyme Linked Immune Sorbent Assay (ELISA) for absence of *T. b. brucei, T. congolense* and *T. vivax* antibodies against the respective whole cell extracts (Katende, et al, unpublished). Then Polymerase Chain Reaction (PCR) for absence of trypanosome DNA with the Internal Transcribed Spacer (ITS) primers (Moser, et al., 1983) [19] was performed to rule out any missed ELISA positive cases including incubating infections. Animals with Packed Cell Volume (PCV) levels not less than 30% were selected from among those negative for trypanosome infection by both ELISA and PCR. The selected animals were dewormed, sprayed with acaricide and prophylactically treated against common haemo-parasites and placed under quarantine for a month. During the quarantine period, the animals were monitored clinically and microscopically (parasite detection) at weekly intervals. Thus, prior to the commencement of the experiment, the animals were in good health and free from inter-current infections. The animals were kept in a fly-proof animal house at COVAB, Makerere University. They were fed on hay (12kg/animal/day) and concentrates (3kg/animal/day). Water and mineral licks were given ad libitum.

Experimental design

Two separate experiments were conducted. One was a baseline post mortem study carried out to determine the typical lesions caused by *T. b. brucei* (UTRO 010291B strain) in fully susceptible Ankole long horn cattle. The other experiment was a post mortem study carried out on the long-time survivors of the tubulin vaccine candidate immunized and challenged animals to determine whether they were recovering from the lesions or not. Comparison of the two post mortem studies determined the effect of tubulin vaccination on the nature and severity of lesions due to trypanosomiasis in Ankole cattle.

Monitoring of temperature, parasitaemia, PCV and other clinical changes

Unless otherwise mentioned, animals were subjected to clinical and laboratory evaluations from the time of challenge with trypanosomes up to the time of sacrifice for post mortem analysis using standard procedures. Temperature, parasitaemia and PCV among other things were monitored. Clinical parameters were monitored daily in the baseline post mortem study while in the immunization-post mortem experiment, the rectal temperatures were taken daily during the first week but subsequently twice a week, using a digital thermometer. Jugular blood samples were examined daily for the presence of trypanosomes starting day 4 post infection (dpi) until animals became patent. After patency, blood was examined twice a week.

The presence of parasites was monitored by dark ground buffy coat technique (Murray, et al., 1977). The parasitaemia intensities were determined as described by Paris, et al (1982) [20]. The PCV, to access the degree of anaemia, was estimated using standard micro-haematocrit method (Jain, 1986). When parasites could not be detected upon several samplings, blood from suspected cattle was inoculated into two mice per animal (200μl/mouse intramuscularly) for more sensitive infection status evaluation in the immunization study.

Baseline post mortem study

This study consisted of three groups (A, B and C), each group containing three female Ankole calves 1-2 years of age and weighing 120-200kg. Two animals in each group were each infected intra-dermally in the neck region with $5x10^4$ trypanosomes/200μl dose. One animal in each group was maintained as an uninfected control. All animals were subjected to clinical, parasitological and haematological examinations as described above. Animals in groups A, B and C were sacrificed at 14, 28 and 84dpi respectively. The 14, 28 and 84dpi represented the acute, transient and chronic phases of the disease respectively. Systematic physical (ante mortem) examinations were carried out prior to the sacrifice of the animals for post mortem analysis.

Immunization-post mortem study

This study involved the post-mortem of long-time survivors from three experimental groups (A, B & C) of nine female Ankole calves. The sex, age, weights and maintenance of animals were the same as in the baseline post mortem study. The trypanosome β-tubulin recombinant antigen (source) used to immunize cattle in group A was emulsified in Quil A adjuvant (Sigma-Aldrich, Germany) while that used to immunize cattle in group B was emulsified in a mixture of Quil A and Alhydrogel (Sigma-Aldrich) adjuvants. These animals were challenged with $5x10^4$ trypanosomes/200μl/dose intra-dermally three (3) days after the 2nd (last) boost of immunization. Animals in Group C were not immunized but challenged with a similar dose of parasites. Clinical, parasitological and haematological data were collected twice a week. Animals were also weighed once every week. A complete physical examination (ante mortem) was carried out prior to the sacrifice of the animals for post-mortem analysis.

Sacrifice and gross examination procedures

For sacrifice, animals were first put on lateral recumbency and totally restrained with ropes. The animals were ex-sanguinated by jugular lavage to facilitate complete drainage of blood from all the organs. The gross pathology (necropsy) evaluation was carried out as per the guidelines of Necropsy of the CL Davis DVM foundation (King, 2005). Briefly, the animal was put on dorsal

recumbency and the skin flayed off to expose the subcutaneous tissues. The state of hydration and any other lesions of the cutis and the sub-cutis were evaluated at this stage. Incisions were made along the costal arch, dorsal flank and the pelvic rim to allow exposure of the abdominal cavity and viscera. A stab was made in the upper most part of the diaphragm to evaluate the pneumo-status of the thorax. The entire upper side of the diaphragm along the costal arch was cut to expose the thoracic viscera and also determine the fluid status. Using a bone cutter, the ribs were cut along the sternum and along the vertebrae to expose the thoracic viscera.

Gross evaluation of the brain was achieved by cutting a transverse line through the frontal bone caudal to the zygomatic processes of the frontal bone. Incisions were made sagitally medial to the occipital condyles. Putting a knife in the incision in the frontal bone the skull cap was pried off to expose the brain. The brain was excised and detached from the spinal cord to enable close examination of all the surfaces. The following organs were examined grossly and samples taken for impression smears and histological analysis: brain, liver, kidney, lymph nodes (pre-scapular, pre-crural and mesenteric), bone marrow (femur), heart, lungs, spinal cord (adjacent to brain), pancreas and spleen. The rest of the carcass was disposed-off by incineration.

Impression smears examination

Impression smears were prepared from freshly cut organs immediately after sacrifice and gross examination. Air dried impression smears were fixed in 100% methanol for three (3) minutes, air dried, stained for 45 minutes in 6% Giemsa stain, followed by washing the slides in Phosphate Buffered Saline (PBS) pH 8.0 and air drying. Microscopic examination for the detection of trypanosomes was done at X40 and X100 magnifications using a light microscope (Olympus).

Histopathological techniques

Whole organs or sections thereof were fixed in neutral-buffered 10% formalin. Tissue blocks were embedded in paraffin wax and sections cut at 5µm using rotary microtome (Leica RM 2235 Germany) and stained in Maryer's hematoxylin and eosin (Sigma-Aldrich, Germany). Microscopic examination for the detection of trypanosomes and histological changes were done using X40 and X100 objectives of an inverted light microscope (Olympus).

In an attempt to make quantitative comparisons between animals the severity of lesions was scored in the kidneys and brains. The quantitative assessment was standardized by counting the positivity/lesions in ten microscopic fields, one field apart in a 10 x 10 graticule (x40 objective). The five (5) point grading scale was developed for each region/organ (Kennedy et al., 2003) [21]. The grades ranged from 0 to 4. Grade 0 was given when there were no pathological lesions and was not included on the visual aid.

When the distribution of lesions was seen in seven or more fields, it was set as the upper limit of grade 4. Grade 1 was the lowest count above zero with lesions in two or less fields while Grade 2 showed lesions in three or four fields and Grade 3 showed lesions in five or six fields. The repeatability of all counts was assessed by the same observer by re-counting 10% of all cases.

Results

Histopathological findings for baseline post mortem study

Impression smears: Trypanosomes were detected in impression smears of the liver, spleen and pre-scapular lymph nodes but not in other organs at 14 and 28 dpi but absent at 84 dpi (Table 1). Parasites were particularly easily seen in spleen and pre-scapular lymph nodes smears (Figure 1). Many parasites were seen on day 14 and scanty parasites were seen on day 28 post-infections.

Organ	14dpi	28dpi	84dpi
Brain	-	-	-
Kidney	-	-	-
Liver	+	-	-
Spleen	+	±	-
Pre-scapular ln	+	±	-
Heart	-	-	-
Mesenteric ln	-	-	-
CSF	-	-	-
Pre-crural ln	-	-	-
Bone marrow	-	-	-

Table 1: Positivity of organ impression smears for trypanosome parasites.

Key: **+**; Trypanosomes seen, **-** ; Trypanosomes not seen, **±**; Trypanosomes seen or not seen; CSF; cerebral spinal fluid, ln; lymph node, dpi; days post infection.
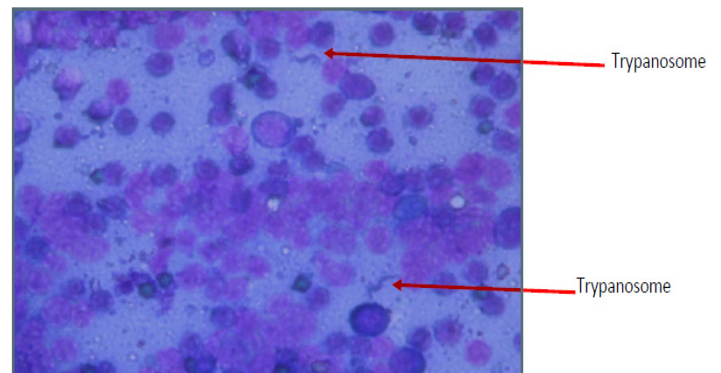

Figure 1: Trypanosomes in impression smear of the pre-scapular lymph node from cattle 14 days' post-infection. Impression smears were prepared from the cut surfaces and stained with 6% Giemsa for microscopic examination. Trypanosomes (red arrow) appear in inter-cellular spaces (x100 objective).

**Histopathological lesions in the baseline post mortem study:** Remarkable microscopic lesions were observed in the lymph nodes, spleen, kidney, heart and brain. Depending on the organ, either lesions healed, persisted or were replaced with different lesions as the infection progressed towards the chronic phase (84dpi).

Lymph nodes: At 14dpi, there was severe increased cellularity of the medulla of the pre-scapular lymph nodes and trypanosomes could be seen in the medulla sinuses (Figure 2b). The cellular reaction was moderate by 28dpi but trypanosomes could still be seen in the medulla sinuses. However, by 84dpi cellular reactivity had ceased and trypanosomes were not detectable. The other lymph nodes (pre-crural and mesenteric) remote to site of parasite inoculation exhibited no lesions and no parasites.


(a) Uninfected x200   (b) Infected x200
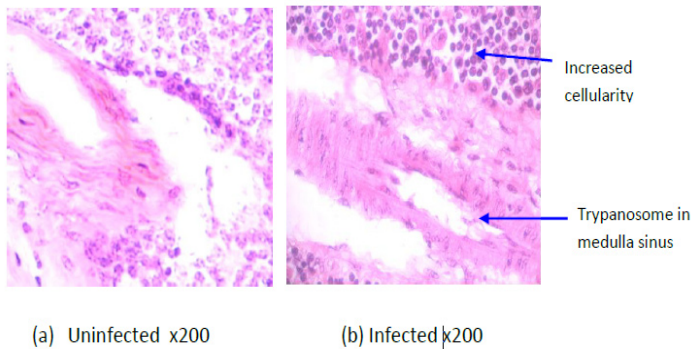
Figure 2: Histological appearance of pre-scapular lymph node in calves 14 dpi with *T.b.brucei*. Panel a: Normal distribution of cells of uninfected control animal. Panel b: Increased cellularity with mononuclear cells and presence of trypanasomes in the medulla sinuses of infected animal.

Spleen: At 14dpi, the increase in cellularity was severe in the spleen and trypanosomes were numerous (Figure 3b). The trypanosomes appeared intravascularly, peri-vascularly and interstitially. Trypanosomes located interstitially appeared ellipsoidal in shape and lacked free flagella. At 28dpi, the number of trypanosomes was moderate and the degree of cellularity was mild (data not showed). At 84dpi the trypanosomes were very few and the number and distribution of the cells were similar to the uninfected control animal (data not shown).

(a) Uninfected x 400   (b) Infected x 400
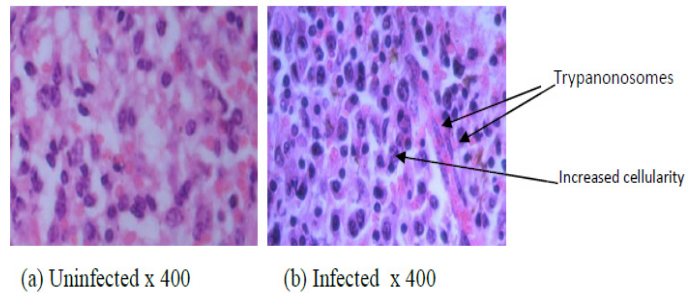
Figure 3: Histological appearance of the spleen from calves 14dpi with *T. b. brucei*. Panel a: Normal distribution of cells in the uninfected control animal. Panel b: Increased cellularity of the mononuclear cells and presence of trypanosomes in infected animal.

Kidney: The early lesions in the kidney were interstitial mononuclear cell infiltrations (Figure 4b) and glomelular contractions with associated enlargement of glomelular space (Figure 5b). Unlike the organs discussed above, the kidney acute phase lesions persisted to the chronic phase and became more severe with time. At 14dpi there was mild glomerular contraction and mild increase in glomerular space of only a few glomeruli. At 28dpi, moderate glomerular contraction and moderate increase in glomerular space were observed. In addition to the above lesions, moderate focal tubular necrosis had set in. At 84dpi, the severe glomerular contraction and severe increase in glomerular space were observed (Figure 5b) and severe (diffuse) tubular necrosis was observed in the renal tissues (Figures 5c, 6b).

(a) Unifected x 10   (b) Infected 14dpi x10
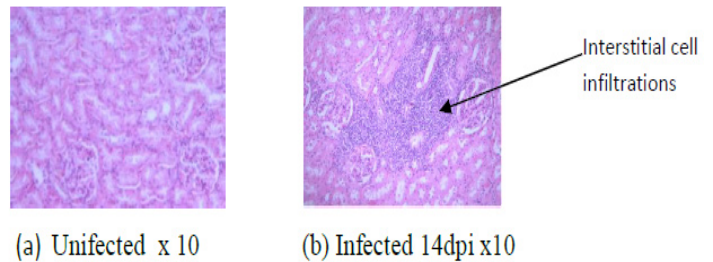
Figure 4: Histological appearance of the kidney of calves infected with *T. b. brucei* at 14 dpi. Panel a: Normal distribution of interstitial cells of the uninfected control animal. Panel b: Severe interstitial cellular infiltrations (arrow) in the kidney of infected animal.

(a) Unifected x20   (b) Infected 14dpi x20   (c) Infected 84dpi x20
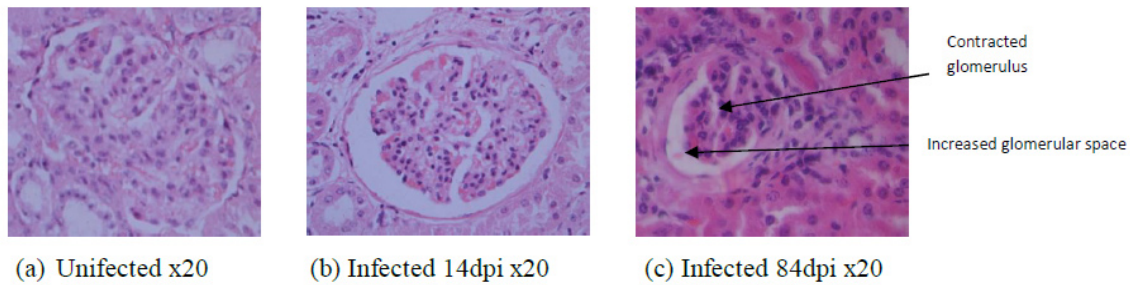
Figure 5: Histological appearance of glomeruli of the kidneys of calves infected with *T. b. brucei* at 14dpi and 84dpi Panel a: Normal structure of the glomerulus in the uninfected control animal. Panel b: Increased glomerular space and mild glomerular contraction at 14dpi. Panel c: Severe glomerular contraction and relatively increased glomerular space at 84dpi.


(a) Unifected x100   (b) Infected 84dpi x100
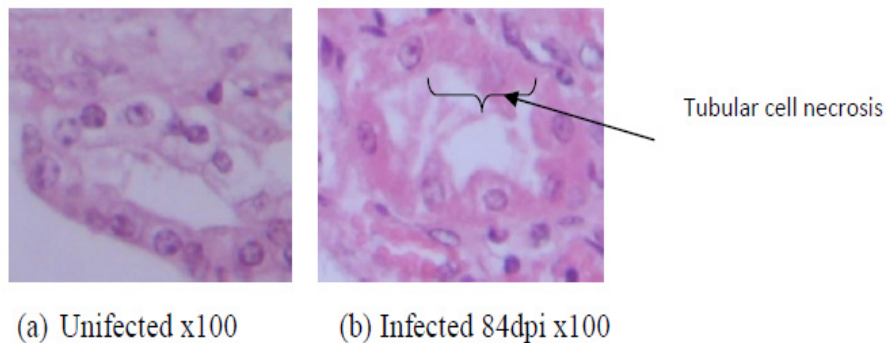
Figure 6: Histological appearance of the renal tubules at 84dpi in calves infected with *T. b.brucei* Panel a: Normal tubular cell morphology and distribution in the kidney of uninfected control animal. Panel b: Severe tubular cell necrosis in the kidney of the infected animal.

Heart: Haemorrhages on the endocardium were the only lesions in the heart and involved mainly the ventricles. Haemorrhages were mild at 14dpi, became moderate at 28dpi and severe at 84dpi (data not shown).

Brain: Although trypanosomes disappeared with time post infection, they appeared to be more persistent in the brain than in other tissues. They also appeared to change location with time being located intravascualry or perivascualry in the early phase and in the parenchyma in the late phase. The main microscopic lesions in the brain were cell infiltrations and necrotic degenerative changes. The lesions were progressive whereby they were more severe in the late/chronic phase (Figure 7). At 14dpi numerous trypanosomes were seen in the blood vessels of the cerebrum and cerebellum (Figure 7a). accompanied with mild cell infiltrations around blood vessels (perivascular cuffing) (Figure 7b). At 28dpi the trypanosomes localization had not changed but the cellular infiltrations were more prominent around the blood vessels (Figure 8b). At 84dpi trypanosomes were much fewer and mainly located in the parenchyma (Figure 9a); the cellular infiltrations were severe and could be observed around blood vessels of the meninges (Figure 9b).

(a) Infected x20

(b) Infected x10
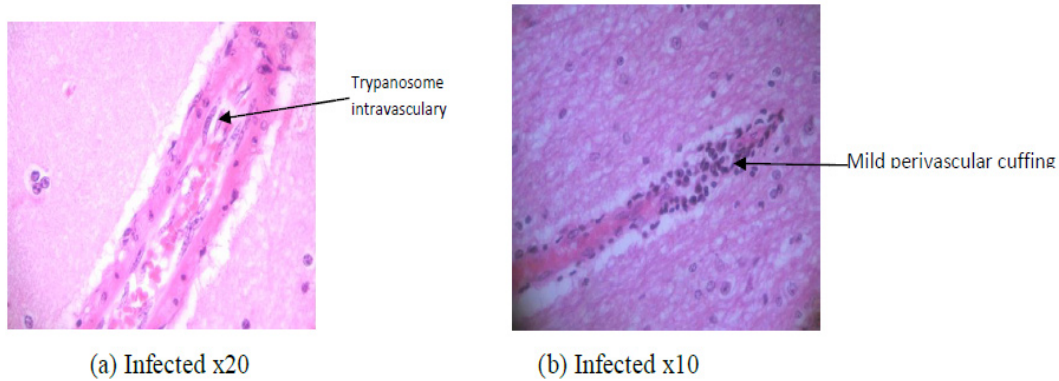
Figure 7: Histological appearance of the brain at 14dpi in calves infected with *T. b. brucei* Panel a: Trypanasomes are seen intravasculalry (arrow). Panel b: Presence of mild perivascular cell infiltrations (perivascular cuffing).

(a) Infected x40

(b) Infected x10
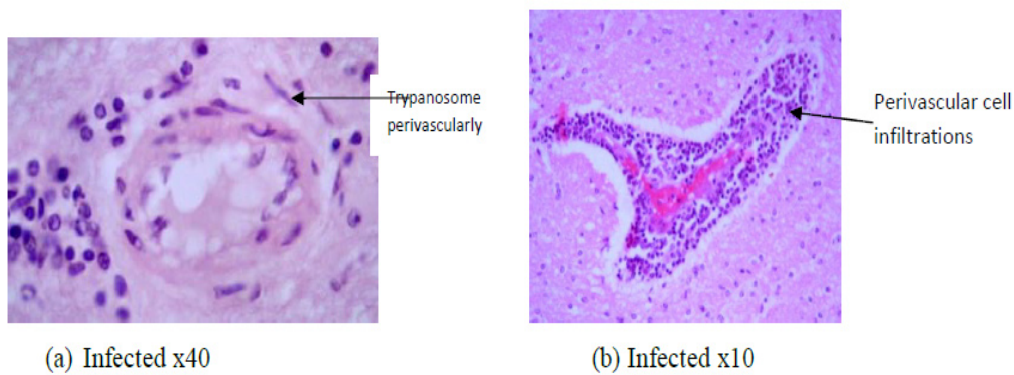
Figure 8: Histological appearance of the brain at 28dpi in calves infected with *T. b. brucei* Panel a: Trypanosomes are seen perivascularly (arrow). Panel b: Presence of diffuse/severe perivascular cell infiltrations (perivascular cuffing).

(a) Infected x40

(b) Infected x10
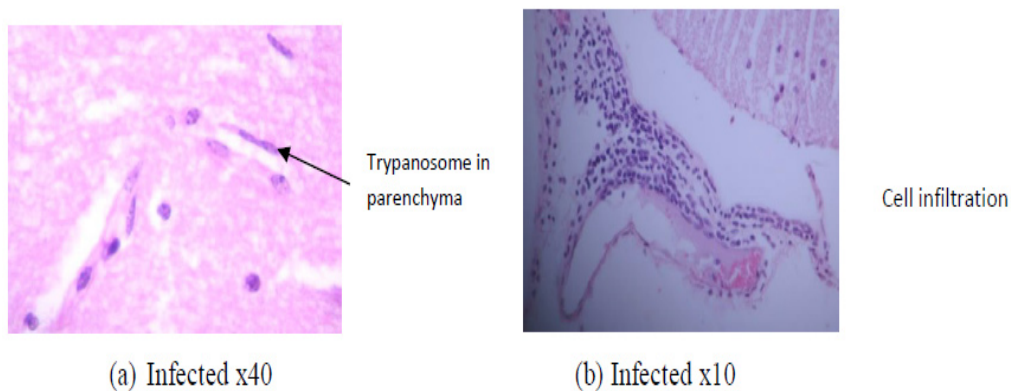
Figure 9: Histological appearance of the brain at 84dpi in calves infected with *T. b. brucei* Panel a: Trypanosomes are seen in the brain parenchyma. Panel b: Presence of moderate perivascular cell infiltrations (perivascular cuffing).

Pathological findings in health survivors of the immunized-infected cattle

The animals were observed all through the clinical disease and were monitored for parasites and other clinical parameters were evaluated. The animals were observed to gradually recover from the disease. Over time, the body conditions of the animals were observed to improve and the animals eventually gained the good lustre of the skin and gained weight. These animals were sacrificed at four different time points (247,254,295 and 336dpi). Uninfected control animals were included at each sacrifice for comparisons. Grossly all carcasses were in good condition. Organs afflicted or not afflicted with some form of lesions were similar as for the non-immunized infected calves except for the liver. Furthermore, the lesions were mild or absent with increased survival time.

Gross pathological findings for the immunization post mortem study

Two out of four immunized animals sacrificed showed mild enlargement of the pre-scapular lymph nodes. The lymph nodes in the other animals were not enlarged. Three out of four spleens had mild enlargement and opaque capsules. The spleen of the animal which was sacrificed last was of normal size and consistence. The opaque capsule of the spleen was not observed in the uninfected control animals. In three of the vaccinated animals the kidneys had mild enlargement with few whitish foci. The animal that was sacrificed last (336dpi) had no gross lesions in the kidney. The animals had haemorrhages on the heart endocardium except the animal that was sacrificed last. There were mild congestion and mild haemorrhages and/or exudates in the brains of two out of the four animals. The lesions were observed to reduce with time post infection. The animal sacrificed last (336 dpi) had no gross lesions in the brain.

Histopathological findings for the immunization post-mortem study

No pathological findings were seen in the lymph nodes during the chronic stage except in one immunized animal which had slight oedema. The spleens of two of the four immunized animals (sacrificed at 247dpi and 254dpi) showed moderate cellularity with average of one parasite per 10 fields while one animal (sacrificed 295 dpi) showed mild cellularity and no parasites. No parasites were observed in the spleen of the animal sacrificed at latest time point. The livers of three of the animals showed mild cellular infiltrations. These were animals sacrificed at earlier time points. Glomerular contraction of the kidneys was found to be mild in those animals that were sacrificed earlier. The lesions were scarcely interspersed within the normal renal tissue in those animals that were sacrificed. The kidney glomerular contractions were observed to become fewer with time and at the end of the experiment, did not find any glomerulus with the contraction. Mild haemorrhages of the heart were seen in three animals but absent in the animal which was sacrificed last. Trypanosomes were seen in the brain parenchyma of three of the immunized animals. No trypanosomes were seen in the one immunized animal that was sacrificed last. Mild cellular infiltrations around brain capillary blood vessels (perivascular cuffing) were observed in the animals that were sacrificed at early time points but in the animal that were sacrificed at the last time point, there were almost no cellular infiltrations.

The 2 adjuvant regimens (Quil A or Quil A plus Alhydrogen) used for immunizations did not have any influence on the clinical and pathological outcomes of trypanosomiasis. Similar findings were obtained in the two vaccinated groups where the adjuvant regimens were varied. Thus, both adjuvants are good agents for tubulin vaccine delivery.

Discussion

The purpose of this study was to examine the pathological lesions associated with African trypanosomiasis in susceptible tubulin vaccine candidate vaccinated or non-vaccinated Ankole Long horn calves using a *T. b. brucei* UTRO 010291B strain. The parasite strain produced acute and chronic infections which allowed a study of progressive pathology in non-vaccinated cattle. In this respect, the model is similar to natural infections of humans and domestic animals where acute and chronic phases of the disease occur (Goodwin, 1970; Losos and Ikede, 1972) [22,23]. Also, this strain reliably produces a central nervous infection, an important aspect of the course of the disease in humans (Jemlings, et al., 1977) [24]. The acute stage was characterized by relapsing parasitaemia similar to that seen in natural infections and the second stage was a prolonged chronic wasting phase where the animals remained non parasitaemic (no parasite detection in blood). In the non-vaccinated cattle, the kidney and brain exhibited marked proliferative responses which were maximal in animals sacrificed 84dpi. Similar findings were reported in these organs during the chronic phase of the disease (Masake and Morrison, 1981) [25]. Trypanosomes of the brucei group are not strict plasma parasites but are capable of invading interstitial spaces of tissues of infected animals (Losos and Ikede, 1971, 1972). The observations of the present study that *T. b. brucei* localized both intra and extravascularly in cattle is consistent with previous findings (Losos and Ikede, 1972) and produced lesions similar to those reported in small ruminants (Losos and Ikede, 1970; Ikede and Losos, 1970) [26], laboratory rodents (Goodwin, 1970; Losos and Ikede, 1970) [22,26] and cattle (Losos and Ikede, 1972). This study also revealed that *T. b. brucei* is a significant parasite of cattle with acute and chronic clinical disease which was similar to previous findings where the disease is very pathogenic for almost all laboratory animals, equine, canine and swine (Uilenberg, 1998) [8].

There are neither gross nor histopathological lesions that are pathognomonic for trypanosomiasis. The striking pathological lesions are degenerative changes in the brain and kidney but these varied in different animals. This was in line with published data that *T. b. brucei*. infections present with inflammatory, degenerative and necrotic changes that are associated with cellular invasion (mononuclear cell infiltration) and oedema of various organs (Ikede and Losos, 1972; Moulton and Sollod, 1976) [11]. Compared with the control (infected, non-immunized) animals, the degenerative changes of tubulin vaccinated cattle were mild. Clinically ill animals (infected controls) had profound lesions in the kidneys where contractions of the glomeruli and cell infiltrations were seen. In contrast, the glomerular contractions became fewer with time post-infection and were absent in the animal that was sacrificed last. This agrees with earlier studies whereby severe pathological changes that are degenerative occur in kidney (Ikede and Losos, 1972; Moulton and Sollod, 1976) [11].

In all the brains of the immunized animals, few trypanosomes were seen in the parenchyma, but in the non-vaccinated animals, trypanosomes were detected in capillary blood vessels, perivascularly and in the parenchyma at different sacrifice periods. At the experimental end point (336dpi) in the vaccinated cattle there were no parasites, minimal perivascular cuffing and encephalitis contributing to a significantly improved neuropathology score. This was probably a consequence of the lower CNS parasite burden in the tubulin vaccinated cattle. Similar findings in the tubulin-vaccinated mice were reported (Lubega, et al., 2002; Li, et al., 2007) [14,15]. This study demonstrates the potential for modulation of the critical step in the progression and pathogenesis of clinical African trypanosome infections, namely the invasion of the CNS and development of neuropathology using vaccine candidates. It seems unlikely that the study findings could be directly applied for vaccination against African trypanosomiasis, rather, they demonstrate the potential for effective vaccinations using more refined immunogens and further emphasizing the importance of host-inflammatory response polymorphisms in understanding the diverse spectrum of progression observed in African trypanosomiasis [27,28].

Conclusions and recommendations

The tubulin subunit vaccine candidate may have prevented severe/pronounced pathological lesions and also prolonged the lives of cattle beyond those of the non-vaccinated infected controls. Consequently, it could be a potential vaccine candidate in the management of African trypanosomiasis. *Trypanosoma brucei brucei* UTRO 010291B is a significant pathogen in Ankole Long horn cattle. *Trypanosoma b. brucei* and cattle can be used as models for evaluating vaccine and drug candidates for African trypanosomiasis Analysis.

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