Recombinant Protein from *Trypanosoma Congolense* a Good Diagnostic Antigen in Immunochromatographic Test

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Abstract

Trypanosomes are hemo-flagellate protozoan parasites that cause disease in humans and livestock called sleeping sickness and nagana, respectively. So far, a vaccine has been elusive because of the immunodominant variant surface glycoprotein (VSG) in a phenomenon known as antigenic variation. According to amino acid domains structure, Tc38630 was assumed as a *Trypanosoma congolense* orthologue of the *T. brucei* invariant surface glycoprotein (ISG) therefore, a potential diagnostic antigen. Recombinant protein Tc38630 was successfully expressed, characterized and found to be antigenic. Among other serological tests, immunochromatographic test (ICT) has an advantage as a one-step rapid analysis, thus making it a convenient and sensitive diagnostic test .BALB/c mice were immunized with rTc38630 protein and serum was collected for IgG polyclonal antibodies purification for use as control line in ICT. The antigen-antibody reaction was detected by colloidal gold conjugated rTc38630 protein at the test line. The ICT result was found to be consistent with rTc38630 protein-based ELISA. For vaccine studies, five groups of BALB/c mice, were randomized and assigned as recombinant protein, recombinant GST, MCF homogenate, BSF homogenate and PBS and

immunized respectively. They were challenged with a lethal dose of *T. congolense*. The recombinant protein did not protect the mice (survival analysis, P=0.09).
Key words: Immunochromatographic test, Nagana, Trypanosome, Vaccine

Introduction

African trypanosomosis has been known to be a serious constraint to livestock sector development in sub-Saharan Africa. The animal form also known as nagana is caused by tsetse-transmitted protozoan parasites, *Trypanosoma brucei, T. congolense*, *T. vivax* among others (OIE, 2013). The parasites may also be transmitted mechanically by biting flies (*Stomoxys spp*, Tabanidae). Despite the disease's economic losses and having been studied for a long time, there are still challenges in diagnosis and treatment (Magez and Radwanska, 2009). According to amino acid domain structures, Tc38630 was assumed as a *T. congolense* orthologue of *Trypanosoma brucei brucei* invariant surface glycoprotein (ISG) and further found it to be a novel diagnostic antigen in experimental mouse model (Mochabo *et al.*, 2013). The use of non-variable surface proteins such as ISG75 is promising alternative in the improvement of diagnostic tests (Hutchinson, *et al*., 2004).

Several diagnostic tests including PCR, IFAT, ELISA and microscopy are used for trypanosome detection and vary in their sensitivity and specificity. In addition, these tests vary in their cost and ease of application (OIE, 2013). It is advisable that proper diagnosis may be achieved by combining appropriate diagnostic tests. Microscopy detection, still considered as a gold standard, has low sensitivity whereby if trypanosomes are 100 parasites per ml or less cannot be detected (Chappuis *et al.,* 2005) and cannot be deployed for large scale screening. In sero-diagnosis, there is detection of either circulating antigens or antibodies and if an assay is able to detect the former, then it would demonstrate an active infection (Hutchinson *et al*., 2004).

Since the ELISA method is labour-intensive and time-consuming, and also requires equipment and trained personnel to carry out, there is need for a simple and rapid test which could be used to preliminary screen the disease by veterinarians or pen-side detection of the disease in livestock (Huang *et al.,* 2004a; Kim *et al*., 2008). Thus, a convenient, rapid, and sensitive diagnostic test, such as an immunochromatographic test (ICT), a nitrocellulose membrane (NC)-based immunoassay which detects antibody and does not require any instrument, is desired (Chandler *et al*., 2000; Richardson *et al*., 2002).

Immunochromatographic test (ICT), which utilizes the model of chromatography, was first introduced in late 70s and became popular in late 80s, (Paek *et al*., 2000; Tanaka *et al.,* 2006; Jia *et al*., 2007). It was first commercially produced for a home-based test for pregnancy (May, 1991). Due to its simplicity and quick results, it has been extensively applied for different purposes such as diagnosis of human and animal diseases and detection of target compounds (aflatoxins, drug metabolites e.g. trypanocides) (Chandler *et al*., 2000; Posthuma-Trumpie *et al*., 2009). Specifically, the ICT has been developed for tropical diseases like malaria (Wongsrichanalai *et al*., 2007), kinetoplastids (leishmaniasis and Chagas disease) (Sundar *et al.,* 1998; Chappuis *et al*., 2006; Roddy *et al,* 2008), schistosomiasis (Bosompem *et al*., 1997), babesiosis (Huang *et al.,* 2004a; Kim *et al*., 2008; Luo *et al.,* 2011; Goo *et al*., 2012),toxoplasmosis (Huang *et al.,* 2004b) and neosporosis (Liao, *et al*., 2005).

Currently, there are few drug regimens with some reportedly toxic while others are becoming alarmingly resistant. Therefore, eventual vaccine development would be tremendously beneficial (Magez and Radwanska, 2009). So far, this has not been possible due to the trypanosome's surface coat ability to avoid the immune responses known as antigenic variation (Donelson *et al*., 1998; Donelson, 2003). However, Wei *et al.,* (2011) differ and offer the opinion of immunosuppression induced by trypanosomal infections. Since the description of genomic organization (Ziegelbauer *et al.,* 1995) of a gene family for the invariant surface glycoproteins (ISG) from *T.*
b. brucei parasites, attempts have been made to use them as a DNA vaccine by Lança *et al.,* (2011) in experimental model. A different approach in the search for vaccine to combat this menace based on 'anti-disease' rather than an anti-parasite strategy has been adopted following observations of trypanotolerance phenomenon in a non-sterile condition whereby pathogenic factors expressed during infection are counteracted by drugs and/or vaccine but not target the parasite (Boulangé *et al*., 2001; Schofield, 2007; AntoineMoussiaux *et al*., 2009).

Recombinant proteins have been used in experimental setting in cattle against *T. congolense* infections whereby trypanotolerance was exhibited in the otherwise trypano susceptible boran cattle (Paling *et al*., 1991). Since the immunodominant VSG has failed as a vaccine, focus has shifted to characterizing invariant glycoproteins as alternative antigens for potential vaccines. These ISGs are embedded under the VSG that include ISG64 (Jackson *et al.,* 1993), ISG65 (Ziegelbauer and Overath 1992), ISG75

(Ziegelbauer *et al*, 1992), ISG100 (Nolan *et al*., 1997). An ideal vaccine candidate has to activate a strong, protective and long lasting immune response (Rajput *et al*., 2007; Kateregga *et al.,* 2012).

Therefore, the aim of this study was to find a rapid diagnostic test using recombinant protein (Tc38630) for the diagnosis of *T. congolense* infections and evaluate the protein as a potential vaccine candidate against trypanosome infections.

Materials and Methods

Parasites and Animals

T. congolense IL3000 savannah strain isolated near Kenya/Tanzania border was used. PCF and EMF were propagated at 27 ºC using *Trypanosoma vivax* medium (TVM)-1 composed of Eagle's minimum essential medium

(EMEM, M4655, Sigma–Aldrich, St. Louis, MO, USA) supplemented with 20% heat-inactivated foetal bovine serum (FBS), 2 mM L-glutamine and 10 mM L-proline. PCFs were routinely maintained by diluting 3ml of log-phase parasite suspension with 7ml of fresh medium every 2 days while the plasticadherent EMF cultures were maintained by replacing the entire culture supernatant with fresh medium every 2 days. BSF was maintained at 33 ºC using HMI-9 medium modified from Iscove's Dulbecco's MEM (IMDM, I3390, Sigma–Aldrich, St. Louis, MO, USA). The modification was done by supplementing the medium with 0.05 mM bathocuproinesulphonate, 1.5 mM L-cysteine, 0.12 mM 2-mercaptoethanol, 1mM sodium pyruvate, supplemented with 20% heat-inactivated foetal bovine serum (FBS) (Hirumi and Hirumi 1991; Sakurai *et al.,* 2008). The BSFs were maintained daily by splashing and replacing the entire supernatant with fresh medium at logphase.

Eight-week-old female BALB/c (Clea, Japan) mice were used for sera production and infections. This experiment was conducted in accordance with the Standards Relating to the Care and Management of Experimental Animals of Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan (No. 24-135).

Recombinant Protein Production

Expression and purification of rTc38630 protein in *Escherichia coli* as a fusion protein with glutathione S-transferase was conducted as described previously (Mochabo *et al.,* 2013).

Immunization and Production of Anti-rTc38630 Sera

Three female BALB/c mice (eight-week-old) purchased from Clea, Japan, were immunized with a recombinant protein using 100µl (100µg) emulsified in equal volume of TITERMAX® Gold (TiterMax USA Inc., Norcross, GA, USA). The immunizations were done intraperitoneally (i.p.) for primary and two boosters at a two-week interval. Two other mice were immunized with GST while one mouse was used as a negative control. One week after last booster injection, blood was collected by cardiac puncture at terminal anaesthesia. Sera were prepared by centrifugation at 17,000 x *g* for 10 min at 4^{o}C and stored at -30^{o}C until use. The concentration of the polyclonal antibody was determined by BCA Protein Assay Kit (PIERCE Chemical Company, Rockford, IL, USA). Final purified polyclonal antibodies were stored at -30^{o}C until use. This, plus the recombinant protein were used in the assembly of ICT.

Preparation of Gold Colloid-Conjugated Antigens and ICT Strip

All of the materials used for ICT (glass fibre, absorbance and nitrocellulose membranes) were purchased from EMD Millipore Corporation (Billerica, MA, USA). Purified rTc38630 antigen fused with GST was diluted to an optimal concentration of 500 µg/ml with 5 mM phosphate buffer (pH 7.0) and conjugated with gold colloid particles (British BioCell International, UK) as described previously (Huang *et al.*, 2006) with some minor modifications. Briefly, rTc38630 (500 µg/ml) was conjugated with a gold colloid (British BioCell International, Cardiff, UK) at pH 6.5 by gentle mixing (1:10, vol/vol) and incubation at room temperature for 10 min. Polyethylene glycol 20,000 (PEG) at 0.05% and bovine serum albumin (BSA) at 1% were then added to stabilize and block the conjugate particles. After centrifugation at 18,000 × g for 20 min, the supernatant was discarded and the pellet was resuspended, sonicated and washed with phosphatebuffered saline containing 0.5% BSA and 0.05% PEG. After the second centrifugation, the pellet was resuspended in phosphate-buffered saline with 0.5% BSA and 0.05% PEG. The conjugate was diluted in 10 mM Tris-HCl (pH 8.2) with 5% sucrose, sprayed onto glass fibre (Schleicher & Schuell, Inc., Keene, NH, USA), and dried in a vacuum overnight. rTc38630 immunized serum was purified for IgG polyclonal antibodies using protein G affinity chromatography.

(Bio-Rad Laboratories, Hercules, CA, USA), dialyzed in PBS and used as control line in ICT. rTc38630 (500 µg/ml), rGST (200 µg/ml) and IgG (1,500 µg/ml) were linearly jetted onto a nitrocellulose(NC) membrane (Schleicher & Schuell, USA) as the test, GST, and control lines, respectively, using a BioDotBiojet 3050 quanti-dispenser (BioDot, Inc., CA, USA) (Huang *et al.*,

2004b; Liao *et al.,* 2005).Then the membrane was dried at 50^{o}C for 30 min and blocked by using 0.5% casein in a 50 mM boric acid buffer (pH 8.5) for 30 min. After a washing with 50 mM Tris-HCl (pH 7.4) containing 0.5% sucrose and 0.05% sodium cholate, the membrane was dried in air overnight. The NC membrane, absorbent pad, conjugate pad, and sample pad were assembled sequentially on an adhesive card (Schleicher & Schuell, NH, USA) in a manner to effect capillary action and cut into 3-mm-wide strips using a BioDot cutter (BioDot, Inc., CA, USA).Detection was performed by pipetting 10µl of the diluted serum (1:5 in PBS) on the sample. The result was judged 15-20 min after the application of serum samples. The presence of a control band alone indicated a negative result, whereas the presence of two bands (control and test bands) indicated a positive result. If a GST line appeared, the test was declared nonspecific (Huang *et al.,* 2004b; Liao *et al*., 2005) and if no band was visible after 20 min, the result was considered invalid. The strips were stably stored with dehumidification in foil pouches at ambient temperature until use.

Mice Infections and Sera

Mice infections and production of sera were described previously (Mochabo *et al.,* 2013).

Immunization of Mice for Vaccine Evaluation

Five groups of seven – eight weeks old female BALB/c (Clea, Japan) mice, five per group, were used in the experiment. Five groups of BALB/c mice (highly susceptible to *T. congolense* infections), were randomized and assigned as recombinant protein, recombinant GST, MCF homogenate, BSF homogenate and PBS. They were immunized respectively using 10µg in 100µl PBS emulsified in equal volume of TITERMAX® Gold (TiterMax USA Inc., Norcross, GA, USA) adjuvant. The immunizations were done subcutaneously (sc) for primary and after one week, with first booster. Second booster was done at a two-week interval after the first boost. Responses to immunization were assessed by ELISA (Authié *et al*., 2001). Briefly, the ELISA plates (Nunc Marxisop®) were coated appropriately with rTc38630 antigen, rGST, BSF homogenate and MCF homogenate in 50 mM carbonate-bicarbonate buffer, pH 9.6 and incubated at 4^{o}C. The plates were blocked with 1% BSA in PBS containing 0.1% Tween-20 (blocking buffer) before incubation with serum dilutions in blocking buffer for one hour. The second antibody was anti-mouse IgG conjugated to horseradish peroxidase (Sigma) while tetramethylbenzidine (TMB) was used as substrate. All washing steps were done with PBS

containing 0.5 Tween 20. Titres to the antibody to the respective antigens were measured up to dilution of 6,400 times.

Endotoxin also known as lipopolysaccharide (LPS) was removed from the recombinant protein using Pierce® High-Capacity Endotoxin Removal Resin Kit (Thermo-Scientific, USA) and the level was measured using Endospecy® ES-50M Kit (Seikagaku Corporation, Japan). A 0.57 EU/ml level of LPS in protein was achieved against the recommended level of 0.250.5 EU/ml equivalent to 0.25-0.5 ng endotoxin / ml (Brito and Singh 2011).

Homogenate Preparation

Metacyclic form parasites (MCFs) were purified from EMF stage *in vitro* cultures by passage through DEAE cellulose (DE-52; Whattman) column chromatography and elution with PSG (PBS, pH 8.0+1.5g glucose/L) (Lanham and Godfrey 1970). BSF stage *in vitro* cultures were harvested and washed three times in PBS as a pellet by centrifugation (1500 x g, 10 min, 4^{o}C). The isolated pellets were freeze-thawed three times and homogenized in PBS. Protein concentrations for MCF and BSF homogenates were determined by bicinchoninic acid (BCA) Protein Assay Reagent (PIERCE Chemical Company, Rockford, IL, USA).

Trypanosome Challenge and Parasitaemia Monitoring

Trypanosomes for experimental challenge were first expanded in BALB/c mice which were later sacrificed. The concentration was determined using a Naubauer hemocytometer and trypanosomes were estimated for challenge of five groups of BALB/c mice. About 5,000 parasites were used i.p. per mouse in the second week after the last boost. After challenge, the mice were monitored daily to determine the pre-patent period.

Parasitaemia was monitored daily via tail blood by wet blood smears and when count was over 50 trypanosomes per field, haemocytometer was used to estimate trypanosomes. The vaccine evaluation was repeated with a larger number of mice, eight BALB/c per group.

Data Management and Analysis

The data were entered and analysed in both MS Excel 2007 and Graph Pad Prism Version 5.04 using descriptive statistics at 95% confidence interval. Differences were considered statistically significant at $P < 0.05$. Survival analysis was done using log-rank tests to compare differences in survival curves. Period of survival was defined as the number of days after challenge, the infected animals remained alive.

Results

In the present study, the rTc38630-based ICT was replicated following a successful rTc38630-based ELISA as the *T. congolense* orthologue of the *T. brucei* ISG as it was found to be a potential diagnostic antigen (Mochabo *et al.*, 2013). As shown in Figure1, sera from the experimentally infected mouse model were used to check the potential use in ICT. The result of ICT was similar to previous rTc38630-based ELISA in the experimental mouse model. The trypanosome antibodies were detected from day 7 suggesting that this assay might be used for early diagnosis of trypanosomosis. Only two bands were visible after 20 minutes and sera did not react with rGST line in all the four mice sera from day 7. Only one band was observed from day zero as expected for pre-infection sera and similarly, only one band for day two and day four post infection which demonstrated a negative result. Serum from mouse 4 demonstrated faint bands on a test line which was consistent with low parasitaemia observed in the previous study.

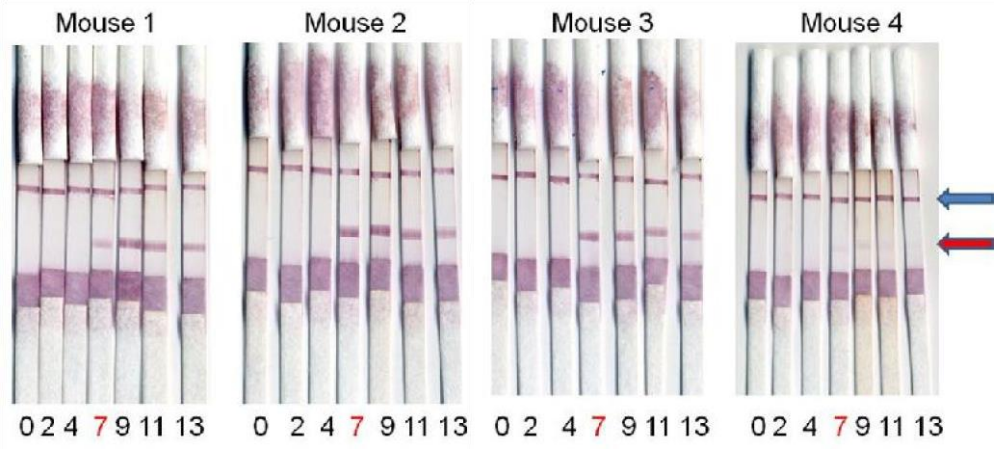

Figure 1: Detection of IgG responses against r38630 antigens on immunochromatographic test showing consistency with Figure 4A on infected mouse experimental sera. 0-13 = Days post infection; Control line = Blue arrowed; Test line = Red arrowed.

For vaccine studies, five groups of BALB/c mice (highly susceptible to *T. congolense* infections), five per group, were randomized and assigned as recombinant protein, recombinant GST, MCF homogenate, BSF homogenate and PBS and immunized respectively. They were challenged with a lethal dose of expanded bloodstream form of *T. congolense* i.p. This is the commonly used route to induce experimental infections, whereas the SC route and use of MCFs would be ideal in mimicking the natural infection (Bannai *et al*., 2003). Use of MCFs through i.p. was attempted, but BALB/c mice were resistant as they

cleared the parasitaemia. The endotoxin, a liposaccharide (LPS) was removed and the levels measured. A 0.57EU/ml level of LPS in protein was achieved against the recommended level of 0.250.5EU/ml equivalent to 0.25-0.5 ng endotoxin/ml.

In the first vaccine trial, the rTc38630-GST immunized mice showed slightly long pre-patent period, higher survival rates compared with control groups, but no significant difference was observed between the groups ($p = 0.09$) (Figure 2).

Recombinant Protein from Trypanosoma Congolense

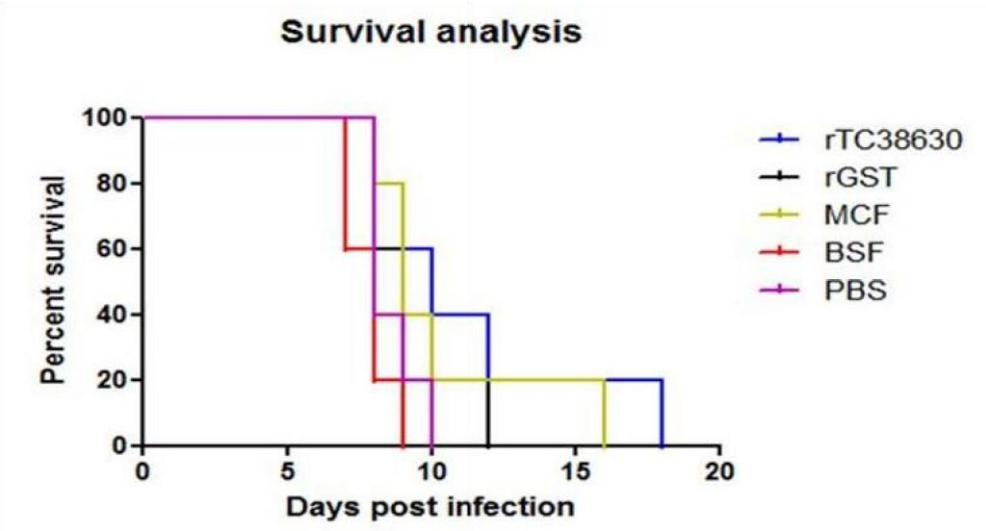

Figure 2: Survival analysis of five groups of immunized BALB/c mice. Overall there was no significant difference (P=0.09） between rTc38630 and control groups. rTc38630 = recombinant protein; rGST = recombinant glutathione S-transferases; MCF = metacyclic form homogenate; BSF = bloodstream form homogenate; PBS = Phosphate buffered saline.

One mouse in the rTc38630 immunized group however, survived longer than the rest for about 17 days and the median survival time (10 days) (Figure 3) was one day longer than in control groups.

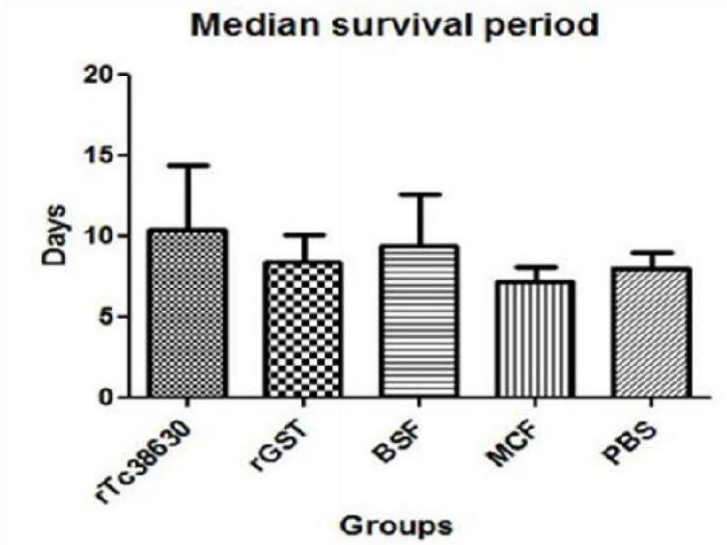

Figure 3: Median survival period for five groups of immunized BALB/c mice.

Overall there was no significant difference (P>0.05） between rTc38630 and control groups. rTc38630 = recombinant protein;

Parasitaemia was monitored daily after challenge of the mice and one mouse in rTc38630-immunized group survived one day longer as shown by a drop of parasitaemia at day 10 compared to control groups (Figure 4A). The median survival time was not any different between the groups ($p>0.05$).

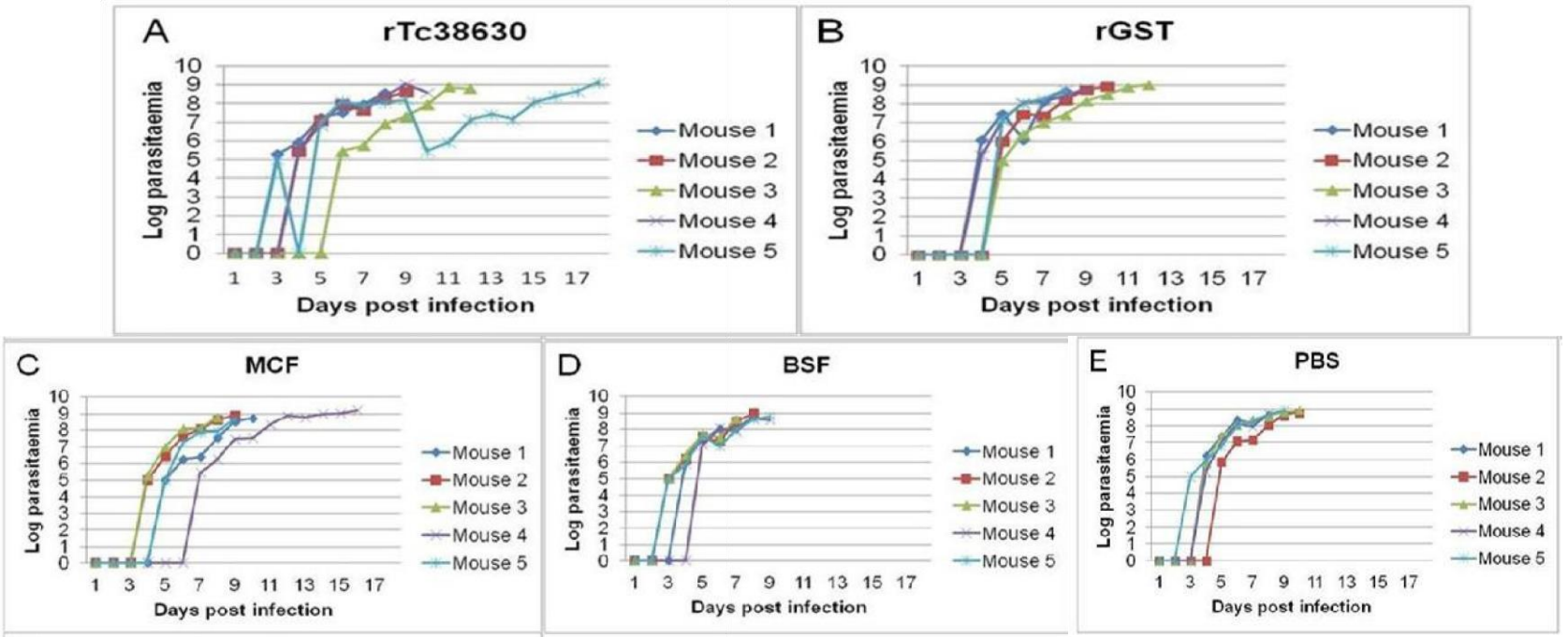

Figure 4: Parasitaemia trends of immunized BALB/c mice in five groups. One mouse in rTc38630 survived one day longer shown by a drop of parasitaemia at day 10 compared to control groups. rTc38630 = recombinant protein; rGST = recombinant glutathione Stransferases; MCF = metacyclic form homogenate; BSF = bloodstream form homogenate; PBS = Phosphate buffered saline.

The mean pre-patent period for all the groups was 3 days with no significant difference between groups (p = 0.11) (Figure 5). In the second trial, one mouse in the MCF homogenate immunized group survived longer and was consistent with the first trial (data not shown).

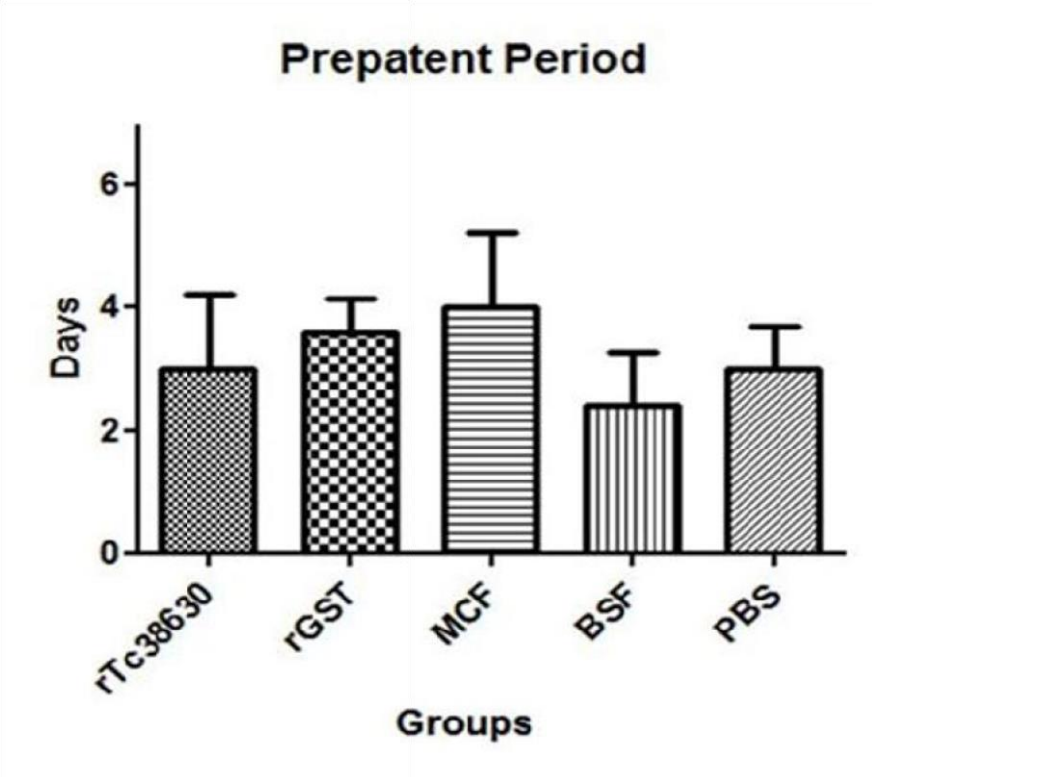

Figure 5: Mean pre-patent period for five groups of immunized BALB/c mice.
Overall there was no significant difference (P=0.11） between rTc38630 and control groups. rTc38630 = recombinant protein; rGST = recombinant glutathione
S-transferases; MCF = metacyclic form homogenate; BSF = bloodstream form homogenate; PBS = phosphate buffered saline.

Discussion

In any disease control strategy, a sensitive and reliable diagnostic test is crucial. An attempt has been made to utilize ICT on AAT using a recombinant ribosomal P0 protein (Cheng *et al*., 2012). Recently, an evaluation of ICT and recombinant antigen ELISA assays for animal trypanosomosis prevalence were done in South Africa and the results were favourably good and only needed further improvement on sensitivity
(Nguyen *et al*., 2015).Various ISGs have been identified and characterised that include ISG 64 (Jackson *et al.,* 1993), ISG 65/70 (Ziegelbauer and Overath, 1992), ISG 75 (Ziegelbauer *et al.,* 1992) and ISG 100 (Nolan *et al.,* 1997). In particular, ISG 75 has been used for molecular diagnosis (Rudramurthy *et al.*, 2013). In serodiagnostics, an ideal antigen should be immunogenic (Manful *et al*., 2010) and all antigens of trypanosomes are potentially immunogenic including the cytosolic

ones because they are released as a result of immune-mediated cell lyses. The use of recombinant antigens in the diagnosis will eventually replace the native ones as they can easily be standardized.

ELISA requires equipment and expertise to conduct, on the other hand, rapid diagnostic tests (RDTs) are rapid (10-20 min), require no capital investment, are simple to perform and easy to interpret (Tanaka *et al.*, 2006; Wongsrichanalai *et al*., 2007). Among the diseases caused by kinetoplastid protozoa, ICT has been used to diagnose leishmaniasis (Sundar *et al*., 1998) and Chagas' disease (Roddy *et al*., 2008). Indeed, Huang *et al.*,(2006) notes that the ICT is an immunoassay in which nitrocellulose (NC), migration membrane that relies on capillary mechanism in its assemblage (should be in a continuous manner from the sample pad, conjugate pad, NC to absorbent pad). The antibodies are captured on the immobile test line of the antigens whereby antigen-antibody reaction develops as a coloured line. The performance of the test is simple as strip is dipped into a sample fluid, and the result can be determined in a few minutes with the naked eye. In addition, no instrument or testing skills are required. The ICT strip is quite stable during long storage under ordinary conditions and is rapid taking less than 20 min to complete. Therefore, this test is more practical to use in the field than any other test. Pen-side immunoassays would be an advantage in making therapeutic decisions and therefore ICT will go a long way to solve this. Taking together the above advantages, ICT was replicated after the antigen showed immunoreactivity in ELISA using a mouse model. By and large, in the validation of any new diagnostic test, often there is a drawback as there is no gold standard or reference test and is difficult in endemic areas to have animals with no known infection status (Bossard *et al.*, 2010). The next plan is to apply the gains in ICT for clinical/field samples as has been conducted for the recombinant rTc38630-based ELISA (Fleming *et al*., 2014).

In our study, the LPS known to provoke non-specific immunity (Magez *et al*., 2010), was removed. The mouse that had a drop in parasitaemia, but failed to clear the infection once it was established, eventually succumbed. This might be attributed to immunosuppression (Vickerman and Barry 1982; Wei *et al*., 2011; Tabel *et al*., 2013). Without any treatment, when infected i.p. with 1 x 10^3*T. congolense* parasites, BALB/c mice survive for 8.5 ± 0.5 days (Tabel *et al.*, 2008). Owing to the fact that developing a conventional anti-parasite vaccine has been elusive due to antigenic variation (Donelson, 2003), and more recently immunosuppression (Wei *et al.*, 2011; Tabel *et al*., 2013), an anti-disease approach strategy was proposed (Authié *et al.*, 2001). This idea was borrowed following observations of trypanotolerance in cattle (Naessens 2006) and in humans (Jamonneau *et al*., 2004). Tolerant animals normally maintain trypanosome levels below 10^3, but tolerance breaks when they reach about 10^7 parasites/ml of blood (O'Beirne *et al*., 1998).

An attempt has been made to utilize metacyclic stage proteins as a vaccine (Esser *et al*., 1982) and they were found out to have a limited protection. Several invariant molecules have been used as vaccine with mixed results which include tubulin, actin, microtubule-associated proteins (MAPs), flagellar pocket (FP) and recombinant ISG75 (Balaban *et al*., 1995;Mkunza*et al*., 1995; Lubega *et al*., 2002a, 2002b; Li, *et al.*, 2007,2009). In an ideal experimental design, there is need to challenge the mice three to six months after the last immunization and check whether protection is elicited (Magez *et al*., 2010). A single bite by tsetse fly can inject about

10^4metacyclictrypomastigotes; therefore in an estimate of 5 x 10^3 parasites were adequate for the challenge through i.p. This regime has been used experimentally for ISG75 but the mice were not protected when challenged (Magez *et al.,* 2010). Other workers, Ramey *et al,* (2009), used an estimation of 10^6 parasite load with no protection reported. Again, blood samples (≈100 µl) were collected from the mice's tail that ensured anaemia was not due to
sampling rather from the infection of the parasites. The target of vaccination often, is to induce B cell memory (Radwanska *et al.,* 2000). The orthologue ISG Tc38630 protein potential as a vaccine candidate was investigated while the ISG from *T. brucei* has been used as a DNA vaccine whereby it produced a partial protection in experimental model (Lança *et al.,* 2011). Although rTc38630 protein was not significantly protective, further work needs to be done that may include the combination of different antigens.

In using conventional approaches to vaccinology as concluded by Mora *et al.,* (2003), some proteins may be immunogenic *in vivo* but not necessarily protective, therefore, the reverse vaccinology would address this concern. Vaccines work by priming the antigen-specific T and B cells which will undergo apoptosis, but a small number will convert to the memory cells that will control subsequent infections by the invader targeted by the vaccine. In future, vaccine development for trypanosomosis should involve reverse vaccinology (Rappuoli 2000; 2001), now that the *T. congolense* genome has been completed (Jackson *et al*. 2012). As further work, the immune responses involved after parasite challenge need to be investigated. The current study on the use of recombinant technology in diagnostics and vaccine will serve as a benchmark for more research to improve on sensitivity and specificity of assays and for effective anti-disease vaccine approach, respectively, in the control of African trypanosomosis.

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