Development of tools to improve the detection of *Trypanosoma evansi* in Australia

Presented By

Celia Margaretha Smuts

BVSc, MVS

This thesis is presented for the degree of

Doctor of Philosophy

School of Veterinary and Biomedical Sciences
Division of Health Sciences
Murdoch University
7 July, 2009
Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

Celia M. Smuts

7 July, 2009
Abstract

The aim of this study was to evaluate new methods to improve detection and investigation of the effects of chronic or subclinical infection with *Trypanosoma evansi* in various mammalian species. Some of the more resistant host species, including pigs and buffaloes, are present in large feral populations in the northern parts of Australia, the area where *T. evansi* is most likely to gain entry to the country. Existing tests are not sufficiently reliable to detect all cases of disease and they cannot distinguish acute from chronic infections. Furthermore, the tests have different sensitivities in different host species.

Surveillance for trypanosomiasis in Australia is problematic because of the need to work in remote parts of northern Australia where provision of a cold-chain for traditional blood and serum storage is difficult. An existing dried blood storage system was modified by treating cotton lint filter paper (Whatman #903) with a commercial post coating buffer (TropBio, Queensland). This treatment increased the longevity of antibodies to *T. evansi* in serum and blood stored on the paper (detected using an antibody-detection ELISA) compared to samples stored on plain paper, especially when the papers were stored under humid conditions and at high ambient temperatures.

Attempts were made to improve the diagnostic utility and repeatability of antibody-ELISAs through the use of 2 recombinant *T. brucei* antigens (PFRA and GM6) and to optimize a competitive ELISA using RoTat 1.2 variable surface antigen and its monoclonal antibody. Antibody-detection using the two recombinant proteins was not sufficiently specific to enable their use for the detection of *T. evansi*. The RoTat 1.2 cELISA had good sensitivity and specificity (75% and 98% respectively) when used to test serum from cattle and
buffaloes experimentally infected with *T. evansi* and uninfected animals. However, the test was not able to detect anti-*T. evansi* antibodies in serum from wallabies, pigs, a dog or a horse that were experimentally infected with *T. evansi*. The inability of the cELISA to detect anti-*T. evansi* antibodies may be due to the small number of samples tested or the lack of RoTat 1.2 specific antibodies in the animals tested.

The feasibility of using an enzymatic test to detect trypanosome aminotransferase or antibodies to this enzyme was evaluated. Prior publications suggested that the detection of TAT was an appropriate diagnostic tool for the detection of *T. evansi* infection in camels. However, the results from this study did not support the use of this test for the detection of *T. evansi* infection in cattle or buffaloes with low to moderate parasitaemia.

Trypanosomiasis is an immunological disease that affects most of the body’s organs, with more severe disease developing over time. Attempts were made to determine key cytokine and biochemical patterns that would distinguish infected from uninfected animals and acute from chronic infections. The results from this study showed that there was no specific pattern in serum cytokines or serum biochemistry that could be used to distinguish infected from uninfected animals, or different stages of disease.

Immunohistochemistry was used on tissues from buffaloes and mice experimentally infected with *T. evansi* and *T. brucei gambiense* respectively to characterise the cellular immune response that was present. The immune response was predominantly cell mediated, with CD3+ T lymphocyte and macrophage infiltration occurring in most tissues. In end-stage disease there was often suppression of the immune system with disruption of the architecture of the spleen and a decrease in B lymphocytes in the circulation.
Trypanosomes were rarely visible in the tissues and were only seen in those animals with high parasitaemia. Lesions generally became more severe over time, but there was a large variation between animals, which suggests that immunohistochemistry is unsuitable as a diagnostic tool.
# Table of Contents

**DECLARATION** II

**ABSTRACT** III

**TABLE OF CONTENTS** VII

**LIST OF TABLES** XIV

**LIST OF FIGURES** XVI

**ACKNOWLEDGEMENTS** XXI

**ABBREVIATIONS** XXIII

1 **GENERAL INTRODUCTION** 1

2 **LITERATURE REVIEW** 3

   2.1 **BACKGROUND** 3

      2.1.1 Morphology 3

      2.1.2 Hosts ranges and vectors 4

      2.1.3 Dynamics of *Trypanosoma evansi* infection in livestock populations 7

      2.1.4 Clinical signs and outcomes of infection 8

      2.1.5 Geographic distribution and economic impact 9

      2.1.6 Variable surface antigen 9

      2.1.7 Treatment and control 11

2.2 **TESTS FOR DETECTING *TRYPANOSOMA EVANSI*** 13

      2.2.1 Parasitological tests 13

      2.2.2 Serological tests 15

      2.2.3 Trypanosome antigens used for detecting trypanosomiasis 17
2.2.3.1 RoTat 1.2
2.2.3.2 GM6
2.2.3.3 Paraflagellar rod protein A
2.2.4 Molecular techniques
2.2.5 Enzyme detection – tyrosine aminotransferase

2.3 IMMUNE RESPONSE

2.3.1 Introduction
2.3.2 Immune response
2.3.3 Antibodies
2.3.4 Cytokines
   2.3.4.1 IFN-γ
   2.3.4.2 IL-6
   2.3.4.3 IL-8
   2.3.4.4 TNF-α
   2.3.4.5 IL-2
   2.3.4.6 IL-4
   2.3.4.7 IL-10
   2.3.4.8 IL-12
2.3.5 Immunopathology

2.4 SURVEILLANCE FOR Trypanosoma evansi in Australia

2.5 PAPER-BASED BLOOD COLLECTION

2.6 AIMS

3 MODIFIED PAPER-BASED BLOOD COLLECTION

3.1 INTRODUCTION
3.2 MATERIALS AND METHODS

3.2.1 Details of serum and blood samples
3.2.2 Optimisation of preservatives used to coat filter paper
3.2.3 Validation of filter paper-based serum collection system
3.2.4 Effect of humidity on persistence of antibody activity
3.2.5 Preservation of whole blood on preservative-treated filter paper
3.2.6 Data analysis

3.3 RESULTS

3.3.1 Optimisation of preservatives used to coat filter paper
3.3.2 Validation of filter paper-based serum collection system
3.3.3 Effect of humidity on persistence of antibody activity
3.3.4 Preservation of whole blood on preservative-treated filter paper

3.4 DISCUSSION

4 EVALUATION OF ALTERNATIVE SEROLOGICAL TESTS TO DETECT ANTIBODIES TO TRYPANOSOMA EVANSI

4.1 INTRODUCTION
4.2 MATERIALS AND METHODS

4.2.1 Animal serum and additional data
4.2.2 RoTat 1.2-based competitive ELISA
4.2.3 Indirect ELISA using the non-variant antigen GM6
4.2.4 PFRA
4.2.5 Data analysis

4.3 RESULTS
4.3.1 RoTat 1.2 competitive ELISA
4.3.2 GM6 indirect ELISA
4.3.3 PFRA
4.4 DISCUSSION

5 TYROSINE AMINOTRANSFERASE

5.1 INTRODUCTION
5.2 MATERIALS AND METHODS
  5.2.1 Animal serum
  5.2.2 Trypanosome preparation
  5.2.3 Tyrosine aminotransferase
  5.2.4 Data analysis
  5.2.5 Results
DISCUSSION

6 INVESTIGATION OF IMMUNOHISTOPATHOLOGICAL CHANGES IN
   TRYpanosome-infected animals

6.1 INTRODUCTION
6.2 MATERIALS AND METHODS
  6.2.1 Preparation of Trypanosoma brucei gambiense
  6.2.2 Animal infections
    6.2.2.1 Mouse experiment 1
    6.2.2.2 Mouse experiment 2
    6.2.2.3 Mouse experiment 3
    6.2.2.4 Buffalo experiment
6.2.3 Trypanosome DNA detection
6.2.4 Detecting antibodies to trypanosomes
6.2.5 Histological examination of formalin-fixed tissues from mice and buffaloes experimentally infected with trypanosomes
6.2.6 Immunohistochemistry on formalin-fixed tissues from mice and buffaloes experimentally infected with trypanosomes
   6.2.6.1 Trypanosome antigen detection in formalin-fixed tissues from mice and buffaloes experimentally infected with *T. b. gambiense* and *T. evansi* respectively
   6.2.6.2 CD3+
   6.2.6.3 CD79+
   6.2.6.4 CD45/B220
   6.2.6.5 Tissue iron
6.2.7 Data analysis
6.3 RESULTS
   6.3.1 Mouse experiment 1: Tissue changes in different mouse breeds affected with end-stage trypanosome infection
   6.3.2 Mouse experiment 2: Clinical infection with *T. b. gambiense*
      6.3.2.1 Spleen
      6.3.2.2 Liver
      6.3.2.3 Lung
      6.3.2.4 Kidney
      6.3.2.5 Heart
      6.3.2.6 Skeletal muscle
      6.3.2.7 Brain
   6.3.3 Mouse experiment 3: Subclinical infection with *T. b. gambiense*
6.3.4 Buffalo experiment 135
6.3.5 Detection of trypanosome DNA in tissues from infected animals 137
6.3.6 Antibodies to trypanosomes 137

6.4 DISCUSSION 137

7 IMMUNOLOGICAL AND BIOCHEMICAL MARKERS IN SERUM INDUCED BY INFECTION WITH TRYPANOSOMA EVANSI 148

7.1 INTRODUCTION 148

7.2 MATERIALS AND METHODS 148

7.2.1 Mouse experiment 148
7.2.2 Buffalo experiment 149
7.2.3 Serology 150

7.2.3.1 Anti-\textit{T. evansi} antibody ELISA 150
7.2.3.2 Detection of IgM and IgG in mouse plasma 150

7.2.4 Cytokines 150

7.2.4.1 Bovine IFN-\gamma 150
7.2.4.2 IL-6 and IL-8 151
7.2.4.3 Mouse cytokines 152

7.2.5 Serum biochemistry 152

7.2.6 Data analysis 153

7.3 RESULTS 153

7.3.1 Serology 153
7.3.2 Cytokines 159
7.3.3 Serum biochemistry 161

7.4 DISCUSSION 161
List of Tables

Table 3-1: Substances evaluated for their ability to preserve antibodies to *Trypanosoma evansi* in serum from an infected calf and uninfected cattle dried on filter paper. 59

Table 3-2: OD results from testing positive control serum dried onto different preparations of filter paper using an antibody-detection ELISA. 66

Table 3-3: OD results from testing positive control serum dried onto different preparations of filter paper using an antibody-detection ELISA. 67

Table 3-4: Summary of results from testing samples stored on plain and PCB-treated filter paper at different humidities at 37°C with an ELISA to detect antibodies to *Trypanosoma evansi* compared to frozen serum samples over a 12 week period. 74

Table 5-1: Optical densities (at 331nm) obtained from testing tyrosine aminotransferase activity present in mouse liver extracts containing PLP. 103

Table 5-2: Optical densities (at 331nm) obtained from testing tyrosine aminotransferase activity present in whole cell lysate of *Trypanosoma brucei rhodesiense*. 103
Table 5-3: Optical densities obtained from testing trypanosomes tyrosine aminotransferase activity in the presence of serum from cattle and buffaloes infected with *Trypanosoma evansi* and uninfected cattle. 104

Table 6-1: Schedule used to sacrifice mice. 111

Table 6-2: Summary of lesion severity in mice clinically infected with *T. b. gambiense*. 119

Table 6-3: Summary of results from mouse Experiment 2. 131

10-1: Results from immunohistochemistry results from mouse Experiment 3. 174

10-2: Results from cytokine detection from uninfected mice and mice infected with *T. b. gambiense*. 177

10-3: Biochemistry results from uninfected buffaloes and buffaloes infected with *T. evansi*. 180
List of Figures

Figure 3-1: Diagram showing the typical layout of one of the humidity chambers used to test the longevity of anti-*Trypanosoma evansi* antibodies in cattle serum dried onto plain filter paper and filter paper treated with post coating buffer (TropBio, Queensland). 63

Figure 3-2: Scatter plots of the difference between the ELISA OD from dried serum samples from cattle infected with *T. evansi* stored on plain and PCB-treated filter paper at ambient temperatures compared to frozen serum after (a) 1 day, (b) 12 weeks and (c) 24 weeks. 69

Figure 3-3: Scatter plots of the difference between the ELISA OD from dried serum samples from uninfected cattle stored on plain and PCB-treated filter paper at ambient temperatures compared to frozen serum after (a) 1 day, (b) 12 weeks and (c) 24 weeks. 70

Figure 3-4: Results from testing serum from a calf infected with *Trypanosoma evansi* stored on plain and PCB-treated filter paper at 37°C at (a) 19%RH, (b) 39%RH, (c) 79%RH and (d) 96%RH compared to frozen serum using an antibody-detection ELISA. 73
Figure 3-5: Comparison of ELISA ODs from serum samples stored on plain filter paper, PCB-treated filter paper, casein-treated filter paper and albumin-treated filter paper for 6 weeks at 19%RH and 79%RH.

Figure 3-6: Scatter plot of the difference in ELISA ODs between dried whole blood samples stored on PCB-treated filter paper at ambient temperature for over 9 months, compared to matched frozen serum samples.

Figure 4-1: ROC curve showing the sensitivity and specificity, with 95% confidence intervals, of the RoTat 1.2 competitive ELISA at various cut-off points.

Figure 4-2: Frequency histogram of the results obtained from the RoTat1.2 cELISA showing the distribution of results from testing serum from *T. evansi*-infected Indonesian cattle and uninfected cattle from Australia.

Figure 4-3: ROC curve showing the sensitivity and specificity, with 95% confidence intervals, of the GM6 ELISA at possible cut-off points.

Figure 4-4: Frequency histogram of the results obtained from the GM6 ELISA showing the distribution of results from testing serum from *T. evansi*-infected Indonesian cattle and uninfected cattle from Australia.

Figure 6-1: Immunoperoxidase stained sections showing the presence of CD3-positive cells in the spleens of (a) an uninfected mouse (x20), (b) a
mouse recently infected with *T. b. gambiense* (x20) and (c and d) a mouse with clinical disease (20x and 10x magnification respectively).

Figure 6-2: Immunoperoxidase stained sections showing the presence of CD3-positive cells in the liver of (a) an uninfected mouse (40x), (b and c) mice recently infected with *T. b. gambiense* (40x) and (d) a mouse with clinical disease (40x), and (e) the presence of trypanosome antigen in the liver of a clinically infected mouse with massive parasitaemia (100x).

Figure 6-3: Immunoperoxidase stained sections showing the presence of CD3-positive cells in the lung of (a and b) an uninfected mouse (20x and 40x respectively), (c and d) mice recently infected with *T. b. gambiense* (20x and 40x respectively), (e) a mouse with clinical disease (20x) and (f) the presence of trypanosome antigen in the lung of a clinically infected mouse with massive parasitaemia (100x).

Figure 6-4: Immunoperoxidase stained sections showing the presence of CD3-positive cells in the kidney of (a) an uninfected mouse (20x), (b) a mouse recently infected with *T. b. gambiense* (20x) and (c) a mouse with clinical disease (20x). Immunoperoxidase staining of (d) CD3-positive cells in swollen, hypercellular glomeruli (40x) and (e) showing the presence of trypanosome antigen in the kidney of a clinically infected mouse with massive parasitaemia (100x).
Figure 6-5: Immunoperoxidase stained sections showing the presence of CD3-positive cells in the heart of (a) an uninfected mouse (20x), (b) a mouse recently infected with *T. b. gambiense* (20x) and (c and d) a mouse with clinical disease (20x and 40x).

Figure 6-6: Immunoperoxidase stained sections showing the presence of CD3-positive cells in the skeletal muscle of (a) an uninfected mouse (20x), (b and c) mice recently infected with *T. b. gambiense* (20x) and (d) a mouse with clinical disease (20x) and (e and f) immunoperoxidase staining of trypanosome antigen in the skeletal muscle of a mouse with massive parasitaemia (40x and 100x).

Figure 6-7: The mean lesion score observed in the tissues from mice infected with *T. b. gambiense* and uninfected mice.

Figure 6-8: Immunoperoxidase stained sections showing the presence of CD3-positive cells in (a) the heart (40x), (b) spleen (x20), (c) liver (x40) and (d) skeletal muscle (x40) of a buffalo infected with *T. evansi*.

Figure 7-1: Optical density (OD) values from testing the plasma of mice infected with *Trypanosoma brucei gambiense* and uninfected mice with an antibody-detection ELISA using a crude *Trypanosoma evansi* antigen.

Figure 7-2: Optical density (OD) values from testing serum from buffaloes infected with *Trypanosoma evansi* and uninfected buffaloes with an antibody-detection ELISA using a crude *T. evansi* antigen.
Figure 7-3: Total IgM levels measured in the plasma of mice infected with

*Trypanosoma brucei gambiense*, mice infected with *T. b. gambiense*

and treated and uninfected mice.

Figure 7-4: Total IgG levels measured in the plasma of mice infected with

*Trypanosoma brucei gambiense*, and treated and uninfected mice.

Figure 7-5: Total IFN-γ levels measured in the serum of buffaloes infected with

*Trypanosoma evansi*.

Figure 7-6: Mean IL-6 levels measured in the serum of buffaloes infected with

*Trypanosoma evansi*.

Figure 7-7: Mean IL-8 levels measured in the serum of buffaloes infected with

*Trypanosoma evansi*.
Acknowledgements

I dedicate this work to Andrew Paul, who has stood by me and provided support throughout this project. He is not nicknamed Endure for nothing.

I would like to thank my supervisors Ian Robertson, Cassie James and especially Simon Reid for their help. Simon has guided me through seemingly insurmountable obstacles with apparent ease and provided encouragement whenever it was needed. I would also like to extend my gratitude to Rod Campbell, who donated some of his retirement in order to help me and showed me some of the mysteries of pathology and more of the streets of Brisbane than he meant to.

Much of the project was only possible due to the help of others. I would like to thank Derrick Robinson from the University of Bordeaux who provided recombinant paraflagellar rod antigen and antibody as well as technical advice; Philippe Büscher from the Institute for Tropical Medicine in Belgium who donated recombinant RoTat 1.2 antigen and its monoclonal antibody; and Phil Toye from ILRI in Kenya who provided GM6 antigen. Thanks to the staff at Balitvet in Indonesia for collecting blood and serum samples from clinically infected buffaloes for many months and to the people from NAQS, especially Ray Petherick, Emma Watkins and Brian Read, who provided a glimpse into the conditions for sample collection in the Northern Territory.

Thank you to Michael Slaven and Gerard Spoelstra who helped me in their histology laboratory. I must have caused them to despair many times, but they never lost their
patience or good humour. I am also grateful to Murray Lindau who put up with me in his workshop, tried to teach me to hold a hammer like a man and allowed me near power tools.

Last but not least I am pleased to have worked with the Trailer Trash inhabitants, especially Michael Banazis (Herr Loom), Peter Adams (General Consensus) and Yazid Abdad (Food Coma), who answered questions, provided an outlet for frustration and quietly sorted out the cause of my ire without expecting anything in return. I hope we remain in contact in the future.

This project was made possible with financial support from Murdoch University, the Australian Biosecurity Cooperative Research Centre (AB-CRC) and the Australian Centre for International Agriculture (ACIAR).
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab-ELISA</td>
<td>Antibody-detection enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>ABTS</td>
<td>2,2’-azinobis[3-ethylbenzthiazoline-6-sulfonate]</td>
</tr>
<tr>
<td>A/G</td>
<td>Albumin/globulin ratio</td>
</tr>
<tr>
<td>Alb</td>
<td>Albumin</td>
</tr>
<tr>
<td>AQIS</td>
<td>Australia Quarantine and Inspection Service</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate aminotransaminase</td>
</tr>
<tr>
<td>ºC</td>
<td>Degrees Celsius</td>
</tr>
<tr>
<td>CATT</td>
<td>Card agglutination test for trypanosomiasis</td>
</tr>
<tr>
<td>CD</td>
<td>Cluster of differentiation glycoprotein</td>
</tr>
<tr>
<td>CK</td>
<td>Creatinine kinase</td>
</tr>
<tr>
<td>DAB</td>
<td>3,3’-diaminobenzidine</td>
</tr>
<tr>
<td>DEAE</td>
<td>Diethylaminoethyl cellulose</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediamine-tetraacetic acid, tri-potassium salt</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>et al.</td>
<td>And others</td>
</tr>
<tr>
<td>GGT</td>
<td>Gamma glutamyl transpeptidase</td>
</tr>
<tr>
<td>GIS</td>
<td>Geographic information systems</td>
</tr>
<tr>
<td>Glob</td>
<td>Globulin</td>
</tr>
<tr>
<td>HCT</td>
<td>Haematocrit centrifugation test</td>
</tr>
<tr>
<td>IgA</td>
<td>Immunoglobulin A</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>ISG</td>
<td>Invariable surface glycoproteins</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilodalton</td>
</tr>
<tr>
<td>l</td>
<td>Litre</td>
</tr>
<tr>
<td>LAMP</td>
<td>Loop-mediated isothermal amplification</td>
</tr>
<tr>
<td>μ (prefix)</td>
<td>Micro (10^{-6})</td>
</tr>
<tr>
<td>m (prefix)</td>
<td>Milli</td>
</tr>
<tr>
<td>MAECT</td>
<td>Mini-anion exchange chromatography</td>
</tr>
<tr>
<td>MHCT</td>
<td>microhaematocrit centrifugation</td>
</tr>
<tr>
<td>MI</td>
<td>Mouse inoculation</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>NAQS</td>
<td>Northern Australia Quarantine Strategy</td>
</tr>
<tr>
<td>Nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PCB</td>
<td>Post coating buffer</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PCV</td>
<td>Packed cell volume</td>
</tr>
<tr>
<td>PFRA</td>
<td>Paraflagellar rod protein A</td>
</tr>
<tr>
<td>PLP</td>
<td>Pyridoxal-5-phosphate</td>
</tr>
<tr>
<td>PNG</td>
<td>Papua New Guinea</td>
</tr>
<tr>
<td>Prot</td>
<td>Protein</td>
</tr>
<tr>
<td>qPCR</td>
<td>Quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>RPMI</td>
<td>Roswell Park Memorial Institute</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecyl sulphate polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>Abbr.</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>-------------</td>
</tr>
<tr>
<td>sp.</td>
<td>Species</td>
</tr>
<tr>
<td>TAT</td>
<td>Tyrosine aminotransferase</td>
</tr>
<tr>
<td>TBil</td>
<td>Total bilirubin</td>
</tr>
<tr>
<td>TBS</td>
<td>Tris (hydroxymethyl methylamine) buffered saline</td>
</tr>
<tr>
<td>TBS-T</td>
<td>Tris buffered saline with 0.05% Tween 20 (v/v)</td>
</tr>
<tr>
<td>TEN</td>
<td>Tris, EDTA and sodium chloride</td>
</tr>
<tr>
<td>TEN-T</td>
<td>Tris-EDTA-sodium chloride with 0.05% Tween 20 (v/v)</td>
</tr>
<tr>
<td>TEN-TC</td>
<td>TEN with 0.05% Tween 20 (v/v) and 0.2% casein (w/v)</td>
</tr>
<tr>
<td>VAT</td>
<td>Variable antigen type</td>
</tr>
<tr>
<td>VSG</td>
<td>Variable surface glycoprotein</td>
</tr>
<tr>
<td>x</td>
<td>Times</td>
</tr>
</tbody>
</table>