Consistent detection of *Trypanosoma brucei* but not *T. congolense* DNA in the faeces of infected cattle

Saldanha, I.[1], Betson, M.[2], Matthews, K.R.[3], Paxton, E., Vrettou, C.[4], Morrison, L.J., Torr, S.J.[1], Cunningham, L.J.[5]

[1]Liverpool School of Tropical Medicine, Dept. Vector Biology; [2]University of Surrey, School of Veterinary Medicine; [3]University of Edinburgh, Institute of Immunology and infection; [4]University of Edinburgh, Roslin Institute; [5]Liverpool School of Tropical Medicine, Dept. Tropical Disease Biology

WHY FAECES?

Species of *Trypanosoma* transmitted by the tsetse fly (*Glossina*) vector are responsible for clinically significant diseases in both human and animal populations. Although significant advances have been made in the control of human African trypanosomiasis (HAT)[1], animal **African trypanosomiasis (AAT) remains a disease of significant economic burden in sub-Saharan Africa**. Current AAT **surveillance tools suffer from poor sensitivity and specificity** [2,3], with **serological methods also requiring animal restraint and blood collection by trained personnel**. Molecular tools have greatly improved the sensitivity and specificity of AAT surveillance, however high cost and limited field applicability remain a barrier for use in affected regions [2]. **Faecal sampling is an option for more accessible sample collection and screening**. Successful detection of blood-borne protozoan DNA in human and wildlife faeces has been demonstrated previously[4–9]. However detection of *Trypanosoma* DNA in livestock faeces has not yet been explored.

This study set out to determine **whether it is possible to detect DNA of *T. brucei* and *T. congolense* in the faeces of experimentally-infected cattle**, and **how these results compare to those obtained from matched blood samples collected** over a ten-week study.

Figure 2: (clockwise from left) infected calves in vector-proof containment; cattle faeces on substrate; collection of faeces using wooden spatula

METHODS

Experimental Infections: Experimental infections were carried out at Roslin Institute (Home Office Project License number PE854F3FC, Roslin Institute Animal Welfare Ethical Review Board number L447) for the purposes of a separate project, however the opportunity was taken to collect environmental samples or utilise surplus sample materials. **The infection study and sample collection was carried out as indicated in Fig.1**, with samples linked to individual calf ID where possible. Faecal samples were taken passively (Fig.2) and **stored in RNA*Later*™**. Blood samples were taken from jugular vein by trained personnel. All samples were subsequently stored at -80°C.

DNA Extraction: Faecal samples were processed using Quick-DNA Fecal/Soil Microbe DNA Miniprep kit (Zymo Research). Blood samples were processed using Dneasy 96 Blood and Tissue kits (QIAGEN).

PCR and qPCR: All samples were screened using *Typanosoma* species-specific PCR assays (TBR-PCR and TCS-PCR) and novel probe-based qPCR assays targeting *Trypanozoon*-specific (TBR-qPCR) and *T. congolense savannah*-specific (TCS-qPCR) repeat regions in kinetoplast minicircle DNA respectively.

TBR-PCR Product Sequencing: TBR-PCR target products (Fig.3) from four faecal samples were excised and purified using an Exo-CIP™ Rapid PCR Cleanup Kit (New England Biolabs). Sanger sequencing was performed by Source BioScience Ltd. Sequence clean-up and alignments were performed in BioEdit v7.2. Resultant sequences were subject to BLAST™ nucleotide analysis (NCBI).

Figure 3: An image displaying results of products resulting from TBR-PCR screening on DNA extracted from 25 post-inoculation cattle faecal samples. LAD = 100bp molecular ladder, NTC = no-template control (nuclease-free water), NFC = negative faeces control. TBB = *T. brucei* M249 DNA 1ng/µL (positive control). White arrow indicates 173bp TBR-PCR target product.

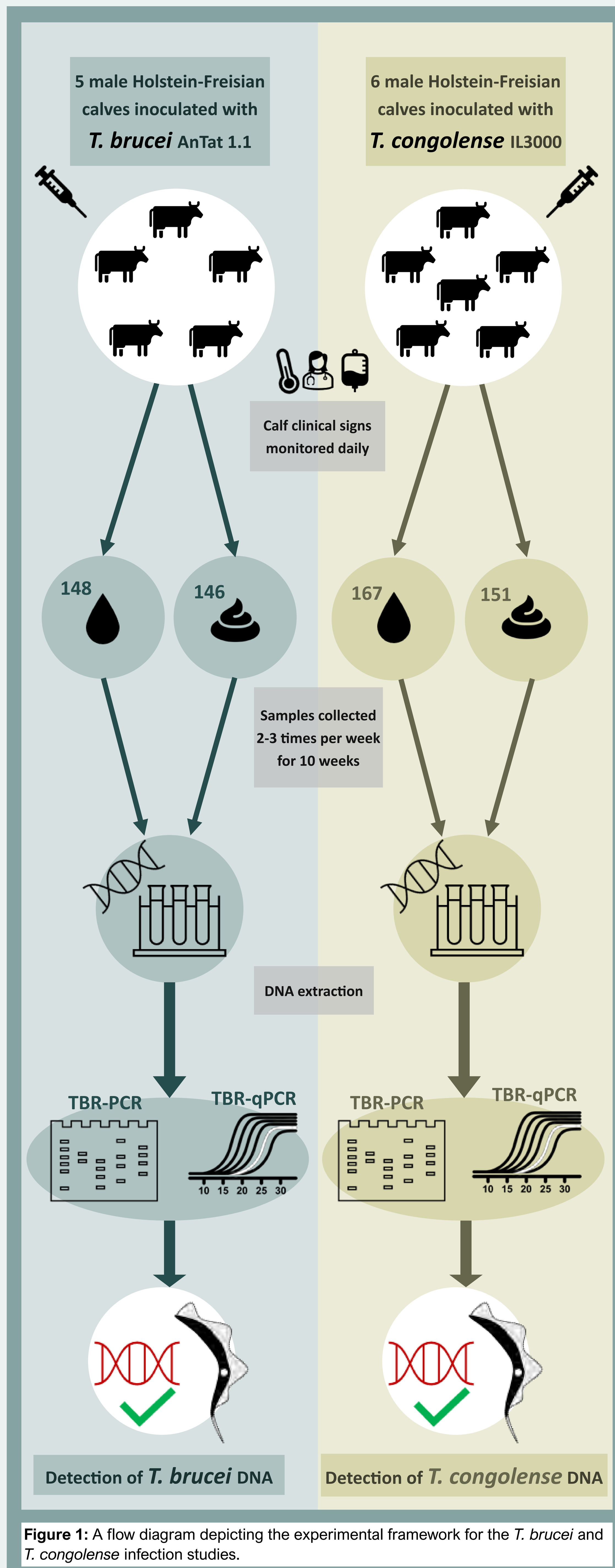
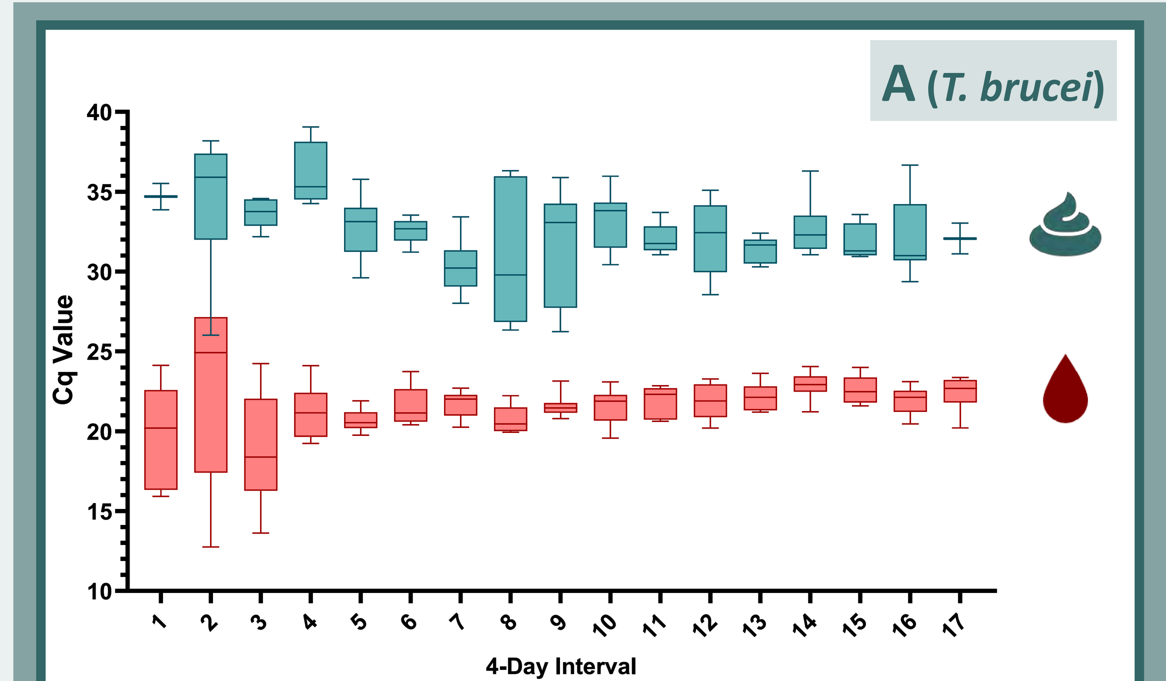


Figure 1: A flow diagram depicting the experimental framework for the *T. brucei* and *T. congolense* infection studies.



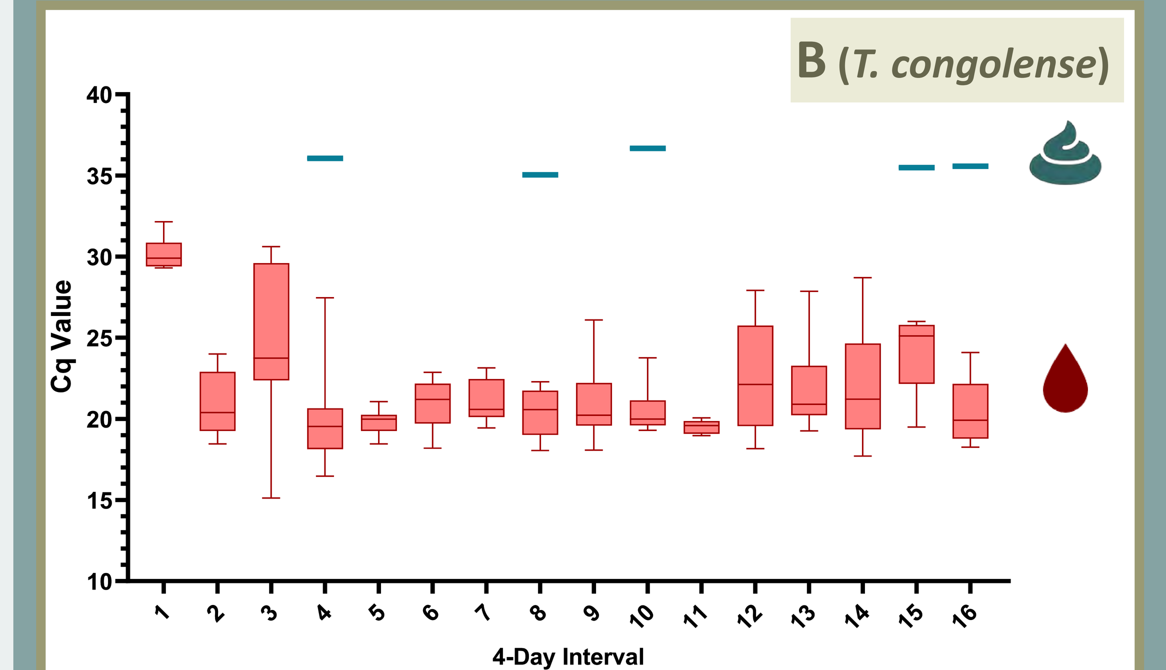


Figure 4: Box plot charts displaying Cq values obtained from screening respective blood and faecal samples with TBR-qPCR (A) and TCS-qPCR (B) over consecutive 4-day interval periods.

RESULTS

Consistent detection of T. brucei DNA in faecal samples: Target DNA was successfully detected in 85% (n=114) of post-inoculation faecal samples by TBR-qPCR and 50% (n=67) by TBR-PCR (Fig.3). Target DNA was detected in faecal samples collected between four days post-inoculation (dpi) to 66 dpi by both TBR-qPCR and TBR-PCR. In post-inoculation blood samples, target DNA was detected in 100% (n=138) of samples by TBR-qPCR.

Poor detection of T. congolense DNA in faecal samples: Target DNA was detected in only 3% (n=5) of post-inoculation faecal samples by qPCR and none by PCR (n=146), despite same LOD for TBR and TCS assays. *T. congo* DNA was detected in 100% (n=155) of post-inoculation blood samples by TCS-qPCR and TCS-PCR.

Relationship between T. brucei DNA quantity in faecal and blood samples: Cq values from faecal samples were consistently higher (mean=32.54) than for blood samples (mean=21.54) (Fig.4). Linear regression analysis revealed a weak yet statistically significant positive relationship ($p=0.0354$, $R^2=0.06345$) between Cq values obtained from matched blood and faecal samples (Fig.5).

Confirmation of T. brucei DNA detection: This was confirmed by Sanger sequencing of 4 PCR products in faecal samples. BLAST™ analysis revealed average percentage identity of 91.59% (±2.834 SD) to *T. brucei* s-l minicircle DNA (accession number K00392.1).

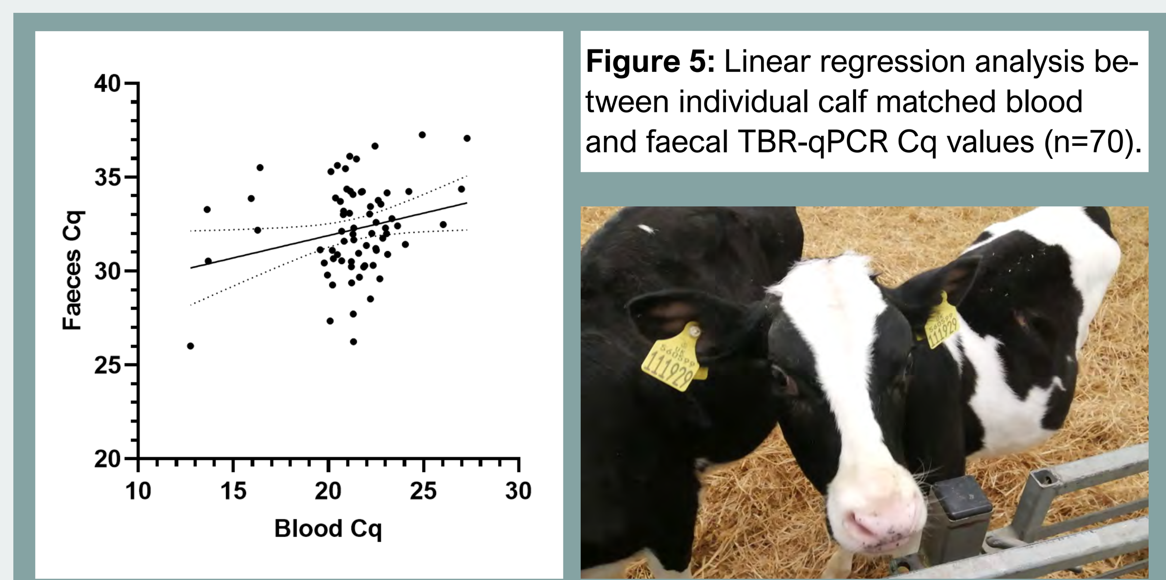


Figure 5: Linear regression analysis between individual calf matched blood and faecal TBR-qPCR Cq values (n=70).

- Able to **consistently detect *T. brucei* DNA in the faeces of infected cattle.**
- However **cannot reliably detect *T. congolense* DNA** in faeces
- Perhaps due to **differences in *Trypanosoma* sp tissue distribution—*T. brucei* is capable of tissue invasion** whilst ***T. congolense* is restricted to the blood circulatory system**
- This study also showcases interdisciplinary collaboration and being able to utilise *in vivo* research animals for several parallel studies, thereby **reducing the number of animals used in research.**
- Future research should focus on refining this novel diagnostic method in **field and wildlife samples** to broaden ***T. brucei* surveillance**.

References: [1]Barrett, M. P. The elimination of human African trypanosomiasis is in sight: Report from the third WHO stakeholders meeting on elimination of gambiense human African trypanosomiasis. *PLoS Negl. Trop. Dis.* **12**, e0006925 (2018). [2]Eshetu, E. & Begejo, B. The Current Situation and Diagnostic Approach of Nagana in Africa: A review. *J. Nat. Sci. Res.* **5**, 117–124 (2015). [3]Eisler, M. C., Dwinger, R. H., Majiwa, P. A. O. & Picozzi, K. Diagnosis and epidemiology of African animal trypanosomiasis. in *The Trypanosomiases* 253–267 (CABI, 2004). doi:10.1079/9780851994758.0253. [4]Jirků, M. *et al.* Wild chimpanzees are infected by Trypanosoma brucei. *Int. J. Parasitol. Parasites Wildl.* **4**, 277–282 (2015). [5]Hamad, I. *et al.* Wild Gorillas as a Potential Reservoir of Leishmania major. *J. Infect. Dis.* **211**, 267 (2015). [6]de Assis, G. M. P. *et al.* Detection of Plasmodium in faeces of the New World primate Alouatta clamitans. *Mem. Inst. Oswaldo Cruz* **111**, 570 (2016). [7]Jirků, M. *et al.* Detection of Plasmodium spp. in Human Feces. *Emerg. Infect. Dis.* **18**, 634 (2012). [8]Al-Shehri, H. *et al.* Non-invasive surveillance of Plasmodium infection by real-time PCR analysis of ethanol preserved faeces from Ugandan school children with intestinal schistosomiasis. *Malar. J.* **18**, 1–8 (2019). [9]Votýpka, J. *et al.* An unexpected diversity of trypanosomatids in fecal samples of great apes. *Int. J. Parasitol. Parasites Wildl.* **7**, 322 (2018). [10]Moser, D. *et al.* Detection of Trypanosoma congolense and Trypanosoma brucei subspecies by DNA amplification using the polymerase chain reaction. *Parasitology* **99 Pt 1**, 57–66 (1989).

Acknowledgements: *Many thanks to Adrian Ritchie and Peter Tennant for their invaluable assistance in the collection of biological samples and clinical data from calves taking part in the infection study at Roslin Institute. Many thanks also to Wendy Gibson and Annette Macleod for providing Trypanosoma sp. DNA for use in specificity testing. Funding provided by Biotechnology and Biological Sciences Research Council (BBSRC) and Bill & Melinda Gates Foundation. Photographs provided by Liam Morrison and author's own.*