A comparison between manual count, flow cytometry and qPCR as a means of determining *Babesia rossi* parasitaemia in naturally infected dogs

De Villiers L.[1]; Quan M.[2]; Troskie M.[2]; Fosgate G.T.[3]; Leisewitz A.L.[1]

[1] Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa

[2] Vector and Vector-Borne Diseases Research Programme, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa

[3] Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa

Introduction

Parasite quantification is crucial to understanding disease pathogenesis. An automated method of determining parasite density would facilitate higher throughput and provide results that are more objective.

Objectives

The study objectives included: a) validating the use of flow cytometry to detect and quantify *Babesia rossi* nucleic acid; b) comparing *B. rossi* parasite density in venous blood quantified by manual count, flow cytometry and quantitative real-time polymerase chain reaction (qPCR) in the same dog; and c) comparing the *B. rossi* parasite density in capillary blood (quantified by manual count), with the *B. rossi* parasite density in venous blood, as determined by manual count, flow cytometry and qPCR in the same dog.

Methods

Peripheral capillary and central venous blood was sampled from 40 naturally *B. rossi*-infected dogs and 10 healthy control dogs. Samples were analyzed by reverse line blot to confirm mono-*B. rossi* infection. Capillary blood parasite density was quantified using light microscopy (manual counts) and venous blood parasitaemia quantified using manual counts, flow cytometry and qPCR.

Results

Flow cytometry, using SYBR Green I staining, showed promise in quantifying *B. rossi* nucleic acid in venous blood. Non-parametric methods were used for preliminary statistical analysis. Spearman's rho revealed a significant correlation between the venous manual counts and qPCR (rs = -0.813; $P < 0.001$), as well as a significant correlation between the capillary manual counts compared to the venous manual counts (rs = 0.793; $P < 0.001$) and qPCR (rs = -0.760; $P < 0.001$). Preliminary data analysis suggest a correlation between flow cytometry and capillary manual counts, as well as venous manual counts, but issues of background and reticulocyte count need to be resolved.

Conclusion

Preliminary results demonstrate that qPCR is of value as an alternative to the gold standard (manual count) for quantifying *B. rossi* parasitaemia in canine whole blood and that flow cytometry may be useful with further refinement.

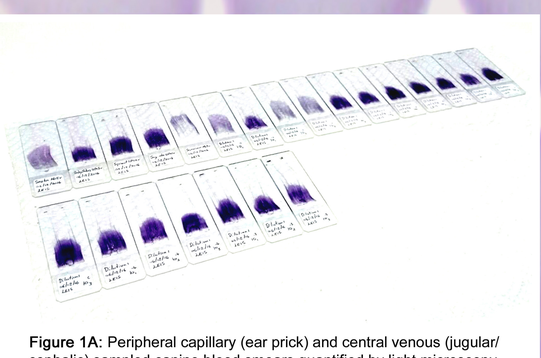

Figure 1A: Peripheral capillary (ear prick) and central venous (jugular/ cephalic) sampled canine blood smears quantified by light microscopy.

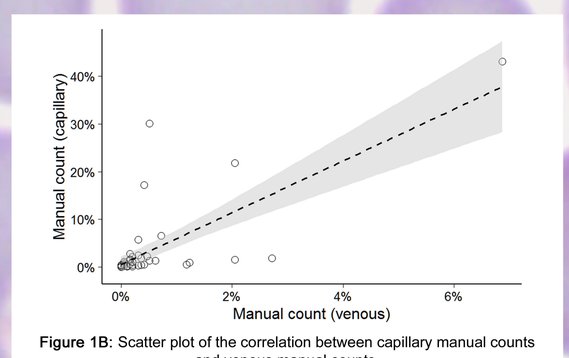

Figure 1B: Scatter plot of the correlation between capillary manual counts and venous manual counts.

Figure 2A: Accuri C6 flow cytometer used to, by means of a SYBR Green I nucleic acid dye, record and quantify *B. rossi*-infected canine red blood cells.

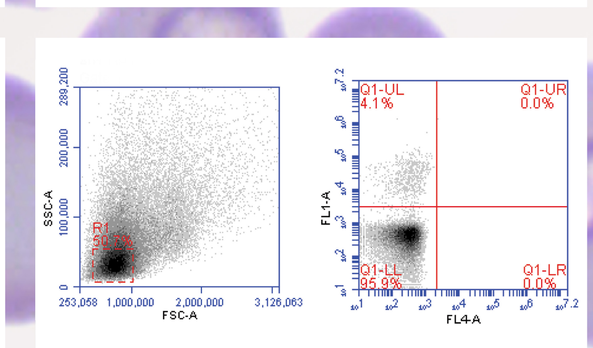

Figure 2B: Flow cytometric dot plots of the optimized red cell gate that enabled detection of FL-1 positive events (SYBR Green I stained pRBC).

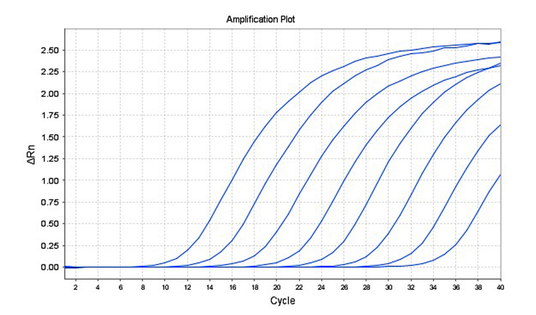

Figure 3A: Quantitative real-time PCR fluorogram of canine *B. rossi*-infected samples, demonstrating positive amplification curves.

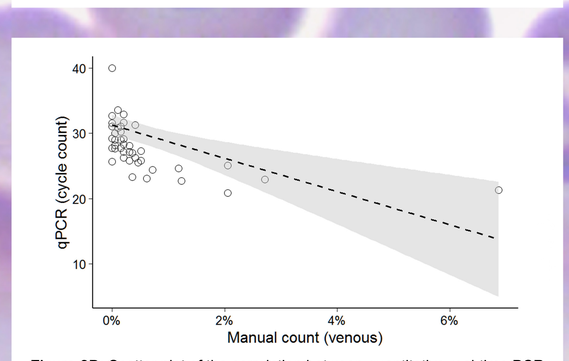

Figure 3B: Scatter plot of the correlation between quantitative real-time PCR and venous manual counts.

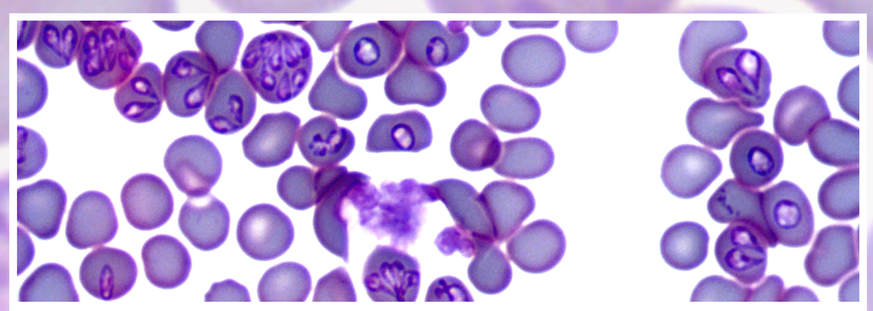

Figure 4: *B. rossi*-infected canine red blood cells. Both the characteristic teardrop (merozoite) and round (trophozoite) life cycle stages of the *Babesia* parasite can be seen on the blood smear made from this sample.

Contact details:

Researcher: Dr Lourens de Villiers (BSc, BVSc), lourensdevilliers@yahoo.com
Supervisor: Prof Andrew Leisewitz (BVSc (Hons), MMedVet(Med), ECVIM-CA, PhD), andrew.leisewitz@up.ac.za
Co-supervisor: Prof Melvyn Quan (BVSc, MSc, PhD), melvyn.quan@up.ac.za
Co-worker: Prof Geoffrey Fosgate (BSc, DVM, PhD, DipACVPM), geoffrey.fosgate@up.ac.za
Co-worker: Mrs Milana Troskie (MTech), milana.troskie@up.ac.za

NRF National Research Foundation

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UNIVERSITY OF PRETORIA
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