Evaluation of an improved Giemsa staining technique for blood borne parasite in thick and thin blood film from dogs

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Abstract

Many a times the use of rapid diagnostic tests for blood borne parasites like trypanosomiasis and Babesiosis is increasingly being used, but the gold standard for its detection is still the use of microscopy both as reference and confirmatory diagnosis. To determine the effectiveness of the adjusted stock giemsa staining technique over the conventional methods. Venous Blood samples were collected from 10 dogs in EDTA and then used for the simultaneous preparation of two thin and thick smear slides, one stained according to Giemsa 1:20 dilution for 30 mins while the other was stained using the Stock Solution for 30seconds the diluted with buffered saline for 20seconds and rinsed. Fixation of the thin smear was done in a covered staining jar containing anhydrous methanol for 1 to 2 min, after which the slides were air-dried. From the result obtained from 10 dogs blood samples gotten from the veterinary clinic, the adjusted giemsa staining technique showed a positive differentiation when compared to the 1:20 dilution, a total of 7 blood samples tested positive for blood borne parasites, *Trypanosoma evansi*, *Babesiosis cani* and Heart worm. The highest percentage occurrence was *T.evansi* (40%), *Babesiosis cani*(20%) and Heart worm (10%).The adjusted Giemsa staining technique serves as a fast, easy and less complex alternative to the 1:20 dilution, where the solution has to be diluted from the stock solution and then stained, although the only disadvantage to this technique would be easy contamination of the stock solution, but the advantages here is that it saves time, quicker result output and better differentiation microscopically.

Keywords: *Trypanosomaevansi*; *Babesiosiscani*; Heart worm; Giemsa

1. Introduction

For early and prompt diagnosis of parasitological diseases in man and animals is the corner stone of prevention control and treatment. Many a times the use of rapid diagnostic tests for blood borne parasites like trypanosomiasis and Babesiosis is increasingly being used, but the gold standard for its detection is the use of microscopy both as reference and confirmatory diagnosis (Alexander et al., 2010). The use of microscopy has the advantage over other serological method by providing a quantitative assessment of peripheral blood parasitaemia and parasite stages, as well as information on the impact of the parasite on the other blood elements (Bejon et al., 2006). Although the arguments on the use of Real Time PCR has been questioned based on the cost as it is much more expensive compared to the conventional microscopy and its but its sensitivity is over 50 times more than the normal microscopy, all these are dependent on the technique employed (Bowers et al., 2009). The use of an accurate microscopy technique would require an appropriate staining technique which has the capacity to differentiate the parasite from other blood elements and also the background. This staining technique must be cheap, efficient, precise, accurate and reproducible. In diagnosis the time factor is considered as a longer staining technique would results in delayed treatment being most microscopic.
analysis are onsite (Jury et al., 2012). The use of conventional Romanowsky stains has become a norm, like the Leishman and the Giemsa stains that are alcohol based, these stains are readily adapted over thick and thin blood films for the detection of parasites (Giemsa 1904). The Giemsa stains are readily adapted in the laboratories here in Nigeria this may be due to its almost 99% staining accuracy and its ability to differentiate the blood parasites from the blood parameters (Mens et al., 2006; Sanghamitra et al., 2014). The Giemsa stain adapted over time has at least 30 minutes of staining when diluted at 1:20 from the stock solution, either in a thick or a thin blood film. The Giemsa stain has also been used to stain smear gotten from the buffy coat for the detection of trypanosomiasis, blood flukes and filarial worms. The time for the staining matters as cases of poor differentiation may come from longer/shorter period of staining and as such the time is sacrosanct to achieving an accurate result. In this current study, the use of the stock solution compared to the diluted 1:20 solution of Giemsa stain is used to determine the accuracy of differentiation and detection of Trypanosoma evansi and Babesiosiscani in thick and thin film comparing the short time exposure with the stock solution and the long time with the diluted 1:20 solution.

2. Material and methods
This study was carried out in the Department of Veterinary anatomy, Faculty of Veterinary Medicine, in the University of Benin, Benin city, Edo state, Nigeria.

In the current study, EDTA anti-coagulated blood samples were collected from 10 dogs showing visible signs of illness Samples were processed with- out delay to avoid morphological alteration of parasites related to storage (Sanghamitra et al., 2014). A sample of 10 μL EDTA blood was then used for the simultaneous preparation of two thin and thick smear slides, one stained according to Giemsa 1:20 dilution for 30 mins while the other was stained using the Stock Solution for 30seconds the diluted with buffered saline for 20seconds and rinsed. Fixation of the thin smear was done in a covered staining jar containing anhydrous methanol for 1 to 2 min, after which the slides were air-dried. Giemsa working solution (Qualigens, product no 39382, batch no NL 06766308S) was diluted 1:20 with phosphate buffered water (pH7.2).

2.1. Statistical Analysis
Comparative analysis using the descriptive statistics Mean±Standard Error, using Graph Pad Prism 7.0.

3. Results
From the result obtained from 10 dogs blood samples gotten from the veterinary clinic, the adjusted giemsa staining technique showed a positive differentiation when compared to the 1:20 dilution, a total of 7 blood samples tested positive for blood borne parasites, Trypanosoma evansi, Babesiosiscani and Heart worm. The highest percentage occurrence was T.evansi (40%), Babesiosiscani (20%) and Heart worm (10%) as seen in Table 1.0, and figure 1.0, 2.0 and 3.0 The thin film and the thick film showed proper differentiation of the parasite but the parasiteamia level was higher in the thick film plate 1.0, 2.0, 3.0 and 4.0.

Figure 1 Comparing the Positive cases of blood borne Parasite using the adjusted Stock solution
Table 1 Percentage occurrence of blood borne parasite microscopically detected using the adjusted Giemsa staining technique

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Stock solution (%)</th>
<th>1:20 dilution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypanosoma evansi</td>
<td>10</td>
<td>4 (40)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Babesia canis</td>
<td>10</td>
<td>2 (20)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Heart Worm</td>
<td>10</td>
<td>1 (10)</td>
<td>1 (10)</td>
</tr>
</tbody>
</table>

Key: n= total number of samples tested, %=Percentage

Figure 2 Comparing the efficacy of detection and differentiation using the adjusted stock solution and the 1:20 dilution

Figure 3 Differentiation percentage of the Adjusted stock solution to the various detected blood borne parasite
Figure 4 and 5 Comparing the adjusted stock solution (2) and the conventional 1:20 dilution (1), the differentiation of *Trypanosoma evansi* in the adjusted stock solution for 50 seconds has a better differentiation.

Figure 6 and 7 Showing Heart worm and Babesiosiscani from the adjusted Giemsa stock solution.

4. Discussion

This result showed that the adjusted Giemsa staining technique is a suitable alternative over the conventional 1:20 dilution used as it requires a lesser time and has a better differentiation when viewed under the microscope. *Trypanosoma evansi* which is a common specie of trypanosomiasis that infects dogs can readily be identified using both the leishman stain, but the Giemsa stain shows a better differentiation (Mens, *et al.*, 2006; Shute *et al.*, 1988). The adjusted Giemsa staining technique further simplifies this technique as it is suitable for field work and without delay due to its short time of staining, this is seen in our study as a plus to the staining techniques for blood borne parasites. Both the thin and thick film stained with the adjusted Giemsa staining technique had a positive morphological differentiation microscopically as seen in this study also shows the detection rate of other blood borne parasites like *Babesiosiscani* and Heart worm from dogs, although the differentiation when compared with other staining technique like the leishman stain could easily be used as a good alternative where leishman and field stain A and B are not available. This Adjusted Giemsa stain, specimen could be stained for just fifty seconds (50 Sec), with all the parasite present easily differentiated morphologically.
5. Conclusion

The adjusted Giemsa staining technique serves as a fast, easy and less complex alternative to the 1:20 dilution, where the solution has to be diluted from the stock solution and then stained, although the only disadvantage to this technique would be easy contamination of the stock solution, but the advantages here is that it saves time, quicker result output and better differentiation microscopically.

Compliance with ethical standards

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Disclosure of conflict of interest

There was no conflict of interest during this study.

References


