Haematology of N’Dama and West African Short Horn cattle herds under natural *Trypanosoma vivax* challenge in Ghana [version 1; peer review: 1 approved, 1 approved with reservations, 1 not approved]

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Abstract

**Background:** Animal trypanosomosis is a major cause of economic loss in livestock production in Africa. A suggested control measure is to use breeds with traits of trypanotolerance. The study examines the effect of natural *Trypanosoma vivax* challenge on haematological parameters in two trypanotolerant cattle [N’Dama and West African Short Horn (WASH)] herds.

**Methods:** *T. vivax*-specific primers were used to diagnose *T. vivax* infection in an N’Dama herd at Cape Coast in southern Ghana and a WASH herd at Chegbanu in northern Ghana from May to July 2011 in a cross-sectional study. Levels of haematological parameters comprising packed cell volume (PCV), haemoglobin (Hb) concentration and total red blood cell (RBC) and white blood cell (WBC) counts; differential WBC counts (neutrophils, lymphocytes, eosinophils, monocytes and basophils); and RBC indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined in blood samples and then compared between infected and uninfected cattle.

**Results:** We found that haematological indices for infected and uninfected animals in both breeds were within the normal range. However, the mean PCV values for *T. vivax*-infected WASH and N’Dama were lower in infected compared to uninfected animals. The difference was significant (*p* < 0.05) in N’Dama but not in WASH. The RBC indices were higher in infected N’Dama compared to infected WASH with a significant difference in total RBC (*p* < 0.05).

**Conclusion:** We conclude from our findings that despite the presence of infection by *T. vivax*, N’Dama and WASH cattle maintained their haematological parameters within acceptable normal ranges, and this
underscores the need for routine diagnosis and treatment so that such trypanotolerant cattle do not serve as potential reservoirs of trypanosome parasites.

**Keywords**
Haematology, cattle, trypanotolerance, trypanosomosis, N'Dama, West African Short Horn

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**Author roles:**
- **Ganyo EY:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing
- **Boampong JN:** Supervision, Writing – Review & Editing
- **Masiga DK:** Conceptualization, Resources, Supervision, Validation, Writing – Review & Editing
- **Villinger J:** Formal Analysis, Funding Acquisition, Methodology, Resources, Supervision, Validation, Writing – Review & Editing
- **Turkson PK:** Conceptualization, Data Curation, Formal Analysis, Methodology, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

**Competing interests:** No competing interests were disclosed.

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Introduction

Animal trypanosomosis, caused by trypanosomes mainly transmitted by tsetse flies results in annual economic losses in Africa in the range of US$ 1.0 - 1.2 billion in cattle production alone, and more than US$ 4.75 billion in terms of agricultural Gross Domestic Product (Enyaru et al., 2010). Among species of trypanosomes that cause nagana, Trypanosoma vivax is the predominant species in Ghana (Adam et al., 2012; Mahama et al., 2004; Turkson, 1993).

The usual consequence of trypanosome infection is anaemia, which is often accompanied by poor growth, weight loss, low milk yield, infertility, abortion and paralysis (Dagnachew et al., 2015; OIE, 2013; Steverding, 2008; Trail et al., 1984). Death may result within a few weeks to several months after infection. Past and current control methods are limited, and it is unlikely that a vaccine will become available in the foreseeable future (Vale, 2009).

Trypanotolerant breeds, although equally susceptible to initial infection by trypanosomes, possess the ability to survive, reproduce and remain productive in areas of high tsetse challenge without the need for the control of vector or drugs to control the parasite (Dayo et al., 2009; Maganga et al., 2017; Rege et al., 1994; Yaro et al., 2016), where other breeds rapidly succumb to the disease (Murray & Dexter, 1988). The trypanotolerant trait is generally attributed to the taurine breeds of cattle in West and Central Africa, namely, the N’Dama and the West African Short Horn (WASH) (Roelants, 1986). Similar observations have been made for the Orma Boran X Maasai Zebu (Orma Zebu) crossbred cattle in East Africa (Maichomo et al., 2005; Mwangi et al., 1998a; Mwangi et al., 1998b). Studies have shown that the basis of this trait was associated with the capacity of these animals to develop less severe anaemia in the face of infection (Murray et al., 1982; Murray & Dexter, 1988).

We previously reported natural T. vivax challenge in N’Dama and WASH cattle herds in Ghana using a sensitive PCR approach (Ganyo, 2014). The current study being reported here examines the effect of natural T. vivax challenge on haematological parameters in these trypanotolerant cattle herds.

Methods

Animals, sampling and blood collection

Fifty-five animals each were sampled from an N’Dama herd at Cape Coast in southern Ghana and a WASH herd at Chegbani in northern Ghana from May to July 2011 in a cross-sectional study. The herds were chosen purposively since these were herds with the breeds of interest. From each animal, about 4 ml of blood was collected from the jugular vein using standard operating procedure that required no sedation and transferred into vacutainer tubes containing EDTA as anticoagulant. The vacutainer tubes were then placed in a coolbox containing ice packs for transportation to the laboratory, where they were refrigerated the same day for subsequent analysis.

Trypanosome detection

DNA was extracted from 200 μl of blood of each animal according to the protocol of Bruford et al. (1998) following red blood cell (RBC) lysis (Bieler et al., 2012). The procedure for DNA amplification and diagnosis of T. vivax infection has been described elsewhere (Ganyo, 2014). Briefly, amplifications were carried out targeting the 170-base pairs (bp) satellite DNA monomer of T. vivax. The PCR was carried out in a total volume of 20 μl containing 10 pmoles of each primer i.e. TVW_A (5’-GTGCTCATGCCCCAGTGTG-3’) and TVW_B (5’-CATATGCTCTGGGAGCGGGGT-3’) (Masiga et al., 1996), 4.0 μl 5X HF Buffer (Finnzymes), 10mM dNTPs, 1 unit Taq polymerase (Finnzymes) and 1 μl of DNA template. Cycling conditions for the PCR were accomplished in a 96-well thermocycler (PTC-100 Programmable Thermal Controller, MJ Research, Gaithersburg) as follows: initial denaturation at 98°C for 30 sec, followed by 35 cycles of denaturation at 98°C for 10 sec; annealing at 68°C for 30 sec, primer elongation at 72°C for 15 sec, and a final extension at 72°C for 7 min. PCR products were mixed with loading dye and samples were loaded alongside a molecular weight DNA marker as well as known positives and negatives into 1.5% agarose gel, stained with 50 mg/μl ethidium bromide. Electrophoresis was set at 75 volts for 1 hr 20 min, followed by visualization of the DNA under UV-illumination.

Determination of haematological parameters

Packed cell volume (PCV) was determined by the microhematocrit centrifugation technique while haemoglobin (Hb) concentration was measured spectrophotometrically by the cyanmethaemoglobin method (Jain, 1986). Total RBC and white blood cell (WBC) counts were done manually using a haemocytometer, according to the procedure outlined in Merck Veterinary Manual (Merck Veterinary Manual, 1986). Differential WBC counts were obtained from air dried thin blood smears stained with Giemsa stain according to the battlemount method (Merck Veterinary Manual, 1986). RBC indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae.

Statistical analysis

Data analysis was performed using the R statistical software version 2.3.7.1 (R Development Core Team, 2013). The one way analysis of variance (ANOVA) test was used to compare the means for haematological parameters in infected and uninfected cattle. Tests of significance were done at α = 0.05.

Results

Seven of the N’Dama samples (n=55) and 4 animals from the WASH samples (n=55) were positive for T. vivax infection. The mean haematological values for trypanosome-positive and negative cattle are shown in Table 1. For the N’Dama cattle, significant differences were observed in PCV (p < 0.05), total RBC count, MCV and MCH (p < 0.01) values between infected and uninfected cattle, with PCV, MCV
and MCH values being significantly higher in uninfected compared to infected cattle. The other parameters were similar for both groups (Table 1). For the WASH cattle, the PCV, Hb and RBC values for uninfected cattle were higher than those for infected cattle (Table 1).

When the mean haematological values for *T. vivax*-positive N’Dama and WASH cattle were compared (Table 2), significant differences were observed in the total RBC count (*p* = 0.01), MCV (*p* = 0.04), MCH (*p* = 0.02) and eosinophil values (*p* = 0.04). The RBC and eosinophil values were significantly

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### Table 1. Within-breed comparison of haematological parameters (mean ± SD) of *Trypanosoma vivax* infected and uninfected N’Dama cattle at Cape Coast and West African Short Horn cattle at Chegban, Ghana.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infected N’Dama (n=55)</th>
<th>Uninfected N’Dama (n=55)</th>
<th>F statistic</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>30.7 ± 4.4</td>
<td>36.5 ± 4.0</td>
<td>5.50</td>
<td>0.023*</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>11.8 ± 1.9</td>
<td>13.1 ± 1.6</td>
<td>3.55</td>
<td>0.065</td>
</tr>
<tr>
<td>RBC (×10⁶mm⁻³)</td>
<td>9.1 ± 2.1</td>
<td>6.9 ± 1.9</td>
<td>7.18</td>
<td>0.010**</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>35.8 ± 11.0</td>
<td>53.1 ± 14.1</td>
<td>9.63</td>
<td>0.003**</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>13.8 ± 4.5</td>
<td>20.0 ± 5.2</td>
<td>8.82</td>
<td>0.004**</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>38.6 ± 4.6</td>
<td>40.6 ± 12.0</td>
<td>0.04</td>
<td>0.845</td>
</tr>
<tr>
<td>WBC (×10³mm⁻³)</td>
<td>7.0 ± 2.9</td>
<td>8.6 ± 3.4</td>
<td>1.43</td>
<td>0.238</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>38.7 ± 12.8</td>
<td>39.1 ± 13.6</td>
<td>0.01</td>
<td>0.943</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>39.6 ± 15.6</td>
<td>40.6 ± 12.0</td>
<td>0.04</td>
<td>0.845</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>21.4 ± 13.7</td>
<td>4.8 ± 1.7</td>
<td>5.65</td>
<td>0.042</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>0.3 ± 0.8</td>
<td>0.2 ± 0.6</td>
<td>0.05</td>
<td>0.829</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.09</td>
<td>0.760</td>
</tr>
</tbody>
</table>

n represents number of samples in each category

*Indicates level of significance at 5% level (*p* < 0.05)

**Indicates level of significance at 1% level (*p* < 0.01)

PCV, packed cell volume; Hb, haemoglobin; RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cells.

### Table 2. Across-breed comparison of haematological parameters (mean ± SD) of *T. vivax* infected N’Dama cattle at Cape Coast and WASH cattle at Chegban, Ghana.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infected N’Dama (n=7)</th>
<th>Infected WASH (n=4)</th>
<th>F statistic</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>30.7 ± 4.4</td>
<td>28.3 ± 1.5</td>
<td>1.16</td>
<td>0.31</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>11.8 ± 1.9</td>
<td>11.5 ± 1.1</td>
<td>0.13</td>
<td>0.727</td>
</tr>
<tr>
<td>RBC (×10⁶mm⁻³)</td>
<td>9.1 ± 2.1</td>
<td>5.5 ± 1.1</td>
<td>9.48</td>
<td>0.013*</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>35.8 ± 11.0</td>
<td>53.4 ± 2.8</td>
<td>8.82</td>
<td>0.039*</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>13.8 ± 4.5</td>
<td>21.5 ± 4.7</td>
<td>7.32</td>
<td>0.024*</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>38.6 ± 4.6</td>
<td>40.5 ± 2.0</td>
<td>0.53</td>
<td>0.468</td>
</tr>
<tr>
<td>WBC (×10³mm⁻³)</td>
<td>7.0 ± 2.9</td>
<td>11.5 ± 1.4</td>
<td>8.46</td>
<td>0.017*</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>38.7 ± 12.8</td>
<td>47.5 ± 19.5</td>
<td>0.834</td>
<td>0.385</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>39.6 ± 15.6</td>
<td>47.5 ± 18.5</td>
<td>0.58</td>
<td>0.466</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>21.4 ± 13.7</td>
<td>4.8 ± 1.7</td>
<td>5.65</td>
<td>0.042*</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>0.3 ± 0.8</td>
<td>0.3 ± 0.5</td>
<td>0.007</td>
<td>0.935</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

n represents number of samples in each category

*Indicates level of significance at 5% level (*p* < 0.05)

WASH, West African Short Horn; PCV, packed cell volume; Hb, haemoglobin; RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cells.
In conclusion, the study found that in spite of the presence of natural Trypanosoma vivax infection, the haematological parameters of N’Dama and WASH cattle herds were within acceptable normal ranges. Since such healthy cattle could serve as a potential source of infection for trypanosusceptible cattle and other domestic animals, the study underscores the need to incorporate routine diagnosis and treatment for trypanosome parasites in the management of trypanotolerant cattle herds.
Owners of animals gave their consent before the animals were bled. Prior to jugular venipuncture, the body of the animal was manually restrained by assistants to avoid injury to the animal. Further, the head of the animal was turned by another assistant at a 30-degree angle to the side by holding the animal under its jaw; this is to allow for easy access to the vein, and, to ensure quick, easy and safe collection of the sample causing minimal distress to the animal. To avoid repeated puncturing, time was taken to locate the vein accurately and it was distended by gentle pressure with the fingers before the needle was inserted. After the vein was located, the area was properly cleaned by alcohol to keep bacteria out of the needle insertion site. To ensure that sampling did not result in hypovolemic shock, physiological stress, anaemia and possibly death, only a minimal amount of 4ml of blood was drawn from each animal. To prevent needle-stick injury, a new needle was used for each venipuncture. As soon as blood was removed from the animal, the insertion site was swabbed with alcohol to remove any bacteria that might have entered the area during the drawing of blood. Pressure was applied for 30–60 seconds immediately following withdrawal of the needle; the pressure caused blood to clot, thereby preventing bleeding.

At the time this work was conducted (2011) there was no requirement by the University of Cape Coast for ethical clearance for work with animals. Therefore, we followed internationally accepted procedures such as those outlined in “Guidelines for the Welfare of Livestock from which Blood is Harvested for Commercial and Research Purposes” published by the New Zealand National Animal Ethics Advisory Committee in 2009 (https://www.mpi.govt.nz/dmsdocument/1475-guidelines-for-the-welfare-of-livestock-from-which-blood-is-harvested-for-commercial-and-research-purposes).

Data availability
Dataset 1: Haematological parameters of *T. vivax* infected and uninfected WASH and N’Dama cattle at Chegbanu and Cape Coast, respectively. 10.5256/f1000research.14032.d197325 (Ganyo et al., 2018)

Author information
EYG holds a PhD in Parasitology. JNB is an Associate Professor in the Department of Biomedical and Forensic Sciences, and the Dean of the School of Biological Sciences. JV is a scientist and head of the Molecular Biology and Bioinformatics Unit, International Centre of Insect Physiology and Ecology Nairobi, Kenya. PKT is a Professor of Veterinary Epidemiology and Dean, School of Veterinary Medicine, University of Ghana, Legon, Accra, Ghana.

Competing interests
The authors declare that they have no competing interests.

Grant information
This work was supported in part by an International Centre of Insect Physiology and Ecology (icipe) six-month Dissertation Research Internship Programme (DRIP) fellowship funded by the Swedish International Development Cooperation Agency (Sida); and institutional financial support from UK Aid from the UK Government; the Swiss Agency for Development and Cooperation (SDC); and the Kenyan Government. The views expressed herein do not necessarily reflect the official opinion of the donors.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements
We thank Jerry Oddoye and Abdulai Munkaila of the Veterinary Services Directorate, Ghana, for technical assistance. The help received from managers of the cattle herds at sampling sites is appreciated.

References


Open Peer Review

Current Peer Review Status: 

Version 1

Reviewer Report 01 June 2018

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Sophie Thévenon
UMR INTERTRYP, French Agricultural Research Centre for International Development (CIRAD), Montpellier, France

The manuscript aims at assessing the effect of infections by Trypanosoma vivax on the hematological parameters of N’dama and WASH (West African Short-horn) cattle, raised in two natural environments in Ghana. The purpose is to highlight the trypanotolerant character of these breeds.

Major comments:

The article suffers from several major problems and is not suited for indexing.

The bibliography is quite incomplete: major papers written by Trail et al\(^1\)-\(^2\) and Mattioli et al\(^3\)-\(^4\) are not cited. These authors worked on N’Dama cattle raised in Congo and Gambia respectively and on the relationships between productivity, anemia and infections. Mattioli et al 1998 (Acta Tropica) showed that N’Dama cattle suffered from high tse-tse challenge. Trail et al 1994 showed that N’dama infected by trypanosomes had lower PCV values and lower weight gain than non-infected N’Dama. In addition, an experimental infection published by Berthier et al (2015)\(^5\), presented anemia evolution in 5 cattle breeds of West Africa under T. congoense infection and show of N’Dama and WASH were less anemiated than Zebu Fulani and Borgou.

The experimental design presented in the article does not bring robust elements on anemia control during T. vivax infection and on the comparison between N’Dama and WASH. There is not any susceptible breed that could be compared to N’Dama and WASH. It is thus not possible to know if the T. vivax strains are highly pathogenic or not. Since N’Dama and WASH are not raised in the same area under the same agro-ecological context, it is not possible to compare these two breeds.

Because only 4 and 7 animals were positive to T. vivax PCR, an Anova cannot be used. Only a non-parametric test can be used.

Other comments:
The article of Bouyer et al (2015) must be cited in the introduction concerning control method. I do not agree with the sentence “past and current control methods are limited”: the use of trypanocide drugs may be useful and efficient when their usage is adapted to the context (environment and breeding system).

In the table I and II and in the text, the terms “positive in T. vivax PCR” and “negative in T. vivax PCR” must be used instead of infected or uninfected. Indeed, PCR has a sensitivity around 75-80% and thus some animals considered as negative in PCR may be infected.

In the discussion, the authors propose to incorporate routine diagnosis and treatment. But the problem is that there is not any routine diagnosis, since parasitological methods have a very low sensitivity, and PCR and serology require a well-equipped laboratory with well-trained technicians. Farmers need the support of farmer’s organization and from veterinary public service. The notion of “reservoirs” due to trypanotolerant cattle has never been clearly investigated.

Finally, the raise of trypanotolerant breeds is important in some agro-ecological context, where tsetse challenge is high and in low input systems. In some areas, only trypanotolerant breed can survive.

References

Is the work clearly and accurately presented and does it cite the current literature? Partly

Is the study design appropriate and is the work technically sound? No

Are sufficient details of methods and analysis provided to allow replication by others? Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
No

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** parasitology, genetics, host*parasite interactions, trypanosomoses

I have read this submission. I believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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Animal trypanosomosis is an important disease in both animals and humans and understanding the host's response to such infection is important scientific endeavor. The manuscript describes some basic hematological parameters in these two breeds of animals, which may have some biological significance and lead to some understanding of parameters affecting disease resistance. However, the number of infected animals is quite low in both groups, with only 4/55 WASH and 7/55 N'Dama infected.

The data supports the conclusions presented.

Suggestions:

The use of real numbers for each WB cell subgroup parameter rather than reporting only a %. For instance the Eosinophils in Table 2 could be represented as a number rather than a % and this would give a greater feel for the level of absolute differences. If for instance the Eosinophils were represented as a number then the differences between the breeds would be much clearer. Both absolute numbers and % could be used.

5mg/ul is a considerable concentration of ethidium bromide (needs to be checked). Green and Sambrook recommends use of 0.5ug/ml suggest checking the value.

Concentration of the template is not given and should be.

Suggest checking concentrations of the reagents used in the PCR. For instance, 10mM dNTPs is quite a
considerable quantity of dNTPs or is it x ul of a 10mM dNTP solution. Typical final concentrations in PCR are between 200-250uM.

Is the work clearly and accurately presented and does it cite the current literature? Yes

Is the study design appropriate and is the work technically sound? Yes

Are sufficient details of methods and analysis provided to allow replication by others? No

If applicable, is the statistical analysis and its interpretation appropriate? I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility? Yes

Are the conclusions drawn adequately supported by the results? Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Immunogenetics, Genetics, Molecular Biology

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 25 April 2018

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Yahaya Adam
Tsetse and Trypanosomiasis Control Unit/PATTEC, Ministry of Food and Agriculture, Pong-Tamale, Ghana

Animal trypanosomosis, as reported in the paper, is indeed a major constraint to livestock production systems in Ghana. The theme for the paper is therefore very appropriate. The study design and methods as presented appear to satisfy the standard requirements for a scientific study. The authors suggested the use of these trypano-tolerant animals as control measure for the problem of animal trypanosomosis but I hold a dissenting view to that. The N'dama and WASH cattle, even though are trypano-tolerant as indicated clearly in the study, can not be a solution to the problem for the following reasons:
1) The T. vivax challenge does not mean a 100% free from the impact as demonstrated in the study (PCV of infected slightly lower than that of uninfected for both breeds of the trypano-tolerant animals used in the study).
2) The N'dama and the WASH breeds are not very productive compared to the other breeds of cattle raised in Ghana, probably due to the T. vivax challenge.
3) The two breeds of cattle in the study have the potential status as reservoir of trypanosomes to other breeds of cattle.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 25 Apr 2018

**Paa Kobina,** School of Agriculture, University of Cape Coast, Ghana

We accept the comments of the Referee. We agree that the two breeds are not as productive as other breeds but having them deal better with trypanosomosis is considered by some livestock keepers as an advantage to keep them and therefore may be preferred..

**Competing Interests:** None.
Paa Kobina, School of Agriculture, University of Cape Coast, Ghana

Responses to Second Reviewer’s comments
1. Actual concentration of ethidium bromide and volumes of the PCR components have been provided in the methods section of the revised text, under the ‘Trypanosome detection’ sub-section.

2. A revised Dataset 1 has been provided in which the differential white blood cell counts (Neutrophils, Lymphocytes, Eosinophils, Monocytes and Basophils) are presented as both absolute numbers and %.
   Table 1 in the revised text also has the differential white blood cell counts presented as both absolute numbers and %.

Responses to Third Reviewer’s comments
1. The comment on incomplete bibliography has been fully addressed in the revised text.
2. The comment on absence of susceptible breed that could be compared to N'Dama and WASH has been addressed in the revised text by providing data on trypanosusceptible Sanga and Zebu breeds that we had sampled together with the N'Dama and WASH but did not report in the initial text.
3. We agree with the comment that it is not possible to compare N'Dama and WASH since the two breeds were not raised in the same area under the same agro-ecological context. This aspect has consequently been removed from the revised text.
4. In the revised text, the non-parametric Kruskal-Wallis test, rather than ANOVA, was used.
5. The reviewer made some suggestions which have been incorporated in the revised text.

Normal haematological values in Table 1:
For ease of reference, we have incorporated the normal values of haematological parameters for cattle (Jain, 1993) into Table 1.

Competing Interests: None