RESEARCH ARTICLE

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]

Ebenezer Yaw Ganyo¹, Johnson N Boampong¹, Daniel K Masiga^{id}², Jandouwe Villinger², Paa Kobina Turkson^{id}³

¹Department of Biomedical and Forensic Sciences, School of Biological Sciences, University of Cape Coast, Ghana

²International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya

³Department of Animal Science, School of Agriculture, University of Cape Coast, Ghana

V1 First published: 13 Mar 2018, 7:314
<https://doi.org/10.12688/f1000research.14032.1>
 Latest published: 10 Aug 2018, 7:314
<https://doi.org/10.12688/f1000research.14032.2>

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 Chegbani 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

Open Peer Review

Approval Status

	1	2	3
version 2			
(revision)			
10 Aug 2018		view	view
		↑	↑
version 1			
13 Mar 2018	view	view	view

1. **Yahaya Adam**, Ministry of Food and Agriculture, Pong-Tamale, Ghana

2. **David M Groth**, Curtin University, Bentley, Australia

3. **Sophie Thévenon**, French Agricultural Research Centre for International Development (CIRAD), Montpellier, France

Any reports and responses or comments on the article can be found at the end of the article.

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

Corresponding author: Paa Kobina Turkson (kobbiecc@gmail.com)

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, 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.

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

Copyright: © 2018 Ganyo EY *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

How to cite this article: Ganyo EY, Boampong JN, Masiga DK *et al.* **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]** F1000Research 2018, 7:314 <https://doi.org/10.12688/f1000research.14032.1>

First published: 13 Mar 2018, 7:314 <https://doi.org/10.12688/f1000research.14032.1>

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 use of chemicals to control the 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 (Biéler *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'-GTGCTCCATGTGCCACGTTG-3') and TVW_B (5'-CATATGGTCTGGGAGCGGGT-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 microhaematocrit 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 battlement 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 $\alpha = 0.05$.

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

Table 1. Within-breed comparison of haematological parameters (mean $\pm$ SD) of *Trypanosoma vivax* infected and uninfected N'Dama cattle at Cape Coast and West African Short Horn cattle at Chegiani, Ghana.

Parameter	N'Dama (n=55)				West African Short Horn (n=55)			
	Infected n = 7	Uninfected n = 48	F statistic	p value	Infected n = 4	Uninfected n = 51	F statistic	p value
PCV (%)	30.7 $\pm$ 4.4	36.5 $\pm$ 4.0	5.50	0.023*	28.3 $\pm$ 1.5	31.6 $\pm$ 5.1	1.56	0.218
Hb (g/dl)	11.8 $\pm$ 1.9	13.1 $\pm$ 1.6	3.55	0.065	11.5 $\pm$ 1.1	11.7 $\pm$ 2.0	0.05	0.834
RBC ($\times 10^6/\text{mm}^3$)	9.1 $\pm$ 2.1	6.9 $\pm$ 1.9	7.18	0.010**	5.5 $\pm$ 1.1	5.8 $\pm$ 1.3	0.18	0.671
MCV (fl)	35.8 $\pm$ 11.0	53.1 $\pm$ 14.1	9.63	0.003**	53.4 $\pm$ 12.8	56.6 $\pm$ 11.8	0.27	0.606
MCH (pg)	13.8 $\pm$ 4.5	20.0 $\pm$ 5.2	8.82	0.004**	21.5 $\pm$ 4.7	21.0 $\pm$ 4.7	0.05	0.821
MCHC (g/dl)	38.6 $\pm$ 4.6	38.0 $\pm$ 4.4	0.14	0.710	40.5 $\pm$ 2.0	37.3 $\pm$ 4.6	1.93	0.171
WBC ($\times 10^3/\text{mm}^3$)	7.0 $\pm$ 2.9	8.6 $\pm$ 3.4	1.43	0.238	11.5 $\pm$ 1.4	10.5 $\pm$ 2.2	0.83	0.367
Neutrophils (%)	38.7 $\pm$ 12.8	39.1 $\pm$ 13.6	0.01	0.943	47.5 $\pm$ 19.5	45.0 $\pm$ 12.4	0.14	0.715
Lymphocytes (%)	39.6 $\pm$ 15.6	40.6 $\pm$ 12.0	0.04	0.845	47.5 $\pm$ 18.5	42.1 $\pm$ 13.5	0.56	0.456
Eosinophils (%)	21.4 $\pm$ 13.7	20.0 $\pm$ 11.5	0.09	0.765	4.8 $\pm$ 1.7	9.6 $\pm$ 5.1	3.46	0.069
Monocytes (%)	0.3 $\pm$ 0.8	0.2 $\pm$ 0.6	0.05	0.829	0.3 $\pm$ 0.5	2.9 $\pm$ 3.6	2.13	0.151
Basophils (%)	0.0 $\pm$ 0.0	0.0 $\pm$ 0.2	0.29	0.590	0.0 $\pm$ 0.0	0.0 $\pm$ 0.3	0.08	0.782

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 $\pm$ SD) of *T. vivax* infected N'Dama cattle at Cape Coast and WASH cattle at Chegiani, Ghana.

Parameter	Infected N'Dama n = 7	Infected WASH n = 4	F statistic	p value
PCV (%)	30.7 $\pm$ 4.4	28.3 $\pm$ 1.5	1.16	0.31
Hb (g/dl)	11.8 $\pm$ 1.9	11.5 $\pm$ 1.1	0.13	0.727
RBC ($\times 10^6/\text{mm}^3$)	9.1 $\pm$ 2.1	5.5 $\pm$ 1.1	9.48	0.013*
MCV (fl)	35.8 $\pm$ 11.0	53.4 $\pm$ 12.8	5.80	0.039*
MCH (pg)	13.8 $\pm$ 4.5	21.5 $\pm$ 4.7	7.32	0.024*
MCHC (g/dl)	38.6 $\pm$ 4.6	40.5 $\pm$ 2.0	0.53	0.468
WBC ($\times 10^3/\text{mm}^3$)	7.0 $\pm$ 2.9	11.5 $\pm$ 1.4	8.46	0.017*
Neutrophils (%)	38.7 $\pm$ 12.8	47.5 $\pm$ 19.5	0.834	0.385
Lymphocytes (%)	39.6 $\pm$ 15.6	47.5 $\pm$ 18.5	0.58	0.466
Eosinophils (%)	21.4 $\pm$ 13.7	4.8 $\pm$ 1.7	5.65	0.042*
Monocytes (%)	0.3 $\pm$ 0.8	0.3 $\pm$ 0.5	0.007	0.935
Basophils (%)	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	—	—

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.

($p < 0.05$) higher in N'Dama compared to WASH, while the MCV, MCH and WBC values were significantly ($p < 0.05$) higher in WASH compared to N'Dama cattle.

Dataset 1. Haematological parameters of *T. vivax* infected and uninfected WASH and N'Dama cattle at Chegban and Cape Coast, respectively

<http://dx.doi.org/10.5256/f1000research.14032.d197325>

Discussion

The mean PCVs for infected N'Dama and WASH cattle in this study were lower than those for uninfected cattle, which support other findings (Waiswa & Katunguka-Rwakishaya, 2004), and are consistent with the pathological effect of anaemia in trypanosome infections. A survey conducted in cattle in Ethiopia (Bekele & Nasir, 2011) revealed that the mean PCV of trypanosome-infected animals was significantly lower (20.8 ± 3.2 %) compared to non-infected animals (24.9 ± 3.8 %). A later study in Ethiopia (Dagnachew *et al.*, 2015) in cattle experimentally infected with *T. vivax* isolates also showed that the mean PCV, Hb and total RBC count were lower ($p < 0.001$) in all infected groups than in non-infected control animals. In Nigeria, domestic ruminants that were naturally infected with trypanosomes had significantly lower ($p < 0.05$) PCV and RBC counts compared to uninfected animals (Ohaeri & Eluwa, 2011). Lower herd average PCVs for trypanosome-positive cattle compared to trypanosome-negative cattle have also been reported from Zambia (Marcotty *et al.*, 2008), Cameroon (Achukwi & Musongong, 2009) and Gabon (Cossic *et al.*, 2017).

The mean PCV, Hb and RBC values observed in both infected and uninfected N'Dama and WASH cattle studied were within the established normal reference values (Jain, 1993). The presence of *T. vivax* parasites is associated with a reduction in the mean RBC values below the normal range, an indication of anaemia (Murray & Dexter, 1988; Silva *et al.*, 1999). However, infected cattle in the present study had mean RBC values within the normal range, which could be attributed to their trypanotolerant trait. In a typical trypanotolerance phenomenon, pathogenic *Trypanosoma* species infection does not usually result in anaemia (Murray *et al.*, 1982; Murray & Dexter, 1988). Our findings compare favourably with the report that the mean RBC, Hb and PCV values in natural *T. vivax*-infected trypanotolerant Muturu cattle in Nigeria (Mbanasor *et al.*, 2003) were well within accepted normal values for cattle. An earlier study (Adam *et al.*, 2012) involving trypanotolerant WASH cattle in Ghana also reported normal PCV value despite the presence of trypanosome parasites.

The importance of keeping trypanotolerant cattle as a trypanosomosis control measure in Ghana has long been recognised (Mahama *et al.*, 2003; Turkson, 1993). However, farmers are

increasingly showing a preference for trypanosusceptible Zebu cattle and its Sanga crossbreed, which have a larger body size, higher milk yield and heavier live weight (World Bank, 1992). For example, the last Ghana Livestock Census published in 1997 indicated that the WASH constituted 70% of the cattle population in Ghana (Mahama *et al.*, 2003), while a later study gave a reduced WASH population of 47.5% (Ahunu & Boa-Amponsem, 2001).

The larger bodied trypanosusceptible cattle breeds command higher prices compared to the smaller bodied trypanotolerant cattle, but unlike trypanotolerant cattle, trypanosusceptible cattle cannot survive in areas of high tsetse densities without veterinary intervention or other tsetse control strategies (Mahama *et al.*, 2003; Turkson, 1993).

Although trypanotolerant breeds are equally susceptible to trypanosome infection, they possess the ability to survive, reproduce and remain productive in areas of high tsetse challenge without the need for the use of chemicals to control the vector or drugs to control the parasite (Dayo *et al.*, 2009; Maganga *et al.*, 2017; Rege *et al.*, 1994; Yaro *et al.*, 2016).

As was clearly demonstrated in this study, the infected trypanotolerant WASH and N'Dama maintained their mean RBC values in the normal range, which is an indication of good health. Further, in the present study, informal information revealed that none of the livestock keepers who keep such trypanotolerant breeds use trypanocidal drugs regularly or strategically to control the infection. Since trypanotolerant cattle could serve as potential reservoirs of trypanosome parasites, there is the need for owners of WASH and N'Dama herds in Ghana to incorporate routine diagnosis and treatment of trypanosome infections into their overall management strategy.

The mean WBC counts for the N'Dama and WASH in the present study were within the normal values reported by Jain (1993) but were lower than those reported for *T. vivax*-infected and uninfected Muturu cattle in Nigeria (Mbanasor *et al.*, 2003). The total RBC count for infected N'Dama was significantly ($p < 0.05$) higher than that for infected WASH suggesting that the N'Dama may be better at controlling anaemia compared to WASH.

Conclusion

In conclusion, the study found that in spite of the presence of natural *T. 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.

Statement of animal welfare and ethics

Collection of blood was done as per standard operating procedures to ensure animal welfare (<https://www.dpi.nsw.gov.au/animals-and-livestock/animal-welfare/general/general-welfare-of-livestock/sop/pigs/health/blood-collection>). These are standard operating procedures used in veterinary medicine internationally.

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 Chegbani and Cape Coast, respectively. [10.5256/f1000research.14032.d197325](https://doi.org/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. DKM is the head of Animal Health, 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

- Achukwi MD, Musongong GA: **Trypanosomosis in the Doayo/Namchi (*Bos taurus* and zebu White Fulani (*Bos indicus*) cattle in Faro Division, North Cameroon.** *J Appl Biosci.* 2009; 15: 807–814.
[Reference Source](#)
- Adam Y, Marcotty T, Cecchi G, *et al.*: **Bovine trypanosomosis in the Upper West Region of Ghana: Entomological, parasitological and serological cross-sectional surveys.** *Res Vet Sci.* 2012; 92(3): 462–468.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ahunu BK, Boa-Amponsem K: **Characterization and conservation of the Ghana Shorthorn Cattle. A report submitted to the Animal Production Directorate of the Ministry of Food and Agriculture.** Accra, Ghana. 2001.
- Bekele M, Nasir M: **Prevalence and host related risk factors of bovine trypanosomosis in Hawagelan District, West Wellega Zone, western Ethiopia.**

- Afr J Agric Res.* 2011; 6(22): 5055–5060.

Reference Source

- Biéler S, Matovu E, Mitashi P, *et al.*: **Improved detection of *Trypanosoma brucei* by lysis of red blood cells, concentration and LED fluorescence microscopy.** *Acta Trop.* 2012; 121(2): 135–140.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Bruford MW, Hanotte O, Brookfield JF, *et al.*: **Multilocus and single locus DNA fingerprinting.** In: A. R. Hoelzel Editor, *Molecular Genetic Analysis of Populations: A Practical Approach*, Oxford, IRL Press. 1998; 287–336.
- Cossic BGA, Adjahoutonon B, Gloaguen P, *et al.*: **Trypanosomiasis challenge estimation using the diminazene aceturate (Berenil) index in Zebu in Gabon.** *Trop Anim Health Prod.* 2017; 49(3): 619–624.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

- Dagnachew S, Bezie M, Terefe G, *et al.*: **Comparative clinico-haematological analysis in young Zebu cattle experimentally infected with *Trypanosoma vivax* isolates from tsetse infested and non-tsetse infested areas of Northwest Ethiopia.** *Acta Vet Scand.* 2015; **57**: 24.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Dayo GK, Thevenon S, Berthier D, *et al.*: **Detection of selection signatures within candidate regions underlying trypanotolerance in outbred cattle populations.** *Mol Ecol.* 2009; **18**(8): 1801–1813.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Enyaru JC, Ouma JO, Malele II, *et al.*: **Landmarks in the evolution of technologies for identifying trypanosomes in tsetse flies.** *Trends Parasitol.* 2010; **26**(8): 388–394.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ganyo EY: **Trypanosome infection and genetic variation in major histocompatibility complex DRB3 gene in cattle in Ghana.** PhD Thesis, University of Cape Coast. 2014.
- Ganyo EY, Boampong JN, Masiga DK, *et al.*: **Dataset 1 in: Haematology of N'Dama and West African Short Horn cattle herds under natural *Trypanosoma vivax* challenge in Ghana.** *F1000Research.* 2018.
[Data Source](#)
- Jain NC: **Essentials of Veterinary Hematology.** Philadelphia, Lea and Febiger. 1993; 417.
[Reference Source](#)
- Jain NC: **Schalm's veterinary hematology.** Philadelphia, Lea and Febiger. 1986; 1221.
[Reference Source](#)
- Maganga GD, Mavoungou JF, N'dilimabaka N, *et al.*: **Molecular identification of trypanosome species in trypanotolerant cattle from the south of Gabon.** *Parasite.* 2017; **24**: 4.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Mahama CI, Desquesnes M, Dia ML, *et al.*: **A cross-sectional epidemiological survey of bovine trypanosomiasis and its vectors in the Savelugu and West Mamprusi districts of northern Ghana.** *Vet Parasitol.* 2004; **122**(1): 1–13.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Mahama CI, Mohammed HA, Abavana MA, *et al.*: **Tsetse and trypanosomiasis in Ghana in the twentieth century: a review.** *Rev Elev Med Vet Pays Trop.* 2003; **56**(1–2): 27–32.
[Reference Source](#)
- Maichomo MW, Ndung'u JM, Ngare PM, *et al.*: **The performance of Orma Boran and Maasai Zebu crossbreeds in a trypanosomiasis endemic area of Nguruman, south western Kenya.** *Onderstepoort J Vet Res.* 2005; **72**(1): 87–93.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Marcotty T, Simukoko H, Berkvens D, *et al.*: **Evaluating the use of packed cell volume as an indicator of trypanosomal infections in cattle in eastern Zambia.** *Prev Vet Med.* 2008; **87**(3–4): 288–300.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Masiga DK, McNamara JJ, Laveissière C, *et al.*: **A high prevalence of mixed trypanosome infections in tsetse flies in Sinfra, Côte d'Ivoire, detected by DNA amplification.** *Parasitology.* 1996; **112**(Pt 1): 75–80.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Mbanasor UU, Anene BM, Chime AB, *et al.*: **Haematology of normal and trypanosome infected Muturu cattle in southeastern Nigeria.** *Nig J Anim Prod.* 2003; **30**(2): 236–241.
[Publisher Full Text](#)
- Merck Veterinary Manual: **The Merck Veterinary Manual. A handbook of Diagnosis, Therapy, and Disease Prevention and Control for the Veterinarian.** 1986; 882.
- Murray M, Morrison WI, Whitelaw DD: **Host susceptibility to African trypanosomiasis: trypanotolerance.** In JR. Baker and R. Muller Editors. *Adv Parasitol.* London, Academic Press, 1982; **21**: 1–68.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Murray M, Dexter TM: **Anaemia in bovine African trypanosomiasis. A review.** *Acta Trop.* 1988; **45**(4): 389–432.
[PubMed Abstract](#)
- Mwangi EK, Stevenson P, Gettinby G, *et al.*: **Susceptibility to trypanosomosis of three *Bos indicus* cattle breeds in areas of differing tsetse fly challenge.** *Vet Parasitol.* 1998a; **79**(1): 1–17.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Mwangi EK, Stevenson P, Ndung'u JM, *et al.*: **Studies on host resistance to tick infestations among trypanotolerant *Bos indicus* cattle breeds in East Africa.** *Ann N Y Acad Sci.* 1998b; **849**(1): 195–208.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ohaeri CC, Eluwa MC: **Abnormal biochemical and haematological indices in trypanosomiasis as a threat to herd production.** *Vet Parasitol.* 2011; **177**(3–4): 199–202.
[PubMed Abstract](#) | [Publisher Full Text](#)
- OIE: **Terrestrial Manual, Chapter 2.4.18 Trypanosomiasis (Tsetse-transmitted).** 2013.
[Reference Source](#)
- R Development Core Team: **R: A Language and Environment for Statistical Computing.** R Foundation for Statistical Computing, Vienna, Austria. 2013.
[Reference Source](#)
- Rege JE, Aboagye GS, Tawah CL: **Shorthorn cattle of West and Central Africa. I. Origin, distribution, classification and population statistics.** *World Anim Rev.* 1994; **78**: 1–14.
[Reference Source](#)
- Roelants GE: **Natural resistance to African trypanosomiasis.** *Parasite Immunol.* 1986; **8**(1): 1–10.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Silva RA, Ramirez L, Souza SS, *et al.*: **Hematology of natural bovine trypanosomiasis in the Brazilian Pantanal and Bolivian wetlands.** *Vet Parasitol.* 1999; **85**(1): 87–93.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Steverding D: **The history of African trypanosomiasis.** *Parasit Vectors.* 2008; **1**(1): 3.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Trail JC, Murray M, Wissocq Y: **The trypanotolerant livestock network in West and Central Africa.** *ILCA Bulletin.* 1984; **18**: 16–19.
[Reference Source](#)
- Turkson PK: **Seroepidemiological survey of cattle trypanosomiasis in coastal savanna zone of Ghana.** *Acta Trop.* 1993; **54**(1): 73–76.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Vale GA: **Prospects for controlling trypanosomiasis.** *Onderstepoort J Vet Res.* 2009; **76**(1): 4–45.
[PubMed Abstract](#)
- Waiswa C, Katunguka-Rwakishaya E: **Bovine trypanosomiasis in south-western Uganda: packed-cell volumes and prevalences of infection in the cattle.** *Ann Trop Med Parasitol.* 2004; **98**(1): 21–27.
[PubMed Abstract](#) | [Publisher Full Text](#)
- World Bank: **Staff appraisal report. Republic of Ghana. National Livestock Services Project.** Report No. 11058-GH. Washington DC: The World Bank. 1992; 132.
[Reference Source](#)
- Yaro M, Munyard KA, Stear MJ, *et al.*: **Combating African Animal Trypanosomiasis (AAT) in livestock: the potential role of trypanotolerance.** *Vet Parasitol.* 2016; **225**: 43–52.
[PubMed Abstract](#) | [Publisher Full Text](#)

Open Peer Review

Current Peer Review Status:

Version 1

Reviewer Report 01 June 2018

<https://doi.org/10.5256/f1000research.15253.r33786>

© 2018 Thévenon S. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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 al¹⁻² and Mattioli et al³⁻⁴ 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. congolense* 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)⁶ 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

1. Trail JC, d'Ieteren GD, Feron A, Kakiese O, et al.: Effect of trypanosome infection, control of parasitaemia and control of anaemia development on productivity of N'Dama cattle. *Acta Trop.* 1990; **48** (1): 37-45 [PubMed Abstract](#)
2. Trail JC, Wissocq N, d'Ieteren GD, Kakiese O, et al.: Quantitative phenotyping of N'Dama cattle for aspects of trypanotolerance under field tsetse challenge. *Vet Parasitol.* 1994; **55** (3): 185-95 [PubMed Abstract](#) | [Publisher Full Text](#)
3. Mattioli RC, Faye JA, Büscher P: Susceptibility of N'Dama cattle to experimental challenge and cross-species superchallenges with bloodstream forms of *Trypanosoma congolense* and *T. vivax*. *Vet Parasitol.* 1999; **86** (2): 83-94 [PubMed Abstract](#)
4. Mattioli R, Jaitner J, Clifford D, Pandey V, et al.: Trypanosome infections and tick infestations: susceptibility in N'Dama, Gobra zebu and Gobra×N'Dama crossbred cattle exposed to natural challenge and maintained under high and low surveillance of trypanosome infections. *Acta Tropica* . 1998; **71** (1): 57-71 [Publisher Full Text](#)
5. Berthier D, Peylhard M, Dayo GK, Flori L, et al.: A comparison of phenotypic traits related to trypanotolerance in five west african cattle breeds highlights the value of shorthorn taurine breeds. *PLoS One.* 2015; **10** (5): e0126498 [PubMed Abstract](#) | [Publisher Full Text](#)
6. Bouyer J, Dicko AH, Cecchi G, Ravel S, et al.: Mapping landscape friction to locate isolated tsetse populations that are candidates for elimination. *Proc Natl Acad Sci U S A.* 2015; **112** (47): 14575-80 [PubMed Abstract](#) | [Publisher Full Text](#)

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?

No

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 confirm that I have read this submission and 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.

Reviewer Report 18 May 2018

<https://doi.org/10.5256/f1000research.15253.r33583>

© 2018 Groth D. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

David M Groth

School of Biomedical Sciences, Curtin University, Bentley, WA, Australia

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 confirm that I have read this submission and 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

<https://doi.org/10.5256/f1000research.15253.r33102>

© 2018 Adam Y. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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 confirm that I have read this submission and 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.

Comments on this article

Version 1

Author Response 19 Jul 2018

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

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research