RESEARCH ARTICLE

REVISED Haematology of N'Dama and West African Shorthorn cattle herds under natural *Trypanosoma vivax* challenge in Ghana [version 2; peer review: 2 approved, 1 approved with reservations]

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v2 **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 Shorthorn (WASH)] herds.
Methods: *Trypanosoma 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 red blood cell (RBC) and total white blood cell (TWBC) 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.
Conclusion: Despite the presence of infection by *T. vivax*, N'Dama and WASH cattle maintained their haematological parameters within acceptable normal ranges, which confirms their trypanotolerant trait. This highlights the need for low-input traditional African farmers in medium, high and severe tsetse challenge areas to be educated on the advantages of N'Dama and WASH breeds to increase their utilization in integrated tsetse and trypanosomosis control programmes.

Open Peer Review

Reviewer Status ✓ ✓ ?

	Invited Reviewers		
	1	**2**	**3**
REVISED **version 2** published 10 Aug 2018		✓ report	? report
version 1 published 13 Mar 2018	✓ report	? report	✗ report

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

Keywords
Haematology, cattle, trypanotolerance, trypanosomosis, N'Dama, West African Shorthorn

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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, 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.

How to cite this article: Ganyo EY, Boampong JN, Masiga DK *et al.* **Haematology of N'Dama and West African Shorthorn cattle herds under natural *Trypanosoma vivax* challenge in Ghana [version 2; peer review: 2 approved, 1 approved with reservations]** F1000Research 2018, **7**:314 (https://doi.org/10.12688/f1000research.14032.2)

First published: 13 Mar 2018, **7**:314 (https://doi.org/10.12688/f1000research.14032.1)

REVISED **Amendments from Version 1**

The revised version differs from the previous version mainly on the basis of our responses to comments and suggestions by the second and third reviewers.

In the **methods** section, we have provided values of concentrations of ethidium bromide, the template and other PCR reagents as recorded in the lab notebook. Based on the suggestions of one of the reviewers, 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 %.

More references have been added in bibliography in the revised text. The **introduction, results** and **discussion** sections have been rewritten to highlight the trypanotolerant character of N'Dama and WASH breeds. We agree with the reviewer's comment on impossibility 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, including a Table 2 with the heading: 'Across-breed comparison of haematological parameters (mean ± SD) of *T. vivax* infected N'Dama cattle at Cape Coast and WASH cattle at Chegbani, Ghana', which was captured in the **results** and **discussion** sections of the previous text, has consequently been removed from the revised text. In the revised text, we used the nonparametric Kruskal-Wallis test, not ANOVA, for the statistical analyses of our results.

We have incorporated the normal values of haematological parameters for cattle in the revised text as reference. We have also corrected the parameter WBC in the previous Dataset 1 to TWBC in the revised Dataset 1.

See referee reports

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) agreeing with reports by Losos (1986) that *T. vivax* predominates in West Africa.

The usual consequence of trypanosome infection is anaemia, which is often accompanied by poor growth, weight loss, low milk yield, infertility, abortion and paralysis (Berthier *et al.*, 2015; Dagnachew *et al.*, 2015; Mattioli *et al.*, 1999; Steverding, 2008; Trail *et al.*, 1990). Death may result within a few weeks to several months after infection. The use of prophylactic and curative drugs has remained the most popular method for the management of animal trypanosomosis in sub-Saharan Africa (SSA) (Kinabo, 1993; Mahama *et al.*, 2003; Turkson, 1993). However, the continued use for more than half a century of a limited number of closely related trypanocidal drugs has led to the emergence of drug resistant trypanosomes (Delespaux *et al.*, 2008; Matovu *et al.*, 2001; Sow *et al.*, 2012). It is unlikely that a vaccine will become available in the foreseeable future (Nyame *et al.*, 2004; Vale, 2009). Control of tsetse flies, the cyclical vectors of trypanosomes in SSA, is a significant factor in managing the disease in a holistic manner (Allsopp, 2001; Bouyer *et al.*, 2015; Kgori *et al.*, 2006; Leak *et al.*, 1995; Mulla & Rickman, 1988; Vreysen *et al.*, 2000). Nonetheless, local eradication successes have been limited to less than 2% of the infested area due to either resurgence of residual populations that were omitted from eradication campaigns or reinvasion from neighbouring infested areas (Bouyer *et al.*, 2015). Landscape friction maps based on tsetse fly genetic distances and remotely sensed environmental data have identified natural barriers to isolated clusters of *Glossina palpalis gambiensis* in West Africa (Bouyer *et al.*, 2015). This represents a major advance in locating and eradicating isolated tsetse populations without risk of reinvasion. However, this innovation has yet to be replicated at the continental level under the auspices of the Pan African Tsetse and Trypanosomoses Eradication Campaign (PATTEC) (Bouyer *et al.*, 2015).

The use of trypanotolerant cattle has not been given adequate consideration in integrated tsetse and trypanosomosis control programmes (Agyemang, 2005; Hendrickx *et al.*, 2004). 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; Mattioli *et al.*, 1998; Rege *et al.*, 1994; Trail *et al.*, 1990; Yaro *et al.*, 2016), where other breeds rapidly succumb to the disease (Berthier *et al.*, 2015; Mattioli *et al.*, 1998; 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 shorthorn (WASH) (Hoste *et al.*, 1992; Maganga *et al.*, 2017; Mattioli *et al.*, 1998; Mattioli *et al.*, 1999; Roelants, 1986; Trail *et al.*, 1990; Trail *et al.*, 1994). 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 (Berthier *et al.*, 2015; Mattioli *et al.*, 1998; Murray *et al.*, 1982; Murray & Dexter, 1988; Trail *et al.*, 1990).

We previously reported natural *T. vivax* challenge in N'Dama and WASH cattle herds in Ghana using a sensitive PCR approach (Ganyo, 2014). This study 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. Within the same study, 55 animals were sampled from a Sanga herd at Aveyime in the coastal savanna agro-ecological zone and 38 Zebu cattle were sampled in herds at Pong Tamale in the Guinea Savanna agro-ecological zone. The herds were

chosen purposively, since these were herds with the breeds of interest. Whereas owners of Sanga and Zebu cattle used trypanocidal drugs regularly to control trypanosome infection, none of the owners of N'Dama and WASH cattle used trypanocidal drugs to control trypanosome infection. 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-nucleotide (nt) satellite DNA monomer sequence of *T. vivax*. The PCRs were carried out in 20 µl reaction volumes with 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), 0.4 µl of 10mM dNTPs, 0.2 µl (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 0.5ug/ml 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). Red blood cell (RBC) and total white blood cell (TWBC) 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

The means and standard deviations per breed for haematological parameters for *T. vivax* positive individuals and *T. vivax* negative individuals were calculated using standard formulae.

The non-parametric Kruskal-Wallis test was used to compare the means for haematological parameters in *T. vivax* positive and *T. vivax* negative cattle with the R statistical software version 3.4.2 (R Development Core Team, 2017). 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. None of the 55 Sanga and 38 Zebu cattle sampled tested positive for *T. vivax*. 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 ($p < 0.01$) 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). For the N'Dama cattle, the mean TWBC counts for infected animals were lower than that for uninfected animals. The mean values of neutrophils, lymphocytes and eosinophils were lower in infected N'Dama compared to uninfected N'Dama. The WASH cattle had higher mean TWBC counts for infected animals compared to uninfected animals. The mean values of neutrophils and eosinophils were higher in infected WASH compared with uninfected.

Dataset 1. Haematological parameters of *T. vivax* infected and uninfected WASH and N´Dama cattle at Chegbani and Cape Coast, respectively

http://dx.doi.org/10.5256/f1000research.14032.d213201

Discussion

The mean PCVs for infected N'Dama and WASH cattle in this study were lower than those for uninfected cattle, which is consistent with the pathological effect of anaemia in trypanosome infections. Our observations support the findings of Berthier *et al.* (2015) that N'Dama and Zebu cattle experimentally infected with *Trypanosoma congolense* had lower PCVs than uninfected animals. Trail *et al.* (1994) also showed that N'Dama naturally infected by trypanosomes in Zaire (now Congo) had lower PCV values and lower weight gain than non-infected N'Dama. A study in Ethiopia (Dagnachew *et al.*, 2015) in Zebu cattle experimentally infected with *T. vivax* isolates showed that the mean PCV, Hb and total RBC count were lower in infected groups than in non-infected control animals. Additionally, an earlier survey conducted in cattle maintained under traditional management system in 8 villages in Hawagelan district, western Ethiopia (Bekele & Nasir, 2011) revealed that the mean PCV of trypanosome-infected animals was significantly lower than that of non-infected animals. Lower herd average PCVs for trypanosome-positive cattle compared to trypanosome-negative cattle have also been reported in Ankole cattle in Uganda (Waiswa & Katunguka-Rwakishaya, 2004), Angoni cattle in Zambia (Marcotty *et al.*, 2008), Doayo and

Table 1. Within-breed comparison of haematological parameters (mean ± SD) of *Trypanosoma vivax* positive and negative N'Dama cattle at Cape Coast and WASH cattle at Chegbani, Ghana.

	N'Dama (n = 55)			WASH (n = 55)			
Parameter	**Positive n = 7**	**Negative n = 48**	***p* value**	**Positive n = 4**	**Negative n = 51**	***p* value**	**Reference range[a] (mean)**
PCV (%)	30.7 ± 4.4	34.6 ± 4.0	0.027*	28.3 ± 1.5	31.5 ± 5.1	0.167	24.0 - 46.0 (35.0)
Hb (g/dl)	11.8 ± 1.9	13.1 ± 1.6	0.140	11.5 ± 1.1	11.7 ± 2.0	0.746	8.0 - 15.0 (11.0)
RBC (x10^{6}mm^3)	9.1 ± 2.1	6.9 ± 1.9	0.017*	5.5 ± 1.1	5.8 ± 1.3	0.795	5.0 - 10.0 (7.0)
MCV (fl)	35.8 ± 11.0	53.1 ± 14.1	0.004**	53.4 ± 12.8	56.6 ± 11.8	0.486	40.0 - 60.0 (52.0)
MCH (pg)	13.8 ± 4.5	20.0 ± 5.2	0.010**	21.5 ± 4.7	21.0 ± 4.7	0.974	11.0 - 17.0 (14.0)
MCHC (g/dl)	38.6 ± 4.6	38.0 ± 4.4	0.762	40.5 ± 2.0	37.3 ± 4.6	0.086	30.0 - 36.0 (32.7)
TWBC (x10^{3}mm^3)	7.0 ± 2.9	8.6 ± 3.4	0.177	11.5 ± 1.4	10.5 ± 2.2	0.250	4.0 - 12.0 (8.0)
Neutrophils (x10^{3}mm^3)	2.8 ± 2.0	3.5 ± 2.1	0.405	5.3 ± 1.6	4.7 ± 1.5	0.399	0.6 - 5.6 (2.2)
Lymphocytes (x10^{3}mm^3)	2.9 ± 1.5	3.5 ± 1.8	0.614	5.6 ± 2.6	4.5 ± 2.0	0.218	1.8 - 9(4.6)
Eosinophils (x10^{3}mm^3)	1.3 ± 0.4	1.5 ± 0.8	0.479	0.6 ± 0.2	1.0 ± 0.6	0.089	0.0 - 2.4 (0.7)
Monocytes (x10^{3}mm^3)	0.0 ± 0.1	0.0 ± 0.1	0.967	0.0 ± 0.1	0.3 ± 0.4	0.077	0.1 - 0.8 (0.3)
Basophils (x10^{3}mm^3)	0.0 ± 0.0	0.0 ± 0.0	0.586	0.0 ± 0.0	0.0 ± 0.0	0.779	0.0 - 0.2 (0.0)
Neutrophils (%)	38.7 ± 12.8	39.1 ± 13.6	0.940	47.5 ± 19.5	45.0 ± 12.4	0.733	15.0 - 47.0 (28.0)
Lymphocytes (%)	39.6 ± 15.6	40.6 ± 12.0	0.820	47.5 ± 18.5	42.1 ± 13.5	0.322	45.0 - 75.0 (58.0)
Eosinophils (%)	21.4 ± 13.7	20.0 ± 11.5	0.970	4.8 ± 1.7	9.5 ± 5.1	0.042*	0.0 - 20.0 (9.0)
Monocytes (%)	0.3 ± 0.8	0.2 ± 0.6	0.967	0.3 ± 0.5	2.9 ± 3.5	0.071	2.0 - 7.0 (4.0)
Basophils (%)	0.0 ± 0.0	0.0 ± 0.2	0.586	0.0 ± 0.0	0.0 ± 0.3	0.779	0.0 - 2.0 (0.5)

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 ($p < 0.01$)

[a]Jain, 1993

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

Zebu White Fulani cattle in Cameroun (Achukwi & Musongong, 2009) and Zebu cattle in Gabon (Cossic *et al.*, 2017). In Nigeria, Ohaeri & Eluwa (2011) observed that in addition to cattle, other domestic ruminants that were naturally infected with trypanosomes had significantly lower ($p < 0.05$) PCV and RBC counts compared to uninfected animals.

The mean PCV, Hb and RBC values observed in both infected and uninfected N'Dama and WASH cattle in current study 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 had mean RBC values within the normal range, which could be attributed to their trypanotolerant trait.

With typical trypanotolerance, cattle have the capacity to develop less severe anaemia in the face of pathogenic *Trypanosoma* species infection, as assessed by packed cell volume (Berthier *et al.*, 2015; Mattioli *et al.*, 1998; Murray *et al.*, 1982; Murray & Dexter, 1988; Trail *et al.*, 1990). Our findings are in agreement with the report (Mbanasor *et al.*, 2003) that the mean RBC, Hb and PCV values in natural *T. vivax*-infected trypanotolerant Muturu (WASH) cattle in Nigeria were well within accepted normal values for cattle. An earlier study involving trypanotolerant WASH cattle in Ghana also reported normal PCV value despite the presence of trypanosome parasites (Adam *et al.*, 2012).

In this study, we sampled trypanosusceptible Sanga and Zebu cattle in the same agro-ecological zones as the trypanotolerant N'Dama and WASH. However, none of the trypanosusceptible cattle tested positive for *T. vivax*. Consequently, there was no *T. vivax* positive Sanga and Zebu for which haematological data could be generated for comparison with *T. vivax* positive N'Dama and WASH. Thus, it is not possible to know if the *T. vivax* strains that infected the N'Dama and WASH are highly pathogenic or not. However, such absence of positives is not surprising. Livestock keepers who kept the trypanosusceptible Zebu and Sanga used trypanocidal drugs regularly to control the infection. In contrast, none of the livestock keepers who kept trypanotolerant N'Dama and WASH cattle used trypanocidal

drugs to control trypanosome infection. This could explain why some of the N'Dama and WASH tested positive for *T. vivax*, while all the Sanga and Zebu tested negative for *T. vivax*.

Although the importance of keeping trypanotolerant N'Dama and WASH cattle as a trypanosomosis control measure in SSA has long been recognized (Hoste *et al.*, 1992; Mahama *et al.*, 2003; Pierre, 1906 cited in Agyemang, 2005), these breeds constitute only a small proportion (6%) of the cattle population of Africa and only 17% of the total cattle population in the affected areas (Agyemang, 2005). The low number of these trypanotolerant animals is attributed in part to the prevalent notion that they are not productive because of their relatively small size. Indeed, in market oriented livestock production systems, livestock keepers by reason of access to cash and consequently to veterinary inputs tend to increase the size of their cattle through crossbreeding trypanotolerant N'Dama and WASH females with trypanosusceptible Zebu bulls. This phenomenon has resulted in the emergence of stabilized crosses such as Sanga in Ghana, Borgou in Togo and Benin, Keteku in Nigeria, Djokoré in Senegal, Bambara in Central African Republic, and Méré in Côte d'Ivoire, Burkina Faso and Mali (Hendrickx *et al.*, 2004; Hoste *et al.*, 1992). Agyemang (2005) reviewed productivity and economic data from numerous studies (including Agyemang *et al.*, 1991; Agyemang *et al.*, 1997; Lhoste, 1986; Otchere, 1983; Republic of Gambia, 2002; Shaw, 2003; Trail *et al.*, 1979a; Trail *et al.*, 1979b; Wagenaar *et al.*, 1986 and Wilson, 1989) and reiterated the findings that trypanotolerant N'Dama and WASH compare favourably with Zebu in terms of productivity and economic indices even in zero- to low-tsetse challenge areas. Besides the threat that tsetse transmitted trypanosomosis poses to livestock, the productivity of more than one third of the total land area in West Africa is hampered by major physical constraints, the most important being soil fertility (Hendrickx *et al.*, 2004). Studies indicate that integration of crop and livestock production or mixed crop–livestock farming is the most sustainable means of increasing land productivity in SSA (McIntire *et al.*, 1992; Winrock, 1992). Accordingly, trypanotolerant cattle have been recommended for low-input traditional African farming systems in areas where tsetse challenge is considered to be medium, high or very severe (Hendrickx *et al.*, 2004; Mattioli *et al.*, 1998; Shaw & Hoste, 1987; Snow & Rawlings, 1999).

As was clearly demonstrated in the current study, infected trypanotolerant WASH and N'Dama maintained their mean RBC values in the normal range, which confirms their trypanotolerant trait. Further, none of the livestock keepers who kept such trypanotolerant breeds used trypanocidal drugs regularly to control the infection. According to various researchers (Dayo *et al.*, 2009; Maganga *et al.*, 2017; Mattioli *et al.*, 1998; Rege *et al.*, 1994; Yaro *et al.*, 2016), trypanotolerant breeds 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, despite being equally susceptible to trypanosome infection. This study thus calls attention to the need to deploy trypanotolerant WASH and N'Dama cattle in integrated tsetse and trypanosomosis control programmes in low-input traditional African farming systems in medium, high and severe tsetse challenge areas. A key component of such programs should be education of farmers on advantages of N'Dama and WASH cattle to establish and rapidly increase their numbers.

The mean values of TWBC, neutrophil and lymphocyte counts for *T. vivax* infected N'Dama in the present study were lower than those for the uninfected N'Dama. Authie (1993) observed that leukopenia is induced by trypanosomosis. Silva *et al.* (1999) reported that the main haematological changes produced by *T. vivax* infections in cattle in the Bolivian Wetlands and Brazilian Pantanal were anaemia and severe leucopenia. Additionally, the natural occurrence of *T. vivax* in cattle in Mosul, Iraq, resulted in leucopenia due to lymphopenia and neutropenia in comparison with normal range for cattle (Rhaymah & Al-Badrani, 2012). Paling *et al.* (1991) and Berthier *et al.* (2015), however, observed leukocytosis in N'Dama cattle due to trypanosome infection. The mean TWBC, neutrophil and lymphocyte counts for *T. vivax* infected WASH cattle in this study were higher than those for the uninfected. Similar observations of leukocytosis due to trypanosome infection have been made in rabbits (Emeribe & Anosa, 1991), vervet monkeys (Kagira *et al.*, 2006) and Zebu cattle (Berthier *et al.*, 2015; Van Wyk *et al.*, 2014). Ilemobade *et al.* (1982) suggested that trypanosome-induced severe leukopenia could compromise the protective immunity of cattle to other diseases. High levels of neutrophils, lymphocytes and eosinophils may indicate an active infection, whereas low counts may indicate a compromised immune system (Davidson *et al.*, 1998; Spivak, 1984). The mean TWBC counts reported in this study were within the normal values for cattle (Jain, 1993), but were lower than those reported in both *T. vivax* infected and uninfected Muturu cattle in Nigeria (Mbanasor *et al.*, 2003), which were higher than the normal values for cattle. The differential WBC counts in this study were within the normal range (Jain, 1993) and agree with what has been reported by Mbanasor *et al.* (2003).

Conclusion

We found that in spite of the presence of natural *T. vivax* infection, the haematological parameters of N'Dama and WASH cattle were within acceptable normal ranges, which confirms their trypanotolerant trait. This underscores the need for low-input traditional African farmers in medium, high and severe tsetse challenge areas to be educated on their advantages in order to establish and rapidly increase their numbers for effective deployment in integrated tsetse and trypanosomosis control programmes.

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 needlestick 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.d213201 (Ganyo *et al.*, 2018).

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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 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.

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Current Peer Review Status: ✔ ✔ ?

Version 2

Reviewer Report 26 September 2018

https://doi.org/10.5256/f1000research.17131.r36986

✔ **David M Groth**
School of Biomedical Sciences, Curtin University, Bentley, WA, Australia

This version is acceptable for indexing.

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.

Reviewer Report 29 August 2018

https://doi.org/10.5256/f1000research.17131.r36985

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

The manuscript has been improved.
Some terms must still be corrected: “infected” and “uninfected” must not be used in the manuscript and must be replaced everywhere by T. vivax PCR-negative and T vivax PCR-positive
Results and discussion section: there is not any significant differences between positive and negative animals, concerning blood formula, thus the figures must not be over-interpreted and they should not be discussed so widely.

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 confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 1

Reviewer Report 01 June 2018

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

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. 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.

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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 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.

Reviewer Report 18 May 2018

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

?

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 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

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

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 breads 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.

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