

Human and animal Trypanosomiasis in Lambwe Valley Foci, Kenya– current situation and latent trypanotolerance

ABSTRACT

Background: Human and animal trypanosomiasis are threats to both animal and human health in Sub-Saharan Africa. Numerous integrated vector control programs in Lambwe Valley foci have not been in tandem with insights aimed at investigating the prevalence trends besides assessing possible trypanotolerance in cattle.

Methodology: The cross-sectional survey enlisted human participants and cattle for examination.

Results: Prevalence of trypanosomiasis in animal subjects was 9.2%. and Blood parameters such as white blood cells, red blood cells, lymphocytes and granulocytes etc. blood parameters were significantly lower among the parasitaemic as compared to the aparasitaemic animals except for monocyte proportions. Red blood cells indices were also depressed in trypanosomiasis.

Conclusions: Viewed together, the presented findings point to animal trypanosomiasis cases in Lambwe Valley with chronic anemia and depressed white blood cell levels persisting as the most common features of the disease. There is a need to mitigate the likely clinical and veterinary cases profiling and management challenge of underestimating the neglected tropical disease burden based on the current widely available and applied prognosis/diagnostic strategies. High throughput molecular-based techniques currently not accessible should be factored-incorporated into national and regional governance control programs as well as monitoring and evaluation strategies, and this should be applied at both veterinary and clinical settings.

Key-words: trypanosomiasis; sleeping sickness; trypanotolerance; sub-Saharan Africa; anaemia

1. INTRODUCTION

African trypanosomiasis is a vector-borne parasitic disease that affects both animal and human health in Sub-Saharan Africa. Trypanosomes, transmitted via the bite of infected *Glossina* tsetse fly as a primary vector are the responsible multi-host parasites with the capacity to infect humans, wildlife and a wide range of domestic species (that may also constitute a reservoir) [1, 2]. The parasitic disease

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29 predominantly affects remote rural area communities where people get exposed to tsetse bite during their
30 daily activities [3].

31 The classic human African trypanosomiasis (HAT) or sleeping sickness which is normally fatal if
32 untreated is caused by two distinct subspecies of the African trypanosomes: *Trypanosoma*
33 *brucei gambiense* (shown to account for about 95% of all reported HAT) is linked to the chronic
34 Gambiense form of the disease in central and west Africa, with the Democratic Republic of the Congo
35 (D.R. Congo), Central African Republic, South Sudan and Chad prevailing as the most affected countries
36 today [2, 4]. Beside the classical symptom of sleep/wake cycles reversal, the disease is also associated
37 with anemia, immunosuppression, infertility and endocrine disorders. A person can be infected for months
38 or even years without major signs or symptoms and when these emerge, the patient is often already in an
39 advanced disease stage involving the central nervous system. In eastern and southeastern Africa
40 (including Tanzania, Malawi, Zambia, and Uganda), *Trypanosoma brucei rhodesiense* is implicated in the
41 acute Rhodesiense form of the disease with first signs and symptoms being observed a few months or
42 weeks after infection [4, 5]. Malawi and Uganda are the most affected countries with Uganda reported to
43 support both *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* [4]. In livestock,
44 animal African trypanosomiasis (AAT) related parasites: *Trypanosoma brucei brucei*,
45 *Trypanosoma congolense*, *Trypanosoma vivax*, *Trypanosoma evansi* and *Trypanosoma equiperdum*
46 have been shown to cause wasting form of the diseases resulting in major economic losses in the
47 concerned nations [1, 5]. *Trypanosoma* species that have been reported to be pathogenic to domestic as
48 well as wild animals include *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei*
49 *brucei* [6] whereas *Trypanosoma brucei gambiense* can infect both domestic and wild animals although
50 their role as a reservoir of the disease is contested [4]. Available reports show that sustained control
51 efforts of the parasite and/or reduction of the vector have succeeded in lowering the number of new cases
52 of *Trypanosoma brucei gambiense* and *rhodesiense* in quite a number of countries [5, 7]. In 2009,
53 reported human cases in Africa were about 10,000 which dropped to 7216 cases in 2012 [8-12] and in
54 areas where the disease is endemic, *Trypanosoma brucei rhodesiense* is apparently associated with
55 fewer cases (less than 2% of the total reported cases).

56 In East Africa, trypanosomiasis has been categorized together with the neglected and possibly re-
57 emerging zoonotic tropical diseases [13] after it was thought to be fully controlled to very low rates in the
58 80s. The transmitting vector, *Glossina pallidipes*, which was thought to have disappeared from the region
59 [14], has been reported to re-invade the southeastern Uganda [15] leading to significant increase in the
60 prevalence of animals trypanosomiasis in particular. The re-invasion premise points to a contested basis
61 for re-emergence of both human and animals' trypanosome infections due to the interconnections of the
62 tsetse fly belts in the East African region. It has also been reported that the Lambwe vector population is
63 more genetically diverse suggesting that these vectors have survived the numerous control efforts over
64 the years [13]. Following the re-invasion premise and associated contentions, the present study

65 investigated the current prevalence and trend of human and animal trypanosomiasis besides assessing
66 potential animal trypanotolerance in Lambwe valley foci.

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68 2. METHODOLOGY

69 2.1 Study design and settings

70 The cross-sectional survey was conducted in the Lambwe Valley of HomaBay County, Kenya
71 from August 2014 to April 2015. Lambwe Valley is located approximately within latitudes 0° 30' South
72 and longitudes 34° 20' East. The area's elevation is about 1100-1400m above sea level and experiences
73 a typical climate of two rainfall seasons with the long rains coming between March and May and short
74 rains between September and November of every year. Daily temperatures range between 260_C to
75 280C. The dominant livestock in the area includes cattle, sheep and goats and people living in the Valley
76 undertake mixed livestock and crop farming as their main source of income. The present study targeted
77 people and domestic animals in the catchment area of Sindo District Hospital in Lambwe Valley of Homa-
78 Bay County within the study period. The study participants were drawn from three health facilities; Sindo
79 district hospital, Ponge and Nyadenda dispensaries which are both located within Ruma National Game
80 Park. The study setting is as detailed in figure 1. The area is one of the disease foci in East Africa and
81 offers a good environment for tsetse flies and wildlife which are the major reservoirs for HAT.

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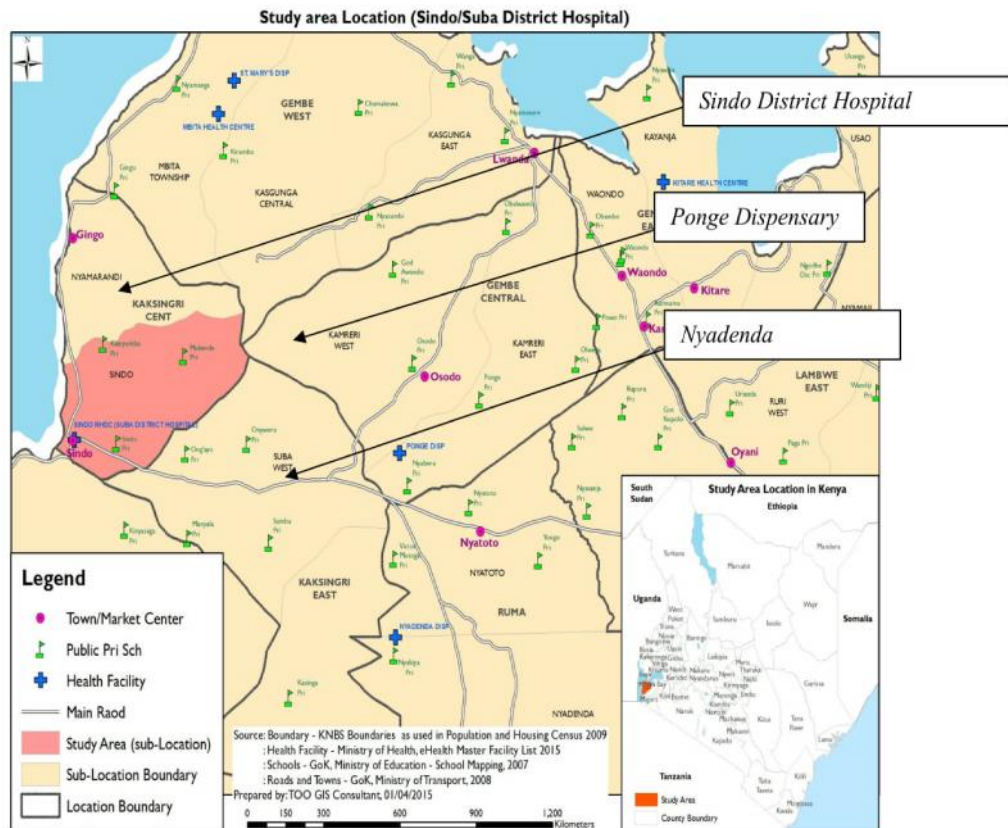


Fig. 1. Study areas within Lambwe Valley, Homa-Bay County

A sampling frame for the catchment area of focus was developed from the current (2009) National Sample Survey and Evaluation Program V (NASSEP V) frame in consultation with the local authorities. NASSEP V is a master sampling frame maintained by the Kenya National Bureau of Statistics (KNBS) and provides a basis for the implementation of household surveys in Kenya. In cognizance of the devolved system of government, NASSEP V utilizes the county boundaries and it stratifies the population into divisions, locations, sub-locations, clusters, and households. Briefly, domestic cattle in the catchment area of Sindo District Hospital and the respective feeder Ponge and Nyadenda dispensaries which are all located within Ruma National Game Park study coverage area that informed the sampling frame were sampled through consecutive sampling [16, 17]. It allowed for the inclusion of all eligible cattle subjects per eligible household within the sampling frame. Eligible households were those that keep cattle and which were identified through community leadership structure in line with the sample frame under the

96 care of the research project enumerators duly trained. Due to the local reality of the community cultural
97 orientation, family households exist in two subcategories: one-family households or multiple-family
98 households. Thus in total, twenty family households keeping cattle were enrolled with between 5-30
99 animals per family household. For inclusion, first, eligible household head informed consent to participate
100 in the project was requested and only those who voluntarily agreed to consent were involved in the study.
101 Second, at the household level, all indigenous cattle of both sexes and all ages -were enrolled in to the
102 study except suckling calves and exotic breeds. Suckling's calves were considered to be those ese aged
103 ≤ 14 months s [18, 19]. Overall, most farmers started weaning and/or stopped suckling between 6-14
104 months s and the majority were not willing to consent suckling calves enrolment due to the perceived
105 delicate nature of this category of cattle. Exotic breeds were excluded as they are not part of dominant
106 cattle breeds domesticated by most farmers in the study setting per veterinary records within the local
107 government authority. Three hundred and four (304) cattle were included in the study and- assessed for
108 signs indicative of nagana [20] by a specialized veterinary officer incorporated in the project and having
109 experience of service in the study setting. In addition, veterinary advice was provided to respective
110 farmers at no costs on a measure of caution, signs to watch out for and any other nutritional and
111 treatment guidance necessary for their livestock.

112 For the human subjects, fifty-fifty two (52) human participants were drawn from the
113 aforementioned health facilities through purposive sampling [16] for the period of study. Pre-study
114 analysis of the selected facilities past health records pointed to between 1-5 suspected cases attended to
115 per month hence the preference for purposive sampling approach. In summary, enrolled participants were
116 among individuals seeking treatment in the named health facilities and were recruited on the basis that
117 they presented with signs and symptoms of trypanosomes infections[21, 22]. The signs were in two
118 categories as part of standardized clinical guidelines at the facilities; classical symptoms: sleeping
119 disorders (insomnia and daytime sleepiness), unexplained drowsiness, fever (that is resistant to malaria
120 treatment), headache (that is resistant to malaria treatment), and swollen lymph nodes; other symptoms:
121 general malaise, muscle weakness, anorexia, confusion, general body pain, chancre, restlessness,
122 pruritus, convulsions, reduced libido (for adults), coma and stupor. Patients' examination was done by a
123 qualified resident physician linked to the project. Patients who presented with all the classical symptoms
124 outlined above and any one or more of the symptoms in the other symptoms category were recruited into
125 the study following a written consent. The project did provide an option for any participant who tested
126 positive for sleeping sickness to be put on treatment (Suramin; 20 mg/Kg body weight 5-7 days for early-
127 stage cases, Melarsoprol; 3.6 mg/Kg body weight, repeated every seven days for four weeks, for late
128 stage cases) [23] and to be followed up by the study Clinician who was based at the Sindo District
129 Hospital.

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2.2 Hematological and Parasitological analysis

Blood samples (3-5_mLs) were collected from human participants into BD® vacutainers (BD Biosciences, San Jose, CA) and from animals (using sterile disposable non-pyrogenic syringes (CATHY YOUGO®, France) then transferred into heparinized vacutainer tubes) and were stored in a cool box at ambient temperature prior to being processed in the laboratory. The samples were processed for hematological indices including, hematocrit, hemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), mean cell volume (MCV) and mean cell hemoglobin (MCH) using a Coe Coulter counter hemoanalyzer (BC-3000 plus, Auto Hematology Analyzer, Mindray Co. Ltd, China). Trypanosome species detection was done by microscopy (B300, Prior Scientific Ltd) following a Giemsa staining of thin blood film preparation according to [24] and by buffy coat method [25].

2.3 Statistical analysis

Reference ranges for clinical hematology were based on the guidelines in the Clinical and Laboratory Standards Institute (CLSI: formerly National Council of Clinical Laboratory Services) guideline document C28-A2 and further validated in Kenya context and in other African populations [26, 27]. Conventional hematological reference ranges as earlier developed and applied were adopted for cattle [See additional file 1] [28, 29]. The collected data were analyzed by a paired Student's t-test or one-way analysis of variance using SPSS version 20.0 software package. For all symptomatic (parasitaemic and aparasitaemic animals), the emphasis was put on changes in red blood cells, hematocrit and hemoglobin for the assessment of the extent of red blood cells lysis and trypanotolerance[30-32]. Differences in mean corpuscular volume and mean corpuscular hemoglobin were used to examine general effects on the red blood cells quality while total white blood cells and the differential count values were used to assess the levels of nonspecific immunity[32, 33]. For all statistical tests, $P < 0.05$ was considered significant.

3. RESULTS

The presented results points to insights of human and animal trypanosomiasis as observed in both the human and animal subjects, respectively in Lambwe valley. A total of 22 male and 30 female participants were enrolled, 21% of these were aged 0-21 yrs, 29% (21-30 yrs), 25% (31-40yrs), 17% (41-50 yrs) and 8% (over 50 yrs). The mMajority (17) of the female participants were adults aged below 30 yrs while for the male participants 50% were aged above 30 yrs (13). All the study participants exhibited all the classical signs of trypanosomiasis while the other signs manifestation was diverse (Table 1). General malaise, muscle weakness, and confusion were the most common among all the study participants. There was no study participant of either gender who was in coma or stupor during the research phase and only one case (male) with reduced libido was reported. Microscopic examination of all blood smears was negative.

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Table 1. Observed symptoms and parasitaemia status in trypanosomiasis symptomatic individuals from Lambwe valley

Gender and symptoms	Male	Female
Classical signs		
Sleeping disorders (insomnia and daytime sleepiness)	22	30
Unexplained drowsiness	22	30
Fever (that is resistant to malaria treatment)	22	30
Headache (that is resistant to malaria treatment)	22	30
Swollen lymph nodes	22	30
Other signs		
General malaise	15	20
Muscle weakness	10	13
Anorexia	5	8
Confusion	0	3
General body pain	9	12
Chancre	0	3
Restlessness	1	4
Pruritus	0	2
Convulsions	0	5
Reduced libido (for adults)	1	0
Coma	0	0
Stupor	0	0
Parasitaemia	Nil	Nil

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Regarding the blood parameters, all the white blood cell indices were within normal range according to the clinical reference ranges (Table 2) while the RBC indices were normal except for a decline in the hematocrit in both male (35.4 ± 1.3 ; reference values 40-50%) and female (32.0 ± 0.9 ; reference values 30-50%) patients. The blood picture showed normocytic and normochromic features of the RBCs. No further analysis was carried out on the human data since the study did not detect trypanosome parasites in all the blood samples.

Table 2. Observed Means \pm SEM of white blood cell, red blood cell and platelet indices of trypanosomiasis symptomatic individuals from Lambwe Valley

Gender and Indices	Males (N=22)	Female (N=30)
a) White blood cell indices		
White blood cells (WBC) $\times 10^3/\mu\text{L}$	7.8 \pm 0.8	6.9 \pm 0.5
Lymphocytes (LY) [%]	38 \pm 3.6	37 \pm 3.0
Monocyte (MO) [%]	8.3 \pm 0.9	7.6 \pm 0.9
Granulocytes (GR) [%]	53.1 \pm 3.8	53.5 \pm 3.3
Lymphocytes (LY) $\times 10^3/\mu\text{L}$	3.3 \pm 0.6	2.8 \pm 0.4
Monocyte (MO) $\times 10^3/\mu\text{L}$	0.6 \pm 0.09	0.5 \pm 0.06
Granulocytes (GR) $\times 10^3/\mu\text{L}$	3.8 \pm 0.5	3.7 \pm 0.3
b) Red blood cells indices		
Red blood cells (RBC) $\times 10^6/\mu\text{L}$	4.7 \pm 0.2	4.3 \pm 0.2
Hemoglobin (Hb) [g/dL]	12.3 \pm 0.4	11.0 \pm 0.3
Hematocrit (Hct) [%]	35.4 \pm 1.3	32.0 \pm 0.9
Mean Corpuscular Volume (MCV) [fL]	74.9 \pm 3.2	76.4 \pm 2.7
Mean Corpuscular Hemoglobin (MCH) [pg]	26.0 \pm 1.0	26.2 \pm 0.9
Mean corpuscular Hemoglobin Concentration (MCHC) [g/dL]	34.8 \pm 0.3	34.4 \pm 0.2
Red cell distribution width (RDW) [%]	15.7 \pm 0.8	16.0 \pm 0.9
Platelet (PLT) $\times 10^3/\mu\text{L}$	202.4 \pm 19.2	207.0 \pm 17.4
Mean platelet volume (MPV) [fL]	7.3 \pm 0.3	7.6 \pm 0.2

Of the 304 cattle that were investigated in this study, 115 (37.8%) exhibited the animal trypanosomiasis signs indicative of nagana [20] including emaciation, lacrimation, and presence of swollen lymph nodes. Although the other 189 (62.2%) animals were asymptomatic, all the study animals were subjected to parasitaemia investigations. Out of the 304 animals, 28 cases were found to be parasitaemic thus suggesting a prevalence rate of 9.2%. Generally the symptomatic cattle exhibited significantly low total WBC count as compared to the asymptomatic cattle (10.1 \pm 0.34 vs 11.3 \pm 0.27; $p=0.005$) and granulocyte proportions (29.9 \pm 1.16 vs 34.4 \pm 0.80; $p=0.001$) with elevated monocyte proportion (11.2 \pm 0.44 vs 9.1 \pm 0.30; $p=0.000$) and count (1.1 \pm 0.05 vs 1.0 \pm 0.03; $p=0.016$). Furthermore, all the red blood cell indices were significantly different between the symptomatic and asymptomatic cattle except for the case of red cell distribution width and platelets count (Table 3) while the symptomatic animals showed notable mean corpuscular volume of (59.7 \pm 1.91 vs 52.4 \pm 0.16; $p=0.000$).

197 **Table 3. Observed Means \pm SEM of white blood cell, red blood cells and platelets indices of**
198 **trypanosomiasis symptomatic and asymptomatic cattle**

Health status and Indices	Asymptomatic cattle (N=189)	Symptomatic cattle (N=115)	<i>p</i> -value
a) White blood cell indices			
White blood cells (WBC) $\times 10^3/\mu\text{L}$	11.3 \pm 0.27	10.1 \pm 0.34	0.005
Lymphocytes (LY) [%]	56.6 \pm 0.80	57.7 \pm 1.03	0.388
Monocyte (MO) [%]	9.1 \pm 0.30	11.2 \pm 0.44	0.000
Granulocytes (GR) [%]	34.4 \pm 0.80	29.9 \pm 1.16	0.001
Lymphocytes (LY) $\times 10^3/\mu\text{L}$	6.7 \pm 0.32	5.8 \pm 0.24	0.068
Monocyte (MO) $\times 10^3/\mu\text{L}$	1.0 \pm 0.03	1.1 \pm 0.05	0.016
Granulocytes (GR) $\times 10^3/\mu\text{L}$	3.8 \pm 0.1	3.3 \pm 0.28	0.084
b) Red blood cells indices			
Red blood cells (RBC) $\times 10^6/\mu\text{L}$	5.7 \pm 0.04	4.3 \pm 0.06	0.000
Hemoglobin (Hb) [g/dL]	10.6 \pm 0.09	8.4 \pm 0.13	0.000
Hematocrit (Hct) [%]	29.9 \pm 0.19	24.8 \pm 0.12	0.000
Mean Corpuscular Volume (MCV) [fL]	52.4 \pm 0.16	59.7 \pm 1.91	0.000
Mean Corpuscular Hemoglobin (MCH) [pg]	18.4 \pm 0.13	19.6 \pm 0.21	0.000
Mean corpuscular Hemoglobin Concentration (MCHC) [g/dL]	35.4 \pm 0.23	33.9 \pm 0.50	0.003
Red cell distribution width (RDW) [%]	35.3 \pm 0.53	36.2 \pm 0.55	0.238
Platelets (PLT) $\times 10^3/\mu\text{L}$	289.4 \pm 7.60	302.1 \pm 12.14	0.352

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200 When the blood indices were compared between the parasitaemic (n=28) and aparasitaemic
201 (n=276) cattle it was observed that white blood cells (WBC) (8.7 \pm 0.7 vs 11.1 \pm 0.2; $p=0.001$), lymphocytes
202 (LY) (4.5 \pm 0.3 vs 6.5 \pm 0.2; $p=0.009$) and granulocytes (GR) (2.7 \pm 0.3 vs 3.7 \pm 0.10; $p=0.015$) were
203 significantly lower among the parasitaemic group (Table 4). Monocyte proportions were however
204 significantly elevated among the parasitaemic cattle as compared to the aparasitaemic group (13.3 \pm 1.2
205 vs 9.5 \pm 0.3 $p=0.000$). All the red blood cells (RBC) indices appeared depressed except for mean
206 corpuscular volume (MCV) (72.1 \pm 7.4 vs 53.4 \pm 0.2; $p=0.000$) which was elevated among the parasitaemic
207 animals. The parasitaemic animals' red blood cells hemoglobin, hematocrit, mean corpuscular
208 hemoglobin and mean corpuscular hemoglobin concentration were significantly lower as compared to the
209 aparasitaemic animals (Table 4). On determining the severity of anemia in the parasitaemic animals, the
210 calculated RBC and Hb cutoff levels were 3.97 $\times 10^6/\mu\text{L}$ and 7.44 g/dL while the observed levels were 3.7
211 $\times 10^6/\mu\text{L}$ and 6.7g/dL (Table 4), respectively which were still below the hematological references ranges
212 applied. The presented data point to severe anemia among all the parasitaemic animals. The blood
213 picture showed macrocytic and normochromic RBCs. The blood platelet numbers (368.4 \pm 35.1 vs
214 286.7 \pm 6.2; $p=0.000$) were significantly higher among the parasitaemic cattle as compared to the
215 aparasitaemic group (Table 4).

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217 **Table 4: White blood cells, red blood cells and platelets indices of trypanosomiasis**
 218 **parasitaemic and aparasitaemic cattle cases**

Trypanosomiasis status and Indices	-VE (N=276)	+VE (N=28)	p-value
a) White blood cell indices			
White blood cells (WBC) $\times 10^3/\mu\text{L}$	11.1 \pm 0.2	8.7 \pm 0.7	0.001
Lymphocytes (LY) [%]	57.3 \pm 0.6	53.7 \pm 2.5	0.091
Monocyte (MO) [%]	9.5 \pm 0.3	13.3 \pm 1.2	0.000
Granulocytes (GR) [%]	32.9 \pm 0.7	30.7 \pm 2.3	0.351
Lymphocytes (LY) $\times 10^3/\mu\text{L}$	6.5 \pm 0.2	4.5 \pm 0.3	0.009
Monocytes (MO) $\times 10^3/\mu\text{L}$	1.0 \pm 0.00	1.1 \pm 0.1	0.359
Granulocytes (GR) $\times 10^3/\mu\text{L}$	3.7 \pm 0.10	2.7 \pm 0.3	0.015
b) Red blood cells indices			
Red blood cells (RBC) $\times 10^6/\mu\text{L}$	5.3 \pm 0.04	3.7 \pm 0.2	0.000
Hemoglobin (Hb) [g/dL]	10.1 \pm 0.08	6.7 \pm 0.3	0.000
Hematocrit (Hct) [%]	28.3 \pm 0.2	24.2 \pm 0.3	0.000
Mean Corpuscular Volume (MCV) [fL]	53.4 \pm 0.2	72.1 \pm 7.4	0.000
Mean Corpuscular Hemoglobin (MCH) [pg]	18.9 \pm 0.1	18.1 \pm 0.3	0.037
Mean corpuscular Hemoglobin Concentration (MCHC) [g/dL]	35.6 \pm 0.2	27.5 \pm 1.0	0.000
Red cell distribution width (RDW) [%]	35.8 \pm 0.4	34.2 \pm 0.8	0.234
Platelets (PLT) $\times 10^3/\mu\text{L}$	286.7 \pm 6.2	368.4 \pm 35.1	0.000

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4. DISCUSSION

It was evident all the study participants demonstrated classical signs for trypanosomiasis while parasitaemia assays were negative whereas 37.8% of the cattle exhibited animal trypanosomiasis signs indicative of nagana [20] including emaciation, lacrimation, and presence of swollen lymph nodes while the rest were asymptomatic. It would be worth noting, there is a likely underestimated transmission levels due to lack of advanced routine clinical molecular-based diagnostic/screening techniques at the clinical level necessary for profiling of potential cases [3-5]. In relation to *Trypanosoma* species that have been reported to be pathogenic to both domestic and wild animals [6], with their role as reservoir being contested [4], it would be of interest for further research in sub-Saharan context to aim at monitoring the African trypanosomiasis epidemiologic patterns in a consistent trend. The monitoring efforts should target complete eradication without underestimating low transmission burden for every life towards better health matters in tune with the sustainable development goals three[3, 34].

The main intervention approaches against sleeping sickness have been active and passive case-finding for early detection of cases followed by treatment, vector control, and animal reservoir management. This should be continued alongside proper disease surveillance programs anchored on modernized molecular diagnostic approaches at the most localized primary health care level (health centres in Kenya) that serve the majority of the affected locals. Therefore, successful strategies for controlling African trypanosomiasis should be pegged onto the precision of techniques applied at early diagnosis and treatment, as well as vector control. Land use and land cover should be adopted for vector eradication as the communities in Lambwe Valley also modify their cattle grazing and watering points in order to reduce the animals' interaction with the vectors. In addition, National and Regional Control Initiatives such as by the Pan African Tsetse and Trypanosomosis Eradication Program (PATTEC) should be greatly enhanced to continue improving human and animal health in most parts of Lambwe Valley through integrated vector control and follow-ups.

Trypanosomiasis pathology is mainly associated with the disease's effects on blood and particularly the fact that the trypanosomes parasitize the RBCs in addition to invading the CNS [35, 36]. The depressed levels of WBC, LY and GR in all the parasitaemic cattle in this study were suggestive of chronic parasitic infection. Due to the trypanosome parasites' antigenic variations abilities and host immune systems evasion, the disease is usually associated with bouts of changes in white blood cell numbers and proportions [6, 37]. The WBC numbers will tend to increase [38] at the onset but decrease with time depending on the duration of the infection. The leucopenia and especially lymphopenia in the study animals may have been compounded by the infection status and anorexia nervosa as the animals appeared emaciated and lethargic. All the parasitaemic animals would also have suffered the deficiency of some of the hemopoietic requirements such as depressed endocrine, nutrients and cytokine factors [32]. In particular, Iron deficiency has been associated with lymphopenia in some conditions.

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256 The severe anemia in cattle reported in this study is suggestive of the fact that the animals in
257 Lambwe valley may not be trypanotolerant. From previous reports severity of anemia has been used to
258 measure the domestic animal's tolerance to trypanosomes infection. This is because anemia is
259 recognized as the main consequence of trypanosomiasis and is a reliable indicator of the disease [39]. It
260 has also been reported that the severity of anemia correlates well with the productive performance of
261 trypanosome-infected cattle [40, 41]. Previous studies have reported that some breeds of cattle, sheep,
262 and goats have the capacity to develop less severe anemia during trypanosomes' infection. Such animals
263 are regarded as trypanotolerant and may be used to improve animal productivity in areas such as
264 Lambwe Valley. The MCV was however higher in the parasitaemic animals and the blood picture was that
265 of microcytic and normochromic RBCs. These findings show that the parasitaemic animals exhibited
266 normochromic anemia associated with leucopenia [42] which attributed such outcomes to the cleavage of
267 erythrocyte sialic acid of cattle infected with *T. congolense*. The RBCs in such a case become prone to
268 phagocytosis by mononuclear phagocyte system in addition to parasitization from the trypanosomes.

269 The study findings presented in the context of real-world clinical protocols at the study site
270 remain alive to the sensitivity debate of the techniques applied as a limitation, particularly in human cases
271 profiling and absence of anaplasma assessment. Incorporating additional molecular screening
272 approaches was a constraint due to limited resources to mobilize and operationalize the same, as despite
273 robust molecular diagnostics having been developed in the recent past, few are yet to reach the patients
274 at primary point of care or attain incorporation into the national control programs whereas the disease
275 burden is prominent in mostly remote settings. Of note, this limitation among other realities, is not just a
276 challenge for the current context and study but a discourse of intense debate between the neglected
277 tropical disease support and financing agencies vis-a-vis science and public health experts as observed
278 [3-5, 7]

279 5. CONCLUSION

280 The findings point to animal trypanosomiasis cases in the Lambwe Valley with chronic anemia and low
281 white blood cell levels persisting as the most common clinical features. There is a need to mitigate likely
282 underestimation of human and veterinary trypanosomiasis cases burden based on the current, widely
283 available and applied clinical screening/diagnostic strategies. Therefore, high throughput molecular-
284 based screening/diagnostic techniques currently not accessible should be factored-incorporated into
285 national and regional governance control programs as well as monitoring and evaluation strategies and
286 be applied at both veterinary and human clinical medicine settings. It would be worthwhile for intervention
287 partners' efforts in Lambwe valley to attempt focusing towards pilot research programs on the viability of
288 trypanotolerant domestic animals for sustainable one-health.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This study received Ethical approval from the Institutional Review Ethics Committee (IREC), Moi Teaching and Referral Hospital (MTRH) Kenya. Written informed consent for voluntary participation was obtained from all human participants. Further permission to get blood samples from domestic animals was obtained from the owners.

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Comment [B04]: What is this?

424 **Additional file 1: Hematologic Reference Ranges**

	Conventional (USA) Units	SI Units	Dog	Cat	Cow	Horse	Pig	Sheep	Goat	Rabbit	Llama	Vietnamese Potbellied Pig	Ostrich
	Conventional (USA) Units	SI Units	Dog	Cat	Cow	Horse	Pig	Sheep	Goat	Rabbit	Llama	Vietnamese Potbellied Pig	Ostrich
PCV	%	× 10 ⁻² L/L	35–57	30–45	24–46	27–43	36–43	27–45	22–38	33–50	29–39	22–50	32
Hgb	g/dL	× 10 g/L	11.9– 18.9	9.8– 15.4	8–15	10.1– 16.1	10–16	9–15	8–12	10–17	12.8– 17.7	7.8–16.2	12.2
RBCs	× 10 ⁶ /μL	× 10 ¹² /L	4.95– 7.87	5.0– 10.0	5.0– 10.0	6.0– 10.4	5–8	9–15	8–18	5–8	11.3– 17.7	3.6–7.8	1.7
Reticulocytes	%	%	0–1.0	0–0.6			0–0.1						
Absolute reticulocyte count	× 10 ³ /μL	× 10 ⁹ /L	<80	<60									
MCV	fL	fL	66–77	39–55	40–60	37–49	50–68	28–40	16–25	58–67	20.9–28	55–71	174
MCH	pg	pg	21.0– 26.2	13–17	11–17	13.7– 18.2	17–21	8–12	5.2–8			18–24	61
MCHC	g/dL	× 10 g/L	32.0– 36.3	30–36	30–36	35.3– 39.3	30–34	31–34	30–36	29–37	43.1– 46.6	31–36	33
Platelets	× 10 ³ /μL	× 10 ⁹ /L	211– 621	300– 800	100– 800	117– 256	200– 500	800– 1,100	300– 600	250– 650		204–518	
MPV	fL	fL	6.1– 10.1	12–18	3.5– 6.5	4.0–6.0							
WBCs	× 10 ³ /μL	× 10 ⁹ /L	5.0– 14.1	5.5– 19.5	4.0– 12.0	5.6– 12.1	11–22	4–8	4–13	5.2– 12.5	7.5– 21.5	5.2–17.9	5.5
Neutrophils	%	%	58–85	45–64	15–33	52–70	28–47	10–50	30–48	20–75	60–74	0–63	63 ^a
(segmented)	× 10 ³ /μL	× 10 ⁹ /L	2.9– 12.0	2.5– 12.5	0.6– 4.0	2.9–8.5	2–15	0.7–6.0	1.0– 7.2	1–9.4	4.6–16	0–11.4	3.4
Neutrophils (band)	%	%	0–3	0–2	0–2	0–1	0–4	0	rare		0–1	0–1	
Lymphocytes	× 10 ³ /μL	× 10 ⁹ /L	0–0.45	0–0.3	0–0.1	0–0.1	0–0.8				0–0.35	0–0.19	
	%	%	8–21	27–36	45–75	21–42	39–62	40–55	50–70	30–85	13–35	15–55	34
	× 10 ³ /μL	× 10 ⁹ /L	0.4– 2.9	1.5– 7.0	2.5– 7.5	1.2–5.1	3.8– 16.5	2–9	2–9	1.6– 10.6	1–7.5	0.8–9.8	1.87
Monocytes	%	%	2–10	0–5	0–8	0–6	2–10	0–6	0–4	1–4	1–4	0–13	2.8
	× 10 ³ /μL	× 10 ⁹ /L	0.1– 1.4	0–0.9	0–0.9	0–0.7	0–1	0–0.75	0–0.55	0.05– 0.5	0.05– 0.8	0–0.67	0.15
Eosinophils	%	%	0–9	0–4	0–20	0–7	0.5– 11	0–10	1–8	1–4	0–15	0–12	0.3
	× 10 ³ /μL	× 10 ⁹ /L	0–1.3	0–0.8	0–2.4	0–0.8	0–1.5	0–1	0.05– 0.65	0.05– 0.5	0–3.3	0–0.73	0.02
Basophils	%	%	0–1	0–1	0–2	0–2	0–2	0–3	0–1	1–7	0–2	0	0.2
	× 10 ³ /μL	× 10 ⁹ /L	0–0.14	0–0.2	0–0.2	0–0.3	0–0.5	0–0.3	0–0.12	0.05– 0.9	0–0.4	0–0.61	0.01
M:E			0.75– 2.5	0.6– 3.9	0.3– 1.8	0.5–1.5	1.2– 2.2	0.77– 1.7	0.7– 1.0				
Plasma proteins	g/dL	10 g/L	6.0– 7.5	6.0– 7.5	6.0– 8.0	6.0–8.5	6–8	6–7.5	6–7.5	5.4–8.3		5.4–7.5	
Plasma fibrinogen	mg/dL	g/L	150– 300	150– 300	100– 600	100– 500	200– 400	100– 500	100– 400	200– 400	100– 400	100–400	

Data on various species compiled and adapted in part from multiple sources, including Latimer KS, *Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology*, 5th ed., Wiley-Blackwell, 2011; and Weiss DJ, Wardrop KJ, *Schalm's Veterinary Hematology*, 6th Ed., Wiley-Blackwell, 2010. Reference ranges vary between laboratories. Values provided by the reference laboratory should be used.

MCV = mean corpuscular volume

Conventional (USA) Units	SI Units	Dog	Cat	Cow	Horse	Pig	Sheep	Goat	Rabbit	Llama	Vietnamese Potbellied Pig	Ostrich
Conventional (USA) Units	SI Units	Dog	Cat	Cow	Horse	Pig	Sheep	Goat	Rabbit	Llama	Vietnamese Potbellied Pig	Ostrich

MCH = mean corpuscular hemoglobin

MCHC = mean corpuscular hemoglobin concentration

MPV = mean platelet volume

M:E = myeloid:erythroid ratio

^a Heterophil

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