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RESEARCH

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Major differences in the dynamics of haematological and biochemical variables between trypanotolerant and susceptible cattle during *Trypanosoma congolense* infection

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Abstract

Background Animal African Trypanosomosis, caused by protozoan blood parasites mainly transmitted by *Glossina*, is a threat to livestock health in Africa. Whilst African zebu and European taurine suffer from trypanosomosis, West African taurine, such as N'Dama, exhibit trypanotolerance and remain productive in enzootic areas. Trypanosomosis affects haematological and biochemical variables in cattle. However, joint characterization of the temporal dynamics of these variables during trypanosomosis in tolerant or susceptible cattle had yet to be carried out. The purpose of this study was to show potential differences in the dynamics of profiles of haematological and biochemical variables between trypanotolerant and susceptible cattle breeds during an infection by *Trypanosoma congolense*. Three cattle breeds comprising N'Dama, West African Fulani zebu, and crossbred cattle (West African zebu x European taurine) were infected with *Trypanosoma congolense* and were monitored for six months post-infection.

Results As expected, N'Dama controlled anaemia and parasitaemia better than Fulani zebu and crossbred cattle. Lymphocytosis and monocytosis were observed in N'Dama, while lymphocyte and monocyte counts remained unchanged in Fulani zebu and crossbred cattle. Granulocyte counts decreased in Fulani zebu and crossbred cattle, but remained stable in N'Dama. Cholesterol and glucose concentrations decreased significantly after inoculation with trypanosomes, but relative variations were observed depending on the breed, with rapid recovery of cholesterol levels in N'Dama. The relative variation in haematological variables was positively correlated with those of cholesterol and glucose, and negatively correlated with parasitaemia. Parasitaemia was also negatively correlated with variations in cholesterol and glucose levels.

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Conclusion This study highlighted some striking differences in the evolution of white blood cells and certain biochemical traits in trypanotolerant N'Dama cattle in comparison with susceptible breeds. These associations indicated likely physio-pathological links between trypanotolerance, characterized by the mitigation of symptoms, anaemia and parasitaemia, an efficient immune response, exemplified by white blood cell profiles, and limitation of some metabolic disorders.

Keywords Trypanosomosis, Trypanotolerance, Cattle, *Trypanosoma congolense*, Haematology, Biochemistry, Leukocytes, Cholesterol, Glucose

Introduction

African Animal Trypanosomosis (AAT) remains a major infectious disease posing a serious threat to livestock health in sub-Saharan Africa [1, 2]. *Trypanosoma* (*T.*) *congolense*, *T. vivax* and, to a lesser extent, *T. brucei brucei* are the main trypanosome species affecting millions of cattle, causing high morbidity and significant mortality each year, seriously reducing productivity [1]. Trypanosomes are mainly transmitted by tsetse flies (genus *Glossina*) in endemic areas. No vaccines have yet been developed for this disease. Vector control [3] and use of trypanocidal drugs [4] effectively control AAT, but require constant efforts and investment in most regions.

The diversity of cattle breeds in Africa is high [5] and variability in the susceptibility to AAT has long been observed in the field and documented in the literature [6, 7]. Both African zebu (*Bos taurus indicus*), which arrived in Africa less than 2,000 years ago [8], and European taurine [9], recently introduced to boost African cattle productivity, are generally susceptible to AAT. Conversely, West African taurine breeds (*Bos taurus taurus*), which have been present in West Africa for around 4,000 years [8], can tolerate trypanosome infections and their associated pathogenic effects, while remaining productive in tsetse-infested areas; hence the term trypanotolerant [10]. They are represented by the N'Dama (longhorn taurine), Lagune and Baoulé (shorthorn taurine) [11]. It has been well documented that trypanotolerant cattle exhibit improved control of anaemia and parasitaemia, as well as better body weight gain during trypanosomosis [10, 12], but the biological bases of trypanotolerance remain elusive.

Significant changes in haematological and biochemical parameters are known to occur in trypanosome-infected cattle [13, 14]. However, to the best of our knowledge, these variables have not been jointly monitored and compared in a single experiment involving tolerant and susceptible cattle breeds. On the one hand, changes in blood cell counts occur, but the pattern of leukocyte response is not constant between experiments. Leukopenia, due to neutropenia, eosinopenia, lymphocytopenia and monocytopenia, was observed both in trypanosusceptible Boran and trypanotolerant N'Dama infected by *T. congolense*, although N'Dama displayed a less pronounced decrease in immune blood cell counts [15].

Lymphocytopenia and neutropenia were also observed in Boran zebu, but lymphocytosis was observed in N'Dama in another experimental infection [16]. Furthermore, leukocytosis was found both in trypanosusceptible African zebu and in trypanotolerant West African taurine breeds, but with a more pronounced leukocytosis in taurine [11]. On the other hand, biochemical modifications such as hypoglycaemia, hypocholesterolemia, and hypoalbuminaemia were highlighted in infected trypanosusceptible cattle [17–19], but to the best of our knowledge, only one study compared susceptible zebu to tolerant Baoulé and reported a similar drop in cholesterol in both breeds [20]. Surprisingly, a comparative assessment of biochemical parameters between N'Dama and zebu is still lacking, although these biochemical variables are crucial for cell metabolism in general and more particularly for cellular growth, proliferation and effector functions of immune cells [21]. In addition, Peyllhard et al. [22] recently explored the blood cell transcriptome of infected cattle and reported an early activation of immune cell response associated with strong activation of cell energy production in N'Dama, while zebu displayed strong modifications of pathways linked to cholesterol metabolism during *T. congolense* infection.

While we now know that leukocyte dynamics and metabolism are interlinked, a joint comparative and accurate characterization of the temporal dynamics of haemato-biochemical variables during trypanosomosis in tolerant or susceptible cattle is lacking [23]. Moreover, to the best of our knowledge, the phenotypes of crossbred cattle (African zebu x European taurine) during trypanosomosis has not yet been assessed, even though these animals are now used by breeders to increase milk production in tsetse-infested areas [24]. The purpose of this study was to show potential differences in the dynamics of profiles of various haematological and biochemical variables between trypanotolerant and susceptible cattle breeds during an experimental *T. congolense* infection.

Materials and methods

Ethical considerations

The experimental protocol was reviewed and approved by the external ethics committee (No: 01_12/2022/CE-CIRDES) of the *Centre International de Recherche-Développement sur l'Élevage en zone Subhumide* (CIRDES).

In addition, the study was carried out according to the ARRIVE guideline (<https://arriveguidelines.org/arrive-guidelines>), and ARRIVE checklist was completed as an additional file 1. For cattle, critical points, at which the experiment was stopped, were defined and used during experimental monitoring as follows: a deterioration in the animal's general condition associated with a pack cell volume (PCV) of 15% or less, a reduction in weight to 20% of the initial value, a respiratory rate >60 cycles/min, or a decubitus over 24 h. Any cattle displaying these conditions was treated intramuscularly with 7 mg/kg of diminazene aceturate (Veriben®) and withdrawn from the study. NMRI mice, an outbred laboratory strains, born and reared in the CIRDES animal facility, were used in this study.

The experiment was implemented by qualified and experienced staff under the supervision of a veterinarian. At the end of the experiment, 177 days post-infection (DPI), all the cattle were treated with Veriben®, tested negative by parasitological and molecular diagnostics to confirm treatment efficacy, and moved from the cowshed to the CIRDES animal park.

Experimental animals

Three cattle breeds were used in the study, with a total of 24 animals: 7 N'Dama (NDA), 9 West African Fulani zebu (ZFU), and 8 crossbred (CRB) cattle. The number of animals used in this experiment was chosen according to previous experiments published by Paling et al. [25], Dagnachew et al. [17], Berthier et al. [11] and Peylhard et al. [22]. These articles demonstrated that having from 6 to 10 animals per breed allows to significantly discriminate main variables between groups. The CRB cattle resulted from artificial insemination of West African zebu with European taurine semen (Holstein, Montbéliarde, or Brune des Alpes). NDA cattle were purchased at the Ouaninou livestock market on the border between Guinea and Côte d'Ivoire. ZFU and CRB cattle were bought from tsetse-free areas in the Sahel region [26] and peri-urban farms of Bobo-Dioulasso, respectively, both in Burkina Faso. Animals were selected according to the following criteria: phenotypes corresponding to the breed of interest, between one and two years old, male sex, and negative for trypanosome infection according to microscopic observation of the buffy coat using the Murray method [27], PCR [28], and indirect Enzyme Linked Immuno-Sorbent Assay (ELISA) to detect IgG antibodies directed against animal trypanosome species (*T. brucei* s.l., *T. congolense*, *T. vivax*) [29, 30]. Apart from these species of trypanosome, the animals were not tested for other diseases.

Upon arrival at CIRDES, the animals were kept in a cowshed under a mosquito net. They were seen by an external veterinary technician to assess the health of each

animal. The animal's gait, respiration, and external organs (skin, muzzle, eyes) were the main points observed by the veterinary technician. All the cattle were treated with an anthelmintic (Albendazole 2,500 mg Vermitan®) per os and those with ticks were deticked by hand. In addition, all the cattle were treated intramuscularly with Veriben® at a dose of 7 mg/kg five weeks before infection. Fodder (*Panicum maximum* and *Andropogon gayanus*) and cotton seed cake were used to feed the cattle. Licking stones and water were supplied ad libitum. An acclimatization period of at least five weeks was observed before infection. All the animals were in good health before the trypanosome challenge.

Cattle genotyping

In order to genetically assign each animal to its corresponding expected group of breeds and check ancestries in CRB cattle, the 24 animals were genotyped using the EuroG MD SNP microarray designed by EuroGenomics, which provided data for 42,806 single nucleotide polymorphisms (SNPs). Genotyping was carried out at the Aveyron Labo platform (Rodez, France) on DNA extracted from cattle peripheral blood mononuclear cells using the Dneasy Blood and Tissue kit according to the manufacturer's recommendations (#69,504-Qiagen, France). Once converted into PLINK format, the genotypes were uploaded into a Web-Interfaced next generation Database dedicated to genetic Diversity Exploration (WIDDE) [31], which stored public and referenced genotypes of 146 bovine populations. This enabled us to place the investigated individuals on a principal component analysis (PCA) featuring the platform's breeds that best represent worldwide cattle diversity, spanning 1,310 individuals from 42 breeds including European taurine, African taurine, Indian zebu, African zebu and admixed breeds. The PCA, computed and visualized online, was calculated using SNPs that were common to the two datasets (investigated and reference), totalling 28,980 positions. In addition to the global PCA containing the whole data set, three PCAs were run separately to acquire a more accurate insight into the positions of the three cattle groups used in this experiment. A PCA was run with the NDA cattle with a reference data set comprising three sub-populations of N'Dama (NDA1, NDA2 originating from Burkina Faso [32] and ND4 from Guinea [33], and Lagune, Baoulé, Somba and Fulani zebu [31]). A PCA was run with the zebu cattle from our experiment with a reference data set comprising Fulani zebu, East African zebu, Bororo zebu, zebu from Madagascar and Kuri. Finally, a PCA was run with the CRB cattle with a reference data set comprising zebu and Bororo zebu, and ten European taurine breeds. Moreover, supervised clustering using $k=3$ was run using Admixture [34] v1.23 on a subset of the merged dataset used for the PCA, which

was downloaded from WIDDE in EIGENSTRAT format and converted to PLINK format. The dataset comprising the individuals from this study and reference individuals was sorted and filtered in order to group those adopted into 3 categories: West African taurine, containing 140 individuals from four breeds (N'Dama, Lagune, Baoulé and Somba), European taurine, containing 575 individuals from 24 breeds, and Indian zebu, containing 107 individuals from five breeds (Brahman, Gir, Nelore, Ongole and Sahiwal), to which African zebu and admixed breeds were added.

Trypanosomal challenge

T. congolense IL1180, a double clone derivative of Ser/71/STIB/212 which was isolated from a lion in the Serengeti National Park, Tanzania, in 1971 [35] was used in this study. This strain has been used in a previous experiment and has medium pathogenicity [11]. A dose of 10^7 trypanosomes in 0.2 mL phosphate-buffered saline solution was injected intraperitoneally into 5 NMRI mice, which had been immunosuppressed the day before by a subcutaneous injection of 300 mg/kg cyclophosphamide (0.2 mL/mouse). Mice were females, aged 4 to 5 weeks and weighing between 25 and 30g. Parasitaemia was monitored daily. After 6 days, 2 mice with parasitaemia at 10^8 trypanosomes/mL of blood were deeply anaesthetized with 50 mg/mL ketamine hydrochloride (0.2 mL/mouse), and then were exsanguinated via intra-cardiac puncture to obtain blood with trypanosomes in accordance with AVMA guidelines (<https://www.avma.org/resources-tools/avma-policies/avma-guidelines-euthanasia-animals>). The parasites were purified from the blood using anion exchange chromatography with di-ethyl amino ethyl (DEAE) cellulose (Cytiva, France), in a phosphate-buffered saline containing 1% glucose (PSG), according to the method of Lanham and Godfrey [36]. Purified parasites were quantified using Quick Read™ Precision Cell (Dominique Dutscher, France). After trypanosome viability was confirmed by microscopic observation [37], a dose of 2×10^5 trypanosomes in 500 μ L of PSG was injected intradermally at two separate points on the animal's neck, half a dose per flank.

Monitoring plan and blood sampling

During the infection period (from 3 December 2022 to 29 May 2023), animals were examined daily and clinical signs were recorded. Rectal temperature (TEMP) and body weight (BW) were recorded on days 12, 8, and 3 prior to infection, then once per week during infection using a digital thermometer and a TAL-TEC® scale, respectively.

During the monitoring period, blood samples were taken from the jugular vein of the animals, in ethylene diamine tetra acetic acid (EDTA) tubes and in lithium

heparin tubes for laboratory analysis. Blood samples in EDTA tubes were used for parasitological and haematological assessments. Trypanosome screening in the blood was carried out 3 times per week from 4 to 177 DPI. Haematological variables were monitored on days 12, 5, 3 and 1 before infection, then 3 times per week from days 4 to 177 during infection. It should be noted that, at some time points for haematological measures, half of each cattle breed was sampled one day and the second half the day after, due to other measures being carried out in parallel (phenotyping of immune cells by flow cytometry, not presented here). Blood samples in lithium heparin tubes were centrifuged at 3,000 rpm for 10 min at 4 °C. The plasma was collected in Eppendorf tubes and stored at 4 °C for quick biochemical parameter analysis and at -20 °C for subsequent total IgG antibody assessment, as detailed below. Before infection, biochemical variables were monitored on days 12, 8 and 3, and total IgG antibodies on days 12, 5 and 1. During infection, biochemical variables were monitored on days 4, 9, 13, 18, 23, 27, 32, 39, 46, 53, 60, 67, 74, 88, 102, 116, 130, 144, 158, and 172. IgG antibodies were assessed on the same days as biochemical variables, except days 4, 18 and 32.

Parasitological test and assessment of biological variables

Trypanosomes in cattle blood were assessed using the buffy coat (BC) technique according to Murray and McIntyre [27]. For a BC-positive result, parasitaemia was scored following a matching method [37] and expressed as trypanosomes/mL of blood.

The KHEMA 4 automatic haematology analyser (Kitvia, France) was used to measure haematological parameters according to the manufacturer's instructions. Haematological variables, namely PCV, Red Blood Cells (RBC), Haemoglobin (HB), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Platelets (PLT), Mean Platelet Volume (MPV), Lymphocytes (LYM), Monocytes (MON), Eosinophils (EOS), and Granulocytes (GRA) were evaluated with 30 μ L of blood taken by the analyser.

Biochemical parameters were evaluated with 220 μ L of plasma for each animal using the KBIO 5 automatic biochemistry analyser (Kitvia, France) and Bilan Diagnostic II kits (#W2BCMWD0648-Skylla, Taiwan) according to the manufacturers' instructions. Plasmatic Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Total Bilirubin (TBIL), Albumin (ALB), Creatinine (CREA), Glucose (GLU), Gamma-glutamyl Transferase (GGT), Cholesterol (CHOL), Lipase (LIPA), Globulin (GLOB which was obtained by estimation of total protein minus albumin) and Urea were assessed in this study.

Total IgG antibodies were assessed using the indirect ELISA technique according to Desquesnes [29,

30] (Additional file 2). Results were expressed as the relative percentage of positivity (RPP) as described by Desquesnes [38]. The RPP of each sample was calculated from the optical density of the sample in relation to optical densities of positive and negative standard plasmas. Any sample with an $RPP \leq 20\%$ was considered negative to *T. congolense* clone IL1180 infection, while a sample with an $RPP > 20\%$ was considered positive.

Statistical analysis

Statistical analyses were carried out with R version 4.2.0 (2022–04- 22 UCRT, URL <https://www.R-project.org/>).

The first step consisted in analysing data collected before trypanosome inoculation. A breed effect on the different response variables was tested with a linear mixed model using the *lme* function of the R nlme package [39]. The animal was considered as a random effect and the breed as a fixed effect, with the trypanotolerant NDA being the breed of reference in the test. For some variables, heteroscedasticity among breeds was also considered in the model. Following the recommendations made by Pinheiro and Bates [40], diagnostic plots were edited to check model assumptions (homoscedasticity, normality of the residues). Several models were tested using a Maximum Likelihood (ML) algorithm and the one that seemed to best fit the data was computed using Restricted Maximum Likelihood (REML). For MON data, which did not follow a normal distribution, the median value of each animal was estimated and the breed effect on the medians was tested using the Kruskal–Wallis test (KW test). For a global overview of variables, animal dispersion and potential correlations between variables, a PCA was performed using the R FactoMineR package [41] with default parameter (reduced and centred variables). The use of redundant variables, e.g., RBC and HB, were removed and seventeen variables (corresponding to the medians of the values before infection) were included in the PCA (TEMP; haematological variables: PCV, GRA, LYM, MON, PLT; biochemical variables: ALB, ALP, ALT, CHOL, CREA, GGT, GLOB, GLU, LIPA, TBIL and Urea).

The effect of trypanosome inoculation and its interaction with the breed on the different variables were then analysed. For parasitaemia, three indicators were calculated for each animal, then compared between breeds using the KW test and a pairwise Wilcoxon (pw-WC) test with the Benjamini & Hochberg correction. These indicators were the prepatent period (i.e., the number of days between the inoculation and the first detection of trypanosomes by BC observation for each animal), the proportion of time points an animal was detected positive in parasitaemia and the average parasitaemia over the experiment (both estimated from $DPI > 0$). One CRB

animal (CRB_1968) died at 27 DPI and was removed from the analyses, except for the prepatent period analysis.

The monitored variables widely differed in their dynamics during the course of infection and required an ad hoc adaptation of the indicators. Simple indicators were first calculated and used to assess a potential breed effect. These indicators were: i) the mean value calculated per animal from $DPI = 1$ to the end of the experiment, ii) the maximum, or minimum value depending on the variable dynamics, calculated as the median of the three highest (or minimum) values per animal, iii) the final value calculated as the median of the last three recorded values, iv) the maximum decrease calculated as the lowest value minus the pre-infection value, v) maximum increase calculated as the maximum value minus the pre-infection value (the latter two indicators being used for variables with different pre-infection values between breeds), and finally vi) the relative variation, calculated as the mean value minus the pre-infection median divided by the pre-infection median. The KW test and the pw-WC test with the Benjamini & Hochberg correction were used to estimate a breed effect on these indicators. A paired Wilcoxon test (pd-WC) was used to compare the final value to the pre-infection median of each animal. For these synthetic variables, the mean and standard deviation were indicated.

In addition, as most variables presented non-linear patterns, generalized additive models (GAMs) were run with the *gam* function of the of the R mgcv package [42, 43]. GAMs can be used to fit non-linear temporal effects with smoothing parameters and consider the repeated data at animal level. Parametric breed effects on the intercept and smoothing factors on DPI were assessed, and the cattle effect was adjusted as random smooths on DPI; NDA was the reference for the contrasts and a Gaussian family was used. GAMs were run for variables that did not present significant differences in pre-infection values.

Moreover, as young animals in the growth phase were considered in this experiment, a mixed linear model was used to adjust weight using the *lme* function of the nlme package [39]. As growth graphically started to be impacted by trypanosome inoculation three weeks post-infection, data from 23 DPIs were used to fit the model. Weight was modelled as a polynomial with a quadratic effect of DPI (DPI^2) and a breed effect in interaction with DPI, allowing an estimation of daily growth, and the animal was considered as a random effect.

Lastly, in order to assess potential links between variables during the course of infection, a second PCA was performed on 20 variables measured over this period. As the variation profiles differed between variables, the indicators used for the PCA were: the relative variations of sixteen variables (TEMP, PCV, GRA, LYM, PLT,

ALB, ALP, ALT, CHOL, CREA, GGT, GLOB, GLU, LIPA, TBIL, Urea), the relative weight gain (WG) ((final body weight – initial body weight)/initial body weight), the average RPP of antibodies, the average monocyte count, and the mean of log₁₀ parasitaemia.

Results

Cattle genotypes

Genetic assignment of the experimental cattle to known local breeds (WIDDE data) was confirmed by individual-based PCA and unsupervised hierarchical clustering (Additional files 3 to 6 and Additional file 7, respectively). ZFU animals were placed with ZFU reference data on the global and the specific PCA and had, on average, 67% of indicine ancestry (S.D.=1%) and 33% of West African taurine ancestry (S.D.=1%), as the reference ZFU data set [44]. NDA were placed within the West African taurine group (AFT) with, on average, 93% of AFT ancestry (S.D.=6%) and 7% of indicine ancestry (S.D.=6%) as in [44]. Two NDA cattle (NDA_1942 and NDA_1945) were slightly admixed with zebu since their percentages of indicine ancestry were 15%, but they were still close to other NDA cattle and the specific PCA showed that the NDA used in the experiment were placed within NDA subpopulations. Finally, CRB animals were placed between ZFU and European taurine (EUT) and their average proportions of admixture were 58% of EUT ancestry (S.D.=5%), 27% of indicine ancestry (S.D.=4%), and 16% of AFT ancestry (S.D.=3%). EUT values ranged from 51% (CRB_1962) to 68% (CRB_1966). According to the specific PCA, 6 CRB were placed between ZFU and the Holstein reference panel, and two other animals between a group containing Brune des Alpes, and Monbéliarde and ZFU.

Overview of the biological variables before infection

A synthesis of statistical analyses performed on pre-infection data and normal reference values for haematological variables [45], as well as biochemical variables [46], are presented in Additional file 8. Average BW before infection was not significantly different between NDA cattle and ZFU cattle ($p=0.64$), while average BW in CRB cattle was significantly higher than that of NDA cattle ($p<0.001$).

The average TEMP between NDA and ZFU cattle was not significantly different ($p=0.19$). In contrast, the CRB group had a significantly higher rectal TEMP than NDA ($p=0.0007$). No significant difference was observed in haematological variables between the breeds. Only a tendency for higher MCV and MCH was found in CRB cattle in comparison with NDA and ZFU cattle (p -values of t -test=0.06 and 0.05 for MCV and MCH, p -values F -test=0.009 and 0.01 for MCV and MCH, *lme* models). In contrast, the mean initial values of biochemistry

variables such as CHOL, GLU, ALB, GLOB, CREA and Urea showed significant differences ($p<0.05$, KW test) between the breeds. Specifically, the initial concentration of CHOL in NDA cattle was significantly lower than that in ZFU cattle ($p=0.0003$, *lme* models) and CRB cattle ($p=0.05$, *lme* models). Similarly, the initial GLU level in NDA cattle was significantly lower compared to both ZFU and CRB cattle ($p<0.0001$, *lme* models, both for NDA vs. ZFU and NDA vs. CRB). Considering ELISA results, RPP were below 20% in all breeds before infection, but pre-infection values were nevertheless slightly higher in NDA (8.9%, S.E.=1.2) than in CRB (effect -2.4, S.E.=1.6, $p=0.15$) and ZFU (effect -6.8, S.E.=1.2, $p=0.001$, *lme* models).

The PCA provided an overview of data dispersion on the factorial plans and of the relationships between these variables (Fig. 1). Axis 1 and axis 2 explained 22% and 15% of the total variance. Axis 1 distinguished cattle according to the breeds by separating NDA from CRB and ZFU cattle, and it was mainly explained by six biochemical variables, GLU, ALB, GLOB, CHOL, Urea and CREA, which were at higher concentrations in ZFU and CRB cattle than in NDA cattle, except for GLOB whose concentration was higher in NDA cattle. Axis 2 roughly separated CRB cattle from NDA and ZFU cattle and was mainly explained by TEMP, ALT and TBIL.

Development of clinical manifestations during infection

All the animals acquired trypanosome infection. The results of the BC tests throughout the course of the infection are displayed in Fig. 2A for each breed of cattle. The pre-patent period ranged from 9 to 18 days, with median values of 11, 13, and 16 days for NDA, ZFU and CRB, respectively. The pre-patent period was not significantly different between breeds ($p=0.06$, KW test). The animals always tested mostly positive in terms of parasitaemia in the first three months of infection, but parasitaemia started to decrease slightly after two months, then fluctuated up to the end of the experiment (Fig. 2B). Over the experiment, the average proportions of time points at which cattle tested positive for parasitaemia were not significantly different between breeds ($p=0.17$, KW test) (Table 1). However, average parasitaemia values were significantly different between breeds ($p=0.001$, KW test), with lower parasitaemia in NDA in comparison with CRB and ZFU ($p<0.001$ for pw-WC). In the first months of infection, animals showed occasional clinical signs. Bristled hair and reduced feed intake were observed in some cattle across the three breeds. In addition, some ZFU and CRB presented lacrimation, recumbency and restlessness. One animal in the CRB group, CRB8, suddenly died at 27 DPI. This animal presented a sharp rise in parasitaemia, an early peak of GGT, a drop in PLT (PLT = 18×10^3 /

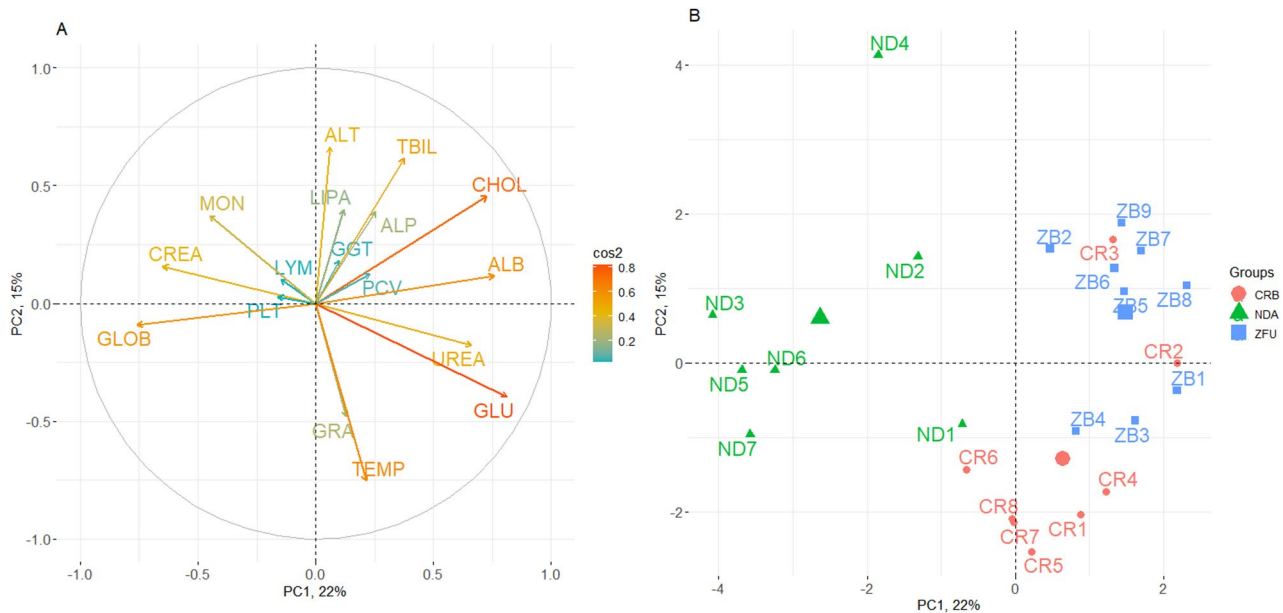


Fig. 1 Principal component analysis of the synthetic variables before infection. **A** Correlation circle of the variables used in the PCA for the principal components (PC) 1 and 2. **B** Factorial plan for PC1 and 2 showing the coordinates of the individuals

μL before death), associated with sudden weakness, while anaemia was not pronounced ($\text{PCV} = 28.5\%$).

TEMP increased in all breeds after parasite inoculation, reaching its first peak between 15 and 30 DPI, and fluctuated with an overall return to pre-infection levels at the end of the study (Additional file 9). The average rectal TEMP in NDA cattle was significantly lower than that in ZFU cattle ($p = 0.002$, GAM) and CRB cattle ($p = 1 \times 10^{-6}$, GAM) (Table 1). The same applied to the maximum temperature, but relative variation was not significantly different between breeds, as NDA had a lower TEMP before infection.

Graphically, cattle growth started to be affected by trypanosomosis in CRB and ZFU cattle two weeks post-infection (Additional file 10). Some ZFU and CRB individuals showed stagnation in their weight, and one ZFU animal, ZFU5, lost weight. Overall, no significant difference in WG was observed between cattle breeds ($p = 0.36$, KW test). However, ZFU cattle had a significantly lower growth rate than NDA cattle ($p = 0.001$, *lme* model), while the growth rate between CRB and NDA was not significantly different ($p = 0.09$, *lme* model).

Haematological changes during infection

The results of the analysis of haematological variables during the study are presented in Table 2. Haematological variables were modified by the infection and displayed different dynamics depending on the breeds. As expected, PCV dropped in all cattle breeds following parasite detection in the blood (Fig. 3). However, after one month of infection NDA cattle mitigated the drop of PCV, and the average PCV gradually increased

to reach pre-infection values. One animal, NDA6, displayed a sudden and one-off decrease in its PCV to 13% from 37 to 46 DPI, without any severe clinical signs or weakness, and subsequently recovered normally. In contrast, the PCV of the ZFU cattle breed and CRB group continued to decline as the disease progressed, stabilizing at the lower threshold and an increase was eventually observed towards the end of the study. However, the CRB group did not recover its initial PCV value at the end of the study. The average PCV in the NDA breed was significantly higher than that in the ZFU breed, ($p = 0.001$, GAM), and in the CRB group ($p = 0.004$, GAM). The dynamics of RBC and HB (Additional files 11 and 12) mirrored those of PCV and were thus excluded from further analyses. MCHC showed a similar slight increase in all breeds and reached a plateau roughly one and a half months post-infection (Additional file 13). No significant difference was observed in the average MCHC in the NDA cattle compared to the other breeds; MCHC final values were above the initial values in all breeds ($p = 10^{-5}$ pd-WC). MCV and MCH presented an increase 1–2 months post-infection and then gradually returned to their pre-infection values, except for ZFU, in which they remained slightly above pre-infection values (Additional files 14 and 15). The mean MCV in the NDA cattle was not significantly different from that in ZFU cattle ($p = 0.14$, GAM), but it was significantly lower than that in the CRB group ($p = 0.008$, GAM). The mean MCH in NDA cattle followed a similar trend to that of MCV, presenting no difference compared to ZFU ($p = 0.07$, GAM), but a significant difference compared to the CRB group ($p = 0.003$, GAM). PLT concentration decreased in all

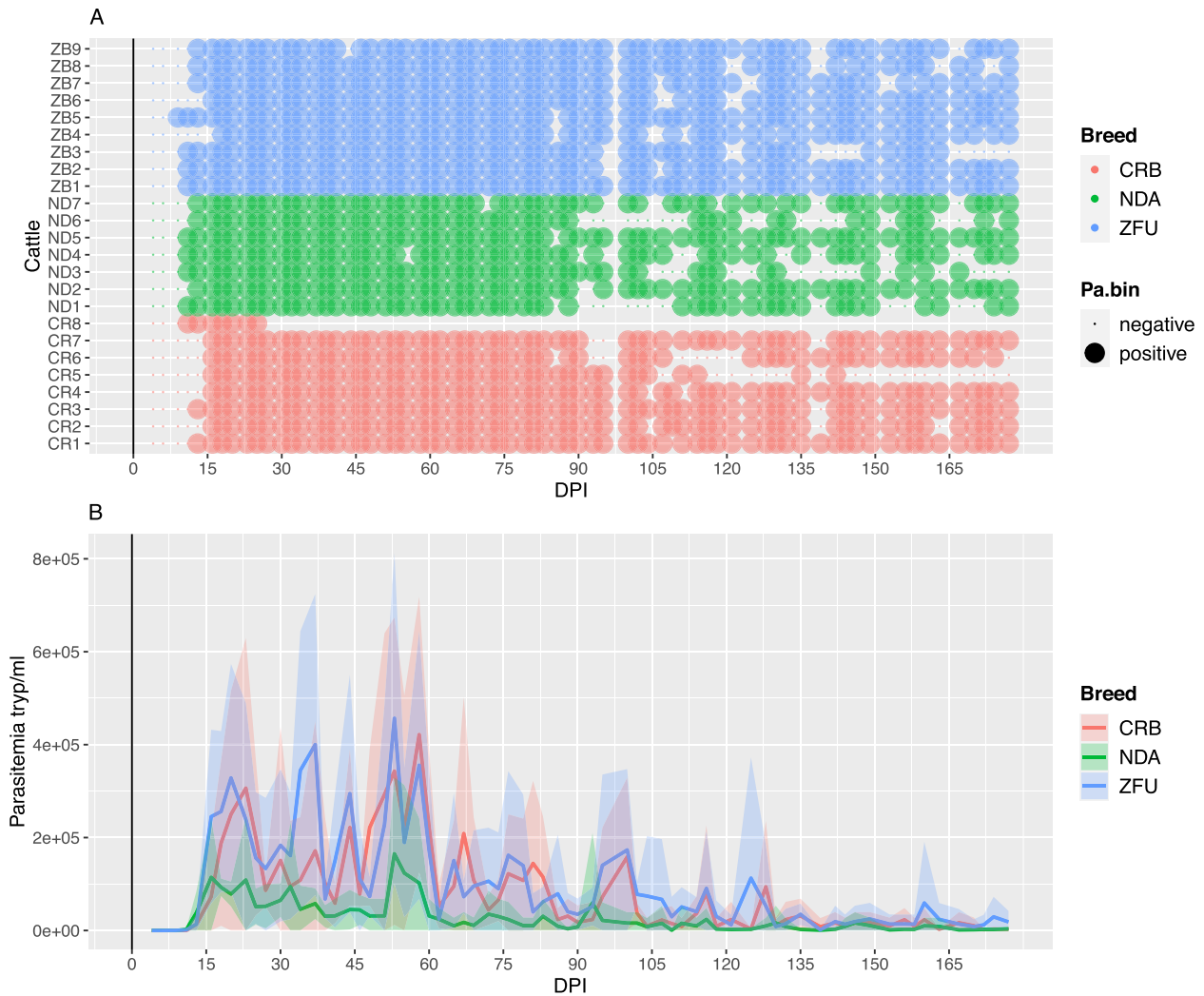


Fig. 2 Change in parasitaemia by animal and breed. **A** Individual buffy coat technique results of the infected cattle during the experiment. Animal identifiers are indicated on the left. **B** Average parasitaemia according to the breed and DPI. Standard deviation intervals are underlined. DPI: days post infection

Table 1 Summary statistics for parasitaemia, temperature and growth during infection

Traits	Indicator (unit)	NDA mean (SD) (SE if gam)	ZFU mean (SD) (SE if gam)	CRB mean (SD) (SE if gam)	Global <i>p</i> -value	Test
PA	mean (× 1000 tryp/mL)	28 (10)	100 (29)	79 (35)	0.001	KW
	proportion of positive time points	0.79 (0.10)	0.89 (0.05)	0.84 (0.12)	NS	KW
TEMP	mean (°C)	37.8 (0.1)	38.2 (0.1) 0.002	38.5 (0.1) 1. E-06	***	gam <i>p</i>
	maximum	38.9 (0.3)	39.4 (0.6)	39.6 (0.3)	0.01	KW
	final versus pre-infection (<i>p</i>)	0.02	0.4	0.93	0.11	pd-WC
Growth	RV	0.01 (0.01)	0.02 (0.01)	0.01 (0.01)	0.29	KW
	mean (kg/day)	0.28 (0.05)	0.12 (0.05) 0.001	0.19 (0.05) 0.09	**	lme <i>p</i>
	WG	0.32 (0.08)	0.22 (0.14)	0.32 (0.22)	0.36	KW

NDA N'Dama, ZFU Fulani zebu, CRB Crossbred cattle, KW Kruskal–Wallis test, pd-WC Paired-Wilcoxon test, RV Relative variation, NS Not significant

**one highly significant contrast or two slightly significant contrasts with gam model

***two highly significant contrasts with gam model

Table 2 Summary statistics for haematological variables

Traits	Indicator (unit)	NDA mean (SD) (SE if gam)	ZFU mean (SD) (SE if gam)	CRB mean (SD) (SE if gam)	Global p-value	Test
PCV	mean (%)	31.6 (1.2)	26.3 (1.7) 0.001	26.7 (1.7) 0.004	***	gam p
	minimum (%)	24.8 (5.5)	19.8 (4.6)	21.6 (1.4)	0.03	KW
	final versus pre-infection (p)	0.81	0.13	0.03	0.02	pd-WC
	RV	-0.03 (0.14)	-0.20 (0.14)	-0.21 (0.09)	0.02	KW
MCHC	mean (g/dL)	27.8 (0.2)	28.1 (0.3) 0.4	27.1 (0.3) 0.5	NS	gam p
	final versus pre-infection (p)	0.02	0.009	0.02	1.E-05	pd-WC
	RV	0.02 (0.04)	0.02 (0.03)	0.02 (0.05)	0.6	KW
	MCV	mean (fL)	43.1 (1.6)	46.2 (2.0) 0.14	48.9 (2.2) 0.008	**
MCH	maximum (fL)	51.2 (17.1)	58.2 (11.4)	56.7 (4.1)	0.12	KW
	final versus pre-infection (p)	0.69	0.004	0.81	0.07	pd-WC
	RV	0.06 (0.11)	0.22 (0.09)	0.11 (0.12)	0.02	KW
	mean (pg)	12.0 (0.4)	13.0 (0.5) 0.07	13.7 (0.6) 0.003	**	gam p
PLT	maximum (pg)	14.4 (4.4)	16.4 (2.8)	16.2 (1.0)	0.12	KW
	final versus pre-infection (p)	0.08	0.004	0.16	0.0005	pd-WC
	RV	0.11 (0.12)	0.27 (0.08)	0.16 (0.13)	0.03	KW
	mean (*1000/ μ L)	313.6 (18.6)	195.3 (24.7) 1.E-06	169.0 (26.1) 1.E-07	***	gam p
MPV	minimum	86.7 (27.0)	61.2 (28.2)	52.9 (33.2)	0.1	KW
	final versus pre-infection (p)	0.30	0.07	0.22	0.006	pd-WC
	RV	-0.27 (0.26)	-0.33 (0.49)	-0.41 (0.35)	0.22	KW
	mean (fL)	5.6 (0.1)	5.9 (0.1) 0.02	5.8 (0.1) 0.01	**	gam p
LYM	maximum (fL)	6.3 (0.2)	6.7 (0.4)	6.6 (0.4)	0.1	KW
	final versus pre-infection (p)				0.23	pd-WC
	RV	0.04 (0.04)	0.05 (0.05)	0.06 (0.04)	0.64	KW
	mean (*1000/ μ L)	11.8 (0.8)	6.9 (1.0) 1.E-06	7.7 (1.0) 3.E-04	***	gam p
GRA	final (*1000/ μ L)	11.8 (1.3)	8.5 (3.8)	6.7 (1.4)	0.03	KW
	final versus pre-infection (p)	0.02	0.36	0.08	0.13	pd-WC
	RV	0.47 (0.12)	-0.07 (0.23)	-0.04 (0.24)	0.001	KW
	mean (*1000/ μ L)	3.8 (0.2)	1.8 (0.3) 1.E-12	2.3 (0.3) 4.E-07	***	gam p
MON	final (*1000/ μ L)	3.6 (0.7)	2.2 (0.7)	2.8 (0.9)	0.02	KW
	final versus pre-infection (p)	0.94	0.05	0.08	0.03	pd-WC
	RV	0.18 (0.41)	-0.45 (0.29)	-0.36 (0.18)	0.001	KW
	mean (*1000/ μ L)	0.9 (0.2)	0.1 (0.2)	0.2 (0.2)	4.E-04	KW
	final versus pre-infection (p)				0.20	pd-WC

NDA N'Dama, ZFU Fulani zebu, CRB Crossbred cattle, KW Kruskal–Wallis test, pd-WC paired-Wilcoxon test, RV Relative variation, NS Not significant

**one highly significant contrast or two slightly significant contrasts with gam model

***two highly significant contrasts with gam model

breeds following infection development and persisted at extremely low levels throughout infection, with a mild increase at the end of infection (Fig. 4). The average PLT during the study in NDA cattle was significantly higher compared to ZFU cattle ($p = 10^{-6}$, GAM) and CRB cattle ($p = 10^{-7}$, GAM), though the relative variations were not significantly different. MPV increased post-infection in all breeds and gradually returned to pre-infection

values (Additional file 16). NDA cattle showed significantly lower MPV compared to both ZFU cattle ($p = 0.02$, GAM) and CRB cattle ($p = 0.01$, GAM).

Immune blood cells showed very different profiles throughout infection between NDA cattle on the one hand, and ZFU and CRB cattle on the other hand. LYM increased 3 weeks post-infection and presented lymphocytosis up to the end of the experiment in NDA cattle.



Fig. 3 Change in pack cell volume by breed during the course of the study. PCV is expressed as a percentage of blood volume. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 24 and 46 indicate the limits of usual values for PCV in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

In contrast, the LYM count remained roughly constant throughout the study in ZFU and CRB cattle (Fig. 5). The average LYM in NDA cattle was significantly higher than in ZFU cattle ($p = 10^{-6}$, GAM) and CRB cattle ($p = 3 \times 10^{-4}$, GAM). A decrease in GRA levels was observed in ZFU and CRB cattle, with GRA counts falling even below normal values for a majority of ZFU, while NDA cattle presented constant GRA levels, though with high fluctuations between individuals and between time-points within individuals (Fig. 6). GRA was significantly higher in NDA than in ZFU cattle ($p = 10^{-12}$, GAM) and in the CRB group ($p = 4 \times 10^{-7}$, GAM). MON levels were relatively constant and low in the first 3 months, then monocytosis occurred up to the end of the experiment in NDA cattle. In contrast, MON counts were mostly undetectable throughout the study in ZFU and CRB cattle (Fig. 7). The average MON level differed significantly between breeds ($p = 0.0004$, KW test). EOS counts were generally

undetectable, but were nevertheless visible more often in NDA than in CRB and ZFU (Additional file 17).

Serological changes during infection

Following infection, the RPP level in the cattle increased as expected. The serological response appeared to occur earlier in NDA cattle compared to ZFU and CRB cattle, with the 20% RPP level being reached at a median of 23 DPI (S.D. = 0) in NDA cattle, while it was at 39 DPI (S.D. = 13.1) in ZFU and 27 DPI (S.D. = 7.6) in CRB cattle ($p = 0.04$, KW test). RPP increased throughout the study and roughly reached a plateau 3–4 months post-infection (Additional file 18). At the end of the study, no significant difference was observed between breeds ($p = 0.12$, KW test).

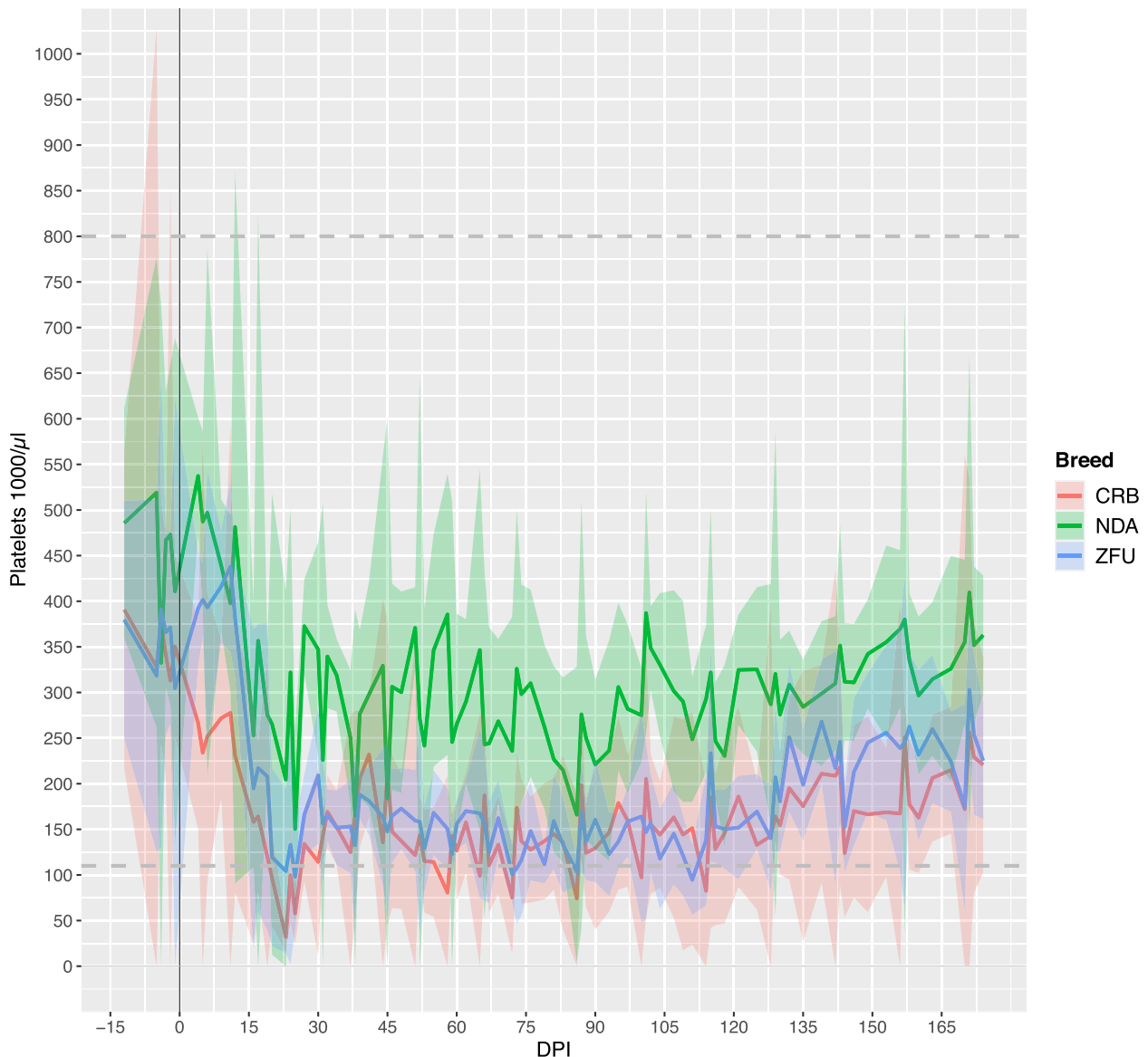


Fig. 4 Change in platelet count by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 110 and 800 indicate the reference limits for the platelet count in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

Changes in plasma biochemical variables during infection

The results from the statistical analyses of the biochemical variables measured during the study are summarised in Table 3. In all cattle, the profiles of the biochemical variables varied during the course of infection. However, the dynamics of the CHOL, GLU, ALB, ALT and ALP variables varied between cattle breeds, while the dynamics of GLOB, CREA, Urea, GGT, LIPA and TBIL were very similar from one breed to another. CHOL concentration started to fall from two weeks post-infection in all breeds, but the extent of the decrease and the temporal profile differed substantially between the NDA breed on the one hand, and ZFU and CRB cattle on the other hand (Fig. 8). Hypocholesterolaemia was observed in ZFU and

CRB cattle, but not in the NDA breed. Indeed, in NDA cattle a gradual increase in CHOL levels was observed from 2 months post-infection up to the end of the study, and by the end of the study the CHOL level was higher than that of the pre-infection level. Recovery was slower in CRB and ZFU cattle, in which CHOL displayed a gradual increase only around 4 months post-infection. Finally, while average CHOL did not differ between breeds ($p=0.34$, KW test), relative variations were significantly different ($p=0.0005$, KW test). Similarly, GLU concentrations decreased in all cattle with trypanosome infection. In ZFU and CRB cattle, GLU levels decreased and remained below pre-infection levels throughout the course of infection. In NDA cattle, GLU, whose initial



Fig. 5 Change in lymphocyte count by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 2.5 and 7.5 indicate the reference limits for the lymphocyte count in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

concentration was lower than in ZFU and CRB cattle, showed a slight increase by the end of the study (Fig. 9). In connection with differences in pre-infection concentrations, the average GLU concentrations during the infection period were significantly different between breeds ($p=0.0005$, KW test), with a lower value in NDA, but the relative variation was more mitigated in NDA ($p=0.0005$, KW test). ALB presented different kinetics between breeds. The ALB concentration started below the limit considered normal for bovine in NDA cattle and remained constant over 2 months of infection. After that, it increased and was within usual values at the end of the experiment. Conversely, ALB concentrations, which were within normal limits in ZFU and CRB cattle, decreased

simultaneously with parasite detection, leading to hypoalbuminemia in the ZFU breed. A return to pre-infection levels was observed in both breeds before the end of the study (Additional file 19). Average ALB concentrations during the infection period were significantly different between breeds ($p=0.03$, KW test). The ALT levels first decreased simultaneously with parasite detection in all cattle, then showed a gradual increase, whose precocity and magnitude seemed to depend on the breed (Additional file 20). Indeed, in NDA cattle from parasite detection up to 60 DPI ALT levels decreased and were around the lower reference bovine limit. A gradual increase was then observed, and the ALT levels were higher than the pre-infection level by the end of the study, but remained

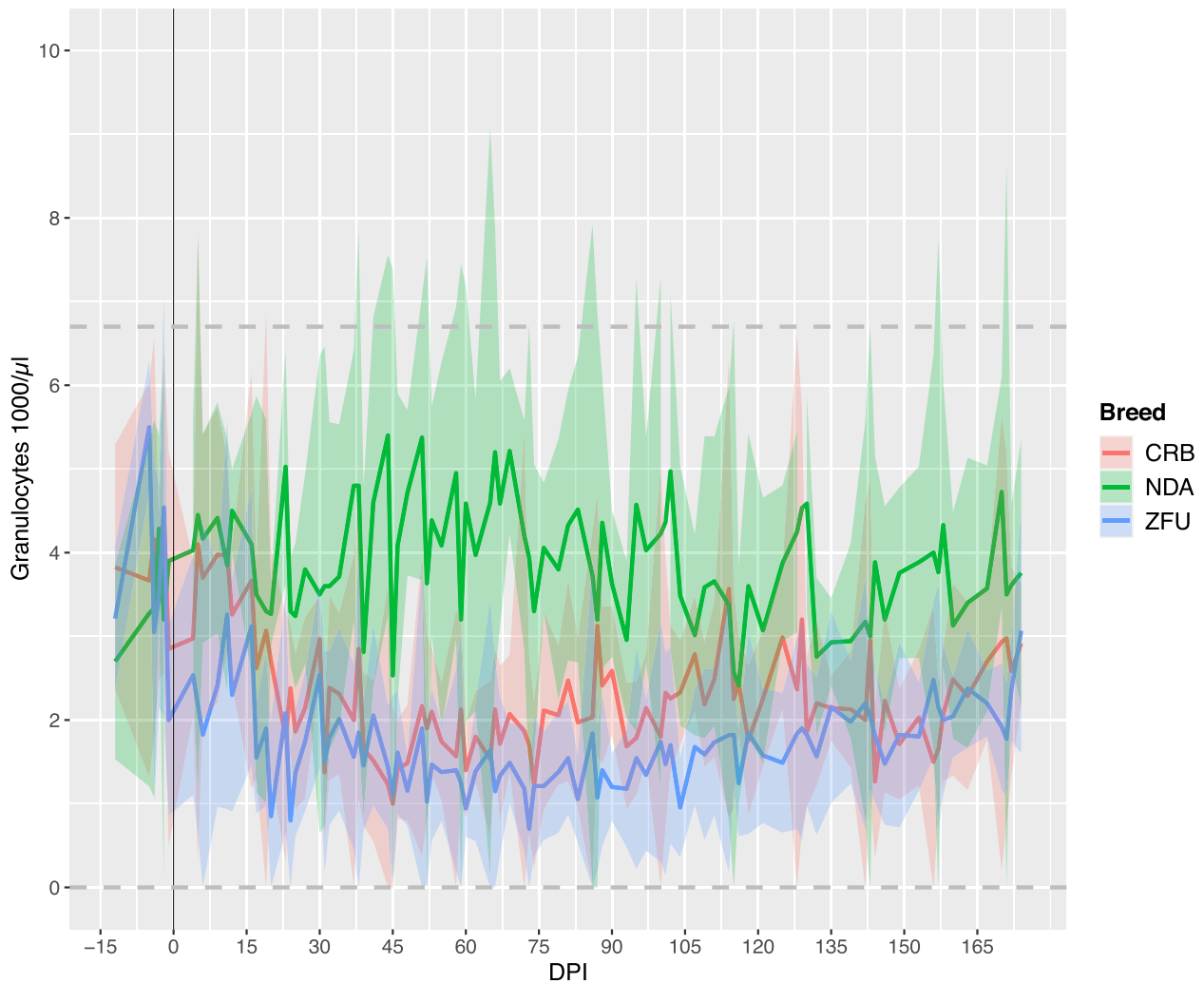


Fig. 6 Change in granulocyte count by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 0 and 6.7 indicate the reference limits for the granulocyte count in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

within the reference limits. In ZFU and CRB cattle, from parasite detection to 120 DPI, ALT levels decreased and were below the limit considered normal for bovines, then returned to the normal range but remained lower than in NDA cattle. Average ALT concentrations during the infection period were significantly different ($p=0.005$, KW test) between breeds. ALP levels roughly showed a similar pattern in all breeds, with an increase post-infection, while remaining within reference limits, followed by a gradual return to pre-infections levels. However, the increase was more pronounced in ZFU cattle than in CRB and NDA cattle (Additional file 21). Average ALP concentrations during the infection period were significantly different between the breeds ($p=0.003$, KW test).

Regarding variables that varied according to infection but differed only slightly between breeds, we found that GLOB concentrations decreased in all breeds with parasite detection in the blood. Thereafter, a gradual

increase was observed in all breeds (Additional file 22). The average GLOB concentrations were significantly different during infection ($p=0.03$, KW test), linked to pre-infection values. CREA levels fluctuated within the normal range for bovines throughout the entire experimental period and showed similar values (Additional file 23). No significant difference was seen in average CREA levels between breeds ($p=0.33$, KW test). Urea gradually decreased in all breeds before stagnating from 2–3 months post-infection (Additional file 24). No significant difference was observed in average urea between breeds ($p=0.64$). GGT showed highly variable profiles depending on individuals, but did not depend on the breed (Additional file 25). In fact, some cattle showed a transient increase several weeks post-infection, but no significant difference was seen between breeds for average GGT concentrations ($p=0.13$, KW test). TBIL concentrations decreased in all breeds and stagnated at low

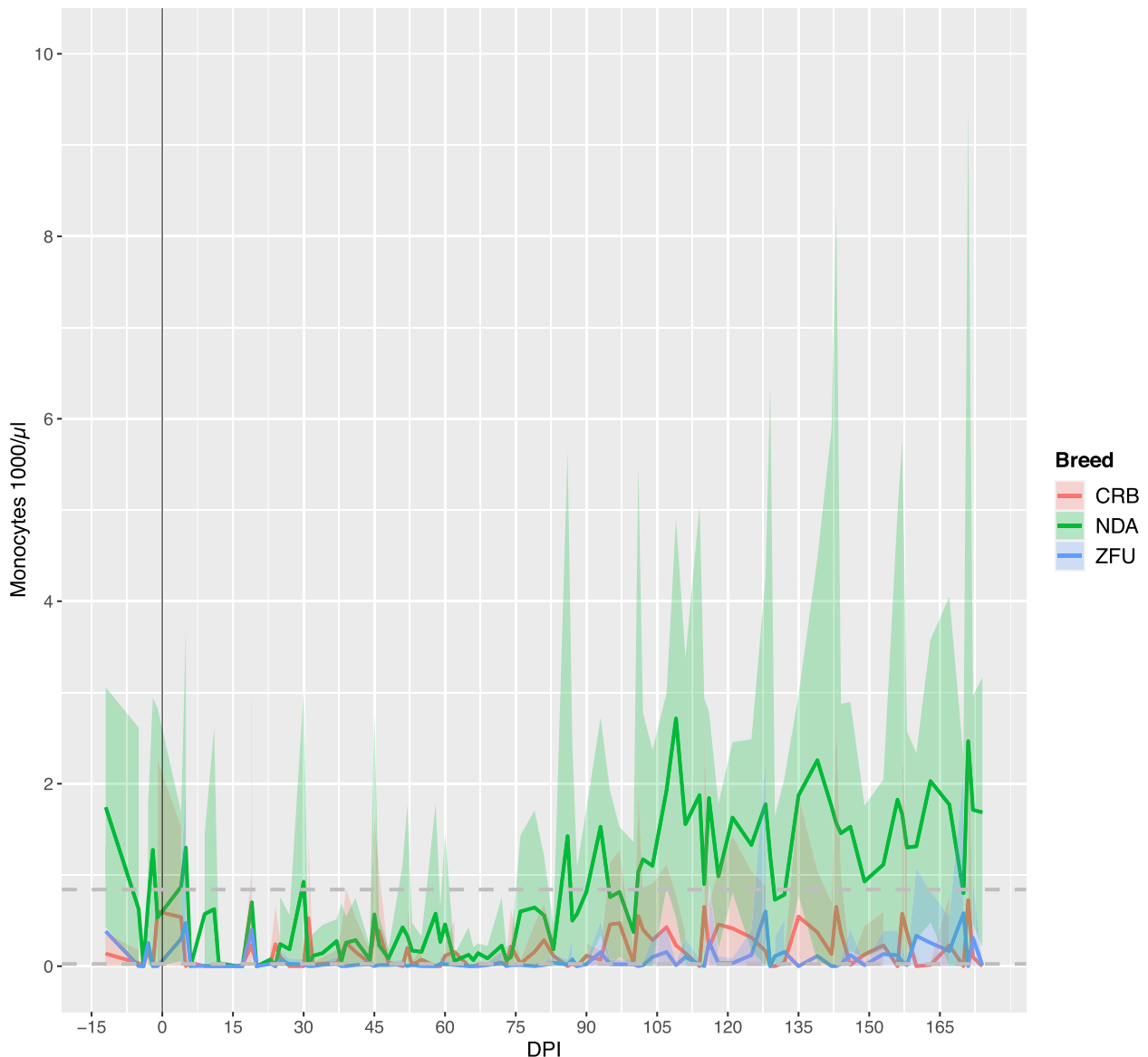


Fig. 7 Change in monocyte count by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 0.025 and 0.84 indicate the reference limits for the monocyte count in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

levels throughout infection (Additional file 26), with a tendency to higher values in NDA compared to the other breeds ($p=0.04$). LIPA concentrations fluctuated substantially in all breeds, with a tendency to increase after 3 months of infection in NDA cattle (Additional file 27). No significant difference was observed in average LIPA concentrations between breeds during infection ($p=0.07$, KW test).

Relationship between the biological variables during infection

The PCA run on one indicator per variable (mainly relative variation prior to pre-infection values) was used to estimate correlations between the variables throughout

the course of infection and their relationships with the individuals (Fig. 10). Axis 1 and axis 2 explained 40% and 12% of total variance. Axis 1 was mainly explained by six variables: CHOL, LYM, parasitaemia (PA), MON, GLU, and PCV, and axis 2 was explained by RPP, ALP and PLT. Strong correlations were found between variables explaining axis 1, while poor correlations were observed for the variables associated with axis 2 (Fig. 11). Indeed, we found that CHOL was positively and significantly correlated with PCV, GRA, MON, LYM, and GLU. PA was negatively and significantly correlated with MON, LYM, CHOL, PCV, and also with other variables, i.e., ALB, TBIL, GRA, relative WG, GLU, and it was positively correlated with CREA. In addition to correlations with

Table 3 Summary statistics for biochemical variables

Traits	Indicator (unit)	NDA mean (SD)	ZFU mean (SD)	CRB mean (SD)	Global p-value	Test
CHOL ^a	mean (mg/dL)	107.2 (43.3)	101.2 (14.5)	93.0 (8.5)	0.34	KW
	minimum (mg/dL)	82.4 (44.4)	63.9 (19.8)	67.6 (13.0)	0.74	KW
	maximum decrease (mg/dL)	-6.7 (12.7)	-76.8 (27.5)	-45.9 (20.1)	0.0005	KW
	final versus pre-infection (p)	0.02	0.02	0.15	0.55	pd-WC
	RV	0.24 (0.25)	-0.27 (0.12)	-0.17 (0.10)	0.0005	KW
GLU ^a	mean (mg/dL)	55.0 (5.1)	65.5 (4.1)	71.2 (3.7)	0.0005	KW
	minimum (mg/dL)	44.9 (5.0)	55.0 (4.1)	61.0 (4.5)	0.0009	KW
	maximum decrease (mg/dL)	-14.8 (7.4)	-23.2 (7.4)	-22.7 (4.2)	0.05	KW
	final versus pre-infection (p)	0.93	0.009	0.02	0.0002	pd-WC
	RV	-0.08 (0.07)	-0.16 (0.03)	-0.15 (-0.04)	0.03	KW
ALB ^a	mean (g/dL)	3.0 (0.2)	3.0 (0.2)	3.2 (0.1)	0.03	KW
	maximum decrease (mg/dL)	-0.1 (0.1)	-0.4 (0.2)	-0.1 (0.3)	0.01	KW
	final versus pre-infection (p)	0.02	1	0.12	0.01	pd-WC
	RV	0.04 (0.03)	-0.04 (0.03)	0.02 (0.06)	0.004	KW
ALT	mean (U/L)	34.4 (7.5)	25.8 (5.2)	22.4 (4.4)	0.005	KW
	maximum decrease (U)	-9.3 (6.2)	-16.7 (9.0)	-9.7 (8.6)	0.23	KW
	maximum increase (U)	19.4 (8.2)	4.7 (7.0)	10.0 (6.2)	0.009	KW
	final versus pre-infection (p)	0.02	0.48	0.16	0.02	pd-WC
	RV	0.09 (0.11)	-0.19 (0.20)	0.02 (0.27)	0.02	KW
ALP	mean (U/L)	167.6 (68.9)	261.8 (74.0)	148.3 (35.4)	0.003	KW
	maximum (U/L)	235.9 (88.7)	365.9 (111.5)	201.6 (55.1)	0.007	KW
	final versus pre-infection (p)				0.94	pd-WC
	RV	0.28 (0.20)	0.66 (0.34)	0.22 (0.09)	0.01	KW
GLOB ^a	mean (g/dL)	4.4 (0.3)	4.0 (0.7)	4.0 (0.3)	0.03	KW
	maximum decrease (U)	-1.4 (0.4)	-1.2 (0.3)	-1.2 (0.3)	0.31	KW
	final versus pre-infection (p)	0.02	0.51	0.04	0.002	pd-WC
	RV	-0.14 (0.05)	-0.11 (0.06)	-0.11 (0.04)	0.4	KW
CREA ^a	mean (mg/dL)	1.3 (0.1)	1.2 (0.1)	1.4 (0.2)	0.33	KW
	final versus pre-infection (p)	0.55	0.009	0.02	0.0004	pd-WC
	RV	-0.7 (0.13)	0.13 (0.07)	0.15 (0.10)	0.01	KW
UREA ^a	mean (mg/dL)	44.6 (5.1)	48.3 (8.3)	50.1 (9.8)	0.64	KW
	final versus pre-infection (p)	0.58	0.004	0.02	0.00001	pd-WC
	RV	0.15 (0.36)	-0.34 (0.10)	-0.19 (0.09)	0.006	KW
GGT	mean (U/L)	21.5 (6.5)	17.3 (5.5)	15.6 (9.7)	0.13	KW
	final versus pre-infection (p)	0.55	0.91	0.07	0.72	pd-WC
	RV	4.11 (8.80)	0.85 (1.31)	0.36 (0.80)	0.06	KW
TBIL	mean (mg/dL)	0.3 (0.1)	0.2 (0.1)	0.2 (0.9)	0.04	KW
	final versus pre-infection (p)	0.04	0.01	0.02	0.00004	pd-WC
	RV	-0.51 (0.34)	-0.86 (0.06)	-0.79 (0.11)	0.01	KW
LIPA	mean (U/L)	31.6 (0.7)	31.1 (0.8)	30.6 (0.6)	0.07	KW
	final versus pre-infection (p)	0.03	0.05	0.78	0.58	pd-WC
	RV	0.01 (0.02)	-0.03 (0.02)	-0.01 (0.06)	0.06	KW

NDA N'Dama, ZFU Fulani zebu, CRB Rossbred cattle, KW Kruskal–Wallis test, pd-WC paired-Wilcoxon test, RV Relative variation

^asignificant differences before infection

CHOL and PA, PCV was positively and significantly correlated with GRA, LYM, MON and GLU. GLU was also positively and significantly correlated with LYM, MON and GRA. ALB and WG were positively and significantly correlated. For axis 2, only slight negative correlations were found between ALP with GLOB and RPP. Axis 1 distinguished between animals by breed: the NDA cattle

were clearly separated from the ZFU cattle along this axis, and CRB animals were split between NDA and ZFU. The position of NDA cattle highlighted the best control of anaemia and parasitaemia, as well as their lymphocytosis and monocytosis and higher relative variations of concentrations for CHOL and GLU. In contrast, ZFU cattle were on the left-hand side of axis 1, indicating strong

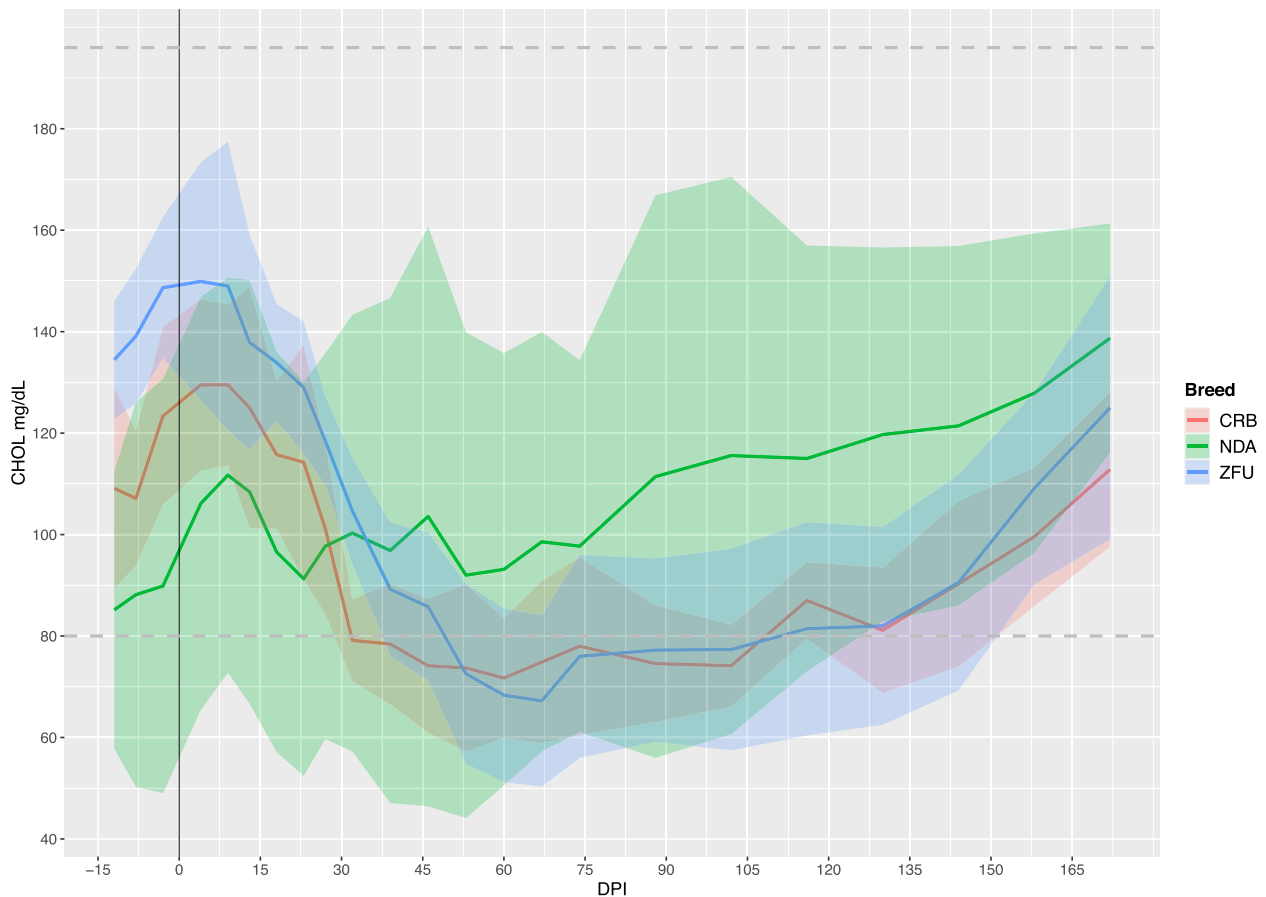


Fig. 8 Change in cholesterol levels by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 22 and 64 indicate the reference limits for the cholesterol levels in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

anaemia and parasitaemia. Animals from the NDA and ZFU breeds were roughly dispersed along axis 2, while CRB animals were placed rather on the top of axis 2, and were positively associated with RPP, but negatively with the relative variations of ALP and PLT. The ACP also confirmed strong inter-individual variability within the breeds.

Discussion

Trypanosomosis causes major haematological and biochemical changes in cattle. Our experimental infection accurately described modifications in blood haematological and biochemical variables in West African cattle infected with *T. congolense* and revealed the main differences that discriminated between trypanotolerant and susceptible cattle.

Basic profile of breeds prior to infection

Before inoculating the parasites and analysing the dynamics of the recorded variables during infection, we looked into potential baseline differences between breeds. As expected, the weight of CRB cattle was significantly

higher than NDA and ZFU cattle, as CRB animals are derived from crosses between ZFU and European taurine which have higher body weights, from birth to adult age, than both local breeds [47, 48]. A higher average temperature was recorded in the CRB cattle compared to NDA, whereas ZFU cattle had similar temperatures to NDA. This difference could be related to a poorer adaptation of cross-bred animals with European ancestry to a hot tropical environment [49]. While basal haematological variables were very similar between breeds, initial differences were recorded for a set of biochemical variables, CHOL, GLU, ALB, GLOB, CREA and Urea levels, which globally distinguished NDA cattle from ZFU and CRB cattle, even though the animals received the same diet. High variability in such variables, e.g., ALB and GLOB levels, has already been documented both between individuals and breeds, and depended on intrinsic factors (breed, age), as well as extrinsic factors, especially food, season and stress [50]. In this study, the observed differences in certain biochemical variables may have been due to genetic factors, as differences in gene expression between N'Dama and zebu breeds were highlighted

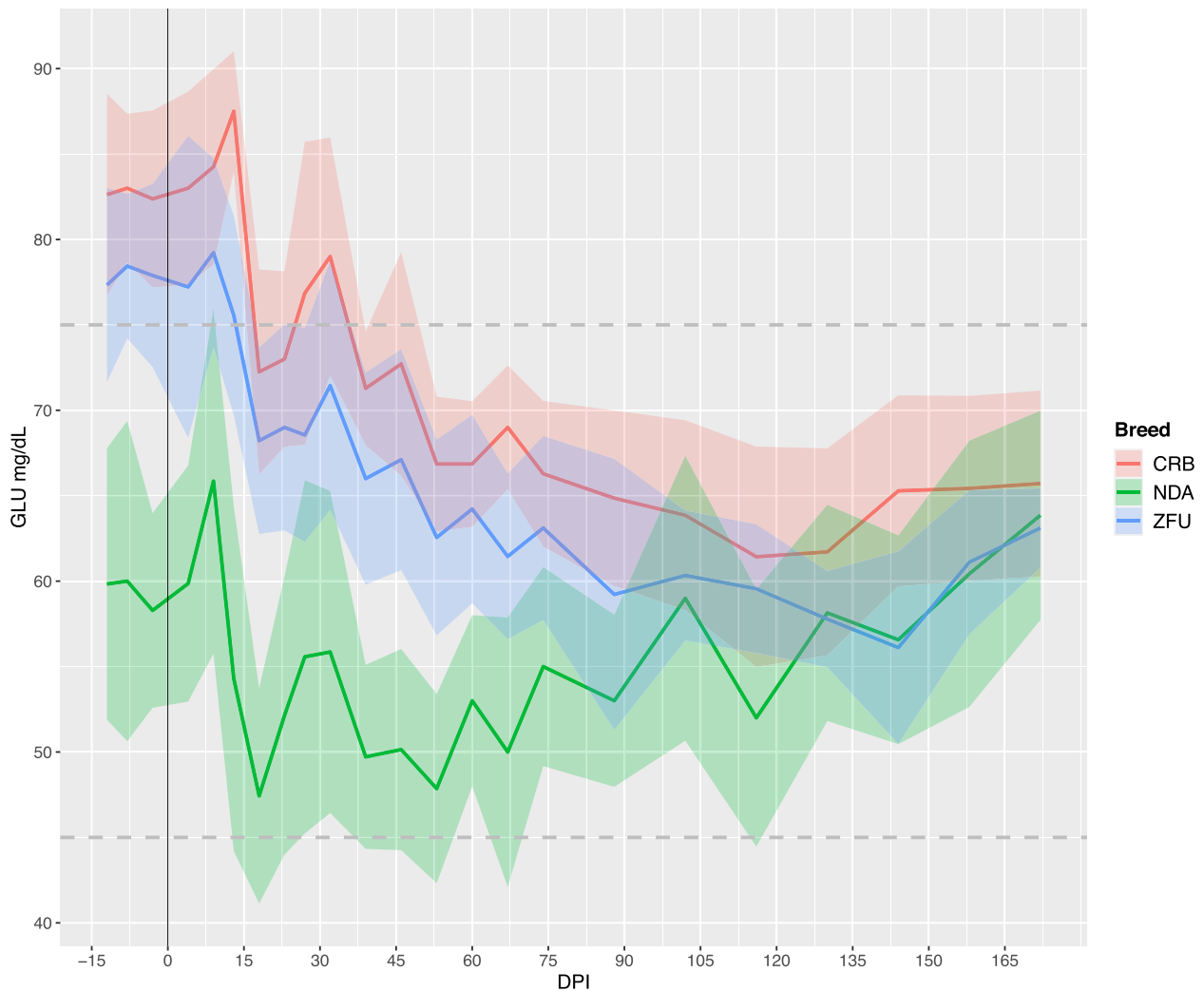


Fig. 9 Change in glucose levels by breed during the course of the study. The Vertical line indicates the day of infection for all animals. Horizontal dashed lines at 45 and 75 indicate the reference limits for the glucose levels in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

before trypanosome infection [22], or to environmental factors, since the animals came from different agro-ecological zones. It should be stressed that no reference data are available for local African breeds reared in extensive farming systems to support either of these hypotheses. In any case, pre-infection differences in our experiment should be taken into account when comparing the evolution of the different variables during the course of infection. Genotyping confirmed the ancestry of the animals that were first assessed according to phenotypic observations and breeders' knowledge. Two N'Dama (NDA_1942 and NDA_1945) showed a slight introgression with indicine ancestry, but in a proportion similar to that already observed in some N'Dama populations [44].

Infection development

All the cattle were equally susceptible to the establishment of infection, and the incubation period did not

differ between breeds, which is consistent with other reports [11, 25]. All the animals showed parasites in their blood, though NDA cattle were less affected by the infection than ZFU and CRB cattle. Indeed, only two out of seven NDA cattle (NDA_1941 and NDA_1946) showed mild clinical symptoms, without this being linked to a stronger indicine introgression for these 2 individuals. The introgressed animals (NDA_1942 and NDA_1945) did not exhibit any notable symptoms or severe anaemia, despite their relatively high parasitaemia. Conversely, almost all ZFU and CRB cattle were severely affected. Significant inter-breed differences with respect to clinical manifestations has been recorded between West African taurine such as Baoulé and zebu breeds during trypanosomosis [51]. The clinical signs reported in this study were associated with an increase in rectal temperatures in all cattle. However, NDA cattle were significantly less febrile than ZFU and CRB cattle. This is consistent with

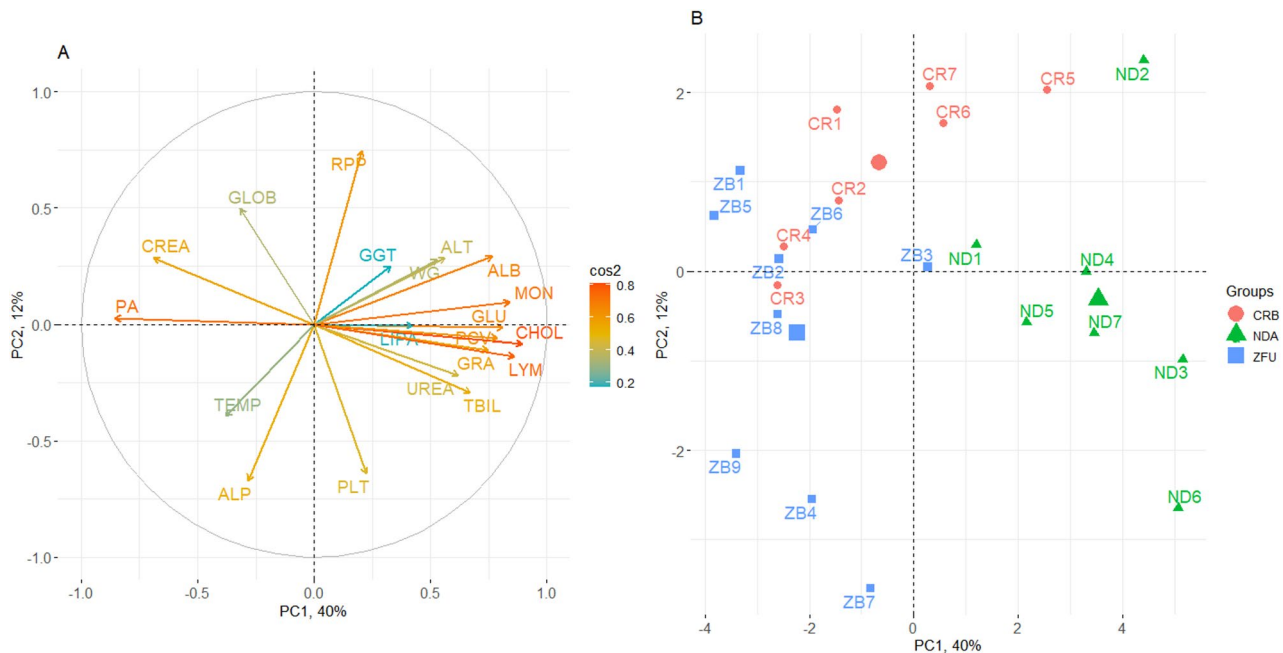


Fig. 10 Principal component analysis (PCA) of the biological variables during infection. **A** Correlation circle of the variables used in the PCA for the principal components (PC) 1 and 2. **B** Factorial plan for PC1 and 2 showing the coordinates of the individuals

the findings of Murray [52], who reported a lower rectal temperature in N'Dama than in zebu during *T. vivax* infection. Better controlled inflammatory responses associated with low parasitaemia, or differences in the thermoregulatory system [53], might explain the lower rectal temperature observed in N'Dama.

The monitoring of PCV, reflecting anaemia, parasitaemia and growth, which are commonly used to define trypanotolerance [54], gave consistent results with previous experiments that compared N'Dama and other West African taurine breeds to zebu populations [6, 11, 16]. Indeed, the NDA cattle breed showed a better response to infection by controlling anaemia and parasitaemia, as well as maintaining a positive growth rate, while the ZFU and CRB cattle groups were anaemic with high parasitaemia. Nevertheless, it should be noted that none of the cattle breeds in the study showed clearance of parasites, as parasites were still detected at the end of the study in a majority of animals, whatever the breed. This suggests that, in absence of an efficient treatment, cattle are long-term carriers of trypanosomes, which sustains the epidemiological cycle. Although one CRB animal succumbed, the crossbred cattle group was, on average, no more susceptible to trypanosomosis than ZFU, contrary to what might have been expected. Anaemia and parasitaemia showed highly similar profiles between CRB and ZFU but, on average, the growth of CRB cattle was not significantly more affected by infection than NDA cattle, and CRB were spread from ZFU to NDA on the PCA run on post-infection data. An important point that might

explain the result is the higher initial weight that could have mitigated the impact of infection in CRB cattle.

Blood cell changes

The sharp drop in PCV, which coincided with the presence of parasites in the blood, was triggered by trypanosome multiplication. PCV and parasitaemia were highly negatively correlated during infection, as already observed in previous studies [6, 55]. The erythrocyte indices (MCHC, MCV, MCH) showed variations over the course of the infection, but MCV and MCH nevertheless remained within the usual range of values, allowing the type of anaemia that occurred in our study to be described as normocytic and normochromic. This is consistent with the study by Akinbamijo et al. [56]. The ability of NDA cattle to control the early decline in PCV and subsequently recover normal values, unlike susceptible cattle, was previously reported [15, 16]. The control of anaemia is considered the most important trait for overall productivity [57] and it could be linked to a greater response of the haemopoietic cells line, according to Naessens et al. [16], or reduced haemolysis. Haemolysis in trypanosomosis is hypothesised to be triggered by several mechanisms [58], including intravascular haemolysis [59], and extravascular haemolysis, primarily through erythrophagocytosis [60, 61]. Overall, our study was not specifically designed to characterize causes of anaemia and its control in N'Dama. However, the results obtained in the current study could support the hypothesis of enhanced haematopoiesis, as proposed by

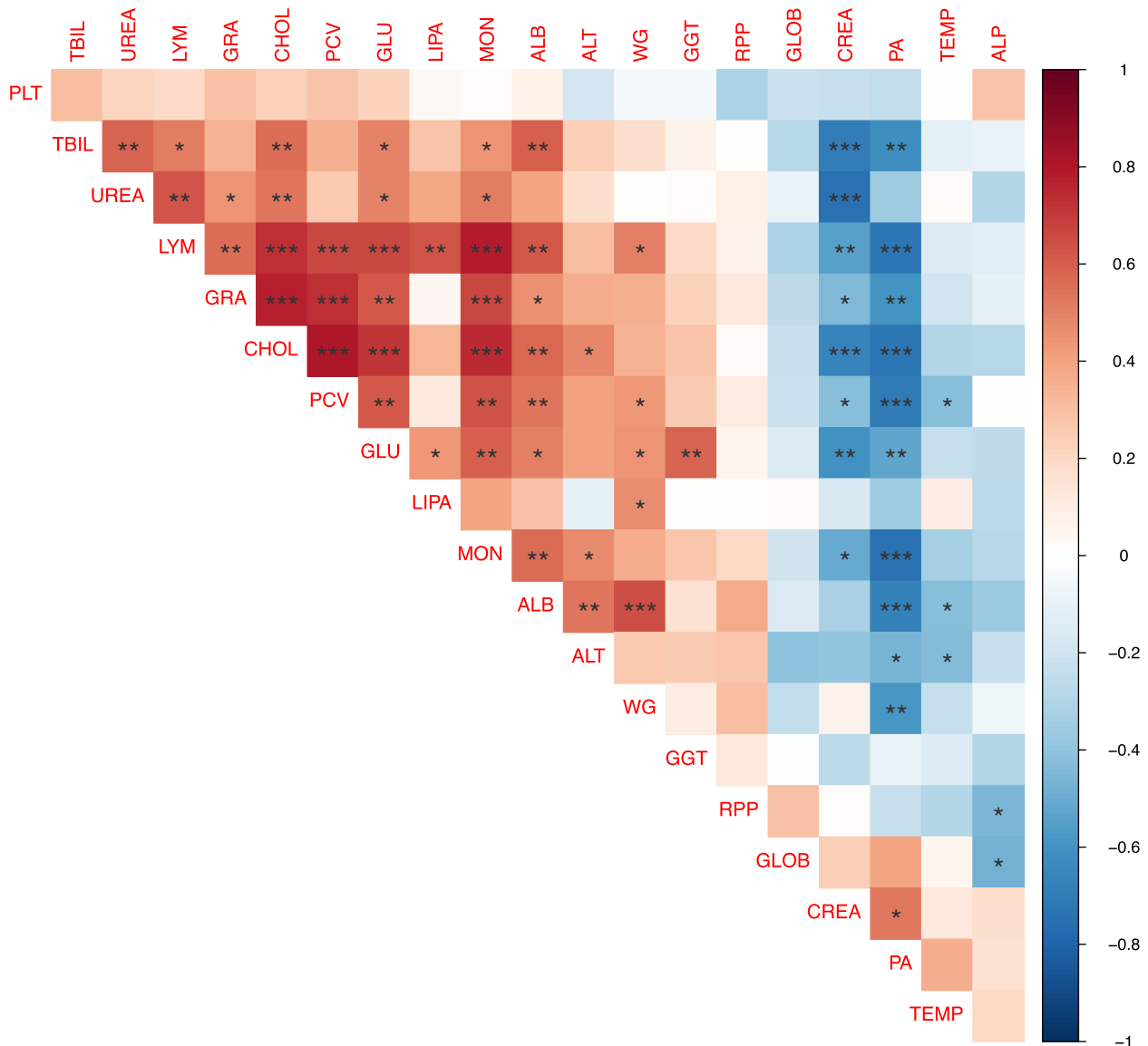


Fig. 11 Correlation between variables over the study. The colour scale shows the direction of correlations and strength of correlation, red indicates a positive correlation, while blue indicates a negative correlation. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Logan-Henfrey et al. [15], and Naessens [62], with a better overall ability of N'Dama to produce several cell lineages at the origin of red blood cells and immune cells, as well as reduced haemolysis. The reduction in haemolysis could be the result of a better and more suitable immune response, capable of limiting parasitaemia and the associated induced erythrophagocytosis.

A strong decrease in PLT counts was seen in all the cattle, with greater thrombocytopenia in ZFU and CRB cattle than in NDA cattle. A fall in platelet counts has already been reported in infected cattle [25, 63] and, interestingly, a very recent study reported modifications in the expression of genes associated with the coagulation pathway [64]. In relation with the drop in PLT, MPV increased post-infection. An increase in MPV is

associated with enhanced megakaryocyte growth in response to thrombopoietic stress, particularly when there is peripheral platelet destruction [65]. MPV values remained low, though it was difficult to interpret as they started below what are considered as normal values for European cattle. The early decrease in platelet counts may have been due to consumption of platelets resulting from the formation of microthrombi induced by parasites [66], given that *T. congolense* tends to bind to the walls of blood vessels [67], or may have resulted from platelet phagocytosis by macrophages in bone marrow [68]. The relative variation in PLT was not correlated to any other variables and PLT did not return to pre-infection values in any of the cattle, contrary to PCV in NDA cattle, highlighting a continuous process leading to

thrombocytopenia or some impairment to replenish the stock of platelets.

A striking difference was observed between the cattle breeds regarding the different populations of white blood cells, lymphocytes, monocytes and granulocytes. Highly significant negative correlations were observed between lymphocytes, monocytes and granulocytes with parasitaemia. Lymphocytosis and monocytosis were observed in NDA cattle, while lymphocyte and monocyte counts did not respond to infection in ZFU and CRB cattle. Lymphocytosis has been reported in N'Dama cattle and lymphocytopenia in Boran cattle [16, 25]. An increase in lymphocyte sub-populations, such as $\gamma\delta$ T cells during *T. vivax* infection in crossbreed Girolando cattle in Brazil [69], or B cell populations in *T. congolense*-infected N'Dama and Boran [70], has been reported. The observed lymphocytosis may have been due to an increase in one or more lymphocyte sub-populations, most likely B cells [70]. Monocyte counts presented the highest negative correlation with parasitaemia. In addition, monocyte counts were especially raised in the late phase of infection, roughly 3 months after parasite inoculation, which corresponded to the waning of parasitaemia. This suggests a physio-pathological link between both variables. Monocytes have been shown to directly phagocytose trypanosomes [71] and initiate the immune response by secreting co-stimulatory cytokines [72]. In addition, they also play a pivotal role by enhancing the haemopoietic system [73]. Granulocyte counts decreased in the ZFU and CRB cattle, while remaining normal in the NDA cattle. Granulocytes are involved in the clearance of opsonized trypanosomes in infected cattle [74]. The loss of these cells from their circulation may therefore severely impair the ability of ZFU and CRB animals to clear opsonized parasites. In general, it is suggested that greater numbers of lymphocytes, monocytes, granulocytes and eosinophils in their circulation endow trypanotolerant cattle with a greater capacity than trypanosusceptible cattle to control parasitaemia by phagocytosis and immune-mediated mechanisms [15], and this is strongly supported by our study. Trypanosomes trigger the production of antibodies, which plays a crucial role in protecting against infection, as the host does not survive in the absence or reduced level of antibodies [75]. In this study, total IgG appeared earlier in NDA cattle compared to ZFU and CRB cattle, and gradually increased throughout the study in all the cattle. However, we did not find any significant differences between NDA and ZFU or NDA and CRB, or a negative correlation between parasitaemia and the relative percentage of IgG positivity. According to Naessens et al. [62], the differences in antibody titres between trypanotolerant and trypanosusceptible cattle may be a consequence and not a cause of differences in parasitaemia and there is no link

between total IgG and trypanotolerance. Nevertheless, more specific studies on antibodies directed against specific parasite proteins would be needed, as Authié et al. [76] proposed that a specific antibody response could be responsible for trypanotolerance.

Plasma biochemical changes

An overall impact of infection was observed on the biochemical variables whatever the breed. Indeed, infection greatly disrupted cholesterol and glucose concentrations in plasma. This confirmed earlier studies, since it is known that bloodstream forms of trypanosomes are unable to synthesize cholesterol, although they require it for growth and synthesis of their membranes [20]. In this study, NDA cattle showed better control of cholesterol levels than ZFU and CRB cattle. In light of the aforementioned importance of cholesterol and significant differences observed in the expression of genes involved in cholesterol regulation during trypanosomosis [22], we suggest that cholesterol could be a key variable involved in better control of clinical disorders, associated with trypanotolerance. A drop in glucose levels was observed during infection, but NDA cattle returned to their pre-infection levels at the end of the study, unlike ZFU and CRB cattle. The use of glucose as an energy source, on the one hand by trypanosomes [17] and on the other hand by immune cells [21], might explain its decrease in the blood. Unlike cholesterol, a further study is needed to better decipher the potential role of glucose in trypanotolerance, as glucose levels were significantly higher in ZFU and CRB cattle than NDA cattle before infection, which could mitigate their decrease during infection.

A change in plasmatic enzymes associated with muscle has diagnostic value in assessing the extent of metabolism disruption and tissue injury [14]. ALT, which is primarily produced by muscle in cattle, differed between NDA cattle and ZFU and CRB cattle. ALT level profiles were shown to be highly variable in previous experiments [17, 77]. In our study, the variations in ALT, both temporal and between breeds, may have been due to an impact of trypanosome infections on protein metabolism, with an indirect effect on muscular tissue. By assessing the impact of infection on protein metabolism, such as albumin and globulin, we observed hypoalbuminemia in ZFU, and decreased globulin in all breeds at the onset of infection, before returning roughly to pre-infection levels. A transitory decrease of albumin level was reported in zebu cattle [17], and in Girolando cattle [18] infected with *T. vivax*. The latter authors also reported increased globulin levels, unlike our study. The decrease in these two proteins may have been due to a reduced protein intake linked to undernourishment during the acute phase of the disease, caused by the inflammation response or anaemia [78].

A temporary increase in liver enzymes, ALP and GGT, was observed, though they remained within normal limits. This could mark transient liver abnormality, which nevertheless did not lead to a severe release of enzymes into the blood in our study. Total bilirubin levels decreased to the range of usual values throughout the infection in all cattle breeds. This suggests that, though red blood cell destruction may be taking place, the liver retained its ability to conjugate the excess bilirubin produced during this period [79]. The assessment of renal function via urea and creatinine did not reveal any significant differences between the breeds, and the recorded values were within the range of normal values, suggesting the absence of noticeable kidney damage. Overall, liver and renal tissues did not appear to be significantly affected by infection in our study. Ultimately, it is worth emphasising that strain IL1180 of *T. congolense* used in this experiment is known to be moderately pathogenic in cattle. Genetic and phenotypic variations have been reported in *T. congolense* [80] and the impacts of more pathogenic strains of *T. congolense* could be greater on tissue lesions and result in greater changes in biochemical variables.

Conclusion

Our study provided the first joint comparative characterization of the dynamics of haematological and biochemical variables involving N'Dama cattle, Fulani zebu, and zebu crossed with European taurine, all experimentally infected with *T. congolense*. Positive variations in lymphocyte and monocyte counts, and control of the decrease in granulocyte count, packed cell volume, cholesterol and glucose concentrations were positively correlated to each other and negatively correlated with parasitaemia. The drop in platelet counts was also an important but often overlooked pathological effect of *T. congolense* infection. Lastly, our study highlighted a major differential evolution of white blood cells and of certain biochemical variables, particularly cholesterol, depending on the breed and in association with trypanotolerance. These associations indicated likely physio-pathological links between trypanotolerance, characterized by the mitigation of symptoms, anaemia and parasitaemia, an efficient immune response, exemplified here by white blood cell profiles, and limitation of metabolic disorders, especially the control of hypocholesterolaemia and hypoglycaemia. Beyond statistical associations, experiments and mechanistic models combining genomic, transcriptomic and detailed phenotyping of the immune response should be undertaken to decipher the link between parasitaemia and anaemia control, immune response and metabolic pathways, in order to elucidate the molecular and cellular bases of trypanotolerance.

Abbreviations

AAT	African Animal Trypanosomosis
AFT	African taurine
ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
BC	Buffy coat
BW	Body weight
CHOL	Cholesterol
CIRDES	Centre International de Recherche-Développement sur l'Élevage en zone Subhumide
CRB	Crossbreed
CREA	Creatinine
DEAE	Di-Ethyl Amino Ethyl
DPI	Day post infection
EDTA	Ethylene Diamine Tetra Acid
ELISA	Enzyme Linked Immuno-Sorbent Assay
EOS	Eosinophils
EUT	European taurine
GAMs	General Additive Models
GGT	Gamma-glutamyl transferase
GLOB	Globulin
GLU	Glucose
GRA	Granulocytes
HB	Haemoglobin
IgG	Immunoglobulin G
KW	Kruskal-Wallis
LIPA	Lipase
lme	Linear mixed model
LYM	Lymphocyte
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
ML	Maximum Likelihood
MON	Monocyte
MPV	Mean platelet volume
NDA	N'Dama
PA	Parasitaemia
PCA	Principal component analysis
PCV	Packed cell volume
Pd-WC	Paired Wilcoxon
PLT	Platelets
PSG	Phosphate-buffered saline Glucose
Pw-WC	Pairwise Wilcoxon
RBC	Red blood cells
REML	Restricted Maximum Likelihood
RPP	Relative percentage of positivity
SD	Standard deviation
SE	Standard error
SNPs	Single Nucleotide Polymorphisms
TBIL	Total bilirubin
TEMP	Temperature
WIDDE	Web-interface next generation Database dedicated to genetic Diversity Exploration
ZFU	Zebu Fulani

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12917-025-05235-4>.

Additional file 1: ARRIVE Compliance Questionnaire

Additional file 2: Indirect Enzyme Linked Immuno-Sorbent Assay (ELISA) to detect IgG antibodies directed against *T. congolense* IL1180

Additional file 3: Principal component analysis of the genotypic data (large data set). The individuals are plotted on the first two principal components according to their coordinates. Each breed is designated by a specific colour. The individuals genotyped in this study are labelled as NDA-CBT, ZFU-CBT and CRB-CBT. The other breeds are: ABO (Abondance), ANG (Angus), AUB (Aubrac), AYR (Ayrshire), BAO (Baoulé), BOR (Borgou), BRA (Brahman), BRU (French brown Swiss), CGU (Creole from Guadeloupe), CHA (Charo-

lais), EAZ (East African shorthorn Zebu), GAS (Gascon), GBV (Gelbvieh), GIR (Gir), GNS (Guernsey), HOL (Holstein), JER (Jersey), KUR (Kuri), LAG (Lagune), LIM (Limousine), MAN (Maine-Anjou), MAR (Maraichine), MON (Montbéliarde), ND1 to ND4 (N'Dama), NEL (Nelore), NOR (Normande), NRC (Norwegian Red Cattle), ONG (Ongole), PMT (Piemontaise), RAN (Red Angus), RMG (Romagnola), SAH (Sahiwal), SAL (Salers), SGT (Santa Gertrudis), SHO (Shorthorn), SOM (Somba), TAR (Tarine), VOS (Vosgienne), ZBO (Bororo Zebu), ZEB (Fulani Zebu), ZFU (Fulani Zebu), ZAM (Zebu from Madagascar)

Additional file 4: Principal component analysis of the genotypic data (focus on the ZFU data set). The individuals are plotted on the first two principal components according to their coordinates. Each breed is designated by a specific colour. The individuals genotyped in this study are labelled as ZFU-CBT. The other breeds are EAZ (East African shorthorn Zebu), KUR (Kuri), ZBO (Bororo Zebu), ZEB (Fulani Zebu), ZFU (Fulani Zebu), ZAM (Zebu from Madagascar)

Additional file 5: Principal component analysis of the genotypic data (focus on the NDA data set). The individuals are plotted on the first two principal components according to their coordinates. Each breed is designated by a specific colour. The individuals genotyped in this study are labelled as NDA-CBT. The other breeds are BAO (Baoulé), LAG (Lagune), ND1 to ND4 (N'Dama), SOM (Somba), ZFU (Fulani Zebu)

Additional file 6: Principal component analysis of the genotypic data (focus on the CRB data set). The individuals are plotted on the first two principal components according to their coordinates. Each breed is designated by a specific colour. The individuals genotyped in this study are labelled as CRB-CBT. The other breeds are ABO (Abondance), AUB (Aubrac), BRU (French brown Swiss), CHA (Charolais), HOL (Holstein), JER (Jersey), MAR (Maraichine), MON (Montbéliarde), SAL (Salers), VOS (Vosgienne), ZEB (Fulani Zebu), ZFU (Fulani Zebu)

Additional file 7: Table of the results of the supervised hierarchical clustering of the 24 individuals genotyped in this study, plus 925 individuals stored in the WIDDE database, with an inferred number of clusters $k = 3$ obtained with ADMIXTURE 1.23. For each individual, the proportions of each cluster were interpreted as representative of EUT, AFT and ZEB ancestries. The readme sheet specifies the name of the breeds

Additional file 8: Table showing the results of statistical analyses of the different variables measured before infection. The first column is the name of the variable, the second is the unit of measure, the third is the mean breed effect, with NDA as the reference of the contrast and ZFU and CRB effects relative to NDA, as assessed by a linear mixed effect model with t-test p-value, the fourth column gives the ANOVA p-value (F-test), and the last shows the usual values in cattle when available

Additional file 9: Change in rectal temperature by breed during the course of the study. The vertical dashed line indicates the day of infection for all animals. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 10: Change in body weight by breed during the course of the study. The vertical line indicates the day of infection for all animals. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 11: Change in red blood cell count by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 5 and 10 indicate the limits of usual values for red blood cells in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 12: Change in haemoglobin concentration by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 8 and 15 indicate the limits of usual values for haemoglobin in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 13: Change in mean corpuscular haemoglobin concentration by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 30 and 36 indicate the limits of usual values for MCHC in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 14: Change in mean corpuscular volume by breed during

the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 40 and 60 indicate the limits of usual values for MCV in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 15: Change in mean corpuscular haemoglobin by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 11 and 17 indicate the limits of usual values for MCH in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 16: Change in mean platelet volume by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 6 and 8 indicate the limits of usual values for MPV in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 17: Change in eosinophil count by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 0 and 2.4 indicate the limits of usual values for eosinophil in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 18: Change in percentage of positivity by breed during the course of the study. The vertical line indicates the day of infection for all animals. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 19: Change in albumin levels by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 3 and 3.6 indicate the limits of usual values for albumin levels in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 20: Change in alanine aminotransferase levels by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 25 and 74 indicate the limits of usual values for ALT levels in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 21: Change in alkaline phosphatase levels by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 0 and 589 indicate the limits of usual values for ALP levels in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 22: Change in globulin levels by breed during the course of the study. The vertical line indicates the day of infection for all animals. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 23: Change in creatinine levels by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 0.9 and 2 indicate the limits of usual values for creatinine levels in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 24: Change in urea levels by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 16 and 39 indicate the limits of usual values for urea levels in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 25: Change in gamma-glutamyl transferase levels by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 22 and 64 indicate the limits of usual values for GGT levels in cattle. Confidence intervals of the mean are underlined. DPI: days post infection

Additional file 26: Change in total bilirubin levels by breed during the course of the study. The vertical line indicates the day of infection for all animals. Horizontal dashed lines at 0 and 0.5 indicate the limits of usual values for total bilirubin levels in cattle. Minimal and maximal values are underlined. DPI: days post infection

Additional file 27: Change in lipase levels by breed during the course of the study. The vertical line indicates the day of infection for all animals. Confidence intervals of the mean are underlined. DPI: days post infection

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Authors' contributions

ST, DB-T and IC designed the study, developed the experiment and supervised study implementation. GFS, MO, and HS collected and analysed the samples. ST, GS and GFS analysed the data. ST and GFS were major contributors to the writing of the manuscript. GS performed the analysis of genotypes. MS, MBS, GKD ensured the smooth implementation of the experiment and provided much advice during the experiment and for the writing of the manuscript. All the authors read and approved the final manuscript.

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Data availability

The datasets generated and analysed during the current study, as well as the R scripts used for statistical analyses are publicly available in the CIRAD DataVerse repository at the URL [<https://doi.org/10.18167/DVN1/7Q91RE>].

Declarations

Ethics approval and consent to participate

The experimental protocol was reviewed and approved by the *Centre International de Recherche-Développement sur l'Élevage en zone Subhumide* (CIRDES) external ethics committee (No: 01_12/2022/CE-CIRDES).

Consent for publication

"Not applicable".

Competing interests

The authors declare no competing interests.

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