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Hematological and serum biochemical changes in gilts experimentally infected with *Trypanosoma brucei*

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ABSTRACT

The aim of this experiment was to study the hematological and serum biochemical changes in domestic cross breed female gilts infected with *Trypanosoma brucei* parasites. Twelve gilts were purchased from piggeries in Zaria Nigeria and housed in clean, fly proof pens in two groups of six infected and six controls. The gilts in the infected group were inoculated with approximately 1.8×10^6 *Trypanosoma brucei* parasites. All the inoculated gilts developed clinical trypanosomosis after a prepatent period of three (3) days. Significant differences ($P<0.05$) were observed in the mean values of Packed Cell Volume (PCV), total white blood cells and differential leukocytes count, serum proteins, aspartate amino transferase, creatine kinase, potassium, inorganic phosphates and calcium between the infected gilts and the controls. The implication of these findings in the pathology of trypanosomosis in this species is discussed.

Key words: *Trypanosoma brucei,* gilts, hematology, serum chemistry

Introduction

Trypanosomosis is a major constraint to pig production in Nigeria (MADUBUNYI, 1988). Natural infections of pigs with trypanosomes have been reported in many parts of the country (AGU and BAJEH, 1986; ONAH, 1991; OMEKE and ONUORA, 1992; OMOTAINSE et al., 2000).

When animals become infected with trypanosomosis, their physiology alters (BIRYOMUMAISHO et al., 2003). This is due to the wide range of blood biochemical

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changes (KATUNGUKA-RWAKISHAYA, 1996) and hematological aberrations that occur (ANOSA and ISOUN, 1980).

The evaluation of blood indices and parameters helps to determine the health status of animals (COLES, 1986), and also to establish the degree of damage to hosts tissues as well as the severity of the infection (OTESILE et al., 1991).

This study was therefore conducted to determine the hematological and biochemical changes that occur in gilts inoculated with *Trypanosoma brucei,* in order to understand the pathological bases for trypanosome infection of the animal.

Materials and methods

Experimental animals. Twelve domestic cross breed female piglets aged eight weeks were purchased from two piggeries in Zaria and housed in clean, fly proof pens at the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. The piglets were ear notched for identification and screened for endo, ecto and hemoparasitic infections. Baseline hematological data were obtained and the piglets were treated against nematodes and ectoparasites, such as mites, with Ivermectin (Ivomec ®) at the dose rate of 200 µg/kg body weight sub-cutaneously. The piglets were fed on a compounded diet of 18% crude protein comprising of maize, 36.8%; soya bean, 5%; ground nut cake, 23.5%; rice bran, 30%; beniseed cake, 2%; bone meal, 2%; premix, 0.2%; table salt, 0.5%, with water provided *ad-libitum* throughout the period of the study.

*Trypanosomes.*The *Trypanosoma brucei* used in this experiment was originally isolated from a pure natural infection in cattle in Federe, Kaduna state in 1995. It was obtained from The Nigerian Institute of Trypanosomiasis Research, Vom, Nigeria where it was inoculated into two mice and transported to the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. On arrival at the Faculty, the levels of parasitemia in the mice were determined using the method described by WOO (1969). The infected blood of the mice was collected at high parasitemia and inoculated into five rats. At high parasitemia, the rats were anesthetized in a jar containing chloroform and bled through cardiac puncture when the pigs were due to be inoculated. The infected blood of the rats was then pooled with ethylene diamine tetra acetic acid (EDTA) as anticoagulant and inoculated into the gilts.

Experimental design. At seven months old, the pigs were randomly divided into two groups, six infected and six uninfected controls.

Inoculation of animals. The piglets were monitored very closely for two months, after which those in the infected group were inoculated with two milliliters each of the infected blood containing $1.8x10^6$ parasites via the anterior vena cava. The gilts in the control group were not inoculated.

Clinical examination and blood sampling for parasitemia. Following inoculation, the pigs were clinically examined and their rectal temperatures were monitored daily. Their blood was also examined daily until parasites were eventually seen in the blood of all the infected pigs. Subsequently, physical examination was conducted on all the pigs and their blood was obtained to determine the levels of parasitemia. This continued throughout the period of the experiment.

Thin blood films for trypanosome identification. About one micro liter of blood was placed on clean grease-free glass slides and thin smears were made with the aid of another microscope slide. The slides were air dried and fixed in methanol for three minutes. They were then stained in 10% Giemsa, air dried and examined under oil immersion using a microscope at ×1000 magnification. Identification of parasites was done using morphological description of HOARE (1972).

Determination of Packed Cell Volume (PCV). Microhematocrit capillary tubes containing approximately 70 μL of blood in anti-coagulant were centrifuged at 906 g for 3 minutes. The PCV was determined using a Hawsley microhematocrit reader (Gelman, Hawsley Ltd, England).

Hematological and serum biochemical analysis. About 8 milliliters of blood were obtained from all the pigs in both the infected and the control groups via the anterior vena cava once every week. Two milliliters of the blood was transferred into test tubes containing anticoagulant for hematological analysis while 6 milliliters was transferred into tubes that do not contain anticoagulant. The tubes without anticoagulant were kept in a refrigerator overnight. The serum that separated out of the clotted blood was then transferred into serum vials and was subsequently used for serum biochemical analysis.

Statistical methods used. The students t-test statistical analysis was used to analyze all the results obtained.

Results

Clinical signs. All the inoculated pigs developed clinical trypanosomosis, which was characterized by fever, pale mucous membranes, anorexia, dullness, reduced weight gain, weight loss, emaciation, recumbency, short and moist cough, moist rales, un-coordinated movements, mucopurulent ocular discharges, hyperemia of the skin, lethargy, reduction in feed intake, posterior paresis and death of two infected pigs.

Parasitemia. All the infected pigs developed parasitemia on the 3rd day post infection (p.i.). Peak parasitemia was attained between day 7 and 42 p.i. Thereafter, there were fluctuations in the levels of parasitemia which continued until the end of the study (Fig. 1). Two pigs died on days 42 and 92 p.i., during which time their levels of parasitemia were (4+ and 3+) respectively.

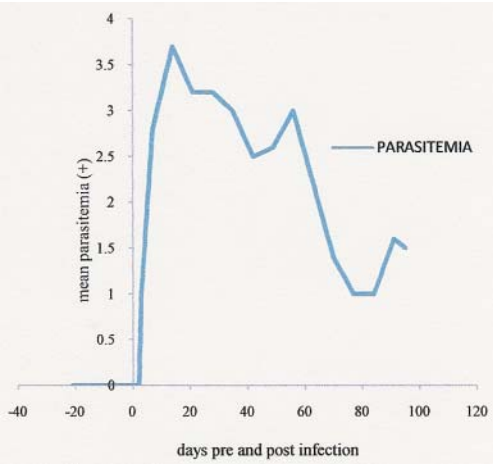

Fig. 1. Mean parasitemia of *T. brucei* infected gilts

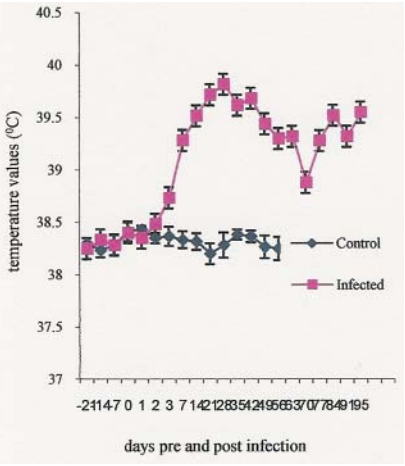

Fig. 2. Mean temperature changes of *T. brucei* infected and control gilts

Temperature changes. The mean rectal temperatures of the infected pigs started rising from the 3rd day p.i. The rise in temperature of the infected animals was followed by fluctuations. The highest mean temperature noticed during the study was 39.8 °C, on day 28 p.i. The temperatures of the infected animals were significantly higher than those of the control ($P<0.05$), (Fig. 2).

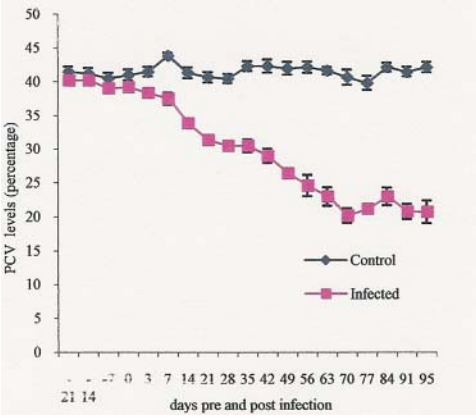

Fig. 3. Mean packed cell volume of of *T. brucei* infected and control gilts

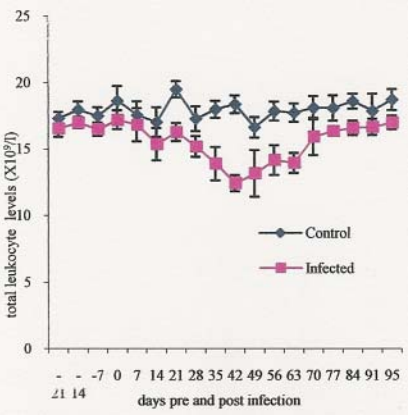

Fig. 4. Mean leukocytes levels of *T. brucei* infected and control gilts

Packed cell volume (PCV). There was a gradual decrease in the mean PCV values of the infected pigs. This started from day 14. The lowest mean PCV value recorded was 20.2%., which was noticed on day 70 p.i. The mean PCV values of the infected animals were significantly lower than those of the control ($P<0.05$), (Fig. 3).

Mean total and differential white blood cell counts. A drop in the mean total leukocytes count started on day 35 p.i. in the infected gilts, until the lowest mean value of 12.4×10^9/L was attained on day 42. This was followed by a gradual increase in the total leukocyte count, which started from day 70 p.i. This increase rose to almost pre infective values (Fig. 4). The total leukocyte count of the infected gilts was significantly lower than that of the control ($P<0.05$).

A drop in the mean neutrophil levels of the infected animals was observed from day 35p.i. The mean values remained low until the termination of the experiment (Fig. 5). The mean neutrophils levels of the infected gilts were statistically different ($P<0.05$) from those of the control.

High levels of mean lymphocyte count were observed in the infected gilts during the course of the experiment. The increase in the levels started from day 7 p.i. (Fig. 6). The mean lymphocyte count of the infected animals was significantly different from that of the control animals ($P<0.05$).

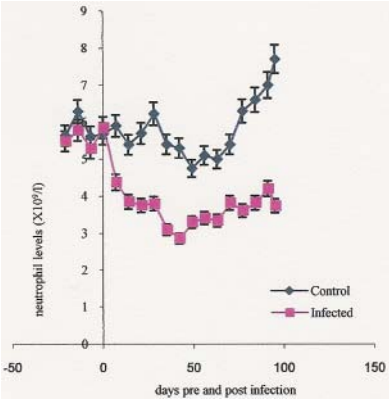

Fig. 5. Mean neutrophils levels of *T. brucei* infected and control gilts

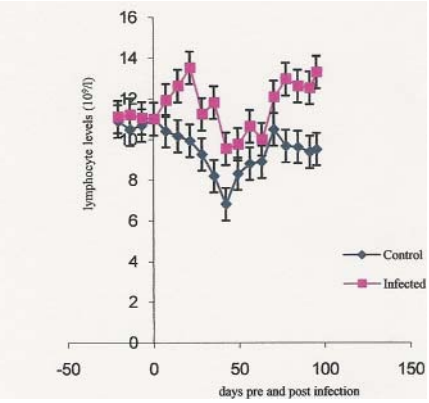

Fig. 6. Mean lymphocyte levels of *T. brucei* infected and control gilts

The mean levels of eosinophils were consistently low in the infected gilts during the course of the infection (Fig. 7). The levels were significantly lower than that of the control ($P<0.05$).

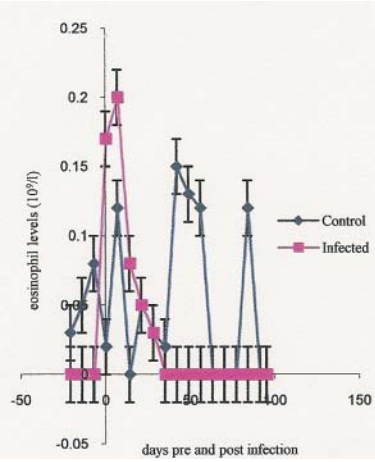

Fig. 7. Mean eosinophils levels of *T. brucei* infected and control gilts

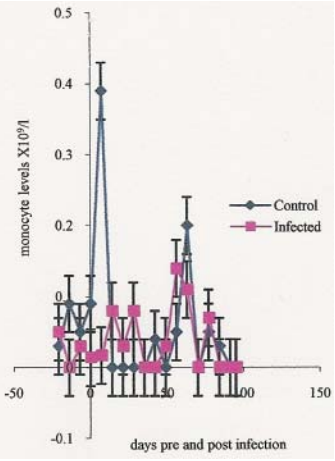

Fig. 8. Mean monocytes levels of *T. brucei* infected and control gilts

No significant change was observed in the mean total monocyte (Fig. 8) and band-neutrophil counts of the infected animals (Fig. 9). The mean levels of total monocytes and band neutrophils were not significantly different from those of the control animals ($P<0.05$).

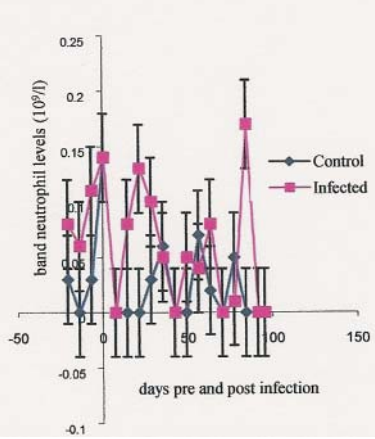

Fig. 9. Mean band neutrophils levels of *T. brucei* infected and control gilts

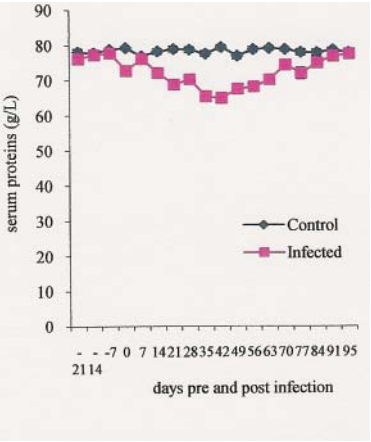

Fig. 10. Mean total proteins levels of *T. brucei* infected and control gilts

Serum biochemical changes. A gradual decrease in the mean values of total proteins was observed in the infected animals. This decrease was noticed on day 35 p.i. with mean values of 65.5 g/L and continued to day 56 p.i. On day 63 the mean values of total protein started to rise. The values rose beyond pre infective levels (Fig. 10).

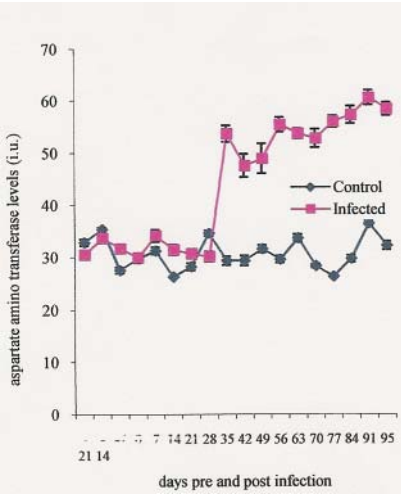

Fig. 11. Mean aspartat amino transferase levels of *T. brucei* infected and control gilts

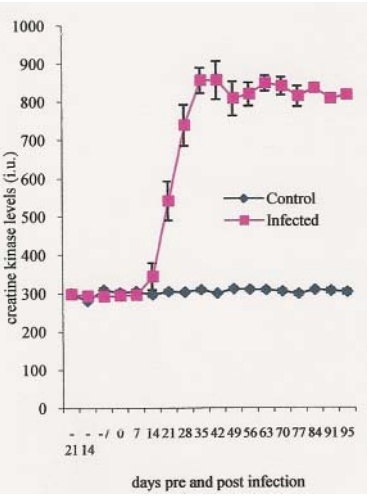

Fig. 12. Mean creatine kinase levels of *T. brucei* infected and control gilts

There were increases in the mean levels of the serum enzymes of the infected animals that were analyzed. The levels of the enzymes were increasingly significant as the disease progressed in all the infected gilts ($P<0.05$). The levels of enzymes in the control animals were all within normal range. Mean aspartate amino transferase (AST) concentrations of the infected pigs increased significantly from 35 day p.i. and the levels remained elevated until the end of the study. The highest mean level of 60.6 I.U/L was recorded on day 91 p.i. (Fig. 11).

The mean serum levels of creatine kinase (CK) increased progressively from 21 days p.i. until the end of the experiment in the infected animals. The highest mean level recorded for this enzyme was 856.7 I.U/L on 42 day p.i. The values of CK were `statistically significantly different ($P<0.05$) from those of the control animals (Fig. 12).

The mean serum values of potassium in the infected gilts increased progressively during the study. This increase started on day 14 p.i. The highest mean level recorded was 8.6 mmol/L on day 63 p.i. These levels were statistically different ($P<0.05$) from those of the control animals (Fig. 13).

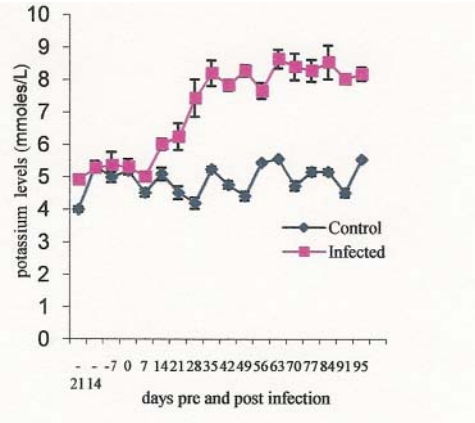

Fig. 13. Mean potassium levels of *T. brucei* infected and control gilts

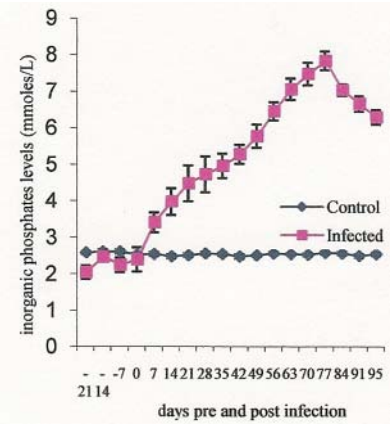

Fig. 14. Mean inorganic phosphate levels of *T. brucei* infected and control gilts

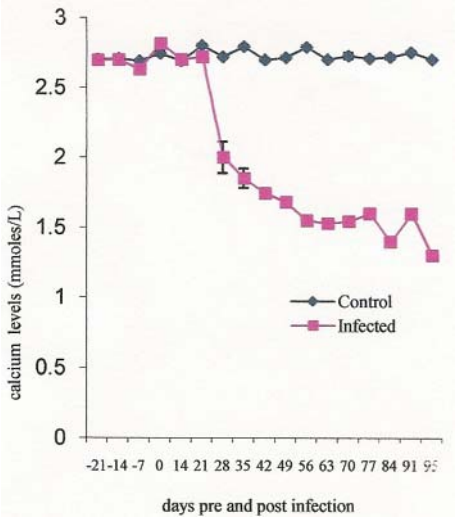

Fig. 15. Mean calcium levels of *T. brucei* infected and control gilts

The mean levels of inorganic phosphates were elevated, while those of calcium were depressed in the infected animals during the course of the infection. The elevation in the levels of inorganic phosphates started from day 56 p.i. and continued to rise until the end of the study (Fig. 14). Whilst the decrease in the levels of calcium started on day 35 p.i., calcium levels remained low until the end of the experiment (Fig. 15). The mean levels

of inorganic phosphates and calcium were statistically different ($P<0.05$) from those of the control animals.

Discussion

The *Trypanosoma brucei* used in this experiment caused clinical trypanosomosis in all the infected gilts, characterized by persistently high rectal temperatures and parasitemia, which were later followed by fluctuations. Anemia indicated by a drop in PCV values was observed during the course of this infection. It is the most consistent feature of trypanosomosis caused by *T. vivax, T. congolense* and *T. brucei* (ANOSA, 1983a, and 1983b). The etiology of this anemia is complex, but the most important factor is said to be hemolysis based on a reduction in red cell mass and life span and also on the occurrence of erythrophagocytosis, hemosiderosis and sometimes hyperbilirubinemia (ANOSA, 1983a and 1983b).

The depression in the mean leukocyte count and the eventual elevation observed during this infection agrees with findings by SAROR (1975). The depression of leukocyte levels could have been a result of immuno-suppression, which usually co-exists with trypanosomosis. The eventual rise in its levels can be attributed to the apparent recovery of the animals immunologically as previously reported by ANOSA and ISOUN (1980).

The decrease in the values of neutrophils seen in this study agrees with findings by ANOSA and ISOUN (1980), but disagrees with CHAUDHARY and IQBAL (2000), who observed an increase in the levels of neutrophils in camels infected with *T. evansi.* The decrease could have been a result of overwhelming secondary bacterial infection that comes about as a consequence of immuno-depression in animals infected with trypanosomosis. Also massive amounts of circulating neutrophils are mobilized into the organs that have ongoing severe inflammatory reactions as noticed in this study (LORDING and FRIEND, 1991).

The persistent increase in the mean lymphocyte levels contradicts observations by CHAUDHARY and IQBAL (2000), who reported a decrease in lymphocytes in camels infected with *Trypanosoma evansi.* In the present study, the lymphocyte count increased significantly. The observed increase in the lymphocyte count could be a result of the relative depression of neutrophils during the infection or as a result of the immune response by the animals during the chronic phase of the infection. The eosinopenia noticed in this study was also observed in goats and sheep infected with *T. vivax* by ANOSA and ISOUN (1980) and in mice infected with *T. brucei* (ANOSA, 1975). The eosinopenia could have resulted from the chronic nature of trypanosomosis (LORDING and FRIEND, 1991).

The gradual decrease in the mean values of total plasma proteins, observed in the infected animals during this study, agrees with previous findings (BIRYOMUMAISHO et al., 2003; KATUNGUKA-RWAKISHAYA, 1996), but contradicts observations made in sheep

infected with *T. brucei by* TAIWO et al. (2003), who observed no change in levels of total plasma proteins from pre-infected values at the initial stage of the infection, but in the later stage the levels increased significantly above pre-infection levels. Protein levels usually drop in trypanosome infections as a result of depressed albumin levels. The increase in protein levels during the chronic phase of the infection is usually due to the increase in globulin levels. This is as a result of the immune response by the animals to the infection (ANOSA, and ISOUN, 1976; SINGH and GAUR, 1983; RAJORA et al., 1986).

The decrease in the levels of calcium and the elevated levels of inorganic phosphates that were observed in this study agrees with findings in cattle infected with *T. congolense* (FIENNES et al., 1946), and also in sheep infected with *T. brucei* (OGUNSANMI et al., 1994). This is said to be due to the deficiency in the parathyroid hormone as a consequence of the destruction of the parathyroid glands or a decrease in serum carriers, which in this case happens to be albumin.

The increase in AST levels noticed in this study agrees with the results obtained during an infection in sheep by *T. brucei* (TAIWO et al., 2003), *T. vivax* infection of cattle and sheep (GRAY, 1963), *T. congolense* infection of goats (ADAH et al., 1992), and also dogs infected with *T. brucei* (OMOTAINSE et al., 1994). However it contradicts observations made by TAIWO et al., (2003) during an infection of sheep with *T. congolense.* The causes of the elevation of AST levels in the serum of animals are necrosis of the liver, skeletal muscles and kidneys (LORDING and FRIEND, 1991).

The increase in the levels of CK agrees with results obtained in a *T. cruzi* infection in mice (CANO et al., 2000), but disagrees with results obtained by LUCKINS, 1992; CHAUDHARY and IQBAL (2000), who observed no change in CK values in animals infected with trypanosomosis. CK is increased in skeletal muscle disease, myocardial injury or necrosis and cerebral cortical necrosis.

The hyperkalemia noticed is suggestive of massive leakages of this electrolyte cells that are destroyed in trypanosome infections.

Conclusion

Trypanosoma brucei infection caused major changes in the hematological and biochemical parameters in the gilts. These changes were responsible for the devastating effects of the disease on the animals.

Acknowledgements

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SAŽETAK

Cilj je bio istražiti hematološke i biokemijske promjene u serumu križanih nazimica invadiranih nametnikom *Trypanosoma brucei*. U istraživanje je bilo uključeno 12 nazimica nabavljenih na svinjogojstvima u Zariji u Nigeriji. Nazimice su bile podijeljene u pokusnu i kontrolnu skupinu te smještene u nastambe potpuno zaštićene od krilatih kukaca. U pokusnoj skupini bile su invadirane s približno $1,8 \times 10^6$ parazita *Trypanosoma brucei*. U svih invadiranih nazimica očitovali su se klinički znakovi tripanosomoze nakon prepatentnog perioda od tri dana. Značajne razlike ($P<0,05$) bile su primijećene u srednjim vrijednostima hematokrita, leukocita, diferencijalne krvne slike, serumskih proteina, aspartat aminotransferaze, kreatin kinaze, kalija, anorganskih fosfata i kalcija. U radu je raspravljeno značenje tih nalaza u patologiji tripanosomoze u svinja.

Ključne riječi: *Trypanosoma brucei,* nazimice, hematologija, biokemijske promjene, serum