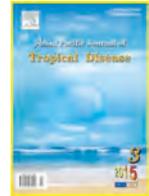




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Sensitivity of Nigerian field isolates of *Trypanosoma vivax* and *Trypanosoma congolense* to commonly available trypanocides

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PEER REVIEW

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This is an *in vivo* study in which the authors evaluated the susceptibility of one isolate of *T. vivax* and one isolate of *T. congolense* against the three main drugs usually used for the treatment and prevention of African animal trypanosomiasis. The results are useful for the contribution to the better prevention of the diseases.

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ABSTRACT

Objective: To assess Nigerian field isolates of *Trypanosoma congolense* (*T. congolense*) and *Trypanosoma vivax* (*T. vivax*) to commonly available trypanocides (isometamidium chloride, homidium chloride and diminazene aceturate).

Methods: Thirty-six Yankasa sheep of both sexes were used for the study. The sheep were screened for ecto and endoparasites, and acclimatized for 2 weeks before the commencement of the experiment. The animals were randomly divided into six groups of six sheep each. Two groups (A and B) were infected intravenously with phosphate buffer solution dilute infected blood of approximately 2.0×10^6 of *T. vivax* and two other groups (E and F) were also infected intravenously with blood containing the same number of *T. congolense*. The remaining two groups served as uninfected controls. Of the two *T. vivax* infected groups, one was further divided into three sub-groups of two animals each and each of the sub-groups was treated with different trypanocides 9 days after the establishment of parasitaemia. The same treatment was carried out on the *T. congolense* infected groups. The other infected groups were left untreated. All the animals were monitored for 8 weeks post infection. Prepatent period of 6 d was recorded for both parasite species.

Results: *T. vivax* was found to be resistant to homidium chloride but sensitive to diminazene aceturate and isometamidium chloride at the recommended doses. Conversely, *T. congolense* was found to be resistant to all the three trypanocides used at the recommended doses.

Conclusions: Although, resistance to trypanocides exists, it was observed to be more prominent with *T. congolense* than *T. vivax* infected sheep.

KEYWORDS

Trypanosoma, Infection, Trypanocides, Treatment, Parasitaemia**1. Introduction**

Trypanosoma vivax (*T. vivax*), *Trypanosoma congolense* (*T. congolense*) and to a lesser extent *Trypanosoma brucei* (*T. b. brucei*) are the main species responsible for

African animal trypanosomiasis (AAT) in West Africa[1]. The disease causes about 3 million deaths annually and has a marked impact on agriculture in Sub-Saharan and South American endemic countries, leading to annual livestock production losses of about 1.2 billion US dollar[2,3]. *T.*

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vivax accounts for up to half of total prevalence of AAT in West African especially in Nigeria where it is considered to be the predominant parasite for domestic animals[2]. This could be ascribed to the mechanical transmission or shorter development cycle in the anterior part of the tsetse fly[2]. *T. vivax* and *T. congolense* infect large variety of domestic and wild animals[4,5].

The cardinal clinical sign observed in AAT is anemia[2]. Within 1 week of infection with the haematic trypanosomes (*T. congolense* and *T. vivax*), there is usually a pronounced decrease in packed cell volume, hemoglobin, red blood cell and white blood cell counts, and within 2 months, they may drop to almost half of the pre-infection values[6]. Also, intermittent fever, oedema and loss of condition are invariably present. Abortion may be seen, and infertility of males and females may be the resultant effects. The severity of the clinical response is dependent on the species, the breed of affected animal as well as the dose and virulence of the infecting trypanosome. Stress, such as poor nutrition or concurrent disease, plays a prominent role in the disease process, and under experimental conditions, stress may be markedly reduced; it is difficult to elicit clinical disease[7].

Control of African trypanosomosis is dependent largely on vector control and use of drugs (chemotherapy and chemoprophylaxis). In animals, the drug control of trypanosomosis is dependent on three compounds: isometamidium chloride, homidium bromide or chloride and diminazene aceturate[6].

The use of drugs for the prevention and treatment of trypanosomosis has been of importance for many decades, but the rapidity that the trypanosomes develop resistance to each introduced drug has tremendously complicated this approach to control the disease[8,9]. In spite of this, some of older chemoprophylactic drugs such as the quinapyramine derivatives, antrycide and antrycide prosalt are still used and give effective protection against *T. b. brucei* infection in horses, camels, and cattle for up to 3 months. The drug pyriminidyl bromide is useful in the prophylaxis of *T. vivax* and *T. congolense* infections in cattle, sheep, and goats and can give protection for up to 5 months[10]. The most widely used of the newer chemoprophylactic drugs is isometamidium chloride[11]. This drug has been in use for over three decades and sold under the trade names samorin, trypanidium, and M&B 4180A; it is excellent for the prophylaxis of all the three species of African animal trypanosomes, and gives protection for 3–6 months. Homidium bromide has also been found to be an effective chemoprophylactic drug in Kenya, and the newly

introduced arsenical compound, cymelarsan, is effective in the treatment of *T. b. brucei* infection. A very widely used chemotherapeutic drug is diminazene aceturate (Berenil[®]), which is effective against all the three African animal trypanosomes such as *T. congolense*, *T. vivax* and *T. b. brucei*. Although chemoprophylaxis has been extensively used in trypanosomosis control, it is an expensive, time-consuming, and thus an unsatisfactory long-term solution to the problem of AAT.

This study was therefore undertaken to assess the sensitivity of the two important species of trypanosomes (*T. vivax* and *T. congolense*) to the commonly available trypanocides.

2. Materials and methods

The study was carried out in Makurdi, Benue State, Nigeria and Idon, Kaduna State, Nigeria. Makurdi is the state capital of Benue State located in Southern Guinea Savanna Region. It has 6 months (April–October) of rain fall and 5 months (November–March) of dry season. Idon is located in Kajuru Local Government Area in southern part of Kaduna State in Northern Guinea Savanna Zone. It has 5 months of rain fall and 5 months of dry season. Cattle farms of about seventy cattle were purposively selected following the report that some of the animals in the farms had pica and reduced appetite. Blood samples were collected from these animals.

2.1. Field isolations of the *T. vivax* and *T. congolense*

T. vivax was isolated from a white Fulani breed of cattle in a sedentary farm in Benue State, Nigeria while the *T. congolense* was isolated from white Fulani breed of cattle in a farm located in Idon Village in Kachia road, Kaduna, Kaduna State, Nigeria. The two isolates were identified using Giemsa stained thin blood smears and PCR as described by Desquesnes and Masiga *et al*[12,13].

2.2. Source of experimental sheep

Thirty-six Yankasa sheep aged between 2–3 years were purchased from an open market at Karfur in Katsina State, Nigeria. On arrival, the animals were screened for ecto, endo and haemoparasites. Physical examination was conducted on each of the animals for presence of ectoparasites. Two milliliters of blood was obtained from

the jugular vein of each of the sheep, and examined for haemoparasites as described by Wool[14]. Three grams of faeces was scooped from the rectum of each sheep using a clean polythene bag and examined as described by Coles[15]. All the experimental sheep were dewormed using albendazole® at the dose rate of 7.5 mg/kg. Ecto-parasites infestations were treated and controlled with deltamethrin® pour-on preparation and asuntol® spray. Those found with *Anaplasma* infections were treated with oxytetracycline long acting at the dose rate of 20 mg/kg body weight. Amprolium was given to those that were infected with *Coccidia* for 5 d. The sheep were also vaccinated each with 1 mL subcutaneous injection of monoclonal *pestes de petit ruminantes* vaccine against *pestes de petit ruminantes*. They were then introduced into arthropod-free pens in the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria and pre-conditioned for 2 weeks before the commencement of the experiment.

2.3. Experimental design

The 36 sheep were tagged and randomly assigned into six groups (A, B, C, E, F and 2C) of sheep each. Base line data were obtained from each of the animal in all the groups daily for a period of 1 week prior to infections. Each sheep in Groups A and B was infected intravenously (*i.v.*) with 2 mL of infected blood containing approximately 2.0×10^6 *T. vivax* while sheep in Groups E and F were infected with the same quantity of *T. congolense* through the same route as above. The trypanosomes were quantified using the improved Neubauer haemocytometer[16]. Groups C and 2C served as uninfected controls. Groups A and E animals were further divided into three sub-groups each of two sheep each (A1, A2, A3, E1, E2 and E3). Following the establishment of massive parasitaemia (++++), each of the three sub-groups was treated with diminazene aceturate at 3.5 mg/kg, isometamidium chloride at 0.5 mg/kg and homidium chloride at 1 mg/kg respectively. All the sheep in the groups were fed with cotton seed cake, maize offal, groundnut husk and *Digitaria* hay. Salt licks and water were given *ad libitum*.

2.4. Parasitaemia

Parasitaemia was determined every two days using simple wet mount and concentration method as described by Murray and Trail[17].

3. Results

3.1. Post-infection observations

3.1.1. Incubation period of *T. vivax* and *T. congolense* infections

All the infected sheep became positive to the parasite 6 days post-infection. But the levels of parasitaemia differed in the sheep.

3.1.2. Daily parasitaemia

There was relapse in the *T. vivax*-infected, homidium chloride treated animals on Days 8 and 18 post treatment (PT) respectively. The two relapses were treated with diminazene aceturate which cleared the parasite from the peripheral blood within 24 h PT and the animals remained negative throughout the period of observation. The experiment was terminated after monitoring for 8 weeks post-infection (Figure 1).

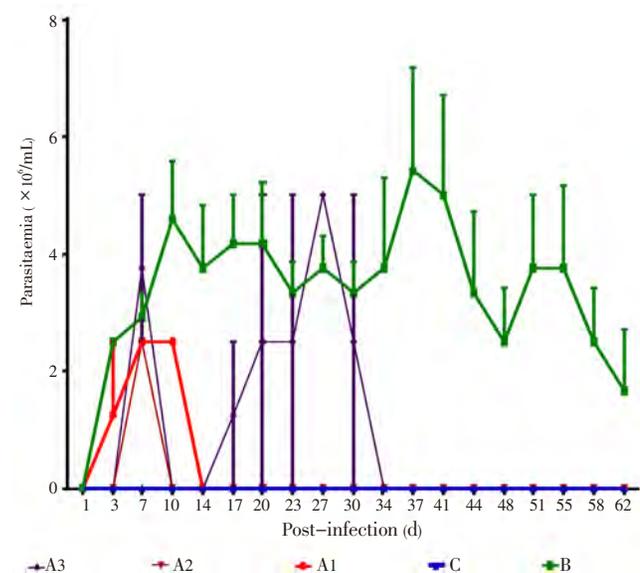


Figure 1. Daily parasitaemia of *T. vivax* infected Yankasa sheep (mean±SE). A1: Infected–diminazene aceturate treated; A2: Infected–isometamidium chloride treated; A3: Homidium chloride treated; B: Infected, untreated; C: Uninfected control.

In the *T. congolense* infected Yankasa sheep, all the treated sheep had relapses on Day 27 (PT). They were then treated for the second time with diminazene aceturate 2 days post relapse. A second relapse occurred Day 6 post secondary treatment and all the relapsed animals were later treated for the third time (third treatment) with homidium chloride but all the sheep became parasitaemic PT resulting in the occurrence of third relapse which was then treated again with diminazene aceturate at the dose rate of 3.5 mg/kg. The parasite disappeared from the peripheral circulation after 24 h PT and the experiment was terminated at Day 56

post-infection (Figure 2).

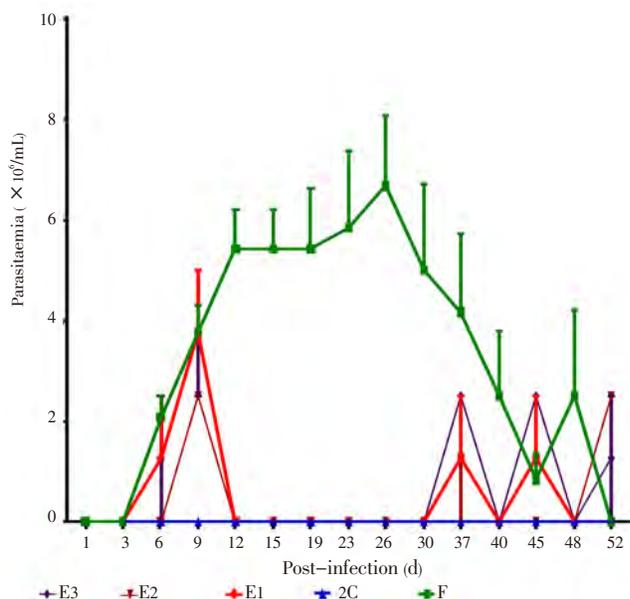


Figure 2. Daily parasitaemia of *T. congolense* infected Yankasa sheep (mean \pm SE).

E1: Infected–diminazene aceturate treated; E2: Infected–homidium chloride treated; E3: Infected–isometamidium chloride treated; F: Infected, untreated; 2C: Uninfected control.

4. Discussion

The relapse observed by the two sheep infected with *T. vivax*–treated with novidium chloride was an indication of the resistance of the isolate to the recommended therapeutic doses of the drug which is in support of the earlier reports by other authors, and of the resistance of trypanosomes to most of the commonly used trypanocidal drugs in many African countries[6,10]. The resistant problem was seen to be more serious with *T. congolense* since more than three relapses were experienced in all the *T. congolense* infected animals that were treated with the three different trypanocides. This shows that the strain of *T. congolense* used for the study is resistant to all the commonly used trypanocides unlike the strain of *T. vivax* used that is resistant to homidium chloride only.

Over the years, diminazene aceturate and isometamidium chloride have been used as the best therapeutic and prophylactic trypanocides respectively[10]. The former was reputed as the only drug to which trypanosomes do not easily develop resistance because of its rapid elimination from the system when compared with the more persistent prophylactic drug and isometamidium chloride[11]. However, the findings from the present study are suggestive of necessary update of the isolates used in this experiment which develop resistance to all the trypanocidal drugs

since the findings disagree with the previous reports that resistance is difficult with diminazene aceturate. Treatment failures with shortened prophylaxis have been observed and attributed to infections with drug–resistance trypanosome species[6,9]. The outcome of this work confirms the reports of other researchers in East Africa that high drug resistance are encountered in the field with *T. congolense* rather than with *T. vivax*[18,19]. Most of these cases of resistance may be due to constant exposure of the animals to sub–therapeutic doses by farmers or non veterinarians in the field or as a result of under estimation of the animal’s weight in the field by veterinarians.

The outcome of this study requires that more work should be done on the area of chemotherapy to review the current dosage and treatment regimens of the commonly used trypanocidal drugs to stem the rising problem of drug–resistance.

The study revealed that novidium chloride was not effective in the treatment of *T. vivax* and *T. congolense* at the recommended therapeutic dosage but diminazene aceturate and isometamidium chloride still maintained their efficacy at the recommended dosages for *T. vivax* but not for *T. congolense*.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

T. vivax and *T. congolense* are two of the species responsible for African animal trypanosomiasis. The control of the disease depends on vector control and chemotherapy or chemoprophylaxis. In this sense, it is important to know the isolates susceptibility of trypanocidal drugs normally used and will help veterinarians for better management and propose new strategic treatment of the disease.

Research frontiers

Studies are being performed in order to determine the

susceptibility of *T. vivax* and *T. congolenses* isolates against trypanocidal drugs, normally used for the treatment or chemoprophylaxis of the diseases in Nigeria.

Related reports

Other reports evaluate the susceptibility of trypanocidal drugs *in vivo* studies. Usually these studies used higher number of isolates and higher number of animals in the *in vivo* experiments.

Innovations & breakthroughs

This paper presents the susceptibility results of one isolate of *T. vivax* and one isolate of *T. congolense* against the three main drugs used for the treatment of African animal trypanosomiasis in Nigeria. And this result proposed some recommendations.

Applications

The results are important for the prevention and control of the African animal trypanosomiasis in Nigeria.

Peer review

This is an *in vivo* study in which the authors evaluated the susceptibility of one isolate of *T. vivax* and one isolate of *T. congolense* against the three main drugs usually used for the treatment and prevention of African animal trypanosomiasis. The results are useful for the contribution to the better prevention of the diseases.

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