Comparative experimental studies on *Trypanosoma* isolates in mice and response to diminazene aceturate and isometamidium chloride treatment

Muluken Yayeh, Shimelis Dagnachew, Meseret Tilahun, Achenef Melaku, Tadegegn Mitiku, Mohamed Yesuf, Zewdu Seyoum, Habtamu Kefyalew.

Comparative experimental studies on *Trypanosoma* isolates in mice and response to diminazene aceturate and isometamidium chloride treatment

Heliyon 4 (2018) e00528.

do: 10.1016/j.heliyon.2018.e00528

Abstract

The current study was undertaken from December 2015 to May 2016 with the aim of determining and comparing the pathogenicity and response to diminazene aceturate (DA) and isometamidium chloride (ISM) treatment in experimentally infected mice with trypanosome isolates from Jawi and Birsheleko areas of northwest Ethiopia. A total of 42 mice were used for the experiment. These mice were randomly assigned in to 7 groups of 6 mice per group. Three of the groups (Group 1, 4 and 5) were inoculated with trypanosome isolated from Jawi and three other groups (Group-2, 6 and 7) were inoculated with trypanosome isolated from Birsheleko and the remaining one group (Group 3) was negative control. Each experimental mice were received 0.3 ml of positive blood at the $10^5$ parasites/ml from donor animals intraperitoneally while negative control group were received 0.3 ml sterile water. The mice were clinically observed daily during the study period. Parameters including level of parasitaemia, body weight,
PCV and hemoglobin value were recorded once per week for ten consecutive weeks post infection. Trypanocidal treatment was given on day 21 post infection when peak parasitaemia was detected in groups (Group 4-DA-Jawi, 5-ISM-Jawi, 6-DA-BRSH and 7-ISM-BRSH). The treatment doses for DA was at 28 mg/kg and for ISM at 4 mg/kg. In all experimental groups during study period when the mice showed severe clinical signs and at the end of the experiment they were euthanized with 70% ethanol for gross and histopathological examinations. The parameters measured during the study period revealed markers leading to pathological changes in all infected groups. Parasitaemia were detected early in the Jawi isolate infected groups compared to the Birsheleko groups. All infected mice showed clear clinical manifestation of depression, weight loss, reduction in feed intake and huddled together in the corner of the cage. Significant ($P < 0.05$) reduction was observed in the mean PCV and hemoglobin value of s infected mice compared to the negative control. The mean PCV values of Birsheleko isolate infected group was significantly ($P < 0.05$) lower than Jawi isolate infected group. This study showed that treatment with either DA or ISM were unable to clear parasitaemia indicating the presence of drug resistance problems for both isolates. Relative improvement in clinical and pathological changes was observed as compared with untreated infected groups. Gross and histopathological lesions were observed in infected groups. In conclusion, the current study suggests the presence of strain difference in virulence between isolates and the drugs unable to cure infections indicating the presence of resistance problems necessitate further molecular characterization of the strains and drug resistance detection in the natural host.

Keyword: Infectious disease

1. Introduction

African trypanosomiasis is a disease complex caused by unicellular protozoan parasites belonging to the genus *Trypanosoma*. Pathogenic s cause clinical disease in humans and are an important factor limiting development of domestic livestock in Africa (Vreysen, 2006). *Trypanosoma congolense* causes the most economically important animal trypanosomosis in Africa. *T. congolense*, although the smallest of the species, remains the most pathogenic to animals (Bengaly et al., 2001). Tsetse-transmitted s infective to livestock cause huge economic losses to the livestock industry and tsetse flies (*Glossina* spp.) currently infest over 10 million square kilometers of fertile land distributed among 37 countries within the African continent (Shaw, 2009).

In Ethiopia, animal trypanosomosis is among the most important diseases limiting livestock productivity and agricultural development due to its high prevalence in
the most arable and fertile land of southwest and northwest part of the country (Dagnachew et al., 2005). Over 6 million head of cattle and equivalent number of other livestock species are at risk of contracting the diseases. More than 20,000 head die per annum, and annual loss attributed to the disease is estimated to be over US$236 million, whereas loss due to reduce meat, milk and draft power is not applicable to this figure (OAU, 2002).

Controls of trypanosomosis depend in the foreseeable future on the use of the existing trypanocidal drugs, however prolonged use of these agents may lead to the development of resistance. Determination of trypanocidal sensitivity or resistance of trypanosomes isolated from the field will help veterinarians for better management and strategic treatment of trypanosomiasis in the country. In Ethiopia, the presence of drug resistance at lower and higher doses of commonly used trypanocidal drugs has been reported by several researchers (Mulugeta et al., 1997; Afework et al., 2000; Ademe and Abebe, 2000; Tewelde et al., 2004; Miruk et al., 2007; Dagnachew et al., 2015a,b; Moti et al., 2012; Dagnachew et al., 2017). However, research works on the different isolates of T. congolense pathogenicity and treatment response is insufficient in Ethiopia. Therefore, the pathological conditions observed in experimentally infected mice and response of mice to the commonly used drugs; diminazene aceturate and isometamidium chloride is essential for the effective control programs of tsetse transmitted trypanosomosis in northwest Ethiopia.

2. Materials and methods

2.1. Study area

The present experimental study was conducted in the University of Gondar at Tewodros campus from January to May 2016. Gondar is located between 12°36′N and 33°28′E at an altitude of about 2300 meter above sea level with an average temperature of 20 °C and an average annual rainfall of 1800 mm. For the experimental study field isolates of trypanosomes were collected from Trypanosomosis prevalent areas of Birsheleko and Jawi in northwest Ethiopia. Birsheleko is located 397 km Northwest of Addis Ababa in West Gojjam Zone whereas Jawi is located at 670 km northwest of Addis Ababa in Awi Zone. The climate alternates with long winter dry season (October—May) and summer rain fall (June—September) with mean annual rain fall of 1569.4 mm. The mean temperature varies between 16.68 °C—37.6 °C and altitude ranges from 648—1300 m.a.s.l. (CSA, 2013).

2.2. Experimental animals

Swiss white mice 12—13 weeks old and weighing 30—40 gram were used obtained from laboratory animal farm’s in the College of Veterinary Medicine and Animal Sciences of University of Gondar. Mice were maintained under standard conditions
with access to palliated feed and water ad libitum. Caging was kept so as to prevent entrance of field rodents and other insects and housed in light and temperature controlled environment. All experimental procedures followed the ethical standards for investigation of experimental pain in animals. All protocols and experimental procedures were reviewed and authorized by the Research and Ethics Review Committee of the College of Veterinary Medicine and Animal Sciences and followed the international council guidelines for animal experimentation (CIOMS, 1985). During the study period when mice showed severe clinical signs and at the end of the experiment all animals were euthanized using intraperitoneal injection of ethanol alcohol.

2.3. Parasite isolation

The trypanosomal parasites were isolated from cattle found in Jawi and Birsheleko areas of northwest Ethiopia which are endemic trypanosomosis prevalent areas in Amhara region, northwest Ethiopia. The presence of s in the screened cattle were detected from blood samples collected from the peripheral ear of the animals. Blood from positive cattle with estimated parasitaemia of $10^6$ s/ml were collected by heparinized vacutainer tube and 0.3 ml of the blood was inoculated via IP route to a mouse and 3 ml to goats through IV as a donor animals. The parasites were confirmed as s by doing thin blood smear with Giemsa staining technique (Murray et al., 1977). However, the absence of molecular characterisation is the limitation of the current study in regards to the identification of the parasites.

2.4. Experimental groups

Forty two mice were used for the experiment and grouped randomly in to seven equal groups of six animals. Group-1 was infected with isolate from Jawi, Group-2 was infected with trypanosome isolate from Birsheleko, Group-3 was negative control, Group-4 was infected with trypanosome isolate from Jawi and treated with DA, Group-5 was infected with isolate from Jawi and treated with ISM, Group-6 was infected with trypanosome isolate from Birsheleko and treated with DA, and Group-7 was infected with trypanosome isolate from Birsheleko and treated with ISM (Table 1).

2.5. Parasite inoculation

Donor mice infected with trypanosome from Birsheleko area were scarified with 70% ethanol consequently blood was collected intracardially and inoculated into the three groups of mice (Group 2, Group 6 and Group 7) through intera peritoneal (IP) route. Similarly donor mice infected with trypanosome from Jawi area were scarified with 70% ethanol consequently blood was collected intracardially and inoculated into the three groups of mice (Group 1, Group 4 and Group 5). Each mice in the infected
groups received 0.3 ml infected blood from donor mice whereas the negative control received 0.3 ml sterile distilled water at the same time. The experimental inoculums size of 0.3 ml was estimated to contain with $10^5$ trypanosome (Eisler et al., 2001).

2.6. Trypanocidal drug treatment

Trypanocidal drug treatment were given for Groups 4, 5, 6 and 7 after observation of peak parasitaemia by wet film examination (Murray et al., 1977). Drug injection was given by IP route, each mice received diminazene aceturate at a dose of 28 mg/kg and isometamidium chloride at a dose of 4 mg/kg based on their body weight respective of groups. Diminazene aceturate-2.36 g Verben made in Libourne-France (lot 753A1 exp.07/2017) and isometamidium chloride-125 mg (Veridium) manufactured in Libourne-France (Lot 198A1 EXP-06/17) were used by dissolving in 15 ml and 12 ml sterile water, respectively.

2.7. Clinical and parasitological examination

During the experimental period (from January to May 2016), experimentally infected mice were clinically examined daily. The presence of parasites, determination of PCV and hemoglobin values, body weight measurement were done one day’s interval for the first one week and weekly until the end of the experiment. Weight measurement was taken using digital balance. Wet film and Buffy coat technique were used to determine the degree of parasitemia and detection of relapse level of parasitemia according to Paris et al. (1982). Packed cell volume (PCV) determination was also done using the microhaematocrit technique. Blood samples were obtained from tail and collected directly into heparinized capillary tubes (Murray et al., 1977). After detection of the parasite in the blood of experimental mice thin blood smears

---

**Table 1.** Experimental groups of mice for pathogenicity and treatment response studies of mice infected with *T. congolense* isolates from different sites of northwest Ethiopia.

| Group | Infection status | Source of isolates | Treatment status |
|-------|------------------|--------------------|------------------|
|       |                  | Birsheleko         | DA at 28 mg/kg   |
| Group-1| Exposed          | _                  | _                |
| Group-2| Exposed          | X                  | _                |
| Group-3| No exposed       | _                  | _                |
| Group-4| Exposed          | X                  | X                |
| Group-5| Exposed          | X                  | _                |
| Group-6| Exposed          | _                  | X                |
| Group-7| Exposed          | _                  | X                |

DA- Diminazene aceturate, ISM- Isometamidium chloride
were prepared and Giemsa stained for observation of morphology via microscopy as described by Kagira et al. (2007). The level of parasitemia was assessed using rapid matching method as described by Herbert and Lumsden (1976).

2.8. Postmortem examination and histopathological analysis

Postmortem examinations were done at the end of the experiment and in severely suffered mice during the experimental period as well as negative controls were humanely euthanized with intraperitoneal injection of 0.5 ml of 70% ethanol (Lord et al., 1991). The animals were evaluated immediately after death. On external examination weight, coat color, eye color, body condition and external lesions were examined. Other abdominal masses or abdominal fluid were palpated. All internal organs were examined systemically for gross lesions or abnormalities (heart, liver, spleen, brain, lungs and kidneys) were taken and preserved in 10% neutral buffered formalin for histopathology (Bush, 1975). The preserved tissue was dehydrated in ethanol and embedded in paraffin wax and sectioned at 5 μm using a microtome (Leica Co., USA). The thin sections were stained using the haematoxylin and eosin method (Paris et al., 1982), examined under a microscope and photomicrographs taken. Histological differences in these tissues between groups of mice were determined.

2.9. Statistical analysis

All data were entered into an Excel spread sheet and imported into SPSS version 20 statistical software. Descriptive statistics were used to summarize the data. Differences in haematological variables, and body weight loss measured between groups were assessed by a one-way repeated measure ANOVA. The significant level was set at \( P < 0.05 \).

3. Results

3.1. Clinical findings and development of parasitemia

The first parasitemia in the field infected mice were observed on day 17 and day 21 pi for Birsheleko and Jawi isolates, respectively and reaches peak parasitemia on day 28 post inoculation for both isolates. In the experimental infected mice the detection of parasitaemia was observed on day 5 post infection (pi) in Birsheleko isolate infected groups and on day 7 pi for Jawi isolate infected group. The parasitemia reached at peak load on day 21 pi for both isolate infected groups as indicated in Fig. 1. All infected mice developed acute form of trypanosomosis, which was characterized by depression, weight loss, reduction in feed intake and huddle together on the corner of the cage.

There was no observed clinical signs of improvement after trypanocidal drug treatment for both Birsheleko and Jawi trypanosome isolates infected groups. In addition
there was no complete clearance of the parasite as shown in Fig. 2 and hence the relapse and presence of low level of parasitaemia in all treated groups of mice clearly indicates the occurrence resistant strains of trypanosome against both drugs.

The mean body weight measurement of negative control (Group-3) mice was significantly higher than the mean body weight of Groups-1, 2, 4, and 6 (\(P < 0.001\)) respectively. The mean body weight of Group-2 was also significantly (\(P = 0.001\)) lower than the mean body weight of Groups-4, 5 and 7. The mean body weight measurement observed in the study did not showed a significant difference (\(P > 0.05\)) up to day 35 pi between infected and negative control groups (Fig. 3).

### 3.2. Haematological findings

Following infection with s, there was variable degree of reduction in mean PCV and Hgb concentration until they attained peak parasitaemia (day 21 pi) as summarized in Figs. 4 and 5 and in Table 2. The mean PCV and Hgb value of trypanosome infected mice were significantly (\(P < 0.00\)) lower than negative control groups as shown in Figs. 4 and 5. Significantly (\(P = 0.004\)) lower mean PCV and Hgb value were also observed in the Birsheleko isolate infected group than Jawi isolate infected group. Group-2 infected mice was further showed significantly (\(P < 0.001\)) lower mean PCV values than Groups 3, 4, 5 and 6 respectively.
3.3. Pathological changes

The spleen, liver, kidney, lung, heart and brain were observed grossly and histopathologically for any abnormality in mice experimentally infected with trypanosome isolates and also in treated groups with DA and ISM. Post-mortem examinations were done at the end of the experiment and during the study period on mice showed

**Fig. 3.** The mean body weight measured during the study period in mice experimentally infected, treated and negative control groups (Group-3) with trypanosome isolates from Jawi (Group-1, Group-4/DA-Jawi, Group-5/ISM-Jawi) and Birsheleko (Group-2, Group-6/DA-BRSH, Group-7/ISM-BRSH) areas of northwest Ethiopia.

**Fig. 4.** The mean PCV values measured in different groups of mice experimentally infected, treated groups and negative control (Group-3) with trypanosome isolates from Jawi (Group-1, Group-4/DA-Jawi, Group-5/ISM-Jawi) and Birsheleko (Group-2, Group-6/DA-BRSH, Group-7/ISM-BRSH) areas of northwest Ethiopia.

**Fig. 5.** The mean Hgb values measured in different groups of mice experimentally infected, treated groups and negative control (Group-3) with trypanosome isolates from Jawi (Group-1, Group-4/DA-Jawi, Group-5/ISM-Jawi) and Birsheleko (Group-2, Group-6/DA-BRSH, Group-7/ISM-BRSH) areas of northwest Ethiopia.
severe abnormalities by euthanizing them with 70% ethanol. The negative control group of mice showed no any pathological changes.

### 3.3.1. Gross pathological changes

**Spleen**: There was clear abnormality grossly observed in major organs of the infected groups of mice (Fig. 6). In all infected groups of mice spleen showed enlarged in size, characterized by marked splenomegaly and congestion. This abnormality was relatively very large in Group-2 Birsheleko isolate infected mice compared to

**Table 2.** Mean PCV and Hgb values of mice experimentally infected, treated groups and negative control (Group-3) with *Tr. congolense* isolates from Jawi (Group-1, Group-4/DA-Jawi, Group-5/ISM-Jawi) and Birsheleko (Group-2, Group-6/DA-BRSH, Group-7/ISM-BRSH) areas of north west Ethiopia.

| Group | Mean PCV ± SD | 95% CI for mean PCV | Mean Hgb ± SD | 95% CI for mean Hgb |
|-------|---------------|---------------------|---------------|---------------------|
|       | Upper | Lower | Upper | Lower | Upper | Lower |
| 1     | 41 ± 6.57*  | 39.038 | 42.96 | 9.286 ± 0.9474* | 8.761 | 9.810 |
| 2     | 35.9 ± 6.96*| 34.064 | 37.736 | 8.462 ± 1.0592* | 7.965 | 8.958 |
| 3     | 49.09 ± 4.41| 47.123 | 51.048 | 11.771 ± 1.2142 | 11.247 | 12.296 |
| 4     | 41.4 ± 4.52*| 39.55 | 43.236 | 9.275 ± 1.9633* | 8.784 | 9.766 |
| 5     | 41.46 ± 4.73*| 39.49 | 43.42 | 9.143 ± 1.5328* | 8.618 | 9.667 |
| 6     | 40.18 ± 6.44*| 38.34 | 42.011 | 9.125 ± 1.7479* | 8.634 | 9.616 |
| 7     | 38.21 ± 6.87*| 36.215 | 40.197 | 8.676 ± 1.5472* | 8.144 | 9.209 |

Significance level compared with Group-3 *P* < 0.05 was indicated by *.

![Fig. 6.](https://doi.org/10.1016/j.heliyon.2018.e00528)

Fig. 6. Major gross pathological findings in mice experimentally infected with trypanosome isolates. A. Haemorrhagic and enlarged spleen of Group-7 (ISM-BRSH), splenomegaly; B. Spleen showing shrinkage and paler colour Group-4 (DA-Jawi) associated with treatment response; C. Splenomegaly (largest shining spleen) of Group-2; D. Small normal coloured spleen of negative control group (Group-3).
the Group-1 (Jawi isolate). Infected and treated groups showed slight enlargement and in comparison to the spleen of negative control groups of mice.

### 3.3.2. Histopathological changes

**Spleen:** Histopathologically spleen of experimentally infected mice with trypanosome showed necrosis and oedema (Fig. 7). But the problem was very pronounced in infected not treated groups. Apart from the above lesion Group-2 Birsheleko isolate infected mice exhibited bleeding and depilation of lymphoid follicle was observed.

**Kidney:** Inflammation with mononuclear cell infiltration on the cortex of kidney was observed in all groups of infected mice (Fig. 8). Glomerulonephritis and mild bleeding were observed in infected non treated groups.

![Fig. 7. Major histopathological findings in spleen of mice experimentally infected with trypanosome isolates. A. ISM treatment group Jawi with mild bleeding and necrosis (low magnification), B. Spleen with severe lymphoid proliferation, depletion of lymphoid follicle and congested with blood (low magnification) (Haematoxylin and eosin staining).](image1)

![Fig. 8. Major histopathological findings in kidney of mice experimentally infected with trypanosome isolates. A. Suppurative glomerulonephritis of Kidney and oedematous with principally characterized by hypercellularity of glomerulus indicate with the arrow or letter at (low magnification), B. Congested kidney with (low magnification) (Haematoxylin and eosin staining).](image2)
Liver: Histopathologically liver showed hepatitis with mononuclear cell infiltration at the portal region in all groups of animals, pyogranuloma in some cases (Fig. 9). The gross and histopathology of liver in non-infected control group were normal in all mice.

Lung: Microscopically most consistent lesion was interstitial pneumonia with proliferation and inflammatory cells which results in severe compression of alveoli in affected region which leads to severe emphysema in the nearby parts in the lung (Fig. 10).

4. Discussion

Trypanosomosis is shown to be the most important constraint for cattle production in western districts of Amhara Region, northwest Ethiopia. The result of the present study has indicated the existence of trypanosome isolates that cause major clinical, hematological and pathological abnormality in experimentally infected mice compared to the negative control group. The early appearance of parasitaemia in the Jawi isolates infected group was observed in the donor mice, however in the continuous passage to the experimental mice the appearance of parasites in the blood was almost within the same range of time in both infected groups needs further investigation.

In the present study there was persistent fluctuating parasitemia. This is the consistent feature of trypanosomiasis and agrees with the idea that fluctuating parasitemia results in continuing cycles of replications, antibody production, immune complex development, and changing of surface-coat glycoprotein (Baral, 2010).

Significant decrease in mean PCV and Hgb values were observed in all trypanosome infected groups compared with the non-infected control group of mice. These results were agreed with Dagnachew et al. (2015a,b) who reported lower PCV and Hgb values in cattle experimentally infected with *T. vivax* isolates. Anaemia indicated

Fig. 9. Major histopathological findings in liver of mice experimentally infected with trypanosome isolates. A. Necrosis of hepatocytes, most hepatocytes in affected area have condensed (pyknotic) nuclei; severe proliferation mononuclear cells, and haemorrhages (low magnification), B. Necrosis of liver hepatocytes appearing pyknotic (high magnification) (haematoxylin and eosin staining).
by a drop in PCV values of experimentally inoculated groups of mice 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 and Kaneko, 1983). The aetiology of this anaemia is complex, but the most important factor said to be is haemolysis. Haemolysis could be caused by mechanical injury to erythrocytes by the lashing action flagella and microtubule-reinforced bodies of the high number of the organisms during parasitaemia (Vickerman et al., 1993). Erythrocyte membrane damage has also been associated with adhesion of erythrocytes and reticulocytes to surfaces via sialic acid receptors leading to damages to erythrocyte cell membranes (Anosa and Kaneko, 1983). In the present study haematological values were decreased on the week after observation of peak parasitaemia on day 28 pi. This agrees with peak parasitaemia associated with pyrexia causes decline in PCV and Hgb value. During this period the huge number of parasites and high body temperature may contribute to the severity of anaemia. Furthermore, dead s can produce various forms of active chemical substances (proteases, phospholipases, neuramidase, etc.) which can elicit erythrocyte injury (Tizard et al., 1977).

![Fig. 10. Major histopathological findings in lung of mice experimentally infected with trypanosome isolates. A. Lung of Group-4, pneumonia and congestion (arrow) of intera-bronchial septae (low magnification), B. Lung of mice Group-2, interstitial pneumonia thickening (arrow) of inter alveolar septae and oedema in the interstitial septae (low magnification), C and D. From Group-1 showed pneumonia with thickening of intra alveolar septae and severe congestion at high magnification and low magnification respectively (haematoxylin and eosin staining).](https://doi.org/10.1016/j.heliyon.2018.e00528)
This study has also revealed that there was significant difference observed on haematological parameters between the two infected groups of trypanosome isolates along with other pathological lesion indicate the presence of strain difference between isolates. Thus the study suggests trypanosome isolates of Birsheleko were more pathogenic in haematological abnormalities than Jawi isolate.

The current study also strongly signify the development of gross lesions and histopathological changes observed in the spleen, liver, lung, heart, kidney of in infected mice whereas the gross and histopathological examination of tissue sections from negative control mice were showed no pathological lesions. Similar pathological findings were reported by several authors in infections (Dagnachew et al., 2015a,b).

Grossly hepatomegaly, splenomegaly, oedematous and haemorrhagic lesions on spleen, liver, heart and congestion in the lung were observed in *T. congolense* infected mice. This gross finding were in agreement with report of organ degenerative changes in animal trypanosomosis and necrotic lesions observed in spleen, kidney and liver were in agreement with the findings of Archivio et al. (2012). In addition, the histopathological investigation in this study showed that acute bronchopneumonia with mononuclear cell infiltration and severe emphysema of the lung and thickening of intera alveolar septae, pulmonary congestion, congestion of the liver, oedema, centrilobular necrosis with mononuclear cell aggregation in the liver, shrunken and congested glomerulus in the kidney agree with the histopathological findings of Taket and Fagbemi (2009) observed on sacrificed *T. congolense* infected rabbits. This all gross and histopathological lesions in the organ agree with the idea it might be immunologic lesions are significant in trypanosomiasis, and it has been suggested that many of the lesions (splenomegaly and glomerulonephritis) in these diseases may be the result of the deposition of immune complexes that interfere with, or prevent, normal organ function. The most significant and complicating factor in the pathogenesis of trypanosomiasis is the profound immunosuppression that occurs following infection by these parasites. This marked immunosuppression lowers the host’s resistance to other infections and thus results in secondary disease, which greatly complicates both the clinical and pathological features of trypanosomosis.

The result of the present study indicated the existence of trypanosome strains that have developed resistance for both diminazene aceturate (DA) and isometamidium chloride (ISM). This might be indicated with treatment with either DA or ISM at a dose of 28 mg/kg and 4 mg/kg, respectively was not totally clear trypanosome from experimentally infected mice of the two isolates. Treatment groups of mice did not show significant improvement on haematological values compared to the infected non-treated groups. The epidemiology of drug resistant populations of *T. congolense* is changing time to time. Once established the incidence has progressively spread within the population. The incidence of recurrent infection was 7% in 1986 and increased to 14% in 1989 in Ghibe valley of Ethiopia (Rowlands et al., 1993) due to the development of resistant
strains. Field observations made in other regions of Ethiopia were in agreement with the present findings. Isometamidium chloride prophylactic efficacy less than 30 days was documented on bovine naturally infected with trypanosome in three villages of Kindo Koysha, Southern Ethiopia (Ademe and Abebe, 2000).

Experimental sensitivity trials conducted on mice using isolates of *T. congolense* taken from Ghibe, Bedele, Sodo and Arbaminch reported failure of trypanocidals to ensure complete clearance using bovine doses (Chaka and Abebe, 2003). Other studies made in Benshangul Gumuz region of northwest Ethiopia reported *T. congolense* isolates refractory to treatment up to 28 mg/kg DA and 4 mg/kg of ISM in mice (Afework et al., 2000). The outcome of the present trypanocidal drug resistance test in mice clearly shows the presence of trypanosomal isolates that have developed drug resistance phenotype to the currently available trypanocides. Consistent with the occurrence of resistant strains and adding to growing evidence that such resistance may be a problem.

5. Conclusion

Trypanosomosis in northwest Ethiopia can cause significant pathology in infected animals and the degree and type of pathological changes might vary with the isolate of strain. Trypanosome infection caused major changes in the clinical, haematological and pathological parameters in mice. These changes were responsible for the devastating effects of the disease on the animals. Moreover, trypanocidal drug treatment of trypanosome infected mice has resulted in short time relief and restoration of clinical signs, however haematological values and histopathological changes were not substantial and the occurrence of resistant strain is more evidenced. Therefore, control of trypanosomosis necessitate characterization of the isolates and application of integrated approach.

Declarations

**Author contribution statement**

Muluken Yayeh: Performed the experiments; Wrote the paper.

Shimelis Dagnachew: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Meseret Tilahun, Habtamu Kefyalew: Contributed reagents, materials, analysis tools or data.

Achenef Melaku: Analyzed and interpreted the data.

Tadegegn Mitiku, Mohammed Yesuf: Performed the experiments.
Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors acknowledges the University of Gondar Research and Publication vice president office supporting this research project into reality. We acknowledge the College of Veterinary Medicine and Animal Sciences support for the logistic and laboratory technicians for their unreserved provision of laboratory facilities and devotion of their time in assisting the experimental procedures during the study period. Our appreciation goes to Bahir Dar Regional Veterinary Diagnostic and Investigation Center staff members their absolute provision of logistic facilities and devotion of time in assisting the isolation of trypanosomes from the field.

References

Ademe, M., Abebe, G., 2000. Field study on drug resistance s of bovine in Kindo Koysha Wereda southern Ethiopia. Bull. Anim. Health Prod. Afr. 48, 131–138.

Afework, Y., Clausen, P.-H., Abebe, G., Tilahun, G., Mehlitz, D., 2000. Multiple-drug resistant Trypanosoma congolense populations in village cattle of Metekel district, northwest Ethiopia. Acta Trop. 76, 231–238.

Anosa, V.O., Kaneko, J.J., 1983. Pathogenesis of Trypanosoma brucei infection in deer mice (Peromyscus maniculatus). Light and electron microscopic studies on erythrocytepathologic changes and phagocytosis. Am. J. Vet. Res. 44, 645–651.

Archivio, S.D., Cosson, A., Medina, M., Lang, T., Minoprio, P., Goyard, S., 2012. Non-invasive in vivo study of the Trypanosoma vivax infectious process consolidates the brain commitment in late infections. PLOS NTD. 7, 1976.

Baral, T.N., 2010. Immunobiology of African trypanosomes: need of alternative interventions. J. Biomed. Biotechnol, 389153.

Bengaly, Z., Sidibe, I., Ganaba, R., Desquesnes, M., Boly, H., Sawadogo, L., 2001. Comparative pathogenicity of 3 genetically distinct types of Trypanosoma
congolesense in cattle: clinical observations and haematological changes. Vet. Parasitol. 108, 1–19.

Bush, B.M., 1975. Veterinary Laboratory Manual. Heinmann Medical Books, London.

Chaka, H., Abebe, G., 2003. Drug resistant s: a threat to bovine production in the Southwest of Ethiopia. Rev. Elevier Med. Vet. Pays Trop. 56, 33–36.

Council for International Organizations of Medical Sciences (CIOMS), 1985. International Guiding Principles for Biomedical Research Involving Animals.

CSA, 2013. The Agricultural Sample Survey, Report on Livestock and Livestock Characteristics (Private Peasant Holding), 2, pp. 8–50. Addis Ababa, Ethiopia.

Dagnachew, D., Tsegaye, B., Awukew, A., Tilahun, M., Ashenafi, H., Tim Rowan, T., Abebe, G., Barry, D.J., Terefe, G., Goddeeris, B.M., 2017. Prevalence of bovine trypanosomosis and assessment of trypanocidal drug resistance in tsetse infested and non-tsetse infested areas of Northwest Ethiopia. Parasite Epidemiol. Control 2, 40–49.

Dagnachew, S., Sangwan, A.K., Abebe, G., 2005. Epidemiology of bovine trypanosomosis in the Abay (Blue Nile) basin areas of northwest Ethiopia. Rev. Med. Vet. Des. Pays Trop. 58, 151–157.

Dagnachew, S., Terefe, G., Abebe, G., Barry, D., McCulloch, R., Goddeeris, B., 2015a. In vivo experimental drug resistance study in Trypanosoma vivax isolates from tsetse infested and non-tsetse infested areas of Northwest Ethiopia. Acta Trop. 146, 95–100.

Dagnachew, S., Terefe, G., Abebe, G., Sirak, A., Bollo, E., Barry, D., Goddeeris, B., 2015b. Comparative clinico-pathological observations in young Zebu (Bos indicus) cattle experimentally infected with Trypanosoma vivax isolates from tsetse infested and non-tsetse areas of Northwest Ethiopia. BMC Vet. Res. 24.

Eisler, M.C., Brandt, J., Bauer, B., Clausen, P.-H., Delespaux, V., Holmes, P.H., Illemobade, A., Machila, N., Mambo, H., McDermott, J., Mehlitz, D., Murilla, G., Ndung’u, J.M., Peregrine, A.S., Sidibe, I., Sinyangwe, L., Geerts, S., 2001. Standardized tests in mice and cattle for the detection of drug resistance in tsetse-transmitted s of African domestic cattle. Vet. Parasitol. 97, 171–182.

Herbert, W.J., Lumsden, W.H.R., 1976. Trypanosoma brucei: a rapid matching method for estimating the host’s parasitaemia. Exp. Parasitol. 40, 427–431.

Kagira, J.M., Ngotho, M., Thuita, J., 2007. Development of a rodent model for late stage rhodesian sleeping sickness. J. Protozool. Res. 17, 48–56.

Lord, R., Jones, G.L., Spencer, L., 1991. Ethanol euthanasia and its effect on the binding of antibody generated against an immunogenic peptide construct. Res. Vet. Sci. 51, 164–168.
Miruk, A., Hagos, A., Yacob, H.T., Asnake, F., Basu, A.K., 2007. Prevalence of bovine trypanosomosis and trypanocidal drug sensitivity studies on Trypanosoma congolense in Wolyta and Dawero zones of southern Ethiopia. Vet. Parasitol. 152 (1-2), 141–147.

Moti, Y., Fikru, R., Van Den Abbeele, J., Büscher, P., Van den Bossche, P., Duchateau, L., 2012. Gibe river basin in Ethiopia: present situation of trypanocidal drug resistance in Trypanosoma congolense using tests in mice and PCR-RFLP. Vet. Parasitol. 189, 197–203.

Mulugeta, W., Wilkes, J., Mulatu, W., Majiwa, P.A.U., Masake, R., Peregrine, A.S., 1997. Long term occurrence of Trypanosoma congolense resistant to diminazene, isometamidium and homidium in cattle at Ghibe, Ethiopia. Acta Trop. 64, 205–217.

Murray, M.P.K., Mcintyre, W.I.M., 1977. An improved parasitological technique for the diagnosis of African trypanosomosis. Trans. Soc. Trop. Med. Hyg. 71, 325–326.

OAU (Organization of African Unity), 2002. Trypanosomosis and tsetse in Africa. The year book report.

Paris, J., Murray, M., Mcodimba, F., 1982. A comparative evaluation of the parasitological techniques currently available for the diagnosis of African trypanosomiasis in cattle. Acta Trop. 39, 307–316.

Rowlands, G.J., Mulatu, W., Authie, E., Leak, S.G.A., Peregrine, A.S., 1993. Epidemiology of bovine trypanosomosis in the Ghibe valley, southwest Ethiopia. Acta Trop. 53, 135–150.

Shaw, A.P., 2009. Assessing the economics of animal trypanosomosis in Africa: history and current perspectives. Onderstepoort J. Vet. Res. 76, 27–32.

Takeet, M., Fagbemi, B.O., 2009. Haematological, pathological and plasma biochemical changes in rabbits experimentally infected with Trypanosoma congolense. Sci. World J. 4 (2), 29–36.

Tewelde, N., Abebe, G., Eisler, M.C., McDermott, J., Greiner, M., Afework, Y., Kyule, M., Münstermann, S., Zessin, K.H., Clausen, P.H., 2004. Application of field methods to assess Isometamidium resistance of s in cattle in western Ethiopia. Acta Trop. 90, 163–170.

Tizard, I.R., Holmes, W.L., York, D.A., Mellors, A., 1977. The generation and identification of haemolysin of Trypanosoma congolense. Experintia. 33, 901–902.
Vickerman, K., Myler, P.J., Stuart, K.D., 1993. African trypanosomiasis. In: Immunology and Molecular Biology of Parasitic Infections, pp. 170–212.

Vreysen, M.J.B., 2006. Prospects for area-wide integrated control of tsetse flies (Diptera: Glossinidae) and trypanosomosis in sub-Saharan Africa. Rev. Soc. Entomol. Argent. 65, 1–21.