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# Drug Sensitivity of T. b. Brucei Stabilates from Livestock in Lamu County, Kenya

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***Abstract:***
*Four trypanosome stabilates previously identified by PCR as T. b. brucei were isolated from livestock in Lamu and tested for drug sensitivity. The stabilates were grown in mice and characterized as either sensitive or resistant using a single dose of; Isometamidium (1.0mgkg$^{-1}$), Diminazene (20mgkg$^{-1}$) or Homidium (1.0mgkg$^{-1}$). Effective and curative doses were noted and evaluated through t-test and bio-assay descriptive graphical analyses. Clinical changes and mortality, Packed Cell Volume (PCV), parasitaemia and weight changes were monitored for 60 days post infection. KETRI 4028 responded to Isometamidium and Diminazene. KETRI 4032, KETRI 3985 and KETRI 3984 were resistant. Results indicated presence of T. b. brucei sub populations circulating in Lamu with multiple resistance to drugs used and 25% of sub-population of T. b. brucei that may be sensitive to Isometamidium and Diminazene. Homidium should be discouraged while use of Diminazene and Isometamidium should be used with caution.*

***Keywords:*** *Lamu County, T. b. brucei, drug sensitivity*

## 1. Introduction

AT has been found responsible for major socio-economic and public health problems in affected regions. In animals, infection with trypanosomes usually result in a chronic, debilitating and more often than not a fatal disease but the outcome of the infection differs between trypanosome species, between and within livestock species, depending on pathogenicity and virulence of trypanosome strains (Connor and van Den Bossche, 2004). *T. congolense, T. vivax* and *T. simiae* are known to be present in Kenyan coast. Boni and Dondori game reserves are an important source of tsetse blood meals, potential reservoir hosts and where livestock encounter a high tsetse and trypanosomiasis challenge. Studies have shown that frequent exposure to drugs in the field promotes gene mutation among the trypanosome parasites leading to drug resistance (Kagira and Maina, 2007). Records from the veterinary department indicate that there is a high level of trypanocidal drug use in Coastal belt. Since the disease prevalence is still high in domestic animals (Murilla *et al*., 2010) it is not clear whether this is due to drug resistance which has been demonstrated elsewhere in the coast (Murilla *et al*., 2014). Farmers in this region have been experiencing treatment failure attributable arguably to improper trypanocidal drugs use, resistance development and poor

qualities of trypanocidals (Ashiembi, 2013) with which farmers administer themselves. Some other factors which relate to the farmer and the parasite have been identified as contributing to treatment failure (Mdachi *et al*., 2006b) which is key in Coastal region. In an earlier study, despite presence of drug resistant trypanosomes in Lamu District, prophylactic and therapeutic drugs are still effective and hence, by use of quality drugs correctly, trypanosomosis can be effectively controlled (Mdachi *et al*., 2006a).

## 2. Materials and Methods

*2.1. Experimental Animals*

Two hundred normal healthy white Swiss mice weighing between 20-30g were obtained from BioRI-KALRO Small Animal Breeding Unit (SABU). They were dewormed subcutaneously using injectable ivermectin® at 0.01ml per mouse and were housed in cages designed for mice with 6 mice per cage and wood chippings as bedding material. The animals were acclimatized for 14 days during which they were maintained on mice pellets (mice pellets®, Unga Ltd, Nairobi, Kenya) and water provided *ad libitum* at room temperature. All experimental procedures and protocols involving mice were accordingly reviewed by Institutional Animal Care and Use Committee (IACUC) of BioRI-KALRO and approved.

*2.2. Trypanosome Isolates*

Four trypanosome stabilates previously identified by PCR as *T. b. brucei* isolated from a goat (KETRI 4032) and donkeys (KETRI 4028, KETRI 3984 and KETRI 3985) in 2007 and 2014 in different villages in Lamu County were used in this study. The stabilates have been cryopreserved in liquid nitrogen at -196$^0$C at the Kenya Agricultural and Livestock Research Organization-Biotechnology Research Institute- (KALRO-BioRI-) trypanosome bank. The stabilates had been collected from infected donkeys and goats in Lamu County during 2007–2014 epidemiological survey. The four were secondary stabilates (prepared after passaging in mice, restabilated and given the KETRI code).

*2.3. Animal Identification*

Body markings were made to avoid errors during recording of data and ensure accurate records and analysis. Different anatomical areas in the body were marked using picric acid solution using dipsticks with cotton.

*2.4. Multiplication of Trypanosome Stabilates in Donor Mice and WBF Preparation*

For each of the four stabilates, two donor mice were immunosuppressed using cyclophosphamide at 0.2ml per mouse for 3 consecutive days before infection (Kagira *et al* 2005). On the third day of the cyclophosphamide injection, two capillaries of each stabilate were retrieved from liquid Nitrogen in trypanosome cryobank and placed in a beaker containing ice and allowed to thaw slowly. The thawed stabilates were aspirated into a 1ml syringe and made up to 0.4mL using EDTA saline Glucose (ESG–PH 8.0). Wet Blood Films (WBF) on slides were prepared, then Giemsa® (Medic Diagnostic Reagents) stained. These slides were examined under a microscope at high power (40x) and oil immersion (100x) objectives (Waren *et al*., 2011a) to identify motile trypanosomes amongst the four *T. b. brucei* stabilates studied.

Each of the two donor mice were injected intraperitoneally with 0.2 ml of this inoculum. The donor mice were monitored for parasitaemia from day 2 post infection via microscopy (Korir *et al*., 2013). Parasitaemia was scored using the matching method of Herbert and Lumsden 1974 (Kobo *et al.,* 2014). When parasitaemia level reached antilog 8.1-8.4, blood enough to fill a capillary tube was harvested individually (for each mice) from the tail vein and diluted appropriately to make an inoculum for infecting experimental mice.

2.5. Infection and Treatment of Experimental Mice for Drug Sensitivity

At peak parasitaemia, blood was drawn from tail vein donor mice which was diluted ten times (1:10) with ESG at PH 8.0. The number of parasites in the inoculum was quantified using a hemo-cytometer. Further dilutions were made to $5x10^5$ trypanosomes/ ml.

There were 4 groups of 6 mice each per stabilate (Table 1). Each experimental mouse was intraperitoneally injected with 0.2 ml of this inoculum containing $1\times10^5$ trypanosomes/ml. Drug dosage used was as described by Geerts *et a*l 2001. Experimental mice were treated on day 1 post infection with a single dose of Isometamidium Chloride (Samorin®), Diminazene Aceturate (Veriben®) or Homidium Bromide (Novidium®) at 1.0 mg kg$^{-1}$, 20mg kg$^{-1}$and 1.0 mg kg$^{-1}$ respectively (Table 1).

| | Group | Drug | Dose (mg/kg body weight) | No of mice |
|---|---|---|---|---|
| 1 | Isometamidium | 1.0 | | 6 |
| 2 | Diminazene | 20.0 | | 6 |
| 3 | Homidium | 1.0 | | 6 |
| 4 | Controls | distilled water | | 6 |

*Table 1: Dose Regimens for Drug Sensitivity Evaluation*

As described by Korir *et al.,* 2013, a trypanosome stabilate was considered drug sensitive if at least 80% treated mice were cured. In this study if fewer than five mice were cured, the stabilate in question was considered drug resistant. I.e. Resistant stabilates are from 0/6 – 4/6 while sensitive stabilates are from 5/6–6/6 (Kagira and Maina, 2007).

*2.6. Monitoring the PCV, Parasitaemia, Body Weight and Mortality in Experimental Mice*

Pre-infection levels of packed cell volume (PCV), body weight and clinical observations on fur state, feed intake and emaciation for both experimental and untreated control mice were collected once a week for two weeks prior to infection. After the mice were infected and subsequently treated 24 hrs. later, parasitaemia was monitored daily by microscopic examination of wet smears (Kobo *et al.,* 2014) which involved using a drop of blood from the mouse tail placed on a clean slide and covered with a cover slip, parasites were counted under a microscope for the first 14 days then weekly for the rest of 60 days through Buffy Coat Technique (BCT). Parasitaemia was scored according to the commonly used matching method of Herbert and Lumsden 1974 (Kobo *et al.,* 2014). PCV of experimental mice was determined through hematocrit centrifugation technique (HCT) (Shimelis *et al.,* 2008). Blood enough to fill ¾ of a capillary was collected from the tail vein using a heparinized capillary tube and completely sealed with plasticin (Korir *et al*., 2013). The sealed capillaries were centrifuged in a hematocrit centrifuge at 10,000rpm, for 5 minutes. PCV was read using a hematocrit reader then expressed as percentage (%) of the total blood volume (Naessens *et al*., 2005). Body weight of experimental mice was monitored weekly using an analytical balance (Mettler Tolendo PB 302 r, Switzerland) (El-arab *et al*., 2006) and expressed to the nearest grams. Mortality of mice was monitored daily. Surviving mice were monitored for 60 days (Korir *et al*., 2013) and any experimental mice which experienced breakthrough infections during this period were humanely euthanized. Post infection data collected was subsequently entered in Ms Excel spreadsheets and cleaned. Graphs were used to show how trends of PCV, parasitaemia and how body weights unfolded.

## 3. Results

Pre-infection results and wet blood films of donor mice infected with stabilates

During pre-infection, generalized increase in all parameters used in drug sensitivity tests including weight, PCV and parasitaemia was observed. When Wet Blood Films (WBF) were examined 24 h post infection in donor mice for drug sensitivity studies, there was absence of trypanosomes until 48 h post infection for KETRI 4028, KETRI 3984 while the rest of the trypanosome species studied, motile trypanosomes were observed 3 days and 4 days post infection for KETRI 3985 and KETRI 4032 respectively. The following WBF captions (Figure 1) were taken under microscope (Olympus 1 X 51 UTV05XC-3 – Japan and Nikon UFX-DX- Japan).

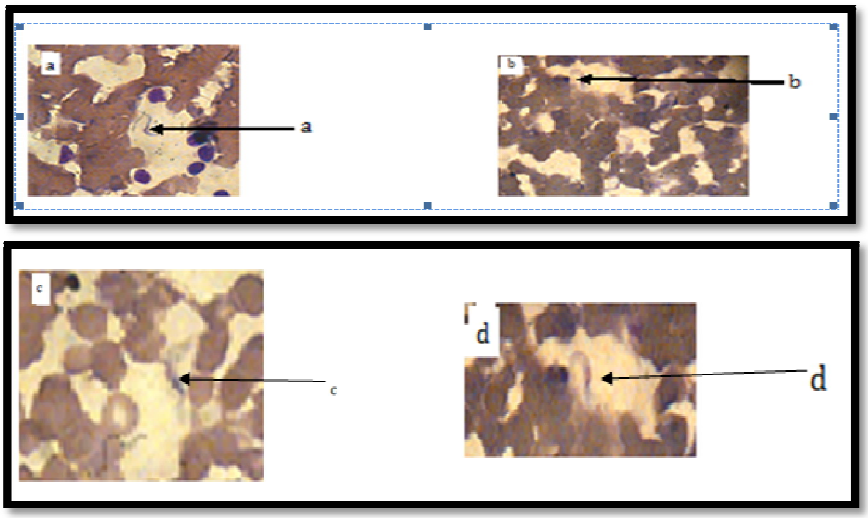


*Figure 1: Wet Blood Smears of Four Viable Trypanosome Stabilates in Experimental Mice Used for Drug Sensitivity Test. Key: A. KETRI 3985; B. KETRI 4032; C. KETRI 4028; D. KETRI 3984*

*3.1. Drug Sensitivity Testing*

Results on drug sensitivity test in mice are summarized in Table 2. Mice infected with KETRI 4028 were cured by Isometamidium1mg/kg and Diminazene 20mg/kg. These two drugs cured 100% of the mice and Homidium cured less than 80% of the mice infected with KETRI 4028. The other three stabilates (KETRI 4032, KETRI 3984 and KETRI 3985) were resistant to all the three drugs. Mice that were not cured following treatment with the three drugs relapsed on different days as shown in Table 2. Homidium treated mice that were infected with KETRI 4028 took significantly longer to relapse (34 days) than the mice infected with the other three stabilates (7, 13 and 20 days).

| Isolate identity | Number of mice cured | | | Relapse time (days ) | | |
|---|---|---|---|---|---|---|
| | Isometamidium 1mg/kg | Homidium 1mg/kg | Diminazene 20mg/kg | Isometamidium 1mg/kg | Homidium 1mg/kg | Diminazene 20mg/kg |
| KETRI 4032 | 2/6 | 1/6 | 1/6 | 13 | 7 | 14 |
| KETRI 3985 | 0/6 | 0/6 | 0/6 | 13 | 20 | 14 |
| KETRI 3984 | 2/6 | 0/6 | 0/6 | 41 | 13 | 27 |
| KETRI 4028 | 6/6 | 4/6 | 6/6 | 0 | 34 | 0 |

*Table 2: Sensitivity of Different Isolates to Different Trypanocidal Drugs*

*3.2. Clinical Signs*

Prior to infection, mice showed normal body conditions but after infection, treatment and relapsing depending on the stabilate and the drug used for treatment, the mice showed varying body conditions. The following plates show captions taken in the course of the study. Mice infected with KETRI 3985 and KETRI 3984 stabilates showed raised hair coat, lethargy, facial and scrotal edema as the disease progressed and also reduced feed intake was observed. Mice infected with KETRI 4032 and KETRI 4028 and treated with different drugs manifested minimal clinical signs of the disease. Isometamidium 1mg/kg treated mice infected with any of the four stabilates were least affected by clinical sign studied as shown in figure 2. The infected mice showed no clinical signs until 96 - 120 h post infection.

*Figure 2*
a. Mouse manifesting minimal symptoms of trypanosomiasis
b. Mouse manifesting moderate symptoms of trypanosomiasis
c. Mouse showing severe symptoms of trypanosomiasis

Figure 2: Caption a. Mouse infected with KETRI 4028 and treated with Isometamidium 1mg/kg manifesting minimal symptoms of trypanosomiasis. Mouse showing a smooth hair coat, and general body condition is normal. The mouse was active and feed intake was high. Caption b. Mouse infected with trypanosome stabilate (KETRI 3985) and treated with Homidium 1mg/kg. The mouse showed shivering, listlessness and huddling in a corner. Caption c. An experimental mouse infected with KETRI 3984 and treated with Diminazene 20mg/kg during a breakthrough infection stage at day 12. The mouse showed raised hair coat, facial edema and emaciation. Feed intake was very low. Mice showed minimal movements inside the cage at advanced stage of trypanosomiasis. Severe clinical signs included shivering and muscle tremors. The mouse died later in the day.

*3.3. Parasitaemia, PCV, and Body Scores in Experimentally Infected Mice*

3.3.1. Parasitaemia

There was significant difference (p-value which was <.001 (<$\alpha$=0.05) between KETRI 4028 and KETRI 3985 stabilates and control but the difference between KETRI 3984 and KETRI 4032 was not significant. On average, the first day of parasitaemia detection in blood for KETRI 4028, Control, KETRI 3985 and KETRI 4032 was 12$^{th,}$ 3$^{rd}$, 5$^{th}$ and 6$^{th}$ day respectively (figure 3). When treated with Homidium 1mg/kg, KETRI 3985 stabilate had the highest mean parasitaemia levels all through the trial followed by KETRI 4032. KETRI 4028 stabilate had the lowest parasitaemia mean.

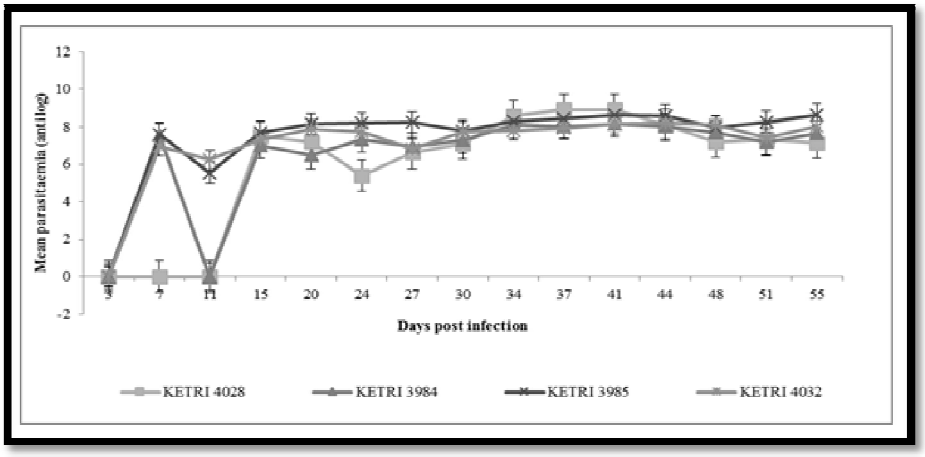


*Figure 3: Mean Parasitaemia Levels Against DPI for The Different Trypanosome Stabilates Treated With Homidium 1mg/Kg*

When treated with Isometamidium 1mg/kg, KETRI 4032 stabilate had the highest mean parasitaemia followed by KETRI 3985. KETRI 4028 stabilate had the lowest (Figure 4).

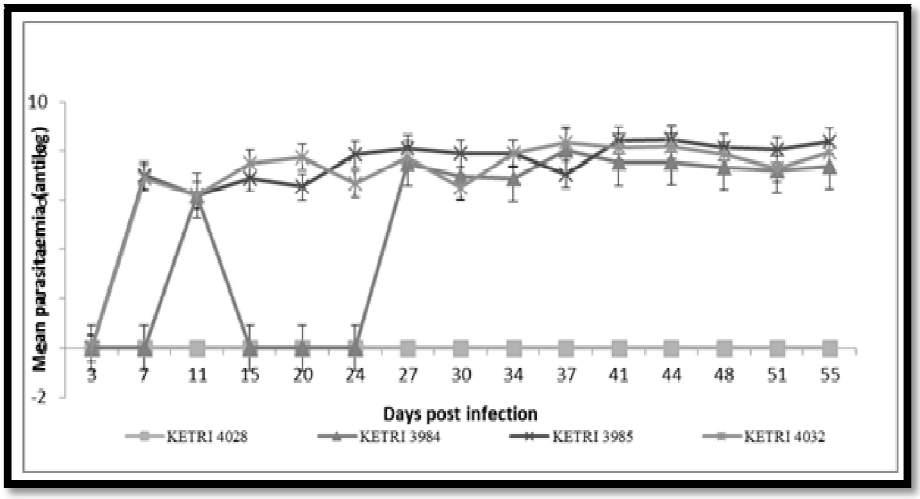


*Figure 4: Mean Parasitaemia Levels Against DPI of the Different Stabilates Treated with Isometamidium 1mg/Kg*

When treated with Diminazene 20mg/kg, KETRI 3985 stabilate had the highest mean parasitaemia throughout the trial followed by KETRI 4032. KETRI 4028 stabilate had the least (figure 5).

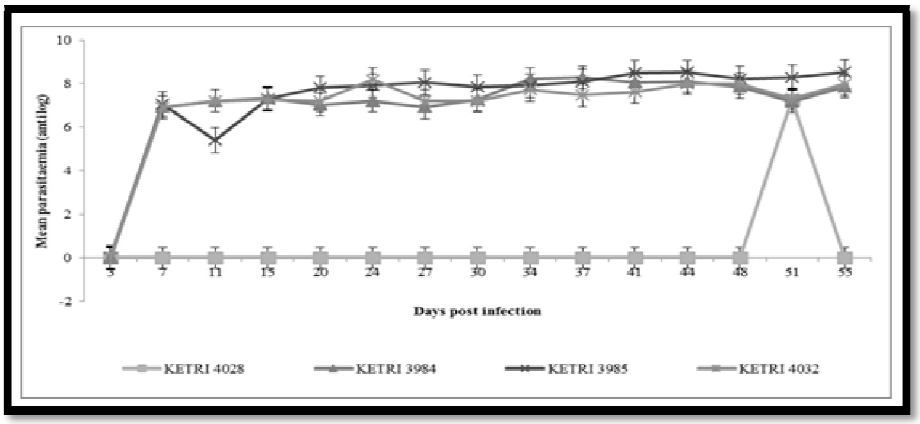


*Figure 5: Mean Parasitaemia Levels Against DPI of the Different Stabilates Treated with Diminazene 20mg/Kg*

When comparing parasitaemia levels of different stabilates in response to different drugs; the control group was most susceptible as it had the highest mean parasitaemia followed by groups treated with Homidium 1mg/kg and Diminazene 20mg/kg. Isometamidium 1mg/kg treated group was the least susceptible to KETRI 4028 isolate infection (figure 6).

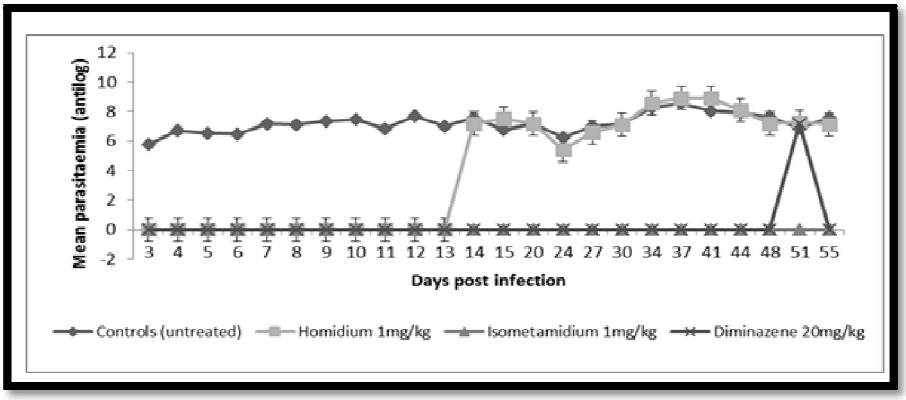


*Figure 6: Mean Parasitaemia Levels Against DPI of KETRI 4028 Isolate Treated With Different Drugs and Control Group*

Isometamidium 1mg/kg treated group was the least susceptible to KETRI 3984 stabilate infection (figure 7). The control group and Diminazene 20mg/kg treated group were most susceptible as they had the highest mean parasitaemia followed by group treated with Homidium 1mg/kg.

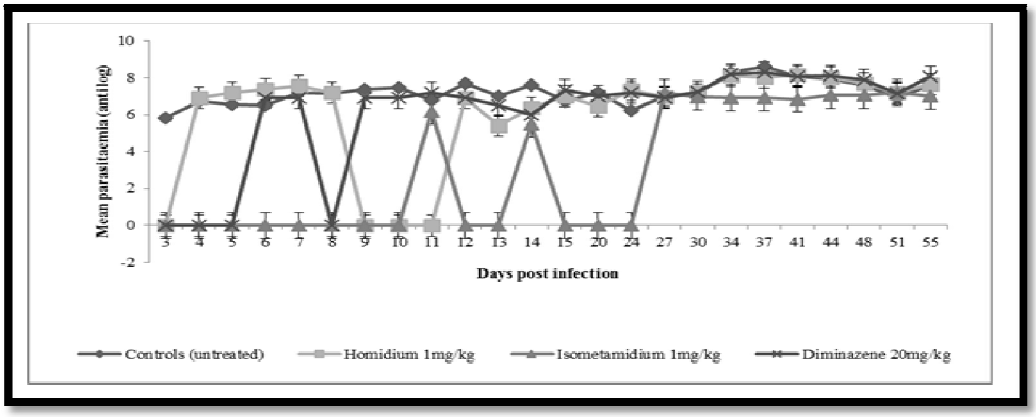


*Figure 7: Mean Parasitaemia Levels Against DPI of KETRI 3984 Stabilate Treated With Different Drugs and Control Group*

The group of mice treated with Homidium 1mg/kg was the most susceptible to KETRI 3985 stabilate as it had the highest mean parasitaemia on average throughout the trial followed by Diminazene 20mg/kg while Isometamidium 1mg/kg treated group was the least susceptible (figure 8).

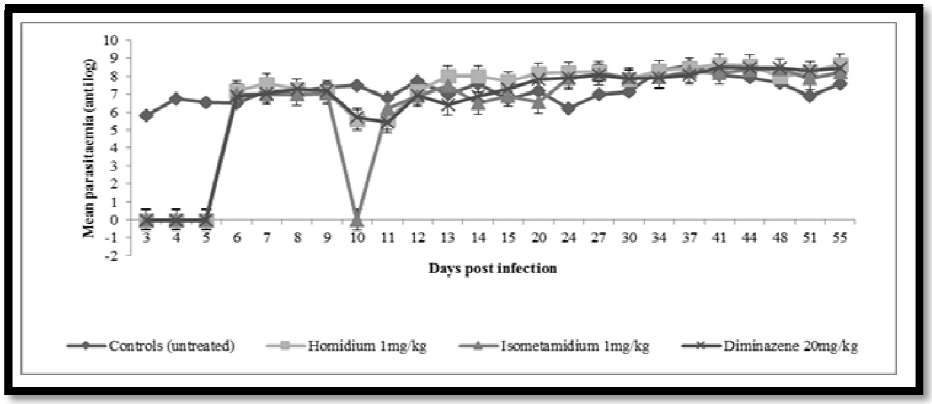


*Figure 8: Mean Parasitaemia Levels Against DPI of KETRI 3985 Stabilate Treated with Different Drugs and Control Group*

The group of mice treated with Homidium 1mg/kg was most susceptible to KETRI 4032 stabilate as it had the highest level of parasitaemia followed by the groups treated with Isometamidium 1mg/kg and Diminazene 20mg/kg (figure 9).

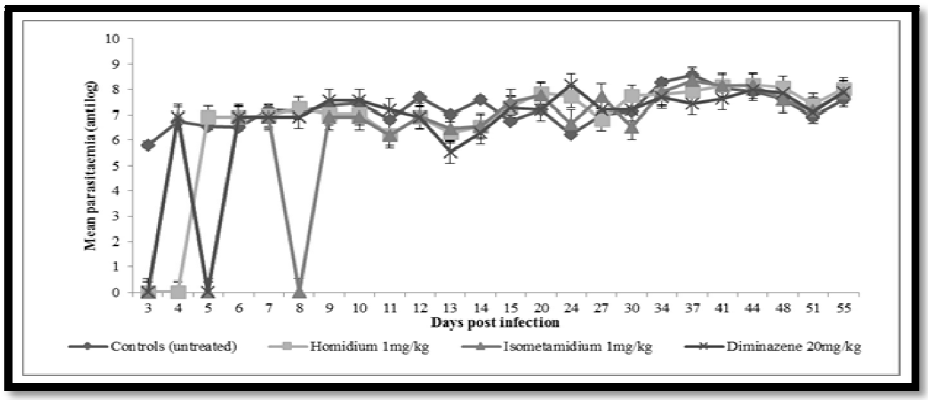


*Figure 9: Mean Parasitaemia Levels Against DPI of KETRI 4032 Stabilate Treated with Different Drugs and Control Group*

3.3.2. PCV Level

There was significant difference in PCV levels of different stabilates, p-value <.001 (<α=0.05). There was a significant change of PCV with time in mice infected with all the four stabilates. When infected with KETRI 3985, KETRI 4028, KETRI 3984 and KETRI 4032, Homidium1mg/kg and Diminazene 20mg/kg treated mice had lower mean PCV compared to those treated with Isometamidium 1mg/kg which had the highest mean PCV during the evaluation period (figure 10, 11, 12).

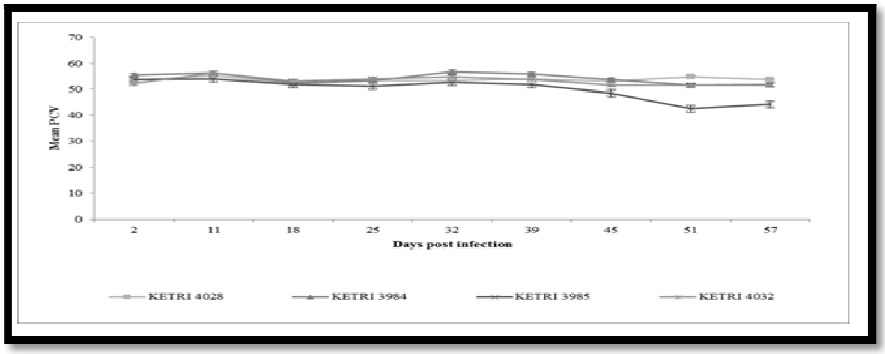


*Figure 10: Mean Pcv Levels Against Dpi of the Four Stabilates Treated With Homidium 1mg/Kg*

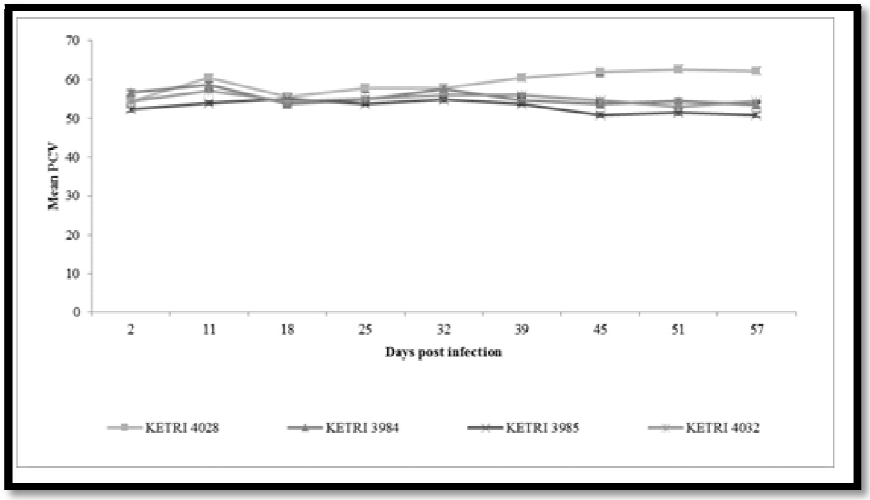


*Figure 11: Mean PCV Levels Against DPI of the Four Stabilates Treated With Isometamidium 1mg/Kg*

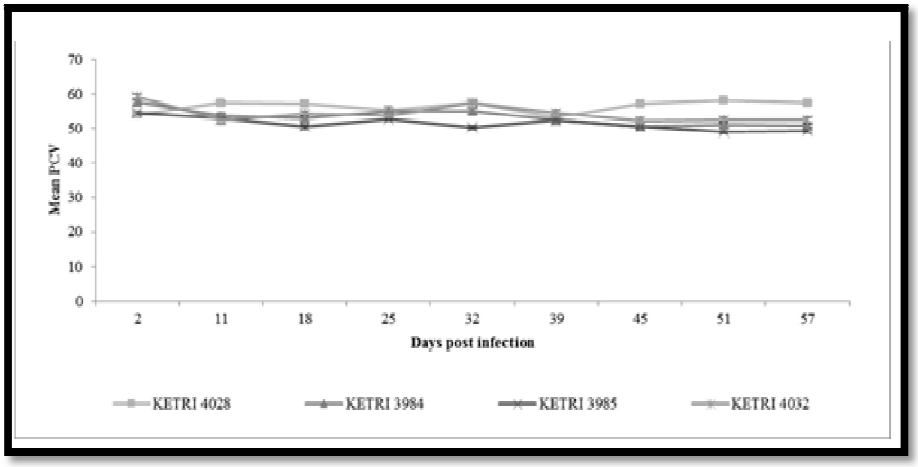


*Figure 12: Mean PCV Levels Against DPI of the Four Stabilates Subjected To Diminazene 20mg/Kg*

The PCV levels of mice infected with different stabilates and treated with the three drugs showed p-value 0.049 (<α=0.05) which indicated a significant difference in PCV levels within groups of mice infected and treated with different drugs. After treatment with all the three drugs, KETRI 3985 and KETRI 4028 infected mice PCV levels were highest followed by KETRI 3984 and KETRI 4032 (figure 13, 14, 15, 16). The following graphs show that mean PCV in controls was significantly different from mice infected with different stabilates p-value <.001 (<α=0.05), as they had the lowest mean PCV.

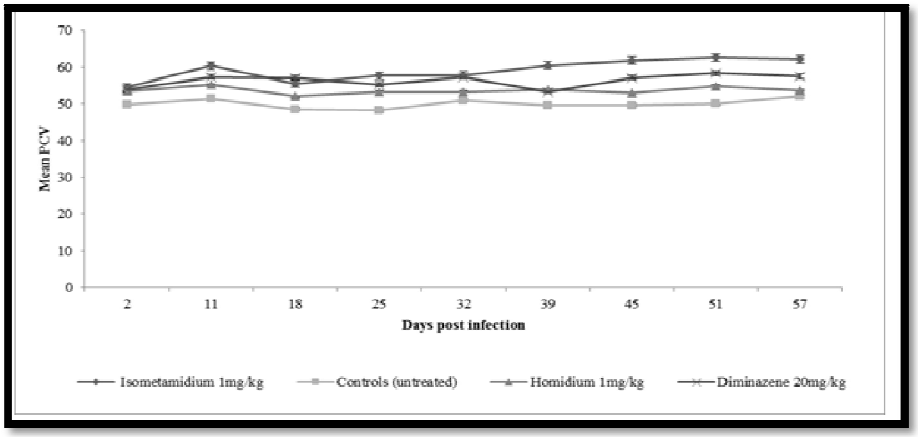


*Figure 13: Mean PCV Levels Against DPI of KETRI 4028 Stabilate Treated With Different Drugs and Control Group*

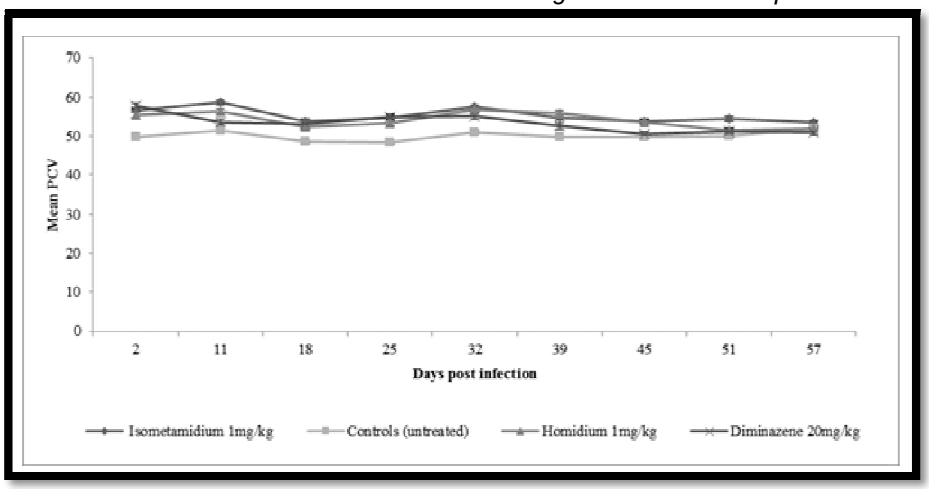


*Figure 14: Mean PCV Levels Against DPI of KETRI 3984 Stabilate Treated with Different Drugs and Control Group*

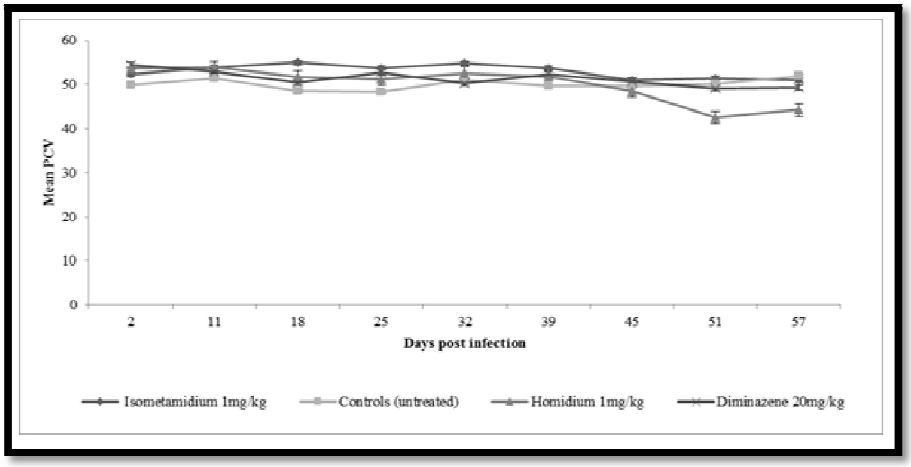


*Figure 15: Mean PCV Levels Against DPI of KETRI 3985 Stabilate Treated with Different Drugs and Control Group*

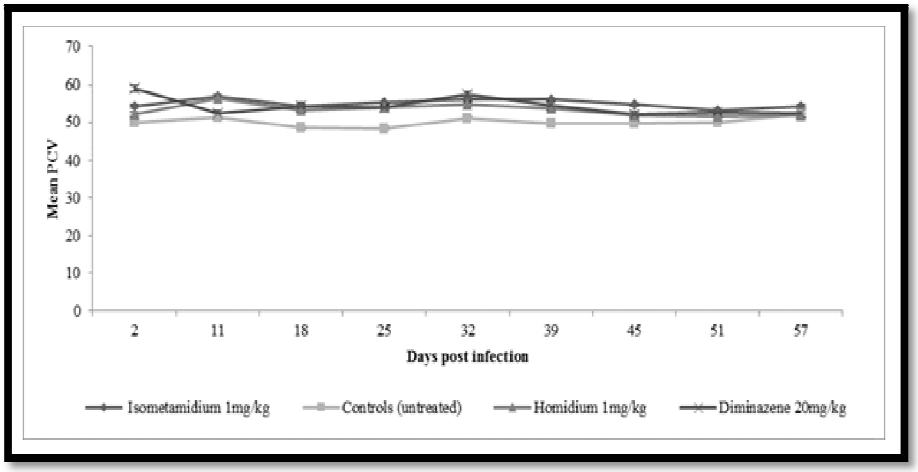


*Figure 16: Mean PCV Levels Against DPI of KETRI 4032 Stabilate Treated with Different Drugs and Control Group*

3.3.3. Body Weight

There was no significant difference in body weights between mice infected with the different stabilates (p-value 0.306 (>α=0.05). The body weights for all stabilates were similarly increasing during the trial except after ten days when KETRI 3985 had a significantly higher mean than the other stabilates when treated with the three drugs, as shown in figure 17, 18, 19.

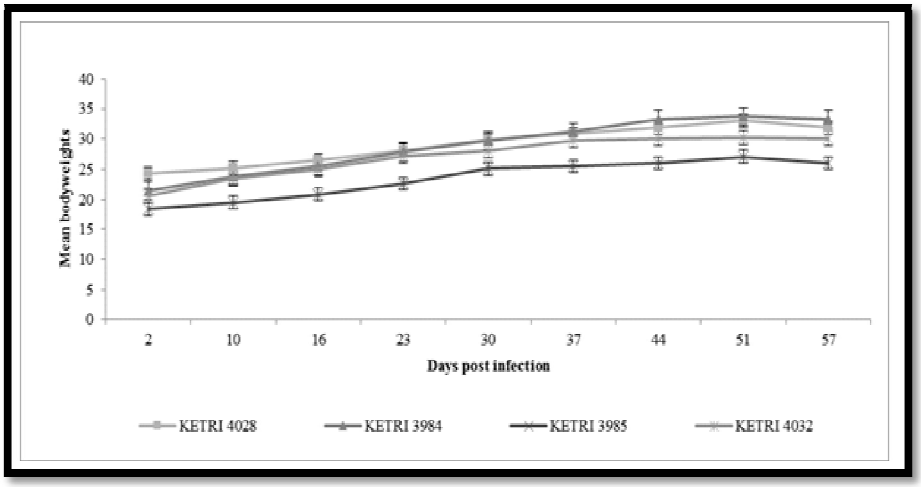


*Figure 17: Mean Body Weights Against DPI of the Different Stabilates Treated With Homidium 1mg/Kg*

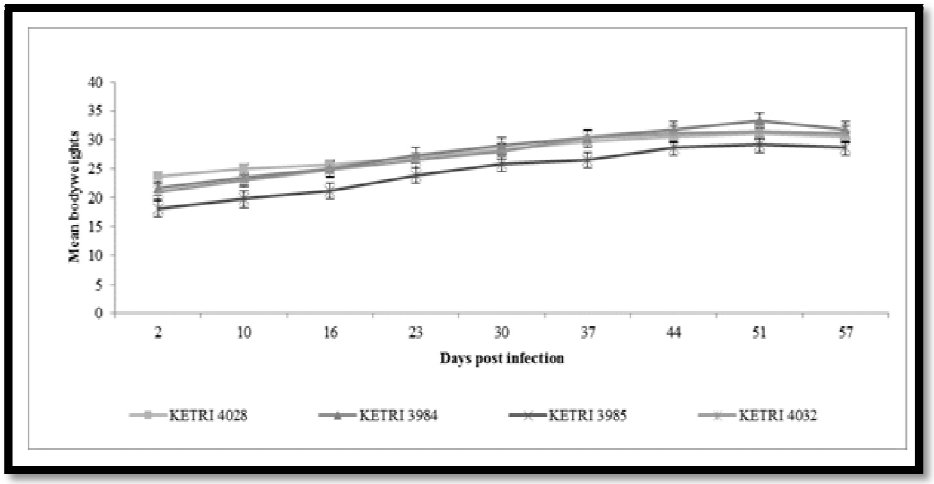


*Figure 18: Mean Body Weights Against DPI of the Different Stabilates and Treated with Isometamidium 1mg/Kg*

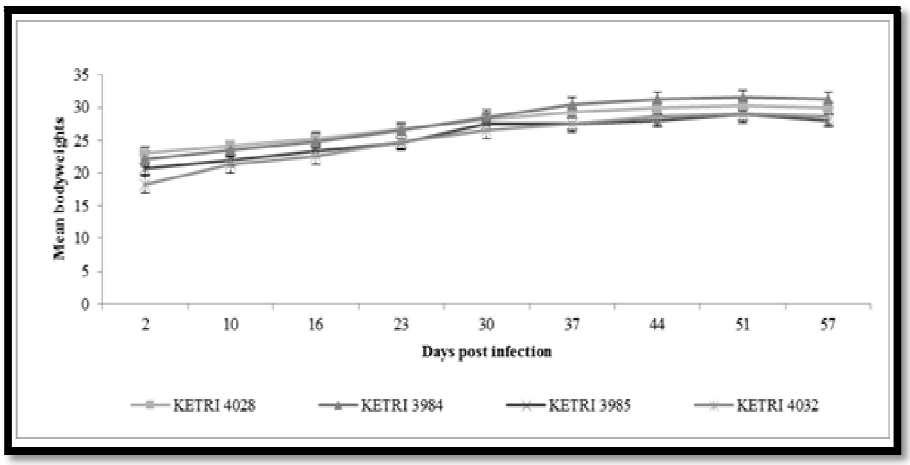


*Figure 19: Mean Body Weights against DPI of the Different Stabilates Subjected To Diminazene 20mg/Kg*

When comparing the three stabilates against different drugs; body weight changes within the time of study p-value was 0.161 (>α=0.05) which means that there was no significant difference in levels of body weights of the different drugs. The body weights of different groups of mice infected with KETRI 3985, KETRI 4028, KETRI 3984 and KETRI 4032 stabilates and treated with Homidium 1mg/kg, Isometamidium 1mg/kg and Diminazene 20mg/kg had a similar increasing trend during the trial period, which was not significantly different from control group (figure 20, 21, 22, 23)

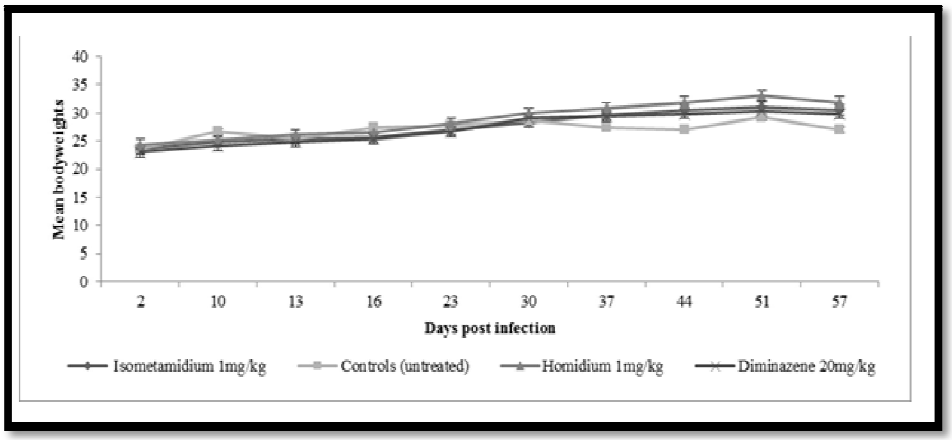


*Figure 20: Mean Body Weights against DPI of Mice Infected with KETRI 4028 Stabilate Treated with Different Drugs and Control Group*

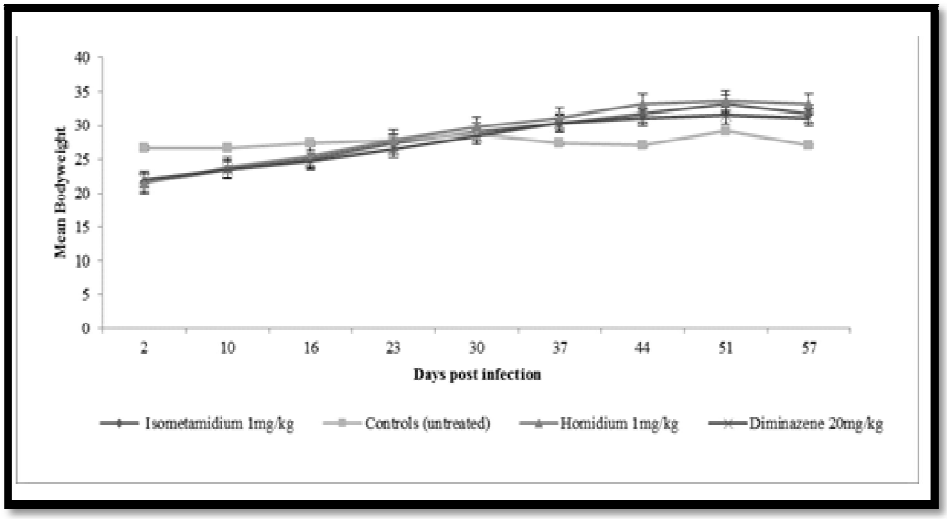


*Figure 21: Mean Body Weights against DPI of Mice Infected with KETRI 3984 Stabilate Treated with Different Drugs and Control Group*

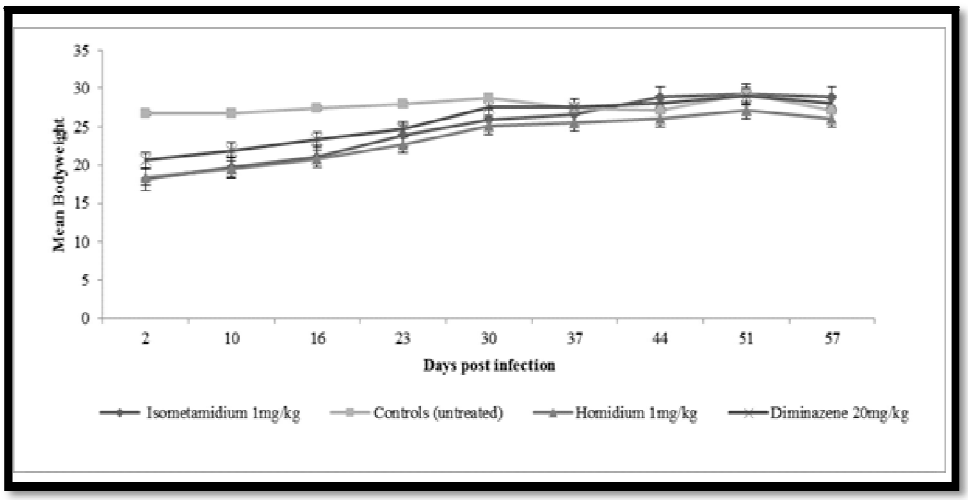


*Figure 22: Mean Body Weights against DPI of Mice Infected with KETRI 3985 Stabilate Treated with Different Drugs and Control Group*

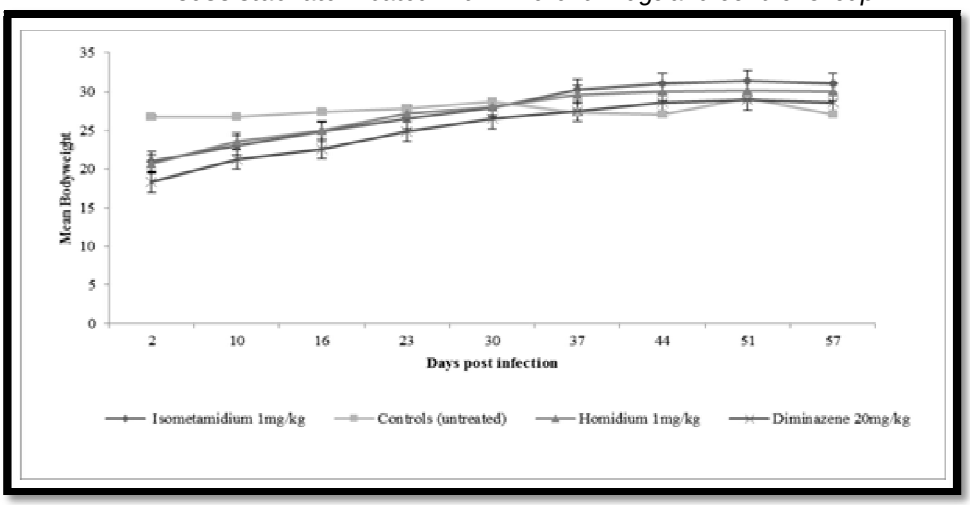


*Figure 23: Mean Body Weights against DPI of Mice Infected with KETRI 4032 Stabilate Treated with Different Drugs and a Control Group*

**4. Discussion**

Clinical signs were characterized by raised hair coats in mice, poor body conditions (lethargy), facial and scrotal edema, loss of appetite, fast short breaths and pus from injured tails which would reduce survival times of mice results interdem with Korir *et al.,* 2013 study. Most of the parasites showed a predominantly slender morphology, a free anterior flagellum with their posterior end being narrow and a sub terminal kinetoplast.

Experimental mice infected with KETRI 4028 and treated with Isometamidium 1mg/kg showed mild clinical signs. This could have been associated with lower virulence of the stabilates unlike in the case of KETRI 3985 and treated with Homidium 1mg/kg which had mild clinical signs. Mice infected with this stabilate had moderate disease symptoms while mice infected with KETRI 3984 and treated with Diminazene 20mg/kg were severely affected by trypanosomiasis as the disease progressed.

Parasitaemia development in KETRI 3984 was faster compared to that in KETRI 4032, KETRI 4028 and KETRI 3985. Mean parasitaemia was the highest in mice infected with KETRI 3985 stabilate and treated with Homidium unlike in all other stabilates treated with the same drug. This could have suggested that KETRI 3985 stabilate was more resistant to Homidium at 1mg/kg than the other stabilates. When treated with Isometamidium 1mg/kg, KETRI 4032 stabilate mean parasitaemia was highest compared to other stabilates treated with the same drug showing that it was more resistant to Isometamidium 1mg/kg than the other stabilates. The same group of mice was resistant to Homidium 1mg/kg as it equally showed a high parasitaemia. Diminazene 20mg/kg treated mice and infected with KETRI 3985 stabilate had the highest mean parasitaemia which suggested that this stabilate was more resistant to Diminazene 20mg/kg compared to the other two stabilates (KETRI 3984 and KETRI 4032). The control group was most susceptible to all stabilates followed by mice groups treated with Homidium 1mg/kg and Diminazene 20mg/kg. Parasitaemia levels of KETRI 3984, KETRI 3985 and KETRI 4032 were higher compared to KETRI 4028. Using Homidium 1mg/kg, KETRI 4028 took 15 days before parasitaemia developed. KETRI 3984 showed waves oscillating from day 7 through day 11 in which it was observed to drop to zero in day 15 when it relapsed. Using Isometamidium 1mg/kg, KETRI 3985 and KETRI 3984 stabilates parasitaemia developed in day 7 and day 11 respectively. The latter had parasitaemia waves oscillating to 27$^{th}$ day and in some instances, it dropped to zero. Using Diminazene 20mg/kg, oscillation was observed in KETRI 4028 at 51$^{st}$ day when parasitaemia developed which later dropped to zero in day 55. In KETRI 3984, slight oscillation was observed which did not drop to zero all through the 60 days of study. Different responses of immune system of mice infected with stabilates used in this study gave the difference in waves of parasitaemia. Different variable antigen types (VATs) of trypanosome stabilates to which immune responses is elicited especially in KETRI 3984, KETRI 3985 and KETRI 4032 could also be associated with the many waves observed in the graphs. PCV has been defined as measure of anaemia level (Kagira *et al*., 2005) in an animal. There was significant difference in PCV levels in the different stabilates treated with different drugs. There was a significant change of PCV with time in mice infected with all the four stabilates. The group of mice infected with KETRI 4028 and treated with the three drugs used in this study had the highest mean PCV indicating that it was sensitive to the drugs and blood PCV remained high. A high PCV level indicates that the parasitaemia levels were lowered by the drugs and blood cells kept high. When infected with KETRI 3985, KETRI 4028, KETRI 3984 and KETRI 4032, Control group of mice had the lowest mean PCV followed by mice treated with Homidium1mg/kg and Diminazene 20mg/kg drugs.

The body weights of all groups of mice increased during the study even in the case of controls. Results from the analysis showed that body weights of mice unexpectedly increased with time despite being infected but were generally lower in KETRI 3984 and KETRI 3985, which is interdem with findings from other studies. This has been explained in other studies by the fact that the mice were still young at the beginning of the experiment when they had just been weaned and now were growing to maturity by the progression of the experiment, thus went on gaining weight (Korir et al., 2013). In the previous studies, general decrease in body weights 12 days post infection (Celine *et al.,* 2005) have been shown which can be related to entry of trypanosomes in the CNS known to control body weight (Darsaud *et al*., 2004), unlike the case in this study. It was observed that infected mice whose parasitaemia levels were high had higher body weights but their PCV levels on the other hand were very low.

The relapse of infection by trypanosomes could occur majorly because the drugs used in this study were not able to target the relevant parts of the body where trypanosomes moved from the blood stream and sequestrated and death occurred in some cases. Where resistance was not a problem, a possible reason for infection relapse would be related to inaccessibility of the drugs to trypanosomes tissue stages of development (Al-Mohammed, 2008). Trypanosomes usually reside in structures including blood vessels of all organs, brain extravascular spaces, lung interstitials (Sudarto *et al.,* 1990) and other extravascular sites (Matovu *et al*., 2003). Sites in which Diminazene aceturate (Diminazene 20mg/kg) in particular is not able to penetrate according to previous studies is the brain spaces (Peregrine, 1993) where relapse was possible. Relapse and death were also seen by some authors in their studies which was also the case in stabilates from Lamu. This study concurs with results from Dargantes (2010), in which treatment with Diminazene 20mg/kg was found to be ineffective because relapse occurred on the 27$^{th}$ day post-treatment when used in rats and goats (Macaraeg *et al.,* 2013) which was the case in this study. Reduced sensitivity to drugs could have been associated with change in genetic constitution, mutation and selection. Drug resistance in *T. brucei* has been shown in other studies (Matovu *et al*., 2001) which were associated with the parasite surviving in high concentrations not tolerable to the host, or the parasite may be naturally resistant due to other factors like

host, vector or parasite. KETRI 4028 stabilate was sensitive to Isometamidium and Diminazene while stabilates KETRI 4032 from goat, KETRI 3985 from donkey and KETRI 3984 from donkey were resistant to the three drugs used in this study. This study has demonstrated the presence of *T. b. brucei* sub population that exhibits multiple drug resistance in mice. Earlier studies in Lamu, Kilifi and Kwale counties of the coastal Kenya have only demonstrated presence of *T.vivax* and *T. congolense* isolated from cattle that have shown multiple resistance to isometamidium, diminazene, homidium and quinapyramine (Mdachi, 2014, Ashiembi, 2013).

**5. Conclusion**

- Trypanosomes isolated from goats are resistant to Isometamidium 1mg/kg commonly used in Lamu market in the recommended dosages according to manufacturers.
- There is presence of *T. b. brucei* sub populations circulating in Lamu that may exhibit multiple drug resistance and only 25% of the sub population may be sensitive to Isometamidium and Diminazene.
- The study recommends that use of Homidium should be discouraged while use of Diminazene and Isometamidium should be used with caution and only in cases that proper diagnosis of the disease has been done.
- Integrated trypanosomiasis control strategies should be advocated in Lamu County.

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