Aggravation of pathogenesis mediated by ochratoxin A in mice infected with *Trypanosoma brucei rhodesiense*

J. K. KIBUGU1,2, J. N. NGERANWA2, J. N. MAKUMI2, J. K. GATHUMB13, J. M. KAGIRA1, J. N. MWANGI1, M. W. MUCHIRI1 and R. E. MDACHI1

1 Kenya Agricultural Research Institute, Trypanosomiasis Research Centre, P. O. Box 362, Kikuyu, Kenya
2 Kenyatta University, Department of Biochemistry and Biotechnology, P. O. Box 43844, Nairobi, Kenya
3 University of Nairobi, Department of Veterinary Pathology, Microbiology and Parasitology, P. O. Box 29053, Nairobi, Kenya
4 Kenya Agricultural Research Institute, Social Economics and Biometrics Division, P. O. Box 00290-57811, Nairobi, Kenya

(Received 12 August 2008; revised 21 September and 28 October 2008; accepted 29 October 2008; first published online 21 January 2009)

**SUMMARY**

Mice fed 1.5 mg ochratoxin A (OTA) per kg body weight and infected with *Trypanosoma brucei rhodesiense* were compared with trypanosome-infected placebo-fed and uninfected OTA-fed controls. Uninfected OTA-fed mice showed fever, lethargy, facial and eyelid oedemas, mild hepatitis and nephritis, and high survival. Infected placebo-fed controls had mean pre-patent period (PPP) of 3.26 days, lethargy, dyspnoea, fever, facial and scrotal oedema, survival of 33–65 days, reduced red cell counts (RCC: 10.96–6.87 × 10^6 cells/μL of blood), packed cell volume (PCV: 43.19–26.36%), haemoglobin levels (Hb: 13.37–7.92 g/dL) and mean corpuscular volume (MCV) of 37.96–41.31 fL, hepatosplenomegaly, generalized oedemas, heart congestion, hepatitis and nephritis. Compared to infected placebo-fed controls, infected OTA-fed mice had significantly (*P* < 0.05) shorter mean PPP (2.58 days), reduced survival (6–47 days), more pronounced fever and dyspnoea. The latter had significantly (*P* < 0.05) reduced RCC (10.74–4.56 × 10^6 cells/μL of blood), PCV (43.90–20.78%), Hb (13.06–5.74 g/dL), increased MCV (39.10–43.97 fL), severe generalized oedemas, haemorrhages, congestion, hepatic haemosiderosis, hepatitis, nephritis, endocarditis, pericarditis and exclusively, splenic macrophage and giant cell hyperplasia, expanded red pulp and splenic erythropagocytosis. It was concluded that OTA aggravated the pathogenesis of *T. b. rhodesiense* infection in mice, and should therefore be taken into consideration during trypanosomosis control programmes.

**Key words:** Ochratoxin A, *Trypanosoma brucei rhodesiense*, pathogenesis, mice.

**INTRODUCTION**

Sleeping sickness is of great medical concern in sub-Saharan Africa where more than 66 million people are at risk (WHO, 2004). The disease, which is caused by *T. b. gambiense* and *T. b. rhodesiense*, and transmitted by tsetse flies, is endemic in areas where other diseases such as HIV-AIDS, parasitic diseases, food-borne diseases (FBD) occur, and whose interaction could lead to serious implications on animal and human health. Mycotoxicosis is an important FBD caused by ingestion of mycotoxins produced by a variety of toxigenic fungi that contaminate food and animal feeds (FAO/UNEP, 1977; Azziz-Baumgartner *et al*. 2005). The most common mycotoxins are aflatoxins, ochratoxins, trichothecenes and zearalenone (Smith and Moss, 1985) and are stable to normal cooking and food processing procedures (Al-Anati and Petzinger, 2006). Through suppression of the immune and haemopoietic systems (Cukrova *et al*. 1991; Williams *et al*. 2004), interference with functions of essential nutrients like vitamins (Anyanwu *et al*. 2004) and toxicity effects on vital organs (Pier and McLoughlin, 1985), mycotoxins influence the course of many diseases. Thus the most common syndromes caused by ingestion of moderate to high levels of mycotoxins range from acute mortality (Azziz-Baumgartner *et al*. 2005), slow growth and reduced reproductive efficiency (Oswald *et al*. 2005) while lesser amounts may result in impaired immunity and decreased resistance to infections (Pier and McLoughlin, 1985).

One of the most potent and frequently encountered mycotoxin is ochratoxin A (OTA) (Kimathi and Siboe, 1994; Bondy and Pestka, 2000) which is a pentaketide secondary metabolite produced by the fungal species *Penicillium cyclosporum* (Smith and Moss, 1985) and *Aspergillus ochraceus* (El-Arab *et al*. 2006). Due to its many adverse effects such as immunotoxicity (Bondy and Pestka, 2000; Assaf *et al*. 2004; Al-Anati and Petzinger, 2006), nephrotoxicity and hepatotoxicity (Smith and Moss, 1985; JECFA 47, 2001), OTA is likely to alter the pathogenesis of trypanosome infections in the field. Also, through its potent nephrotoxicity, this mycotoxin could affect the production of renal-produced erythropoietin.

* Corresponding author: Kenya Agricultural Research Institute, Trypanosomiasis Research Centre, P. O. Box 362, Kikuyu, Kenya. E-mail: jkkibugu@yahoo.com

doi:10.1017/S0031182008005386 Printed in the United Kingdom

Downloaded from https://www.cambridge.org/core, Kenyatta University Library, on 01 Aug 2018 at 11:36:16, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. doi:10.1017/S0031182008005386
leading to aggravation of trypanosome-induced anaemia. Despite the endemicity of both trypanosomosis and mycotoxicosis in the sub-Saharan Africa, the interaction of the two diseases has not been investigated. Therefore, the purpose of the present study was to assess the effects of OTA on the pathogenesis of human infective *T. b. rhodesiense* infection in the murine model.

**MATERIALS AND METHODS**

**Ethics**

All protocols and procedures used in this study were reviewed and approved by the KARI-TRC Institutional Animal Care and Use Committee. The detoxification of mycotoxic waste and safety precautions were carried out as described elsewhere (Scott, 1995). In addition, the droppings and waste bedding material of the OTA-exposed animals were put in a labelled heavy duty plastic container, drenched in diesel and incinerated.

**Materials**

Forty-two days old male inbred adult Swiss White mice from KARI-TRC colony were maintained on mice pellets (Unga Feeds Ltd, Kenya) and water *ad libitum* at a temperature of 21–25 °C. These mice were acclimatized for 7 days before the experiment commenced. Wood-chippings were provided as bedding material. A cryo-preserved trypanosome clone, KETRI 3741, a derivative of KETRI 2537 which was previously isolated from a human host in Uganda in 1972 (Fink and Schmidt, 1980) was used to infect the mice in this study. Purified extracts of OTA (187.5 μg/ml) and a placebo (obtained from Bora Biotech Ltd, Cooper Centre, Nairobi, Kenya) were separately constituted in vegetable oil as described by El-Arab *et al.* (2006). Group A mice were fed on OTA extract for 7 days and then infected with *T. b. rhodesiense*. After infection, the feeding on OTA was continued up to the 30th day. Group B was fed on placebo extract and then infected as in Group A. Group C had the same ochratoxin treatment as in Group A but was not infected with trypanosomes. Groups D and E did not receive the OTA or placebo treatment but the former was infected with *T. b. rhodesiense* while the latter was not. In groups A and C mice, OTA was administered orally through a gavage needle for 30 days at a daily dose of 1·50 mg OTA/kg body weight. Group B mice received the equivalent volume of the placebo. Since higher mortality was expected in groups A, B and C than in D and E, a higher number of mice was assigned to the former. To infect mice, cryo-preserved trypanosome stabilates were first expanded in donor mice that were euthanized at peak parasitaemia and blood harvested as described earlier (Kagira *et al.* 2007a). Groups A, B and D mice were injected intraperitoneally (i.p.) with 10⁴ trypanosomes per mouse (Gichuki and Brun, 1999; Kagira *et al.* 2007a) on day 7 post-commencement of ochratoxin administration.

**Monitoring of parasitaemia and clinical changes**

Body weight of mice was determined weekly as described by El-Arab *et al.* (2006) using an electronic balance (Mettler PM34, DeltraRange®), commencing week 0 pre-mycotoxin exposure (1 week pre-infection) for 7 weeks post-commencement of OTA feeding (6 weeks post-infection duration). All mice as detailed in Table 2 at different intervals were weighed and then bled. Blood (20 μl) from mouse tail snip method was examined daily for parasites from the 2nd day post-infection for the first 2 weeks, every second day for the next 4 weeks and twice weekly for further 4 weeks. The matching technique of Herbert and Lumsden (1976) was used to assess parasitaemia levels. Fifty microlitres of tail blood was collected once a week, analysed by automated Coulter Counter (Beckman Coulter® AC-T diff™) and a full haemogram of each of the experimental mouse obtained. Thin blood smears were prepared, fixed and stained

<table>
<thead>
<tr>
<th>Table 1. Groups of mice used in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mice group</strong></td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td>Group A</td>
</tr>
<tr>
<td>Group B</td>
</tr>
<tr>
<td>Group C</td>
</tr>
<tr>
<td>Group D</td>
</tr>
<tr>
<td>Group E</td>
</tr>
</tbody>
</table>

**Table 2. Number of experimental mice present at different time-intervals**

<table>
<thead>
<tr>
<th>Mice groups</th>
<th>0</th>
<th>1</th>
<th>Inf</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Eu</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td>12</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>8</td>
<td>7</td>
<td>2</td>
<td></td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>2</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Group C</td>
<td></td>
<td></td>
<td>12</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>2</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Group D</td>
<td></td>
<td></td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Group E</td>
<td></td>
<td></td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

Number at different weeks post-mycotoxin exposure (p.m.e.) (Inf = infection time between 1 and 2 weeks p.m.e. *i*=number of mice during infection, Eu = euthanasia between weeks 4 and 5, *s*=number sacrificed.)
with Giemsa stain for differential cell counts (DCC) (Bain and Bates, 2001; Baker et al. 2001).

The pre-patent period (PPP), i.e. the time between infection and appearance of trypanosomes in the blood, for each mouse was determined and recorded. The survival times for each animal i.e. time the animal took to succumb to disease in the absence of chemotherapy, were monitored for 67 days post-trypanosome infection. This time was based on maximum survival time of 54 days observed in T. b. rhodesiense-infected mice (Fink and Schmidt, 1979), to which 2 weeks was added to make sure that time to death event for all mice was observed since the KETRI 3741 clone used in the present study was not well characterized. For animals surviving beyond this period, the survival time was recorded as 67 days and categorized as censored data. The clinical picture was determined daily as described by Gichuki and Brun (1999). Two animals per group were randomly selected, sacrificed on the last day of OTA exposure (30 day post-commencement of ochratoxin administration, i.e. 23 days post-infection), and the spleen weight: body weight ratio recorded. The gross pathology and histopathology of mice were also determined. After euthanasia, autopsy was conducted, major organs were harvested, stored in formalin, and histologically processed and slides stained with Haematoxylin and Eosin (Drury and Wallington, 1980). The prepared slides were then examined under the microscope.

Statistical analysis
Parasitaemia, PPP and spleen weight data were subjected to analysis of variance and mean separation using SAS (SAS Institute Inc., Cary NC, USA, 1999–2001) and StatView (SAS Institute, Version 5.0.1) statistical packages. Skewed haematological data were first subjected to the square root transformation, \( \sqrt{(x+1)} \), and then subjected to repeated measures analysis. Due to the unbalanced nature of the data, least square means were calculated. Survival data analysis was carried out using the Kaplan-Meier method for determination of survival distribution function. Rank tests of homogeneity were used to determine the effect of treatment on early (during early phase of infection) and longer (during late phase of infection) survival times respectively (Everitt and Der, 1998).

RESULTS

Parasitaemia development
The PPP of the T. b. rhodesiense-infected OTA-fed mice (Gp. A) was 2.58 ± 0.2 mean days which was significantly \( (P < 0.05) \) shorter than the T. b. rhodesiense-infected placebo-fed controls (Gp. B; 3.26 ± 0.1 mean days). The pattern of parasitemia is shown in Fig. 1, and was characterized by 2 prominent peaks; the first occurring in the first week of infection followed by a smaller wave that persisted until death. Although there was no significant difference \( (P > 0.05) \) in the level of parasitaemia in the second peak between the groups, the infected ochratoxin-fed mice (Gp. A) showed a higher second peak than the infected placebo-fed controls (Gp. B).

Clinical changes and survival
The clinical signs of all the T. b. rhodesiense-infected mice (Gps A, B and D) included lethargy, dyspnoea, raised hair coat, facial and scrotal oedema. These signs were more pronounced in the infected OTA-fed mice (Gp. A) than in the infected placebo-fed controls (Gp. B), especially dyspnoea. The clinical signs became more severe starting 24 days post-infection and were more pronounced compared to raised hair coat, poor body condition, lethargy, facial and eyelid oedemas observed in the uninfected OTA-fed mice (Gp. C). Fig. 2 shows the body weights of the experimental mice. While weight gain was observed in groups A, C and E, the uninfected naïve controls (Gp. E) maintained higher body...
weights throughout the experiment compared to the infected groups, with the infected OTA-fed mice (Gp. A) having the least values. However, the changes in body weights were not significantly different ($P < 0.05$) between the infected groups (Gps A and B) or between uninfected OTA-fed mice (Gp. C) and uninfected naïve controls (Gp. E).

Fig. 3 shows the survival distribution functions for the trypanosome-infected OTA-fed mice (Gp. A) and the trypanosome-infected placebo-fed controls (Gp. B). The survival for the $T. b. rhodesiense$-infected mice ranged from 6 to 47 days for the OTA-fed group (Gp. A) compared to 33 to 65 days for the infected placebo-fed controls (Gp. B). The OTA-fed group experienced more deaths during the early phase of the disease with the first death occurring on day 8 post-infection (p.i.) compared to day 33 p.i. in the controls. The rank tests of homogeneity showed that the Wilcoxon test $P$-value (0.01) was both significant ($P < 0.05$) and less than that of the Log Rank test (0.15). This indicated that the two groups differed primarily at early survival times with significantly ($P < 0.05$) shorter early survival times in the OTA-fed group (Gp. A) compared to the placebo-fed controls (Gp. B).

Table 2 shows the number of mice sampled at various time-intervals. Groups A, B, C, D, E had 11, 12, 9, 6, 6 mice respectively during infection, and 8, 12, 9, 6 mice respectively at 3 weeks post-mycotoxin exposure (p.m.e.), 5, 10, 7, 4, 4 mice in that order at 5 weeks p.m.e., while 1, 3, 7, 0, 4 mice were left respectively at 9 weeks p.m.e.

**Clinical pathological changes**

There was no difference in pathological changes between the infected placebo-fed (Gp. B) and infected naïve control (Gp. D) groups. Red cell counts (RCC) (Fig. 4), packed cell volume (PCV) (Fig. 5) and haemoglobin levels (Hb) (Fig. 6) dropped while mean corpuscular volume (MCV) increased (Fig. 7) in all $T. b. rhodesiense$-infected mice (Gps A and B) from second week post-infection compared to the uninfected naïve controls (Gp. E). Mild reduction in RCC, PCV and Hb with normal MCV was observed in uninfected OTA-fed mice (Figs 4–7). These
changes were more pronounced in the infected ochratoxin-fed group (Gp. A). The RCC, PCV and Hb were significantly lower (\(P<0.05\)) while the mean corpuscular volume (MCV) was significantly (\(P<0.05\)) higher in the infected ochratoxin-fed mice (Gp. A) than in the infected placebo-fed controls (Gp. B).

Grossly, the infected placebo-fed (Gp. B) and infected naïve (Gp. D) control mice had similar pathology which included emaciation, hepato-splenomegaly, ascites, liver and kidney capsular haemorrhages, heart congestion, cerebral oedema and hydrothorax. Histologically, there was perivascular inflammatory cell infiltration in the liver, heart and kidneys. The kidneys also showed fatty degeneration and necrosis of tubular cells. The gross lesions in the uninfected OTA-fed group (Gp. C) were liver jaundice, ulcerative gastritis and petechiae haemorrhage of kidney capsule. There was histological evidence of minimal inflammatory cell infiltrations, congestion, interstitial oedema, minimal hepatocellular fatty degeneration and coagulative necrosis in the liver of group C mice (Fig. 8). Also in this group, the kidneys had haemorrhages, congestion, tubular degeneration, necrosis and casts within tubular lumen and scanty inflammatory cell infiltrations (Fig. 9).

Mice in the infected OTA-fed group (Gp. A) had similar but more severe lesions than the infected placebo-fed (Gp. B), infected naïve (Gp. D) and uninfected OTA-fed (Gp. C) controls. These were mainly mild hepatosplenomegaly, hydrothorax, hydropericardium, congested liver and pale kidney with white pin-point areas. There was congestion, haemorrhages, fatty changes and massive perivascular infiltration with inflammatory cells (mainly lymphocytes and eosinophils) in the liver (Fig. 10) and kidney (Fig. 11). The liver had haemosiderosis and degeneration of hepatocytes while the kidneys showed degeneration of tubular cells, collapsed tubules, pinkish exudate within the tubules and interstitial oedema. There was endocarditis, pericarditis, perivascular cuffing, necrosis and fibrosis in the heart. The spleen showed macrophage and giant cell hyperplasia, expanded red pulp, and erythropagocytosis. In addition, the infected OTA-fed mice (Gp. A) had significantly (\(P<0.05\)) smaller spleen size than the infected placebo-fed controls (Gp. B) as shown in Table 3.

**DISCUSSION**

The results in this study showed an aggravation of clinical and pathological lesions in *T. b. rhodesiense*-infected ochratoxin-fed mice, indicating that ochratoxicosis synergizes the pathogenesis of the murine trypanosomosis. Increased mean corpuscular volume (indicating macrocytosis), dyspnoea, splenic...
macrophage and giant cell hyperplasia, and erythrophagocytosis in these mice suggested severe erythropoietic crises. Aggravated anaemia observed in the trypanosome-infected ochratoxin-fed animals was expected since anaemia in trypanosomosis and ochratoxicosis is well documented in various animal species. However, anaemia is attenuated by the anti-inflammatory drugs dexamethasone and hydrocortisone in T. brucei-infected mice (Balber, 1974; Halliwell and Gorman, 1989). This shows that while these corticosteroids influence anaemia through immunosuppression (Balber, 1974), a different pathway could be at play for the ochratoxin-mediated exacerbation of anaemia in the present study. The mechanism(s) by which OTA aggravated pathogenesis of anaemia in the present study could involve down-regulation of erythropoietin activity (Naessens et al., 2005) by ochratoxin A. Indeed severe nephritis was observed in the infected ochratoxin-fed mice. Hepatic haemosiderosis and severe haemorrhages in infected OTA-fed mice could also have exacerbated anaemia. This is not surprising since defects in red

Fig. 8

Hepatocellular fatty degeneration (FD) and scanty inflammatory cells (F) in the liver of uninfected OTA-fed mice (Fig. 8) and hepatitis in the liver of infected OTA-fed mice characterized by severe haemorrhages (H), congested vessel (arrow) and massive infiltration with mononuclear cells (M) (Fig. 10). Congestion (arrows) and few inflammatory cells in the kidneys of uninfected OTA-fed mice (Fig. 9) and perivascular inflammatory cell infiltration (F), haemorrhages (H, arrows) and pinkish exudate in renal tubules in the kidneys of infected OTA-fed mice (Fig. 11) (Haematoxylin and Eosin).

Fig. 9

Table 3. Mean spleen size of mice fed ochratoxin and infected with Trypanosoma b. rhodesiense

<table>
<thead>
<tr>
<th>Mice group</th>
<th>Mean spleen weight: body weight ratio (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected naïve controls (clean)</td>
<td>0.007</td>
</tr>
<tr>
<td>Uninfected ochratoxin-fed</td>
<td>0.024*</td>
</tr>
<tr>
<td>Infected placebo-fed controls</td>
<td>0.070*</td>
</tr>
<tr>
<td>Infected ochratoxin-fed</td>
<td>0.029**</td>
</tr>
</tbody>
</table>

* Significant difference in spleen sizes, between infected ochratoxin-fed mice and infected placebo-fed controls.
* Significant difference in spleen sizes, between all treated groups and uninfected naïve controls.
blood cells have been reported in ochratoxicosis (Gupta et al. 1983; Albassam et al. 1987) and trypanosomosis (Kagira et al. 2007b).

Histopathological evidence suggested exacerbation of inflammation in the trypanosome-infected OTA-fed mice in this study. Ochratoxicosis aggravated the inflammation observed in the kidney, liver and heart. The observed lesions have been reported before in trypanosomosis (Stephen, 1986; Maina et al. 2003) and ochratoxicosis (Albassam et al. 1987; JECA 47, 2001; Carlson and Ensley, 2003; El-Arab et al. 2006). OTA is a potent hepatotoxic and nephrotoxic (Smith and Moss, 1985) and could have promoted the degeneration of parenchymal cells in this study as has been observed by other workers (El-Arab et al. 2006). The severe inflammatory cell infiltration in the infected OTA-fed mice could have resulted from the increased number of necrotic cells (Anderson, 1985). The observed inflammatory eosinophils in the liver and kidney (Stephen, 1986) could suggest type I hypersensitivity reactions (Anderson, 1985) which has been reported during the early phase of the infection in the OTA-fed mice causing a more acute disease. This differed with results of similar studies on malarial parasites where, in Plasmodium berghei-infected mice, mycotoxicosis increased the host survival time, an effect that was attributed to direct toxicity of aflatoxin B1 on the parasite (Hendrickse et al. 1986; Young et al. 1988). This difference in the observations may be due to differences in the parasite species and the mycotoxin used. In the present study, ochratoxicosis and trypanosomosis could have worked in synergy to exacerbate the pathological lesions. Budovsky et al. (2006) reported similar cyclophosphamide-mediated aggravation of pathological lesions in T. lec-esi-infected rats, while Sandhu et al. (1998) observed OTA-mediated exacerbation of anaemia and biochemical changes in chicks infected with inclusion body hepatitis virus. Kumar et al. (2003) also observed increased mortality and severity of Escherichia coli infection in OTA-fed poultry. The mild retarded growth rate observed in the infected OTA-fed mice was expected since ochratoxin A and trypanosomosis are known to impede growth in animals (Stephen, 1986; Smith and Moss, 1985).

In conclusion, the study has shown that continuous dosing of mice with OTA aggravated the clinical and pathological aspects of T. b. rhodesiense (KETRI 3741) infection in mice. This aggravation may have some implications on the clinical progression and outcome of sleeping sickness and animal trypanosomosis cases in Africa where the two conditions occur concurrently. Indeed, global climatic changes which could result in favourable conditions for mycotoxin production, and the significance of interaction of mycotoxicoses with various infectious agents that may lead to emergence of new disease patterns has already been recognized (Minakshi, 2005). It is therefore important to consider the effects of ochratoxicosis during control programmes of trypanosomosis and other tropical parasitic diseases in the field. Further, future studies should evaluate the biological mechanisms involved in the exacerbation of pathogenesis of trypanosomosis by ochratoxin A.

Mr R. S. I. Karuku is highly acknowledged posthumously for inspiring the first author to the world of food poisoning, which is the main drive in this communication. We thank the Director, Kenya Agricultural Research Institute (KARI) and the Centre Director, KARI-Trypanosomiasis Research Centre for granting permission to publish this paper. We are particularly grateful to the following KARI-TRC staff: Messrs G. Kimure, B. Wanyonyi, Peter Githiri, A. Mageto, Ms J. Kagendo, Ms S. Kairuthi, Ms S. Nabisingo, Ms C. Kahi, who assisted in data collection and Mr P. Kahuria and Ms T. Kamau who provided the experimental animals. Drs Leonard Munga and John Thuita corrected the manuscript and optimized electronic cell
counting procedures respectively. This work was funded by Kenya Agricultural Productivity Programme (KAPP) through KARI.

REFERENCES


