Evaluation of Intestinal pH and Osmolality Levels in Rats (*Rattus norvegicus*) and Chickens (*Gallus gallus*) Experimentally Infected With *Trichinella zimbabwensis*

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**KEY WORDS:** pH, osmolality, *Trichinella zimbabwensis*, establishment, *Gallus gallus*, *Rattus norvegicus*, small intestines

**ABSTRACT**

The intestinal pH and osmolality levels of *Rattus norvegicus* and *Gallus gallus* infected with *Trichinella zimbabwensis* were evaluated. Forty five 10-week old male rats (*Rattus norvegicus*) and twenty four 3-week old chicks (*Gallus gallus*) were subdivided into 4 groups (Group1 uninfected rats, Group 2 infected rats, Group 3 uninfected chickens, and Group 4 infected chickens). Group 2 rats were each infected with 1,000 *T zimbabwensis* first-stage larvae (L1) and Group 4 chicks were each infected with 1,700 L1 while Group 1 and 3 were used as uninfected control rats and chickens, respectively. Using a standardized post-prandial time, the rats were sacrificed on Days 2, 5, 7, and 10 post-infection and chicks on Days 2 and 5 post-infection. Results in rats showed a significant increase in intestinal pH (*P* < 0.05) in the anterior segment of the intestines at Day 5 post-infection compared with the uninfected controls and a decrease in pH (*P* < 0.05) in the posterior segment at Day 2 post-infection. Osmolality of the anterior segment of the small intestine increased significantly (*P* < 0.05) at Day 2 post-infection before declining at Day 5 post-infection while in the posterior section, osmolality significantly increased (*P* < 0.05) at Day 2 post-infection. In chickens, there was no significant difference (*P* < 0.05) in the level of intestinal pH in the anterior and posterior segments of the infected and control groups. Although there was an increase in osmolality levels in infected chickens at Day 2 and at Day 5 post-infection, it was not significant (*P* > 0.05). It may be concluded that in *T zimbabwensis*-infected rats, intestinal pH and osmolality are altered during developmental stages of the parasite and this alteration might be responsible for facilitating the establishment and survival of the parasite, while no significant changes were observed in infected chickens; this probably explains the poor or non-establishment of the parasite in chickens.
INTRODUCTION
The genus Trichinella has the widest geographical distribution and the largest range of host species.\(^1\) Trichinella spiralis can establish itself in warm-blooded animals with the exception of birds while Trichinella pseudospiralis can establish in both birds and warm-blooded animals.\(^2\) Trichinella zimbabwensis has been reported to survive in a wide range of warm- and cold-blooded vertebrates with the exception of birds\(^3,4\); factors that lead to non-establishment of the species in birds are unknown.

Several factors have been implicated in the influence of habitat selection, establishment of a suitable microenvironment, and survival of Trichinella species in the host.\(^5,6\) Among these factors are intestinal pH, osmolality, and biochemical gradient, which have been implicated to play a role.\(^7,8\)

On one hand, raising the gastric pH has been reported to significantly increase the establishment and fecundity of T spiralis adult worm count in both in vivo and in vitro studies.\(^8\) On the other hand, a decrease in gastric pH induced prior to Trichinella infection led to an improvement in the establishment of the parasite.\(^8\) Chemical reactions that take place in the body have been reported to be sensitive to small changes in pH levels of the body fluids in which they occur,\(^9\) and it has been suggested that rapid changes in caecal pH and osmolality are likely to accompany bursts of ileal outflow,\(^10\) and changes in one or both have been shown to induce colonic propagating pressure waves.\(^11\)

The objective of this study was to determine the intestinal pH and osmolality changes in a susceptible host, Rattus norvegicus, and non-susceptible host, Gallus gallus, infected with T zimbabwensis.

MATERIALS AND METHODS
Parasite Strain
The T zimbabwensis strain used in this study was obtained from muscles of a commercially reared crocodile (Crocodylus niloticus) and maintained under laboratory conditions by periodic passage in Balb C mice. The number of T zimbabwensis larvae in muscle of infected rats was determined by visual enumeration using trichinoscopy.

Experimental Animals
Ten-week-old male rats (Rattus norvegicus) bred and maintained at the animal house unit of the Faculty of Veterinary Science, University of Zimbabwe, were selected for the study, and chickens (Gallus gallus) were bought as day-old chicks from Ross Breeders (Pvt.) Ltd., Zimbabwe, and were raised at the animal house unit. All animals selected for the study were apparently healthy.

The chicks were fed with a chick starter mesh for the first 3 weeks and broiler finisher thereafter bought from Agri-Foods, Zimbabwe, while the rats were fed with commercial mouse comproids from National Foods (Pvt.) Ltd., Zimbabwe.

Experimental Protocol
The chicks and rats were subdivided into groups by simple random sampling as shown in Table 1 and infected with T zimbabwensis at 3 weeks and 10 weeks post-infection.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>Day 2 Post-Infection</th>
<th>Day 5 Post-Infection</th>
<th>Day 7 Post-Infection</th>
<th>Day 10 Post-Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (uninfected rats)</td>
<td>20</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>2 (infected rats)</td>
<td>25</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>3 (uninfected chickens)</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4 (infected chickens)</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

The chicks and rats were subdivided into groups by simple random sampling as shown in Table 1 and infected with T zimbabwensis at 3 weeks and 10 weeks post-infection.
Each chick weighing approximately 350 grams was orally fed 1,700 *T. zimbabwensis* first-stage larvae (L1) while each rat weighing approximately 200 grams was orally fed 1,000 L1 following methods already described. Groups 1 and 3 were kept as uninfected controls for rats and chicks, respectively. Water was available to the rats and chicks ad libitum and food was withdrawn for 17 hours at each interval of slaughter. The rats were anesthetized using ether before slaughter while the chickens were stunned before being sacrificed. The rats were sacrificed at Days 2, 5, 7, and 10 post-infection and chickens at Days 2 and 5 post-infection. The days and frequency of slaughter were selected taking into account the short period of development and survival of *Trichinella* species in the small intestines of host.

**Measurement of pH and Osmolality**

The anterior half of the distance from the pyloric sphincter to the caeca represented the anterior portion of the small intestine while the remaining posterior half represented the posterior portion. Using a calibrated ruler, the anterior half of the small intestines was separated from the posterior half. The contents of each of these segments were collected for the determination of pH and osmolality according to the methods already described. Each portion of the small intestines was divided into 3 sub-portions and representative samples of 0.5 mL of the intestinal contents were collected from each of the sub-portion into Eppendorf tubes that were labeled and tightly closed and placed in ice. The tubes were immediately centrifuged at 13,000 rpm for 35 minutes in a refrigerated centrifuge (Biofuge fresco, Germany) at 0°C. The harvested supernatant was kept at 0°C and pH and osmolality were measured immediately. The pH of the intestinal contents was measured using a Crison pH meter GLP21 (Carburos Metalicos, Barcelona, Spain) and osmolality was determined on a 50-μL sample of supernatant in an Eppendorf tube by freezing point depression (Osmomat 030, Gonotec, Germany).

**Recovery of Adult *T. zimbabwensis* and Measurement of Parasite Length and Sex Determination**

*Figure 1.* Number of adult *Trichinella zimbabwensis* recovered from the small intestine of individual rats at Days 2, 5, 7, and 10 after oral infection with 1,000 larvae/rat.
The intestinal segments of infected rats and chicks were processed for recuperation of adult worms. Each segment was slit open longitudinally and placed in a separate dish. The segments were incubated in 0.85% saline solution at 37°C for 4 hours before the adult worms that had migrated from the intestinal wall into the saline were recovered. The parasites were counted and their sex recorded with the aid of a stereo-microscope. Parasites with a twin terminal appendage and papillae were considered as males while those with a single uterus filled with developing eggs in its posterior region were regarded as females. After the samples were fixed in 70% alcohol, their lengths were measured with the aid of a calibrated stereo-microscope.

Statistical Analysis
The effect of infection on the levels of pH and osmolality were determined by analysis of variance (ANOVA) using the general linear model of the Statistical Analysis Systems and differences were considered significant at $P < 0.05$.

RESULTS
No clinical abnormalities were observed in the infected and control rats and chicks. The number of adult *T. zimbabwensis* recovered from infected rats ranged from 91-416 at Day 2 post-infection (PI), 60-282 at Day 5 PI, 116-282 at Day 7 PI, and 145-255 at Day 10 PI. There was variation in the establishment of *T. zimbabwensis* among individual rats within the infected group. The largest number of recovered parasites from an individual rat was recorded at Day 2 (416 parasites; Figure 1). The distribution of male and female parasites in the anterior and posterior section of the small intestine of rats is shown in Figure 2. More male parasites were recovered from Day 2 to Day 10 in both the anterior and posterior sections of the intestines.

In chickens, a small number of adult parasites were recovered only from the anterior segment of the small intestine at Days 2 and 5 PI. One male and 9 female parasites were observed from the infected group at Day 2 PI and 5 females with no eggs in uterus were observed at Day 5 PI.
The mean lengths of randomly sampled adult male and female *Trichinella zimbabwensis* are shown in Table 2. Significant differences ($P < 0.05$) in the length of female parasite were observed between parasites collected on Days 2, 5, 7, and 10 post-infection; this was an expected outcome. There was a significant increase ($P < 0.05$) in the length of male parasites between Days 2 and 7, Days 2 and 10, Days 5 and 7, and Days 5 and 10 PI. Overall mean parasite lengths in the anterior (1.5 ± 0.04 mm) and posterior (1.6 ± 0.04 mm) sections did not differ significantly.

Table 2. Mean length (±SE) in millimeters (mm) of male and female *Trichinella zimbabwensis* recovered from the small intestine of rats at Days 2, 5, 7, and 10 post-infection.

<table>
<thead>
<tr>
<th>Days Post-Infection</th>
<th>Mean Parasite Length (n)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.9 ± 0.06a (28)</td>
<td>1.5 ± 0.04c (54)</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.8 ± 0.09a (14)</td>
<td>2.0 ± 0.04d (31)</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>1.2 ± 0.11b (8)</td>
<td>2.3 ± 0.04e (30)</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>1.2 ± 0.07b (19)</td>
<td>2.5 ± 0.04f (27)</td>
</tr>
</tbody>
</table>

$n = $ sample size.
Values with different superscript within a column are significantly different ($P < 0.05$).

The pH levels of the intestinal contents of infected and control rats are shown in Table 3. A significant increase in pH ($P < 0.05$) in the anterior segment was observed at Day 5 PI compared with the controls. In the posterior segment of the intestines of the infected rats, there was a significant decrease in pH ($P < 0.05$) at Day 2 PI and a significant increase ($P < 0.05$) at Days 5, 7, and 10 PI when compared to the controls.

In chickens, the pH in the infected group was 6.3 ± 0.07 and 6.2 ± 0.07 at Day 2 PI and Day 5 PI, respectively, while that of the control group was 6.2 ± 0.05 at Days 2 and 5 PI. There were no significant differences ($P > 0.05$) between the intestinal pH levels of anterior and posterior segments of the infected and control group.

Table 4 shows the mean osmolality levels measured from *T. zimbabwensis*-infected and control rats. The mean osmolality level of the anterior segment of the small intestine of the infected rats increased significantly ($P < 0.05$) at Day 2 PI before declining at Day 5 PI when compared to the control group (Table 4). In the posterior section, osmolality was significantly increased in the infected rats as compared to the controls at Day 2 PI. There were no significant changes in osmolality in the posterior section of the

Table 3. Mean pH of the anterior and posterior segments of the small intestines of infected and control rats at Days 2, 5, 7, and 10 post-infection.

<table>
<thead>
<tr>
<th>Day Post-Infection</th>
<th>Number of Rats</th>
<th>Anterior Intestinal Contents pH</th>
<th>Posterior Intestinal Contents pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Infected</td>
<td>Control</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>5</td>
<td>6.1a</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>5</td>
<td>6.1a</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>5</td>
<td>6.1a</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>5</td>
<td>6.1a</td>
</tr>
</tbody>
</table>

Values with different superscript letters within a column are significantly different ($P < 0.05$).
Values within a row without a common superscript letters are significantly different ($P < 0.05$).

Values with different superscript letters within a column are significantly different ($P < 0.05$).
small intestines of infected rats at Days 5, 7, and 10 PI.

In infected chickens, although osmolality levels overall increased from 404.1 ± 15.33 at Day 2 PI to 424.7 ± 19.69 at Day 5 PI, the increase was not significant (P > 0.05) in comparison to the control.

**DISCUSSION**

Previous studies have indicated that immune rejection of worms in secondary infection involves physiologically and presumably immunologically distinct early and late responses, with each response having a different developmental stage of the parasite as its target. In the present study, the levels of small intestine luminal pH in rats significantly changed due to the presence of *T. zimbabwensis*. Raising the gastric pH in rats has been reported to cause a significant increase in *T. spiralis* adult worm count with an increase in their fecundity both in vivo and in vitro. Conversely, lowering gastric pH prior to infection led to a reduction of the adult worm count and their inability to give birth to newborn larvae.

*In vitro* studies have shown that the infective larvae of *T. spiralis* secrete proteases predominantly of the serine type with azocolllytic and elastolytic activities. Degradation of azocasein by the larval excretory/secretory proteolytic enzymes occurs at a broad pH range with peak activity at pH 7 and high activities at pH 5 and 6. The collag-

eolytic activity was maximal at pH 5 while elastolytic was at pH 7. The observed luminal pH range in this study of 6.2-6.6 for the anterior and 5.7-6.9 for the posterior in rats fits well with the peak and high activities of the secreted Trichinella proteinases, which are also likely to be important for the survival of the *T. zimbabwensis* nematode. These enzymes have been implicated to be active in the processes of parasite transformation and counter-immunity, host invasion, and parasitic nutrition as well as evasion of the host’s immune responses to the parasite.

Although very poor establishment of *T. zimbabwensis* was observed in chickens, the pH range at Days 2 and 5 PI was within the survival range reported for most intestinal parasites. There is a likelihood of the pH accommodating establishment of the adult *T. zimbabwensis*, based on the infertile females recuperated but later followed by probably other physiological factors that are detrimental to the survival and ability of the parasite to reproduce in the small intestines.

<table>
<thead>
<tr>
<th>Day Post-Infection</th>
<th>Number of Rats</th>
<th>Anterior Intestinal Contents Osmolality</th>
<th>Posterior Intestinal Contents Osmolality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Infected</td>
<td>Control</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>5</td>
<td>476.3a</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>5</td>
<td>482.6a</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>5</td>
<td>479.8a</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>5</td>
<td>481.5a</td>
</tr>
</tbody>
</table>

Values with different superscript letters within a column are significantly different (P < 0.05). Values within a row without a common superscript letters are significantly different (P < 0.05).
When the larvae are ingested orally, the transit time from the crop to the duodenum under these unfavourable conditions is approximately 2 hours 20 minutes. It is possible that the mechanism of establishing and maintaining some degree of physiological adaptation that should occur between the parasite and the habitat is disturbed thereby limiting the survival of the parasite in the host even in the absence of evolutionary pressures.

In the present study, changes in osmolality of infected rats were observed at Day 2 PI in the anterior and posterior intestinal sections. In vivo osmolality fluctuations in the small intestine due to changes in absorption rates in rats is prevented through effective control of diurnal fluctuations in quantity and osmolality of food reaching the small intestine from the stomach. Osmolality results obtained from this study, except at Day 2 PI, were lower than the reported range of 600-800 obtained from the duodeno-jejunal contents of rats fed with normal solid food while water was available.

Rises in duodenal osmolality (peaking at 430 mosmol/kg) associated with meals, and persisting for hours, have been reported to occur in pigs. Osmolalities of the fluid from the different segments of the digestive tract of a rabbit was reported to be similar at 331 mosmol/kg although slightly hypertonic to the blood plasma of 297 mosmol/kg. In the present study, osmolality in chickens ranged between 400 and 420 mosmol/kg in the posterior section and 449 and 454 mosmol/kg in the anterior section, levels that are slightly lower than the levels reported in normal chickens. Besides a decrease in net lumen-to-tissue fluid movement during primary and secondary infection with *T. spiralis*, changes in osmolality as a result of disruption of fluid secretion associated with rapid expulsion of challenge *T. spiralis* has been reported in rats. Net lumen-to-tissue fluid movement was unaltered when rats were infected with (7 × 103 larvae/rat) and examined 30 minutes later.

Inhibition of gastric emptying by hyperosmolar mannitol has been reported to depend primarily on duodenal resistance, while the inhibitory effect of hyperosmolar glucose depended on nutrient-specific feedback on the stomach more than duodenal resistance. Differences have been observed in the distribution pattern of *T. spiralis* when different sizes of inocula were used, probably as a result of differential flow rates in the small intestine. Changes in motility are likely to combine with other mechanisms, including increased fluid secretion to evict parasites from the gut, rendering the intestine an inhospitable environment for the parasite and leading to a decrease in fecundity and expulsion of the parasite.

*Trichinella spiralis* adult males have been reported to measure approximately 1.0 mm long while females measured approximately 3.0 mm in length. Our findings for the male length range are in agreement with the previously reported results for *T. spiralis*. The difference in length between female *T. spiralis* and female *T. zimbabwensis* could be accounted for by species difference or by the fact that the authors did not indicate at what stage post-infection the parasite measurements were taken.

In this study, the mean adult *T. zimbabwensis* recovery percentages were 21.8% at Day 2, 16.2% at Day 5, 19.0% at Day 7, and 18.8% at Day 10. Although choice of method may reduce the *Trichinella* recovery, recovery percentages have been seen to vary widely within and between experiments with a range of 5%-19% reported in rats. Although the intestinal worm recovery was lower compared to the infection dose in this experiment, it compares well with previous studies reported for *T. spiralis*. The low establishment of *Trichinella* spp in birds other than *T. pseudospiralis* is probably due to physiological factors that resist the activation of infective larvae to the adult stage and the resulting poor reproductive capacity index. Low establishment of *T. pseudospiralis* to ostriches has already been reported. Although muscle tissue of the legs in ostriches were the preferential
site of T pseudospiralis larval distribution, T spiralis was only found in the bird’s muscle tissue after inoculation with a high number (80,000) of L1.34

In this study, it is difficult to explain whether the intestinal pH and osmolality changes observed in rats were as a result of the host responding to the presence of the parasite or the parasite inducing the changes so as to establish a suitable micro-environment. In vitro studies are more likely to give valuable information since critical factors can be controlled while investigating the effects of the parasite to specific physiological factors on specific vital parameters of the host.

REFERENCES


