Host-sex Influences the Establishment of *Trichinella zimbabwensis* in Sprague-Dawley Rats

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ABSTRACT

Males are usually more prone to parasite infections due to behavioural and ecological patterns as well as genetic and physiological differences between males and females. They have associated aggression, dispersal, and assemblage, which increase the chances of contact with both ecto- and endoparasites. In order to determine the effect of host sex on the establishment and development of *Trichinella zimbabwensis*, 50 Sprague-Dawley rats aged 6 weeks and weighing between 130-150g were divided into two groups (25 males and 25 females), and orally infected with *T. zimbabwensis*. On days 5, 10, 15, 20, and 25 post-infection (PI), five randomly selected rats were sacrificed from each group, and the numbers of adult parasites in the intestine as well as larvae in muscles were determined. Results from the study showed a significantly higher amount of *Trichinella* adults and larvae ($P < 0.05$) in male than in female Sprague-Dawley rats (four times higher adult worms and two times higher in muscle larvae in males than in females). The mean number of female adult worms was significantly higher ($P < 0.05$) than male adult worms in male rats on day 5 PI. The mean number of gravid females recovered in male rats on day 5 PI was significantly higher ($P < 0.05$) than mean number of non-gravid females. The female to male ratio of adult worms in male rats increased from 35:1 on day 5 PI to 6:1 on day 10 PI, whilst it was the reverse in female rats. The female to male ratio decreased at day 5 PI from 29:1 to 94:1 at day 10 post-infection. Our study concluded that there were significantly high establishment rates of *T. zimbabwensis* in the intestines and muscles of male Sprague-Dawley rats than in females, and the sex-hormone-linked immunological characteristics could possibly explain these results.

INTRODUCTION

Various factors have been reported to affect the successful establishment of *Trichinella* infection and transmission, depending on the type of species and the respective host involved.[17] These factors vary from environmental, immunological, and physiological, which include the gender of the respective

Table 1. *Mean (± SEM) of Trichinella zimbabwensis adult worms and muscle larvae recovered from male and female Sprague-Dawley rats at different days post-infection and the reproductive capacity index (RCI)*

Groups (N = 5)	Day sacrificed post-infection	Mean ± SEM of total adult worms recovered	Mean ± SEM of number of larvae recovered	RCI
Males	5	$545.8^{a} \pm 88.85$	0	0
Females	5	$84.6^{b} \pm 24.03$	0	0
Males	10	$156^{c} \pm 24.95$	0	0
Females	10	$38.2^{b} \pm 23.20$	0	0
Males	15	0	0	0
Females	15	0	0	0
Males	20	0	0	0
Females	20	0	0	0
Males	25	0	$2366.40^{d} \pm 608.72$	2.37^{f}
Females	25	0	$981.80^{e} \pm 85.99$	0.98^{g}

Different superscript letters in a column indicate significant difference (P < 0.05)
SEM = standard error of means

host and hormones present within the host.[4, 17, 26]

Parasites have been shown to exploit the host's hormonal micro-environment to ensure their survival, with the response being influenced by host species and sex.[4] Host sex has been reported to play a significant role in determining the susceptibility/resistance of a host to a wide variety of protozoan, helminthic, and arthropod infections and this has been attributed to sex hormones.[4, 13, 19]

It has been reported that males are usually more prone to parasite infections due to behavioural and ecological patterns as well as genetic and physiological differences between males and females.[11, 27] Males are commonly involved in behaviours such as aggression, dispersal, and grouping, which then increase the chances of contact with both ecto- and endoparasites.[11] Males are generally larger in size than females, which may make them obvious targets for parasitism.[11] One of the main physiological factors implicated in this male-biased infection is the immune-suppressive effect of testosterone, which increases susceptibility and exposure of host to various parasitic infections.[6, 7]

In a study on the effects of sex hormones on T spiralis, male rats were reported to exhibit greater susceptibility to T spiralis infection than female rats.[13] Male mice have also been reported to be more susceptible to helminths such as *Trichuris muris* and *Schistosoma mansoni*.[8] Plasmodium falciparum merozoites have been reported to produce an increased number of gametocytes after in vitro treatment with testosterone.[4]

In contrast, females have been reported to be less prone to parasitic infections when compared to male hosts.[7] Female hormones are reported to interact with the immune system to elicit a stronger immune response characterized by higher antibody levels and a better adaptive immunity.[7, 8] In a study conducted on *T. spiralis*, male rats injected with stilbestrol, a synthetic estrogenic compound used in treatment of female animals for infertility, displayed a marked decline in larval count as compared to normal male rats. On the contrary treatment of *T. crassiceps* cysticerci with $^{17}\beta$-estradiol increased its

reproduction capacity, whereas testosterone and DHT reduced this function.[4] Physiological changes within the female host will also determine the level of resistance to most parasitic infections.

Although males are reported to be more susceptible than females to many parasites species, there are parasite species for which males are more resistant to than females.[11] According to Hernandez-Bello et al,[9] male mice are less resistant to protozoans like *Plasmodium berghei*, *Tryponosoma cruzi*, and nematodes like Strongyloides sp. However, in vitro exposure of *Entamoeba histolytica* trophozoites to various concentrations of $^{17}\beta$-estradiol, progesterone, testosterone, and dihydrotestesterone (DHT) had little effect on the parasite viability and proliferation.[4]

The objective of this study was to determine the effects of host sex on the establishment and development of *T. zimbabwensis* in Sprague-Dawley rats.

MATERIALS AND METHODS

Study Animals and Design

A total of 50 Sprague-Dawley rats (25 males & 25 females) aged 6 weeks old and weighing 130-150g body weight were used for the study. The animals were housed at the Biomedical Research Unit (BRU) of the University of KwaZulu-Natal (UKZN), in a room subjected to a 12h light/12h dark cycle at a temperature range of 22-24 °C. Food and water were provided ad libitum. Experimental animals were randomly selected into groups of five animals per cage according to sex (Table 1). Cage bedding was changed every second day to ensure a clean and stable environment. Ethical clearance for the study was obtained from the University of KwaZulu-Natal Ethics Committee (069/12/Animal). The animals were weighed weekly from the start of experiment until the experiment was terminated.

Parasite Strain

The *T. zimbabwensis* strain used in our study originated from a naturally infected crocodile (Crocodylus niloticus) and was maintained in Sprague-Dawley rats at the Biomedical Research Unit of the University of KwaZulu-Natal. Carcasses infected with T zimbabwensis were digested using a modified protocol of the HCl-pepsin digestion method (Pozio et al., 2002) and recuperated larvae were used to infect the experimental animals. Muscle tissue from each rat was weighed and for every 100 g of tissue sample, 16 ml of 25% HCl, and 20 g of 0.7 U/mg pepsin (from porcine gastric mucosa); 2L H2O was used to prepare the digestive fluid.

Muscle tissue was digested for 35 min at 37° C in a 2L beaker on a magnetic stirrer. After sedimentation for 40 min in a separating funnel, the larvae were collected by flushing the bottom 40 ml from the funnel into a 50 ml cylinder. After cleaning the digestion fluid with an additional 10 min sedimentation step, the top 30 ml supernatant in the cylinder was removed, and the resulting suspension containing larvae was transferred to a marked petri-dish for quantification of larvae.

The experimental animals were infected by a once-off gastric gavage of 7 larvae per gram (LPG) of animal using an 18 G curved oral dosing needle. On days 5, 10, 15, 20, and 25 post-infection (PI), five animals were humanely sacrificed from each of the two groups using a terminal dose of halothane in order to determine the establishment of infection in the intestines and of larvae in the muscle tissue respectively. Infection was defined as the establishment of larvae in the muscles as a result of successful mating of adult parasites in the intestine and was detected by the digestion of muscle tissue.[23]

Recovery of adult worms from the intestines was achieved using a modified protocol.[15] The small intestines were immersed in 0.85 % saline solution, split open longitudinally using a pair of scissors, and washed with water under 212 µm sieve. After washing, the intestinal tissues were further incubated for 1 hr at 37 ºC and then re-washed under a 212 µm. The washings were viewed under dissecting microscope

Table 2. *Distribution by sex of Trichinella zimbabwensis adult worms recovered from the intestines of male and female Sprague-Dawley rats and reproductive status of the female parasites at different days post-infection*

Groups (N =5)	Day sacrificed post-infection	Mean ± SEM adult worms recovered				Female/ Male Ratio
		Gravid Females	Non-Gravid Females	Females	Males	
Males	5	503.6[a] ±88.1	27.2 d ±1.5	530.8[a] ±89.6	15.0[e] ±3.23	35:1
Females	5	74[b] ±23.8	7.8e ±3.6	81.8[b] ±27.2	2.8[e] ±0.4	29:1
Males	10	106.2c ±11.4	28.6 d ±8.6	134.8[c] ±20.0	21.6[d] ±5.7	6:1
Females	10	37.8b ±23.1	0±0.00	37.8[d] ±23.1	0.4[f] ±0.3	94:1

Different superscript letters in a column indicate significant difference (P < 0.05)
SEM = standard error of means

at 20 X objective for adult worm counts, whereas larvae in muscles were recovered using the modified artificial digestion.18

Data Analysis

Parameters measured include the number of adult *T. zimbabwensis* in the small intestines, reproductive status of adult female parasite, and the RCI. The RCI was calculated as the number of muscle larvae recovered divided by the number of larvae inoculated.[5] Reproductive status of each female parasite in the intestines was evaluated by observing in a female parasite the presence or non-presence of larvae in the uterus. A female parasite was considered as "gravid" when there was presence of larvae in the uterus and as "non-gravid" when there was no presence of larvae in the uterus. All data was expressed as mean ± standard error of means (SEM) using Microsoft Office Excel 2007. Two-way analysis of variance (ANOVA) was used to compare mean values of adult worms and mean values of muscle larvae between male and female rats over time of exposure to infection. One-way ANOVA was used to compare mean number of female, gravid, non-gravid and male adult worms recovered within and between groups of animals. The statistical package IBM SPSS 21 was used and $P < 0.05$ was considered significant.

RESULTS

Establishment of Adult Worms in the Gastrointestinal Tract

The establishment of adult parasites of *T. zimbabwensis* in male and female Sprague-Dawley rats is shown in Table 1. The establishment of adult worms was higher in male rats than in female rats at day 5 and 10 PI, however, the difference was not significant ($P > 0.05$). No adults were observed in both groups thereafter (Table 1).

The mean number (± SEM) of adult female and male of *T. zimbabwensis* and the reproductive status of the female parasites in male and female Sprague-Dawley rats is shown in Table 2. The mean number of female adult worms was significantly higher ($P < 0.05$) than male adult worms in male rats at day 5 PI. The mean number of gravid females recovered in male rats at day 5 PI was significantly higher ($P < 0.05$) than mean number of non-gravid females (Table 2). A similar trend was observed in male rats at day 10 PI (Table 2). The mean number of female adult worms was significantly higher ($P < 0.05$) than male adult worms in female rats at day 5, with a significantly higher ($P < 0.05$) number of gravid females recovered than non- gravid females (Table 2). A similar trend in female rats was observed at day 10 PI. However, no non-gravid females were recovered on that day. The female to male ratio of adult worms in male rats increased from 35:1 at day 5 post-infection to 6:1 at day 10 post-infection, whilst it was the reverse in female rats (Table3). In female rats, the female to male ratio decreased at day 5

post-infection from 29:1 to 94:1 at day 10 post-infection (Table 3).

Establishment of Larvae in the Muscles

The establishment of *T. zimbabwensis* muscle larvae was significantly higher ($P < 0.05$) in male rats than in female rats at day 25 PI. No larvae were recovered on other days of sacrifice except at day 25 PI (Table 2). Reproductive capacity index (RCI) was significantly higher in male rats than in females (Table 2) as indicated by the number of larvae recovered.

DISCUSSION

The results from this study showed that establishment of *T. zimbabwensis* in Sprague-Dawley rats is influenced by host sex, with a higher establishment of adults and larvae in males than in females. This is in agreement with the results on *T. spiralis* where establishment in rats was three times higher in males than females.[13] This suggests sex differences in the response to *Trichinella* infection with susceptibility and intensity more biased towards males than females. Sexual difference in exposure and susceptibility in parasitic infections has also been reported for several other parasite species in different host species with prevalence and intensity biased towards males than females,[1, 10-11] and this has been thought to be associated with genetic, behavioural, and social patterns. Among the behavioural and social factors thought to drive male biased parasitism are sex differential use of habitats,[25] males' aggression during mating,[14] and possible diet differences,[16, 24] all of which lead to differential exposure to parasites. Also courtship exercises and their increased mobility by males during breeding season have been deemed stressful that making males more susceptible to parasitism.[2, 22]

Females have been shown to express more inflammatory, innate, antibody-mediated and cellular responses to parasitic infections when compared to males.[11, 20] The observed sex differences in immune responses and the immune-endocrine interactions to parasitic infections are suspected to be linked to circulating sex steroids. [11, 20] Male testosterone is thought to increase susceptibility of males to parasitic infections, and this is due to the immune-suppressive effect of this hormone.[8, 10] Testosterone mediates its immune-suppressive effects through stimulated production of IL 18, which in turn suppresses Th2 immunity and inhibits IL-13 mediated work expulsion from the host.[8] Testosterone also inhibits translational factors that mediate pro-inflammatory and anti-parasitic cytokines.[11] These inhibitory actions of testosterone probably allow for the successful establishment and production of the parasite in the host. On the other hand, the female hormone, [17] β-estradiol, reportedly acts to enhance Th2 protective immunity against parasitic infections.[12]

These sex hormone linked immunological characteristics could possibly explain the observation from our study where in males there was both greater establishment and greater larval burden in the muscles of infected animals. Similar observations have been observed with infections by Leishmania in mice,[21] and Schistosoma parasites in humans.[3] The role of sex hormones in determining susceptibility of a host to parasitic infection by *T. spiralis* was illustrated[13] wherein injection of testosterone derived compounds in females resulted in an increased larval burden when compared to the group without treatment, whilst injection of estrogenic compounds resulted in significant reduction in larval burden when compared to the untreated group.

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ETHICAL STANDARDS

The authors assert that all procedures contributing to this work were compliant with the University of KwaZulu-Natal animal ethics (069/12/Animal).

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