Comparison of the Infectivity of *Trichinella zimbabwensis* in Indigenous Zimbabwean Pigs (Mukota) and Exotic Large White Pigs

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ABSTRACT

Ten pigs from 2 breeds (5 Mukota, 5 Large White) were each infected with 150,000 larvae of *Trichinella zimbabwensis*. Preinfection whole and clotted blood samples were collected at Day 0 and thereafter weekly for 6 weeks. Differential white cell counts were done on the whole blood while the clotted blood had creatinine kinase and lactate dehydrogenase levels determined. On Day 42, 3 pigs from each group were slaughtered to determine counts of *Trichinella* larvae per gram (LPG) of muscles. The Large White had significantly higher ($P < 0.05$) creatinine kinase levels than Mukota on Days 35 and 42. There were no significant differences in levels of lactate dehydrogenase. The Large White breed recorded significantly higher values of white blood cell count on Days 21 and 28. There were no significant differences between the 2 breeds in the number of LPG obtained in the masseter, snout, and tongue muscles.

INTRODUCTION

Trichinellosis is a parasitic infection of worldwide distribution.[1] *Trichinella* species show a cosmopolitan distribution infecting mammals primarily with scavenging and cannibalistic behaviour.[2] In 1995, *Trichinella* larvae were detected in the muscles of farmed crocodiles (*Crocodylus niloticus*)[3] in Zimbabwe; this represented the first observation of a reptile naturally infected with *Trichinella*.[4] Morphological, biological, biochemical, and molecular studies have been carried out on this isolate and it has been found to be a new non-encapsulated species and was designated *Trichinella zimbabwensis*.[4]

A considerable amount of work has been done on the biology and morphological characteristics of *T. zimbabwensis*.[4-6] Extensive work has also been conducted on *Trichinella* infection in pigs but most studies used *Trichinella spiralis*, the species found in the domestic cycle.

Southern Africa has a fairly large population of indigenous pigs and the Windsnyer (long nosed and razor back) type predominates in Zimbabwe and parts of Mozambique and Zambia.[7] The indigenous pigs in Zimbabwe originate from the Mukota area of northeastern Zimbabwe, thus the name for all indigenous pigs in the country. These are kept mostly in the communal areas of Zimbabwe. Husbandry involves free ranging during the dry season and confinement in simple pig houses during the rainy season.[8] These pigs scavenge for food usually with little or no feed supplementation: these pigs survive under poor standards of hygiene and this would testify their disease resistance.[8]

Trichinella zimbabwensis has been shown to infect both exotic and indigenous pigs *(Sus scrofa)* through several experimental studies.[5,6] However, the differences in the infectivity of this parasite to indigenous and exotic breeds have not been established.

The aim of this study was to compare the infectivity of *T. zimbabwensis* to the Zimbabwean indigenous pig breed, the Mukota, and an exotic breed, the Large White.

MATERIALS AND METHODS

Experimental Animals

A total of 12 pigs (6 Large White and 6 Mukota) aged approximately 3 months old were purchased from the University of Zimbabwe farm piggery unit. These were transported to the animal house unit of the Faculty of Veterinary Science, University of Zimbabwe, where they were housed for the duration of the experiment.

The animals were randomly divided into 4 groups as shown in Table 1 and were fed commercial pig feed. Clean water was supplied ad libitum. Each pig was tagged and housed in an individual pen.

Trichinella Strain and Preparation of Infection Material

A crocodile *(Crocodylus niloticus)*-derived *T. zimbabwensis* was used to infect the experimental pigs. The isolate had been maintained in the laboratory through periodic passages in rats *(Rattus norvegicus)*. To obtain the infection material for the pigs, rats *(R. norvegicus)* infected with *T. zimbabwensis* were euthanized and muscles of each carcass were individually minced. Two-gram samples were obtained from each carcass and these were digested using the HCL-pepsin method and larvae per gram (LPG) of the muscle samples were determined.[9] Based on the LPG value obtained, the muscle samples were divided into portions that contained approximately 150,000 Trichinella first-stage larvae and each portion was fed to the experimental pig.

The pigs were fasted overnight to ensure ingestion of the infected material the next morning. The pigs in Groups 1 and 2 (shown in Table 1) were each fed orally with approximately 150,000 larvae. Groups 3 pigs and 4 were considered as controls.

Table 1. Summary of the Experimental Design.

Group	N	Status	Male	Female	Larvae/Pig, n
1. Exotic	5	Inoculated	2	3	150,000
2. Mukota	5	Inoculated	3	2	150,000
3. Exotic	1	Control	1	-	0
4. Mukota	1	Control	-	1	0

Blood Sample Collection and Analysis

Pre-infection clotted and whole blood samples were collected from the jugular vein and thereafter collection was done every 7 days up to Day 42 post-infection. Differential white blood cell (WBC) counts were done on the whole blood samples. The

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clotted blood samples were centrifuged and sera were collected into serum tubes. The serum samples were analyzed for levels of creatinine kinase (CK) and lactate dehydrogenase (LDH).

Muscle Tissue Examination

On Day 42 post-infection, a total of 6 pigs (3 from Group 1 and 3 from Group 2) were slaughtered. Clotted and EDTA blood samples were also collected before slaughter. Muscle samples were collected from the diaphragm, masseter, intercostal, psoas, snout, and tongue from each of the slaughtered pigs. The samples were processed according to a standard HCL-pepsin digestion method.[9] The larval counts obtained were expressed as LPG by dividing the number of larva counted by the mass of muscle digested.

Statistical Analysis

A student *t*-test was used to determine the differences in levels of CK, LDH, and WBC between the 2 breeds. A 1-way analysis of variance (ANOVA) was performed on the log-transformed data (Log [value + 1]) to determine the difference in LPG obtained from the different muscles.

RESULTS

No clinical signs were observed in the infected animals of either breed. In exotic pigs, mean levels of CK started at 920.3 international units/liter (iuL), dropping to 369 iuL on Day 28, then rising markedly to 1255 iuL on Day 35 and decreasing slightly to 1320 iuL on Day 42. Levels of CK in Mukota pigs reached their peak of 732 iuL on Day 21 and decreased to the normal range towards the end of the experiment (Table 2). There were significant differences (*P* < 0.05) in levels of CK between the 2 breeds on Days 7, 35 and Day 42 with the Large White breed recording higher levels.

Levels of LDH peaked on Day 14 for both breeds (exotic, 616 iuL; Mukota, 624 iuL) then decreased steadily to reach the lowest level on Day 21 (exotic, 452 iuL; Mukota, 510 iuL) (Table 3). There were no

significant differences in levels of LDH throughout the whole period, although the exotic pigs recorded higher levels than the Mukota.

Table 2. Mean (± SEM) CK Levels in Exotic (Large White, n = 5) and Indigenous (Mukota, n = 5) Pigs Experimentally Infected With *Trichinella zimbabwensis*.

Days Post-Infection	Exotic	Mukota
7	920.3[a] ± 357.4	229.8[b] ± 79
14	755.6[a] ± 207.4	596.6[a] ± 227.4
21	942.8[a] ± 374.1	732.2[a] ± 186.2
28	369[a] ± 87.9	357.6[a] ± 69.6
35	1254.8[a] ± 552.7	329[b] ± 34.1
42	1319.8[a] ± 608.7	349[b] ± 32.8

SEM = standard error of the mean.
Values with different superscripts in a row are significantly different (*P* < 0.05).

Table 3. Mean (± SEM) LDH Levels in Exotic (Large White, n = 4) and Indigenous (Mukota, n = 5) Pigs Experimentally Infected With *Trichinella zimbabwensis*.

Days Post-Infection	Exotic	Mukota
7	575.3[a] ± 54.5	496.5[a] ± 25.5
14	616[a] ± 35.7	624.6[a] ± 39
21	452[a] ± 39	510.8[a] ± 44.2
28	515.2[a] ± 44.6	486.2[a] ± 32.7
35	523[a] ± 76.2	478.5[a] ± 28.6
42	542.6[a] ± 73.2	572.2[a] ± 86.4

SEM = standard error of the mean.
Values with similar superscripts in a row are significantly different (*P* < 0.05).

There were significant differences (*P* < 0.05) in the level of WBC between the 2 breeds on Day 21 and Day 28 with the exotic pigs recording higher counts of 26.1×10^9/L and 23.5×10^9/L for the respective days. The Mukota pigs recorded values of 16.8×10^9/L and 14.6×10^9/L on the same days. The inoculated exotic pigs recorded a steady decline in WBC counts throughout the experimental period, while the inoculated Mukota pigs showed a slow increase up to Day 21, then a slight decline at Day 28 before increasing sharply by Day 42 (Table 4).

There were significant differences ($P <$ 0.05) in mean LPG for the diaphragm between the 2 breeds with the Mukota having more larvae than the exotic breed and no significant differences in mean LPG between the breeds for the masseter, snout, intercostal and tongue muscles (Table 5).

Table 4. Mean (± SEM) WBC Counts (×10⁹) in Exotic (Large White, n = 5) and Indigenous (Mukota, n = 5) Pigs Experimentally Infected With *Trichinella zimbabwensis*.

Days Post-Infection	Exotic	Mukota
7	27.7[a] ± 4	15.5[a] ± 2.5
14	26.8[a] ± 2.7	16.8[a] ± 2.4
21	26.1[a] ± 4.2	16.8[b] ± 1.6
28	23.5[a] ± 5	14.7[b] ± 1.8
35	23.2[a] ± 5.3	15.2[a] ± 2.2
42	20.7[a] ± 2.9	19.4[a] ± 4.9

SEM = standard error of the mean.
Values with different superscripts in a row are significantly different ($P < 0.05$).

Table 5. Mean (± SEM) LPG of Muscle From Different Muscles in Exotic (Large White, n = 3) and Indigenous (Mukota, n = 3) Pigs Infected With *Trichinella zimbabwensis* at Day 42 Post-Infection.

Breed	N	Diaphragm	Masseter	Psoas	Snout	Intercostal	Tongue
Exotic	3	0.2[a] ± 0.1	6.7[a] ± 2.8	2.7[a] ± 2.8	1.3[a] ± 1.0	4.6[a] ± 4.1	3.2[a] ± 2.4
Mukota	3	24.6[b] ± 3.5	5.3[a] ± 6.4	0.1[b] ± 0.1	2.1[a] ± 1.9	0.5[a] ± 0.4	6.2[a] ± 6.2

SEM = standard error of the mean.
Values with different superscripts in a row are significantly different ($P < 0.05$).

DISCUSSION

It appears from this study that *T. zimbabwensis* is more infective to the exotic breed of pig (Large White) than to the indigenous breed (Mukota). The exotic breed recorded higher levels of CK than the Mukota pigs throughout the whole period of the experiment. For both breeds, CK levels peaked on Day 21. After the peak, the Mukota recorded decreasing levels up to the end of the experiment. The Large White breed recorded the lowest level on Day 28, and thereafter the CK levels began to rise until the end of the experiment

The damage and increased permeability of the muscle cell membrane result in increased tissue permeability[10] and therefore significant increases in levels of these enzymes in serum. This differs from an experiment where no influence on the muscle enzymes by trichinellosis in raccoon dogs was recorded.[11]

The LDH levels recorded did not significantly vary between the 2 breeds. The trend observed in the levels of CK and WBC indicated that the parasite induced greater reactions in the Large White pigs compared with the Mukota breed. This could be a result of the Mukota breed being tolerant to infection with the parasite.

Leukocytosis is common early in trichinellosis.[12-14] It is correlated with the intensity of the infection in the host, and only the adult stage of *Trichinella* has been shown to elicit eosinophilia.[15] The eosinophils infiltrate the infected portion of intestinal tissue and locate adjacent to the adult worms and then enter into damaged muscle tissue after newborn larvae penetrate them.[16]

The life cycle of *Trichinella* can be separated into an enteric, a vascular, and a muscular phase.[17] It is during the muscular phase that the *Trichinella* parasites enter muscle tissue and cause considerable damage during this process. When there is damage to smooth, heart, or skeletal

musculature, the level of some enzymes in serum such as CK, LDH, and aspartate aminotransferase are elevated.[18] These enzymes are valuable tools used in the early detection of muscle wastage as a result of ischemia, injury, or inflammation.[19]

No clinical signs associated with trichinellosis were seen in any of the animals. Absence of clinical signs in reindeer experimentally infected with *T. spiralis* and *Trichinella nativa* has also been recorded.[20] However, trichinellosis in mice has been reported to cause decreased ambulatory and exploratory activity. This is thought to render the host more vulnerable to predation and thus enhance parasite transmission.[21,22]

Differences in infectivity of isolates of *Trichinella* in different hosts are probably due to the degree of immunological response of the host.[23] Differences in infectivity could be a result of differences of the number of adults that manage to establish during the intestinal phase. But this would have a very marked difference in the mean LPG recorded for the different muscles in the 2 breeds of pigs. Significant differences in mean LPG were observed in the diaphragm and psoas muscles only. Thus, it is highly unlikely that differences in infectivity as measured by CK, LDH, WBC, and LPG are due to differences in the establishment of the parasites in the muscles of the 2 breeds of pigs.

Based on results from this study, it appears that the indigenous Mukota breed is more tolerant to infection with *T. zimbabwensis* than the Large White. However, more studies need to be conducted on this aspect in particular concerning *T. zimbabwensis* infection in the Mukota breed.

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