Evidence of Congenital and Transmammary Transmission of *Trichinella zimbabwensis* in Rats *(Rattus norvegicus)* and its Epidemiological Implications

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ABSTRACT

The occurrence of congenital transmission of *Trichinella zimbabwensis* in rats was investigated by infecting 30 female rats *(Rattus norvegicus)* with *T. zimbabwensis* (2000 L^{-1} larvae/rat) on different days in relation to mating day. Transmammary transmission was investigated by mating 10 rats at 40 days postinfection. Offspring of each rat were checked for *Trichinella* larvae in muscles at weaning. Both congenital and transmammary transmission were observed. Dams infected post-conception had more *Trichinella*-infected offspring than the pre-conception-infected dams. The highest number of infected litters was recorded through the transmammary route.

INTRODUCTION

The role of rodents in the maintenance and transmission of *Trichinella spiralis* has been a source of debate.[1] Brown rats *(Rattus norvegicus)* play an important role in the transmission of *T. spiralis* from one focus to another and in its maintenance in the same focus.[2] In swine farms, the trophic-topical relationships between the rat and swine are extremely complex and often the origin and development of trichinellosis are very unclear.[3] Congenital transmission of *T. spiralis* has been reported as one way that trichinellosis can be transmitted, and its role should not be underestimated.[4]

When illustrating the life cycle of *Trichinella*, the oral route of infection is always shown as the only way to infect the definitive host. However, both oral and congenital transmissions have been demonstrated as alternative routes of transmission of *Trichinella* parasites.[3,4]

The transplacental movement of *Trichinella* larvae has been reported only by a few researchers[5-7] and in only a few species such as pigs, guinea pigs, rats, and mice.[3,8] Guinea pigs and rabbits have no role in the propagation of *Trichinella* infection and hence congenital propagation is of epizootiological importance in pigs and rats only.[3] More recently, congenital infection with *Trichinella* has been demonstrated in Balb C mice.[3,9] *Trichinella* infection in mouse offspring from mothers that had been infected a day after mating has been reported.[9] It was, however, not clear whether infection was transmitted transplacentally or transmammary.

The immunological changes during pregnancy may affect the course of parasitic infections, among which the most studied are those caused by protozoan parasites.[10,11]

The aim of this study was to investigate the occurrence of congenital and transmammary transmission of *T. zimbabwensis* in rats *(Rattus norvegicus)*.

MATERIALS AND METHODS

Assessment of Congenital Transmission

Thirty female rats *(Rattus norvegicus)* aged 4 months, bred at the Animal House unit, Faculty of Veterinary Science, were divided into 6 groups of 5 rats each. All the rats were infected with *T. zimbabwensis* on different days in relation to the mating day at an infection dose of 2000 larva per rat as shown in Table 1.

A crocodile *(Crocodylus niloticus)*-derived *T. zimbabwensis* isolate was used to infect the rats. The parasite was maintained in the laboratory through periodic passages in rats *(Rattus norvegicus)* and mice (Balb C). To obtain the infection material for the experimental animals, rats that were infected with *T. zimbabwensis* were euthanized using ether, skinned, and eviscerated. The rat carcasses were individually minced and weighed. A 2-g sample was obtained from each carcass and digested using the HCl-pepsin method.[12] Larvae per gram (LPG) values of the muscles were determined. Based on the LPG values obtained, muscle samples were divided into portions that contained approximately 2000 first stage larvae each. The rats were placed in individual cages, starved overnight, and fed the inoculum the next morning. Throughout the experiment, the rats were maintained on a commercial diet of mouse comproids and water was available ad libitum.

At parturition of the experimental rats, the date of the birth, number, and identification of each offspring using the identity of the mother was recorded and the offspring were immediately euthanized using ether. Each carcass was minced and digested individually.[12] The samples were preserved in 70% alcohol; larval counts were done using a stereomicroscope.

After the birth of the offspring, the date of conception was calculated by subtracting the average gestation period of rats (20 days) from the date of delivery and the variables, day of infection pre-conception (DIPreC), and day of infection post-conception (DIPostC) were generated. The number of offspring per litter that were positive for *Trichinella* larvae was recorded.

The dams were then slaughtered, and muscle samples collected from the anterior and posterior limbs, diaphragm, and intercostal muscles were weighed, digested, and LPG were calculated.

Assessment of Transmammary Transmission

A group of 10 female rats aged 3 months were infected with *T. zimbabwensis* at a dose of approximately 10,000 larvae per rat. These rats were then mated 40 days post infection. The offspring from the experimental rats were weaned at 21 days of age and together with their mothers were slaughtered to check for *Trichinella* larvae as described above.

RESULTS

From the rats that were infected pre-conception, only 1 rat (10 days pre-conception)

produced one infected offspring as shown in Table 1. After conception, the offspring that tested positive for *Trichinella* were from dams infected at Day 4, 6 and 10 as shown in Table 2. The larval counts of the infected offspring ranged from 1 to 3 larvae per positive offspring.

Only 5 of the female rats from the 10 mated conceived and produced offspring. Rat number 5 did not have any positive offspring while rat 4 had 1 positive offspring and the rest of the rats had 2 positive offspring as shown in Table 3. The larval counts for the positive offspring ranged from 2 to 16.

DISCUSSION

The results show that very few larvae crossed the placenta in a small number of the experimental rats. The rats that were infected 10 days or more pre-conception did not produce any *Trichinella*-positive offspring. This is expected since at this stage most of the larvae will be starting to reach the skeletal muscles. The enteral phase can last up to 6 weeks although larval migration starts 7 days after ingestion of infected meat.[13] Once the larvae settle in the skeletal muscles, they do not reactivate and they wait for ingestion by another host.[14] Following penetration of the muscle cell, there is modulation or redifferentiation in the structure of the muscle cell to form a nurse cell. With the exception of *T. pseudospiralis*,[15] *T. papuae*,[16] and *T. zimbabwensis,*[17] the muscle larval stages of all *Trichinella* form a nurse cell complex, which is then surrounded by a capsule and serves in larval nutrition and in the handling of waste products.[18,19]

Reactivation of hypobiotic larvae occurs with *Strongyloides ransomi* in sows, and *Toxocara canis* and *Ancylostoma caninum* in the bitch.[3,14] These 2 hosts transmit reactivated larvae through mammary tissue and the uterus to multiple litters. This is possible because the hypobiotic larvae of these species do not become intracellular as in the case of some of the *Trichinella* species. No reports, however, have been made of reactivation of encysted *Trichinella* and movement of larvae from the infected muscle of the host to other muscles.[3] Conversely, reactivation and mobilization of *Trichinella* larvae and subsequent infection of offspring of infected dams appears to have occurred in this study. Therefore, both transplacental and transmammary transmission of *T. zimbabwensis* occurred with the transmammary route producing more infected offspring compared with the congenital experiment. Apart from the different timing of infection, successful transmission could also be related to the infection dose. There were differences in the infection doses used for the 2 experiments (congenital experiment, 2,000 larvae per rat; transmammary, 10,000 larvae per rat). The transmammary experiment recorded a higher number of *Trichinella*-positive offspring; this could have been positively correlated with the higher infection dose that the dams in this experiment received. Larval burdens in offspring were lower than in adults, and some offspring in a litter remained uninfected.[5,20]

In this study, the infection of the offspring in the congenital experiment cannot be attributed to lactogenic transmission since the offspring were removed from the dam immediately after birth. Larvae which accidentally reach the uterus are those that do not form a cyst cover and consequently would be able to pass into placental and fetal circulation when the female gets pregnant.[3] In the transmammary experiment, larvae were detected in offspring that were born of dams that were mated long after the life cycle of *Trichinella* had been completed and the larvae had settled in the muscles.

It has been demonstrated that during pregnancy there is an enhanced helminthotoxic effect towards newborn larvae.7 In this study, more positive offspring were recorded from the rats that were infected 6-13 days post-conception. However, despite the immunoactivation caused by pregnancy, vertical transmission of the parasite is possible.[7] Our results are in agreement with work

Table 1. Summary of Congenital Transmission of *Trichinella zimbabwensis* in Rats *(Rattus norvegicus)* Infected Before Conception.

Rat Identification	Larvae per Gram of Muscle of Dam				Congenital Transmission Results			
	Intercostal Muscle	Anterior Limbs	Posterior Limbs	Diaphragm	DIPreC	No. Offspring	No. Positive	Percent Positive
1	205	894	265	726	2	10	0	0
2	515	456	394	1382	4	10	0	0
3	1476	1083	948	935	8	12	0	0
4	290	621	236	1604	9	11	1	9
5	1076	574	986	2018	10	11	0	0
6	693	112	620	2662	10	10	0	0
7	2375	743	3511	7565	11	10	0	0
8	718	954	224	215	12	2	0	0
9	1237	1336	1496	1985	12	8	0	0
10	408	706	824	1065	19	12	0	0

DIPreC = day of infection preconception.

Table 2. Summary of Congenital Transmission of *Trichinella zimbabwensis* in Rats *(Rattus norvegicus)* Infected After Conception.

Rat Identification	Larvae per Gram of Muscle of Dam				Congenital Transmission Results			
	Intercostal Muscle	Anterior Limbs	Posterior Limbs	Diaphragm	DIPostC	No. Offspring	No. Positive	Percent Positive
11	371	470	405	503	0	13	0	0
12	215	569	706	1381	1	9	0	0
13	103	325	240	782	2	12	0	0
14	541	426	401	1479	3	9	0	0
15	112	126	593	849	4	8	1	13
16	99	2189	107	240	4	8	0	0
17	602	1049	715	2409	4	13	0	0
18	179	315	1217	2659	5	11	0	0
19	651	621	1006	800	6	13	6	46
20	917	1385	659	1526	6	10	1	10
21	803	755	1536	2410	7	11	0	0
22	1993	2068	1789	2855	10	9	3	33
23	2083	1400	2156	5046	13	10	0	0

DIPostC = day of infection post conception.

Table 3. Summary of Transmammary Transmission of *Trichinella zimbabwensis* in Rats *(Rattus norvegicus)* Infected After Conception.

Rat Identification	Larvae per Gram of Muscle of Dam				Transmammary Transmission Results			
	Intercostal Muscle	Anterior Limbs	Posterior Limbs	Diaphragm	DMPI	No. Offspring	No. Positive	Percent Positive
1	1221	1774	1338	2903	40	10	1	10
2	1551	1485	1869	5990	40	7	2	29
3	3087	1742	2208	1990	40	6	3	50
4	1269	742	763	2188	40	7	4	57
5	769	1008	974	1667	40	7	5	71

DMPI = day of mating post infection.

done in which there was transplacental migration of *T. spiralis*, and lactogenic transmission was ruled out because the fetuses were obtained by caesarean surgery on Day 17 of pregnancy.[7]

Congenital trichinellosis can be important in the case of human outbreaks. In the Slovak Republic, *Trichinella* larvae were found in the fluid of body cavities, placenta, tissues, and organs of a fetus aborted by a woman who had been infected in an outbreak.[21]

The placental barrier to be crossed by migratory *Trichinella* larvae varies structurally according to animal species.[22,23] Rodents have a hemochorial placenta in which the fetal trophoblast and endothelia have direct contact with the maternal blood and thereby with migrating *Trichinella* larvae.[22] The pig has an epitheliochorial placenta with no direct contact between fetal and maternal blood[22,23] so transplacental movement of *Trichinella* is unlikely.[24] However, experimental congenital *Schistosoma japonicum* infection in pigs has been demonstrated but the invasion mechanism was not clarified.

Transfer of immunoglobins, immune cells, or parasite-derived antigens from mothers to fetuses has been demonstrated in *Trichinella* infection.[26,27] This may elicit some protection against infection in the offspring of infected dams but it may inhibit or down-regulate the immune response of the offspring and thus favor later parasite infection.[24] Vertical transmission has an impact where larval establishment in offspring is high.[28] However, if *Trichinella* transmission to offspring results in abortions or birth of weak individuals, this would make infected animal tissue available to predators or scavengers in the wild and thus influence the epidemiology of *Trichinella*.

Since both transplacental and transmammary transmission of *T. zimbabwensis* have been shown to occur, this represents an efficient way by the parasite to maintain the cycle once infected rats enter a pig-farming premise. For Zimbabwe, this information is important when establishing a biosecurity program in crocodile farms and abattoirs. Strict rodent control needs to be implemented on crocodile and pig farms so as to reduce the risk of exposure to *Trichinella.*[29,30]

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