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INFECTIVITY, REPRODUCTIVE CAPACITY AND DISTRIBUTION OF *TRICHINELLA SPIRALIS* AND *T. PSEUDOSPIRALIS* LARVAE IN EXPERIMENTALLY INFECTED SHEEP

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Twelve Merino sheep were experimentally shown to be susceptible to infection with *Trichinella spiralis* or *T. pseudospiralis* by feeding on infected carcasses of mice or by oral intubation with recovered muscle larvae. The larvae recovered from the sheep showed variable tissue distribution. The diaphragm and tongue were most affected. The viability of the recovered larvae was confirmed by successful passage in mice. The reproductive capacity of *T. spiralis* in sheep was higher than that of *T. pseudospiralis*, and also higher than its reproductive capacity in C57BL/6J mice. The reproductive capacity of *T. pseudospiralis* in sheep at a lower dose was higher than that observed in mice. However at higher doses, it was significantly lower than that in mice. Therefore, it may be concluded that the sheep may be considered a suitable host for both species of *Trichinella*.

Key words: *Trichinella spiralis*, *T. pseudospiralis*, sheep, experimental infection, susceptibility

INTRODUCTION

There has been some concern lately as to the safety of goat and sheep meat sold in Islamic countries with respect to trichinellosis. However no experimental evidence has been provided to demonstrate the susceptibility of sheep to experimental infection with *Trichinella spiralis* or *T. pseudospiralis*, or their role as a possible source of infection to humans. It is rather difficult to consider Islamic countries free of trichinellosis since no surveys of animals or humans have been carried out. Furthermore, some farmers in some Islamic countries such as Egypt, Sudan, Syria, Jordan and others, where raising of pigs is allowed, raise their pigs on the same farm along with sheep and goats.

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Eight Germans were infected with trichinellosis after eating "camel meat" bought in Cairo and illegally imported to Germany in 1980. However the actual source of the meat could not be determined and it was suspected to be beef (Bommer et al., 1985). Moreover, seventeen individuals were reported sick with trichinellosis following the consumption of beef pasterami in Cairo in 1983 (Dr. Hassonah, O., Al Azhar University, Egypt, personal communication) and again the source of the commercially bought meat was not identified. Since horse meat has also been reported to be responsible for the outbreak of trichinellosis (Ancelle et al., 1988 ; Bouree et al., 1979 ; Mantovani et al., 1980 ; Parravicini et al., 1987), herbivore meat as a source of *Trichinella* infection in humans presents a new concept in the public health strategy for the control of the parasite.

In this investigation, the susceptibility of sheep to experimental infection with *T. spiralis* or *T. pseudospiralis,* was studied and their reproductive capacity and tissue distribution *in vivo* measured. Furthermore, the infectivity and viability of the muscle larvae recovered from the experimentally infected sheep were confirmed in mice.

MATERIALS AND METHODS

Parasites : *T. spiralis* and *T. pseudospiralis* used in this study have been previously described by Faubert and Tanner (1971) and Alkarmi and Faubert (1981), respectively. Both parasites were exclusively passaged in C57BL/6J mice every 4–8 months.

Animals :

Mice : Male C57BL/6J mice, five weeks old, were purchased from Bantin and Kingman Ltd. (U.K.). They were housed five/cage and fed Purina chow and water *ad libitum.* These mice were used to propagate the parasite *in vivo* and also to confirm the viability of the larvae from sheep.

Sheep : Twelve Australian Merino sheep, 2 years old, weighing 50 kilograms were purchased locally and divided into 4 groups of three. They were fed Purina chow and water, and supplemented with green grass twice weekly.

Protocol for infection :

Two groups of sheep were fed carcasses of infected mice (1 carcass/sheep) containing approximately either 11,000 *T. pseudospiralis* larvae or 14,400 *T. spiralis* larvae. The other 2 groups were infected orally with 2,000 larvae of *T. spiralis* or *T. pseudospiralis*/kg of body weight (approximately 100,000 larvae/sheep). The sheep were sacrificed on day 50 post-infection and several muscle samples weighing 20–50 grams from the diaphragm, tongue, rector spinae, forelimb and hind limb were collected separately. The remaining muscle tissue was isolated and weighed as grouped above.

Larval recovery and counting :

The muscle samples were digested in pepsin/HCl media (1% pepsin in 0.06 N HCl) for 3 hours at 37°C as has been described by Alkarmi and Faubert (1981).

Larval counts were performed in a nematode egg counting chamber (Hawskly Ltd. U. K.).

Reproductive capacity and expected larval recovery:

The reproductive capacity of *T. spiralis* or *T. pseudospiralis* was measured as described by Alkarmi et al. (1989). The expected larval recovery was calculated on the basis of the average total weight of the muscle or muscle groups from the 12 sheep and the recovered muscle larvae from the same muscle group. The approximate total count was the sum of calculated larvae from the different muscles or muscle groups.

Assessment of larval viability:

The viability of larvae was assessed by morphological criteria. Coiled and intact larvae were considered viable while those that were stretched and broken were considered dead. Inoculation of the recovered larvae from sheep into mice was also carried out to assess larval viability *in vivo*. Furthermore, the activity of the larvae while being observed under closed circuit television (CCTV) was also used to confirm viability.

Results

The data obtained clearly show that *T. pseudospiralis* or *T. spiralis* successfully completes its life cycle in sheep. Viable muscle larvae were recovered from the various tissue samples digested. However, distribution of larvae in host tissue was variable. The different muscle groups showed varying degrees of susceptibility (Tables 1–4). In general, the highest larval recovery per gram of muscle was observed in the diaphragm and the tongue in either infection (Tables 1–4).

The reproductive capacity (RC) of *T. spiralis* and *T. pseudospiralis* was calculated to be 82 and 2, respectively. However, at lower doses, the RC of *T. pseudospiralis* increased to 34 while that of *T. spiralis* dropped to 16 (Table 5).

When mice were inoculated with larvae recovered from the sheep, their infectivity and RC were regained. A RC value of 28 and 16 was observed for *T. spiralis* and *T. pseudospiralis*, respectively.

Discussion

Consumption of scalded mutton leading to an outbreak of trichinellosis in China has been reported (China, Coordinating Group for Prevention and Treatment of Trichinosis, Harbin city, 1981). However, there are doubts as to the true source of the meat involved (Dr. Yamaguchi, T., Hirosaki University, Japan, personal communication).

It has been suggested that *T. pseudospiralis* can also infect humans on the basis of the results of experimental infection with *T. pseudospiralis* in the monkey (Pawlowski & Ruitenberg, 1978). In contrast to *T. spiralis, T. pseudospiralis* can complete its

Table 1. Distribution of *Trichinella pseudospiralis* larvae in different muscles or muscle groups and expected larval recovery in experimentally infected sheep. The infection was established by feeding the sheep with the carcass of an infected mouse containing approximately 11,000 *T. pseudospiralis* larvae. The number in brackets represents the weight of muscle tissue digested from each muscle group.

Muscle tissue	Average wt. of tissue in gm	Mean no. of larvae /gm recovered	Expected total larval recovery
Rector spinae	1,988 (177)	38	75,544
Diaphragm	295 (46)	49	14,455
Tongue	236 (57)	27	6,372
Hind limb	5,080 (410)	46	233,680
Forelimb	2,854 (196)	14	39,956
Total	10,453 (886)		370,007

Table 2. Distribution of *Trichinella pseudospiralis* larvae in different muscles or muscle groups and expected larval recovery in experimentally infected sheep. The sheep were infected with approximately 100,000 *T. pseudospiralis* larvae by oral intubation. The number in brackets represents the weight of muscle tissue digested from each muscle group.

Muscle tissue	Average wt. of tissue in gm	Mean no. of larvae /gm recovered	Expected total larval recovery
Rector spinae	1,988 (323)	10	19,880
Diaphragm	295 (73)	20	5,900
Tongue	236 (61)	48	11,328
Hind limb	5,080 (316)	21	106,680
Forelimb	2,854 (241)	13	37,102
Total	10,453 (1,014)		180,890

Table 3. Distribution of *Trichinella spiralis* larvae in different muscle or muscle groups and expected larval recovery in experimentally infected sheep. The infection was established by feeding the sheep with the carcass of an infected mouse containing approximately 14,400 *T. spiralis* larvae. The number in brackets represents the weight of muscle tissue digested from each muscle group.

Muscle tissue	Average wt. of tissue in gm	Mean no. of larvae /gm recovered	Expected total larval recovery
Rector spinae	1,988 (214)	13	25,844
Diaphragm	295 (53)	44	12,980
Tongue	236 (53)	32	7,522
Hind limb	5,080 (730)	21	106,680
Forelimb	2,854 (313)	24	68,496
Total	10,453 (1,310)		221,552

Table 4. Distridution of *Trichinella spiralis* larvae in different muscles or muscle groups and expected larval recovery in experimentally infected sheep. The sheep were infected with approximately 100,000 *T. spiralis* larvae by oral intubation. The number in brackets represents the weight of muscle tissue digested from each muscle group.

Muscle tissue	Average wt. of tissue in gm	Mean no. of larvae /gm recovered	Expected total larval recovery
Rector spinae	1,988 (206)	503	999,964
Diaphragm	295 (58)	2,413	711,835
Tongue	236 (52)	1,345	317,420
Hind limb	5,080 (290)	887	4,505,960
Forelimb	2,854 (160)	570	1,626,780
Total	10,453 (766)		8,161,959

Table 5. Reproductive capacity (RC) and viability of *T. spiralis* or *T. pseudospiralis* in sheep. The RC in group A is calculated from sheep which were infected with approximately 100,000 larvae by oral intubation while in group B, it was calculated from those which were fed infected mouse carcass as described in materials and methods. The number in brackets represents represents the RC of each parasite in mice.

Parasite	Reproductive capacity	
	A	B
T. spiralis (28)	82	16
T. pseudospiralis (16)	2	34

life cycle in birds. Crows, ducks, herons, kestrels, kites, magpies, owl, pigeons (Miroshnitchenko, 1978), hens (Tomasovicova, 1975), American kestrels (Meerovitch et al., 1982), Japanese quails, seagulls (Bober & Dick, 1983) and finches (Ooi et al., 1985) have all been experimentally infected with *T. pseudospiralis*. The viability and infectivity of the muscle larvae have been retained in these species for a period of 14 months. After the first isolation of *T. pseudospiralis* in Russia by Garkavi (1972) from raccoon, natural infection in the crow (Shaikenov, 1980), Cooper's hawk (Wheeldon, 1983), raccoon (Garkavi and Gineev, 1976), and more recently from Tasmanian wildlife such as the quoll and the Tasmanian devil (Obendorf et al., 1990) have been reported. Mice, guinea pigs, dogs and Syrian hamsters are highly susceptible to experimental infections, while rats and piglets are reported to be more resistant (Bessonov et al., 1978; Garkavi, 1974; Ooi et al., 1985). Although the parasite has shown great adaptability to piglets and the virulence was shown to increase 10–100 times after several passages, it did not approach the level of *T. spiralis* which was always higher (Bessonov et al., 1978; Garkavi, 1974, 1976).

The data presented in this study has shown for the first time that sheep may be experimentally infected with *T. spiralis* or *T. pseudospiralis*, and the infectivity or reproductive capacity of both parasites in sheep is very high. Furthermore, the distribution of larvae in the muscles of sheep is variable. Variable larval distribution in muscles was also reported by Pozio et al. (1985) in swine infected with *T. nelsoni*. However, as observed in this study, the diaphragm and tongue were the most susceptible muscle tissue for either infection.

The reproductive capacity (RC) of *T. spiralis* in sheep (RC 82) was significantly higher than that observed in mice (RC 28), while the RC of *T. pseudospiralis* (RC 2) was significantly lower than that observed in mice (RC 16) (Alkarmi and Faubert, 1981, 1985). However at lower doses, as shown in table 1, the RC of *T. spiralis* approaches the level seen in mice, while the RC of *T. pseudospiralis* increases to 34 which is significantly higher than that observed in mice (Alkarmi and Faubert, 1981, 1985). Unfortunately, the RC of *T. pseudospiralis* in other hosts has not been reported. Therefore, it is rather difficult to assess the true infectivity of the parasite and the susceptibility of other experimental hosts.

It is important to note that sheep may become accidentally infected with *Trichinella* while grazing if parts of dead infected rodents are accidentally ingested. The four sheep in this study ingested the carcasses of mice while feeding on Purina chow. It should also be noted that the larvae recovered from sheep were viable and infective. They retained the same reproductive capacity when inoculated in mice. Thus, infections in sheep may very well be established due to accidental ingestion of infected meat and hence they may become a possible source of infection to humans.

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