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INFECTIVITY OF ISOLATES OF *TRICHINELLA* AND THE ABILITY OF AN ARCTIC ISOLATE TO SURVIVE FREEZING TEMPERATURES IN THE RACCOON, *PROCYON LOTOR*, UNDER EXPERIMENTAL CONDITIONS

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ABSTRACT: The objectives of this study were to determine if the raccoon was a useful experimental animal for infections of *Trichinella* and to determine if the ability of *Trichinella* to survive freezing conditions, known to occur in wild animals, could be duplicated under laboratory conditions. The isolates of *Trichinella* used in this study were from pigs, polar bear, wolverine, arctic fox and *T. spiralis* var. *pseudospiralis* originally isolated from a raccoon in the USSR. The raccoon was found to be a useful experimental host for *Trichinella* as it was easily maintained under experimental conditions and was readily infected. Infectivity indices were lower in raccoons than in laboratory mice. Those isolates of *Trichinella* with the longest association with laboratory mice had the lowest infectivity indices. The isolate of *Trichinella* from an arctic fox retained its ability to survive freezing temperatures when introduced into raccoons held under experimental conditions. The type of host, method of passing the parasite and perhaps a special genetic characteristic of arctic isolates seem to be important factors influencing their ability to survive freezing temperatures.

INTRODUCTION

The ability of *Trichinella spiralis* to survive freezing temperatures in carnivore muscle is well known (Rausch, 1970; Emson et al., 1972; Eaton, 1979; Margolis et al., 1979) and in some studies it was shown that the larvae are infective to mice (Clark et al., 1972; Worley et al., 1976; Dick and Belosevic, 1978; Dies, 1980; Chadee and Dick, 1982). However, larvae isolated from frozen carnivore muscle and infective to laboratory mice did not survive subsequent freezing in mouse muscle (Dick and Belosevic, 1978). Wild carnivores and northern isolates of *Trichinella* seemed to have a unique host–parasite interaction that was lost after the parasite was transferred to laboratory mice. Dick and Belosevic (1978) suggested that a number of factors were important, but to test these ideas controlled experimental conditions and a suitable host were necessary. The raccoon was chosen as the host since it appeared to have a high lipid content in its muscles, was easily maintained in the laboratory, was known to harbor *Trichinella* and uninfected animals could be obtained. A number of isolates of *Trichinella* were used to assess (1) if resistance to freezing by all isolates could be expressed in a suitable host (2) if the type of host used in transferring the parasite was essential for its survival. These are important considerations not only in the epidemiology of trichinellosis but also in the development of a host system to evaluate factors enabling *Trichinella* isolates to survive freezing temperatures.

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MATERIALS AND METHODS

Trichinella isolates used in this study were designated as follows: pig_1, 43°00′N, 81°00′W, 1952; pig_2, 44°00′N, 63°00′W, 1980; polar bear, 58°00′N, 95°00′W, 1976; wolverine, 55°00′N, 100°00′W, 1979; arctic fox_1, 69°15′N, 105°00′W, 1980. Hereafter the strains will be referred to as pig isolate-one, pig isolate-two, polar bear and wolverine isolates and arctic fox isolate-one. The arctic fox isolate is designated as such since other arctic fox isolates from the same geographic region have been referred to in the literature (Dick and Chadee, 1981). The designation of *T. spiralis* var. *pseudospiralis* is discussed elsewhere (Dick, 1983; Dick and Chadee, 1983).

Experimental hosts

Raccoons were trapped in an urban center (Winnipeg, Manitoba) where they were nuisance problems. A total of 35 of these "urban" raccoons were necropsied prior to this study and none had parasites. This was probably related to feeding primarily on garbage and dog or cat food as raccoons collected from the same latitude but from a river bottom habitat (Hedingly, Manitoba) had a wide range of parasites. None of over 60 raccoons examined from both the urban and natural environments harbored *Trichinella* as determined by trichinoscopy and HCl-pepsin digest of pooled samples of tongue, diaphragm and masseters. Furthermore none of the experimental raccoons harbored parasites, other than the experimentally administered *Trichinella* isolates.

Procedures for isolation and administration of larvae

Methods of isolating *Trichinella* from frozen carcasses and mice and infection of mice have been reported elsewhere (Dick and Belosevic, 1978). The procedure for infecting raccoons required removal of frozen muscle from the carcass of an arctic fox and mincing the muscle with scissors as it thawed. This muscle was then ground in a food grinder, mixed thoroughly, a subsample of 10 g digested in 1% HCl-pepsin and counts determined as larvae/g of ground muscle. The remainder of the ground muscle was adjusted to the desired infective dose (3.6 larvae/g). This dose varied between 13,000 and 20,000/individual, depending on a raccoon's weight. Raccoons were fasted overnight prior to placing the ground muscle in a feeding dish. The water dish was removed to prevent washing of the ground muscle by raccoons. Otherwise, raccoons were fed once daily with a mixture of canned and pelleted dog food (Purina) and maintained at a temperature of 20 C and a light/dark cycle of 12/12 hr.

Freezing experiments

Infections in raccoons were terminated 40 days postinfection and raccons were killed, skinned, eviscerated, weighed and examined for intestinal parasites. Each raccoon carcass was sectioned along the medial-sagittal line, the flesh removed from the bones from one-half the carcass and this flesh was minced, ground and digested in 1% HCl-pepsin solution. The infectivity index was calculated and mice infected to determine infectivity (Table 1). The infectivity index was defined as the number of larvae recovered from the digest divided by 50% of the larvae in the original inoculum if one-half the carcass was digested or the total inoculum if the entire carcass was digested. The remaining half of the carcass was frozen at −15 C and random samples of muscle removed from the carcass at selected times postfreezing (PF) (Table 1). Larvae recovered from frozen raccoon muscle were warmed for 20 min at 37 C on a warming plate prior to counting total larvae and determining the percent of worms which were moving. Laboratory mice, Crl COBS CFW (SW) were used to test for viability of larvae frozen for different times.

RESULTS

Tables 1 and 2 outline the results from the freezing trials with the isolates of *Trichinella*. All isolates were initially recovered from frozen carcasses with the exception of the pig isolates and *T. spiralis* var. *pseudospiralis*. Larvae of all isolates which were passed through mice and then through a raccoon (Table 1) did not survive freezing temperatures. Only the arctic fox isolate passed through raccoons retained its ability to survive freezing temperatures. The arctic fox isolate survived freezing for about 9 mo (270 days) in a raccoon after surviving 20 mo in the frozen body of an arctic fox. Furthermore, the ability to survive freezing temperatures is retained in the second passage through raccoons, although at a lower level of infection (Table 2). The number of larvae moving following digestion from frozen raccoon muscle over a period of 30–270 days postfreezing clearly showed a progressive loss of activity over time (Fig. 1). This decline in activity was not related to a decrease in the infectivity index as the value of 15.1 from Table 2 included values ranging from 1.2 to 34.9. Furthermore, values of 21.4 and 27.9 were calculated at 270 days postfreezing.

DISCUSSION

Raccoons appear to be a good experimental host for *Trichinella* but infectivity indices tended to be somewhat lower than for mice (Table 1). This was most pronounced in those isolates (pig isolate-one and polar bear) with the longest association with a mouse host, 27 passages. The exception was *T. spiralis* var. *pseudospiralis* which had been passaged through mice for many generations, at least 10 in this

TABLE 1. Isolates of *Trichinella*, infected hosts and freezing trials (30 days or longer postfreezing).*

Strain	History	*n*	Infected host	Infectivity index
Pig isolate-one	Pig to mouse	30	Mouse	151.0
		1	Raccoon	10.6
Pig isolate-two	Pig to mouse	30	Mouse	80.0
		1	Raccoon	82.0
Polar bear isolate	Polar bear to mouse	30	Mouse	64.0
		1	Raccoon	10.9
Wolverine isolate	Wolverine to mouse	30	Mouse	22.0
		1	Raccoon	21.9
T. spiralis var. *pseudospiralis*	Mouse to mouse	30	Mouse	39.0
		1	Raccoon	49.9

* See Table 2 for data on the arctic fox isolate

TABLE 2. Infectivity index of arctic fox-one isolate (AF_1) from raccoons and mice under different experimental conditions.

Conditions prior to isolation and infection	*n*	Host	Infectivity index
AF_1[a]	6	Mouse[b]	62.0
AF_1[a] to mouse[b]	30	Mouse[b]	37.0
AF_1[a] to mouse[b]	1	Raccoon[b]	18.9
AF_1[a]	2	Raccoon[b]	11.6
AF_1[a] to raccoon (frozen 30–270 days)	27	Mouse[b]	15.1
AF_1[a] to raccoon (frozen 60 days)	1	Raccoon[c]	0.4

[a] Frozen for 20 mo at −15 C.
[b] Unfrozen carcasses.
[c] Frozen for 240 days.

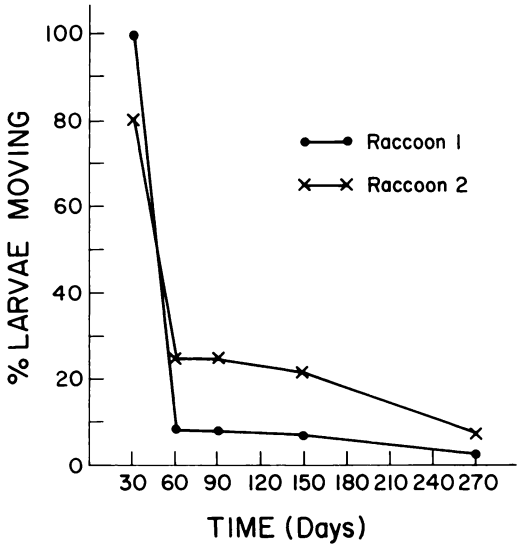

FIGURE 1. Percent of active *Trichinella* larvae from the arctic fox isolate after thawing and digesting in HCl-pepsin. Time indicates days frozen prior to thawing.

laboratory. It is worth noting that *T. spiralis* var. *pseudospiralis* or *T. pseudospiralis* as described by Garkavi (1972) was isolated from a raccoon, *P. lotor*. The possibility that past association influenced current infectivity of *T. spiralis* var. *pseudospiralis* in raccoons is a rather tenuous argument now but will perhaps have more credence as we gain further insight into the effects of host on infectivity of *Trichinella*.

It is clear that some isolates of *Trichinella* can retain the ability to survive freezing temperatures under experimental conditions and that this ability appears to be host dependent. It is also apparent that several of the suggestions made by Dick and Belosevic (1978) are not necessary prerequisites for *Trichinella* to survive freezing temperatures. These factors included mass of tissue, rate of freezing and degree of cyst calcification. Forty-day-old infections in this study were young infections and while cyst formation is complete or nearly so at this time, there is no calcification. Perhaps the decline in movement of larvae over time postfreezing is related to both freezing temperatures and an ageing larval population. The use of movement (coiling and uncoiling) as an indication of survivability may not be a good method to assess viability since infectivity of the arctic fox isolate did not decline over the same time period. Nevertheless *Trichinella* larvae have been shown to survive freezing temperatures under controlled experimental conditions. While this study has not solved the mechanism of freezing resistance in *Trichinella* we are a step closer as we now have a host system that allows some *Trichinella* isolates to retain their ability to survive freezing temperatures under controlled conditions. Perhaps the kind and amount of lipids in the muscle of carnivores and other hosts, such as raccoons, may act as a cryopreservative. It is also possible that the ability to survive freezing temperatures is a specific genetic characteristic of northern and arctic isolates of *Trichinella*. Answers to these questions are now possible.

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