DESIGNATION AND FREEZING RESISTANCE OF ISOLATES OF TRICHINELLA SPIRALIS FROM WILD CARNIVORES

Authors: CHADEE, K., and DICK, T.A.

Source: Journal of Wildlife Diseases, 18(2) : 169-173

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-18.2.169

DESIGNATION AND FREEZING RESISTANCE OF ISOLATES OF *TRICHINELLA SPIRALIS* FROM WILD CARNIVORES

K. CHADEE and T.A. DICK, Department of Zoology, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada.

Abstract: A system to designate and define isolates of *Trichinella spiralis* is proposed. The designation gives the host from which the isolate was recovered, geographic origin, and year of recovery. Isolates of *T. spiralis* recovered from frozen muscles from four species of wild carnivores had low and different infectivity to laboratory mice. Viable larvae of *T. spiralis* were obtained from muscle samples of marten, wolverine, polar bear and arctic fox which had been frozen for 5, 6, 12 and 14 mo, respectively.

INTRODUCTION

Nematodes of the genus *Trichinella* from the northern hemisphere have been recognized both as distinct species (Boev et al., 1979), and as a northern or arctic strain (Rausch, 1970; Dick and Belosevic, 1978). Although geographical isolates of *T. spiralis* are known to differ in their biological characteristics, the taxonomic rank of isolates is unresolved. Recent studies (Dick and Belosevic, 1978; Sukhdeo and Meerovitch, 1977; and Belosevic and Dick, 1979; 1980) on North American isolates of *Trichinella* suggest these isolates are variants or strains of *T. spiralis* and not distinct species since they interbreed and produce viable F_1 hybrids (Belosevic and Dick, 1980).

As early as 1950 it was suggested that northern isolates of *T. spiralis* may be resistant to low temperatures (Brandly and Rausch, 1950). Anecdotal evidence supports this in that viable *T. spiralis* larvae were recovered from previously frozen muscle of wild carnivores (Rausch, 1970; Emson et al., 1972; Eaton, 1979; Margolis et al., 1979). However, relatively few documented cases clearly demonstrate the recovery of viable larvae of *T. spiralis* larvae from frozen tissues (Clark et al., 1972; Worley et al., 1976; Dick and Belosevic, 1978; Dies, 1980). This study reports on larvae of *T. spiralis* recovered from previously frozen muscles of carnivores, their viability after various times post-freezing, and proposes a system to designate and define these northern isolates of *T. spiralis*.

MATERIALS AND METHODS

Carcasses of wild carnivores which had been frozen from 1 to 2 mo, were obtained from trappers and wildlife personnel. Names of hosts and their geographic origins are outlined in Table 1.

TABLE 1. Designation of *Trichinella* isolates.

Wild Hosts	Latitude	Longitude	Year Established
Polar bear *(Ursus maritimus)*	58°00′N	95°00′W	1976
Wolverine *(Gulo gulo)*	55°00′N	100°00′W	1979
Marten *(Martes americana)*	56°00′N	99°00′W	1980
Arctic fox *(Alopex lagopus)*	69°15′N	105°00′W	1980 [1,2,3,4] [a]

[a]Four *Trichinella* isolates.

Carcasses of carnivores were thawed at room temperature (21 C) and examined for cysts of *T. spiralis* by trichinoscope and for larvae by HCl-pepsin digestion. Muscle was separated from fat and bones and ground in a meat grinder. Ground muscle was weighed and placed in flasks containing a 1% pepsin-HCl solution [ratio of meat (gm) to pepsin-HCl solution (ml) was 1:20] for 3 h at 37 C with occasional stirring. A digestion period of 3 h was necessary to free larvae from their cysts. Concentration of larvae from the digest, counting, and procedures of infection are reported elsewhere (Dick and Belosevic, 1978; Belosevic and Dick, 1979; 1980). Outbred white mice [Crl: COBS CFW (SW) Charles River Breeding Laboratories, Wilmington, Massachusetts 01887, USA] were used for all infection studies. Portions of carcasses were refrozen at -15 C and samples of muscle were taken from these refrozen carcasses at various times post-freezing (Table 2) and tested for viable larvae of *T. spiralis*. All larvae recovered from digested muscle were tested for infectivity in mice as described elsewhere (Dick and Belosevic, 1978).

RESULTS

Seven isolates of *T. spiralis* are listed by host, geographical origin and year of isolation (established in laboratory animals) (Table 1).

The level of infection of *T. spiralis* in muscle of wild carnivores varied from 0.008 to 53 larvae/gm (Table 2). In all cases very few calcified cysts were observed.

Results on infectivity and recovery from frozen muscles are summarized in Table 2. Viable larvae of *T. spiralis* were obtained from muscle samples of marten, wolverine, polar bear, and arctic fox which had been frozen for 5, 6, 12 and 14 mo, respectively. All isolates had low but different infectivities in mice examined 40 days post-infection. Larvae/gm of muscle from mice for the isolates were as

TABLE 2. Recovery of infective *Trichinella spiralis* larvae from several carnivores frozen at -15 C.

Hosts	Muscles examined	Time frozen (months)	Larvae/gm of host muscle	Infection Dose larvae/mouse	Larvae recovered from mice	Infectivity Index[a]
Wolverine[b]	Diaphragm	3	1.85	500	3,600	7.20
	Intercostals[c]					
Wolverine	Random	4	6.60	100	1,500	14.60
	Tissue samples[d]					
Wolverine	Posterior half of carcass[d]	5	2.71	400	6,800	17.05
Wolverine	Random	6	1.04	71	75	1.05
	Tissue samples[d]					
Wolverine	Random	7	3.20	50	0	—
	Tissue samples[d]					
Wolverine	Random	8	3.42	60	0	—
	Tissue samples[d]					

TABLE 2. (continued)

Polar bear	Diaphragm[e]	12	1.87	40	2,400	60.00
Marten	Whole carcass[c] (except head)	5	0.008	40	300	7.50
Marten	Tongue[d] Masseters[d]	7	0.70	60	0	—
Arctic fox 1	Half carcass[c]	5	4.81	300	18,600	62.00
Arctic fox 2	Half carcass[c]	5	1.78	200	40	0.20
Arctic fox 3	Diaphragm[c]	5	0.50	10	254	25.40
Arctic fox 4	Diaphragm[c]	5	1.45	29	103	3.55
Arctic fox 1	Random Tissue samples[d]	14	53.00	200	2,280	11.40
Arctic fox 2	Random Tissue samples[d]	14	6.32	100	2	0.02
Arctic fox 3	Random Tissue samples[d]	14	1.47	15	0	—
Arctic fox 4	Random Tissue samples[d]	14	2.98	40	45	1.12

[a]Infectivity index is the number of larvae recovered divided by the number of larvae in the inoculum and was determined on day 40 post-infection from Crl:COBS CFW (SW) mice.

[b]The wolverine carcass was thawed at 3 mo and refrozen, all other samples were taken from the refrozen wolverine carcass.

[c]Carcasses completely thawed at room temperature and refrozen at -15 C.

[d]Samples of muscle taken from frozen carcasses.

[e]From Dick and Belosevic (1978).

follows: from wolverine 97.80; from polar bear, 64.90; from marten, 10.71 and from 4 foxes, 664, 1.42, 9.07 and 3.56, respectively.

DISCUSSION

Due to the controversy over speciation in the genus *Trichinella*, the authors feel it is essential that proper identification of isolates be established. For this reason we have chosen to include name of host, geographic origin, and year of isolation of the larvae. A typical designation is written as follows: wolverine; 55°00'N, 100°00'W; 1979. The designation for arctic fox (arctic fox; 69°15'N, 105°00'W; 1980)[1,2,3,4] refers to four isolates from four individuals from the same geographic region (Table 1). We strongly suggest that if experimental work is done on any isolate a detailed history of each isolate should be kept and should include the strain of experimental host, infectivity index, number of generations in experimental hosts and generation time.

Northern isolates of *T. spiralis* were suggested to be resistant to low temperatures (Brandly and Rausch, 1950). Since then, viable *T. spiralis* have been reported from black bear meat frozen for 81 days at -18 C (Clark et al., 1972), from polar bear meat after storage for 12 mo at -15 C (Dick and Belosevic, 1978), from wolf tissue frozen for 18 mo at -10 C (Dies, 1980), and from grizzly and black bear tissues frozen for 6 mo at -20 C (Worley et al., 1976). In this study muscle type did not affect the ability of *T. spiralis* to survive freezing nor did the location of frozen tissue, whether surfacial or deep inside the carcass. Although, it is not clear how long these larvae can survive freezing, or the range of temperatures they can tolerate in the wild host, we were able to obtain infective larvae in wolverine muscle for up to 6 mo. A decrease of infectivity with time was noted for both wolverine and marten isolates and by 7 mo all larvae were noninfective (Table 2). Tissues completely thawed at room temperature and refrozen for an extended period of time (Table 2) contained infective larvae. Perhaps thawing and refreezing of carcasses could account for the variability in infectivity of larvae from different tissues of the same animal. We also know that larvae recovered from a polar bear and from arctic foxes frozen for 12 and 14 mo, respectively, were infective. High arctic isolates such as those recovered from the polar bear and arctic foxes possibly may survive freezing in the carnivore muscles somewhat longer than do those obtained at somewhat lower latitudes (i.e., high boreal and boreal regions).

All northern isolates in this study had different infectivities in mice and were generally lower when compared to an infectivity index of 151.27 (Belosevic and Dick, 1979) from a pig strain of *T. spiralis* (pig; 43°00'N, 81°00'W; 1952). Although there does not appear to be any initial pattern for level of infection in experimental mice, work in our laboratory shows that isolates stabilize after several generations and infectivity indices become stable and predictable characteristics (Belosevic and Dick, 1979). Possibly localized pressures, such as host and geographical isolation, are selecting for certain biological characters which are stable and predictable under laboratory conditions.

Attempts to determine if northern isolates of *T. spiralis* were resistant to freezing following encystment in the muscles of laboratory mice have been unsuccessful (Dick and Belosevic, 1978). Many factors are important in ensuring survival after freezing in the wild host and are listed elsewhere (Dick and Belosevic, 1978). It appears that resistance to freezing is a biological characteristic of all northern isolates and additional northern isolates of *T. spiralis* should be evaluated for low temperature resistance.

Acknowledgements

The authors thank Mr. R. Stardom, Furbearer Specialist and numerous other staff from Manitoba Department of Renewable Resources and Transportation Services for making available carcasses of marten and portions of the polar bear. We thank Mr. D. Boxer, Wildlife Officer, Government of the Northwest Territories and the Hunters and Trappers Association from Cambridge Bay for the arctic fox carcasses. Financial support initially from the Northern Studies Committee, University of Manitoba and continuing support from the Natural Science and Engineering Research Council of Canada is gratefully acknowledged.

LITERATURE CITED

BELOSEVIC, M. and T.A. DICK. 1979. *Trichinella spiralis:* Comparison of stages in host intestine with those of an arctic *Trichinella* sp. Exp. Parasitol. 48: 432-446.

—— and ——. 1980. *Trichinella spiralis:* Comparison with an arctic isolate. Exp. Parasitol. 49: 266-276.

BRANDLY, P.J. and R. RAUSCH. 1950. A preliminary note of trichinosis investigations in Alaska. Arctic 3: 105-197.

BOEV, S.N., V.A. BRITOV, and I.V. ORLOV. 1979. Species composition of trichinellae. Wiad. Parazytol. 25: 495-503.

CLARK, P.S., E. BROWNSBERGER, A.R. SASLOW, I.G. KAGAN, G.R. NOBEL and J.E. MAYNARD. 1972. Bear meat trichinosis. Ann. Intern. Med. 76: 951-956.

DICK, T.A. and M. BELOSEVIC. 1978. Observations on a *Trichinella spiralis* isolate from a polar bear. J. Parasitol. 64: 1143-1145.

DIES, K. 1980. Survival of *Trichinella spiralis* larvae in deep-frozen wolf tissue. Can. Vet. J. 21: 38.

EATON, P.D.R. 1979. Trichinosis in the Arctic. Can. Med. Assoc. J. 120: 22.

EMSON, H.E., M.A. BALTZMAN and H.E. WEINS. 1972. Trichinosis in Saskatchewan, an outbreak due to infected bear meat. Med. Prac. 106: 897-898.

MARGOLIS, H.S., J.P. MIDDAUGH and R.D. BURGESS. 1979. Arctic trichinosis: Two Alaskan outbreaks from walrus meat. J. Infect. Dis. 139: 102-105.

RAUSCH, R.L. 1970. Trichinosis in the Arctic. In: *Trichinosis in Man and Animals.* S.E. Gould, ed., Charles C. Thomas, Springfield, Illinois. pp. 348-373.

SUKHDEO, M.V.K. and E. MEEROVITCH. 1977. Comparison of three geographical isolates of *Trichinella*. Can. J. Zool. 55: 2060-2064.

WORLEY, D.E., J.C. FOX, J.B. WINTERS, R.H. JACOBSON and K.R. GREER. 1976. Helminth and arthropod parasites of grizzly and black bears in Montana and adjacent areas. Third Inter. Conf. on Bears and Mgt., IUCN Publ. Paper 45. pp. 455-464.

Received for publication 4 May 1981