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Citation	Japanese Journal of Veterinary Research, 34(2), 105-110
Issue Date	1986-04-30
DOI	https://doi.org/10.14943/jjvr.34.2.105
Doc URL	https://hdl.handle.net/2115/2975
Type	departmental bulletin paper
File Information	KJ00002374372.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Jpn. J. Vet. Res., **34**, 105–110 (1986)

INFECTIVITY IN RODENTS AND COLD RESISTANCE OF *TRICHINELLA SPIRALIS* ISOLATAED FROM PIG AND POLAR BEAR, AND *T. PSEUDOSPIRALIS*

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(Received for publication February 21, 1986)

Infectivity in ICR mice and Wistar rats, and resistance to low temperature were compared among *T. spiralis* isolated from a polar bear in Sapporo, Japan (designated p.bear isolate), *T. spiralis* isolated from pig in Poland (designated pig isolate) and *T. pseudospiralis*. The infectivity rate in the ICR mice of the *T. spiralis*-p.bear isolate was the same as *T. pseudospiralis*. However, the *T. spiralis*-pig showed an exceptionally high infective rate not only in mice but also in Wistar rats. *T. spiralis*-p.bear showed an exceptionally low infectivity rate in rats but was comparatively resistant to low temperature treatment, surviving at −20°C for a month. Nevertheless, all three *Trichinella* were not viable after one day at −40°C. Comparison of the length of the muscle larvae recovered 20–24 months after infection in mice showed that it is possible to distinguish the larvae of *T. spiralis*-pig isolate from *T. pseudospiralis* but not *T. spiralis*-p.bear isolate from the other two.

Key words : *Trichinella spiralis*, *T. pseudospiralis*, infectivity, rodent, cold resistance

INTRODUCTION

It has been observed that *Trichinella* isolated from different hosts or geographic entities showed some variation in the infectivity of the various animal hosts.[2,7,10,12,15,16,] These reports have led to the controversy over the speciation of the genus *Trichinella*. Until the discovery of *T. pseudospiralis* by GARKAVI in 1972, *T. spiralis* was thought to be the only species in the genus *Trichinella*. *T. pseudospiralis*, which was isolated from a raccoon in north Caucasus area, has comparatively smaller non-encapsulating larvae in the muscle.[1,9] The larvae of *T. pseudospiralis* is also able to develop to maturity in the muscle of birds[11,17] but not *T. spiralis*.[13]

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This study was supported in part by a Grant-in Aid for scientific research (No. 58370013) from the Ministry of Education, Science and Culture, Japan.

However, two other capsule forming species, namely, *T. nativa* BRITOV and BOEV, 1972, and *T. nelsoni* BRITOV and BOEV, 1972, have also been proposed.[4)]

Quite a number of isolates of *Trichinella* are being maintained in various individual laboratories throughout the world. It is very important that the researchers working in these laboratories characterize the isolates of *Trichinella* that they are handling before using the parasites for immunological or biochemical studies. We report herein the observation of an isolate of *T. spiralis* isolated from a polar bear in 1968 in Maruyama Zoo, Sapporo, Japan (designated p.bear isolate). Its infectivity in ICR mice and Wistar rats and its resistance to low temperature were compared with *T. pseudospiralis* isolated from a raccoon in north Caucasus by GARKAVI and *T. spiralis* isolated from a pig in Poland (designated pig isolate).

MATERIALS AND METHODS

Eight-week-old male ICR mice were orally inoculated with 600 larvae of either *T. pseudospiralis* (kindly supplied by Prof. WONG, Univ. of California, Davis), *T. spiralis*-p.bear isolate or *T. spiralis*-pig isolate (kindly supplied by Prof. YAMAGUCHI, Hirosaki Univ.). The mice were killed 60 days after infection and their muscle larvae recovered by digesting the whole carcass in artificial gastric juice (0.5% pepsin-HCl) for 5h at 37°C. After several washings by sedimentation of the larvae in saline, a sample volume of the larvae suspension was counted under a dissection microscope and the total number of larvae calculated. When the total number of larvae was found to be less than a thousand, all the larvae were counted. For comparison of the length of muscle larvae of the three *Trichinella* isolates, muscle larvae from mice which had been orally infected 20–24 months before were measured with a camera lucida. Fifty male and female larvae of each isolate were measured. Eight-week-old male Wistar rats were similarly inoculated with various doses of larvae as shown in tab. 2.

To test for resistance to low temperature, carcasses of male ICR mice, which were infected 60 days earlier, were stored at either 4°, −20°, −40° or −80°C for 1 to 30 days. After the low temperature treatment, the carcasses were digested in artificial gastric juice and the larvae orally inoculated into male ICR mice in order to confirm the viability of the low temperature treated larvae. The mice were killed on day 9 to check for intestinal adult worms and on day 30 to check for the presence of muscle larvae.

RESULTS

The infectivity rate in the ICR mice of the *T. spiralis*-p.bear isolate was the same as that of *T. pseudospiralis* (tab. 1). However, the *T. spiralis*-pig showed an exceptionally high infective rate not only in mice but also in Wistar rats (tabs. 1 & 2). The *T. spiralis*-p.bear showed an exceptionally low infectivity rate in rats but was comparatively resistant to low temperature treatment, surviving at −20°C for a month (tabs. 2

TABLE 1 *Infectivity of the various Trichinella in ICR mice infected with 600 larvae*

	NO. OF MUSCLE LARVAE RECOVERED	
	Mean**	SD
T. spiralis-p. bear	27,733	5,107
T. spiralis-pig	57,181	14,065
T. pseudospiralis	26,629	5,202

**Average of 7 mice

TABLE 2 *Infectivity of the various Trichinella in Wistar rats*

	DOSE*	NO. OF MUSCLE LARVAE RECOVERED FROM EACH RAT
T. spiralis-p. bear	1,000	204; 103; 20
	3,000	37,165; 56; 20; 6; 2
	6,000	182; 16
T. spiralis-pig	3,000	457,333; 400,260; 312,000
T. pseudospiralis	3,000	200,000; 197,333; 35,262

* No. of infective larvae inoculated orally

& 3). Nevertheless, none of the three *Trichinella* were viable after one day at −40°C. Comparison of the length of the muscle larvae of the three *Trichinella* isolates is shown in fig. 1.

TABLE 3 *Resistance to low temperature treatment among the various Trichinella*

Temperature (℃)	4			−20						−40			−80		
Days Treated	1	15	30	1	2	3	5	15	30	1	15	30	1	15	30
T. spiralis-p. bear	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
T. spiralis-pig	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
T. pseudospiralis	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−

+ : Viability of treated larvae confirmed by successful infection mice
− : Failure of treated larvae to produce infection in mice

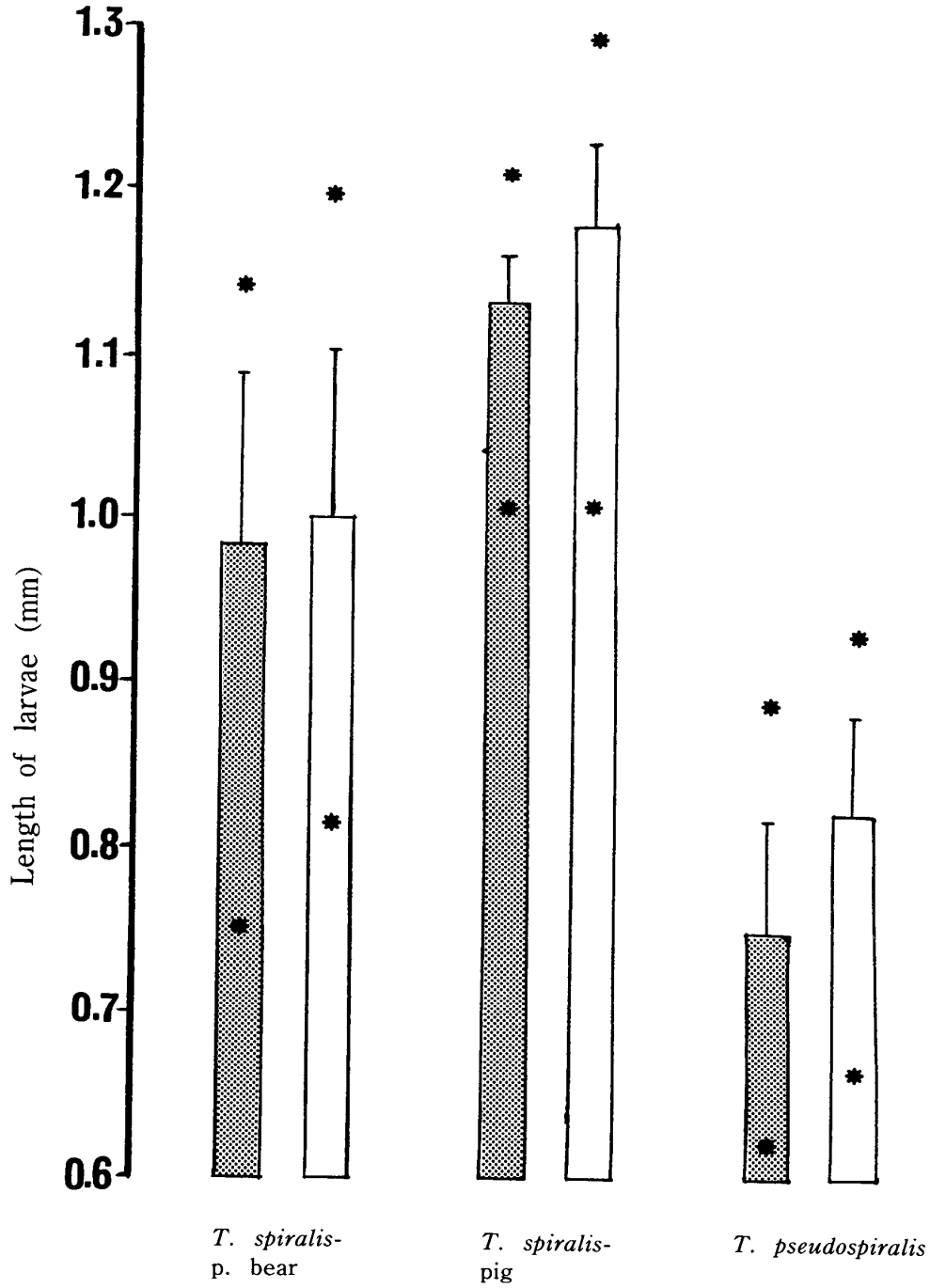

FIGURE 1 *Mean length of the muscle larvae of the various Trichinella*

✱: Range of the length of larvae (maximum and mininum) Bars indicate standard deviation.
▒; Male larvae. □: Female larvae.

Discussion

Our observations confirmed the reports that the pig isolate is highly infective in rodents such as mice and rats.[2,10,12,15] Although it is generally accepted that the rat is refractory to the polar bear isolate of *T. spiralis*, our results showed that in one rat, there was probably a breakdown of immunity that resulted in mass infection. In other rats, the few larvae recovered suggested the presence of mutant individuals in the larval population. Difference in the dose of the infective larvae administered did not have any effect on the refractoriness of the rats to *T. spiralis*-p.bear.

As shown in fig. 1, it is possible to distinguish the larvae of the *T. spiralis*-pig isolate from *T. pseudospiralis* but not the *T. spiralis*-p.bear from the other two *Trichinella*. Thus, attention must be given to this fact when mixed infection is involved.

The ability to survive freezing temperatures has been cited as a criterion for the identification of the proposed *T. nativa*.[5] Since our *T. spiralis*-p.bear isolate was able to survive for a month at −20°C, it is probable that it may belong to the proposed *T. nativa*. However, the validity of *T. nativa* as a species has been questioned because it had been reported to crossbreed with *T. spiralis*.[3] In addition, our *T. spiralis*-p.bear isolate was shown to be able to crossbreed with the *T. spiralis*-pig isolate.[14]

The larvae of *T. spiralis* in the muscle of spontaneously infected polar bear, wolverine, marten and arctic fox have been reported to be viable even after storage at −15°C for more than five month.[6] Dick and Belosevic (1978) reported that the cold resistance of larvae seen in the polar bear muscle was not reproducible in mice. They could not recover viable muscle larvae from the carcasses of mice which had been infected with *T. spiralis* isolated from a polar bear that had been frozen at −15°C for 7–31 days. However, no mention was made of the age of the larvae in the infected mice. Our results showed otherwise. Thus we have demonstrated that even after one and a half decades of passage in laboratory animals, the *T. spiralis*-p.bear still retained its cold resistant characteristic. In many laboratories, the carcasses of infected animals are usually frozen before they are incinerated. That *T. spiralis*-p.bear is still alive even after freezing at −20°C implies that the disposition of infected carcasses must be handled with care.

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