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INTERBREEDING AND FECUNDITY OF A SINGLE PAIR OF TWO STRAINS OF *TRICHINELLA SPIRALIS* IN MICE

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Inter- and intrabreeding with a single pair of two strains of *Trichinella spiralis*, designated as Polish and Sapporo strains, and isolated from a wild pig and a polar bear, respectively, were performed in mice. The mating success was 53% and 47% for the Polish and Sapporo strains, respectively. The number of larvae produced by a single female worm of the Polish strain was 795 ± 294, while those of the Sapporo strain was 275 ± 156. F_1 hybrids produced from a cross between the two strains were fertile. It was concluded that the two strains were not reproductively isolated, although they showed biological differences in fecundity.

Key words: *Trichinella spiralis*, strains, interbreeding, fecundity

Introduction

Until the early 1970's, it was thought that there was only one species of *Trichinella*, that is, *T. spiralis* (Owen, 1835) Railliet, 1895. However, as research on various *Trichinella* isolated from different animals in different geographic entities progressed, it became clear that there were differences in the biological characteristics among these *Trichinella* isolates.[8,11,12] This led to the proposal of a number of new species, namely, *T. nativa*, Britov and Boev, 1972, *T. nelsoni*, Britov and Boev, 1972 and *T. pseudospiralis*, Garkavi, 1972.[3] With the exception of *T. pseudospiralis*, which has a smaller larva size, all the other proposed *Trichinella* species were identical to each other morphologically. Thence the controversy arose as to whether the proposed new species of *Trichinella* were actually valid, or should be considered as varieties, strains or subspecies. The proposal of *Trichinella* species other than *T. spiralis* was made on the basis of the resistance to high or low temperature, manifestation of high or low infectivity and pathogenicity in laboratory rodents, presence or absence of a capsule around the muscle larva and the development in birds.[4]

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The latter two criteria were used specifically for the identification of *T. pseudospiralis*. The strongest evidence for the validity of the proposed new species was the failure to interbreed among themselves. However, despite this, some workers still feel that there is insufficient evidence to raise any of the isolates or varieties to the species level.[6,7,8,9]

Since the manifestation of high or low infectivity dictates the degree of pathogenicity, which is in turn determined by the fecundity of the female worm in the intestine, it would be useful to know the number of larvae produced *in vivo* by a single female of a certain isolate of *Trichinella* species. Moreover, it would be desirable to see if the various isolates maintained in each laboratory will interbreed or not. We present in this study the results of the mating success and fecundity of pairs of two isolates of *Trichinella*, and also an attempt at interbreeding of these two isolates. We designated the two isolates used as *T. spiralis*-Polish strain and *T. spiralis*-Sapporo strain.

MATERIALS AND METHODS

Parasites

Two isolates of *T. spiralis* were used. One was isolated from a polar bear, *Thalarctos maritimus*, at Maruyama Zoo, Sapporo, and since then has been maintained as stock infection in our laboratory. The other was isolated from a wild pig, *Sus scrofa*, in Poland and was kindly donated by Prof. T. YAMAGUCHI of Hirosaki University. The former isolate was designated as Sapporo strain, and the latter, Polish strain. Our preliminary observation revealed that Sapporo strain has a very low, while the Polish strain has a high infectivity rate in rats and that the muscle larvae of both strains are surrounded by a capsule.

Infective larvae were recovered by digestion of the stock mice, which had been infected for at least 8 weeks. The mice were killed under ether anaesthesia, skinned, eviscerated and cut into fine pieces. The carcasses were then digested in artificial gastric juice (0.5% pepsin-0.5% HCl) at 37°C for 3 hours. Undigested materials were filtered off with a coarse metal sieve, and the larvae were collected by repeated sedimentation and washing with physiological saline.

Experimental animals

The strain of mice used was 10 week-old male ICR mice, which were obtained from Shizuoka Agriculture Cooperative Association for Laboratory Animals, Japan. The animals were given commercial feed (CE-2, Nihon Clea) and water *ad lib.*

Sexing of the larvae

The infective muscle larvae were washed in physiological saline at 37°C, placed individually on a slide glass and observed under a microscope at a magnification of 200 times without a cover slip. They were sexed according to the criteria proposed by BELOSEVIC and DICK (1980). Male: distinct crossing of the gonad by the intestine

from the convex to the concave surfaces; longer rectum length of about 50 μm; intestinal bulb usually lying close to the convex surface. Female: no crossing of the gonad by the intestine; intestine always on the concave surface; shorter rectum length of about 30 μm; intestinal bulb lying close to the concave surface. In some male larvae, the intestinal bulb was observed to be close to the concave surface and the intestine first crossed the gonad from the concave to the convex surface and then again from the convex to the concave surface.

The accuracy of sexing was tested by infecting 3 mice, two with 15 male larvae each, and the other with 15 female larvae, respectively. The mice were killed on day 7 postinfection (PI), and the worms in the intestines were recovered for microscopic examination. The diaphragms were macerated in a drop of saline on a slide glass, and a cover slip was placed over the slide for the detection of newborn larvae under the microscope.

Intra- and interstrain breeding

For intrastrain breeding, 15 mice were each inoculated with a single pair of Sapporo strain and Polish strain, respectively. For interstrain breeding, 15 mice were each inoculated with a female Sapporo strain larva and a male Polish strain larva, and vice-versa, respectively.

Each mouse was inoculated orally with a single male and female larvae by means of a stomach tube connected to a syringe. To assure that no larvae remained in the syringe, the syringe was washed repeatedly with saline and the washings examined under the dissection microscope. All the mice were killed on day 42 PI and their diaphragm examined for the presence of muscle larvae. The infected mice were digested individually as described above and the total number of muscle larvae counted.

Results

The result of the intra- and interstrain breeding experiment is shown in table 1.

TABLE 1 *Interbreeding and fecundity of a single pair of Polish and Sapporo strains of T. spiralis*

INOCULUM	NO. OF MICE	NO. OF MICE INFECTED (%)	NO. OF LARVAE RECOVERED MEAN±SD
Pol♂1×Pol♀1	15	8 (53)	795±294
Sap♂1×Sap♀1	15	7 (46)	275±156
Sap♂1×Pol♀1	15	1 (7)	670
Pol♂1×Sap♀1	15	0 (0)	-

Pol: Polish strain
Sap: Sapporo strain

The mating success for Polish and Sapporo strains was 53 and 47%, respectively. However, the mating success in the interstrain breeding experiment was very low, and only one out of the thirty pairs was able to produce the F_1 hybrids. It was clear that the Polish strain had a higher fecundity *in vivo* than the Sapporo strain, and the number of larvae produced by a single female was 795±294 in the former and 275± 156 in the latter.

Fertility of the F_1 hybrid produced from the cross between Polish and Sapporo strains were tested by inoculating orally 50 randomly selected hybrid larvae into 6 mice and examining them for the presence of infective muscle larvae. Backcrossing with the two parental strains was also carried out. The result is shown in table 2.

TABLE 2 *Fertility of F_1 hybrids produced from a cross between Polish and Sapporo strains of T. spiralis*

INOCULUM	NO. OF MICE	NO. OF MICE INFECTED	NO. OF LARVAE RECOVERED FROM EACH MOUSE
F_1 only 50 L	6	6	5,498±1,817#
F_1♀5×Pol♂1	2	2	676; 261;
F_1♂5×Pol♀1	1	1	628
F_1♀5×Sap♂1	2	1	403
F_1♂5×Sap♀1	1	1	677

F_1: First generation hybrids from Polish and Sapporo strains
Pol: Polish strain
Sap: Sapporo strain
#: Mean±SD

F_2 hybrids were recovered from the muscle of all the mice orally inoculated with the 50 F_1 hybrids. Backcrossing of the F_1 with the two parental strains was also successful.

In the sexing accuracy test, 9 female adult worms were recovered from the mouse infected with 15 female larvae. Three male adult worms, 2 and 1 from each of the mice infected with 15 male larvae were recovered. None of the mice had newborn larvae in their diaphragm. The female adult worms had neither embryos *in utero* nor sperms in their seminal receptacles.

DISCUSSION

We showed that the fecundity of the female worm is dependent on the strain of *Trichinella* used. This may account for the difference in the number of progeny produced by a single female, as reported by other investigators. CAMPBELL and YAKSTIS (1969) reported a mating success of only 19% and the recovery of

1,500–2,300 muscle larvae from single pair infections by oral inoculation in mice. BELOSEVIC and DICK (1980) using duodenal injection of a single pair of larvae, were able to obtain a mating success of 42 and 39%, but in their multiple pair breeding experiment, they were able to obtain a very high mating success. Although the use of multiple pair infection will result in higher mating success, the use of single pair infection will completely eliminate the possibility of sexing error.

Trichinella species isolated from the polar bear in the arctic regions had been reported to have a very low infectvity in rats.[1,12] These arctic isolates of *Trichinella* were referred to as *T. nativa*.[3] BESSONOV et al. (1975) also reported a mating success of 72 and 68% and an average recovery rate of 481 and 433 muscle larvae from a single pair infection of *T. spiralis* and *T. nativa*, respectively. Since they were able to produce fertile hybrids from the above-mentioned two "species", they concluded that there were insufficient grounds to consider *T. nativa* as an independent species. The Sapporo strain of *T. spiralis* used in the present investigation was also derived from a polar bear and have low infectivity in rats. Thus, it is highly probable that this parasite is the same as that of the arctic strain. However, our results demonstrated that the Polish strain and the Sapporo strain of *Trichinella* are not reproductively isolated, although they showed a biological difference such as the fecundity of individual females *in vivo*. We deduced that the Polish and Sapporo strains belong to the same species of *Trichinella*, because the F_1 hybrids were able to produce the next generation of F_2 hybrids.

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REFERENCES

1) BELOSEVIC, M & DICK, T. A. (1980): *Trichinella spiralis*: Comparison with an arctic isolate *Exp. Parasitol.*, **49**, 266–276
2) BESSONOV, A. S., PENKOVA, R. A. & USPENSKY, A. V. (1975): On the independence of *Trichinella* species *Wiad. Parazytol.*, **21**, 561–575
3) BOEV, S. N., BRITOV, V. A. & ORLOV, I. V. (1979): Species composition of Trichinellae *Ibid.*, **25**, 495–501
4) BOEV, S. N. & SOKOLOVA, L. A. (1981): Primary diagnostics of sibling-species of Trichinellae *Ibid.*, **27**, 483–387
5) CAMPBELL, W. C. & YAKSTIS, J. J. (1969): Mating success of *Trichinella* larvae administered to mice *Ibid.*, **15**, 526–532
6) DICK, T. A. (1983): The species problem of *Trichinella*. In: Concepts in nematode systematics Ed. STONE, A. R., PLATT, H. M. & KHALIL, L. F., Lond. & New York: Academic Press, 351–360
7) DICK, T. A. (1983): Species, and infraspecific variation. In: *Trichinella* and tichi-

nosis Ed. CAMPBELL, W. C., New York & Lond.: Plenum Press, 31–73

8) KOZAR, Z. & KOZAR, M. (1965): A comparison of the infectivity and pathogenicity of *Trichinella spiralis* strains from Poland and Kenya *J. Helminthol.*, **39**, 19–34

9) MACHNICKA, B., (1979): The problem of geographical strains of *Trichinella spiralis* and their biological properties *Wiad. Parazytol.*, **25**, 197–205

10) MADSEN, H. (1975): The life cycle of *Trichinella spiralis* (OWEN, 1835) RAILLIET, 1895 (syns.: *T. nativa* BRITOV et BOEV, 1972, *T. nelsoni* BRITOV et BOEV, 1972, *T. pseudospiralis* GARKAVI, 1972), with remarks on epidemiology, and a new diagram *Acta Parasitol. Pol.*, **24**, 142–158

11) NELSON, G. S., BLACKIE, E. J. & MUKUNDI, J. (1966): Comparative studies on geographical strains of *Trichinella spiralis* *Trans. R. Soc. Trop. Med. Hyg.*, **60**, 471–480

12) READ, C. P. & SCHILLER, E. L. (1969): Infectivity of *Trichinella* from the temperate and arctic zones of North America *J. Parasitol.*, **55**, 72–73