Comparative infectivity of two isolates of Trichinella spiralis in wild and domestic rodents
by Richard Harding McBee

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
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Abstract:
Experiments were conducted to compare the infectivity of a domestic isolate of Trichinella spiralis from a hog with a wild isolate from a grizzly bear. The experimental hosts consisted of albino mice (Dublin Swiss Webster), Meadow voles (Microtus pennsylvanicus) and deer mice (Peromyscus maniculatus) of which 16, 14 and 10 animals, respectively, were inoculated with 200 larvae of the domestic isolate and 10, 11 and 8 animals, respectively, were inoculated with 200 larvae of the wild isolate. All animals surviving the experimental period were killed 60-65 days postinoculation and the muscle tissues digested to recover the Trichinella larvae. A comparison of the numbers of larvae isolated from the terminated animals showed that significant differences existed between the infectivities of the two isolates in albino and deer mice but not in voles. The wild isolate showed low infectivity for albino mice, whereas the domestic isolate showed low infectivity for deer mice. Both isolates showed low infectivity for meadow voles. Experimental evidence was not sufficient to determine if the wild isolate of Trichinella was naturally indigenous or was of domestic origin and had been altered in its infectivity due to passage in wild hosts. An attempt to induce T. spiralis infections in young deer mice via fecal contamination from their mothers failed*

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Richard H. McBee Jr.

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COMPARATIVE INFECTIVITY OF TWO ISOLATES OF *TRICHINELLA SPIRALIS* IN WILD AND DOMESTIC RODENTS

by

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TABLE OF CONTENTS

LIST OF TABLES

PAGE

TABLE

ABSTRACT

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INTRODUCTION

Trichinella spiralis (Owen, 1835) Railliet, 1895 was first discovered in the muscles of a human by James Paget in 1835. Until recently, it was believed to be maintained in a domestic cycle between rats, pigs and man, with occurrences in wild animals being the exception. In recent years, the likelihood of a world wide enzootic condition has been indicated through surveys showing a high incidence of T. spiralis in wild carnivorous mammals as well as rodents (Gould, 1970a).

Natural infections of T. spiralis have been reported from all continents with the exceptions of Australia and Antarctica (Alicata, 1970). In North America, many of the large carnivores have been implicated as natural reservoirs of infection. Rausch et al. (1956) reported infections in wolves and lynx as well as polar, grizzly and black bears. Within the continental United States, there have been reports of infections in mountain lions (Winters, 1969), wolverines (Zimmermann and Hubbard, 1969) and black bears (Roselle et al.,1965). Asian reports of natural infections have been primarily confined to surveys within the U.S.S.R., where Shikhobalova et al. (1969) reported infections in tigers, lynx, wolves and bears. Kozar (1970) stated that the red fox was the most important reservoir host for T. spiralis in Europe. Surveys for the continents of South America and Africa were not complete. In Argentina, one fox was reported to have been infected

(Neghme and Schenone, 1970), while Nelson and Mukundi (1963) stated that the main hosts for T. spiralis in East Africa were lions, leopards and hyena.

The role of wild rodents in the maintenance and transmission of T. spiralis is not clear. Low incidences of natural infections have been reported in Alaskan ground squirrels and voles by Rausch et al. (1956), in muskrats and beavers by Zimmermann and Hubbard (1969), and in the mice and voles of Russia (Shikhobalova et al., 1969).

Until recently, T. spiralis was regarded as being uniform in morphology, pathogenicity and infectivity. Villella (1970) reported no morphological differences which could be attributed to different strains. Rappaport (1943a, b) was unable to show significant differences in pathogenicity and infectivity in a comparison of three isolates of Trichinella from two humans and one hog.

Strain differences in infectivity were first shown by Nelson and Mukundi (1963) for an isolate of T. spiralis from Kenya which was of low infectivity in rats. This finding was confirmed by Kozar and Kozar (1965) in a comparison of the Kenya strain with one from Poland. Low infectivity for the Kenya strain was again reported by Nelson et al. (1966). This same experiment showed low infectivity of an arctic isolate in rats when compared to an English isolate. Schad et al. (1967) found low infectivity of an Indian strain in rats when compared

to one from Canada. Read and Schiller (1969) reported that Peromyscus leucopus was susceptible to arctic and temperate zone isolates of T. spiralis while laboratory mice and Microtus pennsylvanicus appeared less susceptible to the arctic isolate. Recently, Arakawa and Todd (1971) reported that laboratory mice were relatively refractory to infections with an arctic isolate when compared to an isolate from the temperate part of the United States. In addition, this last experiment showed that after four passages of the arctic isolate in laboratory mice, it no longer differed significantly in infectivity from the temperate isolate.

The following experiment was conducted to compare the infectivity of wild and domestic isolates of T. spiralis from the continental United States in wild and domestic rodents.

MATERIALS AND METHODS

A domestic isolate of T. spiralis was obtained from Dr. N. Kingston of the University of Wyoming. It was originally isolated at the University of North Carolina from a hog and had been maintained in laboratory rats and mice since 1936. The wild isolate was obtained in the Veterinary Research Laboratory, Montana State University, from the tissues of a male grizzly bear (Ursus arctos) killed in Flathead County, Montana, in 1971. The striated muscle tissues of the bear were stored in bulk at 4° C. and wild strain larvae were isolated directly from them as required for experimental infections.

One variety of albino mice and two of native wild mice were used as experimental animals. Laboratory mice were of the Dublin Swiss Webster variety and were approximately 3-6 months old at the time of inoculation. Meadow voles (Microtus pennsylvanicus) and deer mice (Peromyscus maniculatus) were collected locally by live trapping. They were of unknown age and were generally allowed to acclimatize to laboratory conditions for more than one week prior to infection. All mice were housed individually and given food and water ad libitum.

T. spiralis larvae were isolated through a modification of the Winters technique (1969). Approximately 25 grams of tissue was emulsified in 150 ml. of .7% HCl - .8% pepsin artificial digestive solution using a Servall^{R} Omni-Mixer. The mixture was then placed in pint jars and incubated at 37° C. for twelve to sixteen hours on a mechanical shaker to digest all muscle tissues. At the end of the digestion per-

iod, the larvae were washed through a 60-mesh screen, collected on a 200-mesh screen and concentrated by sedimentation. When fewer than 1,000 larvae were present in the suspension, counting was done directly on a lined petri dish under 20 power magnification with a dissecting microscope. Greater numbers of larvae were diluted with water to known volume, placed on a mechanical stirrer and not less than five 0.25 ml. portions were withdrawn and counted. The total numbers of larvae were then calculated from these counts.

Inoculations of 200 larvae were administered orally to each mouse by stomach tube. A total of 16 laboratory mice, 14 voles and 10 deer mice were inoculated with the domestic isolate. In addition, 10 laboratory mice, 11 voles and 8 deer mice were inoculated with the wild isolate of T. spiralis. Infected mice were observed daily for mortality through 60-65 days postinoculation, at which time the surviving mice were killed and examined for the presence of adult and larval Trichinella.

At necropsy, the mice were skinned, eviscerated and then weighed. The entire intestinal tract was cut into sections and shaken in water for 15 minutes to remove any adult worms. Adult worms were separated from intestinal debris by washing the mixture through a 40-mesh screen and collecting them on a 200-mesh screen. The presence or absence of adult Trichinella was verified using a dissecting microscope. The

remaining carcass was subjected to digestion as described above and the total number of larvae counted.

A statistical analysis of the larval counts from animals surviving the experimental period was done using Duncan's multiple range test (Duncan, 1955). Relative infectivity of the two isolates was evaluated by a comparison of the larvae recovered from the mice infected with each strain. Relative susceptibility for a particular isolate was evaluated by a comparison of the larvae recovered from all varieties of mice infected with the same isolate.

Three controls were utilized in this experiment. (1) Samples of mice were collected from the trapping areas and digested to rule out the possibility of prior infection with T. spiralis. (2) Uninfected mice were kept as controls on mortality due to the parasite. (3) Ten laboratory mice were inoculated with the domestic isolate of Trichinella, which had been obtained from rats, in order to determine the effects of larval source on infectivity.

RESULTS

The results of the experimental infections using two isolates of T. spiralis in three varieties of mice are shown in Table I. Analysis of the numbers of larvae recovered from animals reaching the termination of the experiment showed significant differences between the infectivities of the two isolates. The domestic isolate showed a significantly higher rate of infectivity for laboratory mice than did the wild isolate. In deer mice, the wild isolate showed a significantly higher rate of infectivity than did the domestic isolate. The infectivities of both isolates were low for voles and showed no significant differences.

Differences in the susceptibilities of the three varieties of mice to each isolate were also apparent. Laboratory mice showed a higher susceptibility to the domestic isolate than did deer mice and voles. Deer mice were highly susceptible to the wild isolate, whereas voles and laboratory mice were of low susceptibility.

Mortality due to all causes during the course of the experiment was similar for each variety of mouse. The voles exhibited the highest mortality with 45.5% dying during the wild isolate infection and 57% dying during the domestic isolate infection. Laboratory mice showed 10% and 18.5% mortality during the wild and domestic isolate infections, respectively. Deer mice showed no mortality during the entire experiment.

TABLE I. Comparison of the mean recovery values of the domestic and wild isolates of T. spiralis larvae in wild and domestic mice 60 - 65 days after infection with 200 larvae.

Isolate and mouse variety	No. of mice	% mortality	Mean recovery value of larvae*	Standard deviation
Domestic isolate				
1. Laboratory mice	16	18.75%	10,014.07[a]	±6,006.76
2. Meadow voles	14	57.00%	619.75[b]	± 662.10
3. Deer mice	10	------	1,747.55[b]	±2,564.46
Wild isolate				
4. Laboratory mice	10	10.00%	558.51[b]	± 614.64
5. Meadow voles	11	45.50%	864.41[b]	± 931.92
6. Deer mice	8	------	11,772.96[a]	±4,997.67

*The superscripts [a] and [b] refer to Duncan's multiple range test. Any two means not having the same superscript are significantly different ($P < 0.05$).

None of the observed mortality could be directly attributed to Trichinella due to the variation in survival of the mortality controls using uninfected mice. All the controls for deer mice survived the entire experimental period, whereas those for the voles all died prior to termination of the experiment. In the case of the domestic isolate infection, all controls for laboratory mice survived, whereas during the wild isolate infection they all died.

An examination of 49 voles and 11 deer mice from the trapping areas showed no evidences of prior infection of either wild variety of mouse with T. spiralis.

No significant differences were found between the infectivities of the experimental domestic isolate from mice and its control which had been isolated from rats, indicating that the source of the domestic isolate was not a factor in its infectivity for laboratory mice.

DISCUSSION

The experimental results show that significant differences exist between the infectivity of the wild isolate of T. spiralis from a grizzly bear and that of the domestic isolate from a hog and maintained in rats and mice. The wild isolate showed a significantly lower infectivity for laboratory mice than did the domestic isolate, whereas the domestic isolate showed a significantly lower infectivity for deer mice than did the wild isolate. There were no apparent differences in the infectivities of either isolate in voles.

Several workers have reported low infectivities in laboratory animals for strains of T. spiralis isolated from North American bears. Nelson et al. (1966) found that a grizzly bear strain from Alaska was of low infectivity for rats when compared to an English strain from a cat. A comparison of a hog isolate of T. spiralis with a polar bear isolate showed that the latter had reduced infectivity for rats, hamsters and mice (Read and Schiller, 1969). Recently, Arakawa and Todd (1971) reported that another polar bear isolate exhibited low infectivity for white mice when compared to an isolate of human origin.

Work elsewhere with wild isolates of Trichinella has shown that this state of reduced infectivity is apparently transient and will increase markedly with repeated passage of the wild parasite strain in laboratory hosts. Kozar and Kozar (1965) reported that a wild strain, isolated from an African hyena, apparently increased its infectivity

after two passages in laboratory mice. Schad et al. (1967) found increased infectivity of a civet cat strain for rats after nine passages. As previously mentioned, similar findings were noted by Arakawa and Todd (1971) after four passages of their polar bear isolate in white mice.

A similar adaptation of a parasite to a new host was noted by Haley (1961) who found that the rat nematode, Nippostrongylus brasiliensis, increased its infectivity for hamsters after several passages. In addition, this experiment showed that despite repeated passage of the parasite in hamsters, the original high infectivity for rats was retained. It is possible that the wild isolate of T. spiralis, showing high infectivity for deer mice, had been previously passaged in this host in the wild and retained its infectivity. Similarly, the low infectivity of both isolates in voles would suggest that neither strain had been repeatedly passaged in voles under natural conditions.

Differences in infectivities of the two isolates might be attributed to the poor viability of larvae used in the infections due to handling procedures, inherited differences in life span or larval output of the adult worms, or to differences in the host's resistance to parasitic invasion.

The handling procedures for the tissues and larvae are described in the materials and methods section of this paper. Storage of meat at temperatures above freezing is not known to affect adversely the infec-

tivity of Trichinella larvae. Gammon et al. (1968) reported that pork hams showed no decrease in numbers of viable larvae after storage at 4° C. for 60 days. Dykova (1967) allowed samples of infected meat to putrefy for periods of up to 94 days and found that this did not affect the ability of the larvae to infect hamsters. Bear tissues, used as a source of larvae during this experiment, contained viable larvae 112 days after the bear was killed. At that time, a vole fed 200 larvae developed an infection which did not differ significantly from those of the experimental voles. Another sample of bear meat contained active larvae after 124 days of storage at 4° C. The digestion time of twelve to sixteen hours is comparable to the times mentioned by Ransom (1916) and Gould (1970b) as causing no decrease in larval viability. All larvae were utilized for infections immediately following isolation and larval numbers for inoculation were calculated using only those that were mobile or tightly coiled.

Although this experiment was not designed to measure differences in the life spans or larval output of Trichinella adults, other workers have found significant differences in the numbers of larvae maturing to adults in wild and domestic strains. Schad et al. (1967) reported that in rats, a wild strain of T. spiralis from an Indian civet cat produced fewer adults and of these adults a fewer number were males than did a Canadian strain from a hog. In addition, the adults of the wild strain were much smaller than those of the domestic strain. Arakawa and Todd

(1971) also found reduced numbers of T. spiralis maturing from a polar bear strain when compared to a human isolate. Reduced numbers of larvae maturing into adults may account for the lower rates of infection seen in the various mouse varieties in this experiment, but whether these reduced numbers were due to inherited characteristics of the isolates or to variations in host resistance is not known. In this experiment, both isolates apparently were able to produce similar numbers of larvae in a highly susceptible host. Infections with 200 larvae of the domestic isolate produced an average of 10,014 larvae in its presumed most susceptible host, laboratory mice. This figure is not significantly different from the average of 11,773 larvae recovered from the deer mice, which were apparently the most susceptible host for the wild isolate. Therefore the differences seen in the infections were more probably related to host resistance for a particular isolate rather than to inherited abilities of the Trichinella strains to produce an infection in a highly susceptible host.

One of the primary factors in the resistance to T. spiralis infection is the ability of the host to eliminate the adult worms rapidly from its intestinal tract (Catty, 1969). Adult mice, with a slow rate of intestinal emptying, harbor adult Trichinella in the anterior portions of the small intestine, whereas in younger mice, adult worms develop in the posterior portion of the small intestine due to a faster rate of intestinal emptying (Larsh and Hendricks, 1949). Bass and Olson

(1963) stated that the fast rate of intestinal emptying caused a rapid elimination of the adult worms in young mice. The different ages of the wild species of mice used in this experiment may have been responsible for some of the variation seen in susceptibilities to infection with the same isolate.

Elimination of adult worms from the small intestine is facilitated by intestinal inflammation. During the ordinary course of the disease in mice, inflammation of the small intestine due to adult Trichinella peaks between the eighth and eleventh days after infection. This response is characterized by the infiltration of polymorphonuclear leucocytes during the acute phase and by mixed mononuclear cells when inflammation subsides into the chronic or subacute stages (Larsh et al., 1956). Severe inflammation of the gut may restrict the nutrition of the adult Trichinella causing them to leave the intestine before releasing all their larvae (Despommier and Wostmann, 1969). The inflammatory response of the gut may be stimulated by prior infection with another parasite and thereby cause a natural resistance to T. spiralis infection. This was shown by Cox (1952) and Goulson (1958) using the dog hookworm, Ancylostoma caninum, to produce the initial intestinal inflammation. Differences in the times of onset of the inflammatory response and its severity in the different varieties of mice may have influenced their infection rates. In addition, unknown parasitic infections in the two wild species of mice, prior to their capture, may have had unknown effects

on the abilities of these mice to resist infection with T. spiralis.

Differences in host susceptibility to T. spiralis may also be influenced by the conditions under which the host is kept in the laboratory. Davis and Read (1958) found a 50% higher rate of infection with Trichinella in wild mice housed communally than in their controls which were housed individually. They attributed this finding to hypertrophy of the adrenals in the communally housed mice due to fighting and social interaction. This, coupled with cannibalism, could account for the significantly higher rate of infection, average larval count-119,940, found in four survivors of a group of 16 voles which were experimentally infected with 200 larvae each from a second grizzly bear source and housed in a common cage.

During the course of the infections, periorbital swelling and apparent stiffness of movement were observed in the domestic mice from day 13 through day 45. These signs were probably similar to those seen in human patients during the migratory and encystment stages of the infection (Wyrens et al., 1941) (Maynard and Pauls, 1962) (Roselle et al., 1965). Gould (1970c) stated that the periorbital swelling may be due to invasion of the muscle fibers around the eyes, to a larval toxin, or to an allergic response.

The question of the importance of fecal transmission in natural T. spiralis infections has not been answered. Robinson and Olson (1960) found that 6.5% of the larvae fed to mice appeared in the fecal material

within 96 hours. If consumed, these undigested larvae and cysts could cause an infection in another animal (Gould, 1945). This type of fecal transmission was shown by Spindler (1953), who fed hog feces containing cysts to other hogs.

During this experiment, an opportunity to observe possible fecal transmission appeared when three of the deer mice produced litters just prior to their inoculation with the domestic Trichinella isolate. The young were allowed to remain with their mother for 20 days after her inoculation and were then removed from her presence for another 20 days before they were killed and examined for the muscle larvae of T. spiralis. The 13 young mice were all negative for Trichinella. This finding is similar to that of Catron (1937), who found no transmission of infections in mice from mother to young by feces or milk. Without intentional feeding of feces, this type of transmission appears to be rare and is probably insignificant in the maintenance and transmission of the parasite under natural conditions.

These data have shown that there were significant differences in the infectivities of wild and domestic isolates of T. spiralis originating within the continental United States. The differences apparently were due to differences in the host resistance to infection rather than to an inherited inability of the isolates to produce larvae in a susceptible host. The evidence would indicate that the differences seen in this and other isolate comparisons were transient and may disappear

rapidly following association between the parasite and a new host. The evidence submitted here is not sufficient to indicate that the wild isolate of Trichinella is indigenous and not related to the domestic isolates within the continental United States. Certainly, the report by Craighead and Craighead (1971) that the grizzly bears of Yellowstone National Park have had easy access to garbage dumps for the past 80 years would tend to support this domestic origin of the isolate in Montana. It is also tempting to speculate that the wild strain mentioned by Rausch et al. (1956) in the grizzly bear is actually part of a continuum extending the arctic strain of Trichinella southward across Canada into the Northern Rockies of the United States. Further study is needed in the area of epidemiology to give a better indication of the distribution of Trichinella in wild carnivores and rodents of the Western United States. Continued study of the parasite in Montana would also be of value to give a better indication of the incidence of infection in man and domestic animals and to determine what relationship these have to the occurrences of the parasite in wild animals.

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