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Viability of *Trichinella Spiralis* Larvae Stored in Serum[1]

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Abstract. Studies were made to determine the viability of *Trichinella spiralis* larvae stored in immune and nonimmune swine sera at refrigerator and room temperatures.

In trial 1, the trichinae were maintained at room temperature in immune sera for 14 days with only slight reduction in viability, while 7 days storage in nonimmune sera reduced infectivity by nearly 50%. All trichinae were noninfective in both types of sera at 28 days. Room temperature storage in trial 2 gave no loss in vialibility after 7 days storage in nonimmune sera while infectivity for trichinae stored 7 days in immune sera was 33% of the controls. All infectivity was lost in both sera types after 21 days.

Trichinae were maintained at refrigerator temperatures for extended periods in trial 1. Limited infectivity was retained in refrigerated immune sera for 126 days, with 37% viability through 56 days. Trichinae were maintained for 98 days in nonimmune sera, with 50% viability for 56 days. No infectivity was obtained after 28 days storage in either refrigeratde serum type during trial 2.

Apparent viability as indicated by miscoscopic examination gave little correlation with actual transmission studies.

The principal means of maintaining viable trichinae has been either in living animals or in refrigerated infected tissues. Little study has been given to other means or perpetuating or transporting *Trichinella spiralis* larvae. These investigations dealt with the viability of trichina larvae stored in both immune and nonimmune swine sera at refrigerator (4°C) and room (21°-24°C) temperatures.

Oliver-Gonzalez (1940) showed that both antilarval and antiadult precipitins could be demonstrated *in vitro* by incubating trichina larvae and adults in immune serum at 37°C. Mauss (1940) studied the effect of *in vitro* incubation of trichina larvae in both immune and nonimmune rabbit serum. Only one-third as many adult trichinae became established in rats from larvae incubated in immune sera as compared to those incubated in nonimmune sera. Roth (1941) found 2 hours incubation of larvae in immune sera had no deleterious effect upon infectivity of the parasites. Offutt (1941) found that 3-5 days incubation of trichinae in immune sera produced no effect

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on the infectivity of larvae when compared to those similarly exposed to normal sera. A few larvae lived as long as 3 weeks in both immune and nonimmune sera. Levin (1940) maintained trichinae larvae in Tyrode solution at 38°C for up to 11 days, whereas at 5°C the larvae remained coiled and infective for 4 months, when experiment was terminated. The addition of vitamin B did not aid survival but glucose seemed beneficial for long periods.

Materials And Methods

T. spirais larvae were obtained by pepsin-HCl digestion of the ground carcasses of experimentally infected albino rats. The larvae were cencentrated and collected by the Baermann technique and washed 3 times with physiological saline to remove rat protein. After washing, the larvae were treated with a 1:20,000 merthiolate solution for 15 minutes followed by another washing with sterile physiological saline. In trial 1, a portion of the trichinae was suspended in 100 ml of filter-sterilized nonimmune swine serum and the remainder was suspended in 100 ml of filter-sterilized immune serum from a pig which had received 150,000 *T. spiralis* 37 days prior to serum collection. Three rats were infected with each suspension by administration of 1 ml of each serum-larvae combination. These were the control rats for the nonimmune serum and immune serum groups, respectively. The remaining 97 ml of each type was then divided, 30 ml being stored at 4°C while 67 ml were kept at room temperature (21°-24°C). Storage was in serum bottles with puncture type tops.

One ml portions of the larvae-serum mixture from each pool were administered by *gavage* to each of 3 noninfected rats at selected intervals, the interval being 1 week for cultures kept at room temperature. The first two intervals for the refrigerated cultures were 4 weeks followed by intervals of 2 weeks until termination of the experiment. Fifty-six days after infection, the rats were killed, skinned, eviscerated, and the carcasses processed by grinding through meat choppers. Total larvel counts were made for individual animals by the artificial digestion-Baermann method.

In trial 2, the trichinae were again collected by the artificial digestion-Baermann technique. The larvae were counted by a dilution technique and divided into two portions, each containing approximately 150,000 *T. spiralis* larvae. The larvae were then washed and treated with merthiolate, after which 150 ml of filter-sterilized immune serum were added to the first portion and a like quantity of filter-sterilized nonimmune serum was added to the second portion. Each 150 ml quantity was then dispensed aseptically by 6 ml portions into

sterile vials. Thirteen vials of each serum type were stored at room temperature and 12 at refrigerator temperature. On specified sampling dates, 3 rats were each administered 1 ml of the serum-larvae mixture after which the tube was discarded. Sampling days were days 0, 7, 21, 28 and 35 for serum-larvae mixtures stored at room temperature and days 0 and 28 for those stored under refrigerator temperature. Evaluation of viability was made as in trial 1.

In addition to the viability studies made by administering larvae to rats periodically, apparent viability was also determined by microscopic examination at 27x. All worms either moving or coiled were considered viable while those in question mark form or degenerated were considered to be nonviable.

Results

Trial 1. Since division of the pooled larvae was only approximate, 2 groups of control rats were used, 1 group for each type of serum. The mean yield of *T. spiralis* larvae from the 3 control rats in the nonimmune serum group was 90,500 while that for the 3 control rats in the immune serum group was 160,667. Because of the marked difference in initial yield, the results are based both on mean yield and percentage of control yield for the specific serum type. The results will be given under serum type-temperature groupings (Table 1).

Nonimmune serum at room termeprature: The viability of *T. spiralis* larvae was markedly reduced by 7 days storage in nonimmune swine serum kept at room temperature. The main yield of larvae from the 3 rats in this group was 49,000, or 54.2% of the nonimmune serum controls. Storage for an additional 7 days (14 day total) further reduced infectivity, a mean yield of 11,300 (12.5%) being obtained. Nearly all larvae were noninfective after 21 days storage since only 1 to 3 rats contained trichinae, with 7 larvae obtained upon artificial digestion of the carcass. Rats administered larvae-serum mixtures after 28, 35, 42 and 56 days storage were negative.

Microscopic examination of larvae on days of infection provided little correlation with actual results. Examinations on day 7 gave an apparent viability of 98% compared with yield of 54.2%. Similar results were noted for other days. An apparent viability of 89% was noted for day 14, as compared to yield of 12.5%. All trichinae appeared nonviable after day 28.

No growth or development of the larvae was observed for this or the other serum-temperature combinations employed.

Immune serum at room temperature: No loss of viability was found after storage of trichina larvae for 7 days in immune swine serum at room temperature. The mean yield per

Table 1. Effect of serum storage on viability of *T. spiralis* larvae (Trial 1)

	Nonimmune serum				Immune serum			
	Room temperature		Refrigerator temperature		Room temperature		Refrigerator temperature	
Day	Mean Yield	Percent	Mean Yield	Percent	Mean Yield	Percent	Mean Yield	Percent
0 (Control)	90,500	100.0	90,500	100.0	160,667	100.0	160,667	100.0
7	49,000	54.2	.....	....	160,800	100.0	.....	....
14	11,300	12.5	.....	....	149,167	92.8	.....	....
21	2	0.003	.....	....	23,333	14.5	.....	....
28	0	0	90,667	100.0	0	0	134,333	83.5
35	0	0	.....	....	0	0	.....	....
42	0	0	.....	....	0	0	.....	....
56	0	0	45,000	49.8	0	0	59,500	37.0
70	.....	....	5,250	5.8	.....	....	29,667	18.5
84	.....	....	2,750	3.0	.....	....	15,500	9.6
98	.....	....	1,173	1.3	.....	....	5,167	3.2
112	.....	....	0	0	.....	....	1,417	0.9
126	.....	....	0	0	.....	....	59	0.04
140	.....	....	0	4	.....	....	0	0
156	.....	....	0	0	.....	....	0	0

Table 2. Effect of serum storage on viability of *T. spiralis* larvae (Trial 2)

	Nonimmune serum				Immune serum			
	Room temperature		Refrigerator temperature		Room temperature		Refrigerator temperature	
Day	Mean Yield	Percent	Mean Yield	Percent	Mean Yield	Percent	Mean Yield	Percent
0 (Control)	23,000	100.0	23,000	100.00	22,250	100.0	22,250	100.0
7	27,166	118.1	.....	....	7,667	33.4	.....	....
14	1,583	6.9	....	....	630	2.8	.....	....
21	0	0	.....	....	0	0	.....	....
28	0	0	0	0	0	0	0	0
35	.....	....	0	0	.....	....	0	0

rat infected on the seventh day was 160,800 trichinae or 100.0% of the immune serum controls. Only a small reduction in yield was obtained from rats infected on day 14, a mean yield of 149-167 (92.8%) was obtained. The reduction was more marked in rats infected on day 21 when a mean yield of 23,333 was obtained. This was 14.5% of the control yield. No larvae were recovered from rats administered larvae-serum mixture on days 28, 35, 42 and 56.

Microscopic examination gave good correlation on days 7 and 14, with apparent viability of 99% and 92% respectively, compared to actual transmission of 100.0% and 92.4%. The apparent viability for days 21 (93%), 28 (72%) and 35 (6%), were much higher than actual transmission results of 14.5%, 0.0% and 0.0% respectively.

A precipitate similar to that noted in incubation of *T. spiralis* larvae in immune serum was noted on day 14.

Nonimmune serum at refigeration temperature: Storage of excysted trichinae in nonimmune swine serum for 28 days at refrigerator temperature had no apparent effect on the viability of the larvae. The mean number of larvae recovered from the 3 rats infected on day 28 was 90,667 or 100.0% of the nonimmune serum controls. A reduction in viability to 49.3% was found in rats infected on day 56, a mean of 45,000 being recovered. An additional marked decrease occured after 2 additional weeks of storage (day 70) with a mean recovery of only 5,280 (5.8%). Further decreases to 2750 (3.0%) on day 84 and 1173 (1.3%) on day 98 were noted. Negative results were obtained on days 112, 126, 140 and 156.

Microscopic examination again gave little correlation. The apparent viability on day 28 was 86% compared to 100.0% in actual transmission. On day 98, 60% appeared viable while only 1.3% were obtained from rats infected on this day. No transmission was obtained on day 112 while 45% appeared viable miscroscopically.

Immune serum at refrigeration temperature: A slight decrease in mean yield was obtained from rats infected on day 28, the mean yield being 134,333 or 83.5% of the controls. An additional decline in viability was noted in the day 56 group, a mean yield of 59,500 (37.0%) being obtained. This decline continued in the following groups with mean yields of 29,667 (18.5%) on day 70, followed by 15,500 (9.7%) on day 84, 5, 167 (3.1%) on day 98, and 1,417 (0.9%) on day 112. Only slight infectivity was noted on day 112, 1 of 3 rats becoming infected with a yield of 177 larvae. No transmission was obtained on days 140 and 156.

Microscopic viability ratings were 95% on day 28, 48% on day 98, and 30% on day 140. No transmission was obtained on the latter day.

Trial 2. Four control rats were used for each serum type, 2 rats per vial. The mean yield of trichina larvae for the 4 control rats in nonimmune serum groups was 23,000 while that for the 4 control rats in the immune serum group was 22,250 (Table 2).

Nonimmune serum at room temperature: The mean yield of 27,116 larvae for the 3 rats infected after 7 days storage was 118% of the yield for the 4 control rats indicating no apparent loss of infectivity. A marked loss in infectivity then occurred, with the mean yield for day 14 being only 1,583 or 6.9% of the controls. No infectivity was obtained on days 21 and 28.

Microscopic examination indicated 99% alive on day 7, 98% on day 14, 18% on day 21, and 1% on day 28.

Immune serum at room temperature: A rather marked increase in infectivity occurred during the first week of storage with the mean yield of 7,667 being only 33.4% of the immune-serum control rats. This was further reduced on day 14 when the mean yield was 630 (2.8%). No infectivity was obtained on days 21 or 28.

Microscopic examination showed apparent viability of 99% on day 7, 85% on day 14, 37% on day 21 and 2% on day 28.

Nonimmune serum at refrigerator temperature: No transmission occurred after 28 and 35 days storage at refrigerator temperature. Microscopic evaluation showed 26% apparently alive on day 28 and 5% on day 35.

Immune serum at refrigerator temperature: All infectivity was lost after 28 days of storage. Microscopically, 17% appeared alive on day 28 and 3% on day 35.

In order to determine if bacterial contamination was the primary cause of death of the larvae, 6 vials from each serum-temperature group were examined on day 39 for bacterial contamination. Serum samples were streaked on a plate of Difco tryptose blood agar base with 5% defibrinated horse blood added. The plates streaked from vials stored at room temperature showed abundant bacterial growth, primarily Gram positive rods. No bacterial growth was obtained from serum stored at refrigerator temperature.

Discussion

The above results offer evidence that *T. spiralis* larvae can be maintained at room temperature for a 1 week interval with infectivity varying from a moderate loss to no apparent loss.

This method of storage would have little to offer as a routine method of maintenance since infectivity disappeared entirely, or nearly so, by the 21st day. This method does have value when transportation of larvae to distant points is considered. It is difficult and sometimes impossible to adequately cool trichinae-containing tissues for shipment since room temperature contributes to tissue spoilage and prolonged freezing will destroy the trichinae. Shipping of living animals infected with trichinae involves extreme care.

In trial 1, the prolonged survival of trichina larvae in both immune and nonimmune refrigerated serum was highly promising as a method for the maintenance of larval stains in the laboratory for extended periods by use of a minimum number of laboratory animals. Viability was maintained in refrigerated immune sera for 126 days, with 37% viability through 56 days. Viable trichinae were maintained for 98 days in nonimmune sera, with 50% viable for 56 days. However, the complete loss of infectivity of trichinae maintained under refrigeration for 28 days in trial 2 decreases the applicability of this method. Additional studies will be necessary to evaluate this method further.

It is difficult to determine the cause of death for the larvae. Possible explanations may include exhaustion of available nutrients, accumulation of toxic metabolic wastes, or bacterial contamination. The latter was evidenced by prolific growth of bacteria on agar plates streaked with the serum from the serum-larvae combination tubes stored at room temperature in trial 2. However the failure to obtain bacterial growth from tubes stored at refrigerator temperature may dispel the importance of this factor. The procedures followed in trial 2 to eliminate bacterial contamination may indicate that the bacteria present proliferated from the intestinal flora of the trichinae when maintained at room temperature but did not multiply at refrigerator temperature to a level detectable by the culturing techniques utilized.

These studies have shown that microscopic examination alone is not an efficient tool in determining the infectivity of trichina larvae. This is of importance in the examination of pork products such as hams and sausage, which have been subjected to treatment to render trichinae noninfective. Trichinae from these foods may appear to be alive under microscopic examination, but actual transmission studies should be made to determine infectivity.

One aspect of this problem which was not investigated was that of survival selectivity. If any types of strains of *T. spirialis*

exist, certain of these might possibly be more resistant to inactivation than others.

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Trematodes of Passerine Birds from Chickasaw County, Iowa[1]

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Abstract. One-hundred and twenty-five passerine birds were collected in Chickasaw County, Iowa, October, 1959. through August, 1960. Thirteen species of birds (19%) were infected with 14 genera of trematodes. The hosts, helminths and infection sites are tabulated.

Little is known concerning the trematode fauna of Iowa birds belonging to the order Passeriformes. Ward (1901) examined 33 Iowa passerines and reported only one bird harboring a trematode infection.

The present study concerns the trematodes of passerine birds collected October, 1959, through August, 1960, from the Goodale Conservation Area in northwest Chickasaw County, Iowa. This area encompasses approximately 21 acres and contains six major types of habitats: river, marsh, pond, thicket, deciduous woods and meadow.

Previous studies on trematodes of passerine birds indicate varying degrees of infection. In Czechoslovakia Ryšavy (1955a) studied 168 passerines (32 species) and found none of them infected with trematodes. Rankin (1946) as part of a larger helminth survey of birds and mammals in western Massachusetts examined five passerines and found none infected with trematodes. Sulgostowska (1958) examined 33 passerines in Poland and found two (6%) infected with trematodes. According to unpublished data from Iowa Lakeside Laboratory at Lake Okoboji, 161 passerine birds (27 species) have been examined and 59 (37%) were infected with trematodes. In the current study approximately 19% of the birds examined harbored adult trematodes.

Several studies on helminths of a single species of passerines

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