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Citation	Japanese Journal of Veterinary Research, 32(1), 1-7
Issue Date	1984-01-31
DOI	https://doi.org/10.14943/jjvr.32.1.1
Doc URL	https://hdl.handle.net/2115/2304
Type	departmental bulletin paper
File Information	KJ00002374147.pdf

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Jpn. J. Vet. Res., 32, 1–7 (1984)

INNATE RESISTANCE OF CHICKENS TO *TRICHINELLA SPIRALIS* AT THE MUSCULAR PHASE OF THE PARASITE[1]

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(Received for publication September 17, 1983)

Experimental infection of *Trichinella spiralis* in 1-day-old chickens showed that most worms were rapidly expelled from the intestine but those that remained developed to maturity and produced newborn larvae. These newborn larvae were found in the chicken muscles between days 9–21 postinfection (PI) but they showed little or no development. No larvae were detected in the muscles at day 25 PI and thereafter. Although the newborn larvae were able to penetrate the chicken muscle fibers, they were unable to grow normally, which resulted in their death and degeneration. The fact that the newborn larvae, which were shed *in vitro* by female worms retrieved from the chicken intestine, were able to develop into mature larvae when injected intravenously into mice indicated that the death of the newborn larvae in the chicken muscles was not due to any inherent defect of the larvae.

Key words: *Trichinella. spiralis*, chickens, muscle, resistance, newborn larvae.

INTRODUCTION

Although it is widely known that chickens are resistant to *Trichinella spiralis* infection, most of the work done were focussed mainly on the intestinal phase of the parasite. The reasons given for the resistance include the destruction of the adult worms by the mechanical grinding of the gizzard,[1)] the inability of the worms to penetrate the intestinal wall,[6)] the rapid rejection of the intestinal worms[8)] and recently, the sequential digestive effect of the stomach and intestinal secretions.[2)] CRAM (1959) noted that in the chicken, the newborn larvae died soon after they invaded the skeletal muscle but a detailed study of this phenomenon is needed. RITTERSON (1957) also noted that the innate resistance of the Chinese hamster to *T. spiralis* lies in the muscular phase of the parasite. The present study is an attempt to elucidate the innate resistance of the chicken to *T. spiralis* at its muscular phase.

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

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MATERIALS AND METHODS

Parasite

The *T. spiralis* strain used was isolated in 1968 from a polar bear, *Thalarctos maritimus*, at Maruyama zoo, Sapporo, and since then has been maintained as stock infection in mice in our laboratory. Infective stage 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 at a concentration of about 20 ml of the gastric juice to 1 g of the carcass.

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

1-day-old male White Leghorn chickens, commercially known as "Nick Chick", were used throughout the experiments. The chickens were obtained from Hokkaido Chuo Shyukeijo, Japan.

The strain of mice used was 8-week-old male ddY mice which were obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Japan.

The animals were given commercial feed and water *ad lib.*

Experiments

The experimental protocol was divided into three parts: the first, to confirm the intestinal infection of *T. spiralis* in the intestine of the chicken; the second, to study the muscular phase of the parasite in the muscles of the chicken; and the third, to see whether the newborn larvae produced by the female worms in the intestine of the chicken were viable or not in mice.

Experiment 1

After being orally infected with 1,900 infective larvae, the chickens were killed at various intervals and their digestive tracts examined for worms under the dissection microscope.

Experiment 2

Chickens, each orally infected with about 6,000 infective larvae were killed at days 5,7,9,13,17,21,25,27 and 42 postinfection (PI). 0.05–0.15 g of the various muscles were macerated and followed by microscopic examination for the presence of larvae. The muscles examined were the tongue, masseter, pectoral, abdominal, cervical, gastrocnemius, sarcospinal, triceps and intercostal muscles. The length of the larvae was determined by photographing them and measuring their image projected by the negative film. The carcasses of the day 42 PI chickens were digested in artificial gastric juice and examined for the presence of muscle larvae.

A portion of the muscles was fixed in Bouin's fixative, dehydrated in an alcohol series, cleared in xylene and embedded in paraffin wax. 4 μ thick section were made and stained with haematoxylin-eosin for histological examination.

Experiment 3

Mature female worms were recovered from the intestine of chickens at day 7 PI using a modified Bearmann apparatus with physiological saline. They were then incubated in Earle's balanced salt solution (BSS), pH 7.4, which contained 15% bovine calf serum. For each ml of Earle's BSS, 1,000 i.u. of penicillin G were added. Incubation was carried out for 24 hours at 37°C in a Forma Scientific water-jacketed carbon dioxide incubator supplied with 5% CO_2.

The newborn larvae, which were shed during the incubation, were collected by filtering the medium through 2 layers of 63 μ mesh and centrifuging the filtrate at 1,500 rpm. After several washings in warm Earle's BSS, the newborn larvae were injected intravenously into the tail veins of two mice. The mice were killed at day 18 postinjection, and the muscles were digested in artificial gastric juice and then examined for the presence of larvae.

Results

The recovery rate of the *T. spiralis* adults is shown in figure 1. At day 5 PI, the average recovery rate was 8.6% and the females possessed embryos in their uterus. This confirmed the postulation that most of the worms were expelled from the intestine of the chickens but those that remained developed to maturity.

Larvae were recovered between days 9–21 PI from all the muscles of the chickens. The average length of the larvae was 0.10 mm at day 9 PI and 0.16 mm at day 21 PI. Thus the larvae recovered from the muscles of the chickens showed little or no development. No larvae were detected in the muscles of the chickens at day 25 PI and thereafter. Digestion of the chicken carcasses on day 42 PI also produced no larvae. Histological examination of the chicken muscles revealed larvae within the muscle fibers at day 9 PI (fig. 2). Enlargement of the nuclei, basophilic degeneration and disappearance of cross-striation was noted in the parasitized muscle fibers between days 13 to 21 PI (fig. 3). Only slight swelling of the parasitized muscle was observed. From day 13 PI onward, cell accumulation, which consisted mainly of lymphocytes, was seen scattered in the sections (fig. 4). The leucocytic infiltration was seen much more frequently in the muscle sections of day 25 PI and thereafter.

Mature larvae were recovered from the mice which had been injected intravenously with newborn larvae shed *in vitro* by female worms retrieved from the chicken intestine. These mature larvae were morphologically normal and their viability was proved by feeding them to a mouse. A month later, larvae were recovered from the muscle of that mouse.

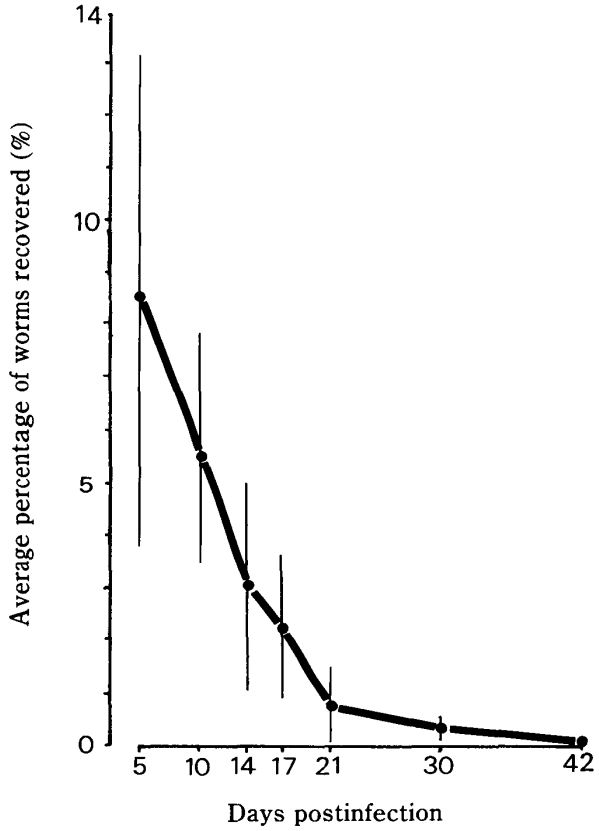

FIGURE 1 *Recovery rate of T. spiralis adults from the digestive tract of chickens infected with 1,900 infective larvae*

7 chickens were examined each time but on day 42 PI, 5 were examined.
Bars indicate standard deviation.

DISCUSSION

Controversial results regarding the susceptibility of chickens to *T. spiralis* infection have been produced by various researchers, with reports ranging from the presence of mature larvae in the muscle of chicken to the presence or absence of adult worms in the intestine.[1,2,6,8,10,17] The strain or species of the parasite used in their experiments may be critical in the establishment of these results. Strains of *T. spiralis* have diverse infectivity for different species of hosts and an uncapsulated parasite, identified as *T. pseudospiralis*, GARKAVI, 1972, which was isolated from a raccoon (*Procyon lotor*) in Northern Caucasus, has been found to be infective for a variety of birds, including chickens.[9,15,16]

One of the criteria for distinguishing *T. pseudospiralis* from other *T. spiralis* strains

is its development into mature larvae in the muscle of an avian host.[3] *T. pseudospiralis* can complete its life cycle in an avian host while *T. spiralis* cannot. Our results confirmed that *T. spiralis* can parasitize in the gut of chickens although we cannot rule out the possibility that rejection due to immunological response may not have occurred because of the use of 1-day-old chickens which may be immunologically immature. The female worms that remained in the intestine were able to produce newborn larvae. These newborn larvae were not congenitally abnormal and were able to develop to maturity in a mammalian host, as proved in experiment 3. Although these newborn larvae were able to penetrate the muscle fibers of the chickens, they were unable to grow normally in that environment, which led to their death and degeneration.

The skeletal muscle of chickens and mammals is reported to be almost morphologically identical[7] but we suggest that sufficient physiological and / or biochemical differences exist to hinder the growth and development of the *T. spiralis* larvae. The death of the newborn larvae of *T. spiralis* in the chicken muscle fibers may also be due to the inability of the larvae to transform the infected muscle cells into the so-called "nurse cell", which is believed to support the growth and development of the larvae.[5,11]

Innate resistance of the Chinese hamster to *T. spiralis* infection can be reversed by cortisone[13] and it is also age dependent.[14] Whether or not the innate resistance of chickens to *T. spiralis* infection at the muscular phase of the parasite is the same as that of the Chinese hamster remains to be answered.

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EXPLANATION OF PLATE

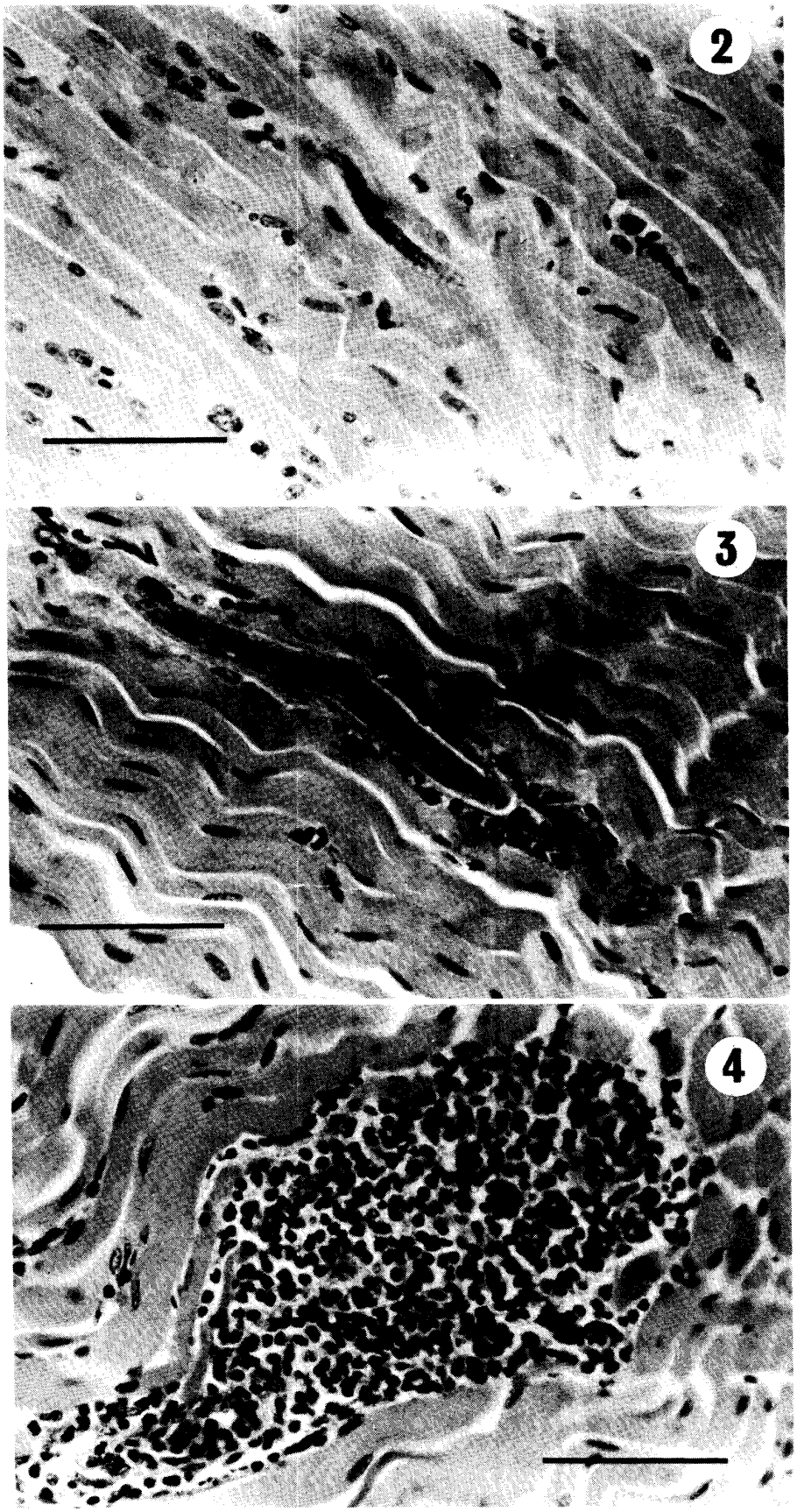
PLATE 1

Bar = 0.05 mm

Fig. 2 Larva in the abdominal muscle of a chicken at day 9 PI

Fig. 3 Larva in the pectoral muscle of a chicken at day 21 PI

Fig. 4 Cell accumulation in the abdominal muscle of a chicken at day 21 PI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