

## The First Host Record of Trichinosis in a Red Fox, *Vulpes vulpes japonica*, from Aomori Prefecture, Northern Honshu, Japan

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**ABSTRACT.** A case of wildlife trichinosis was found in a red fox (*Vulpes vulpes japonica*) captured at Rokkasho, Aomori Prefecture on November 27, 1998. *Trichinella* larvae were obtained from almost all of the muscle tissues except for the masseter. The highest number of larvae per gram of tissue was found in the muscles of the gluteal region and throat. The lowest number was found in the diaphragm and tongue. *Trichina* cysts within the muscle fibers had groups of fatty cells at the poles, and minimal tissue reaction was observed around the cyst. No calcification was found in the cyst. These morphological findings suggested that the considerable time had elapsed since the invasion. This is the first case of trichinosis in a red fox in Japan.

**KEY WORDS:** Aomori Prefecture, trichinosis, *Vulpes vulpes japonica*.

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Trichinosis is one of the most important parasitic diseases in humans and animals. It is caused by infection of the nematodes belonging to genus *Trichinella* which are distributed worldwide. In Japan, the first record of trichinosis was made by Satoh *et al.* [16] and Ohbayashi and Satoh [6] from a dog in Hokkaido. Thereafter, *Trichinella* infection has been reported in a mink (*Mustela vison*) imported from the United States [3], polar bears (*Thalarctos maritimus*), sables (*Martes zibellina*), a tiger (*Panthera tigris*) and a leopard (*P. pardus*) in the Maruyama Zoo of Sapporo [4, 13–15], a black bear (*Ursus thibetanus*) in Aomori [18], a brown bear (*U. arctos*) in Sapporo [7] and a raccoon dog (*Nyctereutes procyonoides*) in Yamagata [12]. Of these animals, the sables, black bear, brown bear and raccoon dog were considered naturally infected indigenous wildlife animals. In addition, the infections in the black bear and brown bear were implicated in the outbreak of human trichinosis in Japan. Human infections associated with the consumption of raw meat of black and brown bears were reported in Aomori and Sapporo [17, 18].

The occurrence of trichinosis in wildlife is important in that wildlife serves as a reservoir for transmission of the parasite to domestic animals and to humans. Here we describe the first record of natural infection of trichinosis in a red fox in Japan.

The male fox, 104 cm long and weighing 6.0 kg, was killed by a hunter at Rokkasho, Aomori Prefecture on November 27, 1998. After the animal was skinned, small samples of muscle tissues collected from the tongue, masseter and diaphragm were routinely examined by artificial digestion and a few *Trichinella* larvae were recovered from the tongue. Therefore, we performed the following examination to determine the more detailed distribution of *Trichinella* larvae in the muscles of the infected fox. Almost all of the muscle tissues (total weight 2,422.3 g) were collected from each part of the animal: head, tongue, throat, neck, tho-

rax, abdomen, back-lumbar region, gluteal region, front and hind legs, diaphragm, and esophagus (*Musculus esophageus*). The muscle samples were minced with scissors and digested individually with artificial gastric juice (pepsin, 1:10,000, 20 g; HCl, 20 ml in 1,000 ml water) at 37°C for 2 hr. Some of the samples were examined after pressing between two glass plates before artificial digestion, and encysted larvae were removed. The digested materials were examined for larvae under a dissecting microscope. In addition, infected muscle samples from the hind legs and esophagus were fixed in 20% buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE).

In total, 6,350 *Trichinella* larvae were obtained from the muscle of the fox. The larvae recovered by artificial digestion were spirally coiled (Fig. 1). Some of them were killed by heating, fixed in 10% formalin and used for morphological observation. The measurements of the excysted larvae are shown in Table 1. The total length was 1.045–1.214 mm in males and 1.118–1.268 mm in females. The esophagus was composed of a slender anterior portion and a posterior stichosome portion. The stichosome portion was surrounded by a single row of stichocytes. The esophagus was followed by the midgut, which led to the hindgut or rectum. The length of rectum was longer in males than in females, measuring 0.052–0.067 mm and 0.025–0.033 mm respectively. In addition, the encysted larvae isolated from the muscles with the aid of needles were ellipsoidal or spindle-shaped (Fig. 2). The size of 30 cysts was 0.356–0.464 (av. 0.401) mm by 0.148–0.220 (av. 0.183) mm. The cyst wall was 0.020–0.046 (av. 0.029) mm in thickness and fatty cells accumulated at the poles of the cysts. Usually, each cyst contained a single, coiled larva. The morphological features as described above closely resembled those of the encapsulated species of the genus *Trichinella*.

Histologically, trichina cysts were found within the mus-

Table 1. Measurements of *Trichinella* larvae recovered from a red fox obtained at Rokkasho, Aomori Prefecture (in mm)\*

	Male	Female
Body length	1.045–1.214 (1.114)	1.118–1.268 (1.176)
Body width	0.034–0.045 (0.038)	0.034–0.043 (0.037)
Length of esophagus	0.709–0.818 (0.758)	0.736–0.891 (0.788)
Length of stichosome	0.532–0.614 (0.561)	0.541–0.682 (0.590)
Length of rectum	0.052–0.067 (0.060)	0.025–0.033 (0.030)

\* All measurements are based on 20 specimens, and the means are given in parentheses.

cle fibers (Figs. 3 & 4). The cyst was situated with its long axis parallel to the muscle fiber, and minimal tissue reaction was observed around the cyst. The homogenous capsule forming the cyst was brightly eosinophilic, and measured 0.012–0.027 (av. 0.019) mm in thickness. Within the capsule, the coiled larva was embedded in a clearly defined basophilic matrix that contained large nuclei. No calcification was found in the cyst. These findings suggested that considerable time (more than 2–3 months) had elapsed since the invasion [2].

The distribution of *Trichinella* larvae in the muscles of the fox is shown in Table 2. The larvae were recovered from almost all of the examined muscles except for the masseter.

The number of larvae per gram of muscle ranged from 8.3 to 0.15, with an overall mean of 2.62. The highest density of larvae was found in the muscle of the gluteal region, and followed by the throat muscle. The diaphragm and tongue, which are the most routinely used site for detecting *Trichinella* larvae, showed the lowest density. In a study of wild foxes (*V. vulpes*) in Scandinavia, the muscles of the legs were heavily infected, while the diaphragm was only moderately infected and no larvae were observed in the muscles of the jaw [1].

In an additional effort to find other infected animals, 5 red foxes, 188 voles (139 *Microtus montebelli*, 42 *Apodemus speciosus* and 7 *A. argenteus*) and 22 shrew-moles (*Urotrichus talpoides*) from Rokkasho were examined by digestion, but all were negative.

The genus *Trichinella* was thought to be monospecific for many years. Recently, however, 5 species (*Trichinella spiralis*, *T. britovi*, *T. nativa*, *T. nelsoni* and *T. pseudospiralis*) and 3 phenotypes of uncertain taxonomic level (*Trichinella* T5, T6 and T8) have been described by Pozio *et al.* [9]. Thereafter a new non-encapsulated species, *T. papuae*, and an encapsulated species, *T. murrelli* (previously identified with the code T5) were reported [8, 11]. In Japan, trichina worms isolated from a black bear and a raccoon dog in northern Honshu have been identified as *T. britovi* by random amplified polymorphic DNA (RAPD) analysis by Pozio *et al.* [10], whereas Nagano *et al.* [5] reported that the two isolates from northern Honshu were considered to be a new genotype (*Trichinella* T9), using polymerase chain reaction based restriction fragment length polymorphism (PCR-RFLP). In addition, it has been suggested that the

Table 2. Distribution of *Trichinella* larvae in the muscles of a red fox obtained at Rokkasho, Aomori Prefecture

Muscles	No. of larvae found	Weight (g) of muscle examined	No. of larvae /g of muscle
Head			
Temporal	45	25.0	1.80
Masseter	0	19.6	—
Others	224	116.6	1.92
Tongue	4	24.4	0.16
Throat	62	8.0	7.75
Neck	51	199.0	0.26
Thorax			
Intercostal	298	153.4	1.94
Others	1,118	248.1	4.51
Abdominal	603	240.8	2.50
Sarcospinal			
thoracic part	227	113.7	2.00
lumbar part	191	98.0	1.95
Gluteal region	322	38.8	8.30
Front legs	1,911	546.7	3.50
Hind legs	1,263	556.8	2.27
Diaphragm	4	27.0	0.15
Esophagus*	27	6.4	4.22
Total	6,350	2422.3	2.62

\* *Musculus esophageus*.

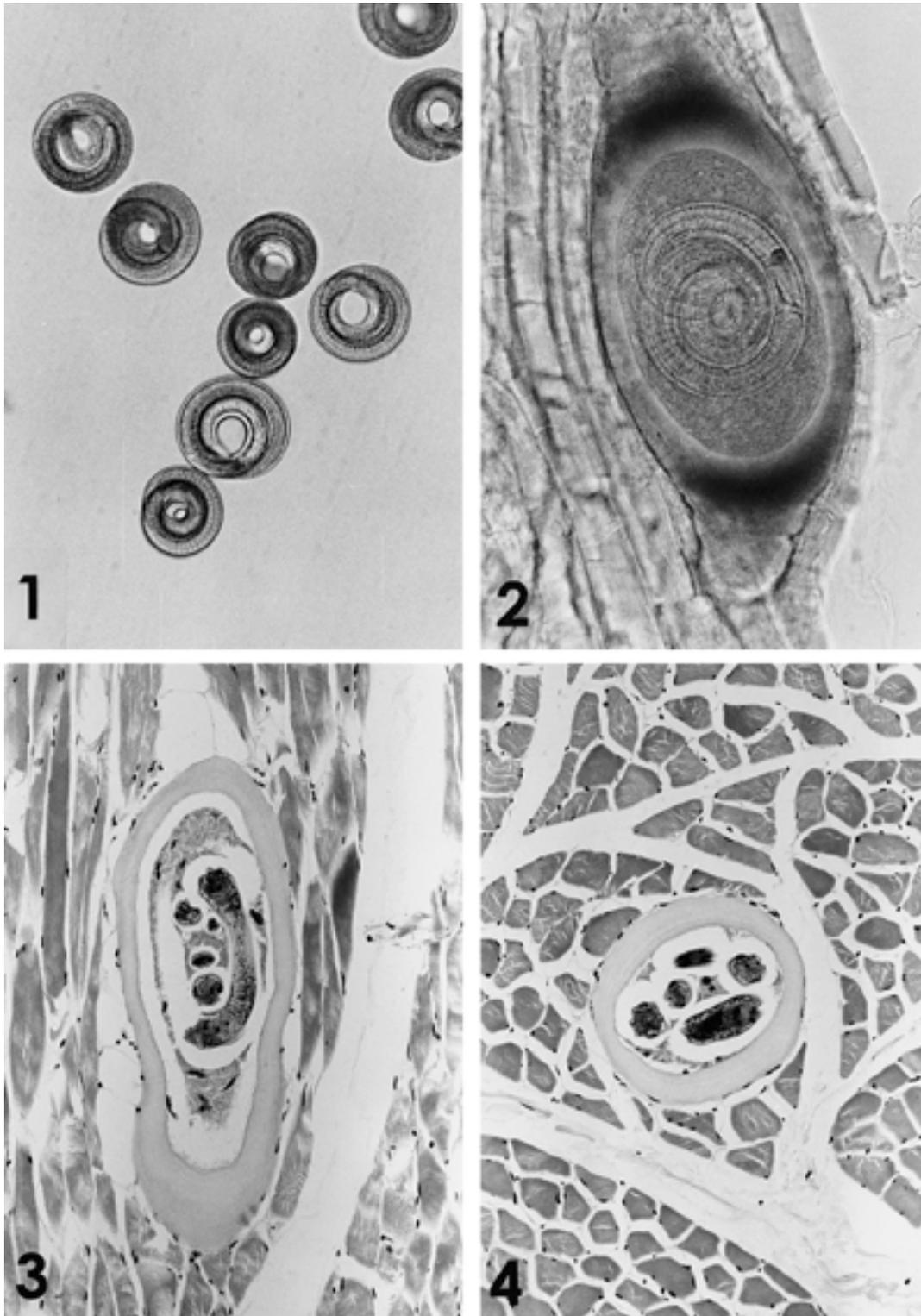


Fig. 1. Excysted *Trichinella* larvae isolated by artificial digestion from the muscle of a red fox.

Fig. 2. A *Trichinella* larva encysted in the muscle fibers of a red fox.

Figs. 3 & 4. *Trichina* cysts in the muscles of esophagus (Fig.3) and a hind leg (Fig. 4). Minimal tissue reaction was observed around the cysts. HE.  $\times 185$ .

parasite found in a brown bear in Hokkaido was thought to be *T. nativa* by the severity of clinical signs in human infection and the long survival of muscle larvae at low temperature [10]. Recently Yimam *et al.* detected *T. nativa* in red foxes (*V. vulpes schrenki*) in Hokkaido using RAPD analysis (personal communication). In the present case, Trichina worms obtained from the red fox in Aomori Prefecture seem to be *T. britovi* or *Trichinella* T9 because of its geographical distribution, although we did not carry out RAPD and/or PCR-RFLP analysis. Further studies are necessary to identify the species of *Trichinella* isolated from this animal.

In Aomori Prefecture, wildlife trichinosis has been recorded from a black bear, which was caught at Iwasaki in the west coast facing the Japan Sea. In our report, *Trichinella* infection was found in a red fox captured at Rokkasho, eastern part of Aomori Prefecture. It is suggested that the trichina worm may be more widely distributed than currently believed among wild animals in Aomori Prefecture.

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