Detection of Muscle Larvae of *Trichinella spiralis* by Enzyme-Linked Immunosorbent Assay

Taisuke KITAMURA* and Toshio ISHII
Department of Parasitology, Nippon Veterinary and Animal Science University, 1-7-1 Kyonan-cho, Musashino, Tokyo 180, Japan
(Received 8 May 1992/Accepted 19 September 1992)

ABSTRACT. ELISA was applied to the detection of *Trichinella spiralis* larvae in infected rat muscles. Anti-muscle larva serum was prepared by inoculating crude extract of muscle larvae to rabbits. Limb muscles of infected rats were emulsified with carbonate buffer and the supernatant was used for ELISA. ELISA values of positive muscular samples were clearly higher than those of negative ones to prove that ELISA can be applied to the detection of muscle larvae. The present method showed positive values to muscles infected with 10 or more larvae per gram of muscle (LPGM)—KEY WORDS: ELISA, muscle larvae, *Trichinella spiralis*.
J. Vet. Med. Sci. 55(1): 123–124, 1993

At present, *Trichinella spiralis* must not be neglected in the field of meat hygiene in Japan, though its infection is rare. Pork imported from other countries is increasing in amounts, where trichinous infections have been prevalent since old days [1], while in Japan, the consumption of raw pork ham tends to increase.

The traditional detection methods of muscle larvae, such as trichinoscopy and the digestion method may miss larvae, especially in inspection of a large quantity of meat [8]. On the other hand, serodiagnosis is not so reliable in the field of meat hygiene because of false negative reactions [4, 6], so it is desirable to develop a new efficient method.

In the present experiments, the antigen, some larval components, was detected by ELISA from infected rat muscles and its applicability to meat inspection was investigated.

Iwasaki strain of *T. spiralis*, supplied by the courtesy of Dr. Tomio Yamaguchi, from the Department of Parasitology, Hirosaki University, has been maintained in the laboratory by serial passage through mice. Larvae were isolated by digestion of the minced carcasses of infected mice in 1% pepsin-1% HCl for 18 hr at 25°C under constant agitation, and the isolated larvae were washed three times with saline.

Crude extract of muscle larvae was prepared as follows; larvae isolated from infected mice (BALB/c, male) were lyophilized, suspended in 0.05M carbonate buffer (pH 9.6) and ground in a teflon homogenizer on ice. The homogenate was repeatedly frozen at −70°C and thawed at 37°C ten times, stirred at 4°C for 2 days and centrifuged at 10,000 × g for 60 min. The supernatant was used as crude extract for obtaining anti-muscle larva serum. The concentrated crude extract (CCE: 3.2 mg/m*l* protein concentration) was inoculated to two rabbits three times each. First, 1 m*l* of CCE was injected into the hind limb pad together with 1 m*l* of Freund's complete adjuvant. Two weeks later, 1 m*l* of CCE was subcutaneously injected into the neck-back. Three weeks after the second inoculation, 1 m*l* of CCE was injected into the ear vein. Seven days later, serum was obtained.

Three male rats, Wistar, were orally infected with 500 larvae per head. Four weeks after infection, larvae were collected from fore and hind limb muscle and the number of larvae per gram of muscle (LPGM) were counted under a microscope to get mean LPGMs of 465 and 574 from 6 fore and 6 hind limbs respectively. These muscle samples were also used to investigate the antigen-detection ability of ELISA from infected muscles (Experiment 1). Subsequently, the sensitivity of this method was investigated. Hind limb muscles were collected 4 and 24 wk postinfection (PI), and LPGM of these muscles was adjusted to 1 to 500 by adding infected or non-infected muscles (Exp. 2).

The ELISA procedure for the detection of antigen (larval components) was as follows; the above muscle samples were well minced and emulsified by adding 10 times volume of carbonate buffer, and the resultant emulsion was centrifuged at 1,500 × g for 10 min. With 100 μl of the supernatant, each well of microtiter plates (flat-bottom, polystyrene, Corning Co.) was coated at 37°C for 2 hr and then maintained at 4°C overnight. Wells were then washed three times with phosphate-buffered saline containing 0.05% Tween 20 and 0.1% bovine serum albumin (PBS-TB), and 100 μl of anti-larva serum diluted to 1:100 with PBS-TB was added to each well. After incubated 1 hr at 37°C, wells were washed three times with PBS-TB and 100 μl of horse radish peroxidase conjugated protein A (Cappel Co.) diluted to 1:100 with PBS-TB was added to each well. Wells were incubated 1 hr at 37°C, washed three times with PBS-TB and developed with 100 μl of substrate containing 0.003% hydrogen peroxide and 0.3 mg 2, 2'-azino-di-(3-ethylbenzthiazoline-6-sulfonate) /m*l* for 1 hr at room temperature. Absorbances were measured by an ELISA reader (MPR 4, TOHSOH). The positive/negative cutoff value was determined by adding three standard deviation to the mean ELISA value (optical density; O.D.) of negative reference muscle samples (hind limb muscles of 10 non-infected rats) [3].

First, the detection ability of ELISA for the larval components in infected muscles was investigated (Table 1). Muscle samples of fore and hind limbs were clearly positive for ELISA values to prove that larval components could be detected from muscles by ELISA. But, in the case of the fore and hind limb muscles used in Exp. 1 which had 465 and 574 LPGM respectively, larvae were easily detected by trichinocopy, so the efficiency of the present method was not definitively concluded. So, the relationship between LPGM and ELISA values was further investigated in Exp. 2.

* CORRESPONDENCE TO: KITAMURA, T., 6-2-3 A-103 Ohizumi-Gakuen-cho, Nerima-ku, Tokyo 178, Japan.

Table 1. Detection of muscle larvae by ELISA from fore and hind limb muscles of infected rats

Infected muscles	LPGM (mean)	ELISA value (O.D.)
Fore limbs	465	1.509
Hind limbs	574	1.680
Cutoff value		0.417

Table 2. The relationship between LPGM and ELISA values of trichinous muscles 4 and 24 weeks postinfection

	ELISA values (O.D.)	
LPGM	4 weeks PI	24 weeks PI
500	1.652	1.563
200	1.422	1.420
100	1.381	1.335
80	1.350	1.308
60	1.192	1.244
40	1.184	1.103
20	0.855	0.855
10	0.815	0.764
9	0.547	0.502
8	0.516	0.431
7	0.433	0.383
6	0.366	0.308
5	0.332	0.314
4	0.396	0.301
3	0.334	0.277
2	0.359	0.288
1	0.353	0.295
Cutoff value	0.417	

Table 2 shows the relationship between LPGM and ELISA values. With the decrease in LPGM, ELISA values showed a tendency to decrease. When muscle samples obtained 4 wk PI were used, ELISA showed positive values of 0.433 or more at 7 or more LPGM, but sufficient positive values of 0.815 or more were shown at 10 or more LPGM. Muscle samples obtained 24 wk PI also showed clearly positive values of 0.764 or more at 10 or more LPGM, although positive values of 0.431 (or more) were shown at 8 (or more) LPGM. In both cases, it was difficult to pass final judgment on the ELISA values at 7 to 9 LPGM. At 10 or more LPGM, positive ELISA values were evidently recognized.

To investigate the influence of calcification on the detection of larvae, muscle samples obtained 4 wk and 24 wk PI were used. The encapsulation of larvae completes 4–5 wk PI, and in the case of rat, the calcification begins 2 months PI [5]. As shown in Table 2, there was little differences between the values of both samples.

Consequently, accurate detection of muscle larvae was possible by the present method, at least 10 or more larvae are present in 1 g of muscle samples.

Trichinoscopy has the missing risk of muscle larvae, because the amount of meat available to this method is too small [2]. The digestion method has a merit in that it can treat much more volume of meat up to 100 g, but, it has also a demerit in that a few infections will be missed in large volume of samples and that dead larvae and calcified cysts may be destroyed by digestion [8]. In order to treat many samples efficiently, the pooled digestion method was devised [7]. In this method, many samples are pooled into some lots, which were digested each, and then all the samples belonging to positive lots are fully examined. The efficiency of the pooled digestion method seems to be influenced by both the infection rate of lots and LPGM of individual samples. The present method can treat many samples simultaneously and individually, and it seems not to be influenced by the calcification of muscle larvae.

The present method was considered to be more efficient and applicable for meat inspection than traditional methods, but the sensitivity must be more enhanced. For this purpose, the anti-muscle larva serum with sufficiently high antibody titer against highly specific antigen should be prepared. The present study made a forward step of a new inspection method of trichinous meat.

REFERENCES

1. Kagei, N. 1987. *Jpn. J. Med.* 97: 1952–1954 (in Japanese).
2. Lehmensick, R. 1970. pp. 437–448. *In*: Trichinosis in Man and Animals (Gould, S. E. ed.), Charles C. Thomas, Springfield, Illinois.
3. Onaga, H., Saeki, H., Hoshi, S., and Ueda, S. 1985. *Avian Dis.* 30: 658–661.
4. Ruitenberg, E. J., Van Knapen, F., and Elgersma, A. 1983. pp. 529–550. *In*: *Trichinella* and Trichinosis (Campbell, W. C. ed.), Plenum Press, New York.
5. Weatherly, N. F. 1983. pp. 173–208. *In*: Trichinella and Trichinosis (Campbell, W. C. ed.), Plenum Press, New York.
6. Yamaguchi, T. 1989. *Trichinella* and Trichinellosis in Japan, Nankodo, Tokyo (in Japanese).
7. Zimmermann, W. J. 1974. pp. 539–548. *In*: Trichinosis (Kim, C. W. ed.), Intext, New York.
8. Zimmermann, W. J. 1983. pp. 515–528. *In*: *Trichinella* and Trichinosis (Campbell, W. C. ed.), Plenum Press, New York.