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Intradermal Test for Field Survey of White Spots in the Liver of Pigs Infected with *Ascaris lumbricoides suum*

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Abstract

The usefulness of intradermal test of immediate type was investigated for diagnosing white spots in the liver of pigs infected with *Ascaris lumbricoides suum*. Protein antigen was accompanied by salt precipitation and polysaccharide by Campbell's method from adult worms of the nematode. These antigens were injected intradermally into the infected animals. The intradermal reaction should be judged as positive when a wheal of over 9 mm in diameter with hyperemia or hemorrhage appears on the skin by injection with 0.1 ml of antigen containing 10 μg of protein at 15 min later. According to this criterion, positive reaction was found in thirteen or 77% of 17 fattened pigs with white spots in the liver.

Key words: intradermal test, *Ascaris lumbricoides suum*, white spots, visceral larval migrans

Introduction

Multiple white spots produced in the liver of fattened pigs have been observed in meat inspection at slaughterhouses in Japan. They are a main cause for liver condemnation. The lesions are caused by migration of *Ascaris* larvae (Jones and Hunt, 1983).

Serologcal tests are useful for diagnosing the visceral larval migrans (Jones and Hunt, 1983). In the previous study (Yoshihara *et al.*, 1987), the authors also examined usefulness of modified direct complement fixation test (MDCFT) for diagnosing white spots in the liver of pigs and reported that an antibody to *Ascaris lumbricoides suum* was found in about 50% of pigs with the liver lesions. However, the test is often inadequate or impracticable for field survey of helminthic disease in domestic animals, when the method requires laborious techniques and efficient facilities.

On the other hand, it is clear that antibodies involved in intradermal reaction of immediate type have been demonstrated in pigs infected slightly with *A. lumbricoides suum* (Yoshihara *et al.*, 1983). Incidentally, these animals were negative in MDCFT.

Therefore, an attempt was made to survey the white spots in the liver of pigs in field by using intradermal test with antigens from adult worms of *A. lumbricoides suum*.

Materials and Methods

Embryonated *A. lumbricoides suum* eggs: The eggs were prepared by the procedure described previously (Yoshihara *et al.*, 1983).

Pigs: Thirty-three pigs (males and females) about 15 weeks of age were used. Six of which were specific pathogen free (SPF) animals. They were produced at the National Institute of Animal Health. The other twenty-seven cross-bred (Hampshire X Duroc) pigs were produced and maintained under sanitary condition at the

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Niigata Livestock Industry Experiment Station and negative for fecal examination. Thirty-one fattened pigs weighing approximately 110 kg from a farm were selected and used for intradermal test at the slaughterhouse.

Inoculation examination: An experiment was carried out by using eight pigs, two of which served as uninfected controls. Pigs used in the other experiment were twenty-five. Fifteen pigs were inoculated with the *Ascaris* eggs. Remaining ten animals were uninfected controls. The number of the eggs and the time intervals between inoculations in these experiments were listed in Table 1 and 2.

Pathology: At autopsy, the liver and intestine from all the pigs used were examined macroscopically in detail. In accordance with Nakagawa *et al.* (1983), white spots in the liver were classified into three scores; mild (I), moderate (II) and severe (III).

Preparation of antigens: Adult worms of *A. lumbricoides suum* were collected from the small intestine of naturally infected pigs at slaughterhouse. Crude extracts were prepared from worms by a modification of the technique described previously (Yoshihara *et al.*, 1983). The concentration of protein antigen (Pt) in the extracts was accomplished by salting out precipitation with specify density of ammonium sulfate. The resulting precipitate was dissolved in physiological saline solution, dialysed and adjusted to various concentrations ranging from 5 μg to 40 μg per 0.1 ml by the Cu-Folin method (Lowry *et al.*, 1951). Polysaccharide antigen (Ps) from adult worms of the nematode was produced by the technique described by Campbell (1936). After the quantitative test by Anthron method (Scott and Melvin, 1953), the concentration was adjusted similarly to that of Pt.

Intradermal test: Before autopsy, the flank of four infected SPF pigs and two crossbred pigs as uninfected control animals was clipped free of hair. Pt and Ps of 0.1 ml at various concentrations were injected intradermally into the skin of six pigs. The same volume of physiological saline solution (Ss) which had been used for the dilution of both antigens was employed throughout this examination as control. In case of remaining two infected SPF pigs, Pt and Ps of 10 μg in 0.1 ml were injected into the skin at the base of the ear. Injections with Pt of 10 μg and Ss were made into the base of the ear of twenty-five pigs and thirty-one fattened pigs.

Judgment of reaction: In the present study, reaction was judged positive when a wheal of over 9 mm in diameter with hyperemia or hemorrhage appeared on the skin by injection with 10 μg in 0.1 ml of Pt at 15 min later.

Results

Reaction in SPF pigs infected with *A. lumbricoides suum*: Fig. 1 shows reaction on the skin of a SPF pig at 15 min after injection. A wheal of over 9 mm in diameter with hyperemia was found distinctly on each injected site except Ss. The injected site with Ss maintained only slight wheal of about 6 mm in diameter at 15 min later. The maximal size of wheals in diameter on the skin of four SPF pigs and two control pigs are summarized in Fig. 2. The reaction to Pt was severer than that of Ps. Stasistically, three concentrations of Pt except 5 μg were suitable for antigen. In case of

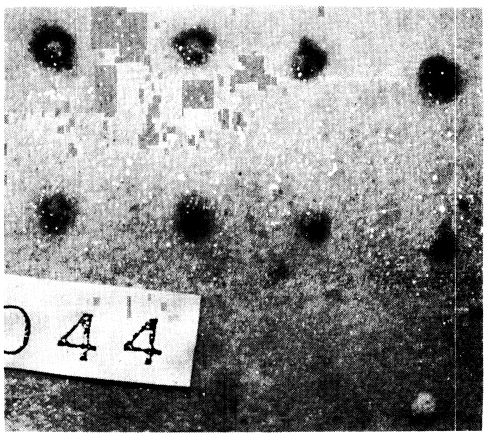


Fig. 1 Intradermal test with Pt (upper) and Ps (middle) of *Ascaris lumbricoides suum* on the skin of infected SPF pig. Lower was control. From right, injected volume was 5 μg, 10 μg, 20 μg and 40 μg.

control pigs, wheals of about 5 mm in diameter were observed on all the injected sites at 15 min later. However, the wheals formed by injection with 20 μg and 40 μg of both antigens were accompanied with slight hyperemia. From these result, it can be concluded that Pt of 10 μg was suitable for antigen of intradermal test.

Fig. 3 illustrates the reaction with Pt and Ps of 10 μg on the skin at the base of the ear of infected SPF pig. Injected site with Pt shows a clear wheal of 11 mm in diameter with hyperemia at 15 min later. Reaction with Ps was weaker than that of Pt. Only a small red point was found on the skin of injected site with Ss. As shown in Table 1, white spot lesions were seen in the liver of all the infected SPF pigs. The lesions were severe (Fig. 4). Adult

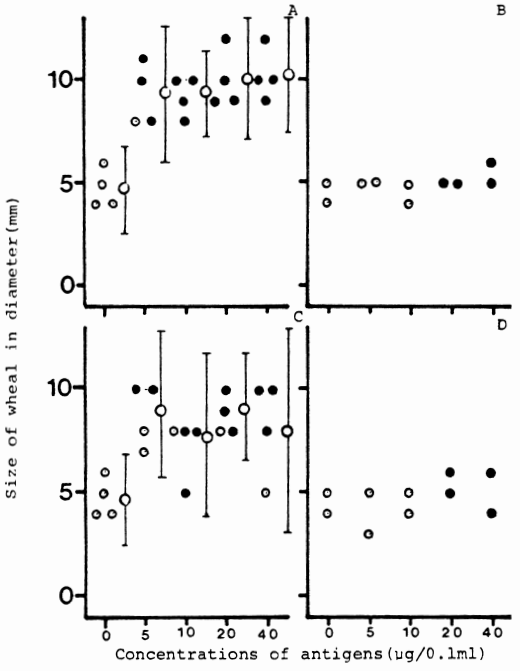


Fig. 2 Result of intradermal test on the skin of the flank of *Ascaris lumbricoides suum* infected pigs. Wheals on the skin of SPF (A) and control pigs (B) formed by injections with Pt and of SPF (C) and control pigs (D) with Ps; ○: Wheals; ●: Wheals with hyperemia; ⊖: Mean and 95% confidence limit.

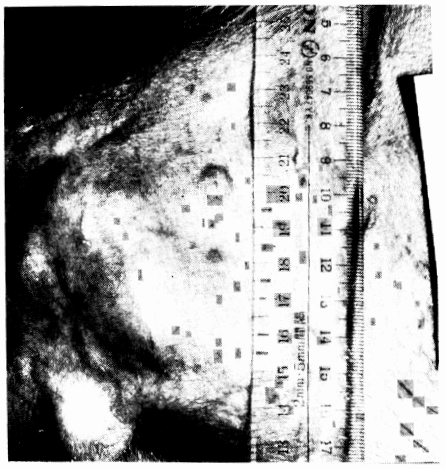
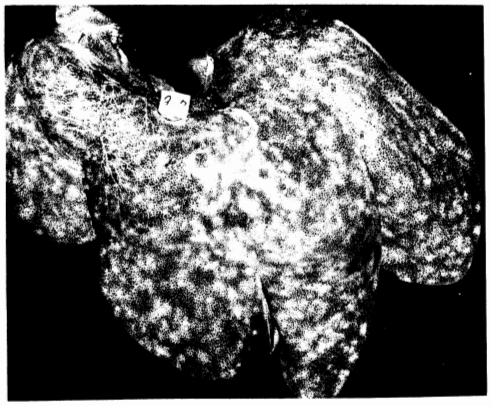


Fig. 3 Intradermal test with 10 μg of Pt (middle) and Ps (lower) on the base of the ear of infected SPF pig. Upper was control.

Table 1 White spots in the liver of pigs experimentally infected with *Ascaris lumbricoides suum*

Pig number	Dose of infective eggs and times	Time intervals between the inoculations (weeks)	Autopsy after the last inoculation (days)	White* spots	Number of adult recovered from the intestine
1	50,000×3	5	2	III	44
2	50,000×3	5	2	III	0
3 (SPF)	50,000×3	5	4	III	1
4	50,000×3	5	4	III	2
5	50,000×3	5	7	III	0
6	50,000×3	5	7	III	0
7				n	0
8				n	0

* Lesion score: III, Liver with severe lesion; n, Normal liver.



worms of *A. lumbricoides suum* were recovered from the small intestine of three infected SPF pigs.

Reaction in crossbred pigs infected with *A. lumbricoides suum*: Pt of 10 μg in 0.1 ml was injected into the base of the ear of fifteen infected pigs and ten uninfected pigs. The results obtained at 15 min later are shown in Table 2 and Fig. 5. Ten of infected animals indicated distinctly wheal with hyperemia.

Fig. 4 Severe white spot lesions on the surface of the liver from injected SPF pig.

Table 2 Result of intradermal test and white spots in the liver of pigs experimentally infected with *Ascaris lumbercoides suum* and uninfected pigs

Pig number	Dose of infective eggs and times	Time intervals between the inoculations (weeks)	Autopsy after the last inoculation (days)	Intradermal test (mm)	White* spots
11	50,000×1		1	5× 5	n
12	50,000×1		7	(8× 7)	II
13	50,000×1		7	4× 3	II
14	50,000×1		14	(11×10)	III
15	50,000×1		14	(10×10)	III
16	50,000×1		21	(20×20)	I
17	50,000×2	2	7	0× 0	III
18	50,000×2	2	7	7× 7	III
19	50,000×2	2	21	(16×15)	I
20	50,000×2	2	21	(20×19)	I
21	50,000×2	4	14	(16×11)	III
22	50,000×2	4	14	(11×10)	III
23	50,000×2	4	14	(18×13)	III
24	50,000×2	4	14	(14×11)	III
25	50,000×2	4	14	(12×12)	III
26				0× 0	NT
27				8× 8	NT
28				7× 7	NT
29				5× 4	NT
30				0× 0	NT
31				0× 0	NT
32				8× 7	NT
33				8× 8	NT
34				0× 0	n
35				0× 0	n

* Lesion score: I, Liver with mild lesion; II, Liver with moderate lesion; III, Liver with severe lesion; n, Normal liver; ( ); Wheals with hyperemia; NT: Not testing.

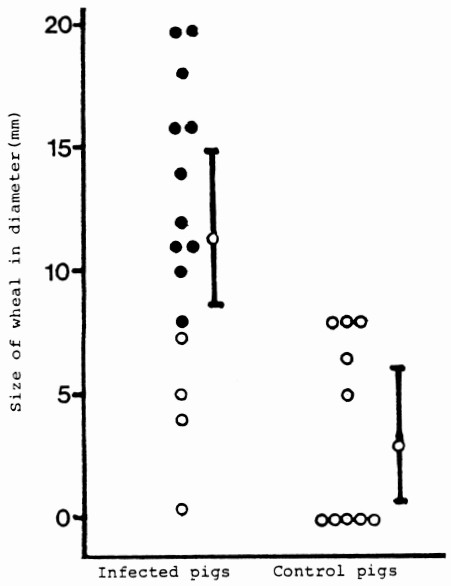


Fig. 5 Result of intradermal test with 10 μg of Pt from *Ascaris lumbricoides suum* on the skin of the base of the ear of pigs. ○: Wheals;●: Wheals with hyperemia; ⊖: Mean and 95% confidence limit.

Four of 5 pigs with weak reaction, however, had moderate or severe lesions in the liver. On the other hand, no severe reaction was observed on the skin of uninfected pigs. From the result, it is considered that intradermal reaction of immediate type should be judged as positive when a wheal of over 9 mm in diameter with hyperemia or hemorrhage appears on the base of the ear of pigs by injection with 0.1 ml of antigen containing 10 μg of Pt at 15 min later.

Reaction in fattened pigs at slaughterhouse: The same test was done by using fattened pigs. The results were summarized in Table 3. According to the criterion mentioned above, positive reactions were observed on the skin of thirteen or 77% of 17 pigs with white spots. On the other hand, positive reaction were also found in 6 (45%) of 14 pigs without the liver lesions. No nematode worms were obtained from the intestine of any of the fattened pigs.

Discussion

The cause of hepatic white spot or multiple chronic parasitic interstitial hepatitis is attributed to the presence of such helminth migration in the liver as ascarid species, *Fasciola hepatica, Stephanurus dentatus* and *Metastrongylus apri* (Nieberle and Cohrs, 1962). In case of Japan, it seems reasonable to consider that the cause of naturally occurring white spot may be attributed to *A. suum* infection (Nakagawa *et al.*, 1983).

These liver lesions are observed severely on the 10th day after oral administration of embryonated *A. suum* eggs and become mild within 25 days (Ferguson *et al.*, 1968) and cured completely on the 35th day (Copeman and Gaafar, 1972; Eriksen *et al.*, 1980). In comparison with the lifetime of the lesions, the prepatent period of the nematode is 60 or more days (Jones and Hunt, 1983). Accordingly, the liver of the animals with mature *A. lumbricoides suum* in the intestine shows sometimes no scars (Ferguson *et al.*, 1968). On the other hand, it is found frequently that worms of *A. lumbricoides suum* could not be detected in the intestine of pigs with severe white spots in the field (Nakagawa *et al.*, 1983). To diagnosing early infection with the nematode and to prove the presence of white spots, therefore, it is necessary to study a simple method without fecal examination. In the previous report (Yoshihara *et al.*, 1983), the authors demonstrated that some of pigs with negative reaction in MDCFT had shown severe reaction in intradermal test of immediate type.

At first, the criterion of judgment was examined in the present study. Since one-tenth ml of saline (protein) extracts were injected intradermally into pigs for diagnosis of ascariasis by Soulsby (1957), this volume of antigens was used in the present examination. From results obtained by using SPF pigs and crossbred pigs, we can say that 10 μg of Pt may be suitable for antigen of intradermal test.

Reaction with Pt was more clear than that of Ps. Soulsby (1957) also mentioned that Ps was less satisfactory than saline (protein) extract. In addition, in comparison with production of Pt, preparation of Ps is not easy. From the viewpoint of usefulness, consequently, Pt may

Table 3 Result of intradermal test in field

Pig number	Intradermal test (mm)	White spots*	*Ascaris lumbricoides suum* in the intestine
1	(13×12)	III	0
2	(12×12)	I	0
3	(11×10)	I	0
4	(11×11)	II	0
5	(12×12)	I	0
6	(15×15)	I	0
7	(10×10)	I	0
8	(12×12)	I	0
9	(12× 9)	I	0
10	(11×10)	I	0
11	(12×10)	I	0
12	(10×10)	I	0
13	(11×10)	I	0
14	(7× 7)	I	0
15	5× 4	II	0
16	7× 7	I	0
17	6× 6	I	0
18	(12×11)	n	0
19	(10×10)	n	0
20	(11×10)	n	0
21	(14×10)	n	0
22	(10×10)	n	0
23	(13×11)	n	0
24	8× 8	n	0
25	8× 8	n	0
26	0× 0	n	0
27	(6× 4)	n	0
28	8× 8	n	0
29	(7× 2)	n	0
30	(8× 7)	n	0
31	8× 7	n	0

* Lesion score: I, Liver with mild lesion; II, Liver with moderate lesion; III, Liver with severe lesion; n, Normal liver; ( ): Wheals with hyperemia.

be more suitable for antigen of the test.

When Ss was injected into the base of the ear of two infected SPF pigs, wheal disappeared within 15 min. In the previous report (Yoshihara *et al.*, 1983), we indicated that a slight wheal remained on the auricle of the ear injected with Ss. These results suggest that the base of the ear may be a suitable site for the

injection.

From the result of intradermal test on the base of the ear of fifteen infected pigs and ten uninfected pigs, it can be concluded that intradermal reaction of immediate type should be judged as positive when a wheal of over 9 mm in diameter with hyperemia or hemorrhage appears on the base of the ear by injection with 0.1 ml of antigen containing 10 μg of Pt at 15 min later. Andrews *et al.* (1976) examined intradermal test for diagnosis of hog trichiniasis and reported that a reaction was considered positive on the development of a reddish-purple, often hemorrhagic area, 10 to 15 mm in diameter, surrounding the injection site with 0.1 ml of antigen at 15 or 30 min later.

The result obtained from fifteen infected pigs suggests that positive reaction may appear on the base of the ear of pigs 14 days following inoculation with *A. lumbricoides suum* eggs. Furthermore, we can assume that if the liver lesions are to be cured in future, positive reaction in the test might continue for a certain period of time.

In accordance with the criterion mentioned above, thirteen or 77% of 17 fattened pigs in field with white spots showed positive reaction in the test. Soulsby (1957) investigated the relationship between the test and presence of white spot in the liver of pigs in field and pointed out that the incidence of positive reaction increased with the advance in severity of the disease in the animals. If the test in the present examination was carried out by using fattened pigs from pigsty contaminated heavily with *A. lumbricoides suum* and during the hot season which had been indicated high incidence of the disease (Nakagawa *et al.*, 1983), so that, rate of positive reaction may also be increased.

We can conclude that intradermal tests lack specificity and thus has limited value for diagnosis of individual cases. However, since this test does not require laborious technique or efficient facilities, this may be useful for field survey or screening of white spots with *A. lumbricoides suum* infection. Further experiments would be thus required to demonstrate the relationship between the test and the liver lesions by using a large number of fattened pigs in field.

Recently, Urban *et al.* (1988) carried out active cutaneous anaphylaxis to *A. suum* antigens from several stages in pigs exposed to the nematode and confirmed the severe reaction on the skin of the flank injected with antigens from larval worms. So, it is also important to examine the antigenicity of larval antigens for intradermal test in future.

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