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Experimental infection of pigs with a *Taenia* species from Korea: parasitological and serological aspects*

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Abstract. Belgian Landrace piglets were experimentally infected with eggs of a *Taenia* sp. of Korean origin. At autopsy, metacestodes were present only in the livers. The proportion of degenerated metacestodes increased from 12%–39% at 5 weeks to 94%–100% at 10 weeks after infection. A sandwich enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies raised against the excretory-secretory products of *T. saginata* metacestodes detected circulating antigen in the sera of the pigs at 1 week post-infection. A good correlation was found between the presence of viable metacestodes and the detection of circulating antigen; the latter disappeared as the metacestodes died off. However, the antibodies were detected only after 3 weeks of infection and onwards until the necropsy of the pigs.

The so-called Taiwan *Taenia*, which behaves differently from *T. solium* or *T. saginata* (Fan 1988), has also been reported to occur in Indonesia, Korea and Thailand (Fan et al. 1989a, b, 1990a) as well as in Madagascar and Ethiopia (Fan et al. 1990c). Pigs, calves, goats and monkeys were shown to be susceptible to experimental infection with the eggs of this *Taenia* species, and the metacestodes developed exclusively in the livers. The adult cestode occurs in man. Additional evidence has recently been presented that Taiwan *Taenia* is a distinct species and not merely a strain of *T. saginata* (Zarlenga et al. 1991). The present experiment was set up to study the evolution of circulating antigen and antibodies using an enzyme-linked immunosorbent assay (ELISA) during the course of an experimental infection of Belgian Landrace pigs with Taiwan *Taenia*.

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Materials and methods

Eight 2-week-old Belgian Landrace piglets were infected per os with 4,000 or 8,000 *Taenia* sp. eggs of South Korean (Cheongju) origin. The eggs were stored at 4° C in tap water containing antibiotics for about 10 weeks prior to infection. The pigs were bled once before their infection and thereafter at intervals of 1–2 weeks. Five animals were killed at 5 weeks and the other three, at 10 weeks post-infection (Table 1). All visceral organs, including the brain, and the muscles of one half of the carcass were cut in thin slices and carefully examined for the presence of viable or degenerated metacestodes.

ELISA for the detection of antibodies

T. saginata metacestodes excised from the muscles of a calf that had been slaughtered at 10 weeks after infection were transferred to an in vitro system to obtain their excretory-secretory (ES) products (Brandt et al. 1992). The ES products diluted 1:20 (v/v) in RPMI-1640 culture medium were used as antigens and incubated in the wells of polystyrene ELISA plates (Maxisorb, Nunc) for 1 h at 37° C and then overnight at 4° C. The pig sera were diluted 1:50 (v/v) in phosphate-buffered saline (PBS, pH 7.2) supplemented with 0.05% Tween-20 and then incubated for 1 h at 37° C. Thereafter, the conjugate (RaSw/IgG, Nordic), diluted 1:1000 (v/v) in PBS-Tween-20, was added and incubated for 1 h at 37° C. Orthophenylenediamine (OPD) and H_2O_2 were used as the substrate. The optical density (*OD*) of the samples was read by an ELISA reader (Titertek, Multiskan) at 405 nm.

ELISA for the detection of circulating antigen

Two monoclonal antibodies (mAb) raised against the ES products of 10-week-old *T. saginata* metacestodes were used in a double-antibody sandwich ELISA according to the method described by Brandt et al. (1992). mAb $12G_5$, was used at 20 µg IgM/ml as a trapping antibody for initial coating of the polystyrene plates, whereas biotinylated mAb $2H_8$ was used at 10 µg IgM/ml as an indicator antibody. The sera were diluted 1:4 (v/v) in PBS-Tween-20. The plates were read as described above after the successive addition of an avidin-biotin peroxidase complex (Dakopatts) and OPD together with H_2O_2 as the substrate.

Serum samples were considered to be positive for circulating antibodies or antigen if the *OD* values significantly differed ($P <$

Table 1. Parasitological and serological results of an experimental infection of pigs with *Taenia* sp. eggs of Korean origin

Pig number	Infection dose (number of eggs)	Autopsy (weeks p.i.)	Number of metacestodes in the liver				Ag	Ab
			S	P	T	D (%)		
1	4000	5	60	80	140	21 (15)	+	+
2	4000	5	8	9	17	2 (12)	+	+
3	4000	5	1	5	6	1 (17)	−	+
4	8000	5	0	0	0	0	−	−
5	8000	5	4	9	13	5 (39)	+	+
6	4000	10	1	5	6	6 (100)	−	−
7	4000	10	2	6	8	8 (100)	−	+
8	4000	10	11	76	87	82 (94)	−	+

S, On the liver surface; P, in the liver parenchyma; T, total number; D(%), number of degenerated cysts and percentage; p.i., post-infection; Ag, Ab, circulating antigen and antibody at autopsy

0.05) from the mean value determined prior to infection in sera from the eight pigs (Sokal and Rohlf 1981).

Results and discussion

Seven of the eight *Taenia*-infected pigs harboured metacestodes in their livers (Table 1). An average of 31% of the metacestodes were found on the surface of this organ just underneath Glisson's capsule, whereas 69% developed in the deeper parenchyma, which confirms the prior findings of Fan et al. (1990b). Metacestodes were not found elsewhere in the body, neither in the musculature nor in any other organ, including the brain. This is in agreement with the results of previous studies (Eom and Rim 1988; Fan et al. 1989b). Degeneration of the metacestodes in the livers occurred rather rapidly, as 12%–39% of them had degenerated by week 5 of the infection. Within 5 weeks therafter, this proportion reached 94%–100%. Although the pigs used in the present experiment were young and degeneration of metacestodes is reported to be slower in young animals (Fan et al. 1990b), it may be that these metacestodes degenerate more rapidly in Belgian Landrace pigs than in other breeds of swine (Fan et al. 1989b, 1990b).

Circulating antigen

At 1 week after the infection, circulating antigen was demonstrated in some of the pigs, including one animal (pig 5) that displayed an infection burden of as few as 8 viable metacestodes (Fig. 1a, b). In cattle in which the circulating antigen has been detected, the lowest viable *T. saginata* metacestode burden has been 88 (Brandt et al. 1992).

A good correlation was found between the presence of viable metacestodes and the detection of circulating antigen (Table 1). A similar correlation has also been observed in studies on *T. saginata* cysticercosis in cattle (Brandt et al. 1992). Between weeks 2 and 5 after infection, the circulating antigen was found in all but three animals; pigs 3, 4 and 8. Pig 4 did not display any metacestodes, whereas pigs 3 and 8 harboured only 5 viable metacestodes each (Fig. 1a, b). However, circulating antigen disappeared from the circulation as the metacestodes degenerated (Fig. 1b). Apparently, the circulating antigen is not detectable in cases in which the viable metacestode burden is 5 or less.

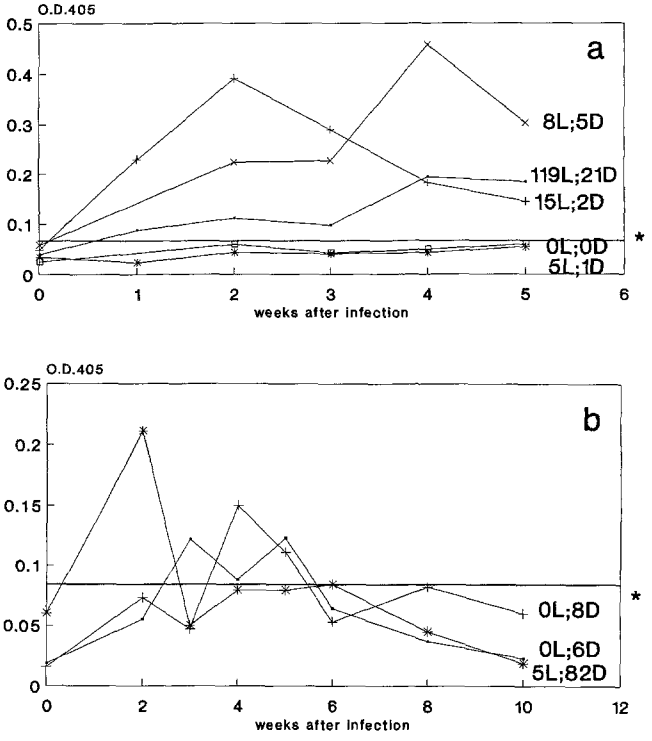

Fig. 1a, b. ELISA values obtained for circulating excretory-secretory antigens in pigs experimentally infected with a *Taenia* species from Korea. *L*, Living cysticerci; *D*, dead cysticerci; * $P=0.05$ (cut-off level). **a** Pigs 1 (−■−), 2 (−+−), 3 (−*−), 4 (−□−) and 5 (−×−). **b** Pigs 6 (−■−), 7 (−+−) and 8 (−*−)

Circulating antibodies

In the infected pigs, the circulating antibodies first appeared somewhat later than the circulating antigen; most of the animals were seropositive at 3 weeks post-infection and remained so until autopsy. Using a fractionated antigen of cyst fluid of *T. hydatigena* metacestodes, Rhoads et al. (1989) also observed that antibodies to Taiwan *Taenia* were first detectable in pigs at 3 weeks post-infection and persisted until necropsy at 32 weeks post-infection. In pig 6, which bore only 6 metacestodes, the antibodies were detected only once at 5 weeks post-infection and were not detectable thereafter. Pig 4 did not show any circulating antigen or antibodies, and we assume that this animal regurgitated the taeniid eggs soon after their oral administration.

Clearly, the mAbs raised against the ES products of *T. saginata* metacestodes are capable of detecting the circulating antigen in pigs harbouring viable Taiwan

Taenia metacestodes. Recently, these mAbs have been shown to cross-react with the circulating antigen of *T. solium* metacestodes (Brandt et al. 1992). It is therefore unlikely that these mAbs can discriminate between the circulating antigen of *T. solium* and that of Korean *Taenia*.

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