

Comparison of single and trickle infections of pigs with eggs of the Asian *Taenia*

E.H.M. Fall, V. Kumar, S. Geerts *, M. Salvoldi, M. Kama

Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen 1, Belgium

Received 2 March 1995; accepted 2 May 1995

Abstract

Two groups of seven Belgian Landrace piglets each were either infected with a single dose of 3000 or with five consecutive doses of 600 Asian *Taenia* eggs at weekly intervals. Nine weeks after the first infection all pigs were autopsied and the number of metacestodes was obtained by slicing the liver. There were no significant differences between the mean number of viable or dead cysts present in both groups of animals. Only very low numbers of living metacestodes were found: 0.4% (3/779) of the total number of cysts present in the single infection group and 1.8% (13/707) in the trickle infection group. Circulating antigens could be detected in only four out of 13 animals and no differences in antibody kinetics were present between the two groups of pigs. The presence of high numbers of degenerated cysts in experimental as well as in field conditions seems to indicate that the biotic potential of the Asian *Taenia* is rather low.

Keywords: *Taenia* sp.; Pig-Cestoda; Trickle infection; Biotic potential

1. Introduction

Fan et al. (1990) have shown that pigs are suitable intermediate hosts of the Asian (Taiwan) taeniid, provisionally identified as a strain of *Taenia saginata* (Cross and Murrell, 1991) or as a distinct species, *Taenia asiatica* (Eom and Rim, 1993). Several experimental infections of pigs have been carried out using different doses of eggs of different geographical origins (Fan et al., 1992). Up to now, however, no trickle infection studies using Asian taeniid eggs have been described. Trickle infection schedules generally simulate better field conditions, where the pigs are exposed to repeated infections with the taeniid eggs. Therefore, we have carried out single and

* Corresponding author.

multiple spaced-dose infections of pigs with the Asian taeniid eggs, especially to compare the effect of the two infection regimens on the viability of the metacestodes in the liver. Such data are important criteria to determine the biotic potential of the parasite.

2. Materials and methods

2.1. Experimental protocol

Fourteen 2-month-old Belgian Landrace piglets were infected orally by gavage with Asian *Taenia* eggs of Korean origin. The eggs were stored in aqua distillata containing antibiotics (1000 IU penicillin and 1 mg streptomycin per ml) and nystatin (1000 IU ml⁻¹) and were 1 month old at the time of first infection. Seven pigs (Group 1) received a single dose of 3000 eggs, whereas seven other pigs (Group 2) received five consecutive doses of 600 eggs at weekly intervals. Blood samples were taken before infection, at weekly intervals during the first 5 weeks post infection (p.i.) and at 7 and 9 weeks p.i.

The autopsy of all the pigs was carried out 9 weeks after the first infection. The liver was cut into thin slices and carefully examined for the presence of viable or degenerated metacestodes. The Mann–Whitney *U*-test was used for the statistical comparison of the number of metacestodes present in both groups.

2.2. ELISA for the detection of circulating antigen and antibodies

A monoclonal antibody (mAb) based sandwich ELISA as described by Brandt et al. (1992) was used for the detection of circulating antigen. The mAb were raised against

Table 1

Number of metacestodes in the livers of pigs after single (1×3000) or trickle (5×600) infection with Asian *Taenia* eggs

Pig No.	Single infection			Pig No.	Trickle infection		
	No. of metacestodes				No. of metacestodes		
	Viable	Dead	Total		Viable	Dead	Total
80	0	36	36	89	0	41	41
82	0	23	23	90	0	291	291
83	3	8	11	91	10	46	56
85	0	32	32	92	0	24	24
86	0	650	650	93	2	105	107
87	0	27	27	94	0	62	62
88 ^a				95	1	125	126
Average (±SD)	0.5 (±1.1)	129 (±233)	130 (±233)		1.9 (±3.4)	99 (±85)	101 (±84)

^a Died shortly after the infection.

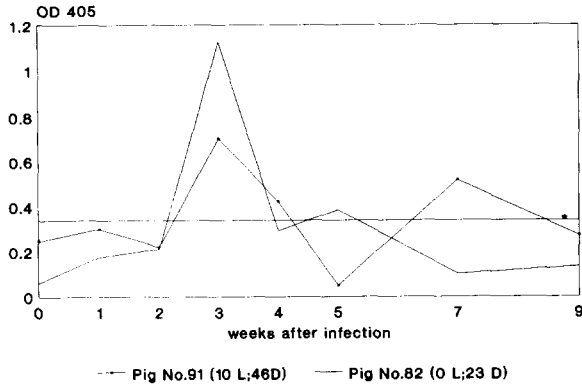


Fig. 1. Evolution of circulating antigens in two pigs after single (No. 82) or trickle (No. 91) infection with eggs of the Asian *Taenia*. —●—, Pig No. 91 (10 L, 46 D); —, Pig No. 82 (0 L, 23 D). L, living cysts; D, dead cysts; *, cut-off.

the excretory–secretory (ES) antigens of *T. saginata* metacystodes. Two mAb (12G5 and 158C11) were used as trapping antibodies and the two others (2H8 and 153G7) as biotinylated indicator antibodies. Serum samples were considered positive for circulating antigen if the optical density (OD) values significantly differed ($P: 0.01$) from the mean OD value of the pre-infection serum samples of the pigs (Sokal and Rohlf, 1981).

Using an indirect ELISA, the presence of antibodies against the ES products of the Asian *Taenia* metacystodes was determined as described by Geerts et al. (1992). The ES antigens were collected after 48 h in vitro culture of 5-week-old viable Asian taeniid metacystodes of Korean origin following Brandt et al. (1992). A similar ELISA was used for the detection of antibodies against somatic antigens. For this purpose, the somatic extract of a heterologous taeniid, *Taenia crassiceps* metacystodes, was used as antigen ($5 \mu\text{g protein ml}^{-1}$). The pig sera were diluted at 1:400 and the conjugate (RaSw IgG-peroxidase) at 1:20 000. Orthophenylenediamine and H_2O_2 were used as

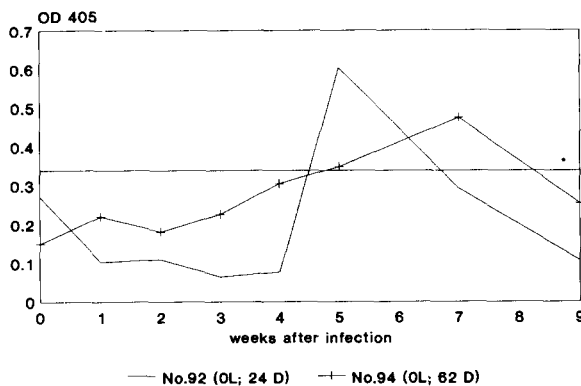


Fig. 2. Evolution of circulating antigens after trickle infection of two pigs (Nos. 92 and 94) with eggs of the Asian *Taenia*. —, Pig No. 92 (0 L, 24 D); —+—, Pig No. 94 (0 L, 62 D). L, living cysts; D, dead cysts; *, cut-off.

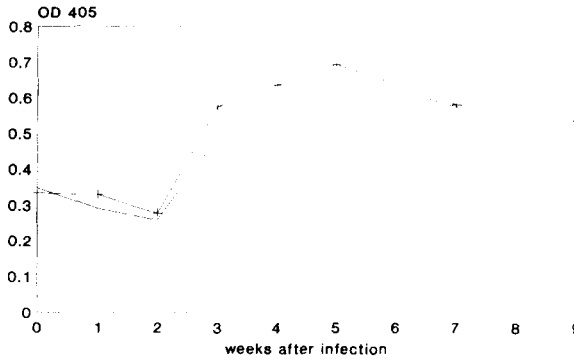


Fig. 3. Evolution of antibodies (mean ELISA values) against ES antigens after single or trickle infections of two groups of pigs with eggs of the Asian *Taenia*. —, single infection; - + -, trickle infection.

chromogen and substrate. The OD of the samples was read by an ELISA reader (Titertek Multiscan) at 405 nm.

3. Results

The numbers of dead and viable metacestodes harvested from the livers of pigs are summarized in Table 1. There were no significant differences between the mean number of dead or viable metacestodes present in either group of animals. Despite a high variation in total metacestode burden in individual pigs, the number of viable metacestodes was very low in both groups; only 0.4% (3/779) of the total number of metacestodes present in the single infection group and 1.8% (13/707) in the trickle infection group of pigs were viable.

During the course of the infection detectable levels of circulating antigens were present in only four out of 13 animals (Figs. 1 and 2). At the end of the experiment,

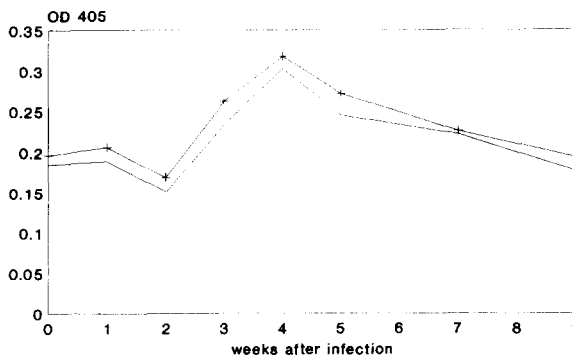


Fig. 4. Evolution of antibodies (mean ELISA values) against somatic antigens after single or trickle infections of two groups of pigs with eggs of the Asian *Taenia*. —, single infection; - + -, trickle infection.

circulating antigens could not be demonstrated in any of the pigs. The evolution of the antibodies against ES or somatic antigens in both groups of pigs is presented in Figs. 3 and 4. There was no significant difference in the antibody kinetics between the two groups of pigs.

4. Discussion

The absence of significant differences between the mean number of cysts in the single and trickle infection groups might be interpreted as an indication of the lack of acquired immunity to superinfection. This would mean that the Asian *Taenia* is an exception within the family of the taeniids, where the development of acquired immunity in the intermediate host is the rule. It is premature, however, to draw such a conclusion because of several reasons. Firstly, nothing is known about the time period needed before acquired immunity against the Asian *Taenia* is fully expressed. In the case of *Taenia hydatigena*, for instance, it takes up to 7 weeks before sheep acquire strong resistance (Gemmell and Johnstone, 1977). Secondly, a lack of significant differences between the two groups (Table 1) has to be interpreted with caution because of the large variation observed in both groups. Thirdly, observations similar to those described here have been reported with other taeniids. Although acquired immunity is known to develop against *T. hydatigena*, no significant differences were present between the number of cysts which developed after infection with a single dose of 1000 eggs or when the same number of eggs was given in ten divided doses at weekly intervals (Gemmell and Johnstone, 1977).

The results of this experiment confirm previous observations of Eom et al. (1992), Fan et al. (1990, 1992) and Geerts et al. (1992), in which low numbers of viable metacestodes were found in pigs receiving a single infection of different doses of Asian *Taenia* eggs. The metacestodes seem to degenerate quite rapidly in the liver. It was surprising to find smaller numbers of viable metacestodes in the single infection group than in the trickle infection one. In a similar experiment in cattle, using a single infection dose of 30 000 *T. saginata* eggs versus trickle infections (12×2500 eggs), more viable metacestodes, were found in the former case than in the latter (Bogh et al., 1995). In the ovine host, Gemmell and Johnstone (1977), however, found no significant differences between the number of viable metacestodes infected either with a single dose of 1000 eggs or with ten consecutive infection doses of 100 eggs of *T. hydatigena* or *Taenia ovis*. The presence of very low numbers of viable Asian taeniid metacestodes was also observed in a large-scale survey of slaughter-pigs in Korea; in only 0.01% out of 25 358 animals were viable metacestodes detected (Eom and Rim, 1992). These observations on the occurrence of high numbers of degenerated metacestodes, in experimental as well as in field conditions, have an important bearing on the biotic potential of the parasite, namely, the number of viable metacestodes produced per infected host per day. Based on the results of the present experiment and the available data in the literature (Rhoads et al., 1989; Fan et al., 1992; Geerts et al., 1992) the average proportion of eggs of the Asian *Taenia* transforming into viable cysts was 1.0%, whereas for *T. saginata* this figure is 1.75% (Geerts, 1993). Conceivably, the biotic

potential of the Asian taeniid might be much lower than that of *T. saginata*, which was considered as very high (Gemmell, 1986).

Owing to the small numbers of viable metacestodes available in the pigs, detectable levels of circulating antigens were observed in only a few animals. The mAb based antigen ELISA is shown to detect only animals harbouring viable metacestodes (Brandt et al., 1992). The fact that circulating antigen was detected for short periods during the course of the infection in some animals (Figs. 1 and 2), although the living metacestodes were no longer present at the time of slaughter, indicates that there had been some development of viable metacestodes, which subsequently degenerated during the course of the experiment.

To confirm the validity of these results in the context of the Asian situation, it would be interesting to carry out similar infection experiments using Asian breeds of pigs.

Acknowledgements

This work was financially supported by the National Institute for Scientific Research, Brussels (NFWO, Grant No. 3008094). The authors gratefully acknowledge Professor K.S. Eom for providing the Asian *Taenia* eggs.

References

- Brandt, J.R.A., Geerts, S., De Deken, R., Kumar, V., Ceulemans, F., Brijs, L. and Falla, N., 1992. A monoclonal antibody-based ELISA for the detection of circulating excretory–secretory antigens in *Taenia saginata* cysticercosis. *Int. J. Parasitol.*, 22: 471–477.
- Bogh, H.O., Lind, P., Gronvold, J., Ilsoe, B., Maeda, G.E., Henriksen, S.A., Nansen, P. and Geerts, S., 1995. Long term experimental *Taenia saginata* trickle infections in cattle. *Res. Vet. Sci.*, submitted.
- Cross, J.H. and Murrell, K.D., 1991. The 33rd SEAMEO–TROPED regional seminar on emerging problems in food-borne parasitic zoonoses: impact in agriculture and public health. *Southeast Asian J. Trop. Med. Public Health*, 22: 4–15.
- Eom, K.S. and Rim, H.J., 1992. Natural infections of Asian *Taenia saginata* metacestodes in the livers of Korean domestic pigs. *Korean J. Parasitol.*, 30: 15–20.
- Eom, K.S. and Rim, H.J., 1993. Morphologic description of *Taenia asiatica* sp. n. *Korean J. Parasitol.*, 31: 1–6.
- Eom, K.S., Rim, H.J. and Geerts, S., 1992. Experimental infection of pigs and cattle with eggs of Asian *Taenia saginata* with special reference to its extra-hepatic viscerotropism. *Korean J. Parasitol.*, 30: 269–275.
- Fan, P.C., Chung, W.C., Lin, C.Y. and Wu, C.C., 1990. The pig as an intermediate host for Taiwan *Taenia* infection. *J. Helminthol.*, 64: 223–231.
- Fan, P.C., Chung, W.C., Lin, C.Y., Wu, C.C., 1992. Pig as the favorite intermediate host of possible new species of *Taenia* in Asia. *Memoirs of Emeritus Professor Ping-Chin Fan, Taipei*, pp. 109–129.
- Geerts, S., 1993. The taeniasis–cysticercosis complex in Africa. *Meded. Zitt. K. Acad. Overzeese Wet.*, 38: 245–264.
- Geerts, S., Zorloni, A., Kumar, V., Brandt, J.R.A., De Deken, R. and Eom, K.S., 1992. Experimental infection of pigs with a *Taenia* species from Korea: parasitological and serological aspects. *Parasitol. Res.*, 78: 513–515.
- Gemmell, M.A., 1986. General epidemiology of *Taenia saginata*. In: J. Block, A.H. Havelaar, P. L'Hermite

- (Editors), *Epidemiological Studies of Risks Associated with the Agricultural Use of Sewage Sludge: Knowledge and Needs*. Elsevier Science Publishers, London and New York, pp. 60–71.
- Gemmell, M.A. and Johnstone, P.D., 1977. Experimental epidemiology of hydatidosis and cysticercosis. *Adv. Parasitol.*, 15: 311–369.
- Rhoads, M.L., Murrell, K.D., Cross, J.H. and Fan, P.C., 1989. The serological response of pigs experimentally infected with a species of *Taenia* from Taiwan. *Vet. Parasitol.*, 30: 279–285.
- Sokal, R.S. and Rohlf, J.J., 1981. *Biometry. The Principles of Statistics in Biological Research*. Freeman and Cie, New York, 829 pp.