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Experimental single and trickle infections of cattle with *Taenia saginata*: studies of immunodiagnosis

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SUMMARY

Two groups of 12 calves were given either a single infection dose of 30,000 *Taenia saginata* eggs (group A) or trickle infected with 12 x 2500 *T saginata* eggs (group B). The calves were killed 16, 22 or 54 weeks after infection and the numbers of viable and dead parasites were counted by slicing selected organs and muscles of half of the carcases. The total numbers of cysts and the numbers of viable cysts were higher in group A than in group B. Within the two groups there were no significant differences between the numbers of cysts at the three dates of slaughter. Comparable results were obtained by using an ELISA and a dot blot procedure for the detection of antibody in the serum of the infected calves; an initial increase in response during the first 10 weeks after infection was generally followed by a gradual decline. When using a monoclonal antibody-based ELISA for the detection of circulating antigens, the sera from all the 14 animals which had no viable cysts or only dead cysts at slaughter did not react with the monoclonal antibodies. Calves 122 and 123, which harboured two and 122 viable cysts, respectively, reacted positively during the late phase of the infection to the antigen-detecting ELISA. However, several other animals which harboured between two and 41 viable cysts at slaughter did not react.

THE infection of cattle with the metacestode stage of *Taenia saginata* is of economic significance in many parts of the world and, furthermore, cattle provide the only reservoir for human infection with *T saginata* (Craig and Rickard 1980). Many attempts have therefore been made to detect the infection in living cattle. One approach makes use of heterologous antigens in serological tests. Thus, Hayunga and Sumner (1991) and Hayunga et al (1991a,b) detected *T saginata* metacestode infection by using an ammonium sulphate-soluble fraction of *T hydatigena* as antigen. Recently, Bøgh et al (1995) evaluated three approaches for the immunochemical detection of cattle infected with the metacestode stage of *T saginata*. They concluded that the dot blot procedure, in which minute amounts of a hydrophobic fraction of cyst fluid from *T hydatigena* were dotted on to filter membranes as antigen, was superior to the ELISA and the Western blot procedures studied. However, only sera from early infections were included in these examinations, and previous short and medium term infection studies have demonstrated that the circulating antibody level decreases after an initial rise (Kyvsgaard et al 1991, Smith et al 1991). An investigation of the levels of antibodies to the hydrophobic fraction of *T hydatigena* cyst fluid during the long-term infection of a large group of calves is therefore warranted in order to give a more reliable estimate of the potential of this, or any other antigen, for the diagnosis of metacestodes of *T saginata* in cattle. Furthermore, trickle infections in which cattle are infected on several occasions over a period of time are necessary to mimic more realistically the natural infections which occur in tropical countries. It is also necessary to correlate the results obtained from the diagnostic test systems with the numbers of cysts, viable or dead, present in the calves. The present study was designed to investigate these issues.

Recently, monoclonal antibodies (mAbs) have been used to detect circulating antigens in *T saginata*-infected cattle, in order to detect animals harbouring living cysticerci, information which is important from a public health point of view (Harrison et al 1989, Brandt et al 1992). However, further studies on the sensitivity of the technique, particularly in long-term infections, are necessary, and it was therefore included in the present study.

MATERIALS AND METHODS

Animals and experimental infections

Twenty-four Jersey calves of five to seven months of age, weighing between 96 and 148 kg at the beginning of the experiment, were divided into three groups according to whether they were to be slaughtered 16, 22 or 54 weeks after infection. The calves were housed until the beginning of the experiment. Half of the calves in each of the three groups were infected orally with 30,000 eggs of *T saginata*, four weeks after they were collected from Belgium (group A) whereas the other half were trickle infected by the weekly oral administration of 2500 eggs for 12 weeks (group B). The calves were bled and weighed fortnightly.

At slaughter, the numbers of viable and degenerate cysts were counted as described by Kyvsgaard et al (1990). The heart, masseters, tongue, diaphragm, liver, lungs and brain together with the muscles of one side of the body, including the forelimb, hindlimb and trunk, from each calf were cut in slices less than 0·5 cm thick. The cysts which were transparent and contained visible protoscolices were considered

TABLE 1: The range and mean (SD) numbers of viable and degenerate metacestodes of *Taenia saginata* and the total number of cysts observed in 12 calves after a single infection with 30,000 (group A) and in 12 calves after a trickle infection with 12 x 2500 *T saginata* eggs (group B)

	Group A				Group B			
Weeks after infection	Number	Viable Range, mean (SD)	Dead Range, mean (SD)	Total	Number	Viable Range, mean (SD)	Dead Range, mean (SD)	Total
16	4	0-15 7·0 (8·1)	16-76 37·0 (26·7)	176	4	0-24 6·0 (10·4)	0-17 4·5 (8·3)	42
22	4	0-41 13·3 (19·3)	3-30 12·8 (12·8)	104	4	0-6 2·0 (2·8)	0-30 8·3 (14·5)	41
54	4	0-122 35·0 (58·4)	4-19 11·8 (6·3)	187	4	0 0 (0)	0-4 1·3 (1·9)	5

to be viable and any other cysts were considered to be dead.

Immunological detection assays

ELISA. The procedures used to detect specific IgG_1 antibodies was that described by Bøgh et al (1995). Briefly, a standard indirect ELISA with the antigen derived from a hydrophobic fraction of cyst fluid from the closely related *T hydatigena*, was used. The antigen-coated 96-well microtitre plates (NUNC, Denmark) were incubated overnight at room temperature with sera diluted 1:1000. Subsequently, a horse-radish peroxidase-labeled conjugate sheep anti-bovine IgG_1 (The Binding Site) was added. After a final wash, the enzyme substrate, *o*-phenylene-diamine dihydrochloride, and hydrogen peroxide were added according to the manufacturer's recommendations (KemEnTec). The reaction was stopped after 15 minutes with 1M sulphuric

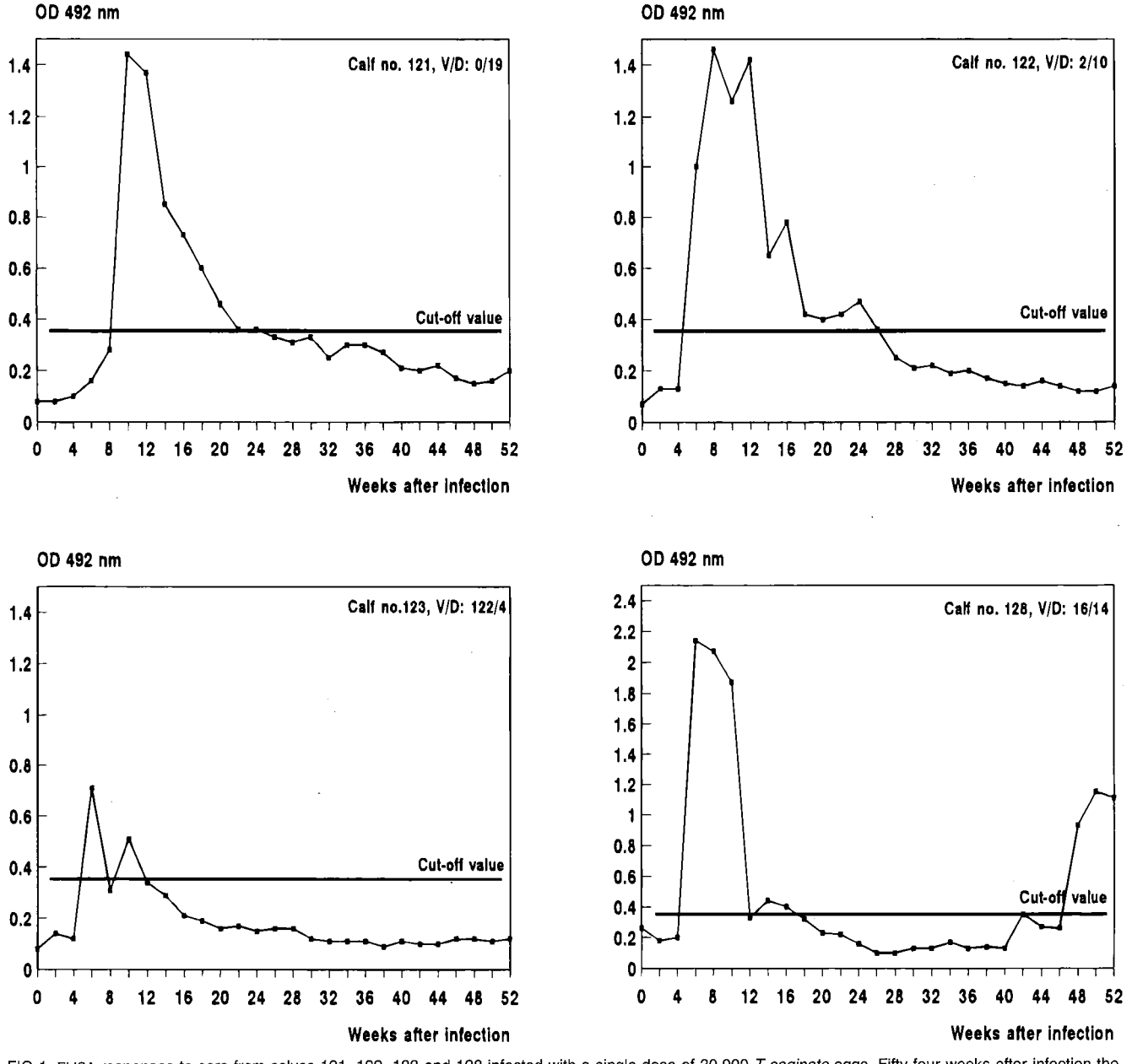

FIG 1: ELISA responses to sera from calves 121, 122, 123 and 128 infected with a single dose of 30,000 *T saginata* eggs. Fifty-four weeks after infection the animals were slaughtered and parasitological data obtained. V/D Number of viable versus dead cysts at slaughter

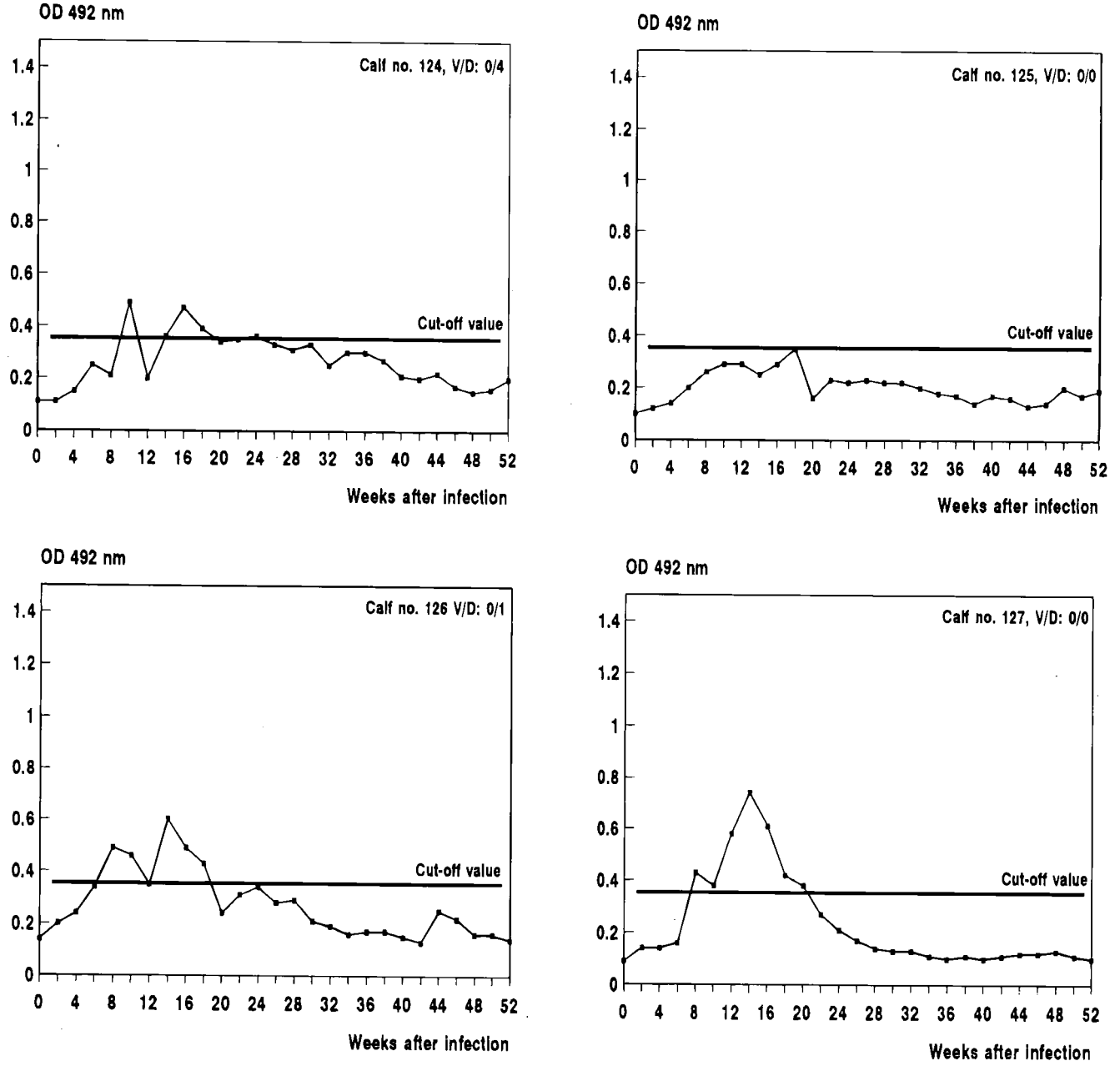

FIG 2: ELISA responses to sera from calves 124, 125, 126 and 127 trickle infected with 12 x 2500 *T saginata* eggs. Fifty-four weeks after infection the animals were slaughtered and parasitological data obtained. V/D Number of viable versus dead cysts at slaughter

acid and read spectrophotometrically at 492 nm. A cut-off optical density (OD) value of 0·371 was calculated by adding three standard deviations to the mean value derived from five uninfected heifers.

To detect the circulating antigens released by *T saginata* metacestodes during the long-term infection of the experimental calves, the method described by Brandt et al (1992) was used, the only modification being the use of a combination of four instead of two different mAbs [12G5, 158C11 (trapping) and 2H8, 153G7 (indicators)]. A sandwich ELISA, using mAbs against the excretory-secretory products of 10-weeks-old cysticerci of *T saginata* was therefore used. The test serum dilution was 1:4. The cut-off value (0·092) was calculated by comparing the OD of each sample with the mean OD of a series of eight negative control samples at a probability of p=0·001 (modified *t* test). The cut-off value obtained in this way was higher than the mean plus three times the standard deviation.

Dot blot. The dot blot procedure was that described by Bøgh et al (1995). Briefly, 1 µl of a pool of hydrophobic fractions of cyst fluid of *T hydatigena* was dotted onto PVDF membranes (Millipore). After the membranes were blocked, they were cut into strips containing the antigen dots. Subsequently, these strips were incubated in 2 ml sera, washed, incubated in sheep anti-bovine IgG_1 conjugate, washed and finally incubated in TMB (membrane peroxidase substract, Kirkegaard & Perry, USA) followed by a wash in deionised water to stop the reaction.

RESULTS

The numbers of viable and degenerate metacestodes, and the total numbers of cysts observed in the 24 experimental calves are shown in Table 1. Eight calves, four in group A and four in group B, were slaughtered 16. 22 or 54 weeks after infection. On each occasion the total number of cysts was higher in group A than in group B. Thus, totals of 176, 104 and 187 cysts were found in group A compared with

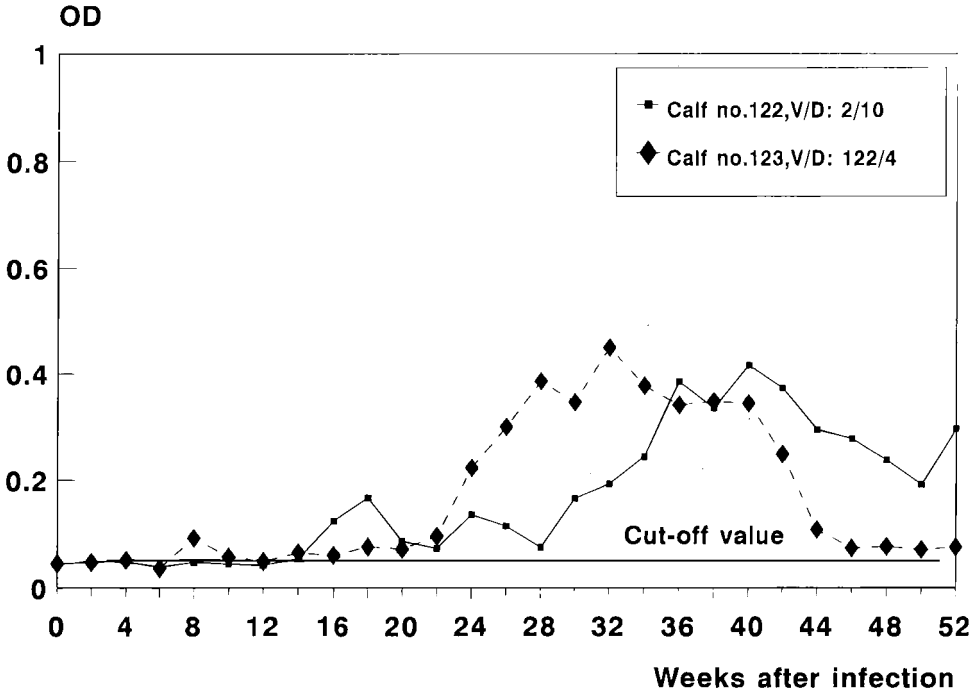

FIG 3: ELISA responses to sera from calves 122 and 123 infected with a single dose of 30,000 *T saginata* eggs, using a monoclonal antibody-based assay for the detection of circulating excretory/secretory antigens. Fifty-four weeks after infection the animals were slaughtered and parasitological data obtained. V/D Number of viable versus dead cysts at slaughter

totals of 42, 41 and five cysts in group B at 16, 22 or 54 weeks after infection. However, the difference between the total numbers of cysts in group A and group B was significant ($P<0{\cdot}01$) only after 54 weeks. Similarly, the numbers of viable and dead cysts, counted separately, were generally higher in group A than in group B, but the differences were not significant, although the P-value approached the 5 per cent significance level on several occasions. Within the two groups there were no significant differences between the numbers of cysts observed on the three dates of slaughter; seven of the 12 calves in group A were infected with a total of 221 viable cysts, whereas in group B, only three of the 12 calves were infected with a total of 32 viable cysts.

The results of the ELISA for the detection of specific IgG_1 antibodies in the eight calves slaughtered after 54 weeks (121-128) are shown in Figs 1 and 2. An increased ELISA response was observed in all eight calves after the infection, and seroconversion was first detected after six weeks in two of the calves in group A. The highest ELISA values were observed in calves 121, 122 and 128, which were infected with a single dose of 30,000 *T saginata* eggs. In the case of calf 125, which had been trickle infected with 12 x 2500 *T saginata* eggs, the ELISA response did not exceed the cut off level of 0·371, and at slaughter 54 weeks after infection neither viable nor dead cysts were found. Overall, the antibody level decreased from approximately 10 weeks after infection and no positive responses were observed 52 weeks after infection, except in calf 128, in which a sudden rise above the cut-off value was observed. Calf 128 harboured 16 viable and 14 dead cysts, whereas the two other calves which harboured viable cysts (calves 122 and 123) at slaughter reacted negatively, although 122 viable cysts were found in calf 123. The increased antibody level did not persist for any longer in group B than in group A.

An ELISA for the detection of circulating excretory-secretory antigens was applied to the sera from all the 24 calves in the experiment. Fourteen of the animals harboured no viable cysts at slaughter and the others harboured two, two, six, 12, 13, 15, 16, 24, 41 and 122 viable cysts. The sera from the 14 animals which had no viable cysts, plus from 0 to 76 dead cysts, did not react with the mAbs against circulating antigens at the time of slaughter. Only two calves, 122 and 123, were detected positive on more than one occasion. Circulating antigens were detected five to six months after infection and persisted for approximately six months (Fig 3); the calves harboured two and 122 viable cysts and 10 and four dead cysts, respectively, 52 weeks after infection.

The results obtained with the dot blot method for the detection of calves infected with *T saginata* were very comparable with the results from the ELISA detection of antibodies (data not shown), and the increases and decreases in the responses clearly followed those obtained with the ELISA. Increased responses were observed early in the infections whereas only slight or no responses were observed as the infection proceeded, except with the serum from calf 128 in which an increase was observed in both the ELISA and the dot blot procedure.

There were no differences in weight gain between the two groups.

DISCUSSION

This study investigated long-term *T saginata* infections in cattle by using recently developed diagnostic tests. Previously, only a few long-term infection studies have been undertaken. However, a thorough investigation of the antibody levels present during long-term infections is needed to provide more reliable information on the potential of using a specific antigen or antibody in test systems (Bøgh et al 1995). Trickle infections, in which the calves are infected for a long time with small doses of the parasite,

probably mimic a natural infection satisfactorily. Furthermore, the correlation of the results of the diagnostic tests with the numbers of viable and dead cysts present at slaughter is also relevant and was assessed.

The numbers of cysts recovered from the infection with a total of 30,000 eggs were very small (Table 1), probably as a result of the low infectivity of the eggs rather than the immune status of the calves, which were only five to seven months of age at the start of the experiment. However, the small numbers of cysts provided a good test of the sensitivity of the diagnostic systems. Larger total numbers of cysts were detected in group A than in group B at 16, 22 or 54 weeks after infection. The development of immunity against *T saginata* infections and closely related species may be important in this respect (Miller 1931, Rickard and Williams 1982). As a result, the penetrating oncospheres of the later trickle infections may have been exposed to a variety of the host's defences, resulting in the death of the developing metacestodes before maturity.

No direct comparison between the effects of single and trickle infections of cattle with *T saginata* has previously been made. However, Gemmell et al (1987) described an experiment in which lambs were given single or repeated doses of *T hydatigena* or *T ovis*. In contrast with the present experiment, no significant differences were observed between the two groups of lambs, indicating that as few as 10 eggs were sufficient to induce resistance to a superinfection (Gemmell et al 1987).

The dot blot results were comparable with the ELISA results using the antibody detection assay, in contrast with the results of Bøgh et al (1995) who found that the sensitivity of the dot blot assay, using heterologous antigens, was superior to that of the ELISA.

The increased ELISA and dot blot responses early in the infection followed by a gradual decrease in the antibody levels correspond with the observations by Smith et al (1991), who studied medium-term *T saginata* infections in calves for 210 days. However, they also observed a secondary increase in antibody levels between 160 and 210 days after infection. In this study a similar observation was recorded only in calf 128 between 48 and 52 weeks after infection. As Smith et al (1991) suggested, this reaction may be induced by the release of metacestode antigens from dying cysts.

Recently, techniques for the detection of circulating antigens in cattle infected with *T saginata* have been developed by Harrison et al (1989) and Brandt et al (1992). In theory, the detection of circulating antigens has the advantage of only viable cysts, which excrete or secrete metabolites, being detected. From a public health point of view, this fact is of great importance (Brandt et al 1992). When using a mAb-based ELISA for the detection of circulating excretory/secretory antigens, two of the three calves which harboured viable cysts (2 and 122 cysts) after 54 weeks of infection could be detected for a long period during the later phase of the infection (Fig 3). This 'late' detection may be ascribed to the use of mAbs raised against antigens secreted by 10-week-old *T saginata* metacestodes (Brandt et al 1992), thereby indicating the presence of stage-specific antigens. Evidence for stage-specific antigens was previously reported for the closely related *T taeniaeformis* (Bøgh et al 1988, Bøgh 1989). This would also explain why seven of the other calves were not detected earlier in the infection despite harbouring viable cysts. The detection of circulating excretory/secretory antigens in calf 122, which harboured only two viable cysts, is unusual because the previously reported detection level was 88 viable cysts (Brandt et al 1992). However, in the present study, a modified method was used, including a combination of four monoclonal antibodies [12G5, 158C11 (trapping) and 2H8, 153G7 (indicators)] instead of two. This combination may have increased the sensitivity of the test and thus the antigen detection level in the two calves. In addition, the modified technique may be useful for studies of the capture of antigen from the serum of these animals, by identifying the structure of the hidden or complex-bound antigen present in the other calves which resulted in a lower sensitivity. The sensitivity of the tests used in the present study needs to be improved and they need to be further evaluated before they can be applied to routine diagnosis or seroepidemiological field studies.

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