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Veterinary Parasitology 76 (1998) 269–274

veterinary parasitology

Improved detection of circulating antigen in cattle infected with *Taenia saginata* metacestodes

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Received 9 July 1997; accepted 18 November 1997

Abstract

In order to improve the sensitivity and the specificity of an existing IgM monoclonal antibody-based ELISA (Brandt et al., 1992) for the detection of circulating antigen in the sera of cattle infected with *T. saginata* metacestodes, a modified sandwich ELISA was developed. Monoclonal antibodies (MAbs) of the IgG isotype were produced against the excretory–secretory (ES)-products of *T. saginata* metacestodes. Since it was shown that the affinity of these IgG MAbs for ES antigen was higher than that of the IgM MAbs, the latter were replaced by two IgG1 MAbs (158C11 and 60H8). Furthermore, heat treatment of the sera significantly increased the OD-values of ES-spiked serum samples as compared to nontreated samples. It also decreased the number of false positive reactions. When the original IgM MAb-based ELISA was compared with the IgG MAb-based ELISA using heat treated sera from animals harbouring more than 50 living metacestodes of *T. saginata*, the sensitivity increased from 56% with the former to 92% with the latter assay. Only a small percentage of animals carrying less than 50 cysts were detected both with the ELISA using IgG or IgM MAbs. The specificity of the IgM- and IgG MAb-based ELISAs was 93.4% and 98.7% respectively. © 1998 Elsevier Science B.V.

Keywords: *Taenia saginata*; ELISA; Circulating antigen; Cattle; Diagnosis; Monoclonal antibodies; Cysticercosis

1. Introduction

Promising results have been obtained using a monoclonal antibody (MAb)-based ELISA for the detection of circulating excretory–secretory (ES) products in *Taenia saginata* cysticercosis (Brandt et al., 1992; Draelants et al., 1995; Harrison et al., 1989;

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PII S0304-4017(97)00226-4

Onyangoabuje et al., 1996). The MAbs which were used, however, were all of the IgM isotype. It is generally accepted that immunoglobulins of the IgM class are less stable than IgG. Furthermore, using the ELISA as described by Brandt et al. (1992), some nonspecific reactions were observed, especially with sera from animals or man infected with parasites, which stimulate a polyclonal B-cell activation such as trypanosomes (unpublished data). Since it was shown that IgG MAbs had a higher affinity for ES antigen of *T. saginata* metacestodes than IgM MAbs, a modified sandwich ELISA was developed using IgG MAbs in order to improve the sensitivity and specificity of antigen detection in bovine cysticercosis.

2. Materials and methods

2.1. Monoclonal antibodies (MAbs)

Eight different MAbs were produced against the excretory–secretory (ES) products of *T. saginata* metacestodes as described by Brandt et al. (1992). During preliminary trials, different immunisation procedures were followed in order to produce MAbs of the IgG isotype. Most MAbs obtained, however, were of the IgM isotype. Four IgM MAbs were used in this experiment: 12G5, 2H8, 41E1 and 3E3. Eventually, IgG MAbs were produced by intrasplenic injection of ES-antigen according to the method described by Spitz et al. (1984). Four IgG MAbs were selected: 158C11, 60H8 and 56D3 belonging to the IgG1 isotype and 59G12 belonging to the IgG2b isotype. Mouse ascites was purified using ammonium sulphate precipitation. Biotinylation of the IgG MAb's was carried out according to the protocol described by Guesdon et al. (1979).

2.2. Affinity of MAbs for ES-antigen

The affinity of four IgM and four IgG MAbs for ES of *T. saginata* metacestodes was compared using an indirect ELISA. The antigen was prepared as described by Brandt et al. (1992) and used for coating of the microtitre plates at a concentration of 5 ng/ml in carbonate buffer (pH 9.6). After blocking by PBS–0.05% Tween-20 containing 1% NBCS (newborn-calf serum) a serial dilution of the MAbs was added. The conjugate was a rabbit anti-mouse (RaM)-IgM– or RaM-IgG–peroxidase (Dako) 1/1000 diluted and orthophenylene diamine (OPD) was used as substrate.

2.3. Serum samples

The serum samples were derived from calves ($n = 72$) experimentally infected with different doses of *T. saginata* eggs (Geerts et al., 1981; Brandt et al., 1992; Bogh et al., 1996). At post mortem, numbers of dead and living metacestodes were estimated by slicing half of the carcass muscles and the complete organs of the infected animals. Thirty preinfection serum samples from these animals were also included. Reference negative serum samples were derived from calves ($n = 8$), which had been reared indoors from birth and never came on pasture. Furthermore serum samples were used

Table 1
Comparison of the sensitivity and the specificity of an ELISA using IgG- or IgM-MAbs for the detection of circulating antigen in bovine cysticercosis

	No. positive/No. tested using	
	12G5-2H8 (IgM)	158C11-60H8 (IgG)
Experimentally infected cattle		
< 50 living cysts	2/47	6/47
> 50 living cysts	14/25	23/25
Heterologous infection	0/46	1/46
Uninfected cattle		
Preinfection sera	5/30	0/30

from calves or adult cattle (mainly *Bos taurus*) experimentally or naturally infected with *Fasciola hepatica* ($n = 7$), *Ostertagia ostertagi* ($n = 6$), *Cooperia* spp. ($n = 8$), *Dictyocaulus viviparus* ($n = 5$), *Theileria parva* ($n = 3$), *Babesia bigemina* ($n = 2$) and *Trypanosoma theileri* ($n = 4$), *T. congolense* ($n = 8$), *T. brucei* ($n = 1$) and *T. vivax* ($n = 2$). These are referred to as heterologous infections (Table 1).

2.4. Heat treatment of the serum samples

In a preliminary study untreated serum samples were compared with heat-treated samples. Heat treatment was done in a warm-water bath at 56°C during 30 min or at 100°C during 15 min, after which the samples were centrifuged at $9650 \times g$ for 5 min and the supernatant recovered. Since the latter treatment resulted in less false-positive reactions than the former, it was used for all tested sera in this study.

2.5. Antigen-ELISA

The following definitive protocol of antigen-ELISA was used. Polystyrene ELISA plates (Nunc® Maxisorb) were coated with 100 μl of the MAb 158C11 (5 μg/ml in carbonate buffer, pH 9.6). Plates were incubated for 1 h at 37°C and overnight at 4°C, washed with PBS + 0.05% Tween 20 (PBS–T20) (five washing cycles in a semiautomatic device) and then blocked with 250 μl of 1% (v/v) NCBS in PBS–T20 for 1 h at 37°C. The test sera diluted 1/4 in PBS–T20 containing 1% NBCS were added and incubated for 1 h at 37°C. The plates were washed as above and 100 μl of biotinylated MAb 60H8 at 1.25 μg/ml in PBS–T20 +1% NBCS was added to each well. After incubation for 1 h at 37°C the plates were washed again and 100 μl of extravidin–horseradish peroxidase (Sigma, No. E2886) diluted 1/1000 in PBS–T20 + 1% NBCS was added and incubated for 1 h. The plates were washed and finally the substrate solution, consisting of OPD (Dako, No. S2000) and H_2O_2, was added. After incubation for 20 min at room temperature, 50 μl of 4 N H2SO4 was added to stop the reaction and the plate was read using an automated spectophotometer (Titertek Multiskan EIA reader) at 490 and 630 nm. The cutoff level was calculated by comparing the optical

density (OD) of each sample with the mean of a series of eight negative samples at a probability level of $P = 0.001$ (Sokal and Rohlf, 1981).

3. Results and discussion

The majority of the ES-antigens of *T. saginata* cysticerci are glycoproteins which, according to Joshua et al. (1988), tend to give rise to antibodies mainly of the IgM isotype. By immunising mice using intrasplenic injection of the antigens, however, it was possible to produce a higher number of MAbs of the IgG isotype.

Fig. 1 clearly shows that MAbs of the IgG isotype have a higher affinity for ES antigen than MAbs of the IgM isotype. In order to identify the optimal combination of IgG MAbs for the double antibody sandwich ELISA, serial dilutions of the four MAbs as trapping antibodies were combined with the homologous and the three heterologous biotinylated detector IgG MAbs in order to detect a given concentration of ES in spiked samples. MAb 158C11 at 5 μg/ml, which performed best as trapping MAb was then combined with serial concentrations of each of the four biotinylated IgG MAbs. A combination of 158C11 at 5 μg/ml (in carbonate buffer, pH 9.6) and biotinylated 60H8 at 1.25 μg/ml (in PBS–T20 + 1% NBCS) gave the best results.

The effect of heat treatment on the sensitivity and the specificity of the test was evaluated by comparing the OD values of treated and untreated negative serum samples, to which ES antigen was added as well as a series of serum samples of animals with heterologous infections. Previously, it has been shown by Draelants et al. (1995) that the epitopes, with which the IgG- and IgM MAbs react, were not destroyed by heat treatment. Fig. 2 shows that the OD values of heat-treated spiked samples were about four times as high as the untreated samples. Out of 15 untreated serum samples from

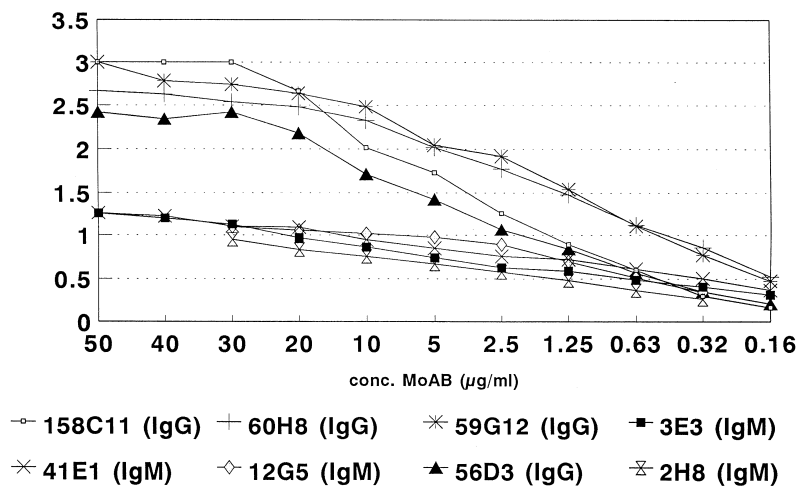

Fig. 1. Affinity of monoclonal antibodies of the IgG (56D3, 59G12, 60H8, 158C11) and the IgM (3E3, 41E1, 12G5, 2H8) isotype for ES antigen of *T. saginata* metacestodes (indirect ELISA).

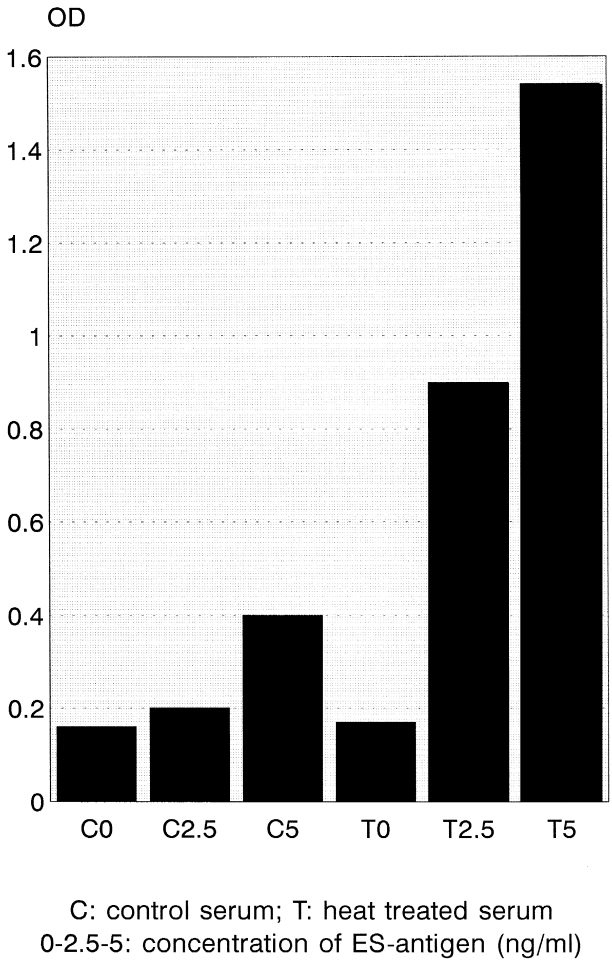

Fig. 2. Comparison of OD values of control and heat-treated serum samples spiked with ES antigen of *T. saginata* metacestodes (sandwich ELISA using IgG MAbs 158C11 and 59G12).

heterologous infections 8 gave a false-positive reaction. Heat treatment reduced the number of false positives to three (data not shown).

Finally the sensitivity and specificity of the sandwich ELISA using the optimal combination of IgG MAbs (158C11-60H8) was compared with the combination of IgM MAbs (12G5-2H8) as described by Brandt et al. (1992) using heat-treated serum samples. The results are summarized in Table 1. Using the IgG MAbs combination a clearcut increase of the sensitivity is obtained, especially with the sera of animals harbouring more than 50 living metacestodes, where the sensitivity increased from 56 to 92%. Only a small fraction of animals carrying less than 50 cysts were detected as well with the combination of IgM as IgG MAbs. The specificity on the other hand only slightly increased from 93.4% (5/76) with IgM to 98.7% (1/76) with IgG MAbs.

It can be concluded that the detection of circulating antigen in bovine cysticercosis is significantly improved by using an IgG-MAb-based ELISA. Other advantages of IgG over IgM were their easier biotinylation and better stability. Further trials are currently in progress to evaluate the performance of the IgG-ELISA for antigen detection in naturally infected bovines.

Acknowledgements

This study was financially supported by the IVK (Instituut voor Veterinaire Keuring), Brussels, Belgium.

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