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Evaluation of an Enzyme-Linked Immunosorbent Assay (ELISA) for Detection of *Taenia saginata* Cysticercosis in Cattle

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Kyvsgaard, N. C., B. Ilsøe, Sv. Aa. Henriksen, N. C. Feld and P. Nansen: Evaluation of an enzyme-linked immunosorbent assay (ELISA) for detection of *Taenia saginata* cysticercosis in cattle. Acta vet. scand. 1991, 32, 233–241. – Serum IgG response of cattle with cysticercosis caused by *Taenia saginata* was studied in an enzyme-linked immunosorbent assay (ELISA) where a *T. saginata* metacestode surface extract was used as antigen. In experimentally infected calves, a sharp rise in specific antibody levels was found 3–4 weeks after the infection followed by a slow decrease. Preinfection levels were reached 2.5 years after infection. The serological level of detection corresponded to about 25 cysts. The ELISA was employed in cattle herds where cysticercosis outbreaks had occurred and also in supposedly uninfected herds. Significantly increased antibody levels were found in the herds with massive cysticercosis cases. The test was not adapted for individual diagnosis as some animals of the uninfected herds, especially within the older age groups, had elevated antibody values. The ELISA was, however, useful in the investigation of outbreaks to determine the extent and pattern of the infection in the herd. The rate of decline in antibody levels in these herds was studied by follow up sampling. The increased antibody levels in the infected herds were also reflected in colostrum-fed calves. This observation was employed to estimate the time of infection.

serological detection; serum IgG; metacestode surface antigen; herd level.

Introduction

Currently, routine diagnosis for bovine *Taenia saginata* cysticercosis is based on visual examination following slaughter. However, the sensitivity of the conventional meat inspection has been found to be low in light infections (*Kyvsgaard et al.* 1989). Therefore, a reliable in vivo test could serve as an alternative test in the abattoir and for epidemiological investigations of outbreaks.

Several immunological tests have been developed to detect *T. saginata* cysticercosis in cattle (*WHO* 1983). Of these methods the enzyme-linked immunosorbent assay (ELISA) is considered to be one of the most suitable techniques for routine laboratory diagnosis as it is readily automated. As diagnostic antigens in ELISA, a number of different homologous or heterologous preparations have been evaluated, e.g. crude extract of homogenized adult *T. saginata* (*Craig & Rickard* 1980), excretory-secretory antigen produced by in vitro cultivation of *T. saginata* metacestodes (*Harrison & Sewell* 1981a), *T. crassiceps* crude antigen (*Geerts et al.* 1981), a 70 % ammonium sulphate soluble fraction of *T. hydatigena* cyst fluid (*Kamanga-Sollo et al.* 1987), and detergent

extract of *T. saginata* metacestode surface antigens (*Harrison et al.* 1989). In most of these studies an indirect ELISA, detecting IgG antibodies, has been employed.

The purpose of the present work was to evaluate the use of an ELISA in experimentally infected calves and in investigations of cysticercosis outbreaks in cattle herds. The antigen preparation employed was an extract of surface antigens from *T. saginata* metacestodes (*Gibbens et al.* 1986, *Harrison et al.* 1989).

Materials and methods

Experimentally infected calves

Blood samples were collected weekly from 15 calves 2 to 6 months old when given a single dose of 11–12.000 *T. saginata* eggs orally. Six calves (referred to as positive controls: a–c and A–C, respectively) were given fresh eggs, whereas 9 calves (d–l) were given eggs from the same batch after the eggs had been exposed to the influence of natural climatic conditions on the soil surface through 13 weeks (d–f), 29 weeks (g–i), or 42 weeks (j–l) (*Ilsøe et al.* 1990a). Twelve calves (a–l) were slaughtered 10–13 weeks after the infection (p.i.), 2 calves 1 year p.i. (A and B), and 1 animal 3.5 years p.i. (C). The number of cysticerci in each animal was determined by thorough slicing of the musculature.

To evaluate potential cross reaction to other helminth infections, sera were obtained from calves with experimental monospecific infections with *Ostertagia ostertagi* (n = 11), *Fasciola hepatica* (n = 2) or *Schistosoma bovis* (n = 1). The age of the calves at sampling was between 6 months and 1 year.

Naturally infected herds and control herds

Blood samples were obtained from 7 herds, 3 of which (herd H, J, and N) had a history of cattle being condemned at slaughter due to massive cysticercosis, i.e. more than 10 cysts had been found on the 'predilection sites': Heart, masseter muscles, diaphragm and tongue. For a description of 2 of these outbreaks (H and J) see *Ilsøe et al.* (1990b).

Herd H was a dairy herd comprising 60 cows and a total of 130 cattle. Of 5 bull calves delivered to slaughter in October 1987, 3 were condemned due to massive cysticercosis and 1 was downgraded because of light infection. No cases were found among animals slaughtered later on, but 1 cow which died accidentially in February 1988 harboured several cysts in the heart. Blood samples were taken from the whole herd in October 1987 and in November 1987, from the calves in January 1988, and from randomly selected animals of all age groups in March 1989.

Herd J, a dairy herd of 90 cows and a total of 200 cattle, experienced a total of 29 animals being condemned or downgraded from July 1986 through 1989. During the first year, both cows and bull-calves were found infected at slaughter, but later on only culled cows were found to be infected. Blood samples were obtained from all cows in October 1986 and from the whole herd in June 1988.

Herd N was a dairy herd with 50 cows and a total of 150 cattle. During January and February 1988 3 bull-calves were condemned and a further 2 calves were downgraded. Condemnations ceased after this time. Blood samples were collected from the whole herd in March 1988.

The last 4 herds (referred to as A, B, C, and D) had no history of bovine cysticercosis.

Antigen preparation and ELISA procedure

The diagnostic antigen was prepared as described by *Gibbens et al.* (1986) from fully developed *T. saginata* metacestodes by extraction of surface antigens by the detergent N-octyl β-d-glucopyranoside.

Specific IgG antibodies were detected in an indirect ELISA: Flat bottomed polystyrene plates (Nunc Maxisorp 4-42404) were coated over-night at 4°C with 100 µl antigen diluted to 1.0 µg protein/ml in 0.1 mol/l-carbonate buffer pH 9.6, per well. The plates were tapped dry and blocked for 1 h at room-temperature (RT) with dilution-buffer which is washing buffer (PBS-T: 0.01 mol/l phosphate pH 7.2, 0.5 mol/l NaCl, 0.1 % Tween-20) supplemented with 1 % gelatine (Difco). Plates were washed 5 times with washing-buffer. This procedure was used for all subsequent washing cycles. A 100 µl volume of testserum diluted 1:160 in dilution-buffer was added to each of 2 adjoining wells and positive and negative control sera to each of 4 adjoining wells. After 2 h incubation at RT the plates were washed and incubated for 1 h at RT with 100 µl per well of peroxidase conjugated purified goat antibody to bovine IgG (γ) (Kirkegaard & Perry Inc.) diluted 1:2000 in dilution buffer. After a final wash, 100 µl of enzyme sustrate (0.05 mol/l citrate buffer pH 5.0, with orthophenylene-diamine-dihydrochloride [0.6 mg/ml] and 30 % hydrogenperoxide [0.5 µl/ml]) were added per well. Colour development was stopped after 15 min with 100 µl per well of 0.5 mol/l H_2SO_4 and the spectrophotometric absorption was read at 490 nm with 650 nm as reference. A relative titer value of each sample was calculated by expressing the mean absorption as a percentage of the mean titer value of the positive controls of the experimentally infected calves.

Results

Experimental calves

In the majority of the heavily infected calves, a sharp rise in specific antibody values was found 3 to 4 weeks after the infection (p.i.) (Fig. 1). In 1 calf (calf c) the rise was found to occur as early as within 1 week p.i. A moderate but distinct increase in titer was found in a calf harbouring only 26 cysts at slaughter 10 weeks p.i. (calf i). The absolute peak titer of this calf was, however, lower than the preinfection values of some of the other calves. A minor response was seen in a calf with 22 cysts (h), whereas no increase was registrated in a calf with 2 cysts (g). Peak values were reached from 5 weeks to 5 months p.i. (based on results from 6 calves) and followed by a slow decrease. In 1 heavily infected animal the titer had decreased to pre-infection level after 2.5 years (C).

No significant cross-reactions were found in the calves with other helminth infection. Three *O. ostertagi* infected calves had values at 25 to 32 % of the positive controls whereas the rest (11 calves) had values below 15 %.

Naturally infected herds and control herds

In the 4 herds, which were assumed to be uninfected, low antibody levels were found among the calves whereas higher values were noted in older cattle (Fig. 2 and Table 1). Within each age group most animals had titers close to the median of the group but a few animals had considerably higher reactions.

In samples taken from herd H immediately after the first animals had been found infected (Fig. 3 and Table 1) very high antibody levels were noted in all categories of animals. The values of the cows and yearlings had decreased moderately in the samples taken 1 month later (Table 1). The distribution of antibody values in the herd 1.5 years after the outbreak did not differ from that of the uninfected herds.

The youngest calves in herd H were tested 3 times during a 3 month period immediately after the detection of the outbreak (Fig. 4). The 4 youngest calves, between 1 day and 1

months of age, were found to have highly elevated antibody values in the first tests. The values of these calves decreased after 1 month and a further decrease was noted following an additional period of 2 months. The antibody levels of those calves, that were under 1 month of age when tested, were found to have decreased at the second testing and further at the third, where all values were below 12 % of the positive control.

In the first testing of herd J, blood samples were only taken from the cows. Highly elevated antibody values were found in this group (Fig. 3). Two years later the antibody values in the herd had decreased considerably. Thirty cows were tested at both occasions. The antibody values had decreased in 29 of these. The mean value of the 30 cows had decreased by 60 % from the first to the second testing.

In herd N, increased antibody values were found only in the section of the stable where the condemned animals had been kept (Fig. 3).

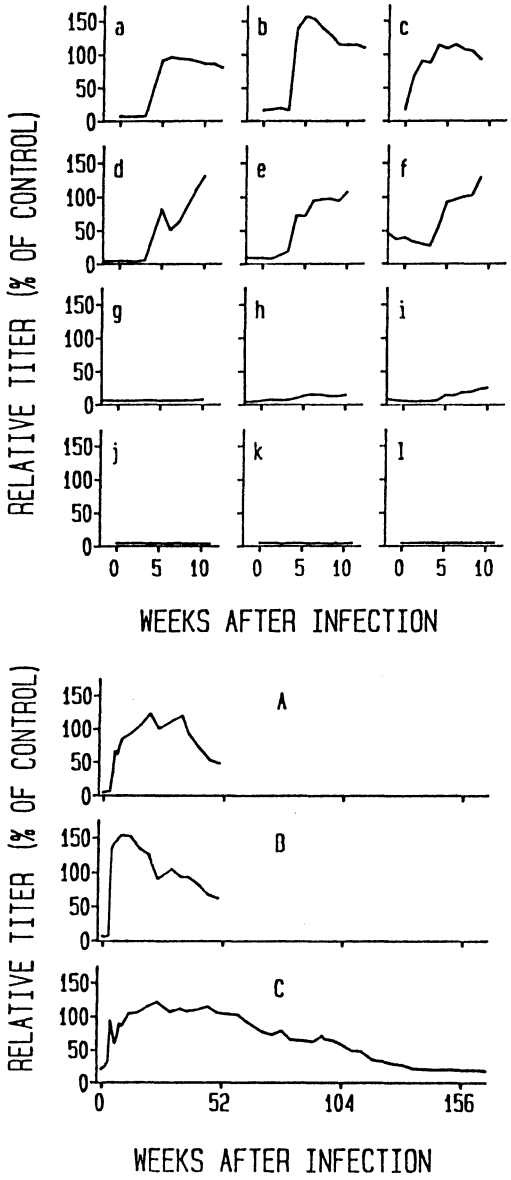

Figure 1. Progressive changes in specific antibody values of 15 calves experimentally infected with 11–12.000 *T. saginata* eggs with different viability.

The calves a–c and A–C were fed fresh eggs, whereas the rest were fed eggs that had been exposed on the soil surface for 13 (d–f), 29 (g–i), or 42 (j–l) weeks, respectively

The number of cysts (viable or degenerated) found at slaughter was:

a) 608, b) 1061, c) 1683,
d) 580, e) 496, f) 240,
g) 2, h) 22, i) 26,
j) 0, k) 0, l) 0.

The calves A, B, and C harboured degenerated cysts only:

A) 254, B) 605, C) 90.

Discussion

The progressive changes in IgG antibody values of calves experimentally infected with *T. saginata* eggs found in the present work were largely consistent with the findings of *Harrison & Sewell* (1981b) and of *Kamanga-Sollo et al.* (1987). As the present assay

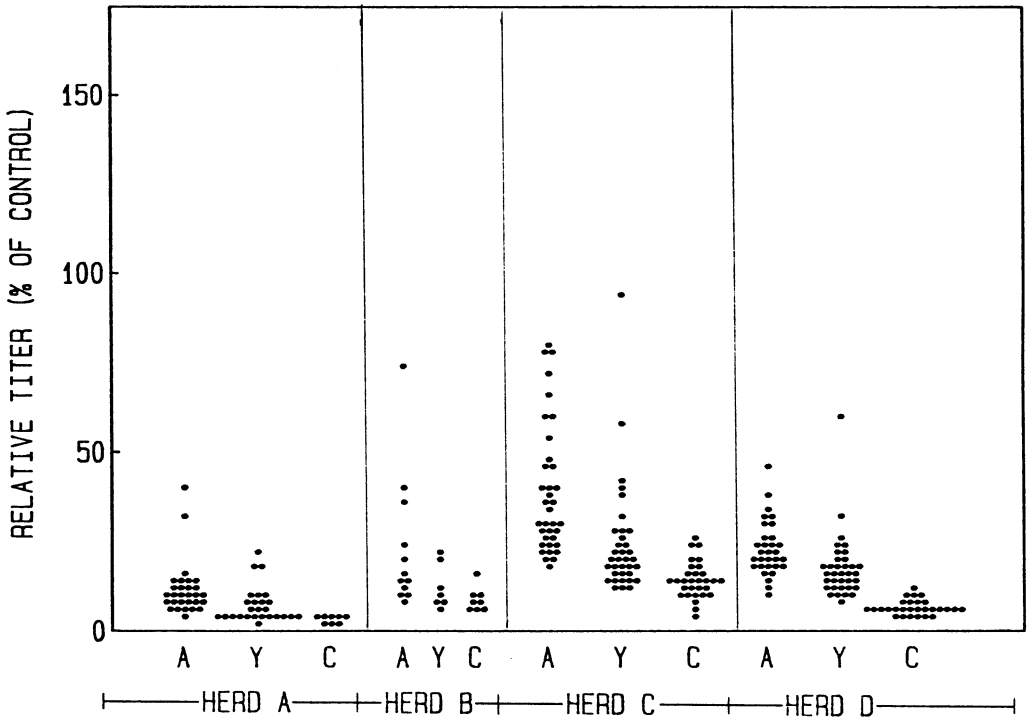

Figure 2. Antibody values of 4 herds that had no history of cysticercosis.
A) Adults, mostly cows
Y) Yearlings, heifers and bull-calves
C) Calves

Table 1. Mean and range of relative ELISA values in infected and supposedly uninfected herds. Each age group of the infected herds was compared to the pool of the corresponding group of the uninfected herds by the Mann-Whitney test.
(* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Herd	Relative ELISA values (%) mean (range)		
	Cows	Yearlings	Calves
Infected:			
H 14 Oct 87	94 (26–170)***	74 (10–156)***	27 (2–82)
H 18 Nov 87	69 (26–150)***	70 (11–140)***	22 (5–70)***
H 6 Mar 89	12 (5– 27)	9 (3– 64)	4 (2–15)
J 13 Oct 86	79 (14–174)***	NT	NT
J 15 Jun 88	28 (6–103)	19 (5– 70)	11 (4–39)
N 10 Mar 88	20 (10– 59)	26 (6– 94)**	9 (5–20)
Uninfected:			
A	12 (4– 40)	7 (2– 22)	3 (2– 4)
B	24 (8– 73)	12 (6– 21)	9 (5–16)
C	39 (17– 79)	24 (11– 95)	14 (4–27)
D	23 (10– 47)	18 (9– 60)	6 (4–12)

NT: Not tested

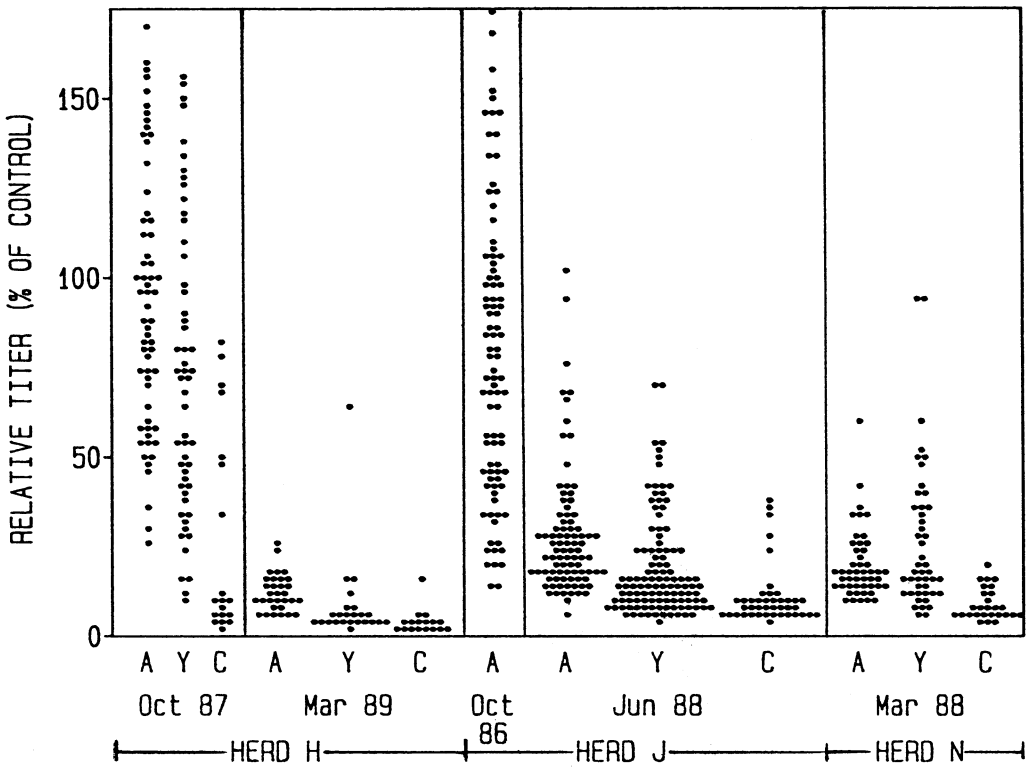

Figure 3. Antibody values of 3 herds with outbreaks of cysticercosis. Two of the herds were tested immediately after the start of the outbreak and again 1.5 years after.
A) Adults, mostly cows
Y) Yearlings, heifers and bull-calves
C) Calves

detected IgG only, and as the IgG/IgM ratio is expected to be low early during the course of the infection, an early IgG response could have been masked by IgM antibodies competing for the same epitopes of the diagnostic antigen. The magnitude of the antibody response was found to be roughly correlated to the number of cysts found at slaughter. In lightly infected calves a moderate but distinct rise in antibody values was found. Senescent eggs present in the older of the egg doses given could have immunized these calves without developing into cysts (*Gemmell & Johnstone* 1977).

The ELISA was found sensitive to detect an increase in titer when repeated samples were taken from experimentally infected calves. The main problem in using the test for individual diagnosis is its specificity. Background antibody levels were found low in young calves, but increased in the older age groups of the uninfected control herds. These reactions are probably caused by cross-reacting antibodies aquired during other infections whether parasitic or bacterial. No cross-reactions were found in the present material in 14 calves with other helminth infections, but in another study *T. saginata*

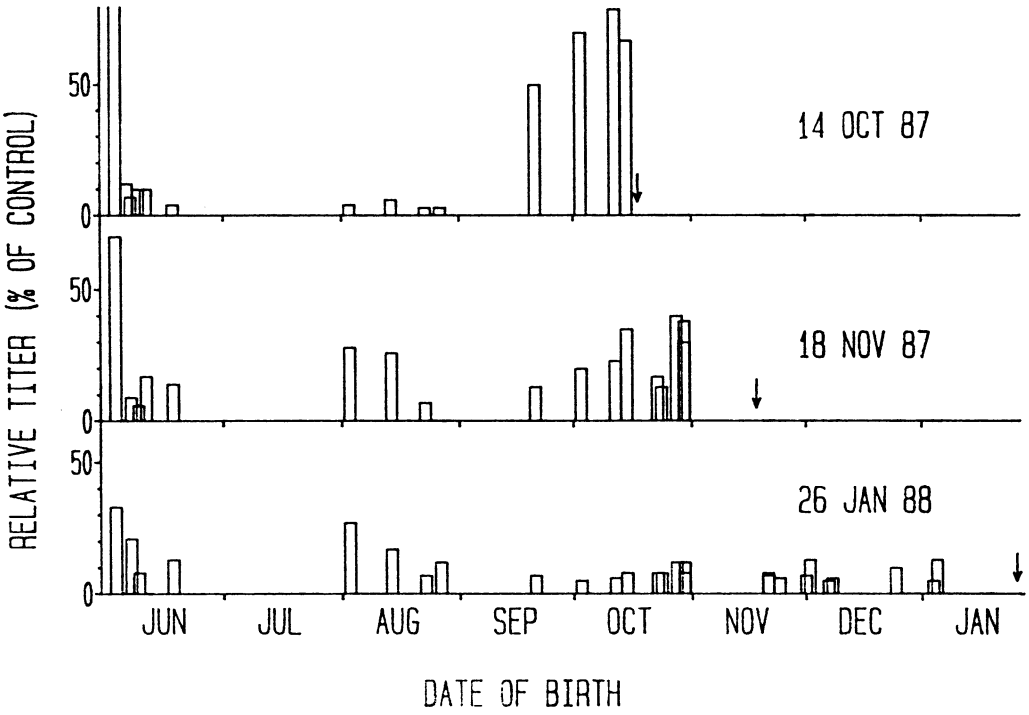

Figure 4. Antibody values of the youngest calves in herd H in relation to their date of birth. Samples were taken at 3 occasions indicated by arrows.

antigens were found to be shared with other helminths commonly found in cattle (*Onyango-Abuje et al.* 1989). Also, cross-reacting antibodies have been demonstrated in ELISA of sera from *F. hepatica* and *T. hydatigena* infected cattle (*Craig & Rickard* 1980) and in sera from *F. gigantica* infected cattle (*Harrison et al.* 1989).

The assay was found useful in cysticercosis outbreaks to determine the extent and pattern of infection within the herd. The ELISA results of herd J and N were in accordance with the findings at meat inspection, indicating extensive infection in herd J and limited infection in herd N. In herd H the ELISA probably gave a better indication of the extent of infection than the meat inspection. Increased titers were found to be widespread whereas condemnations at slaughter were limited to 1 group of cattle.

The decline in specific antibody levels in cattle from the infected herds was comparable with the findings in the experimentally infected calves. The cysts seem to degenerate prior to the disappearance of antibodies, but degenerative cysts may persist longer than antibodies. The overall decline of antibody levels in the herds was caused partly by a decline in the titer of the individual animal and partly by the introduction of animals born after the start of the outbreak, indicating that the infection source had been eliminated.

In 1 infected herd elevated antibody levels were also found among the youngest calves. The reaction observed as early as at 1 day of

age, and the decline of this reaction during the following 3 months, strongly suggests that it was caused by colostrally transmitted immunoglobulins. IgG_1 is the major class of immunoglobulin in bovine colostrum (*Murphy et al.* 1964, *Brandon et al.* 1971). The actual titer of the calf will depend on many variables as e.g. the volume of colostrum actually ingested. The pattern of reactions in the youngest calves might be employed for determination of the time of infection in a given herd. Abscence of specific antibodies in calves below the age, where colostral antibodies would be completely metabolized, could indicate that these calves were born before the potentially infected cows had started excretion of antibodies in colostrum.

Although the ELISA used was not suited for individual diagnosis, it was found to give valuable information during herd outbreaks of cysticercosis. For the interpretation of the results, the age related background reactions of non-infected herds should be kept in mind. Further work on serological tests for bovine cysticercosis should primarily aim at improving the specificity of the test. This may be achieved by the use of monoclonal antibodies to purify the antigen (*Nascimiento et al.* 1987) or to detect circulating parasite products (*Harrison et al.* 1989).

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Sammendrag

Vurdering af en ELISA-metode til påvisning af Taenia saginata hos kvæg.

Ved hjælp af en ELISA-teknik blev IgG-antistofrespons målt i serum fra kvæg med bovin cysticercose. Som antigen anvendtes et *Taenia saginata* metacestode overfladeekstrakt.

I eksperimentelt inficerede kalve sås efter 3–4 uger en markant stigning i titeren af specifikt antistof, efterfulgt af et langsomt fald, hvorved præ-infektionsniveau blev nået 2 1/2 år p.i. Den serologiske detektionsgrænse svarede til et infektionsniveau på omkring 25 tinter/dyr.

ELISA-testen blev afprøvet i besætninger med udbrud af bovin cysticercose, samt besætninger, hvor der ikke var påvist cysticercose i slagtedyr. Testen blev ikke fundet brugbar til individuel diagnostik i besætninger med ukendt smittestatus, da detektionsgrænsen var for høj, og fordi der i formodede uinficerede besætninger fandtes enkelte dyr med forhøjede antistoftitre, især blandt ældre dyr.

Signifikant forhøjet antistofniveau blev påvist i sera fra besætninger med massive cystecercoseudbrud ("tintestorme"), og her fandtes ELISA-testen egnet til at kortlægge smittemønstre og -omfang på besætningsplan. I eet tilfælde blev testen brugt til at fastslå smittetidspunktet, da der fandtes forhøjede titre i kolostrumfodrede kalve født efter en bestemt dato.

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