International Journal for Parasitology 43 (2013) 853–859

Contents lists available at SciVerse ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Multi-test analysis and model-based estimation of the prevalence of *Taenia saginata* cysticercus infection in naturally infected dairy cows in the absence of a 'gold standard' reference test

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ARTICLE INFO

Article history:
Received 12 February 2013
Received in revised form 17 May 2013
Accepted 20 May 2013
Available online 3 July 2013

Keywords:
Taenia saginata
Bovine cysticercosis
Bayesian
Diagnostic
Meat inspection
Prevalence
Epidemiology
Test analysis

ABSTRACT

The diagnostic values of seven serological tests (ELISAs) and of the obligatory European Union-approved routine visual meat inspection for the detection of *Taenia saginata* cysticercosis were investigated. A total of 793 slaughtered dairy cows were selected in three European Union approved abattoirs in Switzerland, an endemic area (apparent prevalence by enhanced meat inspection up to 4.5%) with typically low parasite burdens. ELISAs based on a somatic larval antigen, isoelectric focused somatic larval antigen, larval excretory/secretory antigens, peptide HP6-2, peptide *Ts*45S-10, pooled peptide solution and a monoclonal antibody antigen capture assay were initially screened. As there is no perfect diagnostic 'gold standard' reference test, the obligatory meat inspection and four selected serological tests were further analysed using Bayesian inference to estimate the "true" prevalence and the diagnostic test sensitivities and specificities. The ELISA for specific antibody detection based on excretory/secretory antigens showed highest sensitivity and specificity with 81.6% (95% credible interval: 70–92) and 96.3% (95% credible interval: 94–99), respectively. The Bayesian model estimated the specificity of the ELISA, based on the synthetic peptide *Ts*45S-10 as 55.2% (95% credible interval: 46–65) and sensitivity as 84.7% (95% credible interval: 82–88). The sensitivity of the ELISA based on mAbs, detecting circulating antigen, was 14.3% (95% credible interval: 9–23) with a specificity of 93.7% (95% credible interval: 92–96). The diagnostic sensitivity of the obligatory standard European Union meat inspection procedure for the detection of *T. saginata* cysticercus infection at the abattoir was estimated to be 15.6% (95% credible interval: 10–23). Based on these data, the modelled prevalence of cysticercosis in dairy cows presented at abattoirs in Switzerland was estimated to be 16.5% (95% credible interval: 13–21). These cattle also had a high prevalence of infection with *Dicrocoelium dendriticum* (60.8%) and *Fasciola hepatica* (13.5%).

1. Introduction

Bovine cysticercosis is caused by the larval infection of the zoonotic tapeworm *Taenia saginata*. Humans are the only definitive host and are infected by consumption of cysts from poorly cooked beef. *Taenia saginata* cysticercosis and taeniosis have a worldwide distribution (Murrell, 2005). Based on abattoir reports, prevalences in cattle of between 0.007% and 6.8% have been reported for different countries in Europe (Anonymous, 2005; Dorny and Praet, 2007).

To prevent human infection, the obligatory standard European Union (EU) meat inspection procedure (EC directive N° 854/2004) represents the only control measure in many European countries. Common routine meat inspection is based on palpation and diagnostic incisions of defined muscles, followed by the search for parasitic lesions localised on superficial and cut surfaces of the inspected carcass (Murrell, 2005). The sensitivity of the current routine meat inspection procedure has been estimated at between 10% and 30% (Dewhirst et al., 1967; McCool, 1979; Geerts et al., 1980; Walther and Koske, 1980; Hayunga et al., 1991; Dorny et al., 2000; Murrell, 2005; Eichenberger et al., 2011). The current practice has a substantial economic impact on agriculture through downgrading the value or condemnation of infected carcasses.

Based on the observation of pronounced antibody mediated immunity following taeniid infection, various ELISAs have been developed for the sero-diagnosis of bovine cysticercosis, based on somatic "crude" worm and larval antigens (Craig and Rickard, 1980; Kyvsgaard et al., 1991), homologous and heterologous antigen preparations (Geerts et al., 1981; Harrison and Sewell, 1981a; Smith et al., 1990), excretory/secretory (ES) products from bovine *T. saginata* or murine *Taenia crassiceps* metacestodes (Harrison and

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0020-7519/$36.00
http://dx.doi.org/10.1016/j.ijpara.2013.05.011

Sewell, 1981b; Joshua et al., 1988; Ogunremi and Benjamin, 2010), and synthetic or recombinant peptides from potentially protective immuno-dominant determinants (Harrison et al., 1989; Ferrer et al., 2003; Abuseir et al., 2007). Furthermore, assays for the detection of circulating parasite antigens using monoclonal antibodies (mAbs) against ES products of metacestodes are also available (Harrison et al., 1989; Brandt et al., 1992; Van Kerckhoven et al., 1998).

Sero-epidemiological studies have suggested prevalences of 1.11%, 3.09% and 8.83% for north-eastern Spain, Belgium and Lower Saxony in Germany, respectively (Dorny et al., 2000; Abuseir et al., 2010; Allepuz et al., 2012). Sensitivity and specificity are population-specific parameters that vary not only between natural and experimentally infected animals but between different naturally infected populations. To overcome this limitation in diagnostic test evaluation, multiple tests can be used in parallel on a group of animals and an estimate of prevalence and test characteristics can be made. Such an approach within a Bayesian statistical framework has been applied for test evaluation in porcine *Taenia solium* cysticercosis (Dorny et al., 2004).

The present study was designed to compare and evaluate the diagnostic test characteristics of available ELISAs for the serological diagnosis of *T. saginata* cysticercosis in slaughtered cows. A Bayesian approach was used to estimate the prevalence in cows presented at abattoirs in Switzerland and to estimate the sensitivity of the obligatory standard EU meat inspection procedure for the detection of *T. saginata* cysticercus infection.

2. Materials and methods

2.1. Animals and infection status

Sera were collected from three groups (1–3) of cattle. Serum samples were stored at −20 °C until they were tested.

For Group 1, 61 serum samples were collected from dairy cows kept on three average Swiss farms without any history of *T. saginata* cysticercosis. Farm history was followed up to confirm that cysticercosis had never been observed in any slaughtered cattle from these farms for at least 10 years. These presumed infection-free animals provided material to determine the negative/positive threshold (cut-off) for each antibody ELISA.

For Group 2, 793 serum samples were collected from dairy cows at Swiss abattoirs. Of these, 53 samples were collected from carcasses positive for *T. saginata* cysticercosis confirmed during standard or further enhanced meat inspection (Eichenberger et al., 2011). The further enhanced meat inspection protocol incorporated additional morphological or molecular analysis of lesions using PCR primer Cest3/5 according to Trachsel et al. (2007). Of this group, 775 samples were randomly selected during routine meat inspection. A further 18 confirmed positive samples were added for evaluation of the preliminary test performance (Table 1). These 18 samples were not included in the Bayesian analysis as they would have introduced sampling bias. To obtain data regarding liver fluke infection, bile was taken from gall bladders from 449 randomly selected and inspected carcasses from Group 2 animals. Bile samples were examined for eggs of liver flukes using a washing and sedimentation technique (Rapsch et al., 2006).

Sera from nine experimentally infected animals previously used by Abuseir et al. (2007), kindly provided by Dr. Ch. Epe, Institute for Parasitology, University of Veterinary Medicine Hannover, Germany were used for Group 3.

2.2. ELISA for the detection of specific antibodies

All tests were performed in polystrene 96-well microtiter plates (Nunc Maxisorb, ThermoScientific, Germany). Optimal performances for antibody ELISAs were determined by preliminary titration with regard to dilutions of test-specific antigens, sera and secondary antibodies, respectively. Positive control sera from animals with proven infections and negative control sera from farms without any history of *T. saginata* cysticercus infection were included in all tests to adjust for day-to-day and for plate-to-plate variations. Quantification of the protein concentrations of the antigens used was calculated by the Bradford protein assay using the Bio-Rad Protein assay (Bio-Rad Laboratories, USA).

2.2.1. Somatic "crude" T. saginata metacestode antigen (TsmAg)

Viable cysticerci were dissected from the muscles of naturally infected cows after being detected during routine meat inspection. The cysticerci were thoroughly washed in sterile PBS. Crude extracts of somatic antigen were prepared according to Deplazes et al. (1990) and purified by affinity chromatography based on recombinant protein G sepharose (Protein G Sepharose 4 Fast Flow, GE Healthcare, UK) according to the manufacturer's instructions. Flow from the column containing the antigen was stored at −80 °C until used. The ELISA was performed according to Staebler et al. (2006). Optimal test performance occurred with 10 μg/ml of antigen preparation in coating buffer (0.1 M carbonate/bicarbonate buffer, pH 9.6), a serum dilution of 1:200 in blocking buffer (PBS pH 7.2 containing 0.02% NaN_3, 0.05% bovine haemoglobin (Fluka, USA) and 0.2% (v/v) Tween-20) and alkaline phosphate labelled goat anti-bovine IgG (H + L) antibody (KPL, USA) at a dilution of 1:1000 in blocking buffer.

2.2.2. Protein fraction of TsmAg purified using isoelectric focusing (TsmIEF)

A crude extract of *Ts*mAg was fractionated by isoelectric focusing using the Bio-Rad Rotofor® System for fractionating complex protein samples according to the manufacturer's instructions (Rotofor® Preparative IEF Cell, Bio-Rad Laboratories, USA). The protein fraction with an isoelectric point in the pH-range 4.5–5.0 was further used, since this fraction did not cross-react with sera from cattle infected with *Fasciola hepatica*. Antigen was stored at −80 °C until used. The ELISA was performed as described in Section 2.2.1 for *Ts*mAg. However, in the *Ts*mIEF ELISA, the antigen was applied at a dilution of 1:20 in coating buffer. This optimal dilution was determined by titration in the absence of any possible quantification of the protein concentration.

2.2.3. ES antigens of T. saginata metacestodes (TsmES)

Viable cysticerci were dissected from muscle tissue of naturally infected animals and cultured in vitro according to Ogunremi and Benjamin (2010). Groups of seven cysticerci were cultured in 10 ml of medium at 37 °C and 5% CO2 for 20 days. Culture medium containing the antigen was harvested weekly and centrifuged (2,000*g* for 10 min.). Supernatant was stored at −80 °C until used. ELISA was performed with ES antigens (10 μg/ml) in coating buffer corresponding to Ogunremi and Benjamin (2010). Sample dilutions and secondary antibody conditions were used as described in Section 2.2.1 for *Ts*mAg.

2.2.4. Peptide antigens

Commercial synthesised peptides HP6-2 and *Ts*45S-10 (Ferrer et al., 2003) were included in the study. Peptides had a purity of at least 80% confirmed by the manufacturer (ANAWA Trading SA, Switzerland) using high-performance liquid chromatography and mass spectrometry. Peptide-ELISAs were performed using peptide HP6-2 or *Ts*45S-10 or both peptides pooled (1:1), diluted in coating buffer according to Abuseir et al. (2007). The ELISA procedure was performed as described in Section 2.2.1 for *Ts*mAg, except the blocking buffer contained 1% ovalbumin (Sigma–Aldrich, USA) instead of bovine haemoglobin. Serum samples were diluted 1:100 in the modified blocking buffer.

Table 1
Preliminary evaluation of serological tests for the detection of bovine cysticercosis in 793 dairy cows slaughtered at Swiss abattoirs.

Test	% Sensitivity[a] (95% CI)			% Specificity[b] (95% CI) (n = 740)
	Total (n = 53)	Viable infection status (n = 29)	Died/infection status (n = 24)	
*Ts*mAg	88.7 (76.3–95.3)	89.7 (71.5–97.3)	87.5 (66.5–96.7)	79.9 (76.9–82.7)
*Ts*mIEF	81.1 (67.6–90.1)	86.2 (67.4–95.5)	75.0 (52.9–89.4)	73.1 (69.7–76.2)
*Ts*mES	92.5 (80.9–97.6)	96.6 (80.4–99.8)	87.5 (66.5–96.7)	86.6 (83.9–88.9)
HP6-2[c]	73.6 (59.4–84.3)	79.3 (59.7–91.3)	66.7 (44.7–83.6)	72.3 (68.9–75.5)
*Ts*45S-10[c]	71.7 (57.4–82.8)	79.3 (59.7–91.3)	62.5 (40.8–80.4)	80.5 (77.5–83.3)
PP[d]	28.3 (17.2–42.6)	37.9 (21.3–57.6)	16.7 (5.5–38.2)	90.8 (88.4–92.7)
mAb B158/B60[e]	22.6 (12.7–36.5)	34.5 (18.6–54.3)	8.3 (1.5–28.5)	93.9 (91.5–95.5)

CI, credible interval; *Ts*mAg, *T. saginata* metacestode somatic antigen; *Ts*mIEF, *Ts*mAg purified using isoelectric focusing; *Ts*mES, *T. saginata* metacestode excretory/secretory antigen; PP, pooled peptides; mAb, monoclonal antibody.
[a] Test sensitivities were calculated as a percentage of test-positive sera amongst confirmed positives.
[b] Calculated on abattoir negative population (including false negatives) as judged by enhanced meat inspection (Eichenberger et al., 2011). Test specificities were calculated as percentages of test-negative sera amongst assumed negatives.
[c] Commercial synthesised purified peptide.
[d] Pooled peptides HP6-2 and *Ts*45S-10.
[e] ELISA using a combination of mAb $B158C_{11}A_{10}$ and $B60H_8A_4$.

2.3. ELISA for detection of circulating antigens

The ELISA for detection of circulating antigens (mAb B158/B60 assay) was performed as described by Van Kerckhoven et al. (1998) modified by Dorny et al. (2000) using mAb B158C11A10 as the capturing antibody and biotinylated mAb B60H8A4 as the detecting antibody.

2.4. Determination of cut-off values

To discriminate between *T. saginata* cysticercus-infected and non-infected animals, a single cut-off value was determined for each antibody assay by a two-graph receiver-operation curve (TG-ROC) with an optimal threshold value at maximal Youden's index (Greiner et al., 1995). Serum samples from the 61 animals in Group 1 and the 62 proven positive animals from Groups 2 and 3 were used to determine the cut-off values for the antibody ELISAs. Cut-off values for each antibody ELISA relative to positive and negative control sera were 0.309, 0.453, 0.302, 0.431, 0.387 and 0.486 for *Ts*mAg, *Ts*mIEF, *Ts*mES, peptide HP6-2, peptide *Ts*45S-10, and pooled peptides, respectively. The cut-off for the ELISA using mAbs was determined according to Dorny et al. (2000).

2.5. Preliminary determination of test performance characteristics

Determination of the preliminary specificity for each test was calculated using the 740 animals from Group 2 that were negative on enhanced meat inspection, although this group is likely to have included a number of false negatives. Test sensitivities were calculated as percentages of test-positive sera from confirmed positives. For this analysis, the positive group was split into two groups – animals with viable or animals with dead cysts (Eichenberger et al., 2011). Test diagnostic values from experimentally infected animals (Group 3) were calculated separately from the abattoir population. Test specificities were calculated as percentages of test-negative sera among assumed negative samples.

2.6. Bayesian test performance characteristics and estimated "true" prevalence

Bayesian statistical inference techniques were utilised to estimate the (unknown) diagnostic sensitivity of enhanced meat inspection with PCR confirmation, the antigen detection assay, three selected antibody assays and the estimated prevalence of *T. saginata* cysticercus infection in Switzerland (in the absence of a 'gold standard' reference test) (Joseph et al., 1995; Enoe et al., 2000; Dendukuri and Joseph, 2001). Selection of antibody assays to be incorporated into the Bayesian analysis was based on the preliminary test performance characteristics evaluation. Thus, *Ts*mAg, *Ts*mES and peptide antigen *Ts*45S-10 were included. It was assumed that the specificity of further enhanced meat inspection with PCR confirmation was 100%. Due to bias in collecting positive animals in Group 2, 18 samples were removed from the data set prior to analysis, leaving a data set of 775 randomly selected animals from the abattoir. Detailed description of the statistical model is summarised in Supplementary Data S1. The parameters in each model were estimated using Markov chain Monte Carlo (MCMC) simulation in JAGS (Plummer, 2003). The sensitivities and specificities for each test were assumed to be uniformly distributed between 0 and 1 (except for the further enhanced meat inspection with PCR confirmation where the specificity was fixed at 1.0). Disease prevalence was parameterised within the model as a logistic regression. Covariances between tests were determined algorithmically in JAGS as described in Lewis et al. (2012). To select the best fitting model the Deviance Information Criterion, a Bayesian goodness of fit criterion was used (Spiegelhalter et al., 2002). All usual diagnostic checks were performed, for example the 'Gelman and Rubin convergence diagnostics' (Brooks and Gelman, 1998). A standard stepwise model selection search was then used, commencing from a model where all tests were conditionally independent and then comparing goodness of fit with that of models which included (separately) each possible covariance term until it was no longer possible to improve the model fit. The chosen optimal model – comprising five tests, one prevalence population and two covariances for dependence between tests – was then used to generate (posterior) density estimates and 95% credible intervals (CIs)

for sensitivities and specificities of each of the tests. Finally, based on the modelled data set, positive and negative test predictive values depending on sensitivity, specificity and estimated prevalence were calculated (Brenner and Gefeller, 1997), and an estimate of the sensitivity of the obligatory EU-approved routine meat inspection technique was made.

3. Results

Results of the preliminary analysis of the different diagnostic tests regardless of misclassification in the negative abattoir population are shown in Table 1. This data set was used to determine which tests should be included in the statistical model. Thus *Ts*mAg, *Ts*mES and peptide *Ts*45S-10 assays were included for modelling the estimate of diagnostic test performances and the estimated "true" prevalence. In addition, the mAb B158/B60 assay was included as it is an antigen capture assay rather than an antibody assay and hence was likely to be conditionally independent of the other serological tests.

The Bayesian estimates of diagnostic test performances are shown in Table 2. ELISA based on *Ts*mES antigen for detection of specific antibodies showed the best test characteristics for this group of cows with a sensitivity of 81.6% (95% CI; 70.1–92.0) and a specificity of 96.3% (95% CI: 93.5–99.0). Using sera from nine experimentally infected animals (Group 3), all antibody tests were positive.

The best fitting model indicated that the sensitivities of two sets of tests were conditionally dependent: the diagnosis based on meat inspection and the mAb B158/B60 assay, and a similar dependence between the *Ts*mES and the peptide *Ts*45S-10 assay. Thus, an animal was more likely to test positive by the mAb assay if the animal was also positive at enhanced meat inspection. This can be illustrated by the fact that the mAb-assay was positive in nine of the 32 samples that were also positive at enhanced meat inspection. This gives an estimate of sensitivity in these samples of 28%, (95% CI: 15.5–45.5), which is significantly higher compared with the sensitivity in the general abattoir population of 14.3% (95% CI: 8.7–21.5). In addition, if the potentially biased population of 18 additional positive animals was included, 29 animals had viable cysts. Of these 29, 10 were positive with the mAb test (34.5%, 95% CI: 19.9–52.8). No dependence was detected between the specificities of any tests.

Overall, the prevalence of cysticercosis in this population of dairy cows was estimated to be 16.5% (95% CI: 12.5–21.2) using a Bayesian model. The sensitivity of the EU-approved routine meat inspection was estimated at 15.6% (95% CI: 10.0–23.3), which was increased by additional slices into the heart muscle to 24.2% (95% CI: 16.6–34.2).

Results of potential cross-reacting events against liver fluke infection are shown in Fig. 1. From 793 slaughtered cows (Group 2), bile samples from 449 animals were tested for eggs of liver flukes. Of these, 64.3% (95% CI: 59.7–68.6) were positive. *Fasciola* eggs were diagnosed in 13.5% (95% CI: 11.1–17.1) and eggs of *Dicrocoelium* in 60.8% (95% CI: 56.1–65.3) of the samples, respectively. Of these samples, 41 were *T. saginata* cysticercosis-positive at abattoir inspection and they were therefore not included for the evaluation of cross-reactivity. *Ts*mAg-ELISA showed high cross-reactivity (68.75% against single *F. hepatica*, and 56.5% against simultaneous *F. hepatica* and *Dicrocoelium dendriticum* infection). Interpretation of potential cross-reactivity between the other tests is difficult due to positive reactions in the fluke-negative samples and because the prevalence was estimated to be 16.5% in cattle presented at the abattoir.

4. Discussion

Based on an abattoir population, this study evaluated test diagnostic values for the serological diagnosis of *T. saginata* cysticercus infection in central Europe. Epidemiological data on bovine cysticercosis in this region (McCool, 1979; Geerts et al., 1981; Eichenberger et al., 2011) and data from abattoir reports indicate infected animals typically have low parasite burdens.

Evaluation of diagnostic test results in serological assays can only be analysed if a reliable cut-off value can be determined. In an environment with unknown prevalence (e.g. no observable clinical signs) and absence of a 'gold standard' as a reference test cut-off, determination is difficult (Brenner and Gefeller, 1997). Therefore, in this study the optimal cut-off value was determined by maximal Youden's index, a summary index independent of the prevalence (Youden, 1950; Greiner et al., 1995). Because test diagnostic values are population parameters (Greiner and Gardner, 2000; Leeflang and Bossuyt, 2005), the cut-off values of all but one test were calculated on a homogenous reference population of sera from cows originating from three Swiss farms with no history of *T. saginata* cysticercosis. However, these cattle were exposed to a similar environment as the cattle presented at the abattoir.

First, test characteristics were calculated regardless of potential false negative results in the negative abattoir population. Furthermore, preliminary evaluation included an evaluation of potential cross-reactions to liver flukes. Non-specific reaction to *F. hepatica* using a "crude" larval antigen has previously been demonstrated by Craig and Rickard (1980). Typically, the Swiss cattle population has a high prevalence for *F. hepatica* infections, indicated by 13.5% observed in this study, which is consistent with previous estimations of 18% (Rapsch et al., 2006). The "crude" larval antigen *Ts*mAg

Table 2
Bayesian test performance characteristics: estimates of test diagnostic values for the diagnosis of *Taenia saginata* cysticercus infection in 775[a] dairy cows slaughtered at Swiss abattoirs.

Test	% Sensitivity (95% CI)	% Specificity (95% CI)	% PPV[b]	% NPV[b]
*Ts*mAg	77.7 (65.5–87.4)	87.9 (84.8–91.0)	86.5	95.2
*Ts*mES	81.6 (70.1–92.0)	96.3 (93.5–99.0)	95.7	96.4
*Ts*45S-10[c]	55.2 (45.9–65.1)	84.7 (81.6–87.6)	78.3	90.5
mAb B158/B60[d]	14.3 (8.7–21.5)	93.7 (91.5–95.5)	69.4	84.7
EU routine MI[e]	15.6 (10.0–23.3)	100 (assumed)	100	85.7
Enhanced MI[f]	24.2 (16.6–34.2)	100 (assumed)	100	87.0

PPV, positive predictive value; NPV, negative predictive value; CI, credible interval; *Ts*mAg, *T. saginata* metacestode somatic antigen; *Ts*mES, *T. saginata* metacestode excretory/secretory antigen; mAb, monoclonal antibody; EU, European Union; MI, meat inspection.

[a] Of the 793 animals observed in the abattoir, 18 were removed from the data set prior to analysis as these were biased due to positive selection.

[b] Based on prevalence of 16.5%.

[c] Commercial synthesised purified peptide.

[d] ELISA using a combination of mAb $B158C_{11}A_{10}$ and $B60H_8A_4$.

[e] According to Murrell (2005).

[f] According to Eichenberger et al. (2011).

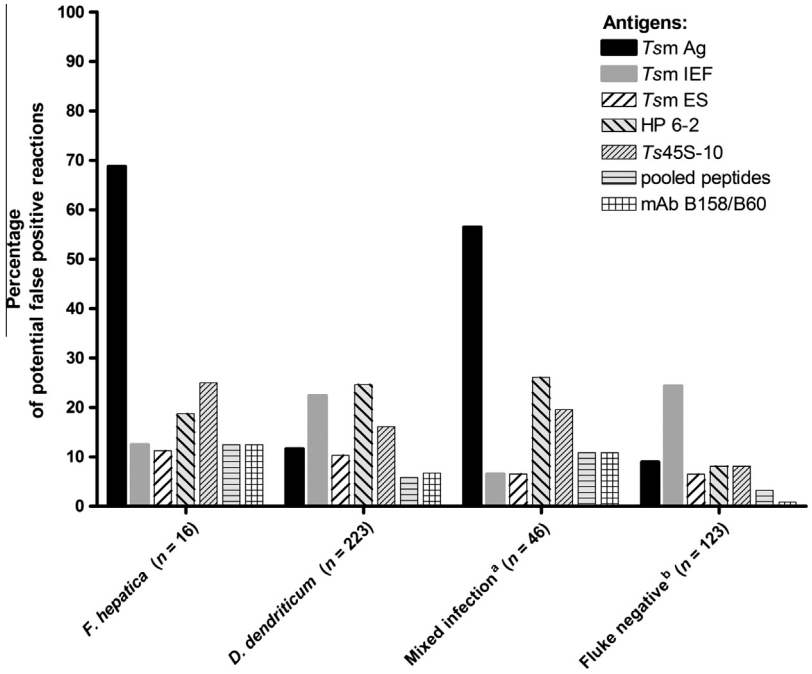

Fig. 1. Degree of potential cross-reactivity (positive results) in different ELISAs for the detection of *Taenia saginata* cysticercosis in 408 sera (of Group 2) from cows examined for liver flukes (*Fasciola hepatica*, *Dicrocoelium dendriticum*, animals harbouring mixed infections and liver fluke negative animals) confirmed by bile examination in cysticercosis negative samples as judged by enhanced meat inspection. [a]Animals infected simultaneously with *F. hepatica* and *D. dendriticum*. [b]Animals diagnosed negative for liver fluke infection. *Ts*mAg, *T. saginat*a metacestode somatic antigen; *Ts*mIEF, *Ts*mAg purified using isoelectric focusing; *Ts*mES, *T. saginata* excretory/secretory antigens; mAb, monoclonal antibody.

gave high unspecific results. An isoelectric focused fraction (*Ts*mIEF) from the *Ts*mAg was sensitive for *T. saginata* cysticercosis and did not show significant cross-reactions against sera from cows infected with *F. hepatica*. Nevertheless, this fraction showed significantly less specificity than the "crude" antigen, suggesting other unspecific epitopes. The preliminary evaluation of the test performances and the cross-reactions were used to decide which test to include for the Bayesian analysis. Thus *Ts*mAg, *Ts*mES, peptide *Ts*45S-10 and the ELISA for detection of circulating antigens were further evaluated, respectively.

Using Bayesian models we estimated the diagnostic performance of a number of serological tests; the sensitivity of the current obligatory standard EU meat inspection procedure for the detection of bovine cysticercosis and estimated the "true" prevalence of cysticercosis. The Bayesian techniques used in analysing the test performance in the abattoir population gave the best available unbiased estimate of test performances in a naturally infected population. Using the one population five test model, there were adequate degrees of freedom to estimate test characteristics assuming that there was no conditional independence of tests. If a test of 100% specificity is included in the analysis then each of the two-way parameters that model of conditional dependence of the specificity of this test and other tests disappears. In addition, tests that directly detect the presence of the parasite are likely to have a sensitivity that is conditionally independent of antibody-based tests, which detect the immune response to the parasite. The study had one test with a specificity fixed at 1 (enhanced meat inspection followed by PCR confirmation). This then resulted in sufficient degrees of freedom to analyse potential conditional dependence between other test specificities and test sensitivities in a pairwise manner. With this approach only two conditionally dependent parameters remained significant in the final model. Firstly, there was dependence between the enhanced meat inspection procedure and the mAb assay. This dependence is explained by the fact that an animal is more likely to be detected by meat inspection if it has a large number of cysts and hence may have greater levels of circulating antigen compared with an animal that passes meat inspection but nevertheless is positive and is likely to have only a few cysts. Secondly, there is a similar correlation between the *Ts*mES and the peptide test. These two parameters were retained to calculate an unbiased estimate of the parameters of interest.

The ELISA based on *Ts*mES applied to naturally infected animals showed the best test result with overall Bayesian sensitivity and specificity estimates of 81.6% and 96.3%, respectively. This result is comparable with other reports of 92.9% and 90.6% in experimentally infected cattle compared with a simulated parasite burden in field infected animals (Ogunremi and Benjamin, 2010). This result is not surprising due to the close involvement in host/parasite interaction of ES proteins (Harrison and Sewell, 1981b; Joshua et al., 1988; Ogunremi and Benjamin, 2010). Nevertheless, *T. saginata* metacestodes are difficult to collect and production of ES antigens from in vitro cultivation is laborious and hence they are not readily available.

It was demonstrated that synthetic peptides have a potential for diagnostic use with highly sensitive and specific test characteristics using experimentally infected calves and uninfected calves reared under experimental conditions. However, application of the peptide *Ts*45S-10 to identify infection in a group of naturally infected cattle showed an estimated specificity of 84.7% and sensitivity of 55.2%. Our results indicate that pooling the peptide results generates a higher specificity but a clearly decreased sensitivity. This is in contrast to the report by Abuseir et al. (2007).

The ELISA for the detection of circulating parasite antigens showed an estimated test specificity of 93.7%. The test sensitivity of 14.3% from the Bayesian analysis in this study without differentiation of viability of the cysts in animals with low cyst burden was comparable with previous reports. In animals harbouring less than

50 living cysts a sensitivity of 12.5% was reported using the identical test (Van Kerckhoven et al., 1998). However, our case definition included viable and calcified cysts and therefore test parameters for antigen detection are not appropriate because this test was designed to detect viable infections only. Indeed in this study the modelled test sensitivity for detecting circulating antigen significantly increases to 34.5% if only animals with proven viable infections were considered. There is also evidence that the ELISA for the detection of circulating antigens is more likely to detect an infected animal if this animal has also been detected by enhanced meat inspection compared with an infected animal that has a false negative result from enhanced meat inspection. Importantly, the current obligatory standard EU meat inspection procedure also defines non-viable cysts or calcified lesions as positive due to the likely coexistence of viable cysts in animals found to be infected (Juranek et al., 1976). So although it can be argued that we used the circulating antigen test beyond what it was designed for, it provided us with important additional information for the Bayesian approach, particularly because it was conditionally independent of the serological tests detecting antibody responses and thus facilitated model convergence without making any unjustified assumptions.

The model presents an evaluation of the obligatory EU-approved routine meat inspection. The estimated sensitivity of 15.6% (95% CI: 10–23) from this study is consistent with previous estimates of 10–30% (Dewhirst et al., 1967; McCool, 1979; Geerts et al., 1980; Walther and Koske, 1980; Hörchner, 1983; Dorny et al., 2000; Eichenberger et al., 2011). Finally, the estimated prevalence of bovine cysticercosis is 16.5%. The prevalence in cows is at least a measure of exposure but does not necessarily relate to the prevalence of viable cysts similar to the observations of Praet et al. (2010). It should be interpreted that it is likely to lie somewhere between 12.5% and 21.2%. Nevertheless, this high value compared with an observed prevalence of 4.5% by an enhanced meat inspection protocol (Eichenberger et al., 2011) indicates a higher contact rate of dairy cows with this parasite, with the potential development of parasitic lesions causing substantial financial losses. Therefore, control strategies have to be reconsidered including risk factors for the transmission of bovine cysticercosis on farms (Flütsch et al., 2008; Jenkins et al., 2013), improved diagnostic tools and highly protective vaccines (Lightowlers et al., 1996).

Acknowledgements

Our thanks go to all involved parties, especially the friendly support of all participating meat inspectors. Special thanks go to Prof. R. Stephan for initiating the abattoir trials. Appreciation is expressed to I. Tanner and L. Kohler for their technical assistance. This study was partially supported by the Swiss Federal Veterinary Office.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpara.2013.05.011.

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