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Research paper

High prevalence of bovine cysticercosis found during evaluation of different post-mortem detection techniques in Belgian slaughterhouses

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ABSTRACT

Bovine cysticercosis (BCC), caused by the helminth *Taenia saginata*, is currently diagnosed solely by official meat inspection (MI) based on macroscopic detection of viable cysticerci or typical lesions of degenerated larvae. MI has a known low sensitivity (< 16%), leading to a large proportion of infected cattle carcasses entering the human food chain and posing a risk to public health. Prevalence in Belgium based on MI results is estimated at around 0.22%. Due to the low sensitivity of MI, alternative techniques to detect BCC should be considered. This study evaluates MI, MI with additional incisions in the heart, specific antibody detection against excretory/secretory (E/S) in the Ab-ELISA and circulating antigens in the B158/B60 Ag-ELISA on 715 (101 MI-positive and 614 MI-negative) samples collected from carcasses at slaughterhouses in Belgium. Full dissection of the predilection sites was considered the reference test.

During the study, mostly carcasses with (very) light infections were detected containing predominantly degenerated or calcified cysticerci and only few viable cysticerci. Dissection of the predilection sites detected 144 (23%) additional infections in the 614 MI-negative carcasses. When sequentially performing first the dissection of the predilection sites, followed by the Ag-ELISA and the Ab-ELISA, an additional 36% of MI-negative carcasses were found positive for BCC, resulting in a prevalence very much higher than the above mentioned 0.22%.

The B158/B60 Ag-ELISA showed a sensitivity of 40% for the detection of carcasses containing viable cysticerci and a specificity of 100%, and detected 70 positive carcasses of which only 14 had been identified as positive during MI. If Ag-ELISA were implemented as a detection technique for BCC in the slaughterhouses, many infected carcasses would still not be detected due to the sensitivity of 40%. But as sensitivity increases with increasing number of cysticerci in the carcass, the infected carcasses passing inspection will be the ones containing only a few viable cysticerci and thus posing a smaller food safety problem. Ag-ELISA is preferred over the ES Ab-ELISA in this study, which had a sensitivity of 13.3% and a specificity of 91.7% in a population with overall low infection burdens.

1. Introduction

Taenia saginata (beef tapeworm) is a worldwide occurring zoonotic cestode, of which the adult tapeworm resides in the intestinal lumen of the human final host (taeniosis), and the metacestode larval stage is found primarily in the muscles of the bovine intermediate host (bovine cysticercosis, BCC). Viable cysticerci consist of a transparent capsule with an invaginated scolex. When cysticerci die, they undergo degeneration, followed by calcification surrounded by host tissue proliferation (lesions). BCC includes both animals with viable and/or degenerated cysticerci. Only consumption of viable cysticerci can lead to the development of a tapeworm in humans (Murrell, 2005).

BCC is responsible for important economic losses in the meat sector, particularly for beef cattle (Wanzala et al., 2002; Scandrett et al., 2009). In the EU, diagnosis of BCC is currently based on official meat inspection (MI) (EC directive 854/2004) and this is the main control measure in all European countries. During MI, the oesophagus and visible muscle surfaces are visually inspected and several incisions are made in

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the predilection sites, overall considered to have high cysticercus densities (heart, masseters, diaphragm, oesophagus and tongue) (Kyvsgaard et al., 1990).

The estimated prevalence of BCC in Belgium, based on MI, was 0.24% in 2012, 0.20% in 2013 and 0.23% in 2014 (annual reports Federal Agency for the Safety of the Food Chain, FASFC, 2012, 2013, 2014). Since MI has a known low sensitivity (< 16%) (Kyvsgaard et al., 1990; Dorny et al., 2000; Eichenberger et al., 2013), alternative techniques to detect BCC should be considered. Currently, these techniques include meat inspection with application of additional incisions in the heart muscle and several serological techniques. Cysticerci are most frequently found in the heart and they degenerate and calcify quicker in this organ, making them become more visible (Juranek and Forbes, 1976; Kyvsgaard et al., 1990; Lopes et al., 2011). A recent study showed a doubling of detected infected carcasses by making extra incisions in the heart (Eichenberger et al., 2011, 2013).

Serological techniques can detect specific antibodies (Ab) or antigens (Ag) related to the presence of BCC. Ab-detecting techniques do not differentiate between viable and degenerated/calcified cysticerci (or past infections). Several Ab-detecting tests have been developed, with varying performance, mostly depending on the type of coating antigen used. Commonly used antigens for detection of cysticercosis are excretory-secretory (ES) antigens. Ogunremi and Benjamin (2010) developed an ELISA detecting IgG1 using excretory-secretory antigens of *T. saginata*, with a sensitivity of 92.9% and a specificity of 90.6%. The use of this test on naturally infected cattle from Canada showed a prevalence of 4.6% instead of the previously detected prevalence of 2.3%, demonstrating the usefulness as a detection technique.

Current Ag-detecting techniques were developed to specifically demonstrate the presence of viable cysticerci (Wanzala et al., 2002). Since this is the only infective stage, Ag-detecting tests demonstrating that carcasses are free of viable cysticerci (thus containing none or only degenerated cysticerci), could approve these carcasses for human consumption without imposing a food safety issue and restrict the value losses for the meat sector. The B158/B60 monoclonal-based Ag-ELISA is a widely-used Ag-ELISA for *Taenia* spp. Originally, IgM monoclonal antibodies (MoAbs) against excretory/secretory antigens of the metacestode stage of *T. saginata* were used in the sandwich ELISA (Brandt et al., 1992). The technique was improved by Van Kerckhoven et al. (1998) by switching to MoAbs of the IgG isotype and further improved in a study in Belgium, detecting a tenfold of infected carcasses with BCC than MI (3.09% versus 0.26% (Dorny et al., 2000)). Similar results were found in other studies: a prevalence of 1.11% was found when using the Ag-ELISA in comparison to 0.02% through MI in Northeast Spain (Allepuz et al., 2012).

The current study evaluates and compares several post-mortem detection techniques for BCC in slaughtered cattle (MI, MI with additional incisions in the heart, Ag-ELISA and Ab-ELISA) in Belgian slaughterhouses by comparing the test results with full dissections of the predilection sites (heart, tongue, masseters, diaphragm and oesophagus). Even though Ag- and Ab-ELISA are not limited to post-mortem detection of bovine cysticercosis, they can be used as such and will be considered a valid post-mortem detection technique in this study. Results will contribute to a better estimation of the sensitivity and specificity of the studied detection techniques and of the prevalence of BCC in Belgium and will guide decision makers on the use of a better post-mortem detection technique that can be implemented in slaughterhouses to decrease the prevalence of BCC and taeniosis in Belgium.

2. Materials and methods

2.1. Sampling design

Samples were collected from slaughterhouses for the determination of the performance of the designated tests (see 2.2). Additional samples were collected from first-grazing season calves on farms for the determination of the specificity of the Ag-ELISA (see 2.3).

2.2. Sampling at the slaughterhouses

Sampling was conducted in three Belgian slaughterhouses during three consecutive 10-month periods between 2012 and 2015. During weekly visits randomly selected carcasses (adult dairy and beef cattle ranging from 2 to 2016 months of age) were sampled at the slaughter line. Samples consisted of a collection of the predilection sites (heart, tongue, masseter muscles, oesophagus and diaphragm) and a blood sample. Sanitel ear tag numbers (Belgian system for computerised management of the identification, registration and control of livestock) were noted as well as the MI result. Since only a small percentage of animals was expected to be positive for BCC on MI (0.22%), samples of all MI-positive carcasses (predilection sites, blood sample, sanitel number, MI result including a muscle sample with the suspected cysticerc) detected in the specific slaughterhouses during the 10-month period of random sampling were collected together with the MI-negative samples. Eventually this lead to 101 MI-positive samples and 614 MI-negative samples. All samples were transported to the laboratory of the Institute of Tropical Medicine (ITM) in Antwerp, Belgium for further testing.

Blood samples were kept overnight at 4 °C and thereafter centrifuged for 20 min. Serum was stored at −20 °C until tested. Meat samples were stored at 4 °C and dissected 1–2 days after collection. Techniques included in the study were meat inspection (MI, Section 2.2.1), enhanced MI (Section 2.2.2), dissection of the predilection sites (Section 2.2.3), Ag-ELISA (Section 2.2.4), Ab-ELISA (Section 2.2.5) and PCR-RFLP (Section 2.2.6) on the suspected lesions.

2.2.1. Meat inspection (MI)

Licenced veterinary inspectors performed the MI at the slaughter line according to standard European Union meat inspection procedures (854/2004). For all animals older than six weeks, the oesophagus and visible muscle surfaces are visually inspected and several incisions are made in the predilection sites. The area of detection and stage (viable, degenerated or calcified) of cysticerci was reported to the ITM.

The lesions were visually inspected by ITM laboratory technicians to confirm or reject the MI result and these results (viable, degenerated, calcified or no cysticercus) were added to the database. Cysticerci found were dissected and stored in 70% ethanol for molecular confirmation.

2.2.2. Routine MI with extra incisions in the heart (enhanced MI)

Six extra incisions were made in the collected hearts as described by Eichenberger et al. (2011). The number and stage of the detected cysticerci was registered. Cysticerci found were dissected and stored in 70% ethanol for molecular confirmation.

2.2.3. Dissection of predilection sites (PS)

Predilection sites (heart, tongue, masseter muscles, oesophagus and diaphragm) were completely dissected making 0.5 cm thick slices. The number and stage of all cysticerci was recorded. Detected cysticerci were dissected and stored in 70% ethanol for molecular confirmation.

2.2.4. Enzyme-linked-immunosorbent assay for the detection of circulating antigens (Ag-ELISA)

The B158/B60 Ag-ELISA was performed as described by Dorny et al. (2002). Repeatability for this test was assessed and considered good (Jansen et al., 2016). Each sample was tested in duplicate, and on each plate, two positive serum samples from cattle with confirmed *T. saginata* cysticercus infections (positive controls) and eight serum samples from *T. saginata* cysticercosis-free cattle (negative controls) were analysed. The plate was read using an automated spectrophotometer (Titertek Multiskan EIA reader).

The optical density of each serum sample was compared with the collection of negative serum samples (N = 8) at a probability level of

P = 0.001 to determine the result in the test (Sokal and Rohlf, 1981).

2.2.5. Enzyme-linked-immunosorbent assay for the detection of specific antibodies (ES ab-ELISA)

An aliquot of all serum samples was sent to the laboratory of the Institute of Parasitology, University of Zurich, Switzerland to perform the antibody-ELISA based on excretory/secretory (ES) antigens. ES antigens were obtained from *in vitro* cultures of viable cysticerci, dissected from muscle tissue of naturally infected animals. The test was performed as described by Ogunremi and Benjamin (2010). Discrimination between *T. saginata* cysticercus-infected and non-infected animals was based on a single cut-off value previously determined by a two-graph receiver-operation curve (TG-ROC) with an optimal threshold value at maximal Youden's index (Eichenberger et al., 2013).

2.2.6. PCR – RFLP

Polymerase Chain Reaction – Restriction Fragment Length polymorphism (PCR-RFLP) was performed on at least one cysticercus found per carcass for confirmation, according to Geysen et al. (2007).

DNA was extracted using a modified Boom method (Boom et al., 1990). The lysis and nuclease-inactivating properties of guanidine thiocyanate combined with the DNA-binding properties of diatomaceous earth form the base of this method. The final pellet is resuspended in Tris-EDTA (10 mM Tris, 1 mM EDTA) and stored at −20 °C.

For DNA amplification, first round primers ITM TnR (5′ CTCAATAATAATCGAGGGTGACGG 3′) and TaenF (5′ GTTTGCCACCTCGATGTTGACT 3′) and second round primers ITM TnR and nTAE (5′ CGTGAGCCAGGTCGGTTCTTAT 3′) were used. Primers are based on conserved regions between a partial *T. saginata* sequence for large subunit ribosomal RNA (LSU rDNA), adjacent to a partial sequence for *T. saginata* 12S ribosomal RNA (12S rDNA) and the *T. solium* 12S ribosomal RNA (12S rDNA) sequence. The amplification products were digested with restriction endonuclease *DdeI* (10 U/μg DNA) (10,000 U/ml; New England Biolabs, Inc., Ipswich, Mass.)

2.3. Sampling from first-grazing season calves

Additionally, to determine the specificity of the Ag-ELISA, serum samples of 154 first-grazing season calves were collected from 11 commercial cattle farms. These animals had been grazing on pastures for at least one grazing season and had been exposed to common parasite species that could possibly cause cross-reactions. The B158/B60 Ag-ELISA was performed as described in Section 2.2.4. In case of positive results, the calves were to be purchased followed by full body dissections to determine the presence of cysticerci in the carcasses.

2.4. Data management and analyses

Results from the various detection techniques were combined in an Excel file. Analyses were done in STATA/MP 14.1 software (Stata Corp., College Station, TX). The result of the dissection of the predilection sites was considered as the reference test, since it was practically not possible to dissect an appropriate number of complete carcasses.

3. Results

3.1. Dissection

3.1.1. Meat inspection positive carcasses

During the three-year sampling period, 101 MI-positive carcasses were collected. All, but one, carcasses were considered to have light infections, since a maximum of three cysticerci was found per carcass during meat inspection and dissection of the predilection sites. One animal with a generalised infection contained multiple (20–30) cysticerci on each cutting area, indicating an infection with thousands of parasites. This animal was considered as a positive carcass in the further analyses and discussion, but when counting the total number of cysticerci found, only one cysticercus was counted for this carcass. A heavily infected carcass will be detected at the slaughter line in any case because cysticerci are visible on the muscle surfaces, and these carcasses will not enter the food chain. When estimating the mean number of cysticerci per carcass and assessing the diagnostic tools, using the real number of cysticerci in this particular carcass would be overpowering the numbers of collected cysticerci in the other carcasses and would interfere with getting clear, useful results. In Belgium, on average 16.2 heavily infected carcasses are found yearly (in contrast to 1428.2 carcasses with light infections (annual reports Federal Agency for the Safety of the Food Chain, FASFC, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014)).

Table 1
Total number of carcasses collected (N) and carcasses found positive for BCC during dissection of the predilection sites (PS+) for both meat inspection negative (MI−) and positive (MI+) carcasses. The number of cysticerci found during meat inspection (MI) and dissection of predilection sites (PS) is given (viable, degenerated, calcified and total number).

	Number of Carcasses		Number of cysticerci			
	N	*PS+*	*Viable*	*Degenerated*	*Calcified*	*Total*
MI+	101	35	12	22	69	103
MI−	614	144	13	62	236	311

Among the 101 MI-positive carcasses, 12 carcasses each contained one viable cysticercus in the predilection sites. Four of these 12 viable cysticerci were not considered viable at visual inspection by ITM lab technicians. A total of 22 degenerated and 69 calcified cysticerci were found during MI in the MI-positive carcasses (Table 1, row 1). In 35 MI-positive carcasses, extra cysticerci were found during the dissection of the predilection sites.

3.1.2. Meat inspection negative carcasses

In the 614 MI-negative carcasses, cysticerci were found in four carcasses when applying extra incisions to the heart muscle, none of which were viable.

Cysticerci were, however, found in 144 carcasses (23%) when dissecting the predilection sites (Table 1, row 2). Most of these carcasses contained degenerated (62 cysticerci) or calcified (236 cysticerci) cysticerci; only ten carcasses contained viable cysticerci (13 cysticerci). The percentage of cysticerci found in each predilection site are given in Table 2 (including the distribution for viable, degenerated and calcified cysticerci).

3.1.3. PCR-RFLP

PCR-RFLP confirmation was done for 92 cysticerci found in the 101 MI-positive carcasses. Seventy of these were positive on PCR (10 in 11 viable, 19 in 20 degenerated and 41 in 61 calcified cysticerci). Of the 178 cysticerci found during dissection of the PP and tested with PCR-RFLP, 130 were found positive (10 in 12 viable, 25 in 36 degenerated and 95 in 130 calcified cysticerci). Thus, similarly to Geysen et al. (2007), molecular analyses of a total of 279 cysticerci found, confirmed 222 cysticerci (80%) (87.0% viable, 78.6% degenerated and 71.2%

Table 2
Distribution of the presence of the different stages (viable, degenerated, calcified) of cysticerci in the predilection sites (%).

Predilection site	% cysticerci	% viable	%degenerated	% calcified
Heart	39.9	4.2	15.8	80
Masseters	36.7	9.2	25	65.8
Diaphragm	15.2	1.6	17.5	81
Tongue	4.6	15.8	36.8	47.4
Oesophagus	3.4	0	21.4	78.6

calcified cysticerci). As PCR should always be positive for viable cysticerci, since *T. saginata* DNA is present, the three viable cysticerci that could not be confirmed may be other structures or DNA material could have been lost when cysticerci were accidently cut open during dissection. For this reason, these lesions were not considered cysticerci in further calculations. In degenerated and calcified cysticerci, there is a possibility that DNA cannot be detected anymore, leading to negative PCR results (Geysen et al., 2007).

3.2. Serological results

The Ag-ELISA identified 70 cysticercus positive carcasses (in 715 carcasses tested). Fourteen of these had also been identified by meat inspection (only three containing viable cysticerci found at MI).

Among the 70 carcasses positive in the Ag-ELISA, 38 were only positive in the Ag-ELISA, two were also positive in the Ab-ELISA and 30 had cysticerci present in the predilection sites (found during MI and dissection of PS). Six carcasses that were Ag-ELISA positive, had viable cysticerci in the predilection sites, found during MI or dissection of the predilection sites, suggesting that the other 64 Ag-ELISA positive carcasses contained at least one viable cysticercus elsewhere in the carcass.

Viable cysticerci were found in 24 of the 715 carcasses in total (including MI-positive as well as PS-positive carcasses). Since for one viable cysticercus PCR-RFLP was not performed (at least one cysticercus per carcass was tested) and three were not found positive by PCR-RFLP, these were excluded for the calculation of the sensitivity of the Ag-ELISA. Eight out of 20 carcasses with a confirmed viable cysticercus were positive in the Ag-ELISA, therefore, the sensitivity of the Ag-ELISA for detection of viable cysticerci was 40% (95%CI: 21.8–61.6).

The specificity of the Ag-ELISA was determined by the study on 154 first-grazing season calves. All animals tested negative in the Ag-ELISA, leading to a test specificity of 100% (95%CI: 97.0–100). Since all calves were found negative, no full carcass dissections were performed.

The Ab-ELISA was performed on all but five samples, because not enough serum was available from these samples. The test identified 71 positive samples in total, of which 18 had been found positive at MI. In twenty-three carcasses (of the 71 Ab-ELISA positive carcasses) cysticerci were found during dissection of the predilection sites (nine carcasses also positive for MI). Thirty-seven carcasses were positive only in the Ab-ELISA.

Considering all carcasses containing cysticerci in the predilection sites and for which Ab-ELISA was performed (240 carcasses), 32 were positive for the Ab-ELISA, generating a sensitivity of 13.3% (95%CI: 9.43–18.45). If we calculate a sensitivity for only viable cysticerci, sensitivity decreases to 10% (95%CI: 1.75–33.13) (20 carcasses with viable cysticerci). We could not exactly determine the specificity of the Ab-ELISA because we could no longer conduct the total carcass dissection of the first-grazing season calves to determine the real infection status at the time of availability of the Ab-ELISA results. Furthermore, positive results can still be expected in the Ab-ELISA when cysticerci have already disappeared from the animal body. As an alternative, we estimated the specificity by considering carcasses negative for all tests (except for the Ab-ELISA) as true negatives. By this parallel interpretation of multiple tests, sensitivity of these tests is increased and we can assume that all individuals negative for all applied tests represent true negative individuals (Thrusfield, 2005; Dohoo et al., 2014). Of the 430 animals, 393 were negative in the Ab-ELISA, indicating a specificity of 91.4% (95%CI: 88.2–93.8).

4. Discussion

This study aimed to evaluate different post-mortem detection techniques for BCC, comparing their outcomes with the results of dissections of predilection sites (heart, masseters, tongue, oesophagus and diaphragm). During dissection of the PS of the meat inspection negative samples (N = 614), cysticerci were detected in 144 carcasses (23%). Most lesions were found in the heart during dissection of the PS and in the masseter muscles during MI, supporting previous observations that cysticerci have a preference to establish in these muscles (Juranek and Forbes, 1976; Kyvsgaard et al., 1990; Lopes et al., 2011). Since 23% of cysticerci present in the PS were not found during MI and many more cysticerci can be located elsewhere in the body, there is a great need for an improved detection technique for BCC, such as serological techniques.

In total, 70 samples were positive in the B158/B60 Ag-ELISA, indicating the presence of viable cysticerci. Fourteen of these had been identified as positive during MI, but in only six, viable cysticerci were found in the PS. The other 64 Ag-ELISA positive carcasses thus potentially contained viable cysticerci in other body parts. The sensitivity of the Ag-ELISA, determined in this study was 40% for the detection of viable cysticerci. An earlier study by Van Kerckhoven et al. (1998) showed a very high sensitivity of the Ag-ELISA for animals infected with more than 50 viable cysticerci (98.7%) and a reduction to 12.8% when less than 50 cysticerci are present. Sensitivity thus increases with the number of cysticerci present in the body. As observed in our study most infections in Belgium are light, therefore, with a sensitivity of 40% a considerable number of infected carcasses will remain undetected when the Ag-ELISA is applied. These carcasses will, however, contain one or few viable cysticerci, thus representing a limited food safety problem, compared to carcasses containing a higher number of viable cysticerci, which are likely to be detected by the Ag-ELISA.

The B158/B60 Ag-ELISA has been tested for cross-reactions against the most common helminth infections on Belgian cattle farms. No cross-reactions were found when testing serum samples of animals experimentally infected with *Ostertagia ostertagi, Cooperia oncophora, Dictyocaulus viviparus, Fasciola hepatica* (non-published data, P. Dorny) and *Sarcocystis* spp. (Ogunremi et al., 2004). Light cross-reactions were described for the *in situ* use of monoclonal antibodies on carcasses with *Echinococcus* spp. hydatid cysticerci (Brandt et al., 1992; Ogunremi et al., 2004) and with other *Taenia* spp. (Dorny et al., 2004). *Echinococcus* and *Taenia* spp. other than *T. saginata* are very uncommon in Belgian cattle (personal communication, P. Dorny). Specificity of this test was previously estimated to be 98.7% (Van Kerckhoven et al., 1998). Results from the current study on first-grazing season calves confirm the high specificity of the B158/B60 Ag-ELISA (100%).

Eichenberger et al. (2013) compared six serological detection techniques with routine MI and an enhanced MI procedure on slaughtered dairy cows in Switzerland and subjected four of the serological techniques to a model-based Bayesian inference approach. The antibody detection ELISA based on ES antigens showed highest sensitivity (81.6%) and specificity (96.3%). The B158/B60 Ag-ELISA (Dorny et al., 2000) had a specificity of 93.7% and a sensitivity of 14.3% for all infected carcasses, but for carcasses containing viable cysticerci, sensitivity rose to 34.5%. In the present study, Ag-ELISA had a sensitivity of 40% and a specificity of 100% for the detection of viable cysticerci, while the Ab-ELISA had a sensitivity of only 13.3% for the detection of all cysticerci and an estimated specificity of 91.4%. The sensitivity of the Ab-ELISA for viable cysticerci decreased to 10%.

The observed difference in test performances between the Swiss and the current study can be due to several factors. The manner of documentation and analyses can play an important role in the values for sensitivity and specificity. For example, in the study of Eichenberger et al. (2013), cysticerci were only collected/looked for during MI and enhanced MI with additional incisions in the heart, while in this study a dissection of the predilections sites was also performed. Many more infected carcasses were thus found in the current study, using better-characterised samples. For the Ag-ELISA, sensitivity and specificity could not be determined using the same population. On the other hand, Bayesian analysis, performed by Eichenberger et al. (2013) is designed to account for the sampling of false negative cases, regardless of individual animal test results, in the absence of reference tests. Both tests thus use very distinct approaches to determine test characteristics,

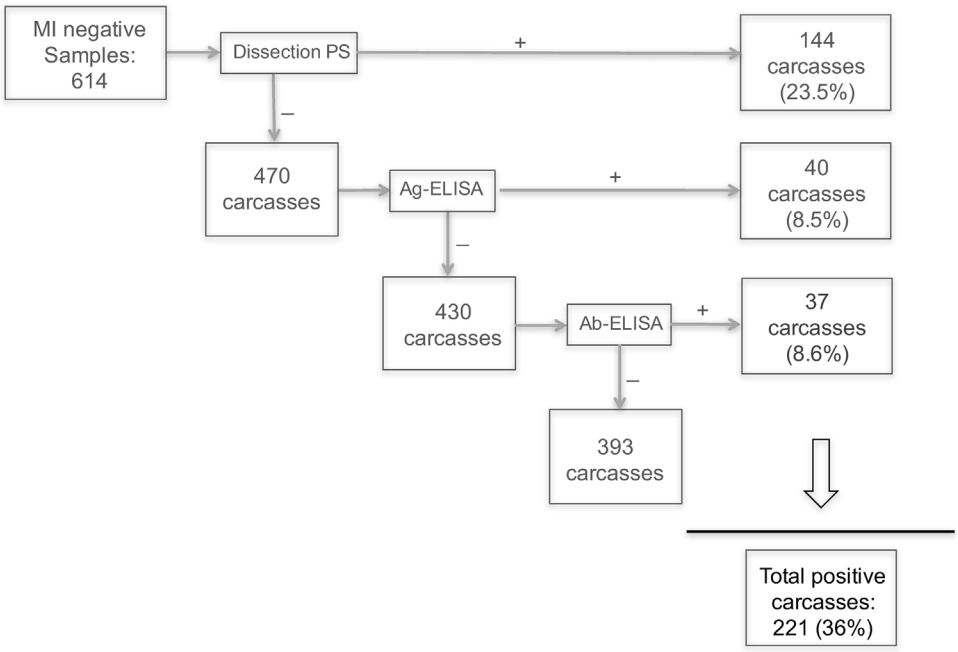

Fig. 1. Simulated numbers and percentages of positive and negative carcasses within the MI-negative (MI −) samples for the different detection techniques when performed sequentially, based on data at hand. Summation leads to the total of positive carcasses found.

possibly giving a difference in these parameters. Furthermore, depending on the definition of a case (current infection or prior exposure), prevalences and test performances of the same test can differ. Ag-ELISA is less sensitive to detect exposure because it will only detect viable cysticerci that are excreting antigens (Praet et al., 2010).

Using the dissection of the predilection sites as the reference test for sensitivity is not completely accurate. By combining results from several experimental infections (Kyvsgaard et al., 1990; Soares et al., 2011 + personal communication; experimental infections P. Dorny/S. Gabriël, unpublished results), we can estimate that on average 23.9% of all cysticerci in a carcass are located in the predilection sites, meaning that 76.1% of cysticerci are located elsewhere in the body and cannot be found during MI or dissection of the predilection sites. While full carcass dissections on naturally infected cattle would provide a better gold standard test, the massive workload and high costs involved make this approach impossible.

Furthermore, tests can perform differently in different epidemiological situations and sensitivity and specificity are population-specific parameters that vary between naturally infected populations (Greiner and Gardner, 2000).

When applying extra incisions to the heart, four positive cases were detected among the 614 MI-negative samples, with no viable cysticerci found. In comparison to the 50% increase in positive carcasses found using enhanced MI in the study of Eichenberger et al. (2011), this is a negligible improvement, even though most (39.9%) cysticerci were found in the heart during dissection of the predilection places. Differences in intensity of infection in both populations might be at the base of this result but a repetition of this study or an analysis of the meat juice from heart tissue would be advised to confirm or reject this result.

To determine the total number of infected animals in the study population and the estimated prevalence, a simulation was done using the data at hand. Fig. 1 shows the results of this simulation as a summation and total of the number of positive carcasses in the MI-negative samples when the different techniques would be performed sequentially (instead of parallel testing). Within the 614 MI negative carcasses, 144 (23.5%) were found positive during the dissection of the predilection sites. Sequentially, out of the remaining 470 negative carcasses, 40 (8.5%) gave a positive test result for the Ag-ELISA. After subtraction of these positive carcasses, 430 negative carcasses remained, of which 37 (8.6%) were positive for the Ab-ELISA. Eventually, 221 of the 614 (36.0%) carcasses found negative at MI, were positive using other detection methods for BCC. As such, the prevalence of BCC in Belgium appears to be much higher than the previously reported prevalence of 0.20–0.24%, based on meat inspection (annual reports Federal Agency for the Safety of the Food Chain, FASFC, 2012, 2013, 2014) and even the prevalence of 3.09% based on a previous study using the B158/B60 Ag-ELISA (Dorny et al., 2000).

Obviously, there is an urgent need to implement improved detection methods for BCC. The results of the current study indicate that the sensitivity of the MI is even much lower than previously estimated (< 15%). It would not be impossible to implement multiple new techniques for post-mortem detection of cysticercosis in slaughterhouses due to financial and time related factors. Applying for example only the Ag-ELISA, will not only lead to a more time-consuming detection process at the slaughterhouses/laboratories, but also to a great increase in economic costs involved. Performing the Ag-ELISA is costlier than performing MI, and since an estimated 9% of carcasses would be diagnosed positive for BCC, this would lead to a much higher number of carcasses that need to be frozen before consumption. However, on the long term, this method should lead to a decrease in human taeniosis cases and sequentially to a decrease in BCC cases. The impact and cost of implementing new detection techniques in the slaughterhouses on short and long terms for the meat sector and public health in Belgium will be determined, using current results. A risk analysis model determining these factors is currently being developed.

Conflict of interest statement

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