Veterinary Parasitology 254 (2018) 142–146

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Research paper

Estimating prevalence and diagnostic test characteristics of bovine cysticercosis in Belgium in the absence of a 'gold standard' reference test using a Bayesian approach

Famke Jansen[a,b,*], Pierre Dorny[a,b], Sarah Gabriël[c], Ramon Marc Eichenberger[d], Dirk Berkvens[a]

[a] Department of Biomedical Sciences, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium
[b] Laboratory of Parasitology, Faculty of Veterinary Medicine, Ghent University, 133 Salisburylaan, B-9820 Merelbeke, Belgium
[c] Department of Veterinary Public Health and Food Safety, Faculty of Veterinary Medicine, Ghent University, 133 Salisburylaan, B-9820 Merelbeke, Belgium
[d] Institute of Parasitology, Vetsuisse-Faculty, University of Zürich, Winterthurerstrasse 266a, CH-8057 Zürich, Switzerland

ARTICLE INFO

Keywords:
Taenia saginata
Cattle
Bayesian model
True prevalence
Belgium

ABSTRACT

A Bayesian model was developed to estimate values for the prevalence and diagnostic test characteristics of bovine cysticercosis (*Taenia saginata*) by combining results of four imperfect tests. Samples of 612 bovine carcases that were found negative for cysticercosis during routine meat inspection collected at three Belgian slaughterhouses, underwent enhanced meat inspection (additional incisions in the heart), dissection of the predilection sites, B158/B60 Ag-ELISA and ES Ab-ELISA. This Bayesian approach allows for the combination of prior expert opinion with experimental data to estimate the true prevalence of bovine cysticercosis in the absence of a gold standard test. A first model (based on a multinomial distribution and including all possible interactions between the individual tests) required estimation of 31 parameters, while only allowing for 15 parameters to be estimated. Including prior expert information about specificity and sensitivity resulted in an optimal model with a reduction of the number of parameters to be estimated to 8. The estimated bovine cysticercosis prevalence was 33.9% (95% credibility interval: 27.7–44.4%), while apparent prevalence based on meat inspection is only 0.23%. The test performances were estimated as follows (sensitivity (Se) – specificity (Sp)): enhanced meat inspection (Se 2.87% – Sp 100%), dissection of predilection sites (Se 69.8% – Sp 100%), Ag-ELISA (Se 26.9% – Sp 99.4%), Ab-ELISA (Se 13.8% – Sp 92.9%).

1. Introduction

Bovine cysticercosis is caused by the metacestode larvae of the globally occurring cestode *Taenia saginata*. The parasite manifests itself in humans as the adult tapeworm in the intestines after consuming raw or undercooked infected beef (taeniosis) (Murrell et al., 2005). It is not considered a serious public health problem or food safety issue but the parasite induces a high economic impact for the meat sector (Wanzala et al., 2002; Scandrett et al., 2009). To prevent taeniosis and bovine cysticercosis from spreading, cattle are inspected at slaughter according to standard European Union meat inspection procedures (854/2004). For all animals older than six weeks, the oesophagus, tongue, diaphragm and visible muscle surfaces are visually inspected and several incisions are made in the heart and masseter muscles.

Meat inspection is the only detection method currently employed in Europe and average prevalence in Belgium based on official data is 0.23%, with annually 1,168 localised infections and 15 generalised infections (FASFC, 2012, 2013, 2014, 2015, 2016). The sensitivity of this technique has generally been estimated to be < 16% (Kyvsgaard et al., 1990; Dorny et al., 2000; Eichenberger et al., 2013), while the specificity is considered to be high (Geysen et al., 2007). This was recently found to be an overestimation for Belgium (Jansen et al., 2017). Surveys in cattle are often based on results of other methodologies, e.g. different antibody or antigen ELISAs (Allepuz et al., 2012; Dorny et al., 2000; Harrison et al., 1989; Ogunremi and Benjamin, 2010). All techniques used have varying test characteristics, making it difficult to compare the results.

Furthermore, in most studies only one technique is used to determine the unknown infection status of an animal. Within a population, the prevalence determined will thus be an "apparent" prevalence and not necessarily the "true" prevalence. Without a gold standard test (complete dissection of the carcase), true infection status cannot be accurately estimated (Berkvens et al., 2006; Speybroeck et al., 2013). Sensitivity (Se) and specificity (Sp) are often considered to be intrinsic

* Corresponding author at: Department of Biomedical Sciences, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium.
E-mail address: fjansen@itg.be (F. Jansen).

https://doi.org/10.1016/j.vetpar.2018.03.013
Received 16 January 2018; Received in revised form 6 March 2018; Accepted 11 March 2018

to the diagnostic test, but in reality these characteristics vary with external factors (cross-reacting organisms or low infection pressure) and populations (Praet et al., 2010). Another common assumption is that tests are independent of each other and of the true disease status, but this is not necessarily true for tests with a similar biological basis (Berkvens et al., 2006; Lesaffre et al., 2007; Speybroeck et al., 2013).

To get a better estimate of the prevalence of a disease when true infection status is unknown, several authors have attempted to create models combining the results of multiple diagnostic tests (Boelaert et al., 1999; Dorny et al., 2004; Eichenberger et al., 2013; Enøe et al., 2000). The problem when using imperfect diagnostic techniques with unknown sensitivity and specificity is that the model requires more parameters to be estimated than allowed by the degrees of freedom. By adding deterministic and/or probabilistic constraints based on expert opinion and literature review, the number of parameters to estimate can be reduced. Deterministic constraints (Dendukuri and Joseph, 2001; Gardner et al., 2000) calculate the probability of different outcomes as a function of test sensitivity, specificity and covariances between them, but prior distributions for the covariances are very difficult to estimate for the experts since it has no relation to real life. This seems also true for sensitivity, because this factor almost always needs to be determined under experimental conditions (not real life settings) and is mainly estimated using a small sample size. Giving expert opinion for probabilistic constraints has proven to be easier since they set a prior distribution for a parameter or prior opinion on a conditional performance of one test given the result of another test (Berkvens et al., 2006; Lesaffre et al., 2007; Speybroeck et al., 2013).

Combining prior (expert) opinion with experimental data can be performed in a Bayesian analysis framework to estimate prevalence and diagnostic test characteristics (Lesaffre et al., 2007). Prior opinion is necessary to reduce the number of parameters to be estimated, while allowing estimation of prevalence and test characteristics, meaning that the information needs to be general enough so it can be applied in the particular situation and precise enough to allow estimation of the parameters. This is usually done with information about specificity (Berkvens et al., 2006; Lesaffre et al., 2007; Speybroeck et al., 2013).

In this study, a Bayesian model was developed to estimate prevalence and test characteristics using a dataset of 612 bovine carcases that were found negative for cysticercosis during meat inspection, collected at three Belgian slaughterhouses. Cysticercosis was detected with four different detection techniques on collected samples: enhanced meat inspection with additional incisions in the heart, dissection of the predilection sites, antibody detection and antigen detection.

2. Materials and methods

2.1. Sampling design

Randomly selected carcases (mix of dairy and beef cattle from 6 to 216 months of age) were sampled at the slaughter line weekly during three consecutive 10-month periods between 2012 and 2015, in three Belgian slaughterhouses. 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) and meat inspection (MI) results were noted. Only a small percentage of animals is positive for BCC on MI (0.22%), so samples of all MI-positive carcasses (predilection sites, blood sample, SANITEL number, MI result including a muscle sample with the suspected cysticerc) detected were collected together with the MI-negative samples in each slaughterhouse during the 10-month time of sampling. 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 enhanced MI (Section 2.2), dissection of the predilection sites (Section 2.3), Ag-ELISA (Section 2.4) and Ab-ELISA (Section 2.5).

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

Six additional incisions were made in the collected hearts as described by Eichenberger et al. (2011).

2.3. Dissection of the predilection sites (PS)

Predilection sites (heart, tongue, masseter muscles, oesophagus and diaphragm) were completely dissected making 0.5 cm thick slices. This test was done sequentially after the enhanced MI. Cysticerci found during enhanced MI were removed and not counted again for the dissection of the predilection sites.

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). Briefly, each sample was tested in duplicate, together with 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) on each plate. 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).

2.5. Enzyme-linked-immunosorbent assay for the detection of specific antibodies (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 on a *T. saginata* negative population of Swiss dairy cows (Eichenberger et al., 2013).

2.6. Statistical analysis

Results collected using the four tests explained above, were fed into a model. Since all MI-positive samples were collected, together with a random sampling of the MI-negative carcasses, these two groups represent different populations. We opted to perform the Bayesian analyses on the population of MI-negative samples only since (a) these still contain many true positives due to (1) the very low sensitivity of the MI (Jansen et al., 2017) and (2) prevalence estimated with MI is only 0.23%, indicating that the MI-negative population is still a good representation of the real population, and (b) the lack of true negatives in the MI-positive population, making a Bayesian model difficult to perform. Total population size was 612, due to the Ab-ELISA not being done on two serum samples because of a limited amount of serum.

Expert opinion and a literature study were used to specify prior information on the diagnostic test characteristics. The expert opinion was obtained from helminthologists at the Institute of Tropical Medicine, Antwerp. Upper and lower limits were provided for various test sensitivities and specificities, to lower the number of parameters to estimate. First, priors that result in an explicit reduction of the number