ESTIMATION OF PREVALENCE AND DIAGNOSTIC TEST EVALUATION THROUGH BAYESIAN MODELING.

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ABSTRACT

The influence of both the data at hand and the choice of prior information on the parameter estimation in a Bayesian model is illustrated, based on prevalence studies for Giardia and Cryptosporidium in calves and based on diagnostic test evaluation in epidemiological and clinical studies. The prevalence of Giardia and the sensitivity and specificity of three diagnostic tests for the detection of Giardia in fecal samples were estimated with only an informative prior on the prevalence. For Cryptosporidium however a four test model was developed and the outcome varied depending on the choice of dataset, i.e. clinical or epidemiological. Furthermore the addition of a dataset of 2 PCR tests resulted in the development of a six test model necessitating extensive prior information for model converging.

SAMENVATTING

De invloed van zowel de dataset als de keuze van prior informatie op het schatten van parameters in een Bayesiaans model wordt uiteengezet op basis van prevalentiestudies naar het voorkomen van Cryptosporidium en Giardia bij kalveren en op basis van test evaluatie voor klinische of epidemiologische studies. De prevalentie van Giardia en de test karakteristieken van drie diagnostische testen werden geschat enkel met behulp van een informatieve prior op de prevalentie. In de Cryptosporidium studies werd een vier-test model ontwikkeld. De bekomen schattingen van de test karakteristieken varieerde tussen epidemiologische en klinische studies. Het bijvoegen van de resultaten van 2 bijkomende diagnostische PCR reacties aan de data resulteerde in de ontwikkeling van een zes-testen model, waarbij uitgebreide prior informatie nodig was om het aantal te schatten parameters terug te dringen.

1. INTRODUCTION

The intestinal protozoan parasites Cryptosporidium and Giardia are commonly identified in the faeces of dairy calves and should be considered in young animals with diarrhea and failure to thrive (12; 5). The wide variation in prevalence described for both parasites is not only due to differences in farm management practice or climate (12), but also to the differences in study design, such as choice of diagnostic test (8). Differences in sensitivity and specificity between diagnostic techniques can result in a substantial variation in prevalence estimates (1). However, diagnostic test characteristics are not known in a bovine population, yet reliable estimates of these parameters are needed to adjust prevalence estimates. Furthermore, the estimates of these test characteristics vary among published validation studies which are generally performed in human medicine. General scepticism is appropriate against the extrapolation of test parameters validated on human samples to bovine samples, because these test characteristics would not apply in populations with a different prevalence (8). Since there is no gold standard for the diagnosis of both parasites in dairy calves, the infection status of the population under study is uncertain and the accuracy assessment of a new test can be seriously biased by the use of an imperfect reference test as gold standard (1; 3).
A relatively new approach used to circumvent this gold standard problem is the Bayesian approach, which has proven its potential in validating diagnostic techniques and providing a reliable estimate of the disease prevalence, when at least three independent diagnostic test results are available (2; 4; 7; 9). In this study, the prevalence of both *Giardia* and *Cryptosporidium* in calves in the province of East-Flanders was estimated using a Bayesian approach. For the evaluation of the diagnostic tests, different approaches were used, illustrating the benefits and potential pitfalls of a Bayesian analysis.

### 2. MATERIALS AND METHODS

Fifty dairy farms in the province of East-Flanders, Belgium, were randomly selected and visited on a single occasion. Faecal specimens were collected rectally from Holstein or Holstein cross calves aged from newborn to 10 weeks. For the detection of *Giardia* a sucrose flotation technique followed by iodine staining (ME), the combined MERIFLUOR *Cryptosporidium/Giardia* kit (IFA) and the TechLab *Giardia* test (Techlab Elsia) was performed. For *Cryptosporidium* a carbolfuchsin smear method followed by microscopical examination (ME), the IFA, the TechLab *Cryptosporidium* test (Techlab Elisa), the Bio-X Digestive ELISA Kit (Tetra) and two PCR reactions targeting an unknown sequence (C-PCR) and the sequence for the *Cryptosporidium* Oocyst Wall Protein (COWP-PCR), was performed. Furthermore 186 samples from calves suspected to have a *Cryptosporidium* infection based on clinical symptoms, were processed in a clinical study using the IFA, Techlab, Tetra and a Dip-stick (Bio-X).

A Bayesian analysis framework was used to draw inferences about the prevalence of *Cryptosporidium* and *Giardia* and the test properties (sensitivity and specificity) of the different tests. Different models were constructed in WinBUGS 1.4 (10). For *Giardia*, a three-test model was constructed, requiring 15 parameters to be estimated. For *Cryptosporidium*, different approaches were used: a four-test approach, requiring 31 parameters to be estimated, and a new six-test approach, requiring 127 parameters to be estimated. These models are in fact not identifiable, since the data provide not enough degrees of freedom to allow all parameters to be estimated. Therefore, the model building strategy consists of incorporating extraneous prior information in a dependence model, such as expert opinion (11). For some parameters no objective prior information can be formulated. Therefore it is necessary to leave prior information on these parameters non-informative, if one wants to maintain a minimum degree of honesty (6). Prior information can also be applied to reduce the possible range of values for a specific parameter. This reduction may affect the possible range of values for other parameters as well.

### 3. RESULTS

In the *Giardia* three-test only prior knowledge on the prevalence had to be included to make the model converge. The sensitivity and specificity of each test is presented in table 1. In the *Cryptosporidium* four-test approach prior information on prevalence and on the specificity of the IFA, Techlab and Tetra had to be included into the epidemiological model to reach convergence. Adding information on the sensitivity of the IFA further improved the model. Additional constraints did however not improve the model. In the clinical study, the same model was used although only prior information on the specificity of the IFA, Techlab and Tetra had to be included to optimise the model. The estimated sensitivity and specificity of each test in these models is presented in table 1. In the *Cryptosporidium* six-test model, prior information on the specificity of both PCR assays (sp=1) was included. The specificity of both PCR assays was confirmed by the sequencing of amplification products of randomly chosen samples throughout the study. The specificity constraints made the model converge and made estimates of test characteristics possible (table 1).

### 4. DISCUSSION

These are the first known studies to use a Bayesian approach to estimate the prevalence of both parasites in dairy calves and to evaluate several diagnostic assays. Since there is no gold standard for the diagnosis of infection, a prevalence estimation based on the results of a single diagnostic assay would have been unreliable. In this study for example, the calf prevalence ranged from 13% to 46 % for *Cryptosporidium* and from 17% to 25% for *Giardia* depending on the technique being used. A Bayesian approach has proven its potential to circumvent this gold standard problem when 3 (9) or 4 tests (4) were used to diagnose infection. Both approaches were also used in these studies.

The results of the present studies illustrate, next to the benefits, the pitfalls of test evaluation and prevalence estimation using Bayesian analysis. Similar to diagnostic test evaluation in a frequentist approach, extrapolation of results on test characteristics beyond the limits of the study cannot be made in a Bayesian approach and results
should always be considered within the limits of the study. In a Bayesian analysis the posterior estimates are the result of both prior information and the data at hand (4). Prior information or constraints are essential in the present analysis in order to reduce the number of parameters to be estimated. In all models the use of prior information was needed to constrain the parameter space, allowing estimation of all remaining parameters. Expert opinion or published results from previous studies can be used as valuable prior information (11). In the Giardia three test approach and in the Cryptosporidium four-test approach the use of wide range constraints, based on previously published estimates of prevalence and test characteristics or on information provided by the manufacturer of the diagnostic assays, was sufficient to reduce the number of parameters to be estimated. In the Cryptosporidium six-test approach however, the use of more stringent constraints was necessary to reduce the high number of parameters. Although the validity of this prior information used in the six-test approach was previously described and further confirmed by sequencing PCR amplification products of randomly selected samples throughout the study, the posterior estimates of both the prevalence and the test characteristics are not stringent parameters, but should be considered as best-possible estimates based on the observations and the prior information. Since the data at hand in the present studies resulted mainly from epidemiological studies including both calves with and without clinical symptoms, and greatly depend on the choice of test for diagnosis, the posterior estimates of the test characteristics must be interpreted taking these limits into account. The rather low sensitivity estimates of some techniques is mostly due to the low excretion in healthy or subclinically infected calves. It does however not imply that these techniques are not reliable for clinical diagnosis, since clinical symptoms correlate with an increased excretion of oocysts, as was confirmed in our clinical analysis estimating higher sensitivity and specificity values were found for the IFA, Techlab and Tetra (see table 1).

### Table 1: sensitivity (Se(%)) and specificity (Sp(%)) estimates obtained by the different models

<table>
<thead>
<tr>
<th></th>
<th>3 test approach Giardia epidemiological</th>
<th>4 test approach Crypto Clinical</th>
<th>4 test approach Crypto epidemiological</th>
<th>6 test approach Crypto epidemiological</th>
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<tbody>
<tr>
<td></td>
<td>Se (%) Sp (%)</td>
<td>Se (%) Sp (%)</td>
<td>Se (%) Sp (%)</td>
<td>Se (%) Sp (%)</td>
</tr>
<tr>
<td>C-PCR</td>
<td>NA NA</td>
<td>NA NA</td>
<td>79 (69-87) 100</td>
<td></td>
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<tr>
<td>COWP PCR</td>
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<td>NA NA</td>
<td>59 (50-67) 100</td>
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<td>ME</td>
<td>56 (39-73) 87 (81-91) NA NA</td>
<td>78 (56-95) 79 (72-87) 40 (31-49) 84 (75-97)</td>
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<td></td>
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<td>IFA</td>
<td>77 (53-97) 95 (91-99) 86 (78-94) 91 (81-99) 78 (54-95) 95 (91-99) 26 (19-34) 94 (88-99)</td>
<td></td>
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<tr>
<td>Techlab Elisa</td>
<td>89 (70-99) 90 (83-97) 88 (81-95) 89 (82-97) 76 (54-92) 89 (84-94) 37 (28-46) 84 (76-92)</td>
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<tr>
<td>Tetra Elisa</td>
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<td>89 (81-95) 89 (81-97) 59 (39-77) 93 (89-96) 30 (21-42) 88 (81-94)</td>
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<td>Dip-stick</td>
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<td>NA NA</td>
<td>79 (71-88) 74 (19-96) NA NA NA NA NA</td>
<td></td>
</tr>
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5. REFERENCES


