

A Bayesian approach for the evaluation of six diagnostic assays and the estimation of *Cryptosporidium* prevalence in dairy calves

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Abstract – The prevalence of *Cryptosporidium* in calves and the test properties of six diagnostic assays (microscopy (ME), an immunofluorescence assay (IFA), two ELISA and two PCR assays) were estimated using Bayesian analysis. In a first Bayesian approach, the test results of the four conventional techniques were used: ME, IFA and two ELISA. This four-test approach estimated that the calf prevalence was 17% (95% Probability Interval (PI): 0.1–0.28) and that the specificity estimates of the IFA and ELISA were high compared to ME. A six-test Bayesian model was developed using the test results of the 4 conventional assays and 2 PCR assays, resulting in a higher calf prevalence estimate (58% with a 95% PI: 0.5–0.66) and in a different test evaluation: the sensitivity estimates of the conventional techniques decreased in the six-test approach, due to the inclusion of two PCR assays with a higher sensitivity compared to the conventional techniques. The specificity estimates of these conventional assays were comparable in the four-test and six-test approach. These results both illustrate the potential and the pitfalls of a Bayesian analysis in estimating prevalence and test characteristics, since posterior estimates are variables depending both on the data at hand and prior information included in the analysis. The need for sensitive diagnostic assays in epidemiological studies is demonstrated, especially for the identification of subclinically infected animals since the PCR assays identify these animals with reduced oocyst excretion, which the conventional techniques fail to identify.

Cryptosporidium / Bayesian / test evaluation / prevalence / calves

1. INTRODUCTION

Cryptosporidium is a protozoan parasite closely related to the gregarine parasites within the *Apicomplexa* [3] and is considered as an important agent in the aetiology of diarrhoea in both humans and livestock [7, 15]. In cattle, the parasite has been associated both with acute and chronic

clinical disease [23], especially in young calves [4]. The prevalence of *C. parvum* in calves aged less than 6 months varies from 2.4% [38] to 54% [27]. This variation in prevalence is not only due to substantial differences in climatological or management conditions, but also to differences in study design, such as differences in the number of farms or animals, the age of the animals under study, sampling strategies or the diagnostic techniques used in the study. The conventional diagnostic assays for the

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diagnosis of *Cryptosporidium* in calves comprise microscopic examination (ME) following Kinyoun acid fast staining, modified Ziehl-Neelsen staining or carbolfuch-sine staining, and immunological assays, such as the immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA). More recently, polymerase chain reaction (PCR) has been shown to be both very sensitive and specific [19]. Accuracy assessment of diagnostic tests is traditionally performed using contingency tables with the test results of one assay or a combination of assays [14] as the gold standard reference. However, due to a lack of knowledge of the true disease status of the tested animals (i.e. no gold standard), diagnostic test evaluation is frequently rendered ineffective leading to an over- or underestimation of the performance of the new test. The accuracy assessment of tests for the diagnosis of *Cryptosporidium* is illustrative of the problem. In some studies ME was used as the gold standard, overrating the ME sensitivity and specificity values compared to ELISA or IFA [13, 21], whereas in other studies IFA [27] or PCR [19] were used as the gold standard, underestimating the relative sensitivity and specificity of ME. Similar reflections can be made for the evaluation of ELISA using IFA as the gold standard [8, 30]. These problems can be partially evaded by using spiked samples to evaluate the sensitivity and specificity of diagnostic test methods. A higher recovery rate for IFA compared to ME has been described [39, 40], although recovery of *Cryptosporidium* oocysts from spiked specimens is known to be low [39]. Furthermore, this procedure can only be used in a controlled evaluation protocol and not for evaluation of diagnostic tests in clinical or epidemiological studies where the true disease status of the subject is unknown. Recently, Bayesian analysis has proven its value in circumventing this gold standard problem [1, 5, 11, 26]. In this study, two different Bayesian approaches

are presented for the estimation of *Cryptosporidium* prevalence in dairy calves in the province of East-Flanders, Belgium, and for the evaluation of six diagnostic tests.

2. MATERIALS AND METHODS

2.1. Study design

Fifty dairy farms in the province of East-Flanders, Belgium, were randomly selected and visited on a single occasion between September 2001 and November 2002. All calves were Holstein or Holstein cross. Faecal specimens were collected rectally from all calves aged from newborn to 10 weeks, present on the farm at the time of the visit. All samples were transported to the laboratory and examined using six different diagnostic assays. The samples were examined within 48 h after collection with ME and IFA. Afterwards, all samples were preserved at -20°C and examined with ELISA and PCR.

2.2. Detection of *Cryptosporidium* oocysts

2.2.1. The carbolfuch-sine smear method followed by microscopical examination

A direct faecal smear followed by carbolfuch-sine staining [12] was used to demonstrate *Cryptosporidium* oocysts in fresh faecal material. Approximately 2 cm^2 of each slide was examined at a $1\ 000\times$ magnification. A sample was considered positive as soon as an oocyst with the correct morphology ($4.6\text{--}5.6\ \mu\text{m}\times 4\text{--}4.8\ \mu\text{m}$) was identified.

2.2.2. Immunofluorescence Assay

The quantitative IFA was developed using the commercial MERIFLUOR *Cryptosporidium* kit (Meridian Diagnostics,

Inc., Cincinnati, Ohio, USA) and based on the method of Xiao and Herd [41]: one gram of faeces was suspended in distilled water and strained through a layer of surgical gauze to withhold large debris. After sedimentation for at least 1 h and centrifugation at 3 000 *g* for 5 min, the sediment was resuspended in 1 mL of distilled water. After thorough vortexing, an aliquot of 10 μ L was pipetted onto a treated IFA-slide. After staining the slide, as instructed by the manufacturer, the entire slide was examined at a 400 \times magnification under a fluorescence microscope. A sample was considered positive if at least one, clearly recognisable *Cryptosporidium* oocyst was identified. The number of *Cryptosporidium* oocysts per gram faeces was obtained by multiplying the total number of oocysts on the slide by 100.

2.2.3. Enzyme-linked immunosorbent assay for the detection of *Cryptosporidium* antigen in the faeces

The TechLab *Cryptosporidium* test (Techlab, Inc., Blacksburg, VA, USA) and the Tetrakit or Bio-X Digestive ELISA Kit (Bio-X diagnostics, Marche-en-Famenne, Belgium) were used to demonstrate the prevalence of *Cryptosporidium* antigen in frozen samples. Both assays were used as instructed by the manufacturer.

2.2.4. PCR diagnostic assays (PCR)

DNA was extracted from faecal samples using the QIAamp[®] Stool Mini Kit (Qiagen, Hilden, Germany). The eluted DNA was subsequently precipitated by the addition of 140 μ L isopropanol and centrifuged for 30 min at 16 000 *g*. Following a wash step with 75% ethanol, the pellet was dissolved in 12.5 μ L ultrapure water. Amplification products of both PCR assays were

randomly selected and sequenced throughout the study to confirm the specificity of both PCR assays.

2.2.4.1. C-PCR

The oligonucleotide primers (CP-CR and O21F) used for detection of *Cryptosporidium* DNA were previously described [17, 18]. They were designed to amplify a 312 bp product from the bovine *C. parvum* genotype. The reaction mixture consisted of a master mix containing Taq DNA polymerase, dNTP mixture, Bovine Serum Albumin, standard Taq buffer, 10 pM of each primer and 2.5 μ L of template cDNA in a total of 25 μ L. Subsequent steps were initial denaturation for 2 min at 94 $^{\circ}$ C, followed by 40 cycles of denaturation for 30 s at 94 $^{\circ}$ C, annealing for 30 s at 58 $^{\circ}$ C and an extension for 30 s at 72 $^{\circ}$ C. Amplification products were subsequently visualised on 1.5% agarose gels with ethidium bromide. A sample was considered positive if a band of 312 bp was visualised. A positive and negative control sample was included in each PCR reaction. The positive control consisted of a plasmid containing the 312 bp rDNA product. This was originally cloned from a *C. parvum* DNA sample isolated from a *C. parvum* positive calf (kindly provided by Simone Caccio). The negative control was purified water.

2.2.4.2. COWP-PCR

The oligonucleotide primers (cry-15 and cry-9) used for detection of the COWP were previously described by [33]. They were designed to amplify a 550 bp product from the *C. parvum* genome. The reaction mixture is similar to the previous protocol. Subsequent steps were 40 cycles of denaturation for 50 s at 94 $^{\circ}$ C, annealing for 30 s at 55 $^{\circ}$ C and an extension for 50 s at 72 $^{\circ}$ C.

2.3. Statistical analysis

The apparent prevalence for each diagnostic assay was calculated as the number of positive animals divided by the total number of animals. A Bayesian analysis framework was used to draw inferences about the prevalence of *Cryptosporidium* and the test properties (sensitivity and specificity) of the different tests. Different models were constructed in WinBUGS 1.4 [35]. Typically, a burn-in phase of 5 000 iterations was used and the models were run for another 10 000 iterations to obtain estimates. The Brooks, Gelman and Rubin convergence statistic [9] was used to assess model convergence and only properly converged models were further considered. Model selection proceeded on grounds of identifiability of the model and through minimisation of the deviance information criterion (DIC) while ensuring a positive number of parameters (p_D) estimated in the model [10, 34]. Furthermore, the Bayesian p -value [10] was used as a measure for the goodness-of-fit of the model (a comparison of the data to the posterior predictive distribution) and essentially represents the proportion of positive differences at consecutive iterations between the deviance for “cell” counts, sampled from the model, and the deviance calculated for the data (a value close to 0.50 signifies a model that does not exhibit lack-of-fit). Since the independence model does not take possible conditional test characteristics into account [11], only fully conditional models were considered. Two different approaches were used to estimate the prevalence and test characteristics. In the four-test approach, a model, based on a multinomial distribution and including all possible interactions between the ME, IFA, Techlab and Tetra was used, requiring 31 parameters to be estimated [5]. Furthermore, a new six-test approach was developed, based on the results of all six individual tests, requiring 127 parameters

to be estimated: the prevalence, the sensitivity and specificity of the first test, two conditional sensitivities and two conditional specificities for the second test, four conditional sensitivities and four conditional specificities for the third test, eight conditional sensitivities and eight conditional specificities for the fourth test, sixteen conditional sensitivities and sixteen conditional specificities for the fifth test and finally thirty-two conditional sensitivities and thirty-two conditional specificities for the sixth test (cf. conditional probabilities, P. 675). This model is in fact not identifiable, since the data only allow 63 parameters to be estimated (64 “classes” of test results with probabilities summing to unity). Therefore, the model building strategy consisted of incorporating extraneous prior information in a dependence model, such as expert opinion [36], in an attempt to reduce the number of parameters to be estimated. This can be done by stating, for example, that the specificity of one of the tests is 1, leading to the exclusion of all parameters pertaining to false positives for this test: e.g. in Conditional probabilities, P. 675, equating the specificity of test 1 to 1 ($P_3 = 1$) automatically means that P_7 and all parameters including P_7 do not need to be estimated, since $Pr(D^- \cap T_1^+) = 0$. For other 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 [10]. All prior information that was used in the present study, is based on expert opinion or previously published data and is therefore empirical information. No data of the present study were used to formulate these priors.

Prior information can also be applied to reduce the possible range of values for a specific parameter [6]. This reduction may affect the possible range of values for other parameters as well. Because of the

CONDITIONAL PROBABILITIES

P1 Prevalence	$Pr(D^+)$
P2 Se ₁	$Pr(T_1^+ D^+)$
P3 Sp ₁	$Pr(T_1^- D^-)$
P4	$Pr(T_2^+ D^+ \cap T_1^+)$
P5	$Pr(T_2^+ D^+ \cap T_1^-)$
P6	$Pr(T_2^- D^- \cap T_1^-)$
P7	$Pr(T_2^- D^- \cap T_1^+)$
P8	$Pr(T_3^+ D^+ \cap T_1^+ \cap T_2^+)$
P9	$Pr(T_3^+ D^+ \cap T_1^+ \cap T_2^-)$
P10	$Pr(T_3^+ D^+ \cap T_1^- \cap T_2^+)$
P11	$Pr(T_3^+ D^+ \cap T_1^- \cap T_2^-)$
P12	$Pr(T_3^- D^- \cap T_1^- \cap T_2^-)$
P13	$Pr(T_3^- D^- \cap T_1^- \cap T_2^+)$
P14	$Pr(T_3^- D^- \cap T_1^+ \cap T_2^-)$
P15	$Pr(T_3^- D^- \cap T_1^+ \cap T_2^+)$
P16	$Pr(T_4^+ D^+ \cap T_1^+ \cap T_2^+ \cap T_3^+)$
...	
P23	$Pr(T_4^+ D^+ \cap T_1^- \cap T_2^- \cap T_3^-)$
P24	$Pr(T_4^- D^- \cap T_1^- \cap T_2^- \cap T_3^-)$
P25	$Pr(T_4^- D^- \cap T_1^- \cap T_2^- \cap T_3^+)$
...	
P31	$Pr(T_4^- D^- \cap T_1^+ \cap T_2^+ \cap T_3^+)$
P32	$Pr(T_5^+ D^+ \cap T_1^+ \cap T_2^+ \cap T_3^+ \cap T_4^+)$
...	
P50	$Pr(T_5^- D^- \cap T_1^- \cap T_2^- \cap T_3^+ \cap T_4^-)$
P51	$Pr(T_5^- D^- \cap T_1^- \cap T_2^- \cap T_3^+ \cap T_4^+)$
...	
P63	$Pr(T_5^- D^- \cap T_1^+ \cap T_2^+ \cap T_3^+ \cap T_4^+)$
P64	$Pr(T_6^+ D^+ \cap T_1^+ \cap T_2^+ \cap T_3^+ \cap T_4^+ \cap T_5^+)$
...	
P103	$Pr(T_6^- D^- \cap T_1^- \cap T_2^- \cap T_3^+ \cap T_4^+ \cap T_5^+)$
P104	$Pr(T_6^- D^- \cap T_1^- \cap T_2^+ \cap T_3^- \cap T_4^- \cap T_5^-)$
...	
P127	$Pr(T_6^- D^- \cap T_1^+ \cap T_2^+ \cap T_3^+ \cap T_4^+ \cap T_5^+)$

Table I. The apparent prevalence and the test properties of the diagnostic tests (95% PI) as estimated by the four test approach and the six test approach.

	4 test approach			6 test approach	
	AP	Se (%)	Sp (%)	Se (%)	Sp (%)
C-PCR	46	ND	ND	79 (69–87)	100
COWP PCR	35	ND	ND	59 (50–67)	100
ME	30	78 (56–95)	79 (72–87)	40 (31–49)	84 (75–97)
IFA	16	78 (54–95)	95 (91–99)	26 (19–34)	94 (88–99)
Techlab	21	76 (54–92)	89 (84–94)	37 (28–46)	84 (76–92)
Tetra	14	59 (39–77)	93 (89–96)	30 (21–42)	88 (81–94)

AP = apparent prevalence; Se = sensitivity; Sp = specificity; ND = not done.

complexity of the model, it is impossible to predict or calculate what level of reduction in the number of parameters to be estimated will result from this prior information. Therefore, DIC is used to assist in model selection. DIC is an information criterion and consists of two components: first an equivalent of a likelihood measure (transformed in such a way that a lower value means a better fit) and secondly a penalty for the complexity of the model (the lower the value, the simpler the model). This DIC is to be minimised during the model building process, attempting to find the best fit with the simplest possible model. The number of parameters (p_D) that was effectively estimated in the model represents the complexity of the model and is an indication of the final reduction in the number of parameters that had to be estimated.

3. RESULTS

3.1. Animals

In total, 234 calves were examined for the presence of *Cryptosporidium* oocysts in their faeces. The mean age was 26.2 ± 1.4 (mean \pm standard error) days ranging from 1 to 70 days. More than 60% of the calves were younger than 1 month. Seventy-three percent of the investigated animals were

female and 27% were male. There were an average number of 5 calves on the farms, ranging from 1 to 14.

3.2. Apparent calf prevalence and Bayesian analysis

The apparent calf prevalence as estimated by the different diagnostic assays, is presented in Table I and ranged from 0.14 in the Tetra ELISA up to 0.46 in the C-PCR. In the four-test approach, a conditionally dependent test model was used. Prior information on prevalence (prevalence constrained between 0.1–0.8) and on the specificity of the IFA, Techlab and Tetra (0.7–1) had to be included into the model to reach convergence. Adding information on the sensitivity of the IFA (0.4–1) further improved the model. Additional constraints did not, however, improve the model (Tab. II). Therefore, this model was used to estimate prevalence and test characteristics of the four assays. The estimated prevalence was 0.17 (95% Probability Interval (PI): 0.1–0.28). The estimated sensitivity and specificity of each test are presented in Table I.

Since a six-test dependence model is in fact not identifiable due to a high number of parameters that have to be estimated compared to the degrees of freedom offered by the dataset, prior information on

Table II. Prevalence (95% PI) and model comparison in the 4 and the 6 test approach based on the deviance information criterion (DIC), the number of parameters (p_D), and a Bayesian P -value (Bayes- p).

	Bayes- p	p_D	DIC	P
4 test approach				
Dependent $P = 0.1-0.8$; $Sp_2 = 0.7-1$	Model did not converge			
Dependent $P = 0.1-0.8$; $Sp_{2,3} = 0.7-1$	Model did not converge			
Dependent $P = 0.1-0.8$; $Sp_{2,3,4} = 0.7-1$	0.5451	8.582	71.481	0.198 (0.11-0.47)
Dependent $P = 0.1-0.8$; $Sp_{2,3,4} = 0.7-1$; $Se_2 = 0.4-1$	0.5642	9.582	70.939	0.167 (0.11-0.26)
Dependent $P = 0.1-0.8$; $Sp_{2,3,4} = 0.7-1$; $Se_{2,3} = 0.4-1$	0.7152	9.011	77.08	0.149 (0.10-0.21)
Dependent $P = 0.1-0.8$; $Sp_{2,3,4} = 0.7-1$; $Se_{2,3,4} = 0.4-1$	0.5288	7.263	79.83	0.137 (0.10-0.19)
6 test approach				
Dependent	Model is not identifiable			
Dependent $Sp_{PCR1and2} = 1$	0.408	29.908	202.9946	0.578 (0.50-0.66)

P = prevalence; Sp = specificity; Se = sensitivity; 2 = IFA; 3 = Techlab ELISA; 4 = Tetrakit ELISA; PCR 1 = C-PCR; PCR 2 = COWP PCR.

the specificity of both PCR assays ($sp = 1$) was included [18, 33]. The specificity of both PCR assays was confirmed by the sequencing of amplification products of randomly chosen samples throughout the study ($n = 25$). All sequences proved to be *C. parvum*, both for the C-PCR and the COWP-PCR. The specificity constraints made the model converge, whereas widening the constraints on the specificity of both PCR assays to a range between 0.8 and 1 prohibited the model convergence. Therefore the model with the more rigid constraints on PCR specificity ($sp = 1$) was used to estimate the test characteristics of all diagnostic assays (Tab. I). The animal population prevalence estimated using this model was 0.58 (95% PI: 0.5-0.66).

3.3. Intensity of oocyst excretion and average prevalence in different age categories

For all the tests, a high number of positive calves was found between the age of 1 and 4 weeks. In calves older than one month, the number of positive calves decreased, except for the PCR as-

says, demonstrating a high number of positive test results in calves up to the age of 10 weeks. The apparent prevalence in calves younger and older than one month as estimated by the C-PCR was 45% and 49% respectively. For the COWP the prevalence was 34% and 41% respectively for calves younger and older than one month.

Since the number of positive calves for the different age categories estimated by ME, IFA and both ELISA were similar, only the data obtained with IFA are presented in Figure 1, in comparison with the data of the C-PCR assay. The number of excreted oocysts was determined on the same samples using IFA and ranged from 200 to 24 000 000 oocyst per gram faeces (OPG). The OPG was the highest in calves between the age of 1 to 3 weeks (Fig. 1). The highest individual OPG was observed in a 12 day old calf.

4. DISCUSSION

This is the first known study to use a Bayesian approach to estimate the

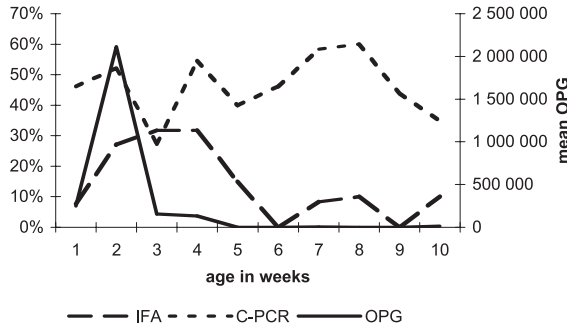


Figure 1. Prevalence and mean oocyst excretion (oocysts per gram faeces: OPG) of *Cryptosporidium parvum* in different age categories (1–10 weeks), as diagnosed by IFA and the C-PCR assay.

prevalence of *Cryptosporidium* in dairy calves and to evaluate diagnostic assays for the detection of the parasite. Since there is no gold standard for the diagnosis of a *Cryptosporidium* infection [14], a prevalence estimation based on the results of a single diagnostic assay would have been unreliable. In this study for example, the apparent calf prevalence ranged from 13% to 46%, depending on the technique being used. Previously a Bayesian approach has proven its potential to circumvent this gold standard problem when 3 [11, 26] or 4 tests [5] were used to diagnose infection. The four-test approach was also used in this study and yielded a *Cryptosporidium* calf prevalence estimate of 17% (95% PI: 0.1–0.28), which is comparable to observations by others in calves of the same age category [16, 20, 25, 32]. The 95% PI might even underestimate the prevalence due to intra-herd correlation not being taken into account. When the test results of two additional PCR assays were combined with the results of the four conventional assays and used to develop the six-test Bayesian approach, the prevalence estimate increased to 58% (95% PI: 0.5–0.66). The higher prevalence was largely due to the detection by both PCR assays of positive calves older than one month that were not detected by the conventional techniques, which is probably due

to the combined effect of the lower oocyst excretion in these calves and the higher sensitivity of PCR compared to the conventional techniques [40]. Similar to previous reports [22, 24, 42], the peak in oocyst excretion in this study was observed in calves between 1 to 4 weeks of age. Although the oocyst excretion decreases in calves older than one month, the prevalence estimated by both PCR assays remained high in calves older than one month, on the contrary to other studies with a decrease in prevalence found in calves in the age category of 4 to 10 weeks [25, 28]. The results of the present study indicate that calves between the age of 4 to 10 weeks can be an important source of infection for susceptible animals, although there is a possible shift in *Cryptosporidium* genotypes with age in calves [31].

The evaluation of the conventional techniques in the four-test approach yielded comparable sensitivity estimates for the ME and two out of the three immunological techniques, confirming previous reports that immunological detection methods are not significantly more sensitive than ME [14, 29]. The specificity of the immunological techniques, however, was higher than the specificity of ME. The speculated higher sensitivity of the PCR assays [19, 33] was confirmed by the six-test approach, with sensitivity estimates for

the C-PCR and COWP PCR of 0.79 and 0.59 respectively, whereas all other techniques had sensitivity estimates ≤ 0.4 . The difference in test characteristics estimates of the conventional assays determined in the four-test and the six-test approach, was due to the addition of two diagnostic techniques with a high sensitivity into the six-test approach: both PCR assays not only confirmed most samples positive by the four conventional assays, but also detected additional positive samples, resulting in a decrease in sensitivity estimates for the conventional techniques, whereas the specificity estimates for the four conventional assays were comparable in both approaches.

These results also illustrate 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 not rigid parameters but estimated variables resulting from both prior information and the data at hand [5]. Prior information or constraints are essential in the analysis in order to reduce the number of parameters to be estimated. Both in the four-test approach and in the six-test approach, 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 [2, 36, 37]. In the four-test approach the use of wide range constraints [6] based on previously published estimates of *Cryptosporidium* prevalence [16, 25, 27] and test characteristics [14] or on information provided by the manufacturer of the diagnostic assays, was sufficient to reduce the number of parameters to be estimated. In the six-

test approach however, the use of more stringent constraints was necessary to reduce the high number of parameters. Although the validity of the prior information used in the six test approach was previously described [18, 33] and 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 study resulted from an epidemiological study including both calves with and without clinical symptoms, and greatly depended 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 in the six-test model of the conventional techniques was mostly due to the low excretion of *Cryptosporidium* oocysts in calves older than one month and to the higher sensitivity of PCR compared to more conventional techniques. It does, however, not imply that these conventional techniques are not reliable for clinical diagnosis, since clinical symptoms are mostly diagnosed in calves younger than one month and correlate with an increased excretion of oocysts. In view of our epidemiological study, however, the results of the six-test approach demonstrate that PCR assays can provide additional information compared to the conventional diagnostic tools.

The results of this study illustrate the benefit of a Bayesian approach in estimating prevalence in a population with unknown disease status and in providing a reliable tool for the evaluation of diagnostic test characteristics, within the limits of the data at hand and the prior information. The need for sensitive diagnostic assays, such as the PCR assays, in epidemiological studies was demonstrated, especially

for the identification of subclinically infected animals who might be an important source of infection for other animals, since the PCR assays identify those animals with reduced oocyst excretion that the conventional techniques fail to identify.

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