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Short Communication

A Bayesian evaluation of four immunological assays for the diagnosis of clinical cryptosporidiosis in calves

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Abstract

A Bayesian approach was used to evaluate four immunological assays for the clinical diagnosis of cryptosporidiosis in calves: an immunofluorescence assay (IFA), two ELISA tests and an immunochromatographic (dipstick) assay. Faecal samples from 287 calves aged less than 6 weeks with clinical signs of gastrointestinal disease were examined for the presence of *Cryptosporidium* spp. The high prevalence (63%) of *Cryptosporidium* spp. indicated the relevance of this agent in the aetiology of diarrhoea in calves. All diagnostic assays were found to be relatively specific (IFA: 94.8%; Tetra ELISA: 95.9%; Techlab ELISA: 92.7%; dipstick assay: 91.5%) and sensitive (IFA: 97.4%; Tetra ELISA: 93.6%; Techlab ELISA: 95.4%; dipstick: 87.8%). Despite a lower sensitivity, the dipstick assay provided a practical alternative to laboratory diagnosis of clinical cryptosporidiosis in calves.

Keywords: *Cryptosporidium*; Calf; Bayesian; Diagnosis; Immunochromatography

Cryptosporidium spp. is a protozoan parasite associated with acute diarrhoea in young calves (de Graaf et al., 1999). Diagnosis is based on clinical signs, age of calves and detection of *Cryptosporidium* spp. oocysts or antigens in faecal samples by microscopic examination (ME), immunofluorescence assay (IFA) or enzyme-linked immunosorbent assay (ELISA). Since these techniques require laboratory equipment and trained personnel, an on-site immunochromatographic assay (dipstick assay) could provide a practical and time-saving alternative.

Although a dipstick assay was found to be both sensitive and specific for the detection of *Cryptosporidium* spp. in human samples (Depierreux et al., 2001; Llorente et al., 2002), evaluation for use in bovine faecal samples is lacking. Despite previous evaluation in an epidemiological study (Geurden et al., 2006), the sensitivity (se) and specificity (sp) of IFA and ELISA for diagnosis of cryptosporidiosis are unknown, since extrapolation of test characteristics to populations with different excretion patterns is not appropriate. Therefore, a Bayesian analysis framework was used to estimate the test properties of an IFA, two ELISAs and a dipstick assay, using a 4-test model in WinBUGS 1.4 (Spiegelhalter et al., 2003).

Faecal specimens were collected from clinically affected calves under 6 weeks of age, and tested using four diagnostic assays: dipstick assay (Bio-X Diagnostics), IFA (MERIFLUOR *Cryptosporidium/Giardia* kit, Meridian Diagnostics Inc.), Techlab ELISA (TechLab *Cryptosporidium* ELISA, Techlab Inc.) and Tetra ELISA (Bio-X Digestive ELISA, Bio-X Diagnostics). Samples were examined by IFA and dipstick assay within 48 h of collection. All samples were preserved at −20 °C and tested later by ELISA. All assays were performed according to the manufacturers' instructions, except for the IFA, which was performed according to a previously described protocol (Geurden et al., 2006). The Bayesian models were run and evaluated as previously described (Berkvens et al., 2006).

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Table 1
Cross-classified test results obtained by the four individual tests (IFA = immunofluorescence; Tetra = Tetrakit ELISA; Techlab = Techlab ELISA; Dipstick = Bio-X dipstick) for the detection of *Cryptosporidium* spp.

IFA	Tetra	Techlab	Dipstick	Number of samples
1	1	1	1	156
1	1	1	0	14
1	1	0	1	0
1	1	0	0	2
1	0	1	1	4
1	0	1	0	3
1	0	0	1	2
1	0	0	0	2
0	1	1	1	0
0	1	1	0	0
0	1	0	1	1
0	1	0	0	1
0	0	1	1	2
0	0	1	0	2
0	0	0	1	3
0	0	0	0	95

The number of samples is presented for that particular combination of test results.

Faecal samples from 287 calves were examined for the presence of *Cryptosporidium* spp. The test results for the different immunological assays are presented in Table 1. Of 287 samples tested, 156 (54%) were positive and 95 (33%) were negative in the four assays. The apparent prevalence was 63.7% by IFA, 60.6% by Tetra ELISA, 63.0% by Techlab ELISA and 58.5% by dipstick assay.

The different Bayesian models and their evaluation criteria used in this study are presented in Tables 2 and 3. Probabilistic prior information was added for the prevalence and for the sp of the IFA, Techlab ELISA and Tetra ELISA and these priors induced model convergence (Table 2: Model M5). Adding information on the se of the IFA, Tetra ELISA and Techlab ELISA did not improve the analysis (Table 2: Model M6–M8).

The prior information for the sp and se of the techniques used in this study was based on previously published information (Geurden et al., 2006). The prior information for the prevalence was based on previous reports (Otto et al., 1995; de Graaf et al., 1999; Constant, 2001; Tartera, 2002). Since there was no reliable prior information on the dipstick assay parameters (Table 5: $p16$–$p31$), no constraints were used. The estimated prevalence of cryptosporidiosis was 63%, with a 95% probability interval (PI) of 57–69%. The estimated se and sp of each test, with 95% PI, are presented in Table 4.

Although no testing was performed for other enteric pathogens, the high prevalence indicates that *Cryptosporidium* spp. should be considered as an important pathogen in the aetiology of neonatal diarrhoea in calves (Otto et al., 1995; de Graaf et al., 1999; Constant, 2001; Tartera, 2002). As in a previous epidemiological study (Geurden et al., 2006), the results of the present Bayesian evaluation confirm that the IFA, Techlab ELISA and Tetra ELISA are specific diagnostic assays (sp > 90%). Previously, IFA and both ELISAs were found to lack sensitivity for the diagnosis of calves with low levels of oocyst excretion (Geurden et al., 2006). However, calves with clinical cryptosporidiosis excrete a high number of oocysts in the faeces (Nydam et al., 2001). In the present study, the IFA and both ELISAs were sensitive (se > 90%) in detecting infection in clinically affected calves.

The dipstick assay was found to be slightly less specific (91.5%) and sensitive (87.8%). Although 14 positive cases confirmed by the three other assays were not recorded as positive in the dipstick assay, the immunochromatographic method is a practical alternative for diagnosis of clinical cryptosporidiosis in calves. The main advantages of the dipstick assay are the rapid on-site diagnosis and user-friendly technique. The lower sensitivity of the dipstick can be countered by multiple samplings, either from different animals on the farm or from the same animal at different time points.

From a statistical perspective, the results of the present study confirm that estimates of test characteristics should be considered within the limits of the study design. In a previous epidemiological study (Geurden et al., 2006), differ-

Table 2
Parameters (p) to be estimated in the eight models (M) that were constructed from the available 'expert' opinion

	M1	M2	M3	M4	M5	M6	M7	M8
$p1$	0–1	0.5–0.7	0.5–0.7	0.5–0.7	0.5–0.7	0.5–0.7	0.5–0.7	0.5–0.7
$p2$	0–1	0–1	0–1	0–1	0–1	0.85–1	0.85–1	0.85–1
$p3$	0–1	0–1	0.9–1	0.9–1	0.9–1	0.9–1	0.9–1	0.9–1
$p4$	0–1	0–1	0–1	0–1	0–1	0–1	0.8–1	0.8–1
$p5$	0–1	0–1	0–1	0–1	0–1	0–1	0–1	0–1
$p6$	0–1	0–1	0–1	0.8–1	0.8–1	0.8–1	0.8–1	0.8–1
$p7$	0–1	0–1	0–1	0–1	0–1	0–1	0–1	0–1
$p8$	0–1	0–1	0–1	0–1	0–1	0–1	0–1	0.7–1
$p9$	0–1	0–1	0–1	0–1	0–1	0–1	0–1	0–1
$p10$	0–1	0–1	0–1	0–1	0–1	0–1	0–1	0–1
$p11$	0–1	0–1	0–1	0–1	0–1	0–1	0–1	0–1
$p12$	0–1	0–1	0–1	0–1	0.7–1	0.7–1	0.7–1	0.7–1
$p13$–$p31$	0–1	0–1	0–1	0–1	0–1	0–1	0–1	0–1

$p1,\ldots,p31$: see Table 5 for the parameter definition. The range (a–b) denotes that a is the lower limit and b is the upper limit of the parameter interval.

Table 3
Model (M) comparison based on the Bayesian *P*-value and two evaluations (parent nodes and multinomial) of the deviance information criterion (DIC) and the number of parameters (p_D)

Model	Bayesian *P*-value	Parent nodes		Multinomial	
		DIC	p_D	DIC	p_D
M1	No convergence				
M2	0.4524	−24.926	−79.442	60.362	9.067
M3	0.4088	66.509	7.976	59.724	9.015
M4	0.413	69.832	7.955	59.900	9.067
M5	0.4184	72.260	8.050	59.803	9.044
M6	0.4112	75.869	7.968	59.859	9.086
M7	0.4114	79.122	7.963	59.692	9.011
M8	0.4132	81.583	7.976	59.805	9.070

Table 4
Sensitivity (se) and specificity (sp) of diagnostic assays for detection of *Cryptosporidium* spp., with the 95% probability interval of the immunofluorescence assay (IFA), the Tetra ELISA (Tetra), the Techlab ELISA (Techlab) and the dipstick assay

	se	sp
IFA	97.4 (91.7–99.9)	94.8 (90.3–99.7)
Tetra	93.6 (87.1–98.3)	95.9 (90.4–99.3)
Techlab	95.4 (90.0–98.6)	92.7 (86.6–97.8)
Dipstick	87.8 (81.9–92.7)	91.5 (85.5–96.5)

Table 5
Conditional probabilities

Prevalence	$\Pr(D^+)$	$p1$
se_1	$\Pr(T_1^+\|D^+)$	$p2$
sp_1	$\Pr(T_1^-\|D^-)$	$p3$
	$\Pr(T_2^+\|D^+ \cap T_1^+)$	$p4$
	$\Pr(T_2^+\|D^+ \cap T_1^-)$	$p5$
	$\Pr(T_2^-\|D^- \cap T_1^-)$	$p6$
	$\Pr(T_2^-\|D^- \cap T_1^+)$	$p7$
	$\Pr(T_3^+\|D^+ \cap T_1^+ \cap T_2^+)$	$p8$
	$\Pr(T_3^+\|D^+ \cap T_1^+ \cap T_2^-)$	$p9$
	$\Pr(T_3^+\|D^+ \cap T_1^- \cap T_2^+)$	$p0$
	$\Pr(T_3^+\|D^+ \cap T_1^- \cap T_2^-)$	$p11$
	$\Pr(T_3^-\|D^- \cap T_1^- \cap T_2^-)$	$p12$
	$\Pr(T_3^-\|D^- \cap T_1^- \cap T_2^+)$	$p13$
	$\Pr(T_3^-\|D^- \cap T_1^+ \cap T_2^-)$	$p14$
	$\Pr(T_3^-\|D^- \cap T_1^+ \cap T_2^+)$	$p15$
	$\Pr(T_4^+\|D^+ \cap T_1^+ \cap T_2^+ \cap T_3^+)$	$p16$
	$\Pr(T_4^+\|D^+ \cap T_1^+ \cap T_2^+ \cap T_3^-)$	$p17$
	$\Pr(T_4^+\|D^+ \cap T_1^+ \cap T_2^- \cap T_3^+)$	$p18$
	$\Pr(T_4^+\|D^+ \cap T_1^+ \cap T_2^- \cap T_3^-)$	$p19$
	$\Pr(T_4^+\|D^+ \cap T_1^- \cap T_2^+ \cap T_3^+)$	$p20$
	$\Pr(T_4^+\|D^+ \cap T_1^- \cap T_2^+ \cap T_3^-)$	$p21$
	$\Pr(T_4^+\|D^+ \cap T_1^- \cap T_2^- \cap T_3^+)$	$p22$
	$\Pr(T_4^+\|D^+ \cap T_1^- \cap T_2^- \cap T_3^-)$	$p23$
	$\Pr(T_4^-\|D^- \cap T_1^- \cap T_2^- \cap T_3^-)$	$p24$
	$\Pr(T_4^-\|D^- \cap T_1^- \cap T_2^- \cap T_3^+)$	$p25$
	$\Pr(T_4^-\|D^- \cap T_1^- \cap T_2^+ \cap T_3^-)$	$p26$
	$\Pr(T_4^-\|D^- \cap T_1^- \cap T_2^+ \cap T_3^+)$	$p27$
	$\Pr(T_4^-\|D^- \cap T_1^+ \cap T_2^- \cap T_3^-)$	$p28$
	$\Pr(T_4^-\|D^- \cap T_1^+ \cap T_2^- \cap T_3^+)$	$p29$
	$\Pr(T_4^-\|D^- \cap T_1^+ \cap T_2^+ \cap T_3^-)$	$p30$
	$\Pr(T_4^-\|D^- \cap T_1^+ \cap T_2^+ \cap T_3^+)$	$p31$

ent se estimates were found for the IFA, Tetra ELISA and Techlab ELISA compared to the present clinical study. This was largely due to differences in data sets, with more negative samples and more samples with different test results for the respective assays in the epidemiological dataset. In the present study, accordance between test results was high, with a large proportion of the samples (87%) testing either positive or negative with the four assays. Similar to the previous Bayesian test evaluation (Geurden et al., 2006), prior information was essential in the present analysis to reduce the number of parameters to be estimated. Although these priors do direct the model to a certain extent, the estimates of prevalence, sensitivity and specificity were obtained by combining both the data at hand and previously published prior information (Otto et al., 1995; de Graaf et al., 1999; Constant, 2001; Tartera, 2002; Geurden et al., 2006).

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References

Berkvens, D., Speybroeck, N., Praet, N., Adel, A., Lesaffre, E., 2006. Estimating disease prevalence in a Bayesian framework using probabilistic constraints. Epidemiology 17, 145–153.

Constant, F., 2001. Les cryptosporidies confirmées *E. coli* toujours plus résistant. Point Veterinaire 219, 16–17.

de Graaf, D.C., Vanopdenbosch, E., Ortega-Mora, L.M., Abbassi, H., Peeters, J.E., 1999. A review of the importance of cryptosporidiosis in farm animals. International Journal for Parasitology 29, 1269–1287.

Depierreux, C., Pé, E., Paquet, A., Raimond, O., Leclipteux, T., 2001. L'immunochromathographie, technique alternative à la microscopie et à l'ELISA, pour la detection rapide, simple, efficace et performante du *Cryptosporidium parvum*. Focus Diagnostica 9, 90–94.

Geurden, T., Berkvens, D., Geldhof, P., Vercruysse, J., Claerebout, E., 2006. A Bayesian approach for the evaluation of six diagnostic assays and the estimation of *Cryptosporidium* prevalence in dairy calves. Veterinary Research 37, 671–682.

Llorente, M.T., Clavel, A., Varea, M., Olivera, S., Castillo, F.J., Sahagun, J., Rubio, M.C., Gomez-Lus, R., 2002. Evaluation of an immunogromatographic Dip-Strip test for the detection of *Cryptosporidium* oocysts in stool specimens. European Journal of Clinical Microbiology and Infectious Diseases 21, 624–625.

Nydam, D.V., Wade, S.E., Schaaf, S.L., Mohammed, H.O., 2001. Number of *Cryptosporidium parvum* oocysts or *Giardia* spp. cysts shed by dairy calves after natural infection. American Journal of Veterinary Research 62, 1612–1615.

Otto, P., Elschner, M., Günther, H., Schulze, F., 1995. Vergleichende untersuchungen zum nachweis von rotaviren, coronaviren, kryptosporidien und enterotoxigenen *E. coli* im kot durchfallkranker kälber.. Tierärtzliche Umschau 50, 80–86.

Spiegelhalter, D.J., Thomas, A., Best, N.G., Lunn, D., 2003. WinBUGS Version 1.4 User Manual, vol. 4. MRC Biostatistics Unit, Cambridge.

Tartera, P., 2002. *Cryptosporidium parvum* est présent chez 20 à 50% des veaux diarrhéiques. L'hebdo Vétérinaire 109, 11–14.