Short Communication

Evaluation of rapid assays for the detection of bovine coronavirus, rotavirus A and *Cryptosporidium parvum* in faecal samples of calves

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ABSTRACT

Rapid immunochromatographic assays for detecting infections with bovine coronavirus (BCV), rotavirus A and *Cryptosporidium parvum* in calf faeces were evaluated using as gold standards a reverse transcriptase polymerase chain reaction (BCV and rotavirus) and a sedimentation-flotation technique (*C. parvum*). Rapid tests for the detection of BCV and rotavirus showed a high specificity (96.4% and 95.3%, respectively), but a relatively low sensitivity (60.0% and 71.9%, respectively). Sensitivity and specificity for detection of *C. parvum* were high (100% and 94.6%, respectively).

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Conventional diagnostic methods to detect enteropathogens in calves require time, experience and specialised laboratory equipment. The practising veterinarian, however, requires rapid, reproducible, sensitive and simple diagnostic tests for quick decisions on therapeutic and prophylactic strategies. Therefore, rapid immunochromatographic assays have been developed to detect different enteropathogens in calf faeces within a few minutes in the field.

Rapid immunochromatographic tests for rotavirus and *Cryptosporidium parvum* were evaluated by Luginbühl et al. (2005) in faecal samples from 60 calves. The sensitivity for detection of rotavirus was low (57%) compared to an antigen enzyme-linked immunosorbent assay (ELISA). The sensitivity (75%) and specificity (100%) of the rapid test for *C. parvum* were relatively high compared to modified Ziehl-Neelsen (MZN) staining. The aim of the present study was to evaluate further these commercial rapid assays for detecting BCV, rotavirus A and *C. parvum* in faecal samples using a reverse transcriptase polymerase chain reaction (RT-PCR) (BCV and rotavirus) and a sedimentation–flotation technique (*C. parvum)* as gold standards.

Faecal samples from 180 calves, aged 1–42 days, from 61 randomly selected farms were collected; 98 calves had diarrhoea. All samples were tested with the following test kits (MegaCor Diagnostik GmbH) according to the manufacturer's guidelines: BCV (FASTest BCV Strip), rotavirus (FASTest ROTA Strip) and *C. parvum* (FASTest CRYPTO Strip). Faecal samples were also examined for the presence of *Cryptosporidium* spp. oocysts by a sedimentation–flotation technique (Bauer, 2006). Samples with discordant results for *Cryptosporidium* spp. were further tested using three different PCRs (Leng et al., 1996; Morgan et al., 1997, 1998) and MZN staining (Bronsdon, 1984).

RT-PCR was used for detection of BCV and rotavirus, as previously described (Tsunemitsu et al., 1991; Schwarz, 2002; Haschek et al., 2006). The genomic location of PCR products were nucleotides 589–1022 of the rotavirus A VP6 gene and nucleotides 92–480 of the BCV nucleocapsid gene. Purified labelled PCR products were analysed with the ABI Prism 310 Genetic Analyser. Nine randomly selected samples, which had revealed a positive RT-PCR result for BCV, but were negative in the rapid test, were retested once by RT-PCR.

RT-PCR for BCV was positive in 70/180 (38.9%) samples, of which 42/70 (60%) were also positive using the rapid assay. These results indicated a sensitivity of 60.0% and a specificity of 96.4% for the rapid assay (Table 1). By retesting RT-PCR positive samples that had negative results in the rapid test, the positive RT-PCR results could be reproduced and the specificity was confirmed by sequencing. All sequences had identities of ≥98% with BCV reference strains (DB2: GenBank DQ811784; OK-0514-3: GenBank AF058944; R-AH-187: GenBank EF424620; E-AH187: GenBank EF424619).

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Table 1
Results of RT-PCR and rapid assay to detect bovine coronavirus

		PCR		Total
		Positive	Negative	
Rapid assay	Positive	42	4	46
	Negative	28	106	134
	Total	70	110	180

Overall agreement = 0.822, sensitivity = 0.600, specificity = 0.964, positive predictive value = 0.913, negative predictive value = 0.791, Cohen's κ = 0.601, $P < 0.001$.

Table 2
Results of RT-PCR and rapid assay to detect rotavirus A

		PCR		Total
		Positive	Negative	
Rapid assay	Positive	23	7	30
	Negative	9	141	150
	Total	32	148	180

Overall agreement = 0.911, sensitivity = 0.719, specificity = 0.953, positive predictive value = 0.767, negative predictive value = 0.94, Cohen's κ = 0.688, $P < 0.001$.

RT-PCR was positive for rotavirus in 32/180 (17.8%) samples; 23 of these 32 samples were also positive using the rapid assay, resulting in a sensitivity of 71.9%; seven samples were positive in the rapid assay but negative in the RT-PCR (specificity 95.3%) (Table 2). The sensitivities and specificities of the rapid assays for detection of BCV and rotavirus did not differ significantly between diarrhoeic and clinical healthy calves ($P > 0.05$).

Using the sedimentation-flotation technique for *C. parvum*, 51/180 (28.3%) samples were positive, all of these 51 samples were also positive in the rapid assay (sensitivity 100%). Seven samples positive in the rapid assay were negative by the sedimentation-flotation technique (specificity 94.6%). One of the seven divergent samples tested positive in the PCR of Morgan et al. (1997), while all other PCR results were in accordance with the sedimentation–

Table 3
Results of microscopic examination and rapid assay to detect *Cryptosporidium parvum*

		Microscopic examination		Total
		Positive	Negative	
Rapid assay	Positive	51	7	58
	Negative	0	122	122
	Total	51	129	180

Overall agreement = 0.961, sensitivity = 1, specificity = 0.946, positive predictive value = 0.879, negative predictive value = 1, Cohen's κ = 0.908, $P < 0.001$.

Table 4
Results of PCR testing of differing samples for *Cryptosporidium parvum* using rapid assays and microscopic examination

	Rapid assay	Microscopic examination	PCR 1[a]	PCR 2[b]	PCR 3[c]	Modified MZN [d]
1.	+	−	−	−	−	−
2.	+	−	−	−	−	+
3.	+	−	+	−	−	+
4.	+	−	−	−	−	+
5.	+	−	−	−	−	+
6.	+	−	−	−	−	+
7.	+	−	−	−	−	+

[a] PCR for detection of *Cryptosporidium* spp. (Morgan et al., 1997).
[b] PCR for detection of *Cryptosporidium parvum* (Morgan et al., 1998).
[c] PCR for detection of *Cryptosporidium parvum* (Leng et al., 1996).
[d] Modified Ziehl–Neelsen staining.

flotation technique. Six of these seven samples were positive on MZN staining (Tables 3 and 4).

Advantages of rapid assays are that a large number of samples can be processed quickly with minimum effort and intervention strategies can be implemented immediately. The need for technological expertise or specialised laboratory equipment is minimal. For evaluation of the rapid assays for BCV and rotavirus A, RT-PCRs were considered to be the gold standards in view of their high sensitivity and specificity (Tsunemitsu et al., 1991; Schwarz, 2002). For the infectious pathogens tested, the rapid assays exhibited varying sensitivities and specificities. Compared to RT-PCR, the sensitivities were low for BCV and rotavirus (60.0% and 71.9%, respectively). The specificities of the rapid tests compared to PCR were 96.4% for BCV and 95.3% for rotavirus.

Evaluation of the same rapid assays by Luginbühl et al. (2005), who compared them to an antigen ELISA, indicated a lower sensitivity (57%) and a slightly higher specificity (100%) for the rotavirus test. In clinical cases, the specificity might be of higher importance than the sensitivity but, if the causative virus cannot be detected in severely diseased animals, the diagnostic advantage of a fast test is questionable. The sensitivity may be increased by testing on a herd basis (three or more animals sampled). Given that the prevalence of BCV was high in the present study, the positive predictive value was also high (0.91). Conversely, in the case of rotavirus, which had a lower prevalence, the negative predictive value was comparably high (0.94).

In contrast to the results for the viral agents, the sensitivity and specificity of the rapid assay for *C. parvum* were high compared to the sedimentation-flotation technique (100% and 94.6%, respectively), but the results could not be confirmed conclusively by additional detection protocols (Table 4). The results of the present study suggest that the *C. parvum* test is sensitive and specific and therefore a helpful, fast and effective tool for the veterinary practitioner.

Conflict of interest statement

Except for one author (A. Kern), none of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper. Samples were processed and results evaluated by independent and uninfluenced people (D. Klein, G. Lapan, V. Benetka, K. Möstl, A. Hassl, W. Baumgartner). Dr. A. Kern is on the scientific staff of the MegaCor Diagnostik company.

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