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TROUSSE ELISA *Cryptosporidium*

BIO K 346/2

Test diagnostique de *Cryptosporidium* chez toutes les espèces.

Les diarrhées néonatales sont une cause majeure de mortalités chez le veau de moins d'un mois. Les gastroentérites néonatales sont des pathologies multifactorielles qui peuvent être causées par des virus (rotavirus, coronavirus), par des bactéries (colibacilles entéropathogènes, *salmonella*) ou par des protozoaires tels que *Cryptosporidium* .
Le diagnostic des causes de diarrhée passe obligatoirement par des tests de laboratoire car il n'est pas possible d'identifier l'agent causal sur base de signes cliniques. Il est possible de mettre en évidence *cryptosporidium* dans les matières fécales de veaux souffrant de diarrhée par la technique de flottaison ou par coloration (Ziehl Neelsen modifié). Ces deux méthodes sont cependant très fastidieuses à mettre en oeuvre lorsqu'un grand nombre d'échantillons doit être analysé. La méthode ELISA est très simple à réaliser. Sa sensibilité et sa spécificité sont très proches de celles obtenues par flottaison ou par coloration.

Fiabilité des résultats

L'utilisation d'un anticorps monoclonal comme conjugué et comme réactif de capture assure une excellente spécificité et permet d'obtenir des résultats très fiables.

Facilité d'utilisation

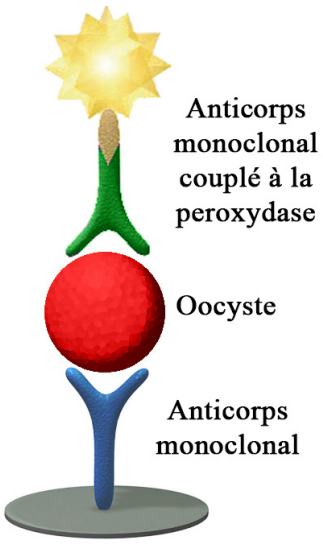
Peu de manipulations sont nécessaires.
Incubation à température ambiante.
Résultats disponibles en maximum 140 minutes. Toutes les solutions sont prêtes à l'emploi

Flexibilité

Les résultats peuvent être interprétés à l'aide d'un spectrophotomètre ou visuellement

Protocole du test

1- La microplaque est sensibilisée par un anticorps monoclonal
2- Ajouter les échantillons et le contrôle positif.
Incuber une heure à 21°C +/- 3°C
Laver
3- Ajouter le conjugué.
Incuber une heure à 21°C +/- 3°C
Laver
4- Ajouter le TMB
Attendre 10 minutes.
Ajouter la solution d'arrêt.
Lire à 450 nm



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Exemple de résultats

Flottaison

ELISA BIO K 346

	+	-	
+	33	6	39
-	1	60	61
	34	66	100

Spécificité: 90,9 %
Sensibilité: 97,1 %

Jours après la naissance

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Veau 1	Crypto						+	+	+	+	+	+	+	+	+			
	Rota																	
	Diarrhée						+	+	+	+	+	+	+					
Veau 2	Crypto							+	+	+	+	+	+	+	+	+	+	+
	Rota					+	+											
	Diarrhée					+	+	+	+	+	+	+	+	+	+		+	

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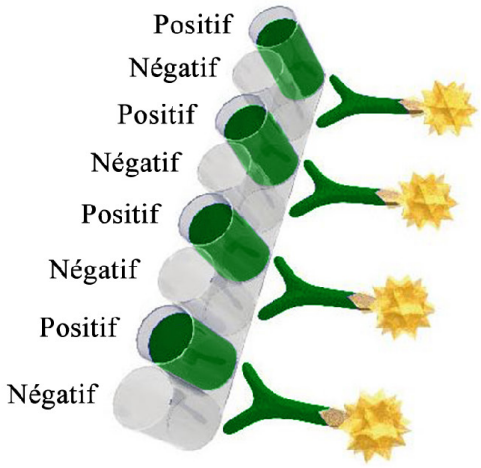
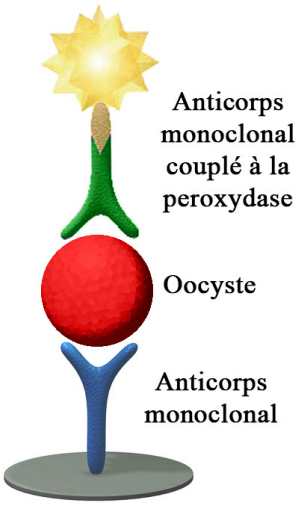
Composition de la trousse

BIO-X TROUSSE ELISA *CRYPTOSPORIDIUM* : BIO K 346/2

	BIO K 346/2
Microplaques	2
Solution de lavage	1 X 100 ml (20 X)
Tampon de dilution	1 X 50 ml (5 X)
Conjugué	1 X 25 ml (1 X)
Antigène de contrôle	1 X 4 ml (1 X)
TMB mono-composant	1 X 25 ml (1 X)
Solution d'arrêt	1 X 15 ml (1 X)

Stabilité : 1 an entre +2°C et +8°C.





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DETECTION OF ENTEROPATHOGENS INVOLVED IN CALF NEONATAL DIARRHOEA:
VALIDATION OF ELISAs AND LATERAL FLOW IMMUNOASSAYS AS COMPARED WITH REFERENCE METHODS

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Key words: Calves; neonatal diarrhoea; diagnosis; enteropathogens; ELISA;lateral flow immunochromatography; PCR

1. Introduction and Objectives

Several pathogens play a role in calf neonatal diarrhoea. The major enteropathogens involved are *Escherichia coli* F5⁺/K99 (*E. coli*), *Cryptosporidium parvum*, bovine enteric coronavirus, bovine rotavirus and bovine viral diarrhoea virus. In our laboratory different methods – e.g. selective culture for *E. Coli* F5⁺/K99, microscopic examination of faecal smears for *Cryptosporidium parvum*, a commercially available latex agglutination test for bovine rotavirus, and a commercially available antigen-detection-ELISA for BVDV are routinely used for detection of these agents. For bovine enteric coronavirus no routine diagnostic method was implemented until now.

The objectives of this study were to evaluate two commercially available antigen-detection-ELISA kits and two lateral flow immunochromatography tests (on site tests) for the detection of four of the above-mentioned pathogens.

2. Materials and Methods

2.1 Samples At necropsy rectal contents were sampled from calves between 0 and 6 weeks of age with diarrhoea (n=216). Samples were investigated by routine procedures and then stored at -20 °C to enable batchwise testing.

2.2 ELISAs Samples were tested in two different ELISA kits according to the instructions of the manufacturers. Samples positive for bovine coronavirus in one or both ELISAs were tested by a coronavirus-specific PCR for confirmation.

2.2 Lateral flow immunochromatography tests. A subset of 100 samples with a more or less equal distribution of positive results for the four pathogens of interest, were tested by two lateral flow strip tests (C and D). Tests A and C were produced by the same manufacturer. All samples of this subset were also tested for bovine coronavirus by PCR.

3. Results

Agreement is presented in table 1. For *E. coli* F5⁺/K99, the number of positives in the reference test and other tests was comparable. For rotavirus and cryptosporidium, slightly more samples were positive in ELISAs and slightly less samples were positive in fast tests then in the reference tests. Agreement between ELISA tests was also good, and correlation coefficients between ELISA results were high for the four enteropathogens evaluated.

Table 1. Level of agreement between different tests for four pathogens associated with neonatal diarrhoea in calves, displayed as κ-values (Kappa)

		Reference method			
		E. coli K99	bovine rotavirus	bovine coronavirus	Cryptosporidium parvum
BIO K 348	ELISA kit A	0.93	0.80	0.55	0.81
	ELISA kit B	0.96	0.72	0.54	0.70
BIO K 156	Fast test kit C	0.89	0.91	0.37	0.85
	Fast test kit D	0.91	0.72	0.05	0.73

For coronavirus all positive samples in ELISA kit A wer confirmed by PCR, whereas ELISA kit B scored some fals positives. In the comparative study on a subset of 100 sample PCR scored 26 samples positive for coronavirus, of which 12 an 14 samples scored positive in ELISA kits A and B, respectivel Fast test C was as sensitive as ELISA kit A, but scored a additional 14 samples positive, discrepant, however, from th additional PCR positives. Fast test D only scored 1 sampl positive.

Fig. 1 shows the numbers of samples for each pathogen detected b ELISA kit A (four pathogens) or routine methods for BVDV an Salmonella typhimurium/dublin. Fig. 2 demonstrates detection c more than one pathogen in 25 % of the samples.

Fig. 1 Frequency distribution of defined enteropathogens in faecal samples of young calves with diarrhoea

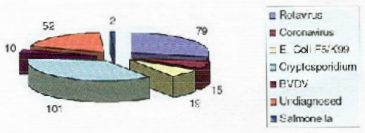
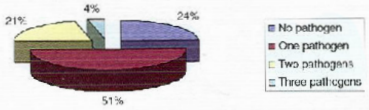


Fig. 2 Simultaneous detection of enteropathogens in faecal samples of young calves with diarrhoea



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Discussion and Conclusions

Hardly any literature is available concerning diagnosti performance of commercially available ELISA kits and lateral flov kits for detection of the major enteropathogens involved in cal neonatal diarrhoea (2, 3). All kits showed satisfactory diagnosti performance for detection of E. coli K99, bovine rotavirus an cryptosporidium parvum, with kits A and C showing the highes kappa-values. For detection of bovine coronavirus, kit D faile almost completely, whereas kappa-values of the other kits wer rather poor. The reference test, however, was PCR. Considering th relative low detection limits of PCRs in general, the clinica significance of these PCR results remain to be seen (1).

Also the significance of – frequently occurring – combinations o enteropathogens in calf neonatal diarrhoea may cause a headach for the veterinary practitioner.

5. References

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