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Verification of Sensitivity and Specificity of Group A Rotavirus Detection in Piglets Faeces with Monoclonal Blocking ELISA Methods

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With 3 tables

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Summary

Monoclonal antibodies to group A rotavirus Vp6 protein were prepared and used for verification of three blocking enzyme-linked immunosorbent assay (ELISA) modifications to detect rotavirus A. Selected competitive blocking ELISA (CB-ELISA) and electron microscopy (EM) were used for examination of 194 field faecal samples of piglets affected with diarrhoea. Rotavirus was detected in 43 samples (22.2%) by CB-ELISA method, whereas in 26 (13.4%) samples by EM examination. However, of 26 samples positive by EM, rotavirus A was detected by CB-ELISA in 19 (73.1%) samples; indicating the share of group A rotavirus in all cases of gastroenteritis caused by rotavirus. The sensitivity and specificity of the CB-ELISA was verified both by inclusion of control samples containing transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhoea virus (PEDV) in each analysis and by comparative examination of samples with the commercial ELISA kit. The CB-ELISA sensitivity was positively affected by examination of samples in the presence of chelating agent.

Introduction

Rotaviruses rank among significant causes of gastroenteritis in the majority of vertebrates. Data on their prevalence in human populations attest to their significance. A total of 110–140 million cases of gastroenteritis caused by rotaviruses with high mortality in children, particularly in developing countries, are annually reported (Bajolet and Chippaux-Hyppolite, 1998; Parashar et al., 2003). It follows that rotaviral infections play a significant role in farm animals kept under much worse hygienic conditions. Besides rotaviruses, two coronaviruses – transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhoea virus (PEDV) – rank among the economically most important viruses causing diarrhoea in pigs. Rotaviruses, which are about 75 nm, with double-stranded RNA genome, are highly resistant to the conditions of external environment and to both chemical and physical agents (Ramos et al., 2000; Fischer et al., 2002). They replicate mainly in enterocytes covering small intestinal villi and are spread via the faecal–oral route (Benfield et al., 1982; Debouck and Pensaert, 1983; Gelberg et al., 1991). Previously, all rotaviruses were considered antigenically related, containing common Vp6 antigen (group A rotavirus) with Mr 41K. Later, rotaviruses without the common antigen were classified as so-called non-group A rotaviruses. Seven antigenically distinct groups of rotaviruses (A–G) were described; four (A, B, C and E) of them are pathogenic for pigs. Group A rotavirus which is the most often (67.8–95.4%) causal agent of rotaviral gastroenteritis (Sigolo de San et al., 1986; Janke et al., 1990; Pongsuwanna et al., 1996) comprises a series of subgroups and serotypes according to antigenic analysis of Vp4 and Vp7 proteins present in the outer capsid of virion. Common group A rotavirus Vp6 antigen is found in the inner capsid of virion, and rotaviral infections caused by that agent may be diagnosed by use of specific Vp6 antibodies regardless of animal species affected.

Rotavirus detection gives more exact information on the cause of gastroenteritis than determination of antiviral antibodies, which are present almost in all blood serum samples. Therefore, both in veterinary and in human medicine, a series of methods are used [latex agglutination, reverse transcription polymerase chain reaction (RT–PCR), enzyme-linked immunosorbent assay (ELISA), electron and immune electron microscopy (EM) etc.]; some of them are available as commercial diagnostic kits (Benfield et al., 1984; Gerna et al., 1987; Al Yousif et al., 2001; Raboni et al., 2002).

The objective of the present study was to compare three modifications of blocking ELISA method for the detection of rotavirus A. The results obtained with selected competitive blocking ELISA (CB-ELISA) method were compared with those obtained by EM and by commercial ELISA kit.

Materials and Methods

Viral and control antigen

The reference strain of rotavirus A (OSU strain, VR-892; serotype G5/Vp7 and serotype P7/Vp4) and three of our own rotavirus A field isolates were propagated in MA-104 cells grown in minimum essential medium Eagle in the presence of trypsin (5 μg/ml, T-4799; Sigma, St Louis, MO, USA). The virus was released from the cells 24–48 hpi by repeated freezing/thawing. After centrifugation ($3000 \times g$ for 15 min) of the culture medium, the pellet was resuspended in phosphate-buffered saline (PBS) in 1/100 of the original volume as crude viral antigen (V-Ag). Control antigen (C-Ag) was prepared similarly from uninfected cells. Purified V-Ag was prepared by ultracentrifugation of supernatant on a cushion of 45% sucrose in PBS (Optima LE-80K Ultracentrifuge; Beckman, Palo Alto, CA, USA). Crude V-Ag and C-Ag were similarly prepared by propagation of TGEV (strain CAPM V-344; Collection of Animal Pathogenic Microorganisms, Brno) and PEDV (strain CV-777) in porcine kidney (PK-15) and

Vero cell lines, respectively. They were used for specificity check of all ELISA methods used.

Polyclonal antisera to rotavirus

Two hysterectomy-derived, colostrum-deprived, 3-week-old piglets were kept in sterile conditions, and orally infected with $3.10^{4,3}$ $TCID_{50}$ of group A rotavirus. Seven weeks post infection, piglets were challenged with $5.10^{4.3}$ $TCID_{50}$ of the virus and killed under total anaesthesia 12 days later. The titre of rotavirus A antibodies in the blood serum obtained (SwSpos.) was determined by indirect ELISA. Rotavirus-negative porcine blood serum (SwSneg.) was obtained by exsanguination of 3-week-old uninfected piglet. The immunoglobulin fraction (SwIgRota) was obtained from positive serum by ion-exchange chromatography and used as a binding antibody in the blocking ELISA methods.

Electron microscopic examination

Rota- and coronaviruses were detected by electron microscopic examination of culture media and faecal samples of piglets after negative staining with 2% ammonium molybdate solution in water, pH 7.0 (Šmíd et al., 1993).

Monoclonal antibodies

Inbred mice of the line BALB/c were repeatedly immunized with purified rotavirus A. Hybridomas were prepared by a standard procedure (Galfrè and Milstein, 1981) by fusion of splenic lymphoid cells with cells of the myeloma line Sp 2/0. Specificity of mAb produced by hybridomas was checked by ELISA, Western blot (WB) and immunoperoxidase (IP) detection of rotavirus A in infected cells. Selected hybridomas were used for preparation of ascitic fluids. Monoclonal antibodies (mAb) purified from ascitic fluids by ion-exchange chromatography were stored at −18°C in 50% glycerol or used for the preparation of peroxidase conjugates (HRPO-mAbRota).

Peroxidase conjugates

Antibodies to swine and mouse immunoglobulins were purified from hyperimmune swine (SwAMoIg) or rabbit (RASwIg, RAMoIg) sera by affinity chromatography. These antibodies and mAbRota were conjugated with horseradish peroxidase (HRPO, type VI-A; Sigma) using the periodate method (Boorsma and Streefkerk, 1979). The stock conjugate solutions were adjusted to 1 mg antibodies/ml. For the IP, WB and ELISA they were diluted 100× to 10 000× in PBS containing 0.05% Tween 20 and 0.5% lactalbumin hydrolysate (PBST-LAH).

Indirect ELISA

Rotavirus A antibodies in swine and mouse sera, in culture media of hybridomas and in purified mAbRota preparations were assayed using the indirect ELISA method. The wells of microtitre plates (Nunc-Immunoplate, PolySorp, Denmark) pre-coated alternatingly with crude rota A V-Ag and C-Ag were incubated (1 h at 37°C) with various dilutions of tested samples. After washing the second identical incubation with conjugates to swine or mouse immunoglobulins followed. The reactions were visualized by incubation with chromogen TMB (3,3′,5,5′-tetramethyl-benzidine; Sigma) solution. After 15 min, the reaction was stopped and absorbances were measured spectrophotometrically at 450 nm. Control wells filled with the diluent (blank) only, or with control negative or positive sera at the first incubation, were included in each examination. Highest samples dilution showing a difference in optical density of at least 0.1 (after subtraction of absorbances of blank wells) between the wells with bound V-Ag and C-Ag were classified as positive.

Blocking ELISA

Sensitivity and specificity of three variants of a blocking ELISA were determined by box titration using faeces of an experimentally infected piglet. The wells of microtitre plates (Nunc-Immunoplate, MaxiSorp, Denmark) were pre-coated overnight with binding antibodies in carbonate-bicarbonate buffer, pH 9.6 (5 μg Ig/ml; 50 μl/well). During the first incubation (2 h at 37°C), pairs of pre-coated wells were filled with 50 μl mixtures of faeces with SwSneg. or SwSpos. PBST-LAH containing 0.5 M NaCl and 3 mM ethylenediaminetetraacetic acid (EDTA)·Na_2 salt was used for sample dilution at the first incubation. After the second incubation (always 1 h at 37°C) with 50 μl of detection antibodies (conjugates HRPO-mAbRota or HRPO-SwIgAMoIg), visualization of the reaction and assessment of absorbances (A) followed, similarly to indirect ELISA. Between incubations, the wells were rinsed with PBST four times. Pairs of wells filled with the diluent (blank) or the mixtures of crude V-Ag (rotavirus A, TGEV, PEDV) and SwS neg./pos. during the first incubation were included in each analysis. The samples were regarded as positive if the net absorbance (NA), i.e. the difference of average absorbances in the wells incubated with SwSneg./pos. was >0.1, and reactions were blocked by $>50\%$ in the wells with SwSpos. Blocking percentages were determined using the formula: $\%B = 100 - [(A_{SwSpos.} \times 100) : (A_{SwSneg.})]$. The following variants of the blocking ELISA method were investigated:

Variant 1

Double antibody sandwich ELISA (DAS-ELISA): Wells pre-coated with binding mAbRota. The first incubation with antigen test samples was performed for 2 h, the second incubation with the detection antibody (HRPO-mAbRota).

Variant 2

Competitive blocking ELISA (CB-ELISA): Wells pre-coated with binding SwIgRota. After 1 h of incubation with the antigen test samples the wells were supplemented with 50 μl diluent containing 5 μg mAbRota/ml, and the incubation continued for another hour. The second incubation was undergone using detection antibody HRPO-SwIgAMoIg.

Variant 3

The DAS-ELISA: Wells pre-coated with binding SwIgRota. The first incubation with the antigen test samples was

performed for 2 h and the second incubation was conducted with the detection antibody HRPO-mAbRota.

Rotavirus A detection by commercial ELISA kit

Commercial kit (Ingezim Rota DAS 1.1.RT.K.2; Ingenasa; Inmunologia y Genetica Aplicada, s.a.; Spain, thereafter DAS-ELISA kit) was used for the detection of rotavirus A in faeces. The kit utilizes biotin-conjugated mAb anti-rota A and streptavidin–peroxidase conjugate. Examinations were carried out according to the manufacturer's instructions. Samples were evaluated as positive at the values of corrected absorbance (cA) > 0.3, dubious at cA = 0.2–0.3 and negative at cA < 0.2 (cA = A of the sample tested − A of negative control antigen). Results of the commercial kit examinations were compared with those obtained by EM and CB-ELISA methods.

Field samples

In total, 194 faecal samples from piglets with diarrhoea were examined. Most samples were from piglets younger than 21 days. After delivery to the laboratory, they were diluted in two to three volumes of Earle's medium, centrifuged (15 min, 3000 × ***g***), and the supernatants immediately examined by EM. Before CB-ELISA examination, the samples were kept at −80°C.

Results

Monoclonal antibodies

After verification of the specificity of hybridomas producing mAb to rotavirus A, two of them were selected for further use. Both mAb of the isotype specificity IgG2a (G6/D4) and IgG2b (B10/F10) reacted with the viral protein Vp6 in WB analysis (results not shown). Indirect ELISA titres of both mAb stock solutions containing 5 mg Ig/ml reached 4×10^5. Cross-reactivity with other viral antigens could not be detected using any of the methods mentioned (Table 2).

Comparison of blocking ELISA methods

Sensitivity comparison of three variants of the blocking ELISA method of rotavirus A detection were performed by box titrations in microtitre plate wells pre-coated with binding antibodies. Mixtures containing the faecal sample of an experimentally infected piglet and SwSpos./SwSneg. fivefold diluted 2–250× and 20–2500×, respectively, were examined. The sample of faeces in the entire range of dilutions was assessed as positive by all of the three blocking ELISA methods. Only slight differences in sensitivity were detected. Results obtained with minimal and maximal dilutions of SwS only are shown in Table 1. Competitive blocking ELISA methods proved to be most effective for the determination of coronaviruses (TGEV: L. Rodák, B. Smid, Z. Nevorankova, L. Valicek and R. Smitalova, unpublished data; PEDV: L. Rodák, L. Valicek, B. Smid and Z. Nevorankova, unpublished data). Therefore, CB-ELISA method (variant 2) was also used for routine rotavirus A detection in field samples; mixtures of faecal samples and SwSneg./pos. were examined in working dilutions 1 : 2 and 1 : 40, respectively. Using diluent supplemented with chelating agent in the first incubation, more satisfactory results were obtained in most of samples in comparison with standard diluent (PBST-LAH).

Net absorbance values (NA > 0.1) and %B > 50 was necessary to obtain for positive evaluation of the samples. Under these conditions, sample evaluation was highly specific; as established by examination of selected positive and negative samples in eight wells (*n* = 8), determination of mean absorbances (mA) and standard deviations (±SD). Calculated coefficients of variation (CV%) were 1.3–10.1%. Only with negative samples giving low absorbance values, the CV% was occasionally higher.

Table 1. Detection of rotavirus A in a faecal sample from an experimentally infected piglet using three variants of monoclonal blocking enzyme-linked immunosorbent assay (ELISA) methods

		Mean absorbance, net absorbance and percentage blocking							
		SwS dilution (20×)				SwS dilution (2500×)			
ELISA variant	Faeces dilution	Negative	Positive	NA	%B	Negative	Positive	NA	%B
DAS-ELISA (mAbRota*; HRPO-mAbRota†)	2×	**1.532**	**0.052**	**1.480**	**96.6**	1.528	1.328	0.200	13.1
	10×	**1.253**	**0.017**	**1.236**	**98.6**	1.479	0.900	0.579	39.1
	50×	**0.943**	**0.004**	**0.939**	**99.6**	**1.016**	**0.363**	**0.653**	**64.3**
	250×	**0.323**	**0.002**	**0.321**	**99.4**	**0.341**	**0.062**	**0.279**	**81.8**
CB-ELISA (SwIgRota*; HRPO-SwAMoIg†)	2×	**1.800**	**0.056**	**1.744**	**96.9**	1.843	1.676	0.167	9.1
	10×	**1.567**	**0.029**	**1.538**	**98.1**	1.416	0.890	0.526	37.1
	50×	**0.877**	**0.015**	**0.862**	**98.3**	**0.906**	**0.182**	**0.724**	**79.9**
	250×	**0.333**	**0.015**	**0.318**	**95.5**	**0.281**	**0.052**	**0.229**	**81.5**
DAS-ELISA (SwIgRota*; HRPO-mAbRota†)	2×	**1.540**	**0.015**	**1.525**	**99.0**	1.485	1.302	0.183	12.3
	10×	**1.342**	**0.024**	**1.318**	**98.2**	**1.218**	**0.517**	**0.701**	**57.6**
	50×	**0.794**	**0.007**	**0.787**	**99.1**	**0.847**	**0.104**	**0.743**	**87.7**
	250×	**0.327**	**0.006**	**0.321**	**98.2**	**0.365**	**0.033**	**0.332**	**91.0**

Dilutions of samples with positive values NA (>0.1) and %B (>50%) are in bold.
*Binding and †detection antibodies used in respective blocking ELISA variant.
NA, net absorbance; differences of mean absorbances (A) in wells incubated in the presence of rotavirus A-negative or -positive blood sera (NA = A SwSneg. − A SwSpos.); DAS-ELISA, double antibody sandwich ELISA; CB-ELISA, competitive blocking ELISA.
%B, percentage of absorbance blocking in wells incubated with SwSpos. in comparison with the wells containing SwSneg.

Comparison of CB-ELISA method and commercial kit

Sensitivity of the CB-ELISA method and DAS-ELISA kit was compared by examination of faecal sample of experimentally infected piglet twofold diluted 2× to 1024×. The results document that the highest sample dilution 64× and 1024× was regarded as positive by DAS-ELISA kit and CB-ELISA respectively. CB-ELISA absorbances (A) of the sample diluted 1 : 64 were 1.058/0.009 (Table 2). It follows that sample with CB-ELISA A < 1.0 will be probably negative by DAS-ELISA kit. Comparative examinations of crude V-Ag (rota A, TGEV, PEDV) proved high specificity of both the methods (Table 2). Also 41 randomly selected field faecal samples, assessed positively and negatively by EM and CB-ELISA, were comparatively examined by DAS-ELISA kit (Table 3). The group of 41 samples comprised: nine (EM pos.; CB-ELISA pos.), 17 (EM neg.; CB-ELISA pos.), five (EM pos.; CB-ELISA neg.) and 10 (EM neg.; CB-ELISA neg.). All 15 CB-ELISA-negative samples were negative by DAS-ELISA kit. However, of the 26 CB-ELISA-positive samples only one half was positive by DAS-ELISA kit. With all remaining 12 DAS-ELISA kit-negative (two EM pos.; 10EM neg.), positive results with expected A < 1.0 (A = 0.850/0.026 and 0.683/0.060) were obtained by CB-ELISA (Table 3).

Table 2. Sensitivity and specificity of rotavirus A detection in control viral antigen samples and in positive faecal sample by CB-ELISA method and commercial DAS-ELISA kit

	CB-ELISA absorbance, NA and %B*					DAS-ELISA kit†	
	Wells incubated with						
Control Ag faeces dilution	SwSneg.	SwSpos.	NA	%B	E	cA	E
DAS-ELISA pos. Ag	**0.772**	**0.022**	**0.750**	**97.1**	+	**1.928**	+
DAS-ELISA neg. Ag	0.012	0.011	0.001	–	–	0.002	–
Rota V-Ag	**1.595**	**0.075**	**1.520**	**95.3**	+	**2.905**	+
TGE V-Ag	0.005	0.005	0.000	–	–	0.001	–
PEDV-Ag	0.006	0.024	−0.018	–	–	0.001	–
Faeces – 2×	**2.154**	**0.155**	**1.999**	**92.8**	+	**3.285**	+
4×	**2.124**	**0.110**	**2.014**	**94.8**	+	**3.251**	+
8×	**1.897**	**0.072**	**1.825**	**96.2**	+	**2.987**	+
16×	**1.764**	**0.056**	**1.708**	**96.8**	+	**1.745**	+
32×	**1.455**	**0.015**	**1.440**	**99.0**	+	**0.817**	+
64×	**1.058**	**0.009**	**1.049**	**99.1**	+	**0.385**	+
128×	**0.689**	**0.007**	**0.682**	**99.0**	+	0.186	–
256×	**0.419**	**0.006**	**0.413**	**98.6**	+	0.083	–
512×	**0.216**	**0.004**	**0.212**	**98.1**	+	0.044	–
1024×	**0.111**	**0.010**	**0.101**	**91.0**	+	0.023	–

*Samples of crude V-Ag (rota A, TGEV, PEDV), and a faecal sample from experimentally infected piglet diluted twofold 2× to 1024× were examined by CB-ELISA in a mixture with SwSneg./SwSpos. diluted 40×. Samples were evaluated as positive at NA values >0.1 and %B > 50.
†Samples examined by commercial DAS-ELISA kit for rotavirus A detection. According to corrected absorbance (cA = A tested sample − A neg. Ag) samples were assessed as positive at cA > 0.3; dubious at cA = 0.2–0.3 and negative at cA < 0.2.
E, final evaluation of samples tested (positive +; negative –); DAS-ELISA, double antibody sandwich ELISA, CB-ELISA, competitive blocking ELISA; TGEV, transmissible gastroenteritis virus; PEDV, porcine epidemic diarrhoea virus; NA, net absorbance; differences of mean absorbances (A) in wells incubated in the presence of rotavirus A-negative or -positive blood sera (NA = A SwSneg. − A SwSpos.); Samples with positive values are in bold.
%B, percentage of absorbance blocking in wells incubated with SwSpos. in comparison with the wells containing SwSneg.

Table 3. Comparison of sensitivity of rotavirus A detection in field faecal samples by electron microscopy (EM), CB-ELISA method and commercial DAS-ELISA kit

	CB-ELISA-positive EM-positive			CB-ELISA-positive EM-negative			CB-ELISA-negative EM-positive			CB-ELISA-negative EM-negative		
	*n**	CB-ELISA†	DAS-ELISA‡	*n**	CB-ELISA†	DAS-ELISA‡	*n**	CB-ELISA†	DAS-ELISA‡	*n**	CB-ELISA†	DAS-ELISA‡
DAS-ELISA-positive	6	1.367/0.221	*2.620*	7	1.296/0.156	*2.324*	0	–	–	0	–	–
DAS-ELISA dubious	1	1.564/0.061	*0.216*	0	–	–	0	–	–	0	–	–
DAS-ELISA-negative	2	0.850/0.026	*0.161*	10	0.683/0.060	*0.060*	5	0.025/0.020	*0.011*	10	0.030/0.025	*0.014*
Total sample (*N*)	9			17			5			10		

*Number (*n*) of faecal samples evaluated as positive, dubious or negative by commercial DAS-ELISA kit.
†Mean absorbances obtained by CB-ELISA method by examination of samples in wells incubated in presence of SwSneg./pos., respectively. Samples were evaluated as positive at the values of NA > 0.1 and %B > 50%.
‡Mean absorbances (in italics) obtained by commercial DAS-ELISA kit by examination of *n* samples in duplicates. Samples were evaluated according to the corrected absorbance (cA) as positive at cA > 0.3; dubious at cA = 0.2–0.3 and negative at cA < 0.2.
DAS-ELISA, double antibody sandwich ELISA; CB-ELISA, competitive blocking ELISA.

Examination of field faecal samples

In total 194 faecal samples from piglets affected by diarrhoea were examined by EM and CB-ELISA methods. Rotavirus A was detected in 43 samples (22.2%) by CB-ELISA, whereas in 26 (13.4%) samples by EM, indicating higher sensitivity of the former method. However, of 26 EM-positive samples, rotavirus A was detected in 19 (73.1%) samples by CB-ELISA. Congruency of both EM and CB-ELISA examinations thus suggest the share of rotavirus A in all cases of gastroenteritis caused by rotavirus. In the remaining seven (26.9%) EM-positive but CB-ELISA-negative samples the presence of non-group A rotaviruses is supposed. This is supported both by identical results of examination of five of these samples by DAS-ELISA kit (Table 3) and by demonstration of group C rotavirus electropherotype (results not shown) in one EM highly positive sample. Determination of electropherotypes in the remaining six samples with low rotavirus contents by EM was unsuccessful.

The average CB-ELISA absorbances of 151 rotavirus A-negative samples in wells with SwSneg./pos. were 0.039/0.030. Elevated absorbances were detected (A = 0.2–0.5) in three samples only, with positivity excluded by blocking reaction (results not shown).

Discussion

Polyclonal and mAb to group A rotavirus were prepared and used as binding or detection antibodies for sensitivity testing of three ELISA variants for rotavirus A demonstration. In similar experiments dealing with TGEV and PEDV detection (L. Rodák *et al*., unpublished data), lower sensitivity of DAS-ELISA variants using conjugated mAb was proven. Therefore, more sensitive variants of CB-ELISA, based on the use of unconjugated mAb, were selected for routine demonstration of both coronavirus and rotavirus A infections. The use of a uniform methodical procedure thus allows detecting simultaneously all three most important causative agents of viral gastroenteritis.

Specificity of examination was confirmed by comparative assessment of V-Ag (rotavirus A, TGEV, PEDV) by CB-ELISA and by commercial DAS-ELISA kit (Table 2). Absence of cross-reactivity of CB-ELISA with bacterial antigens present in field faecal samples confirmed the results of 151 rotavirus A-negative samples.

Higher sensitivity of rotavirus A detection by CB-ELISA in comparison with commercial DAS-ELISA kit was proven. By examination of positive faecal sample twofold diluted 2× to 1024×, at least 10 times higher sensitivity of CB-ELISA method was demonstrated (Table 2). This was also confirmed by comparative examination of 41 randomly selected field faecal samples. All 15 CB-ELISA-negative samples were evaluated negatively by DAS-ELISA kit examination. However, of the remaining 26 CB-ELISA-positive samples, only one half of samples were evaluated as positive by commercial DAS-ELISA kit (Table 3).

The sensitivity of rotavirus A detection by CB-ELISA in comparison with EM is also higher. Of 194 field faecal samples rotavirus was detected in 43 samples by CB-ELISA whereas in 26 samples by EM. On the contrary, of 26 EM positive samples rotavirus A was detected in 19 (73.1%) samples by CB-ELISA. The fact that all groups of rotavirus are detected by EM, while group A rotavirus only is detected by CB-ELISA, can explain this difference. The congruency of EM and CB-ELISA examinations (73.1%) indicates the share of rotavirus A in cases of rotaviral gastroenteritis and correlates with other findings (Sigolo de San et al., 1986; Janke et al., 1990; Pongsuwanna et al., 1996). The assumption that the remaining seven (26.9%) samples likely contain non-group A rotavirus was partly confirmed by demonstration of group C rotavirus electropherotype in one EM highly positive sample. Therefore, more sensitive RT–PCR techniques will be applied in following experiments.

It is well-known that chelating agents destroy the outer capsid of rotavirus and its infectivity (Ward and Ashley, 1980; Fang et al., 1989). Therefore, we presumed that their presence in ELISA examinations might improve accessibility of antigens localized in the inner capsid of virion, particularly the Vp6 antigen. This was confirmed in our study, and better results were obtained in most samples using solution with chelating agent in comparison with the standard diluent.

Highly positive values of NA and %B were obtained in 40 of 43 CB-ELISA-positive faecal samples; it confirms suitability of working dilutions of faeces (2×) and SwSneg./pos. (40×) selected for routine use. The remaining samples assessed as negative because of the %B $<$ 50 were also positive at higher working dilution.

Diagnosis of the causal agents of viral gastroenteritis is a basic prerequisite both for introduction of immunoprophylactic measures (Schaller et al., 1992; Ebina, 1996; Hodgins et al., 1999) and confirmation of their effectivity. The use of uniform methods allowing diagnosing simultaneously rotavirus and coronavirus infections could contribute to this goal, as well as introduction of PCR in following experiments.

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