Electron microscopy in the diagnosis of viral gastroenteritis in hospitalised children in the Czech Republic

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Abstract Our study has been aimed at demonstrating the main role of viruses in the aetiology of acute gastroenteritis in children less than 5 years old and at pointing out the diagnostic potential of electron microscopy in the diagnosis of viral gastroenteritis. A prospective study was conducted to analyse the aetiology of diarrhoeal diseases in children less than 5 years of age admitted to the Department of Infectious Diseases between September 2006 and December 2008. All children were tested by faecal culture, latex agglutination and electron microscopy. A total of 832 children were included in the study. An aetiological agent was detected in 788 children (94.6 %). A bacterial aetiology was found in 22 (2.6 %) children and bacterial–viral co-infection was found in 146 (17.6 %) patients. The most frequent causative agents of gastroenteritis in children were viruses, which were detected in 620 (74.5 %) patients. The main causes of viral gastroenteritis were rotaviruses (detected in 410 children), followed by caliciviruses (42),
coronaviruses (28), adenoviruses (19) and astroviruses (14). Dual viral infections were detected in 107 children, with rotavirus–calicivirus co-infection being the most common. Electron microscopy proved to be a more sensitive method in comparison with the latex agglutination test for the diagnosis of rotaviruses and adenoviruses. The major role of viruses in diarrhoeal diseases among children under 5 years of age in the Czech Republic has been confirmed. The diagnostic potential of electron microscopy, particularly in small outbreaks of gastroenteritis, was clearly shown.

Abbreviations

AGE	Acute gastroenteritis
ELISA	Enzyme-linked immunosorbent assay
EM	Electron microscopy
ETEC O25	Enterotoxigenic *E. coli*
ICH	Immunochromatographic methods
LAG	Latex agglutination
NRL/ELM	National Reference Laboratory for Direct Diagnosis of Viruses, Borrelia, and Foreign Cells in Clinical Specimens
PCR	Polymerase chain reaction
REVEAL	Rotavirus Gastroenteritis Epidemiology and Viral Types in Europe Accounting for Losses in Public Health and Society
RT-PCR	Real-time PCR
VGE	Viral gastroenteritis

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Introduction

Acute gastroenteritis (AGE) is one of the most frequent diseases in children worldwide and remains the main cause of childhood morbidity and mortality, particularly in developing countries. Each year as many as 1.5 million children in the

first 5 years of life die from AGE (Dey et al. 2007; Fabiana et al. 2007; Rahman et al. 2007; Parashar et al. 2006).

Rotaviruses (Fig. 1a and b), classified in the family *Reoviridae*, were first reported in humans in 1973 by Bishop et al., who detected them by electron microscopy (EM). Concern about rotaviruses grew in the following years. New diagnostic methods were developed: latex agglutination (LAG), enzyme-linked immunosorbent assay (ELISA), immunochromatographic methods (ICH) and, most recently, nucleic acid amplification tests (PCR). Using these methods, more than 125 million children worldwide are annually diagnosed with rotavirus diarrhoea (Rahman et al. 2007; Parashar et al. 2006).

In the Czech Republic, AGE patients are routinely tested by faecal culture and LAG. Other methods (ELISA, ICH) are available only in some laboratories. Although rapid and non-selective, EM is not routinely used. Nevertheless, it plays a crucial role in the diagnosis of the aetiology of AGE, as well as in surveillance studies, because it is able to detect all infectious agents and even two or more agents simultaneously, if present in the analysed specimen.

Rotaviruses are considered the main cause of viral gastroenteritis (VGE) in children under 5 years of age who present with severe diarrhoea, often with the need for parenteral rehydration (Steyer et al. 2006; Parashar et al. 2003). The main aetiological agents of severe diarrhoeal disease in children are group A rotaviruses (Parashar et al. 2006; Stupka et al. 2007; Nguyen et al. 2007; Pazdiora and Svecova 2006).

The second most frequent AGE agents in children are viruses of the family *Caliciviridae* (Fig. 1c), including noroviruses and sapoviruses (Gomes et al. 2007). Other causative agents are adenoviruses (Fig. 1d) from the family *Adenoviridae* (serotypes 40 and 41), human astroviruses (Fig. 1e) from the family *Astroviridae* (Nguyen et al. 2008) and intestinal coronaviruses from the family *Coronaviridae* (Fig. 1f).

Materials and methods

Study patients

Eight hundred thirty-two children with AGE from 1-month-to 5-year-old children admitted to the Department of Infectious Diseases of the University Hospital Bulovka in Prague from September 1, 2006, to December 31, 2008, were enrolled in the study.

Fig. 1 a–f Electronograms of faecal isolates from the study subjects: **a–b** rotaviruses, **c** calicivirus (norovirus), **d** intestinal adenovirus, **e** astrovirus and **f** coronavirus (Schramlova et al. 2010)
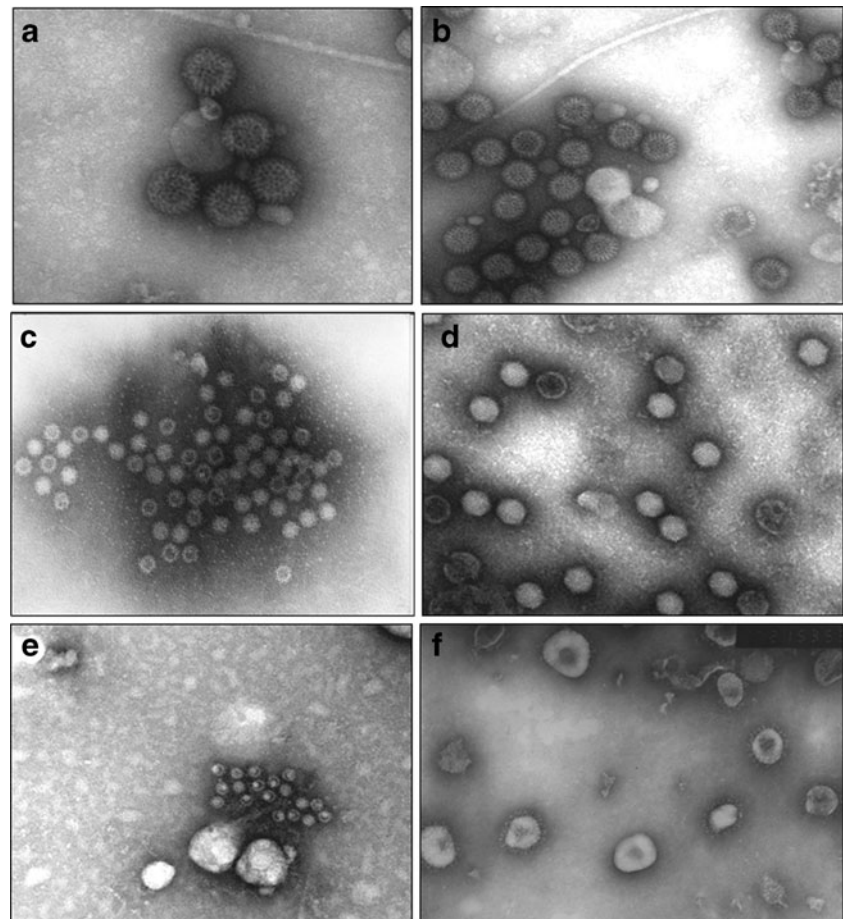
Bacteriological examination of faecal culture

Faecal samples from all admitted children were examined using routine culture at the Department of Clinical Microbiology of the University Hospital Bulovka. Standard solid and liquid culture media were used for the cultures. Faecal samples were cultured in an incubator at 37°C for 24 h. For the detection of *Campylobacter jejuni*, selective media were incubated at 42°C for 48 h. All faecal samples were also tested for pathogenic strains of *Escherichia coli*. When indicated, the detection of *Clostridium difficile* and parasitological analysis of faecal samples were performed.

Latex agglutination test

This test was carried out at the Department of Virology of the Institute of Public Health, Prague. The commercial kits Rotalex and Adenolex (Orion Diagnostica, Czech Republic) were used to detect rotaviruses and adenoviruses, respectively.

Electron microscopy

All examinations were carried out in the National Reference Laboratory for Direct Diagnosis of Viruses, Borrelia, and Foreign Cells in Clinical Specimens (NRL/ELM), National Institute of Public Health, Prague. To prepare samples, 10 % stool suspensions made in distilled water were centrifuged at 1,000 rpm for 5 min, and cell and bacterial fragments were removed. Viruses were adsorbed on 400-mesh formvar carbon-coated electron microscope grids. Two grids, one negatively stained with 2 % ammonium molybdate and the other with 2 % aqueous uranyl acetate, were used for each viral suspension. All steps were strictly carried out in a biohazard hood in compliance with biosafety regulations. The grids were examined under electron microscope Morgagni 268D Philips (FEI Company, USA) at 100,000-fold magnification.

Results

The mean age of children was 1.9 years (median of 1.6 years), and the mean length of hospitalisation was 5.6 days (median of 5 days). The male to female ratio was 426:406. The most frequent diagnosis at admission was acute gastroenteritis, reported in 683 (82.1 %) children, followed by acute enteritis in 85 (10.2 %) children and acute haemorrhagic enterocolitis in 38 (4.6 %) children. Acute haemorrhagic gastroenterocolitis was less frequently diagnosed, in 19 (2.3 %) patients, and 7 (0.8 %) children presented with vomiting without diarrhoea. At admission, 524 (63 %) children were febrile or subfebrile, and 648 (77.9 %) children showed signs of dehydration of different degrees of severity.

Table 1 Causative agents of acute gastroenteritis

Agent	Number of cases	% of cases
Bacterial gastroenteritis	22	2.6
Viral gastroenteritis	620	74.5
Dual bacterial–viral gastroenteritis	146	17.6
Gastroenteritis of unknown aetiology	44	5.4
Total	832	

Six hundred and forty (76.9 %) children needed parenteral rehydration; 574 of them received intravenous rehydration, and 66 were rehydrated using a nasogastric tube. The highest need (80.7 %) for rehydration was reported in children with rotaviral AGE. Laboratory analysis showed elevated liver transaminases in 393 (63.4 %) children.

Aetiology of AGE

Causative agents were detected using the previously mentioned methods in 788 (94.6 %) children (Table 1). Bacterial infections detected in 22 (2.6 %) children were caused most frequently by *Salmonella enteritidis* (17 cases), followed by *C. jejuni* (3 cases) and pathogenic *E. coli* (2 cases). Dual bacterial–viral infection was detected in 146 (17.6 %) children. The most frequent combinations were rotavirus–*S. enteritidis* and rotavirus–enterotoxigenic *E. coli* (ETEC O25). Viral infection was diagnosed in 620 (74.5 %) children.

The most frequently detected viruses were rotaviruses, caliciviruses, coronaviruses, adenoviruses and astroviruses. Co-infections with two or more viruses were found in 107 cases (Table 2). EM and LAG results are summarised in Table 3.

Table 2 Causative agents of viral gastroenteritis

Agent	Number of cases	% of cases
Rotaviruses	410	66.1
Caliciviruses	42	6.7
Coronaviruses	28	4.5
Adenoviruses	19	3.1
Astroviruses	14	2.3
Multiple viruses	107	17.3
Total	620	
Dual viral gastroenteritis	107	
Rotaviruses+caliciviruses	33	30.8
Rotaviruses+coronaviruses	25	23.4
Rotaviruses+astroviruses	18	16.8

Table 3 The incidence of viruses detected by different methods

Agent	Method	Number of cases	% of cases
	Latex agglutination		
Rotaviruses		352	42.3
Adenoviruses		19	2.3
Negative		461	55.4
Total		832	
	Electron microscopy		
Rotaviruses		478	57.5
Caliciviruses		60	7.2
Coronaviruses		36	4.3
Adenoviruses		24	2.9
Astroviruses		20	2.4
Multiple viruses		148	17.8
Negative		66	7.9
Total		832	

Comparison of LAG and EM in the diagnosis of rotaviral and adenoviral AGE

Rotaviruses were detected by both LAG and EM methods in 302 children; 176 children were EM positive, but LAG negative. EM detection of two or more viruses, most often rotavirus in combination with another virus, was not taken into account in this tally, as the actual causative agent remained unclear. None of the patients was LAG positive while EM negative. Thus, the sensitivity of EM was 100 %, and the sensitivity of LAG was only 63.2 %.

Thirteen children were positive for adenoviruses by both methods, whereas 24 children were only EM positive (again, viral co-infections were not taken into account). None of the patients was LAG positive and EM negative. The sensitivity of EM was 100 % again, whereas that of LAG was only 35.1 % (Table 4).

Table 4 Comparison of electron microscopy and latex agglutination in the diagnosis of acute gastroenteritis

Agent	Method	Number of cases LAG +	Number of cases LAG −
Rotaviruses	EM +	302	176
	EM −	0	0
Adenoviruses	EM +	13	24
	EM −	0	0

EM + electron microscopy positive, *EM −* electron microscopy negative, *LAG +* latex agglutination positive, *LAG −* latex agglutination negative

Seasonal trends in viruses causing VGE

The seasonal trend is clearly evident for the most frequently diagnosed viruses, i.e. rotaviruses, which were detected primarily from January to May. The rotavirus incidence curve copies the shape of that for AGE admissions and of that for confirmed VGE (Fig. 2). The seasonal trend of other viruses is difficult to evaluate because of the small number of viruses detected in 2008.

Discussion

The number of fatal cases of rotavirus AGE in children in the world varies from about 440,000 cases to 611,000 cases per year (Fabiana et al. 2007; Rahman et al. 2007; Parashar et al. 2006; Vesikari et al. 2007). In Europe, mortality from rotavirus diseases is not high (about 200–250 deaths per year in children under 5 years of age) (Vesikari et al. 2007; Parashar and Glass 2008; Pazdiora 2007b).

In the Czech Republic, diarrhoeal diseases are the second most common cause of morbidity after respiratory infections in children under 5 years of age; however, a fatal course in children is very rare. Two deaths from rotavirus AGE in children under 5 years of age were reported by the Public Health Information System in 2002, one death in 2004 and no fatal cases in the years 2003 and 2005–2009 (Sasek et al. 2005; Pazdiora 2007a).

Our results clearly demonstrated that viruses were the major causative agents of AGE in the patients enrolled in this study. Similar data have been reported in other European countries (Vesikari et al. 2007; Froggatt et al. 2004; Lennon et al. 2007; Medici et al. 2006, 2004; Wilhelmi et al. 2003; De Grazia et al. 2004; Van der Wielen et al. 2007).

We further demonstrate that EM has a significant potential for the detection of the causative agents of AGE (94.6 %), with our data being superior to those from other countries. Based on a large study comparing the results from different European countries, causative agents are detected in about 40–55 % of AGE patients less than 5 years of age, depending on the method of detection employed (Guarino et al. 2008).

As many as 66.1 % of VGE cases in this study were caused by rotaviruses, which is similar to other studies (Wales, 21.6 %; Ireland, 82.28 %; Italy, 21.1–40.7 %; Germany, 28 %; Sweden, 52 %; France, 61 %). The Rotavirus Gastroenteritis Epidemiology and Viral Types in Europe Accounting for Losses in Public Health and Society (REVEAL) study concluded that the incidence of rotavirus infection in Europe is high. Rotaviruses are responsible for 30–60 % of AGE cases and for 19–54 % of hospitalisations in children less than 5 years of age with AGE (Guarino et al. 2008; Ambrozova and Arientova 2008).

Other viruses were detected with substantially less frequency, which is also consistent with the abovementioned

Fig. 2 Seasonality of viruses in the year 2008. *VGE-total* viral gastroenteritis-total
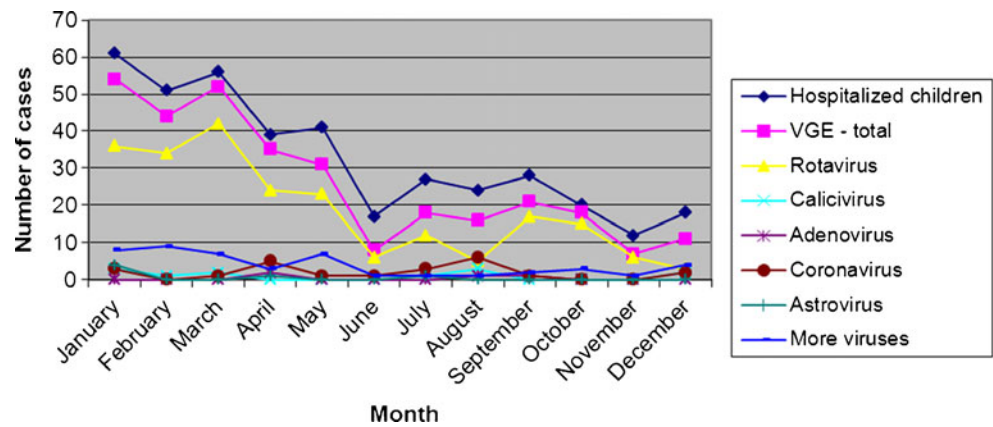
studies. Caliciviruses have been reported as the second most common causative agent of VGE (2–20 %) in practically all published data (Froggatt et al. 2004; Medici et al. 2006, 2004; De Grazia et al. 2004; Van der Wielen et al. 2007; Guarino et al. 2008; Papaventsis et al. 2007).

Adenoviruses ranked after coronaviruses when considered as a possible cause of VGE. Some studies have reported the same rates of detection of coronaviruses in faecal samples from both healthy persons and patients, while other studies detected coronaviruses more frequently in patient samples (Ambrozova 2006). In European studies, adenoviruses (2–10 %) have been reported to be the third most common cause of VGE, after rotaviruses and noroviruses (Medici et al. 2006, 2004; De Grazia et al. 2004; Guarino et al. 2008). Unsurprisingly, astroviruses ranked the last in our study, which is in accordance with previous studies dealing with rates of astroviruses in child AGE from developed countries (2–8 %) such as France, Spain, Australia and the USA (De Grazia et al. 2004; Papaventsis et al. 2007; Savadkoohi et al. 2007).

Co-infection with two or more viruses was detected in as many as 17.3 % of VGE cases. Dual infections have also been reported in sporadic cases of VGE, but in a lower proportion (about 3–6 %), with the rotavirus–norovirus co-infection being the most frequent (Froggatt et al. 2004; Medici et al. 2006, 2004).

Co-infection with two or more pathogens is difficult to interpret because it is impossible to decide which of the detected pathogens is the causative agent of infection or if one, two or more agents are implicated, and to what extent. Similarly, the detection of rotaviruses in combination with another virus can indicate dual infection, but in the case of massive detection of rotaviruses coupled with insignificant detection of another virus, rotavirus is more likely to be the causative agent of the infection (Ambrozova and Schramlova 2005).

Another indicator studied was the seasonal incidence of causative viruses. The most obvious seasonal trend was observed in rotavirus AGE. In accordance with the literature, the highest incidence of rotavirus infection was observed in the cold period of the year (Medici et al. 2006, 2004; De Grazia et al. 2004; Van der Wielen et al. 2007; Guarino et al. 2008).

The clinical picture of AGE included primarily loose stool, without blood, together with vomiting. At admission, 648 (77.9 %) children showed signs of dehydration of different degrees of severity. Moreover, 640 (76.9 %) needed parenteral rehydration. In accordance with previous data (Wilhelmi et al. 2003; Taborska and Pazdiora 2005), the most severe infections were caused by rotaviruses and required parenteral rehydration in 80.7 % of cases. Laboratory analysis revealed elevated liver transaminases in 63.4 % of the children. Parainfectious hepatopathy with transaminase levels of 1–2 μcat/l is almost uniformly found in VGE (Ambrozova and Arientova 2008; Taborska and Pazdiora 2005).

The mean length of hospitalisation was 5.6 days (median of 5 days). In Europe, the mean length of hospitalisation varies from 2 to 9.5 days (median of 4.8 days). In Western European countries, the length of hospitalisation reaches 3–5 days, while in other European countries, it is substantially longer (e.g., 8.3 days in Hungary and 9.5 days in Poland) (Rodrigo 2007).

We have proved EM to be much more sensitive than LAG. EM showed 100 % sensitivity in detection of rotaviruses in the study, while that of LAG was only 63.2 %. For adenoviruses, the respective sensitivity rates were 100 and 35.1 %. These poor results can be partially influenced by frequent finding by EM of rare viruses or their fragments, which cannot be detected by LAG (Ambrozova and Schramlova 2005).

In conclusion, EM demonstrated that caliciviruses and coronaviruses are more frequent than adenoviruses in the stool of children with AGE in the Czech Republic. Also, we detected a relatively high number of co-infections (virus–virus and bacterium–virus). Altogether, the direct EM method has the great advantages of being rapid and non-selective. After a simple and fast negative stain, "open view" of EM allows rapid morphologic identification and differential diagnosis of different agents contained in the specimen.[32]

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