Electron Microscopic Reporting of Gastrointestinal Viruses in the United Kingdom, 1985–1987

Stephan S. Monroe, Roger I. Glass, Norman Noah, Thomas H. Flewett, E. Owen Caul, C. Ian Ashton, Alan Curry, Anne M. Field, Richard Madeley, and Patrick J. Pead

Viral Gastroenteritis Unit, Respiratory and Enterovirus Branch, Division of Viral and Rickettsial Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia (S.S.M., R.I.G.); Communicable Disease Surveillance Centre, Public Health Laboratory Service, 61 Colindale Avenue, London (N.N.); Regional Virus Laboratory, East Birmingham Hospital, Birmingham (T.H.F.); Joint Regional Virus Laboratory, Public Health Laboratory, Myrtle Road, Kingsdown, Bristol (E.O.C.); Public Health Laboratory, Fazakerley Hospital, Lower Lane, Liverpool (C.I.A.); Public Health Laboratory, Withington Hospital, Manchester (A.C.); Virus Reference Laboratory, Central Public Health Laboratory, 61 Colindale Avenue, London (A.M.F.); Department of Virology, The Royal Victoria Infirmary, Queen Victoria Road, Newcastle-upon-Tyne, (R.M.); Public Health Laboratory, St. Mary's General Hospital, Milton Road, Portsmouth (P.J.P.), England

We examined some epidemiological features of the viruses associated with gastrointestinal illness, using national data reported by electron microscopists in the United Kingdom. During the 3 years analyzed (1985–1987), a total of 1,993 positive detections of astroviruses, caliciviruses, coronaviruses, and small round structured viruses (SRSVs) were reported. In 1 year of this period, 8,210 rotaviruses were reported. More than 90% of the astroviruses and caliciviruses were detected in children under 5 years of age, while coronaviruses and SRSVs were detected in adults as well as children. Detections of astroviruses increased in the winter and were infrequent during the summer, a seasonal pattern similar to that observed for rotaviruses. There was some variability between reporting regions in rates of detection of fecal viruses. We have attempted to identify the reasons for this. We make suggestions for improving the detection of human fecal viruses, and we recognize the need for continued surveillance of these agents.

KEY WORDS: astrovirus, calicivirus, classification

INTRODUCTION

Viruses are recognized as frequent causes of sporadic diarrhea in young children and of outbreaks of diarrhea and vomiting in patients of all ages. Those implicated include rotaviruses, adenoviruses, astroviruses, caliciviruses, small round featureless viruses (SRVs), small round structured viruses (SRSVs), and coronaviruses. The SRSVs are a morphological group that includes those viruses most frequently associated with outbreaks: the Norwalk virus and other similar viruses [Caul and Appleton, 1982; Kapikian and Chanock,

1990]. The routine technique for diagnosing these infections is electron microscopy (EM), but the capital cost and technical difficulties have limited the availability of EM to comparatively few diagnostic laboratories. Alternative tests for two viruses (group A rotaviruses and adenoviruses of types 40 and 41) are now available, but EM remains the only catchall technique at present. Not surprisingly, epidemiologic data on most of these viruses are scanty and no national data have been published hitherto.

In the United Kingdom, the laboratories that form the Public Health Laboratory Service (PHLS) are distributed through England and Wales and, augmented by University and National Health Service laboratories in England, Northern Ireland, and Wales, report their positive fecal virus findings weekly to the Communicable Disease Surveillance Centre (CDSC) at Colindale, London. We have analyzed the reports to CDSC, which do not include results from Scotland, for the period 1985–1987, to identify any apparent epidemiological trends. Data on rotaviruses, astroviruses, caliciviruses, SRSVs, and coronaviruses have been included in the analysis. The compilation of these data, resulting from more than 50,000 EM observations annually, allowed a unique opportunity to examine aspects of the epidemiology of viral agents of gastroenteritis on a large scale. Observations from the U.K.

experience have formed the basis for a system about to be introduced in North America [Lew et al., 1990]. Information on the epidemiology of individual viruses could focus future investigations to identify specific risk groups of time, place, and person.

MATERIALS AND METHODS
Source of the Data

The data were compiled from the weekly reports to CDSC for the period January 1, 1985 through December 31, 1987. The specimens were stools from sporadic cases of gastrointestinal illness and from common-source outbreaks. There was no selection by age. The laboratories used a standardized nomenclature based on morphology (Fig. 1) to identify enteric viruses. Small round fecal viruses present particular problems of identification and were classified as astrovirus, calicivirus, or SRSV when compatible morphology was present (Table I). These viruses were distinguishable from other small round viruses, present in human fecal samples, which lacked identifiable surface morphology and were termed small round featureless viruses (SRVs). The featureless viruses have not been monitored in the U.K. reporting system. The PHLS EM Committee monitored the accuracy of the reports by reviewing micrographs submitted by the laboratories and by offering help and advice when it was sought. The same committee has an advisory role in the annual distribution of coded reference specimens provided by the PHLS as part of a national scheme (NEQAS).

Analysis was based on positive results, irrespective of whether they were from sporadic cases or outbreaks. The data are the mean of 3 years of reports (1985–1987), with the exception of the weekly data for SRSVs, which are the average of reports from only 2 years (1986–1987). For comparative purposes, rotavirus detections were analyzed for a single season from July 1986 to June 1987. It is important to note that a proportion of rotaviruses will have been detected by other techniques, such as enzyme-linked immunosorbent assay (ELISA), latex agglutination, and polyacrylamide gel analysis,) as well as by EM. Information on adenovirus detections was not compiled because specific identification of enteric serotypes (40 and 41) was not available.

Seasonal Distribution of Cases

Rotavirus detections in the United Kingdom were highest in the late winter and lowest in the late summer (Fig. 2), a pattern observed in many other temperate climates [Cook et al., 1990]. By comparison, astrovirus detections followed the same general pattern of winter peaks and summer troughs, but with greater scatter because of fewer detections. This pattern was observed in each of the 3 years analyzed (data not shown), although the week of maximum astrovirus detections varied from one year to another. The largest calicivirus peaks occurred during the winter, but peaks of summer activity were also detected, and the numbers

were too small to produce a consistent pattern from year to year (data not shown). SRSV and coronavirus detections showed no distinct seasonal patterns.

Age Distribution of Cases

More than 90% of astroviruses and caliciviruses were detected in children under 5 years of age (Fig. 3), the majority in children under 1 year of age. By contrast, 40% of the SRSVs and coronaviruses were detected in patients and ≥15 years of age.

Regional Detection Frequencies

The data were compiled at CDSC by standard National Health Service Regions. As information on the number of specimens examined was not available, incidence rates for the various agents could not be calculated. However, we have attempted to make comparisons between regions using their respective rotavirus detections as denominators. The derived figures, expressed as proportional detection rates, are shown in Table II. Differences in these ratios could indicate variability between regions in disease incidence or in the efficiency of detection of these viruses, provided that we assume that both the disease incidence and detection efficiency of rotavirus were relatively constant throughout the country.

For the 3-year period, the four other agents as a group were detected annually at 8.1% the frequency of rotavirus (664 vs. 8210 annual detections, Table II). There was considerable variation (up to 14-fold) in the proportional detection rates between regions. In some regions, individual viruses were detected at two to three times the national average, while in others particular viruses were never reported.

CONCLUSIONS

The first attempt to analyze aspects of national epidemiologic data on the viruses associated with gastrointestinal illness is reported. The data are derived from viruses found in the feces mainly of hospital inpatients. Most specimens were sent to the laboratory at the discretion of the clinician and probably represented a considerable underestimate of the true incidence. No attempt was made to allow for severity of illness, asymptomatic excretion, or variations in interest and technique between laboratories. Astroviruses and caliciviruses have morphological features that permit positive identification, but the distinguishing features of SRSVs and coronaviruses are easily damaged, making recognition difficult. These figures therefore represent minimum observations, and only tentative conclusions can be drawn from them.

The proportional detection rates for these viruses varied from one region to another. This may have occurred for several reasons: differences in disease incidence; different research interests; variable commitment to, and facilities for, EM; variable interest and commitment of the clinicians, and the length of the lines of communication. It would therefore be unwise to speculate further. Moreover, it is inappropriate to
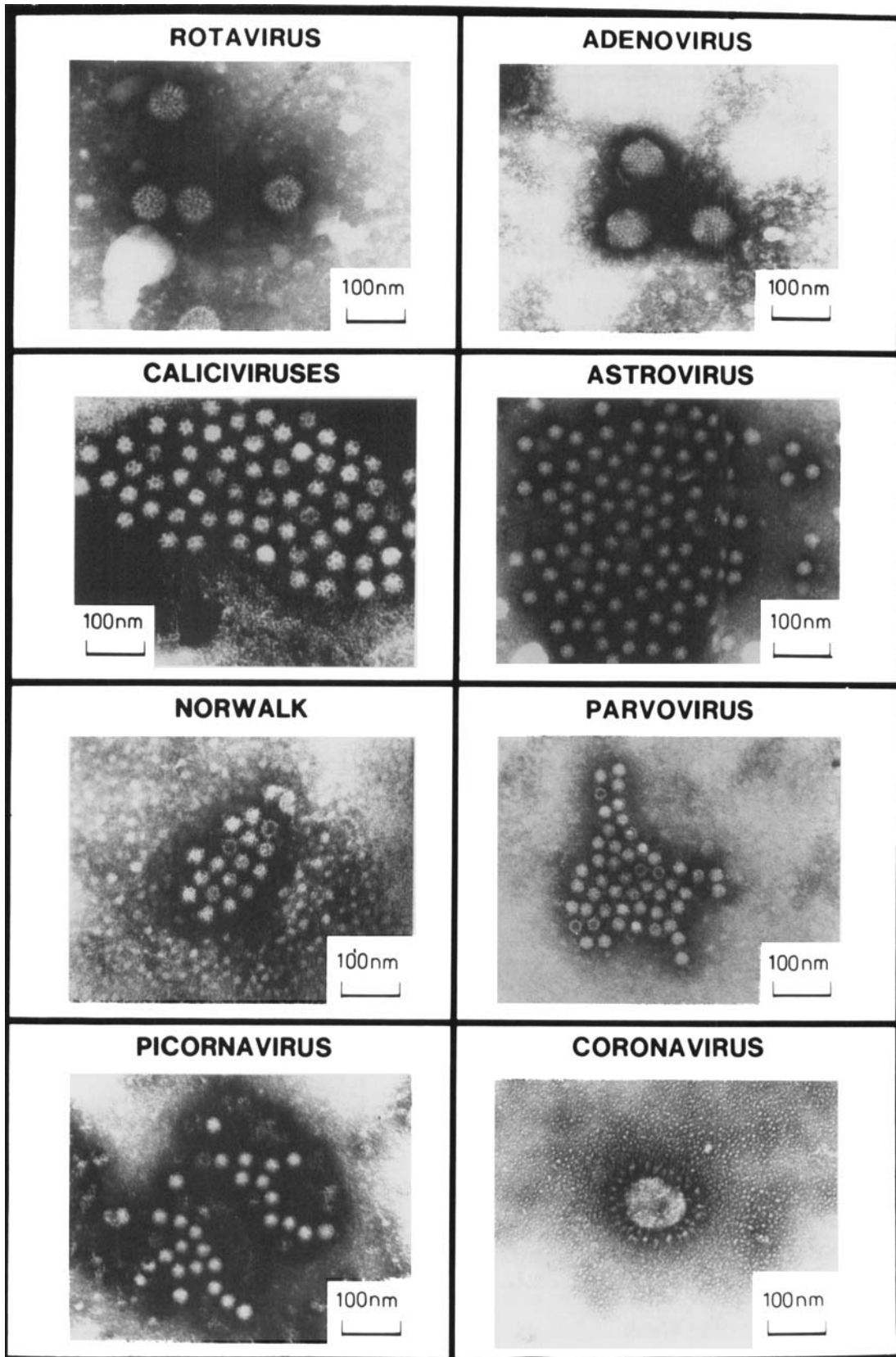
Fig. 1. Electron micrographs of enteric viruses. Micrographs of representative samples of the viruses detected in fecal specimens were prepared as a reference collection. All micrographs were printed at the same magnification. Bar = 100 nm.

TABLE I. Classification Scheme for Small Round Fecal Viruses*

Group	Type	Surface features	Physical properties[a]	Hosts and examples

Featureless viruses
  Smooth entire edge;
  no surface structure
	Enterovirus
	  (SRV)
			20–30 nm
			BD 1.34 g/cm^3
			RNA genome
				Human (poliovirus,
				  hepatitis A)

	Parvovirus (SRV)		18–26 nm BD 1.38–1.46 g/cm^3 DNA genome	Cat, dog, mink, cattle

	Candidate parvoviruses
	  (SRV)
			22–26 nm
			BD 1.38–1.40 g/cm^3
				Human (Wollan,
				  Ditchling, cockle,
				  Paramatta)

Structured viruses
  Surface structure
  and/or ragged outline
	Astrovirus	5–6 pointed
		surface star
			28–30 nm
			BD 1.36–1.38 g/cm^3
			RNA genome
				Human (types 1–5),
				  lamb

	Calicivirus	Surface hollows,
		"star-of-David"
		configuration
			30–38 nm
			BD 1.36–1.39 g/cm^3
			RNA genome
				Human (UK1-UK4,
				  Sapporo), cattle,
				  pig, dog

	Small round
	structured virus
	(SRSV)
		Amorphous surface,
		ragged outline
			27–35 nm
			BD 1.36–1.41 g/cm^3
				Human (Norwalk,
				  Snow Mountain,
				  Hawaii, Taunton
				  Montgomery County)

*Adapted from Caul and Appleton [1982].
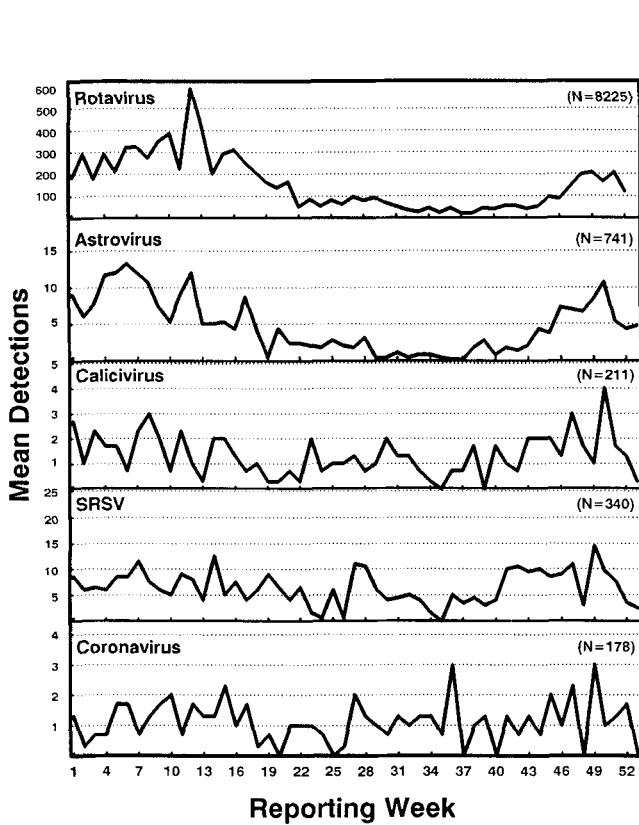[a]BD, buoyant density in cesium chloride.

Fig. 2. Seasonal distribution of enteric virus detections. Data represent the mean of three years of reports (1985–1987) except for SRSVs (2 years 1986–1987) and rotavirus (1 year 7/86–6/87). The total number of detections reported for each virus is indicated (N).
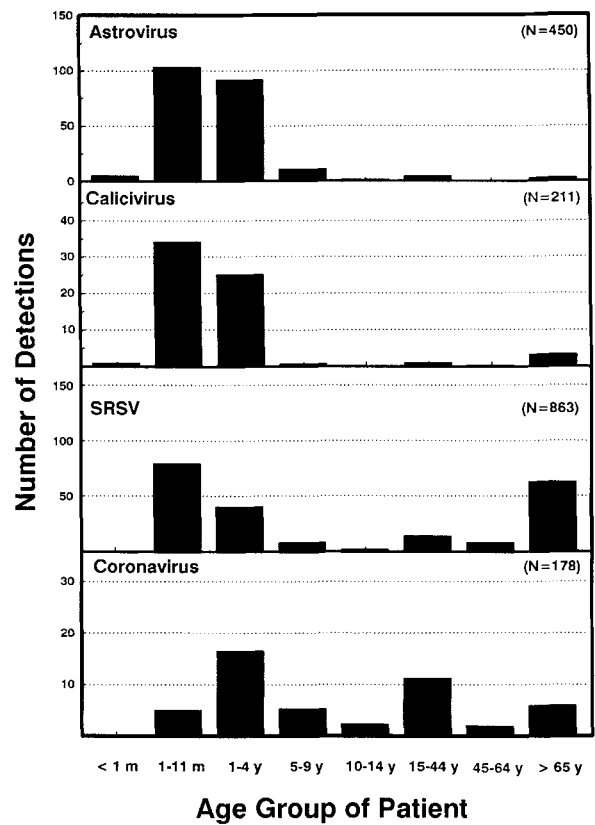

Fig. 3. Age distribution of enteric virus detections. Data represent the total of 3 years of reports (1985–1987). Those detections for which a patient age was unknown are not included. The total number of detections reported for each virus is indicated (N).

TABLE II. Proportional Detection of Novel Viral Agents of Gastroenteritis

Region	Rotavirus 7/86-6/87 N	Proportional detection rate[a] for viruses				
		Astro	Calici	SRSV	Corona	Total
Northern	293	4.8	0.7	2.4	0.2	8.1
Yorkshire	1,087	0.0	0.0	1.2	0.3	1.5
Trent	825	1.3	0.5	1.2	0.4	3.4
E. Anglia	238	0.0	0.8	1.1	0.0	1.9
Thames	2,149	3.2	1.3	2.5	0.7	7.7
Wessex	294	2.6	0.1	3.9	0.0	6.6
Oxford	268	7.5	0.6	12.7	0.4	21.2
S. Western	545	6.5	1.0	11.0	2.1	20.6
W. Midlands	1,197	1.5	0.1	1.9	0.2	3.7
Mersey	213	4.9	0.5	8.6	1.1	15.1
N. Western	853	5.5	2.8	5.3	2.3	15.9
Wales	136	3.4	0.2	2.5	0.0	6.1
N. Ireland	112	10.1	0.0	4.5	0.0	14.6
Overall rate		3.0%	0.9%	3.5%	0.7%	8.1%
Total detections[b]	8,210	741	211	863	178	1,993

[a]Proportional detection rate = mean annual detections × 100/rotavirus detections.
[b]Single season detections for rotavirus. Total of 3-year detections for novel viral agents.

compare these data in detail with Grohmann's [1985] proportional detection rates in Australia, in a study conducted by a single laboratory with standardized techniques.

While EM remains the "gold standard" for detecting all fecal viruses, other techniques have recently been shown to detect viruses in EM-negative specimens. A study of diarrhea among children in Arizona day-care centers showed that although 11 of 375 specimens (2.9%) contained calicivirus detectable by ELISA and confirmed by immune electron microscopy (IEM), no particles were observed by direct EM [Matson et al., 1989]. Similarly, Grohmann [1985] used IEM to examine 800 specimens that were initially negative by direct EM and detected a virus in 205 cases (25.6%). In particular, of the 300 EM-negative samples from adults (aged 15–59 years), 125 (42%) contained "Norwalk-like" (i.e., SRSV) particles detectable by IEM. These results suggest that antibody-enhanced EM methods (IEM) increase sensitivitiy and should be more widely applied, particularly for the examination of outbreaks.

Astrovirus, like rotavirus, occurred more commonly during the winter months, while calicivirus, SRSV, and coronavirus did not exhibit obvious seasonal patterns. A winter increase in astrovirus detections was also observed in the 6-year Australian study [Grohmann, 1985]. Whether this pattern is related to serotypic variations in pathogenicity, seasonal differences in host susceptibility, modes of transmission, or some other environmental factors remains to be determined.

Astrovirus and calicivirus were found almost exclusively in samples from young children, specifically in those under 1 year of age. This finding is consistent with observations that acquisition of antibodies to these agents occurs in early childhood [Kurtz and Lee, 1978; Grohmann, 1985; Kurtz and Lee, 1987; Nakata

et al., 1988]. By contrast, SRSV and coronavirus were frequently found in adults as well as children. In the case of SRSV, the observed age distribution was consistent with these agents being frequently associated with community outbreaks of gastroenteritis among adults [Kapikian et al., 1990]. In such outbreaks in the United Kingdom, it is normal that only a small number of samples is examined and, of these, only a small proportion (10–40%) is positive [Caul, 1988]; consequently, there will be considerable underreporting. A further problem is that many laboratories do not investigate outbreaks but concentrate on childhood diarrhea. It follows that the age incidence of SRSVs plotted in Figure 3 should not be overinterpreted.

The analysis demonstrates that a retrospective reporting system can provide an indication of the prevalence of fecal viruses in symptomatic patients. A more complete understanding of the true incidence of these viruses, particularly in asymptomatic excretion, would require expensive and time-consuming prospective studies. These should include collection of data to define the age-specific incidence of infection, with or without disease, and to establish the common signs and symptoms associated with infection. Progress in enhancing the sensitivity of EM detection of these agents, particularly of SRSVs, depends on the application of immune techniques. The collection of paired serum and stool samples will be essential in establishing standardized protocols for immune-enhanced detection and for the development of new assays. Ultimately, more sensitive diagnostic tests based on immunoassays will be required for the diagnostic clinical laboratories whilst molecular techniques such as hybridization probes or the polymerase chain reaction will be needed as research tools to permit studies on the etiology of gastroenteritis.

Data from routine specimens will always be incomplete as compared with detailed prospective surveys. Nevertheless, the results presented here provide some indications of the disease-associated activity of these viruses. Comparison with the data from the newly instituted North American system will prove both interesting and instructive.

ACKNOWLEDGMENTS

REFERENCES

Caul EO (1988): Small round human fecal viruses. In Pattison JR (ed): "Parvoviruses and Human Disease." Boca Raton, FL: CRC Press, pp 139–163.

Caul EO, Appleton H (1982): The electron microscopical and physical characteristics of small round human fecal viruses: An interim scheme for classification. Journal of Medical Virology 9:257–265.

Cook SM, Glass RI, LeBaron CW, Ho M-S (1999): Global seasonality of rotavirus infections. Bulletin of the World Health Organization 68:171–177.

Grohmann G (1985): Viral diarrhoea in children in Australia. In Tzipori S (ed): "Infectious Diarrhoea in the Young." Amsterdam: Elsevier, pp 25–28.

Kapikian AZ, Chanock RM (1990): Norwalk Group of Viruses. In Fields BN, Knipe DM, Chanock RM, Hirsch MS, Melnick JL, Monath TP, Roizman B (eds): "Virology." New York: Raven Press, Ltd., pp 671–693.

Kurtz J, Lee T (1978): Astrovirus gastroenteritis age distribution of antibody. Medical Microbiology and Immunology (Berlin) 166:227–230.

Kurtz JB, Lee TW (1987): Astroviruses: Human and animal. In Bock G, Whelan J (eds): "Novel Diarrhoea Viruses." Ciba Foundation Symposium 128. New York: John Wiley & Sons, pp 92–107.

Lew JF, Glass RI, Petric M, LeBaron CW, Hammond GW, Miller SE, Robinson C, Boutilier J, Riepenhoff-Talty M, Payne CM, Franklin R, Oshiro LS, Jaqua M-J (1990): Six year retrospective surveillance of gastroenteritis viruses identified at ten electron microscopy centers in the United States and Canada. Pediatric Infectious Diseases Journal 9:709–714.

Matson DO, Estes MK, Glass RI, Bartlett AV, Penaranda M, Calomeni E, Tanaka T, Nakata S, Chiba S (1989): Human calicivirus-associated diarrhea in children attending day care centers. Journal of Infectious Diseases 159:71–78.

Nakata S, Estes MK, Chiba S (1988): Detection of human calicivirus antigen and antibody by enzyme-linked immunosorbent assays. Journal of Clinical Microbiology 26:2001–2005.