

Caliciviruses

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INTRODUCTION

The caliciviruses are a family of hitherto-neglected viruses which include only three well-characterised members, all isolated from animals. All human caliciviruses remain as candidates because it has proved difficult to grow them in cell cultures and consequently many of the typical properties of viruses in the group have not been demonstrated. This situation is exacerbated by the existence of several other viruses which do not show the characteristic virus morphology in the electron microscope (EM), but are likely nevertheless to belong to this virus family. The purpose of this review is to put into context those caliciviruses significant in human disease. Of necessity this must be based on the characteristics of established group members and a review of the animal viruses is therefore appropriate. Characteristics of established and candidate caliciviruses are summarised in Table 1.

Established caliciviruses

Vesicular exanthema of swine virus and San Miguel sealion virus

The history of the calicivirus group dates from 1932 when samples were taken from pigs suffering from an infection similar to foot and mouth disease. These early isolates were poorly characterised and eventually destroyed in the belief that they were in fact foot and mouth disease virus. However, later outbreaks were better characterised, and spread of the virus was attributed to the use of contaminated feed. The last such outbreak in the USA occurred in 1956 and it was thought that the virus had been eradicated. However, in 1972 a calicivirus was isolated from lesions on the flippers of a sealion on San Miguel island, California. This was later termed San Miguel sealion virus (SMSV) and similar viruses have since been isolated from a variety of marine animals including fish, pinnipeds and whales. Antibody to the virus is common in marine mammals living in, or passing through, the area. Antibodies to SMSV were also found in some land animals including potential scavengers such as foxes, and the virus was also obtained from a fish preyed on by sealions thus raising the possibility that SMSV could be transmitted through food.¹ SMSV is serologically distinct from vesicular exanthema of swine virus (VESV), but the viruses are clearly related by RNA homology, and are also similar in terms of the cells in which the virus can replicate in culture. Unlike other caliciviruses which have a relatively narrow host range, SMSV

can infect a variety of species including primates, and can also produce symptoms of vesicular exanthema when inoculated into pigs. Consequently it is possible that VESV spread to pigs through feed contaminated with viruses from marine sources. Indeed, SMSV has been isolated from animal feed containing fur seal meat.²

Feline calicivirus

Feline calicivirus (FCV) was first isolated in 1957 when attempts to isolate the infectious agent of feline panleukopaemia led to the discovery of a virus which was rapidly cytopathic in feline cell cultures. This virus was later shown to be ubiquitous in cat populations, and is the second largest cause (after feline herpesvirus) of feline upper respiratory tract infection serious enough to cause owners to seek veterinary attention for their animals. Infection is associated with a superficial vesiculation and ulceration predominantly of the oral cavity. Some strains of virus are associated with the development of a polyarthritis (limping syndrome), although the reasons for this are not clear. Respiratory infection is milder than that caused by feline herpesvirus, and unless complicated by secondary bacterial invasion, recovery is usually uneventful. However, the virus is able to establish a persistent infection in some animals which may become life-long carriers, with continuous shedding of the virus. The tonsils are likely to be the major, but not the only, site for viral persistence. Levels of virus antigen in tonsil tissue are low compared with the amount of virus shed, and tonsillectomy does not cure a persistent infection.^{3,4}

Candidate caliciviruses

As animal caliciviruses were studied by negative contrast EM, it was soon appreciated that the particles had a characteristic morphology, and their surfaces appeared as if covered in a regular array of dark spots. This appearance (described in more detail later) remains the simplest and most obvious criterion by which caliciviruses can be identified. In 1976, Madeley and Cosgrove⁵ observed particles of this characteristic morphology in the stools of infants suffering from diarrhoea by direct EM (Figure 1). As with other enteric viruses, these particles were also found in the stools of symptom-free infants. Human caliciviruses have since been linked with cases of mild diarrhoea in both infants and children, from all over the world and shown to induce illness in volunteer studies. Originally these agents were referred to as human calicivirus, and abbreviated to

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Table 1. Probable and confirmed caliciviruses

Virus	Serotypes	Infection
San Miguel sealion virus	16 or more	Vesicular
Vesicular exanthema of swine	13	Vesicular (FMDV-like)
Feline calicivirus	1 (numerous related strains)	Respiratory Oral vesiculation
Human calicivirus	5 or more	Enteric
Small round structured viruses	4-9	Enteric
Newbury agent of cattle	2?	Enteric
Porcine enteric calicivirus	?	Enteric
Infectious stunting calicivirus of chickens	?	Enteric
Canine calicivirus	2?	1 enteric 1 vesicular
Amyelosis chronic stunt virus	?	Enteric
Hepatitis E virus	1	Hepatitis
Rabbit haemorrhagic disease virus	?	Hepatitis
Mink calicivirus	?	Respiratory (haemorrhagic pneumonia)

HCV. However, this term has now become established for reference to hepatitis C virus. In order to avoid confusion, we will refer to these human caliciviruses as HuCV in this review.

Other viruses have been identified in human faeces which are thought to be caliciviruses on biochemical grounds but which appear fuzzy in the EM, and do not show the positive identifying features described below. These include the small round structured viruses (SRSV) which are also associated with diarrhoea, and the virus causing enterically transmitted non-A, non-B hepatitis now termed hepatitis E virus (HEV).

Viruses with typical calicivirus morphology have also been observed in faeces from a number of animals with diarrhoea, including the dog: canine calicivirus,⁶ pig: porcine enteric calicivirus,⁷ calf: Newbury agent,⁸ chicken calicivirus⁹ and an insect larva, the naval orange worm: Amyelosis chronic stunt virus (ACSV)¹⁰. A virus causing haemorrhagic hepatitis in the rabbit has also recently been described (rabbit haemorrhagic disease virus; RHDV).

FEATURES OF THE CALICIVIRUS FAMILY

At first all these viruses were considered to be a subgroup of the picornavirus family, but later studies indicated that

they differed from picornaviruses in both the structure and morphology of their capsid, and also in their manner of replication.¹¹ Finally in 1978 the viruses were recognised as distinct from previously identified genera and grouped as a separate new family, the *Caliciviridae*. This comprises at present a single genus, the caliciviruses, whose members have the following properties.

Structure

Under the EM, negatively stained calicivirus particles are 33-35 nm in diameter and their surface structure appears fundamentally different from most other viruses with an icosahedral capsid. The capsid is usually defined by space occupying subunits, often visible as knob-like capsomers (Figure 2). These displace stain and appear white in micrographs. By comparison, the calicivirus surface has an array of dark stain-filled hollows giving a lacy appearance. There are 32 such hollows, located at each apex (12) and face (20) of a regular icosahedron and each is separated from its neighbours by an unstained ridge (Figure 3). These cup-like hollows give the virus group its name (*calyx* = a cup) and form very characteristic patterns when viewed along the two-, three- or five-fold axes of symmetry (Figure 3). Along the two-fold symmetry axis, four dark patches are seen, often in a pronounced rhomboid or diamond pattern,

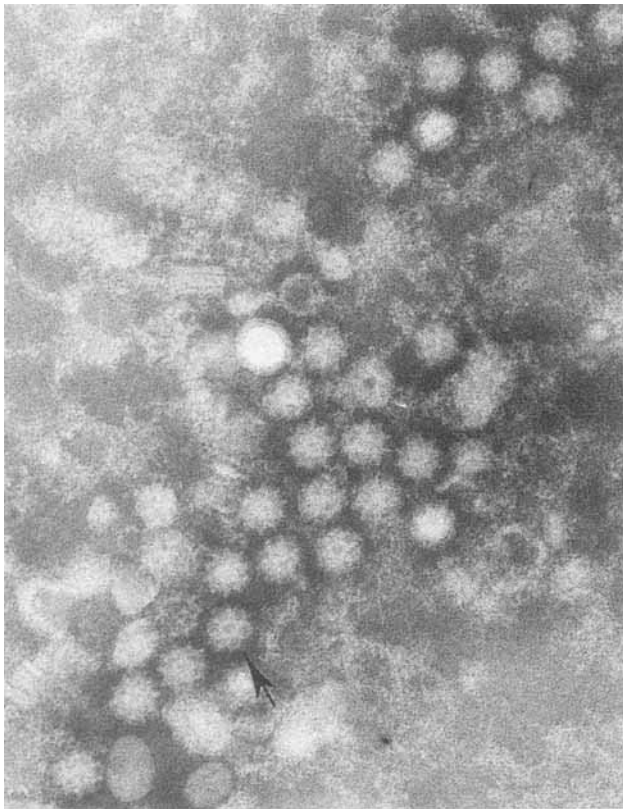


Figure 1. Typical human calicivirus particles from stool. Negative contrasted image prepared with 3% potassium phosphotungstate pH 7.0. Surface hollows are visible on most, but not all, particles. Several show ten peripheral spikes and one (arrowed) shows the six-pointed star of David appearance unique to these viruses. (Magnification $\times 200\ 000$.)

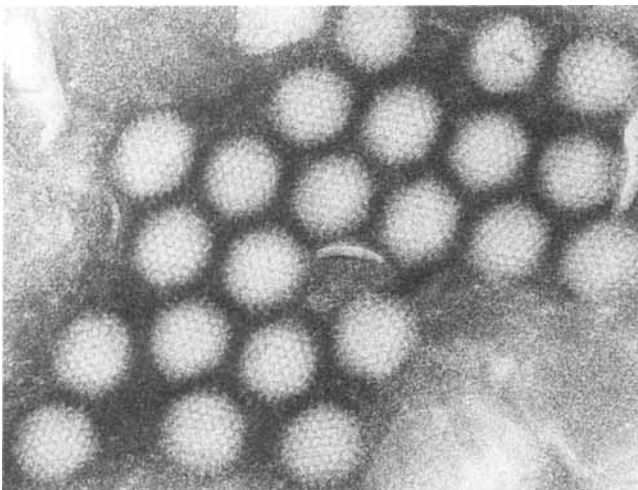


Figure 2. Adenoviruses from a stool extract seen by negative contrast. Compared with Figure 1, the particles are constructed from knob-like space filling capsomers which displace the stain and appear white. There are no hollows on the surface. 3% potassium phosphotungstate pH 7.0. (Magnification $\times 200\ 000$.)

separated by light ridges in the form of an 'H' (Figure 3a). Seen along the three-fold axis, a central dark patch is surrounded by six others, a pattern which is unique to the caliciviruses and often seen as a star of David (Figure 3b). Finally, the view along the five-fold axis shows a central

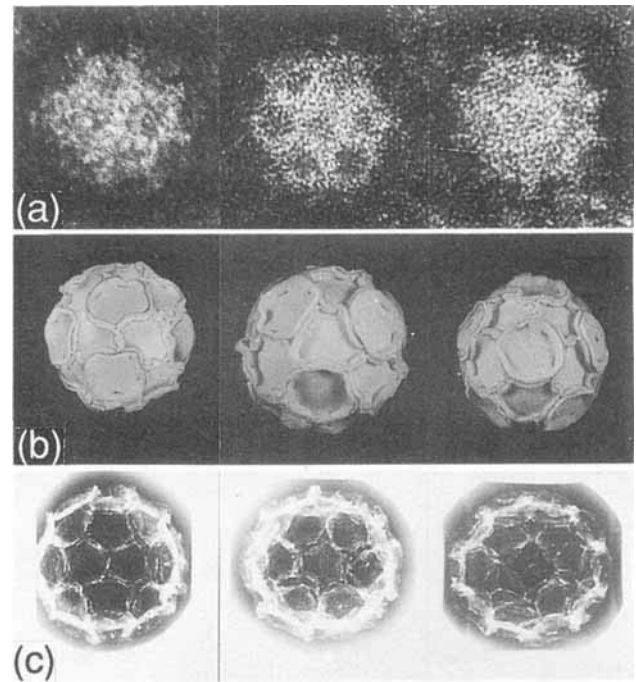


Figure 3. Caliciviruses viewed along two-, three- and five-fold axes of symmetry (columns 1, 2 and 3 respectively). Row (a), negative contrast electron micrographs of calicivirus particles. Row (b), photographs of a foam rubber model constructed from 32 discs fixed together in the form of an icosahedron. Row (c), x-ray image of the model which has been soaked in barium sulphate suspension to provide contrast in the x-ray beam. This process mimics the superimposition of absorbances at different levels in a three-dimensional structure, to form the two-dimensional image as observed in the EM. Note the similarities between, in particular, rows (a) and (c), indicating that the model is a fair representation of virus structure. (Approximate magnification of EM micrographs $\times 600\ 000$.)

dark patch with 10 spike-like projections around the periphery of the particle (Figure 3c). These spikes are interpreted as the rims of the cup-like depressions seen end on. These patterns are visible on only a minority of particles in any preparation but provide the most widely recognised feature for assigning a virus to the family.

A further unique feature of the caliciviruses is that the capsid is constructed from only one type of structural protein. This is generally in the size range $M_r = 60\text{--}70\ 000$. The virus particle is approximately 18% RNA, and has a total M_r in the region of 15×10^6 . Calicivirus strains from many sources have a buoyant density in caesium chloride of 1.3–1.4 g/mL. Biochemical and biophysical properties of some representative caliciviruses are given in Table 2.

Genome

The genome of the virus is a single strand of positive sense RNA. This is 7.3–8 kb long and bears a covalently attached protein, VpG, at its 5' end. This structure appears similar to that of the picornaviruses and, in common with other positively stranded viruses, the isolated genome is infectious. However, unlike picornaviruses, the genome VpG is required for infectivity.¹²

Replication strategy

Intracellular proteins

In general, where information is available, the findings with all cultivable animal viruses are compatible with each other,

Table 2. Biochemical and biophysical properties of some caliciviruses

Virus	Structural protein $M_r \times 10^{-3}$	Buoyant density (g/mL) in CsCl	Size (nm)	Synthesis of subgenomic RNA
Feline calicivirus	62	1.34–8	37	Yes
San Miguel sealion virus	60	1.36–9	34	Yes
Vesicular exanthema of swine virus	65	1.36–8	34	Yes
Chicken calicivirus	?	1.38	36	?
Human calicivirus	62	1.38	30–35	?
Small round structured viruses				
Snow Mountain	62	1.33	27–32	?
Hawaii	?	1.36–41	35	?
Norwalk	59 (soluble 29)	1.38	27–32	?
SRSV-Japan 9	63 (soluble 28)	1.36	33	?
Canine calicivirus	58	1.34	35	?
Amyelosis chronic stunt virus				
2 types of particle	70 (calici-like) 29 (smooth- heterogenous in density)	1.32 1.4	38 28	?
Porcine enteric calicivirus	58 ^a (additional 28)	1.37	30	?
Newbury agent	?	?	33	?
Rabbit haemorrhagic disease virus	60	1.365	27–35	Yes
Hepatitis E virus	?	1.29 ^b	27–30	Yes

? = unknown, no data available.

^aSizes determined under non-reducing conditions.

^bDetermined on glycerol/potassium tartrate gradients because the virion disintegrates in CsCl. This normally gives lower values (cf. VESV, 1:27).

but not all proteins have been reported in all cases and there are discrepancies between the sizes of each reported from different laboratories.

Calicivirus proteins mature by proteolytic cleavage. This process is rapid and the uncleaved forms cannot be detected unless measures are taken to inhibit the action of proteases. The events occurring after FCV infection of feline kidney cells have been examined by western blotting and provide the most complete description of protein synthesis by these viruses.

Following infection a short-lived protein of M_r 73 000 has been observed.^{13,14} This was only detected in low amounts and its existence has not been confirmed. As the infection progresses, proteins of M_r 96 000, 75 000, 62 000, 39 000, 36 000 and 27 000 accumulate in the infected cell. These are believed to be the end products of the proteolytic protein processing pathways. The M_r 62 000 protein forms the capsid of the progeny virus and is

synthesised in greatest amount. Under conditions which inhibit proteolytic processing, additional molecules can be detected. These comprise two large proteins (M_r 125 000 and 123 000), and two which are smaller (M_r 98 000 and 76 000). Only one of these four proteins has been characterised. The M_r 76 000 protein is a precursor to the mature M_r 62 000 capsid protein; the others are presumably precursors for the rest of the virus proteins.

Virus RNA synthesis

Early studies showed that caliciviruses induced multiple RNA species in the infected cell which differentiated them clearly from the picornaviruses. Four such RNAs were found by gel electrophoresis under denaturing conditions.¹⁵ Mapping experiments indicated that these formed a 3' co-terminal nested set very similar to that which had already been observed in coronaviruses and arteriviruses. Subsequent reports indicated that these are

probably replicated independently of the full-sized RNA through a negative sense template for each subgenomic RNA.¹⁶

However, although all subgenomic positive RNAs have been assumed to be messenger RNAs, this function has been demonstrated for only one of them. Furthermore, whilst recent data obtained from the molecular cloning of caliciviruses support the synthesis of at least one subgenomic RNA, they have failed to confirm the others, and this suggests that the current scheme of calicivirus replication may require amendment.

HUMAN ENTERIC CALICIVIRUSES

Caliciviruses of characteristic morphology

The discovery of human caliciviruses has been described above. They were later linked with Winter Vomiting Disease, and with diarrhoea. Since then volunteer studies have confirmed their link with gastrointestinal disease (reviewed in reference 17). HuCV has been grown in primary human embryo cells in culture, and also in a dolphin cell line.¹⁷ However, these methods have not been widely applied for two reasons. The first concerns the host cell; human embryo cells are not widely available, and the dolphin cells rapidly lose their ability to support HuCV. Second, virus growth is poor, and in common with other enteric viruses, HuCV replication is dependent on proteolytic supplements such as trypsin which damage the cells. For these reasons the replication strategy of the virus has not been examined in detail, and investigation still relies primarily on EM methods.

Five serotypes have been defined by immune electron microscopy (IEM). Four were found in the UK and one in Japan. In this review the five serotypes will be termed HuCV-UK1–4 and HuCV-Japan. Although distinguished by IEM, all five serotypes are probably related since a radioimmunoassay (RIA) detects cross-reactions between four of them, and some reactivity to SRSVs was also detected. This suggests the existence of a group antigen and also implies that RIA/enzyme immunoassay (EIA) is not the best method for studies of the separate epidemiology of HuCV and SRSV.¹⁸ These five serotypes have been found worldwide although lack of suitable reagents and adequately equipped laboratories have prevented firm identification and cross-comparisons in most countries. The viruses are responsible for episodic outbreaks of diarrhoea and vomiting, mainly in infants and the elderly, and have been associated with the consumption of contaminated food. Secondary infections are common. In one episode in the UK, infection in an old-persons' home was tentatively linked with illness in a dog kept at the home which developed a gastric upset before the infection appeared in humans. Later serology showed that the dog had antibodies to HuCV-UK3, the virus identified in the outbreak.¹⁹

There seem to be two distinct patterns of illness induced by caliciviruses. In the first, exemplified by HuCV-UK1 and the Japanese agent, illness is limited mainly to infants with relative sparing of adults. Most cases present with

diarrhoea (96%) and/or vomiting (77%). The illness lasts from 1–11 days and is paralleled by the period for which virus is detectable in the stools. Other infections, mainly those associated with strains UK 3 and 4, affect individuals of all ages and the disease pattern is very similar to that of the SRSVs (see below). Disease patterns induced by caliciviruses and SRSVs are similar when infections in the same age group are compared. Antibody to HuCVs is acquired during the first 5 years of life in places as diverse as the UK, Saudi Arabia and Japan. Levels of seropositivity reach 80% in the 6–12 year age group, suggesting that exposure at least to some serotypes of the virus is common. The frequency of recorded HuCV infections decreases rapidly above this age, presumably because of developing immunity in the population.¹⁷

Small round structured viruses

There are other viruses which are thought to be caliciviruses on biochemical grounds but lack the features typical of caliciviruses, for example, those associated with gastroenteritis and collectively referred to as the SRSVs (Figure 4). The prototype virus of this group is Norwalk virus. This virus cannot be grown in culture, and all samples for analysis have therefore been obtained from clinical infections. Morphology should therefore be interpreted against the background of the possible complications due to antibody which could bind to the surface of the virus and obscure structure, or limited degradation which could have occurred in the faeces before samples are taken.

Norwalk virus was discovered by Kapikian and colleagues²⁰ in 1972 when the technique of IEM was used to capture and concentrate any virus particles present in samples from an outbreak of gastrointestinal illness which had occurred in Norwalk, Ohio in 1968. This virus is now known to have a worldwide distribution. The particles were originally reported to be smaller than typical caliciviruses at 27 nm, although this has since been amended upwards as the original view, that the particles were coated with a layer of antibody, has been revised. SRSVs have no clear surface structure; they are more fuzzy, the dark spots are less well defined and do not display a regular pattern. The surface appears lighter and retains less stain. Indications that a cup-like structure may underlie this surface appearance are sometimes visible at the periphery where a spike-like pattern similar to that referred to above is discernible. The whole surface structure is less regular than that of the typical caliciviruses, although it is not clear whether this is due to attachment of antibody, damage to the surface or is a true feature of the virion.

Purified virions possess a single capsid protein M_r 59–62 000 (Table 2) as expected for a member of the calicivirus family. In addition, Norwalk virus and the enteric SRSVs in general seem always to be associated with large amounts of a protein M_r 29 000. This is not a virion component and is generally referred to as a 'soluble' protein. An intriguing possible explanation is raised by studies of the insect calicivirus ACSV. Hillman and colleagues¹⁰ clearly demonstrated the proteolytic degradation of typical 38 nm calicivirus particles composed of a M_r 70 000 protein, to form smooth, heterodense 28 nm particles composed of a

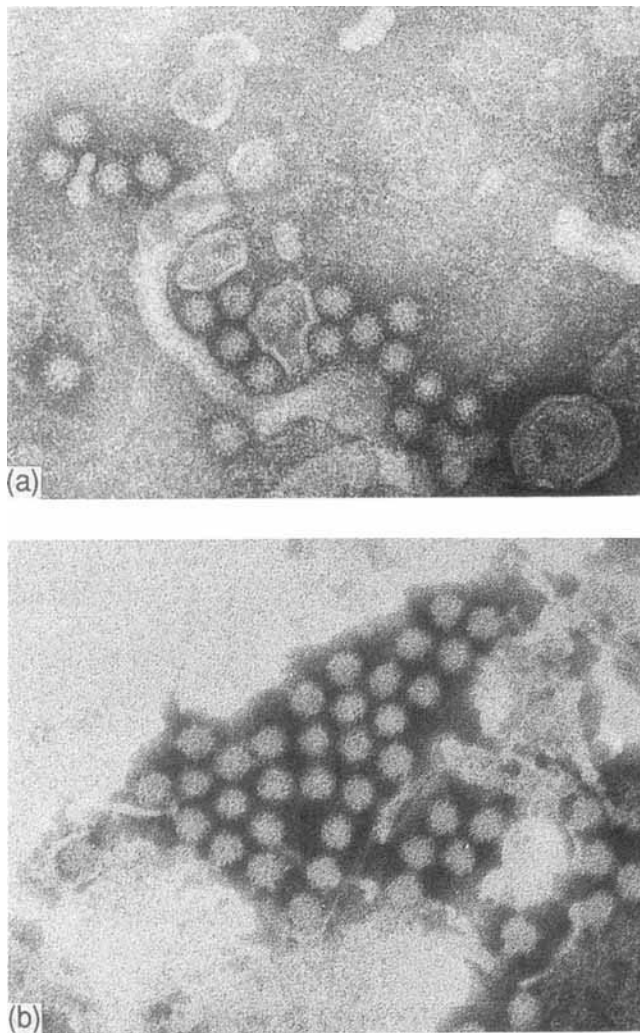


Figure 4. Typical SRSVs seen by negative contrast in extracts of faeces from patients with symptoms of gastrointestinal illness. Panel a, from an endemic case. Panel b, from an outbreak. Particles appear similar regardless of their origin from endemic or outbreak cases. They are 33–35 nm in diameter, have a 'hairy' appearance but lack any clearly visible surface stain-filled hollows. While not incompatible with caliciviruses in general appearance and size, they do not show any positively identifying features. Negative contrast with 3% potassium phosphotungstate, pH 7.0. (Magnification $\times 200\ 000$.)

M_r 29 000 protein. If this process were to continue, it is possible that these particles themselves might disintegrate to form a large amount of 'soluble' protein.

There are thought to be four or more serotypes of SRSV defined by IEM, although characterisation is incomplete. In particular there has been little cross-characterisation of isolates from the UK and Japan with those from the USA. Recently an attempt has been made to rectify this situation and selected SRSVs were grouped into four preliminary serological groups by IEM.²¹ Recent data from Japan suggest there may be as many as nine antigenic types.²²

Data on the relationships between viruses are difficult to interpret. The nomenclature used is mainly geographical and several outbreaks of HuCV and SRSV have been reported from the same city (Sapporo). Furthermore, systematic names used in Japan and the UK (SRSV-1, 2, etc) are not compatible, and the source of each virus must be specified. However, it is possible to assemble the data into groups of presumptive similarity (but not, by any means,

Table 3. Deduced relationships among small-round structured viruses

Norwalk group SRSV-UK 2, SRSV-Japan 2, (SRSV-Japan 4,5 ^a)
Hawaii group SRSV-UK 3, Otofuke, Sapporo, SRSV-Japan 6,7
Snow Mountain group SRSV-UK 4, SRSV-Japan 8,9
Taunton group SRSV-UK 1

There are no data to allow the two distinct viruses, SRSV-Japan 1 and 2 to be assigned to this table.

^aSRSV-Japan 4 and 5 are provisionally assigned to the Norwalk group, but data are incomplete.
Compiled primarily from data in references 21 and 22.
Inclusion in a particular group implies cross-reactivity with the named group representative by IEM, and is not indicative of identity. Cross-reaction between groups by other techniques is common.

identity) as illustrated in Table 3. This analysis of the reports suggests that there are four distinct groups of similar viruses reported from several sources. There are no data addressing a comparison of SRSV-Japan 1 and 2 with SRSV-UK1. However, since SRSV-Japan 1 and 2 are distinct from SRSV-Japan numbers 3–9, and from each other, they cannot both be classified with UK1. Consequently we can deduce 5–6 such virus groups in total.

Other reports have sometimes used different techniques to compare SRSVs and this can make the data difficult to interpret. IEM seems to detect type-specific antigens and differentiates well between viruses. However, western blotting, RIA and EIA find increased cross-reactivity, even between SRSV and HuCV and may reflect the presence of group antigens.

Illness associated with SRSVs has been reported more commonly in older children and adults than in infants. There is as yet no satisfactory explanation for this, especially as it seems likely that children are often exposed to the virus before they have fully developed antibody responses. In a longitudinal study of children in Bangladesh the prevalence of antibody to Norwalk virus increased from 7% to 80% between the ages of 6 months and 5 years. Not all rises in titre were associated with episodes of diarrhoea. Most of the increases in serum titre took place between the ages of 14–36 months.²³ Broadly similar results were obtained in studies performed in Ecuador and the Philippines.^{24,25} Volunteers infected with Norwalk virus commonly develop vomiting and diarrhoea, often associated with headache and abdominal discomfort.²⁶ Illness generally lasts 24–48 hours. Norwalk virus infection causes flattening and broadening of the jejunal villi similar to that caused by other diarrhoea-associated viruses. There is also mucosal inflammation and crypt hypertrophy during repair.^{27,28} However, these changes occurred even in the absence of diarrhoea, and were accompanied by

decreased levels of brush border enzymes and malabsorption of fats and xylose.²⁹ Such changes will adversely affect those children who already have an impoverished diet even if they do not develop overt disease.

In the developed world antibody is detected in relatively few children, but increases in prevalence to reach 50–75% in adults. This increase takes place later than in the third world and occurs mainly during adolescence.²⁴ This is later than the acquisition of antibody to rotavirus in the same locations which suggests a different pattern of transmission for these two enteric viruses. Further, levels of antibody do not appear to correlate with protection and the whole question of disease production and the development of immunity requires more investigation. Only 50% of volunteers challenged with Norwalk virus became ill. Surprisingly, pre-existing serum or intestinal fluid antibodies actually predisposed individuals to the development of symptoms rather than protected them. Such persons were subsequently resistant to rechallenge in the short term (1–2 months) but were reinfected and developed symptoms again if rechallenged later (2–4 years). One volunteer out of four was again ill after a third challenge. In contrast individuals lacking pre-existing antibody were resistant to disease production on the first challenge and remained so on subsequent occasions. The antibody titres which did develop were low.³⁰ Two suggestions have been put forward to explain these unusual results. First, it has been postulated that there is a natural genetic resistance (or susceptibility) to Norwalk virus infection in some cases. There is some evidence of familial clusters of disease within populations exposed similarly to the virus which would support such a hypothesis.³¹ An alternative suggestion proposes that multiple sequential challenge with the virus is required before any individual can succumb to infection and begin to build up antibody titre. This suggestion would explain why infections appear to be mild in persons with lower levels of antibody, and why prevalence of antibody eventually reaches such high levels.

Both HuCV and SRSV are causes of epidemic gastroenteritis in the community. SRSVs in particular accounted for 42% of such outbreaks investigated in the USA over a 4-year period, and were implicated in a further 23%.³² Most episodes whose origin could be traced were spread by contaminated water supplies rather than by food. Shellfish are often implicated in food-borne outbreaks of HuCV- and SRSV-associated gastric illness. Shellfish such as oysters can be expected to acquire viruses by filter feeding from seawater. At least one calicivirus (SMSV) is relatively stable in seawater.³³ However, whilst the capacity of shellfish to spread pathogenic enteric bacteria is lost rapidly (24–48 h), they remain able to transmit viruses such as enteroviruses for a much longer period (4–6 weeks). The possibility that viruses replicate in these organisms deserves consideration. However, these viruses account for only a small percentage of cases admitted to hospital with diarrhoea (SRSV, 1.6%; HuCV, 0.9–3%) which probably reflects the generally mild and self-limiting nature of the disease.^{34,35,36} Both human enteric caliciviruses and the SRSVs have recently been reviewed.^{17,37}

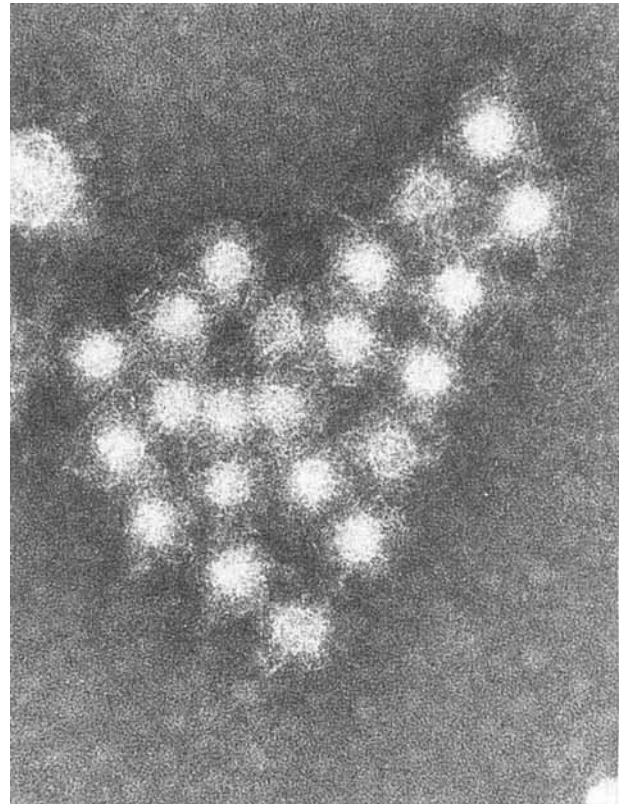


Figure 5. Hepatitis E virus particles visualized by immune electron microscopy. No surface structure is visible through the heavy coating of antibody but some impression of surface hollows can be gained on some of the particles. (Magnification $\times 200\,000$.) Micrograph kindly provided by Dr D. W. Bradley, Hepatitis Branch, CDC, Atlanta, USA.

HEPATOTROPIC CALICIVIRUSES

The candidate caliciviruses with a fuzzy appearance in the EM now include the hepatitis virus (HEV) responsible for enterically transmitted non-A, non-B hepatitis in man (Figure 5), and RHDV. Like other SRSVs, these viruses cannot be grown in culture, and all samples examined have been obtained from infected animals or humans.

Hepatitis E virus

Enteric non-A, non-B hepatitis is an acute disease generally occurring in epidemics in developing countries, and may account for up to 50% of clinical hepatitis, making this possibly the most common form of acute hepatitis in parts of the developing world.³⁸ It is transmitted by the faecal oral route,³⁹ often through sewage contamination of water supplies. The disease has an incubation period of 2–9 weeks with a mean of 6 weeks and a fatality rate of 1–2%.⁴⁰ This is ten times higher than that of an hepatitis A epidemic. Pregnant women are particularly at risk, and among this group the disease is fatal in 10–20% of cases. Death is caused by a fulminant hepatitis. The mortality rate is highest during the third trimester of pregnancy and lowest during the first trimester.³⁸

The infectious agent has been identified as a 27–30 nm particle of indistinct morphology (Figure 5).^{41–45} Few viruses are found in acute phase stools by IEM, either due to low virus titre or poor specimens after peak virus shedding. Solid phase IEM can dramatically increase the efficiency of detection.⁴⁶

IEM has been used to analyse sera from cases occurring in different regions and different years. This has shown that enterically transmitted non-A non-B hepatitis is due to one virus (HEV) or a group of serologically related viruses.⁴⁷ The virus is extremely labile and unable to withstand high salt concentrations. Even pelleting the virus can lead to disruption of the particles. The particles have a buoyant density of 1.29 g/mL in potassium tartrate/glycerol gradients which is comparable with other caliciviruses in this sedimentation medium.⁴⁸

HEV can be transmitted to primates and pigs by intravenous inoculation of stool extracts.^{39,49} Reyes and colleagues⁵⁰ infected cynomolgus macaques and used infectious bile to obtain a recombinant complementary DNA (cDNA) clone. Cloned cDNA hybridised to three RNA species in infected liver, and to the largest (7.7 kb), in faecal samples from infected humans.

Further molecular characterisation has shown that the virus has a polyadenylated, positive stranded genome of 7.6 kb, which has been sequenced, although not published. Consequently both the genomic organisation and replication strategy (via the synthesis of multiple subgenomic RNAs) are consistent with the virus being a member of the *Caliciviridae*.

Rabbit haemorrhagic disease

Rabbit haemorrhagic disease was first reported in China between 1984 and 1986.^{51,52} Though the disease does affect wild rabbits, it mainly attacks animals raised under intensive conditions.

Virus has been obtained from infected liver and has been variously classified a picornavirus,^{53,54} a parvovirus⁵⁵ and a calicivirus.⁵⁶ Detailed analysis of the virus has been hampered by the inability to grow the virus in cell cultures, but it has been purified from infected liver. It was found resistant to ether, exposure to pH 3.0 and heating to 50°C, but was inactivated by treatment with 1% sodium hydroxide⁵⁴ and 0.4% formaldehyde. Formaldehyde had no effect on the immunogenicity of the particles and has been used to produce a killed virus vaccine. The virions have a buoyant density of 1.365 g/mL in caesium chloride and appear similar to caliciviruses in the EM.^{57,58}

Ohlinger and colleagues⁵⁸ identified a single antigenic protein of M_r 60 000 from purified virus also observed by Parra and Prieto.⁵⁶ The capsid contains a single-stranded positive RNA of 8 kb in length, polyadenylated at the 3' end and infectious on intrahepatic injection.⁵⁸ Infected liver RNA has been analysed in northern blots using a radioactive cDNA probe. This identified a 2 kb subgenomic RNA species similar to that found in calicivirus-infected cells. Consequently, capsid structure, genome composition and replication strategy all indicate that RHDV is most likely a calicivirus.

The disease appears very similar to European brown hare syndrome, an acute necrotising hepatitis of the hare, which has been reported in a number of European countries including the UK.^{59,60} The disease has been associated with virus-like particles, which are non-enveloped, icosahedral structures, 30 nm in diameter. However, no cross-reactivity has been established between this virus and

RHDV, and the viruses do not cross-infect each other's host animals. At present there is no definitive evidence to suggest that the virus from hares is also a calicivirus.

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