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Astroviruses: human and animal

J.B. Kurtz and T.W. Lee

Virology & Public Health Laboratory, John Radcliffe Hospital, Oxford OX3 9DU, UK

Abstract. The name astrovirus was used by Madeley and Cosgrove in 1975 to describe a small round virus (approximately 28 nm diameter) with star-like appearance on electron microscopy. It was first seen in faeces from a few children with gastroenteritis. An aetiological role in gastroenteritis has since been confirmed. The virus causes a mild illness after an incubation period of 3–4 days. Antibody studies indicate that infection is widespread and, in Britain, mainly occurs in the 2–5 year age group. Outbreaks occur in, for example, institutions and paediatric wards. The virus usually spreads by the faecal–oral route but food- or water-borne outbreaks have occurred.

Strains of astrovirus have been isolated from many animals including calf, lamb, pig, cat, dog, duck and turkey. The lamb strain can cause gastroenteritis but the bovine strain did not cause diarrhoea in gnotobiotic calves. Infected turkeys have scours, and infection in ducklings causes haemorrhagic hepatitis with a mortality up to 25%. Five human serotypes have been described, all antigenically distinct from the bovine and ovine strains. The human astrovirus does not replicate in conventional tissue cultures but undergoes a non-productive cycle in human embryo kidney cells, and productive replication in the presence of trypsin. It is a positive-strand RNA virus, which is acid stable (pH 3), survives at 60°C for five but not 10 minutes and, like the enteroviruses, resists inactivation by alcohols. It has a density of 1.35–1.37 g/ml in caesium chloride.

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Astroviruses were so called in 1975 (Madeley & Cosgrove) because of their characteristic 5–6-pointed star-like form. Earlier in 1975, Appleton & Higgins described an outbreak of diarrhoea in some infants in a maternity unit which they associated with a virus of this appearance seen in the affected infants' faeces. These reports were of human infection, but in retrospect a distinct type of hepatitis in ducks due to an astrovirus had been described by Mansi et al in 1964. Subsequently, astroviruses have been detected in a wide variety of animals including sheep (Snodgrass & Gray 1977), calf (Woode & Bridger 1978), pig (Bridger 1980), dog (Williams 1980), cat (Hoshino et al 1981), deer (Tzipori et al 1981), turkey (McNulty et al 1980), duck (Gough et al 1984) and mouse (Kjeldsberg & Hem 1985). In some of these species infection is associated with illness; in others the association is, at best, doubtful. Where studies have been

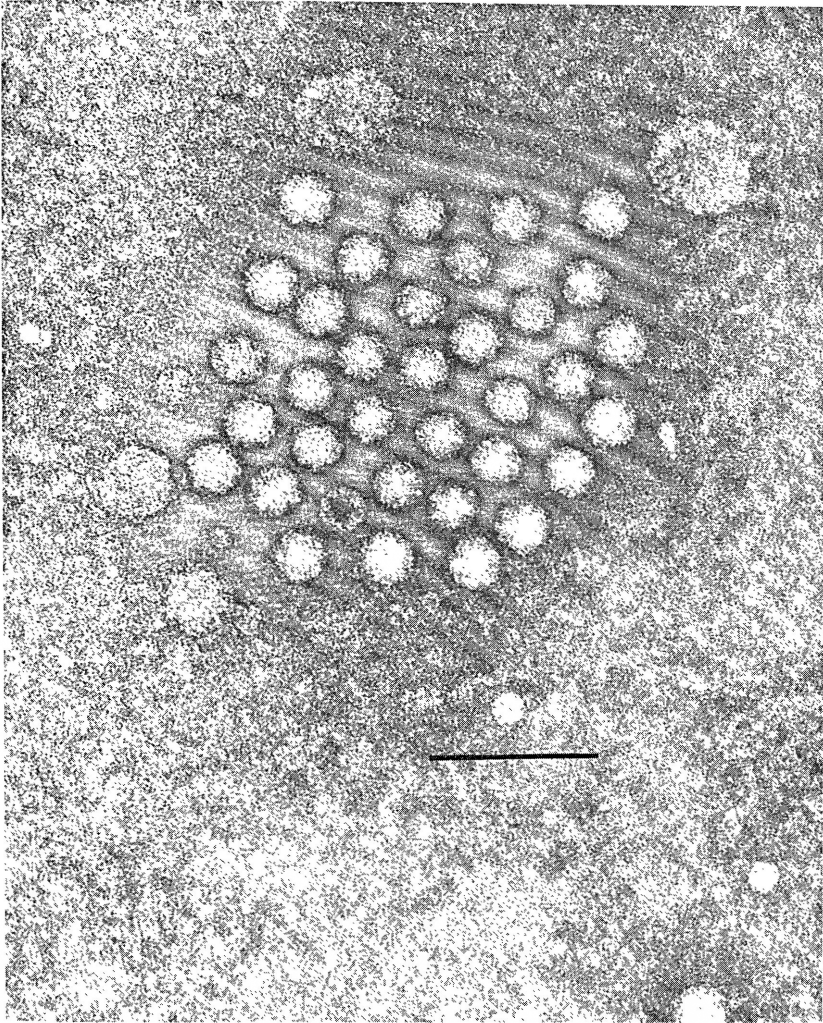


FIG. 1. Negatively stained (methylamine tungstate, pH 6.6) astrovirus particles from a human faecal sample. Bar, 100 nm.

done, no antigenic relationship has been found between viruses from the different species.

The virus

Astroviruses have a smooth or slightly crenated round outline with a solid star-shaped core (Fig. 1). Bridging structures (Snodgrass & Gray 1977)—

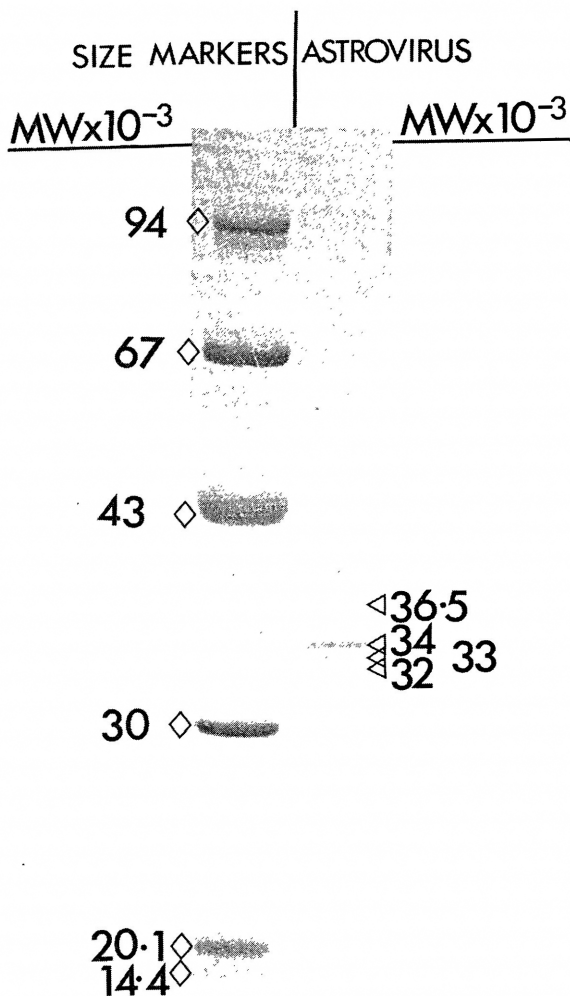


FIG. 2. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of polypeptides from tissue culture-grown human astrovirus (serotype 4). (A. Easton, Warwick University.)

external projections associated with the points of the star—are occasionally seen. Not all particles show the characteristic appearance and in its absence identification may be impossible. Aggregation of the virus with antibody also interferes with its appearance. The diameter of the virus is 28–30 nm. Its density in CsCl is 1.35–1.37 g/ml. The ovine strain (Herring et al 1981) has a single-stranded RNA and only two major capsid polypeptides of similar relative

molecular mass (M_r), 33 000. The human virus likewise has a positive-strand RNA genome, of approximately 7500 nucleotides. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) of the human virus shows it to have four polypeptides of M_r 36 500, 34 000, 33 000 and 32 000 (Fig. 2). The largest of these is very faint and, by analogy with the enteroviral virus polypeptide O (VPO), may represent a precursor to one of the others. As the gel only resolved polypeptides down to M_r 14 000, a smaller polypeptide equivalent to the VP4 (M_r 5500) of enteroviruses could not have been detected (A. Easton, personal communication 1986). This pattern suggests that astroviruses are members of the picornavirus family. Physical characteristics of the human astrovirus include stability to acid (pH 3) and 10 minutes shaking in chloroform, and survival at 60°C for five but not 10 minutes.

The virus does not replicate in conventional tissue culture systems but human strains will undergo a non-productive cycle of replication in primary human embryo kidney (HEK) cells. There is no cytopathic effect but virus may be detected in the cytoplasm by fluorescent antibody techniques in 24–48 h infected cells. When 10 µg/ml crystalline trypsin is included in a serum-free medium, virus is released from infected HEK cells and a productive infection established (Lee & Kurtz 1981). Tissue culture-grown virus is morphologically and antigenically indistinguishable from faecally derived virus and after passage in HEK cells some strains can be adapted to a continuous line of rhesus monkey kidney cells (LLCMK2). In infected cells crystalline arrays of virus are seen in the cytoplasm adjacent to vacuoles (Kurtz et al 1979).

Infections in man

Astroviruses have a world-wide distribution. Infection may occur throughout the year but the peak incidence is in winter/spring in temperate zones. Overt illness is commonest in 1–3-year-old children; an antibody prevalence survey in Oxfordshire showed a rise from 4% in 6–12-month-olds to 64% in 3–4-year-olds and 87% in the 5–10 year age group. Symptomatic infection was found in 62 of 79 (80%) babies infected with astroviruses (Madeley 1979) while 11 (12%) had no diarrhoea and in six (8%) an association was doubtful. The frequency of re-infection and the likelihood of it being symptomatic are not known, but in the presence of detectable serum antibody, infection of volunteers did not result in diarrhoea (Kurtz et al 1979).

There are at least five different serotypes of human astrovirus (Kurtz & Lee 1984), demonstrable by immunofluorescent tests and immunosorbent electron microscopy (ISEM, Roberts & Harrison 1979) using rabbit antisera to various tissue culture-grown strains of the virus. Fig. 3 shows the virus counts of the heterologous reactions as a percentage of the homologous reactions for the five serotypes, using ISEM in which the grids were pre-coated with antisera to increase the adsorption of the homologous virus. Community-acquired strains

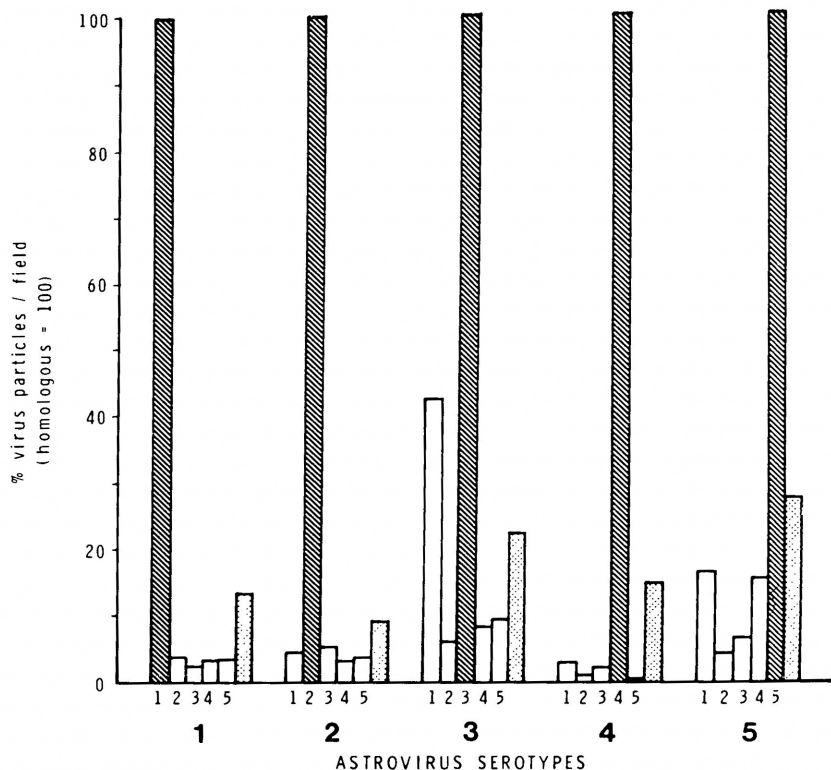


FIG. 3. Immunosorbent electron microscopy of astrovirus strains using anti-astrovirus sera. Cross-hatched bars indicate homologous reaction. Stippled bars indicate adsorption to uncoated grid. (Reproduced from Kurtz & Lee 1984 with permission of *The Lancet*.)

of astrovirus collected since 1975, mainly from the Oxford region, show the following distribution of serotypes: 72% type 1, 8% type 2, 8% type 3, 6% type 4 and 6% type 5.

Virus is transmitted by the faecal-oral route, either directly or via fomites, food or water. Person-to-person spread commonly occurs in the family, nursery and paediatric ward where infection may be endemic. Outbreaks have been associated with eating oysters (E.O. Caul, personal communication 1981) and drinking water from a contaminated culvert (W.D. Cubitt, personal communication 1986).

The clinical features of symptomatic infection include not only diarrhoea but also systemic signs and symptoms (Fig. 4). After an incubation period of 3-4 days, fever ($\geq 37^\circ\text{C}$), headache, malaise, nausea and occasionally vomiting may occur. Astroviruses become detectable in the faeces at this time, to be followed within a day by diarrhoea which is typically unformed or watery. Diarrhoea (2-6

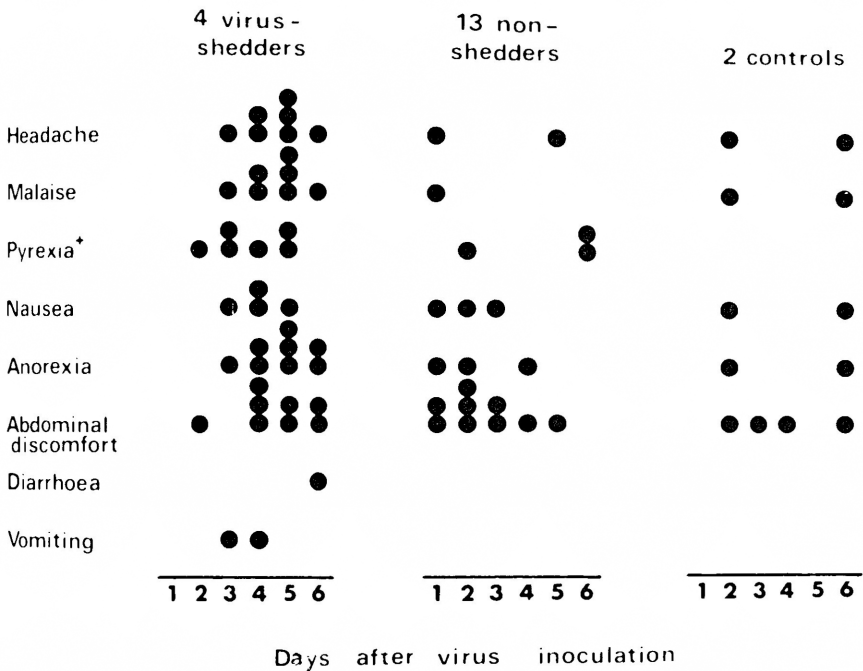


FIG. 4. Clinical responses of 17 volunteers inoculated with astrovirus diluted in orange squash in whom virus shedding was or was not detected, and in two controls who were given diluent alone. Each point represents the recording of a symptom or sign in one volunteer on each day after virus inoculation. Pyrexia⁺ = a temperature $\geq 37.2^{\circ}\text{C}$. (Reproduced from Kurtz et al 1979 with permission Alan R. Liss, Inc.)

motions/day) usually lasts only 2–3 days but may continue for 7–14 days and is accompanied by virus excretion. More persistent infection may occur in immunocompromised patients. Thus, one child with combined immunodeficiency who was given a bone marrow transplant became infected with astrovirus several weeks later. This infection, which was associated with diarrhoea and virus shedding, persisted for more than four months, until the child's death (E. Davies, personal communication 1986).

Duodenal biopsy during astrovirus infection has shown that the virus infects epithelial cells of the lower part of the villi (Phillips et al 1982).

Direct electron microscopy (EM) is the most useful method for detecting astroviruses in the routine laboratory. In the acute illness up to 10^{10} virus particles/ml faeces are present, although viable counts are lower ($10^8/\text{ml}$). This exceeds the lower limit of sensitivity of EM ($10^6/\text{ml}$). An advantage of EM is that other potential pathogens may be found in a specimen. Nazer et al (1982) reported that of 28 children infected with astrovirus 16 were co-infected with another enteric pathogen, 11 with rotavirus, four with bacteria (*Salmonella* spp.



FIG. 5. Astrovirus particles inside autophagic vacuoles and free in the cytoplasm of a mid gut apical enterocyte, 38 h after infection of a gnotobiotic lamb. Bar, 400 nm. (E.W. Gray, Moredun Research Institute.)

or enteropathogenic *Escherichia coli*) and one with both rotavirus and bacteria. If more than one pathogen is found it is often not possible to establish the aetiology of the diarrhoea.

Infections in animals

The first species other than man in which astrovirus infection was observed was the sheep. Snodgrass & Gray (1977) reported an outbreak of diarrhoea in 4–6 week-old Suffolk lambs on a farm. Astroviruses were seen in the faeces of eight of 17 lambs examined. The virus was passaged twice through gnotobiotic lambs in which virus was excreted. It was associated with a yellowish diarrhoea developing on the 4th day after ingestion and lasting for two days in one lamb that was not sacrificed early. Virus excretion persisted from the 3rd to the 9th day in this lamb. After experimental infection (Snodgrass et al 1979), lesions appeared scattered throughout the small intestine by 23 h and were maximal in the mid gut and ileum at 38 h, after which gradual healing led to resolution by the 5th day. Virus replicated only in mature columnar epithelial cells situated in the apical two-thirds of the villi. These cells developed vacuoles and were replaced by immature cuboidal cells from the crypts, resulting in partial villus atrophy. Virus was seen by EM in the cytoplasm of these columnar cells 14–38 h after infection (Fig. 5). It was present as crystalline arrays, in apical pits and tubules in the microvillus border (which were suggested as the site of entry of the virus) and in autophagic vacuoles (Gray et al 1980).

In 1978 a bovine astrovirus was recognized by Woode & Bridger. A mixed infection with small round viruses, one of which was morphologically an astrovirus, caused diarrhoea in a calf. Experimental infection of gnotobiotic calves with this astrovirus produced no illness and the virus was considered non-pathogenic in this species. More recently (Woode et al 1984), single and dual infections (quite common in nature) with either bovine rotavirus or Breda virus 2 in gnotobiotic calves have confirmed the absence of illness with astrovirus infection alone, although the faeces became yellow and soft at the time of virus excretion (2–9 days after infection). Scours developed in the animals with dual infections or with Breda 2 or rotavirus infections alone. Astrovirus infection was confined to specialized M epithelial cells of the domes overlying Peyer's patches in the jejunum and ileum of the calf. As in the sheep, infected cells showed degenerative changes and were sloughed and replaced by cuboidal cells. The limited number of susceptible cells in the bovine gut compared to that of the sheep might well explain the lack of illness in the former. Infection however, is common, as shown in a serological survey in Iowa where 30% of calves had astrovirus antibodies. By cross-neutralization tests using antisera raised in gnotobiotic calves to two USA isolates and one British astrovirus isolate, three serotypes have been identified. More serotypes are suggested by the fact that

when 16 field isolates were tested, five could not be classified by the above system (Woode et al 1985).

Astroviruses have been reported in several other mammals. A feline strain has been reported in a four-month-old kitten with diarrhoea. The illness persisted for two weeks and required hospitalization (Hoshino et al 1981). Astrovirus was seen in five of 56 artificially reared red deer fawns who developed diarrhoea. The deer produced antibody to their virus (Tzipori et al 1981). In the dog, astrovirus was seen in diarrhoeal stools of beagle pups (Williams 1980). Its significance was difficult to ascertain because in each case the faeces contained a variety of viruses. The five pups were infected with astrovirus and typical coronaviruses, both of which disappeared when the diarrhoea stopped. In addition, one pup had a parvovirus, and two had atypical coronaviruses in their faeces which persisted after the pups recovered. An astrovirus has also been detected in three-week-old piglets who developed diarrhoea 3–4 days after weaning (Bridger 1980). Again the faeces contained a number of viruses (astro-, calici- and rotavirus-like, and enterovirus), making attribution of significance impossible.

The same problem of significance occurs in the turkey. Astroviruses have been seen in association with rotaviruses in two outbreaks of scours which caused an increase in mortality in the flock (McNulty et al 1980). Astrovirus has also been detected in the faeces of young chickens with scours but the virus does not appear to cause disease in chicks kept under laboratory conditions (R.E. Gough, personal communication 1986).

The most serious disease caused by astrovirus in the veterinary world is in the duck. Heavy losses, with a mortality rate up to 25% in 3–6-week-old birds, were noticed in fattening ducklings kept on open fields in Norfolk in 1983. The birds died acutely of hepatitis (Gough et al 1984), the livers showing haemorrhages, widespread necrosis of hepatocyte cytoplasm and usually bile duct hyperplasia. Experimental transmission of a virus, morphologically similar to astrovirus, from infected liver and faeces caused a haemorrhagic hepatitis 2–4 days after inoculation in five of 20 2–3-day-old ducklings. Mature ducks were unaffected by the infection. A similar disease caused by an agent serologically distinct from classical duck virus hepatitis virus had been described in 1964 by Mansi et al and was named duck hepatitis (DH) type II virus (Asplin 1965). Cross-protection experiments using sera and vaccines to the classic DH type I and DH type II viruses have shown that the astrovirus is closely related to the DH type II virus. The virus replicates with difficulty in chick embryos but one strain has been adapted to growth in them. Although no wild-life reservoir has been detected it is possible that wild birds may transmit the disease, especially as outbreaks have initially involved ducks kept on open fields. Control by vaccination with the attenuated chick embryo-adapted strain, or by reducing contact with wild birds by housing the ducks, has been successful; nevertheless, the disease continues to be a serious problem in East Anglia.

References

- Appleton H, Higgins PG 1975 Viruses and gastroenteritis in infants. *Lancet* 1:1297
- Asplin FD 1965 Duck hepatitis. Vaccination against two serological types. *Vet Rec* 77:1529–1530
- Bridger JC 1980 Detection by electron microscope of caliciviruses, astroviruses and rotavirus-like particles in the faeces of piglets with diarrhoea. *Vet Rec* 107:532–533
- Gray EW, Angus KW, Snodgrass DR 1980 Ultrastructure of the small intestine in astrovirus infected lambs. *J Gen Virol* 49:71–82
- Gough RE, Collins MS, Borland E, Keymer IF 1984 Astrovirus-like particles associated with hepatitis in ducklings. *Vet Rec* 114–279
- Herring AJ, Gray EW, Snodgrass DR 1981 Purification and characterization of ovine astrovirus. *J Gen Virol* 53:47–55
- Hoshino Y, Zimmer JF, Moise NS, Scott FW 1981 Detection of astroviruses in faeces of a cat with diarrhoea. *Arch Virol* 70:373–376
- Kjeldsberg E, Hem A 1985 Detection of astroviruses in gut contents of nude and normal mice. *Arch Virol* 84:135–140
- Kurtz JB, Lee TW 1984 Human astrovirus serotypes. *Lancet* 2:1405
- Kurtz JB, Lee TW, Craig JW, Reed SE 1979 Astrovirus infection in volunteers. *J Med Virol* 3:221–230
- Lee TW, Kurtz JB 1981 Serial propagation of astrovirus in tissue culture with the aid of trypsin. *J Gen Virol* 57:421–424
- McNulty MS, Curran WL, McFerran JB 1980 Detection of astroviruses in turkey faeces by direct electron microscopy. *Vet Rec* 106:561
- Madeley CR 1979 Viruses in the stools. *J Clin Pathol* 32:1–10
- Madeley CR, Cosgrove BP 1975 28 nm particles in faeces in infantile gastroenteritis. *Lancet* 2:451–452
- Mansi W, Schofield PB, Gonzalez VC 1964 Duckling virus infection. *Vet Rec* 76:740
- Nazer H, Rice S, Walker Smith JA 1982 Clinical associations of stool astrovirus in childhood. *J Pediatr Gastroenterol Nutr* 1:555–558
- Phillips AD, Rice SJ, Walker Smith JA 1982 Astrovirus within human small intestinal mucosa. *Gut* 23:A923–924
- Roberts IM, Harrison BD 1979 Detection of potato leafroll and potato mop-top viruses by immunosorbent electron microscopy. *Ann Appl Biol* 93:289–297
- Snodgrass DR, Gray EW 1977 Detection and transmission of 30 nm virus particles (astroviruses) in faeces of lambs with diarrhoea. *Arch Virol* 55:287–291
- Snodgrass DR, Angus KW, Gray EW, Menzies JD, Paul G 1979 Pathogenesis of diarrhoea caused by astrovirus infections in lambs. *Arch Virol* 60:217–226
- Tzipori S, Menzies JD, Gray EW 1981 Detection of astroviruses in the faeces of red deer. *Vet Rec* 108:286
- Williams FP 1980 Astrovirus-like, coronavirus-like and parvovirus-like particles detected in the diarrhoeal stools of beagle pups. *Arch Virol* 66:215–226
- Woode GN, Bridger JC 1978 Isolation of small viruses resembling astroviruses and caliciviruses from acute enteritis of calves. *J Med Microbiol* 11:441–452
- Woode GN, Pohlenz JF, Kelso Gourley NE, Fagerland JA 1984 Astrovirus and Breda virus infections of dome cell epithelium of bovine ileum. *J Clin Microbiol* 19:623–630
- Woode GN, Kelso Gourley NE, Pohlenz JF, Liebler EM, Mathews SL, Hutchinson MP 1985 Serotypes of bovine astrovirus. *J Clin Microbiol* 22:668–670

DISCUSSION

Caul: In your seroepidemiological work in the Oxford region you found that about 75% of adults had antibody to astrovirus. In the laboratory, we detect astrovirus in perhaps 5–10% of symptomatic neonates or children. There are several possible explanations for this discrepancy. One is that subclinical infection with astrovirus is common. Alternatively, the symptoms are so mild that medical intervention is not warranted. There is support for this view from the Japanese study (Konno et al 1982), where only one of 46 ill children in a kindergarten required treatment. Another possible explanation is misidentification, because the characteristic surface star structure is lost when antibody is added to astrovirus preparations. This brings to mind the Marin County agent in the USA, which caused an outbreak of gastroenteritis in a geriatric population. It was described as Norwalk-like, but it resembles an astrovirus in the published immuno-electron micrographs. Immuno-electron microscopy is a useful tool for establishing an aetiological role, but should not be recommended where precise identification is needed.

Kapikian: The Marin County agent is a 27 nm virus detected by Oshiro et al (1981) in stool specimens from a gastroenteritis outbreak in elderly patients in a convalescent home in 1978, in Marin County, California. We also examined this agent by electron microscopy, and I agree with Dr Caul that it had the appearance of an astrovirus in certain preparations without antibody. However, only a few particles had the characteristic star-like appearance, as we had to examine many particles to observe the infrequent one which had that appearance, reminiscent of the astroviruses. Further studies have been carried out by Drs Midthun, Walsh, Greenberg et al, which Dr Greenberg can describe.

Greenberg: This was work done primarily by Karen Midthun. The Marin County agent seems to be an astrovirus, because its morphological characteristics are similar to those of astroviruses, and also because the purified virus, when iodinated and run on a polyacrylamide gel, has one or two protein bands in the M_r range of 30000. Thus it is not a calicivirus-like particle, because it has smaller molecular weight protein(s) than caliciviruses.

Marin County virus was given to volunteers, with results strikingly similar to those of Dr Kurtz's astrovirus volunteer study (Fig. 4, p 97) where, of 17 volunteers, only one had serious diarrhoeal disease. We gave Marin County virus to 18 volunteers; only one developed a clear-cut diarrhoeal disease, on the fifth and sixth days after inoculation. This contrasts with studies of Norwalk virus, where almost invariably the majority of inoculated volunteers become ill.

Finally, the Marin County epidemic occurred in an old-age home; aside from this outbreak, are there reports of other astrovirus outbreaks in adults, including the elderly? Mostly astrovirus infections seem to be occurring in young children.

Cubitt: We have recently studied an outbreak due to astrovirus type 1 in an

old people's home. Over a period of 22 days, 34 (80%) of the residents and 13 (44%) of the members of staff were affected. The illness was characterized by vomiting, diarrhoea and abdominal pains with a duration of 48 hours. Serological studies showed that patients developed significant antibody responses to astrovirus type 1 (titres rising from 20 to ≥ 160) (J. Gray, T. Wreghitt & W.D. Cubitt, unpublished work).

Kurtz: Astrovirus infections are mainly in children, either in paediatric wards or as sporadic cases; rarely they affect adults. There have been a few food-associated outbreaks of astrovirus diarrhoea in the UK in adults; the first one to be associated with the eating of oysters was described by Owen Caul (personal communication; see p 96).

Caul: Another small round virus from the USA, the Snow Mountain virus, also presents problems of identification as a result of applying the technique of immuno-electron microscopy. Do you know whether that is a Norwalk type of virus, or an astrovirus?

Kapikian: It is a Norwalk-like virus morphologically and has been shown by Madore et al to contain one major structural protein of M_r 62 000 (Madore et al 1986).

Caul: The astrovirus outbreak associated with oyster eating (unpublished observations) was in a naval base after an officers' dinner. About 24 hours after the consumption of the oysters, many of the officers went down with an apparently classical Norwalk virus infection. Disappointingly, the only virus found by electron microscopy was a small round featureless virus (which we call 'parvovirus-like') whose pathogenicity is unproven. Four days later, after recovery from the primary illness, the patients went down again with diarrhoea; this time they excreted large numbers of astroviruses. My feeling is that the first episode was probably due to a Norwalk-like virus which we were unable to detect and that the second episode was due to astroviruses, in a population who were probably susceptible to both viruses.

Bishop: Are you suggesting that they got a dual infection from the oysters?

Caul: Yes. Oysters are known to concentrate Norwalk-like viruses, so I expect that they concentrate other small round viruses as well.

Bishop: And the two episodes were due to the differing incubation periods initially of the two viruses?

Caul: That would be my interpretation.

Appleton: I would agree that this dual outbreak reflects the different incubation periods of two viruses. Shellfish frequently transmit several different viruses at once and mixed infections within one outbreak are quite common. In recent months we have examined specimens from several gastroenteritis outbreaks associated with molluscs and detected at least two different viruses in the majority of these outbreaks; and it is of course not unknown for persons to develop gastroenteritis 24–48 hours after eating shellfish and then hepatitis A three weeks later.

Mixed infections are probably responsible for the wide range of incubation

periods recorded within many other food-borne outbreaks of viral gastroenteritis.

Kurtz: Mixed viral infections are probably far more common than we realize. This makes determining the cause of an outbreak, or a single case, of gastroenteritis very difficult. Mixtures of rotaviruses, adenoviruses, astroviruses and other small round viruses occur in both humans and animals. In fact, in the latter, mixed infections are almost the rule, rather than the exception.

Kapikian: I am interested in the view of the veterinary experts here on the suggested classification of astroviruses as picornaviruses. For an ovine astrovirus, Herring et al (1981) described the presence of two polypeptide species in about equimolar amounts with molecular weights of about 33 000. In addition, Konno et al (1982) have described the density of astroviruses from a kindergarten outbreak of acute gastroenteritis in Japan to be 1.39–1.40 g/cm³ in caesium chloride. Thus, on the basis of morphology, polypeptides and density considerations, I thought that astroviruses were considered to be a separate family of viruses. Is there general agreement that they are picornaviruses? I have never seen a picornavirus with this 'star' appearance of astroviruses.

Kurtz: The molecular weight profile of the proteins in our culture-grown astroviruses looked more like picornaviruses than anything else; that is as far as we have gone.

Horzinek: Is anything known about the genome?

Kurtz: Only that it is positive-stranded RNA.

Horzinek: How do you know? Is it infectious? Has anybody put extracted RNA into cells and found progeny virus?

Kurtz: That has not yet been done.

McCrae: What is the evidence then that the RNA is positive-stranded?

Greenberg: It is polyadenylated, so it is likely to be positive-stranded RNA.

Woode: All known bovine astrovirus isolates share a common immunofluorescent (IF) antigen, but can be subdivided into serotypes by neutralization. We routinely screen for bovine astrovirus in faeces by IF of 24 h infected cell cultures. This is the most sensitive method, as there are few particles observed by electron microscopy but there is an infectivity titre of 10³ to 10⁴. This approach might be useful for the isolation of human astroviruses.

Horzinek: Is there an antigenic relatedness between any of the human serotypes and the duck hepatitis virus?

Kurtz: I don't know if that has been looked at. There is no antigenic cross-reaction between any of the animal and human astroviruses where it has been investigated. We examined ovine/human and bovine/human reactions, but did not find any crossing. Dr Snodgrass has looked at the ovine/bovine reaction.

Snodgrass: Yes. But even with convalescent serum, which you found to have a broader specificity, the lamb, calf and human astroviruses studied by us did not show any cross-immunofluorescence (Snodgrass et al 1979).

Kurtz: The chicken and duck astroviruses are also unrelated.

Greenberg: I have been interested in whether the small round viruses are important causes of mild diarrhoea in children. In young children, Norwalk virus causes a mild diarrhoea that does not take children to hospital and is frequently not seen by a physician. Over time, however, repeated episodes of mild illness may be an important cause of malnutrition. I wonder whether anybody has information on astroviruses, or any of the other small enteric viral pathogens, on the role of such viruses in children in developing countries. Do they cause mild diarrhoea in these countries?

Mathan: When we studied nearly a thousand cases of acute gastroenteritis in children in southern India, presenting at outpatient departments, astroviruses accounted for only 1.7% of cases. All children in whom astrovirus was found had a mild illness.

Bishop: Have you studied many children with malnutrition who may be excreting these small viruses chronically?

Mathan: No: I have no data on such children.

Cubitt: We recently looked at 200 faecal specimens from the Medical Research Council Unit in The Gambia, and found that only three patients with symptoms of diarrhoea were excreting astrovirus.

Kurtz: In immunodeficiency syndromes, some children have excreted astroviruses for several months, with liquid, rather unpleasant motions during that time. In normal children, virus excretion usually continues for only 4–5 days, occasionally for 10 days.

Caul: I am not aware of any report in immunologically normal children that chronic excretion of astrovirus, detectable at the electron microscope level, occurs. It certainly occurs in immunosuppressed children. We had a recent case of a leukaemic child who died from pneumonia. After autopsy we saw more astroviruses by electron microscopy in the small intestinal contents than we had ever seen previously. The child did not have diarrhoea, and there was no villous atrophy.

Bishop: How common are particles with the star-like appearance? Is the star something that you see only when the staining happens to be right, or is it a consistent finding in all astrovirus preparations?

Caul: We find phosphotungstic acid to be the best stain. In our experience, uranyl acetate or ammonium molybdate are not as good. With PTA, 5–10% of particles show the surface star reliably. Whether this is a 'real' star, I don't know!

Woode: In experimental infections, where you know that the virus is there, you always see some recognizably star-centred particles.

Holmes: Everybody who has looked at astroviruses, including Dr Madeley who first named them, has found that only a proportion of the particles show the stars. The few astroviruses that we saw were like that. It is probably a question of how the particles are oriented on the electron microscope grid.

Woode: The frequency of stars is quite high with bovine astrovirus, of one in 8–10 particles or better.

McNulty: In my experience with astrovirus, the stars may not be evident on the electron microscope screen, but in a photographic print it is easier to see the star-shaped profiles.

Flewett: Dr Madeley made that same point.

Wadell: I am wondering about the ubiquitousness of astroviruses. It occurs to me that they could play some part in less symptomatic diarrhoea. Are there any serological studies in populations who eat less shellfish?

Kurtz: We have looked at Zulus in South Africa and also at sera from North America and Australia. Antibody acquisition rates were similar to the rate in Britain.

Holmes: Roger Schnagl's studies of Aborigines in central Australia showed that astroviruses were present in about 2% of Aboriginal samples, whether or not the patients had diarrhoea. That area is a long way from any shellfish! (Schnagl et al 1979.)

Cubitt: We have tested the same batches of gammaglobulin as were used by Dr Bridger in her rotavirus studies. We found that in every country where we could get pooled immune globulin, namely Japan, South Africa, Belgium, France, Switzerland, Canada and the USA, there were very high levels of antibody to astrovirus type 1. The highest titre was in the American gamma globulin (Hyland Laboratories, Batch 2703 M00 AA).

Bishop: Would it be fair, then, to say that we believe astrovirus to be a common infection in animals and humans, and seldom a cause of severe diarrhoea, except perhaps in poultry?

Appleton: Although in general the symptoms are mild, in the first outbreak that we looked at (Appleton & Higgins 1975), in newborn babies in a maternity unit, the original symptoms of gastroenteritis were relatively mild but those babies went on with malabsorption problems for many weeks. At this period bottle-feeding was fashionable, and I think every affected baby was bottle-fed. It was not possible to get many of them back onto full-strength feeds for several weeks. So there may be longer-term effects.

Bishop: Perhaps there is a case, then, for a proper study of astrovirus infection in children in developing countries.

References

- Appleton H, Higgins PG 1975 Viruses and gastroenteritis in infants. *Lancet* 1:1297
Herring AJ, Gray EW, Snodgrass DR 1981 Purification and characterization of ovine astrovirus. *J Gen Virol* 53:47–55
Konno T, Suzuki H, Ishida N, Chiba R, Mochizuki K, Tsunoda A 1982 Astrovirus-associated epidemic gastroenteritis in Japan. *J Med Virol* 9:11–17

- Madore HP, Treanor JJ, Dolin R 1986 Characterization of the Snow Mountain agent of viral gastroenteritis. *J Virol* 58:487–492
- Oshiro LS, Haley CE, Roberto RR, Riggs JL, Croughan M, Greenberg H, Kapikian A 1981 A 27-nm virus isolated during an outbreak of acute infectious non-bacterial gastroenteritis in a convalescent hospital: a possible new serotype. *J Infect Dis* 143:791–795
- Schnagl RD, Morey F, Holmes IH 1979 Rotavirus, coronavirus-like particles and parasites in central Australia. *Med J Aust* 2:115–118
- Snodgrass DR, Angus KW, Gray EW, Menzies JD, Paul G 1979 Pathogenesis of diarrhoea caused by astrovirus infections in lambs. *Arch Virol* 60:217–226