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Astroviruses

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Introduction

Astroviruses are enteric viruses first identified in the feces of children with diarrhea that predominantly impact the pediatric population. Detection was originally based on a five- to six-pointed star morphology of virions by electron microscopy (EM). However, only about 10% of viral particles display these structures; the remaining 90% of particles have a smooth surface and a size similar to other small, round-structured viruses like picornaviruses and caliciviruses. Thus, accurate diagnostics were difficult to obtain and determining the true prevalence of astrovirus within a population was challenging. Development of much more sensitive detection techniques like real time reverse transcription-polymerase chain reaction (RT-PCR), cell culture RT-PCR, and astrovirus-specific enzyme-linked immunosorbent assays (ELISAs) have made detection more accurate and specific, even allowing diagnosis of specific serotypes. Utilizing these techniques, classic human astroviruses are known to be distributed worldwide and are associated with 2–9% of cases of acute, non-bacterial diarrhea in children although incidences as high as 61% have been reported. Astroviruses can also be isolated in a subset of asymptomatic individuals, suggesting that a proportion of infected individuals shed the virus asymptptomatically or for some time after the resolution of other symptoms of infection. Asymptomatic carriers may be a major reservoir for astroviruses in the environment and could contribute to dissemination of the virus.

The release of astroviruses into the environment is a concern due to the extreme stability of the virus. Astroviruses are resistant to inactivation by alcohols (propanol, butane, and ethanol), bleach, a variety of detergents, heat treatment including 50 °C for an hour or 60 °C for 5 min, and UV treatment up to 100 mJ cm⁻². Human astroviruses are known to survive up to 90 days in both marine and tap water, with survival potential increasing in colder temperatures. Studies have described the isolation of infectious virus from water treatment facilities. Furthermore, astroviruses can be concentrated by filter-feeding shellfish like oysters and mussels in marine environments. Astroviruses are transmitted fecal–orally, and contaminated food and water have been linked to astrovirus outbreaks.

History and Classification

Astroviruses were originally observed by Appleton and Higgins in 1975 as a small round virus in stools. Later that year, Madeley and Cosgrove identified the virus in association with diarrhea in children and bestowed the name astrovirus (from the Greek astron, meaning star) for the star-like morphology of a proportion of viral particles seen by EM (Figure 1(a)). Because of genomic

Glossary

Enterocytes Epithelial cells lining the intestines.
Interstitial nephritis Inflammation of the kidney.
Intussusception Obstruction of the intestine.

Poult Young turkey.
Villi Finger-like intestinal projections lined with enterocytes.
similarities, astroviruses were originally thought to belong to either the families Picornaviridae or Caliciviridae. However, the lack of a helicase and use of a frameshifting event during replication (discussed below) distinguish astroviruses so completely that, in 1995, the International Committee on Taxonomy of Viruses (ICTV) classified astroviruses as a unique family, Astroviridae, which is now divided into two genera, Mamastrovirus and Avastrovirus. Since 2008, the number of animal hosts that have been shown to be infected with astroviruses has significantly increased comprising at least 30 mammalian and 14 new avian species increasing the complexity of classification. Originally, classification within each genus was based on the species of the host of origin. At present genera are divided into viral species or genotypes based on either the host range or genetic differences within the complete capsid sequence. On average the mean amino acid distance (p-dist) between both genera is 0.83 and within genera distances are 0.72 and 0.64 respectively. Currently, ICTV recognizes 19 species within the Mamastrovirus genus (MAstV) and 3 within the Avastrovirus (AAstV) genus but following the standardized criteria for classification, the two genera could be further divided into 33 and 11 genotype species respectively (Figure 2). This complexity is likely to increase in the future given the continual isolation of new

Figure 1  (a) Astroviruses have historically been identified by the five- to six-pointed star morphology visible by electron microscopy. Scale = 100 nm. (b) A reconstruction of the astrovirus virion, based on cytoelectron microscopy, along the twofold axis of symmetry. Reprinted from Matsui SM and Greenberg HB (1996) Astroviruses. In: Fields BN, Knipe DM, and Howley PM (eds.) Fields Virology, 3rd edn., pp. 875–893. Philadelphia: Lippencott-Raven.
astrovirus genotypes, identification of recombinant viruses, and due to the fact that it is common to find that a single host species may be susceptible to infection with divergent astrovirus lineages. There is also increasing evidence of cross-species transmission highlighting astroviruses zoonotic potential.

**Epidemiology**

**Humans**

Astroviruses have been detected throughout the world. While the exact incidences of infection vary from study to study, community-acquired astroviruses are found in 2–9% of children with infectious gastroenteritis. In some developing countries, infection rates as high as 61% have been observed. In many cases, astroviruses are one of the most commonly detected viral pathogen in young children after rotavirus and norovirus. Astrovirus infections are identified in up to 2% of asymptomatic
Astroviruses were the first pathogen detected in many flocks and were most commonly detected in birds less than 4 weeks of age. Astroviruses have been found in association with most animals examined, although the effect of infection varies with species (see below). While astroviruses were originally identified in humans, they have since been identified in both marine and land-based, companion and a variety of mammals including mink, bat, rabbits, mice, calves, sheep, piglets, dogs, red-tailed deer, kittens, and a variety of domestic and wild avian species. Like HAstVs, there appears to be large genetic diversity amongst the other MAstV including ovine, bovine, and bat astroviruses. The best epidemiologically characterized animal astroviruses are the turkey astroviruses. Surveillance of turkey flocks in the 1980s isolated astrovirus from 78% of diseased flocks, but only 29% of normal flocks. Astroviruses were the first pathogen detected in many flocks and were most commonly detected in birds less than 4 weeks of age. Similar to human infections, turkey astrovirus was frequently isolated with other pathogens, most commonly rotavirus-like viruses. The early age of infection and the prevalence of co-infections led one group to postulate that astrovirus infection may predispose birds to infection by other viruses.

The early age of infection and the prevalence of co-infections led one group to postulate that astrovirus infection may predispose individuals to infection by other viruses. Because astrovirus disease is generally mild in humans (see the section discussing pathogenesis), hospital cases may represent only a slight proportion of actual infections in the community. In support of this, serological studies have demonstrated that up to 90% of children have been exposed to at least one strain of astrovirus by age 9.

Viral infection occurs with equal frequency in boys and girls and predominantly in children under the age of 2. Infection is not restricted to young children, however, and has been noted in individuals of all ages, including immunocompetent adults and the elderly. Immunodeficient individuals, particularly those that are HIV-positive, appear to be at an increased risk of astrovirus infection.

Astrovirus infection occurs year-round, but with the highest frequency during the autumn and early winter months. In tropical climates, infection correlates with the rainy season. These seasonal correlations likely reflect the indoor confinement of the population as well as the increased stability of astroviruses in cold, damp conditions. Astrovirus outbreaks have also been associated with high-density environments, including childcare centers, primary and junior high schools, military recruiting centers, elderly care centers, and swimming pools. Astrovirus as a cause of hospital-acquired viral diarrhea in young children is second only to rotavirus and norovirus, occurring at rates of 4.5–6%, and, in some studies, surpasses rotavirus in rates of nosocomial infections.

Four phylogenetic clades of human astroviruses (HAstV) have been identified to date including the 8 serotypes of classic HAstV (HAstV 1-8 in MAstV 1) and the novel HAstV-MLB (MAstV 6), HAstV-VA2/4 and HMO-A (MAstV 8), and HAstV-VA1/3, HMO-B/C, and PS (MAstV 9). The classic HAstV 1–8 strains are widely recognized as a common cause of diarrhea in children, with all eight circulating globally to various levels. HAstV-1 is by far the most prevalent serotype, comprising 25–100% of astroviruses in a region, and the most prevalent reactivity of antibodies detected, although serological surveys of all serotypes have not been undertaken. HAstV-6, -7, and -8 are the least frequently detected, although three to four serotypes of HAstV are often detected in a region at any given time. The differing prevalence of serotypes could be a reflection of severity; perhaps HAstV-1 infection results in a higher frequency of hospital visits than other serotypes and is therefore overrepresented in hospital-based epidemiological studies. Alternatively, serotypes may be restricted by region. For example, one Mexican study identified HAstV-1 as the predominant serotype throughout the country, but HAstV-3 and -8 were prominent in select regions. Intriguingly, there appears to be a decrease in classic HAstV incidence in the last few decades. This could be due to differences in detection methodologies or it’s possible that this could be due to displacement of classic HAstV infections by those of the novel HAstV strains.

Infections with the non-classic HAstVs (HAstV-MLB and HAstV-VA/HMO), which are genetically more closely related to animal viruses, were first detected in the stool of children with gastroenteritis. However, a case control study on HAstV-MLB1 and classic HAstVs in India showed that while classic HAstVs were significantly associated with diarrhea, HAstV-MLB1 was not and MLB1 titers in stool did not differ between symptomatic and asymptomatic individuals. HAstV-MLB2 viruses have also been detected in the plasma of a child with upper respiratory infection suggesting that HAstV-MLB pathogenicity may affect extra-enteric tissues and tropism may not be restricted to the gastrointestinal tract. Similarly, the HAstV-VA/HMO viruses have been detected in pediatric gastroenteritis samples from several parts of the world and serological studies suggest that HAstV-VA is a highly prevalent human infectious agent. Recent studies reported that an HAstV-VA1-like strain was detected in a patient with new-onset celiac disease and associated with extra-intestinal dissemination (including neural tissue) in immunocompromised children. Together, these findings highlight the public health need to better understand the impact of different human astrovirus genotypes in human health requires. Importantly, new diagnostic tests are desperately needed that detect all of the HAstV genotypes. Our current assays are primarily limited to detecting the classical HAstV 1–8 strains.

Interestingly, astrovirus infection occurs quite frequently (up to 50%) as a co-infection with other enteric pathogens. The most frequent co-pathogens are noroviruses and rotaviruses, but infections with adenoviruses, parasites, and enteric bacteria are often detected as well. The importance of this in humans is not entirely clear. In a study specifically examining co-infections, astrovirus co-infection with rotavirus increased the duration of diarrhea and vomiting over either virus alone, although whether this difference was statistically significant is unknown. Further, no studies to date have correlated disease severity with the duration of viral load or specific human astrovirus genotype.

Animals

Most animals are not routinely screened for astrovirus infection, so our knowledge of the prevalence of infection is limited to surveillance studies. Through these studies we now know that there is remarkable genetic diversity amongst the MAstVs and AAstVs. Astroviruses have been found in association with most animals examined, although the effect of infection varies with species (see below). While astroviruses were originally identified in humans, they have since been identified in both marine and land-based, companion and a variety of mammals including mink, bat, rabbits, mice, calves, sheep, piglets, dogs, red-tailed deer, kittens, and a variety of domestic and wild avian species. Like HAstVs, there appears to be large genetic diversity amongst the other MAstV including ovine, bovine, and bat astroviruses. The best epidemiologically characterized animal astroviruses are the turkey astroviruses.
Virus Propagation

Attempts at in vitro propagation of astroviruses have been met with varying degrees of success. The most successful techniques utilize cultured cells from the host species and provide exogenous trypsin in the culture. Successful propagation of the classical HAstV 1–8 strains was originally achieved by repeated passage through primary human embryonic kidney cells; it was later discovered that direct passage through the human intestinal cell line Caco-2 would also yield infectious virus. Propagation of porcine, bovine, and chicken astroviruses has been successful in their respective host cells in vitro. However, many astroviruses still have not been adapted to propagation in vitro for unknown reasons, while others lose infectivity with subsequent passages and therefore cannot be maintained continuously. This problem has been circumvented in some systems by passing the virus through an animal system, as is the case for the turkey astrovirus, in which highly concentrated virus can be obtained from infected turkey embryos in ovo.

Molecular Virology and Protein Expression

Astroviruses contain one copy of positive-sense, single-stranded RNA. The genome is approximately 6.8 (6.2–7.8) kb excluding the 3' polyadenylated tail and contains three open reading frames (ORFs), ORF1a, -1b, and -2, as well as 5' and 3' untranslated regions (UTRs) (Figure 3). A VPg protein is covalently linked to the 5' end of the genome and the 5' and 3' UTRs are highly conserved and are believed to contain signals important for genome replication. The classic HAstVs, HAstV-VA/HMO, cat, ovine, porcine and avian AstVs also contain a stem-loop II secondary structure motif at the 3' end of their genomes of unknown function that is also found at the 3' end of the genomes of some members of coronaviruses, noroviruses, and rhinoviruses. The length of the ORFs varies amongst the astrovirus strain with variations due largely to insertions and deletions present at the 3' end of the ORF1a. A new ORF, termed ORFX, overlapping the 5' end of ORF2 in the +1 reading frame has been described in the classic HAstVs and some mammalian astroviruses.

Astroviruses initiate infection by binding to an unknown receptor. Given the susceptibility of different cell lines for HAstV infection (depending on the serotype and genotype) it is possible that astroviruses use a variety of attachment proteins or receptors including carbohydrate moieties. Studies of HAstV infection in the highly permissive Caco-2 cells suggest that HAstV enters the cell via clathrin-mediated endocytosis. RNA uncoating likely occurs upon acidification and maturation of the endosome leading to release of the viral RNA into the cytoplasm where ORF1a and -1b are immediately translated by the host machinery. It has been estimated that the initial binding and virus uncoating steps takes ~130 min. ORF1a is 2.8 kb and encodes a polypeptide of approximately 110 kDa. This polypeptide contains a variety of conserved motifs, including several putative transmembrane domains, a bipartite nuclear localization sequence (NLS), and a serine protease motif. The translated polypeptide is cleaved by both cellular protease(s) and the viral protease into at least five peptides. The actual function of each protein remains largely unknown. The transmembrane domains may localize to the endoplasmic reticulum (ER) membrane to facilitate replication, as all plus-strand RNA viruses have been shown to replicate in association with a membrane. One peptide, NSP1a/4, colocalizes with the viral RNA at the ER membrane; mutations in NSP1a correlate with increased viral titers in vitro and in vivo, suggesting a role for this protein in viral replication. The role for the NLS remains unclear; some reports suggest viral antigen is observed in the nucleus, while others find that it is excluded. Recent studies demonstrated that some of the non-structural proteins may undergo posttranslational modifications including phosphorylation that could modulate viral protein-protein interactions.

The second reading frame, ORF1b, overlaps ORF1a by 70 nucleotides and has no detectable start codon. Intensive research has determined that ORF1b is translated by a frameshift into the −1 frame. This frameshifting event is unique among plus-strand animal RNA viruses and requires a highly conserved shifty heptameric sequence (A3C) as well as a downstream hairpin structure.

Figure 3  The genomic organization of astroviruses (based on HAstV-1), including open reading frames and encoded protein features, is shown. Reprinted from Virus Taxonomy: Sixth Report of the International Committee on Taxonomy of Viruses, 1995, p. 365, Astroviridae, Murphy FA, Fauquet CM, Bishop DHL, et al. (eds.), copyright 1995, with kind permission of Springer Science and Business Media.
This event, which occurs with frequencies up to 25% in cells, results in an ORF1a/1b fusion peptide. Cleavage near the 1a/1b border releases the ORF1b gene product: the viral RNA-dependent RNA polymerase (RdRp). Astrovirus polymerase is a super group 1 RdRp, a group which generally utilizes a VPg to initiate transcription.

Expression of the RdRp results in production of a minus-strand viral template. This generates multiple copies of the plus-strand genome as well as a polyadenylated subgenomic RNA (sgRNA) containing short 5′ and 3′ UTRs and ORF2. ORF2 is in the 0 frame and overlaps ORF1b slightly (four nucleotides) in human astroviruses. Production of the capsid protein from a sgRNA not only temporally restricts capsid production to later in the viral replication cycle, but also allows for massive capsid protein expression; it is estimated that sgRNA is produced in tenfold excess of the viral genome by 12 h post infection (hpi). The sgRNA is about 2.4 kb and encodes the single structural protein of approximately 87 kDa. This peptide is cleaved by an intracellular protease to approximately 79 kDa; mutational analyses suggest that this 8 kDa stretch is required for efficient expression of the capsid protein. Individual capsid proteins multimerize spontaneously to form icosahedral structures of about 32 nm (Figure 1(b)). Positive-sense genomes are packaged into these viral-like particles (VLPs), possibly through interactions with the first 70 amino acids of the capsid protein. The virions are released by an unknown mechanism, which may involve cellular caspases, after which the capsid undergoes an extracellular trypsin-mediated maturational cleavage. This increases infectivity up to 10^3 fold, condenses the virion to approximately 28 nm, and transforms the 79 kDa capsid protein into at least three smaller peptides of approximately 34, 29, and 26 kDa. Computational predictions suggest that VP34 may comprise the core of the virion while VP29 and VP26 form spike-like projections that may be important for viral tropism and receptor binding. This is corroborated by studies suggesting that VP26 is only loosely associated with the virion. These spikes are also thought to be responsible for the star morphology visible by EM (Figure 1(a)). Finally, a subset of mammalian astroviruses contain an additional ORF, ORFX, that overlaps the 5′ end of the ORF2 in the +1 reading frame that could be translated through leaky scanning. The translation product of ORFX has not been confirmed.

Little is known about the role of host cell signaling pathways in astrovirus replication. Interaction of Caco-2 cells with HAstV results in the activation of the extracellular signal-regulated kinase (ERK1/2) and the phosphoinositide-3 kinase (PI3K) pathways both of which are required for effective entry and productive replication. Activation of ERK1/2 is independent of productive viral replication; binding alone is sufficient. Although clearly required for productive replication, the exact mechanism(s) by which these host kinases regulate the astrovirus life cycle remains unknown.

**Evolution**

Examination of nucleotide changes and nonsynonymous amino acid changes from the whole genome and across species suggests that an ancient divergence between avian and mammalian astroviruses occurred approximately 310 million years ago and was followed by divergence within the mammalian viruses due to several cross-species transmissions. The phylogenetic tree topology suggests that inter-species transmissions may occur and that astroviruses have zoonotic potential. Several of the mammalian and avian astrovirus species may infect more than one host indicating that cross-species transmission is a frequent event, especially in birds. Further, it is clear that a variety of HAstV genotypes can infect humans.

As RNA viruses, nucleotide mutations and recombination events are important in evolution. HAstV genome mutation rates are similar to other rapidly evolving ss(+)-RNA viruses for example picornaviruses and estimated to undergo approximately 3.7 x 10^{-3} nucleotide substitutions per site per year; although higher genetic variability occurs in the ORF2 as compared to ORFs 1a and 1b due to selective pressures or distinct evolutionary constraints. Amongst the host species, enhanced accumulation of synonymous substitutions, particularly in ORF2, have been observed in porcine, ovine, mink, and turkey astroviruses compared to those infecting humans.

Finally, recombination events are being increasingly identified amongst the astroviruses. Natural recombinants have been identified between strains belonging to the same genotype or different serotypes of classic HAstV. Whether recombination occurs amongst strains belonging to distinct viral genotypes is unknown although there have been suggested events between classic HAstV and California sea lion astrovirus and classic HAstV and porcine astrovirus. Most recombination points have been described as upstream of the ORF1b/ORF2 junction region, but can be found at areas within each of the ORFs. Further work is needed to understand the impact of recombination on astrovirus evolution.

**Clinical Features, Pathology, and Pathogenesis**

**Mammalian Astroviruses**

Astrovirus infection in mammals presents clinically as gastroenteritis. Disease has been most closely studied in humans and, in volunteer studies, astrovirus-infected individuals develop diarrhea, the most prominent symptom, as well as vomiting, nausea, anxiety, headache, malaise, abdominal discomfort, and fever. HAstV diarrhea is typically milder than those caused by rotaviruses or noroviruses. Onset of symptoms at 2–3 days post infection (dpi) correlates with shedding of the virus in feces, although shedding can continue after resolution of other symptoms. Astrovirus infection has also been associated with intussusception, although a causative role has not been established, and with necrotizing enterocolitis in premature infants. More recently, classic HAstV infection were shown to spread systemically and cause severe disseminated lethal infections in highly immunocompromised children. As described above, the novel HAstV-MLB and HAstV-VA/HMO have also been identified in extra-enteric tissues including...
the upper respiratory tract and brain as well as sera. Further studies are needed to understand the tropism of astroviruses and the role of these viruses in pediatric infections. Additionally, mink and bovine astrovirus infections have been associated with neurological complications including encephalitis.

The earliest studies of astrovirus pathogenesis utilized gnotobiotic sheep and calves as models. In calves, astrovirus infection was localized to the dome epithelial cells overlying Peyer’s patches. These cells appeared flat or rounded and released cells were identified in the intestinal lumen. Astrovirus infection in calves was shown to be specifically targeted to M cells and led to the sloughing of necrotic M cells into the intestinal lumen. Enterocytes were never observed to be infected. Specific tropism of the virus for immune cells suggests that astrovirus may have an immunomodulatory role in calves. While the virus replicated in these animals and could be detected in their feces, the calves displayed no clinical signs. In most bovine studies, viral infection is asymptomatic, although changes in the feces from solid and brown to soft and yellow were noted in one study. Mild villus atrophy and slight changes in villus-to-crypt ratios have been noted but no changes in xylose absorption were observed. Despite the lack of symptoms, viral shedding continued until the termination of the experiment.

Studies in sheep have shed more light on histological changes associated with infection. Astrovirus-infected sheep developed a transient diarrhea as early as 2 dpi, but virus was detected at early as 14 hpi and initially confined to the luminal tip of the intestinal villi. By 23 hpi, virus was observed coating the microvilli and infection had spread to the apical two-thirds of the villi. This correlated with sloughing of degenerate cells from the apical portion of the villi, which continued through 38 hpi. At this time, villus blunting was apparent in the ileum and midgut. Furthermore, normal epithelial cells lining the villi were replaced with immature, cuboidal cells reminiscent of crypt cells. Neither these immature cells nor crypt cells were ever observed to be infected, suggesting that only mature enterocytes are susceptible to infection. By 5 dpi, viral infection had cleared and intestinal histology had returned to normal.

Volunteer studies in humans have not explored the underlying causes of astrovirus pathogenesis; our knowledge is therefore limited to intestinal biopsies taken for other reasons, but generally support the observations described above. In a biopsy from a child shedding large quantities of astrovirus, slight histological changes, including mild villous blunting and irregular epithelial cells, were observed. Infection increased distally through the small intestine. Similarly to animal models, astrovirus infection was restricted to the apical two-thirds of intestinal villi and could be identified in infected cells.

The recent characterization of murine astroviruses may afford a mouse model to study many aspects of astrovirus replication, tropism and immune response, although they did not suffer diarrhea and may not be an ideal model to understand disease pathogenesis.

In vitro studies using differentiated Caco-2 cells demonstrated that HAstV infection leads to reorganization of the cytoskeleton and disruption of the epithelial tight junctions resulting in increased epithelial barrier permeability. Notably, the increased barrier permeability was independent of viral replication; purified recombinant capsid protein alone was sufficient. Similar results were obtained in turkey pouls administered purified recombinant turkey astrovirus type-2 capsid protein. Inoculated pouls exhibited disruption of cellular tight junctions and epithelial barrier permeability and acute diarrhea suggesting that the astrovirus capsid protein may act as a novel viral enterotoxin. Although this appears to be independent of increased cell death, particular strains of HAstV have been shown to induce apoptosis late during the course of infection. Further studies are required to understand the mechanism(s) for the increased barrier permeability and the role in disease pathogenesis.

Avian Astroviruses

In avian species, astrovirus infection has a much broader range of disease than in mammals. While astrovirus does cause gastroenteritis in turkeys and chickens, it can also cause nephritis in chickens and a severe, often fatal, hepatitis in young ducklings.

Turkey astrovirus was the first discovered avian astrovirus and remains the best characterized in terms of pathogenesis, due in part to the development of the turkey as a small animal model. In these animals, virus could be detected from 1 to 12 dpi in the intestines. Viral replication was limited to the enterocytes on the apical portion of the villi, but the virus could be detected throughout the body, including the blood. The development of viremia is rare among enteric viruses and its function remains unclear. Infected turkeys developed a yellow, frothy, gas-filled diarrhea from 1 to 12 dpi. Diarrhea occasionally contained undigested food, but never blood. The intestines of infected birds became thin walled, flaccid, and distended. Despite these changes, histological examination suggested that only mild changes occur during infection. A mild crypt hyperplasia and shortening of the villi were noted from 4 or 5 to 9 dpi, and single degrading enterocytes could be identified. However, TUNEL staining suggested that the amount of cell death in infected intestines is similar to control birds. D-xylose absorption, a measure of intestinal absorption, was significantly decreased from 2 to 5 dpi in one study and up to 13 dpi in another. This effect was exacerbated in the presence of another enteric pathogen, turkey coronavirus. Astrovirus infection also caused a significant growth depression in turkey pouls by 5 dpi; infected birds never recovered from this, leading to flock unevenness. Infected birds also demonstrated a transient (3–9 dpi) reduction of the thymus, which returned to normal by 12 dpi.

Avian infection by astroviruses can present with nonenteric symptoms as well. Infection of ducklings with duck astrovirus causes a severe hepatitis. Infected birds develop liver hemorrhage, swollen kidneys, and hepatocyte necrosis. On farms, infection leads to mortality rates of 10–25% in adult (4–6-week-old) ducks, but can reach 50% in ducklings under 14 days of age. In chickens, infection with the astrovirus avian nephritis virus (ANV) results in discoloration of the kidney, development of renal lesions, and interstitial nephritis by 3 dpi. Pathogenesis is age dependent, with 1-day-old chicks the most susceptible and adult birds the least. ANV infection can result in mortality rates of up to 33%, although rates appear to be strain specific.
Immune Response

The immunological response to astrovirus infection is poorly defined; however, observations in humans and animal models suggest that both the adaptive and innate responses play important roles in controlling and eliminating the virus.

The humoral immune response likely plays a major role in astrovirus immunity. The biphasic infection pattern of young children and the elderly suggests that antibodies are protective during the middle of life. Indeed, serological studies have indicated that approximately 50% of neonates have maternally acquired antibody to HAstV, which wane by 4–6 months of age. Children then acquire anti-HAstV antibodies rapidly due to astrovirus exposure. By the age of 9, up to 90% of the population has been exposed to HAstV-1. Furthermore, volunteer experiments demonstrate that astrovirus exposure generally leads to an increase in anti-astrovirus antibody titer. While astrovirus antibodies protected individuals from symptoms associated with infection, virus was identified in the feces, suggesting that such antibodies do not necessarily prevent viral replication. Additionally, immunoglobulin treatment has been attempted as a treatment for severe or chronic astrovirus infection. The results have been mixed and difficult to interpret, as the presence of astrovirus-specific antibodies in the immunoglobulin treatment was not always confirmed. Seroprevalence studies also showed that the vast majority of healthy young adults have antibodies against the novel HAstVs.

Cellular immunity may also play a role in controlling and/or preventing astrovirus infection. Studies have demonstrated that most individuals possess HLA-restricted, astrovirus-specific T cells. When stimulated with astrovirus in vitro, these cells produce tumor necrosis factor, interferon gamma, and occasionally interleukin (IL)-5 but not IL-2 or IL-4. These cytokines are typical of the T-helper-type response thought to be important in controlling viral infections. Individuals deficient in T and B-cell functions are unable to control infection, shedding virus to very high titers (≥ 10^14 particles ml^-1) and for extended periods of time (up to 18 months) further supporting the importance of cellular immunity.

Finally, adaptive immunity has been shown to restrict astroviral replication during primary infections in the murine model. Rag1^-/- mice, which are deficient in B and T cells, show significantly higher levels of viral shedding in the feces and higher genomic copies in intestinal and extra-intestinal tissue as compared to wild-type mice suggesting that viral dissemination is restricted by the murine adaptive immune response. Viral replication is also higher in Stat1^-/- highlighting a role for interferon in limiting astrovirus replication.

Experiments in a turkey model demonstrate that the adaptive response is not the only important immunological response. In this model, no increase in T cells (CD4^+ or CD8^+) could be demonstrated after TAstV-2 infection. Moreover, while infected turkeys produced a slight increase in antibody production, these antibodies were not neutralizing and did not prevent against future infection. However, it was noted that macrophages from TAstV-2 infected turkeys produced significantly higher levels of nitric oxide (NO) both in vivo and upon stimulation ex vivo. Inhibition of NO in vivo led to a significant increase in viral production, while addition of exogenous NO decreased viral production to below the detection limit, suggesting that NO is an important factor in controlling astrovirus infection. The importance of macrophages and their role in astrovirus infection has been corroborated by observations in astrovirus-infected lambs, where EM showed virosomes within macrophages. Furthermore, it is possible that astroviruses have a mechanism to combat this response, as macrophages in astrovirus-infected turkeys demonstrate a reduced ability to phagocytose.

Finally, the human astrovirus capsid protein inhibits the complement system, which is an important innate immune response against infection. Purified recombinant capsid protein directly interacts with and inhibits the complement regulatory proteins C1 and MBL suggesting an ability to block both the classical and lectin complement pathways. Intriguingly, a 15-amino acid sequence (residues 79–108) at the conserved N-terminal end of the capsid, which is highly conserved amongst a variety of mammalian astroviruses, is the minimal region required to inhibit complement. More work is needed to understand the role of complement inhibition in astrovirus pathogenesis.

Treatment, Prevention, and Control

Because astrovirus infection is generally mild and self-limiting in humans, treatment is generally restricted to fluid rehydration therapy. This can often be accomplished at home; thus, hospital admittance is rare. No vaccine is yet available for humans, and as noted above, immunoglobulin treatment for immunocompromised individuals has been met with varying degrees of success. More recently, authors have speculated that the use of probiotics may interfere with the biological cycle of enteric viruses and may be useful in treatment; however, more studies are needed to confirm these findings. Additionally, no treatment for astrovirus-infected animals exists. The best solution, therefore, is prevention of transmission, which is best done in humans by conscientious hand and food washing. The stability of astroviruses and their resistance to inactivation make them difficult to eliminate after introduction. This is a significant problem in hospitals, where individuals are generally immunocompromised and therefore more susceptible to infection. One outbreak in a bone marrow transplant ward prompted the hospital to scrub the entire ward with warm, soapy water. However, surveillance of the subsequent inhabitants demonstrated fecal shedding of astroviruses, underscoring the difficulty in removing the virus. This is also a significant problem in commercial farming, where astrovirus infection of animals significantly decreases productivity. Its introduction and maintenance in this environment can mean drastic financial losses. In each of these environments, early detection and thorough disinfection are keys to limiting transmission and controlling infection.
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