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Astroviruses; Pathogenesis and Diagnosis: A Review

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A B S T R A C T

Astroviruses are non-enveloped, single-stranded, positive-sense RNA viruses that can infect a wide range of hosts and are often linked to gastrointestinal sickness. Disease might range from asymptomatic to encephalitis depending on the host and virus genotype. Astroviruses have a significant genetic diversity due to a faulty polymerase and frequent recombination events across strains.

Using diverse approaches and animal models to explore virus-host interactions, researchers have uncovered several notable aspects of astroviruses, including the ability of the astrovirus capsid to behave as an enterotoxin, disrupting the gut epithelial barrier.

Outside of the gastrointestinal tract, astroviruses have been found to cause sickness. More animal models should be developed to study this phenomenon as well as astrovirus pathogenesis, particularly in strains that might cause extraintestinal infection. The current dogma, as with other viral infections of the gut, argues that astroviruses infect in a species-specific manner; however, mounting evidence of cross-species infection of these viruses has called this notion into question.

There are some methods of determining if you have an astrovirus infection. Electron microscopy, cell culture, immunoassays, polymerase chain reaction, and a variety of other molecular methodologies are currently being used in diagnostic and surveillance research. This review will discuss the last updates on pathogenesis and diagnosis of Astroviruses.

Keywords: *Astroviruses, Cell Culture, Electron Microscopy, Gastrointestinal Infection, , Immunoassays, Polymerase Chain Reaction (PCR)*

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1 Introduction

Astroviruses (AstVs) are a type of virus detected in 1975 using electron microscopes in the aftermath of a human diarrhea outbreak (1). Human Astrovirus (HAstV) has now been identified as the third most common cause of viral acute gastroenteritis in children, infecting primarily newborns, young children, immunocompromised hosts, and the elderly. Astroviruses have now been isolated from a variety of mammalian animal species (and are classed as the genus Mammastrovirus) as well as avian species such as chickens, ducks, and turkey poults, in addition to humans (classified as genus Avastrovirus).

Astroviruses are icosahedral viruses having a diameter of 28–35 nm with a five- or six-pointed star-like surface shape as seen under electron microscopy (2). Astrovirus has a non-enveloped icosahedral capsid with a non-segmented, single-stranded, positive sense RNA genome (3). Human astroviruses have been demonstrated in numerous studies to be a major cause of gastroenteritis in young children all over the world (2). In addition, data suggests that Astroviruses in animals induce gastrointestinal infection, as well as encephalitis (in humans and cattle), hepatitis, and nephritis (in birds) (4).

2 Morphology and Classification

Astrovirus was initially detected in the stool of children with gastroenteritis in 1975. It is a non-enveloped positive-sense single-stranded RNA virus. Madeley and Cosgrove named the virus after observing a star-like appearance in a part of the virions under electron microscopy (1)(Figure 1).

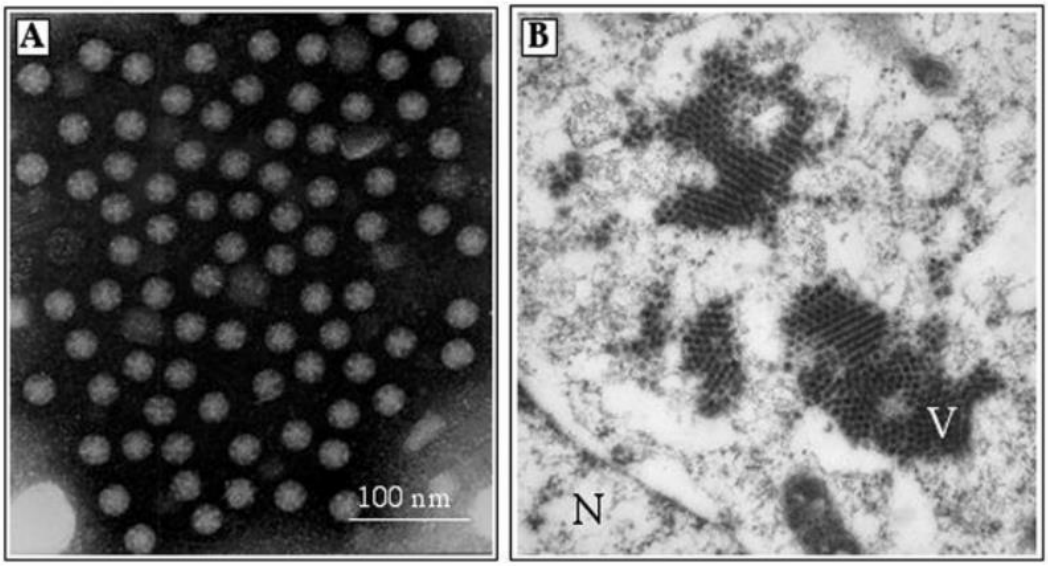

Figure 1. A) Astrovirus has a six- and five-point star-like shape, which may be seen in feces using negative staining and an electron microscope. (B) Infected Caco-2 cells with paracrystalline arrays of human astrovirus particles detected using a transmission electron microscope; virus clusters (V) are generally found at the nuclei's periphery (N). (5)

In 1993, the entire genome sequencing of a virus resulted in the formation of a new family, Astroviridae (6). The Astroviridae family has since been divided into two genera: Avastrovirus and Mamastrovirus, which infect avian and mammalian species, respectively. Based on their genetic relatedness within the hypervariable capsid protein, they were also classified into two genogroups (Figure 2) (7).

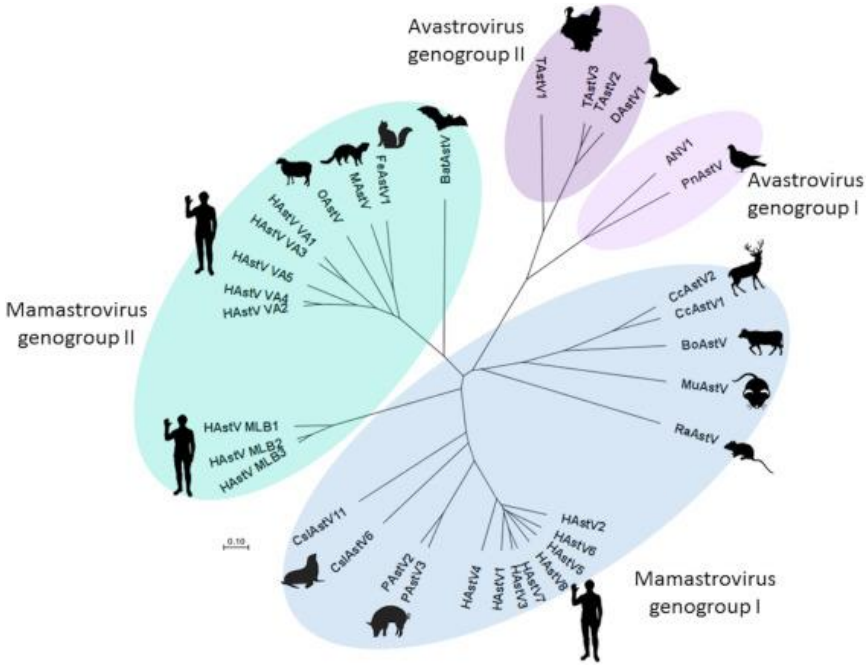

Figure 2. Astroviridae phylogenetic classification. Capsid protein amino acid sequences were used to create the tree. Based on the International Committee on Virus Taxonomy's Ninth Report, published in 2012.

3 Chemical and Physical Agents Susceptibility

Phenolics, acidic pH, chloroform, a variety of detergents, heat, ambient temperatures, quarternary ammonia, most alcohols, and lipid solvents inactivation have almost no effect on AstVs. (8).

AstV has been demonstrated to be destroyed by formaldehyde, -propiolactone, 90 percent methanol, and a disinfectant containing potassium peroxymonosulfate. AstVs isolated from embryonic intestines are exceptionally stable at 4°C, with infectivity lasting several weeks. Virus can be stored at –20°C or –70°C for long-term infectivity. In the laboratory, AstVs can be inactivated using 90 percent methanol or a disinfectant comprising potassium peroxymonosulfate (8, 9).

4 Life Cycle

The initial stage in the virus reproduction cycle is the attachment of the virus capsid to a receptor on the cell surface (Figure 3). The receptor's identity, as well as whether different HAstVs share a similar receptor or whether different serotypes have several receptors, are unknown. Furthermore, unlike many non-enveloped viruses, no post-attachment interactions between the virion and cellular co-receptors have been identified, which are required for virus internalization (10, 11).

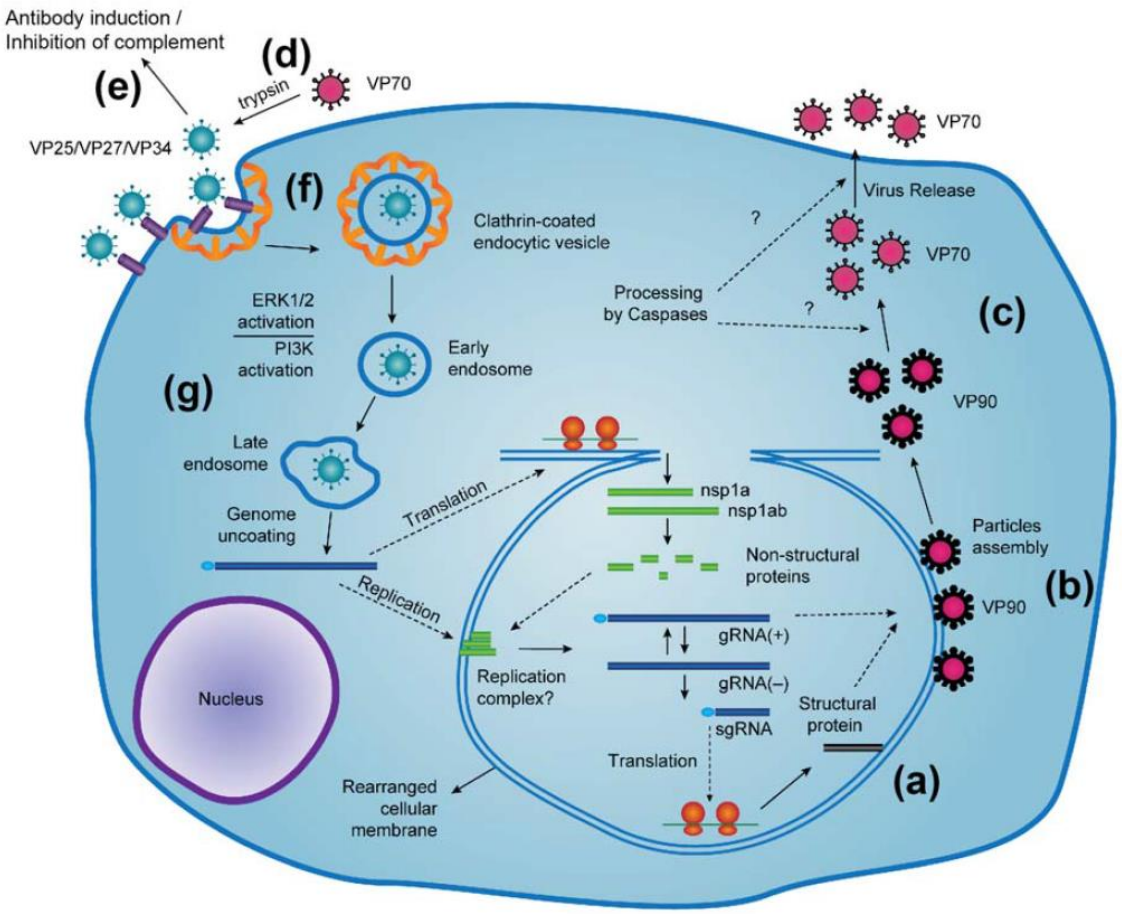

Figure 3. Replication cycle of human astroviruses (adapted from (12)) (a) The 90 kDa human astroviruses (HAstV) capsid protein (VP90) is synthesized from viral subgenomic RNA (sgRNA); (b) VP90 proteins (180 copies) are assembled with viral genomic RNA to form HAstV particles; (c) Caspase-mediated cleavage of VP90 Ctermini to form VP70 and release of immature HAstV particles; (d) Cleavage of immature HAstV particles by an extracellular protease to produce mature, infectious HAstV particles. (e) Extracellular HAstV particles increase host antibody formation and prevent host complement activation; (f) HAstV particle attachment and clathrin-dependent endocytosis; (g) Uncoating of the virus genome in the late endosome During HAstV binding or entrance into the cell, the extracellular signalregulated kinase (ERK1/2) and phosphoinositide 3kinase (PI3K) are activated.

HAstVs show varied replication efficiencies in different cell lines depending on their serotype (13), hinting that they may use different receptors or co-receptors on the cell membrane during virus entry. The existence of post-entry restriction cellular components that limit viral replication in a serotype-specific manner, on the other hand, cannot be overlooked. At the entrance level, cell tropism restriction for HAstV-1 was proven in BHK-21 cells (baby hamster kidney cells), which are resistant to virus infection but become permissive when virus replication is induced in the cells by transfection of in vitro-transcribed viral RNA (14).

HAstV-8 attaches to the surface of MA104 cells (African green monkey kidney cells) just 2 to 3 times as efficiently as it binds to the surface of Caco-2 cells (human colon cancer cells), despite Caco-2 cells being 1000-fold more receptive to infection than MA104 cells. These findings suggest that the virus must first adhere to the cell surface before interacting with post-attachment cell surface components in order to enter the cell (co-receptors) (15).

5 Uncoating and Replication

While the specific pathophysiology for viral uncoating remains unclear, investigations have shown that it is mediated by an endoplasmic reticulum signal motif in the C-terminus of VP90 that is also membrane associated. The durability of recombinant viral-like particles (VLPs) is attributed to divalent cations in the capsid protein, according to research. As a result, both the low cation content in the cytoplasm and the acidic environment of late endosomes should encourage virus uncoating (16).

The first proteins translated once the viral genome is released are nonstructural proteins, which are encoded by ORF1a and ORF1b. The nonstructural polyprotein nsp1a is cleaved into smaller proteins by the viral serine protease and unidentified cellular proteases, some of whose function is unknown. nsp1a/4, a VPg that appears to bind with viral RNA and is expected to facilitate viral replication and maybe transcription from the negative-sense RNA template, is one of the nsp1a proteins. Despite the lack of a direct link between the VPg and the viral genome, pretreatment of viral RNA with proteinase K before transfection into susceptible cells significantly reduced the infectious virus produced, suggesting that a protein moiety is required for successful replication. Capsid protein is synthesized by both genomic and sgRNA, with an excess of sgRNA and enhanced capsid protein production after 12 hours post-infection. This characteristic is present in many positive-sense single-stranded RNA viruses as a mechanism of bypassing host-mediated translation inhibition. While caliciviruses contain a VPg on their sgRNA segments to initiate translation, it is unknown if astroviruses have a similar mechanism (16-18).

Although one study indicated that host proteins are involved in fatty acid and cholesterol synthesis, phosphatidylinositol and inositol metabolism, and RNA helicase activity, all of which are essential for viral replication (18). the actual role of host proteins in astrovirus replication is unknown. Hargest et al., 2017 demonstrated that successful viral replication necessitates a 15-minute increase in phosphorylated ERK1/2 protein following viral binding (7). Others recently asserted that the ubiquitin proteasome system is essential for viral genome replication and sgRNA replication as well (19, 20).

6 Assembly and Release

HAstV4 and HAstV8 appear to assemble in vacuoles near the nucleus, while ovine astrovirus assembles in lysosomes and autophagic vesicles, respectively. In these limited membrane regions, viral capsids can self-assemble into complete virions or VPLs, and viral release can occur via an unknown, nonlytic method without significant cell death. Astroviruses may release themselves using nonlytic ways similar to those used by rotaviruses and polioviruses, or through a type of cell membrane instability. Many questions concerning the astrovirus replication cycle remain unsolved, and further research is needed to understand critical components of each step, such as which receptors and binding proteins are involved, and how viral release happens without causing cell death (7, 19, 20).

7 Cross-Species Transmission

According to an increasing corpus of studies employing both standard sequencing and NGS techniques, cross-species transmission of astroviruses is possible. A recent astrovirus outbreak in mink, for example, revealed a novel clade that is closely linked to astroviruses found in chicken and turkey (21). According to our findings (22) nonhuman primates can carry mammalian and avian astrovirus genotypes, including those connected to human infections. This was the first time HAstV strains had been discovered in non-human animals, and the discovery of a HAstV-MLB2-like virus in a diarrheal sample from a monkey in a Chinese zoo (23) backed up the findings.

The concept that astroviruses are species-specific is challenged by these findings. Serologic evidence of avian astrovirus infections in humans adds to their general character (24); nonetheless, more study is needed to verify the ability of astrovirus transmission across species, with a special focus on whether such infections might cause sickness (18).

8 Diseases in Humans

Humans were thought to be infected by only eight closely related astrovirus genotypes designated as human astrovirus type 1–8 until 2008. (HAstV1-8). The classic HAstV genotypes are made up of these eight genotypes. Eight novel HAstV genotypes were identified with the advent of unbiased full-genome sequencing and new pathogen identification techniques, each given a name based on the city where it was discovered: Melbourne (MLB1–3) and Virginia (VA) (VA1-5). In the same year when the VA genotypes were discovered, the same viruses were discovered in samples from Nigeria, Pakistan, and Nepal. These viruses are commonly referred to as HAstV-HMO due to their similarities to human, mink, and ovine astroviruses; however, for the sake of this study, they will be referred to as HAstV-VA. The new MLB and VA genotypes are more closely related to other mammalian astroviruses than the old genotypes, resulting in three genetically distinct Mammastrovirus clades (7).

Classic HAstV is the second to third most prevalent cause of viral gastroenteritis in young children, accounting for 2 to 9% of non-bacterial infections. Mild diarrhea, which usually disappears in 2-3 days after a 4.5-day incubation period, is

accompanied by vomiting, significant stomach pain, anorexia, and fever (25). It's assumed that antibodies' high protection throughout maturity leads to pauci-/asymptomatic infections at this time, based on the biphasic age distribution of vulnerable individuals. Children have been found to have asymptomatic HAstV infection (26, 27), and while some large case-control studies demonstrate a relationship between HAstV infection and diarrhea (28-32), others do not (33, 34).

Infection can be chronic (35) and/or spread in immunocompromised patients (36). HAstV-1 is the most common serotype, with a seroprevalence of up to 94 percent in children aged 6–9 years old (37), while HAstV-6 and HAstV-7 are rarely seen (seroprevalence of 16 percent and 10 percent, respectively (38)). HAstV-3 appears to be more frequently linked to infections that are chronic and severe (39). Classic HAstV has been found in respiratory samples or associated with respiratory symptoms on rare occasions (40-44), but a definitive link between HAstV and respiratory disease has yet to be established.

9 Diseases in other Mammalian Species

Astrovirus can infect cattle, sheep, pigs, bats, minks, dogs, and cats, as well as marine mammals such as California sea lions, bottlenose dolphins, and Steller sea lions.

Infections with MamAstV in animals aren't necessarily associated with gastrointestinal issues, and symptoms can range from asymptomatic illness to severe disease. With seven genotype species, porcine astrovirus (PoAstV) is one of the most diversified MamAstv viruses (45, 46). PoAstV infections are typically asymptomatic, signaling a potentially lasting infection and making pigs a favorable reservoir for astrovirus transmission, despite a prevalence of up to 80-90 percent. Despite this, a neuroinvasive PoAstV strain (Ni PoAstV-3) has recently been identified as the cause of neurological disease and mortality in newly weaned paraplegic piglets; oddly, the genome of Ni PoAstV-3 was not found in the feces of affected pigs (47). The role of PoAstV in congenital tremor (48) and poliomyelitis in piglets has also been speculated (49).

PoAstV was found in a significant proportion of asymptomatic and symptomatic pigs' respiratory samples (47-50), and also in many other organs outside of the gastro-intestinal tracts; this shows a lack of a clear relationship with clinical disease and a wide tissue tropism (51, 52).

Previously, bovine astroviruses (BoAstV) were separated into four genotype species, none of which were linked to diarrheal disease (53). BoAstV-CH13/Neuro S1 and BoAstV-CH15/-BH89/14 viruses have recently been found in non-suppurative encephalitis cow brain samples (54-57). These strains are distinct from other BoAstV strains and belong to the human-mink-ovine (HMO) clade, which includes human, mink, and ovine strains. BoAstV-CH13/Neuro S1 was found in up to one-third of brain biopsies from cattle with undiagnosed encephalitis disease, including some cases dating back to 1950 (58). Despite a paucity of evidence tying the virus to the disease, astrovirus was discovered in cattle with bovine respiratory disease (59).

The first animal astrovirus, ovine astrovirus (OvAstV), was described in 1977 (60) and a second genotype was recovered from a healthy sheep in 2009 (61). While there is no evidence that OvAstV causes diarrhea, incidences of non-suppurative meningoencephalitis in sheep have been linked to a specific neurotropic OvAstV CH16 genotype species (62-64).

The pre-weaning diarrhea sickness and the so-called shaking mink syndrome (65) are both linked to the mink astrovirus (66).

Bats, like other viruses, serve as a large reservoir for astrovirus diversity, and their asymptomatic infection with a variety of astrovirus variants puts them at risk for recombination events and emerging strains with potential cross-species transmission (67).

Dromedaries (68), foxes (69), marmots (70), rats (71), mice (72-74), rabbits (75, 76), wild boar (77), roe deer (78), and yaks (79) are newest hosts for the new variants discovered recently.

10 Diseases in Avian Species

The astroviruses from the genus Avastrovirus are isolated from birds including chickens, turkeys, ducks, geese, guinea fowl and wild birds (80-82). The detection of AstVs in poultry has grown dramatically in recent years, owing to improved surveillance and diagnostic assays. Two chicken-origin AstVs (avian nephritis virus [ANV] and chicken astrovirus [CAstV]), two turkey-origin AstVs (TAstV1 and TAstV2), and two duck-origin AstVs (DAstV1 and DAstV2) have been identified in poultry to date based on species of origin and viral genome characteristics (Figure 4). When identifying newly reported AstVs from poultry by species, be cautious because ANV has been found in a variety of species, including turkeys (83), ducks, and geese

(84, 85). Ducks and guinea fowl were also found to carry the TAstV1 and TAstV2 viruses, respectively (85-87). According to these findings, AstVs may be able to transcend species barriers. Moreover, the prevalence of TAstV2 antibodies in persons exposed to turkey has recently been revealed in the United States, despite the fact that no evidence of avian AstV replication or related clinical disease was already provided in humans to date (24). As a result, the significance of this finding in terms of public health warrants additional investigation (8).

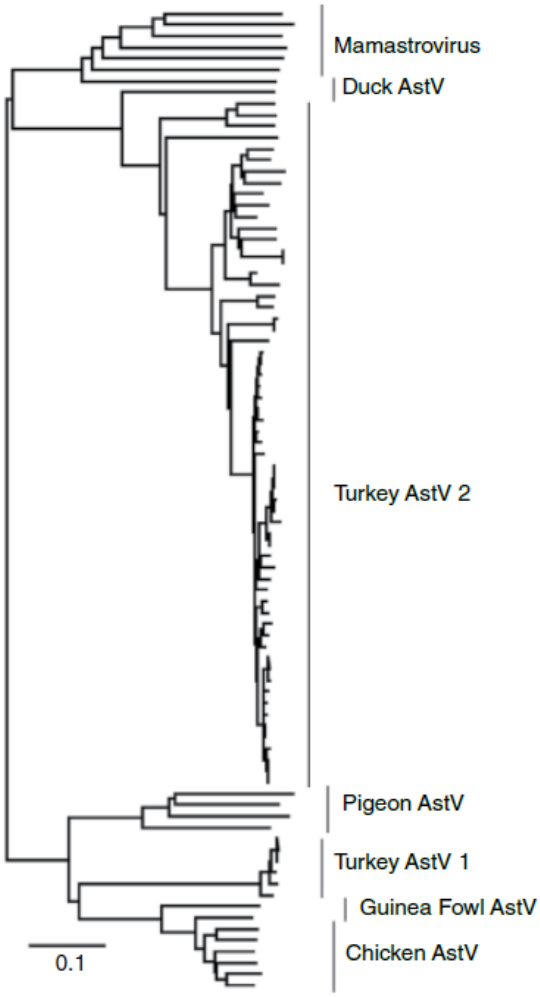

Figure 4. Phylogenetic trees of avian astrovirus capsid sequences. MEGA4 software was used to align the full nucleotide sequence of the astrovirus genome or an 800nucleotide fragment of the ORF2 capsid gene using the NeighborJoining approach and a Kimura 2parameter model with pairwise deletion (88).

Poult enteritis complex (PEC), poult enteritis syndrome (PES), and poult enteritis and mortality syndrome (PEMS) in turkeys are all caused by astrviruses. (Described in (89)) (81, 90, 91). In chickens, AstVs have been associated with growth retardation, nephritis, white chicks hatchery disease, and runting-stunting syndrome (RSS) in broilers (92-94) (Reviewed in (82)). They can, however, be isolated from clinically healthy birds, raising concerns regarding the significance of certain AstV strains in disease pathology (91, 95).

Recently, an emerging novel goose astrovirus has been reported to be associated with a fatal infection of goslings characterized by visceral gout (urate deposition). This virus has been isolated and could successfully reproduce the signs of the disease in experimental infection; demonstrates the etiological role of this AstV. Studies of experimental infection indicated that the isolate was highly pathogenic in goslings, causing clinical signs, growth repression and also death in many cases. Lesions were found primarily in the kidneys of sick birds, according to histopathological testing (96-100).

Table 1. Astrovirus localization in extra-intestinal tissues. RT PCR: Reverse Transcription Polymerase Chain Reaction (101).

Animal/Human	Tissue(s)	Method(s) of Detection
Turkey	Bursa, Thymus, Spleen, Kidney, Liver, Skeletal Muscle, Bone Marrow, Pancreas, Plasma	RT-PCR, immunofluorescence, infectious virus isolation
Duck	Liver	RT-PCR
Cow	Brain	RT-PCR, Sequencing
Mink	Brain	Sequencing
Pig	Blood	RT-PCR, Sequencing
Human	Blood	RT-PCR, Sequencing
Human	Cerebrospinal Fluid	Sequencing
Human	Urine	Sequencing
Human	Brain	Sequencing, Immunohistochemistry
Human	Nasopharyngeal swab	RT-PCR, Sequencing
Human	Pharyngeal swab	RT-PCR, Sequencing

11 Diagnostic Methods

11.1 Electron Microscopy (EM)

The first EM observation of 28–30 nm particles in the stool of newborns with gastroenteritis was reported in 1975 (102). The author quickly coined the term "Astrovirus" (derived from the Greek word "Astron," which means "star") (1) to describe the viruses' star-shaped surface arrangement, and this morphological trait has been widely employed for detecting astrovirus infection in both people and animals ever since and this morphological feature has been extensively used for the detection of astrovirus infection in both humans and animals ever since (103-105).

The fact that even a tiny number of virions have a complete star-shaped structure makes direct EM difficult, hence extensive examination may be required to distinguish astrovirus from calicivirus (106), which are comparable in size. The sensitivity of EM is further affected by high particle concentrations, which are usually approximately 107 per gram of stool (107).

Immunoelectron microscopy approaches based on particular antibodies or convalescent sera can improve detection sensitivity (108) and aid in typing (109) and the discovery of novel viral agents (110). Because of the limitations outlined above, molecular approaches have largely replaced EM in the diagnosis of viral infections, and it is now relatively uncommon in clinical laboratories.

11.2 Virus Isolation

Astroviruses, like other gut viruses, can be difficult to replicate in standard cell cultures. Lee and Kurtz were the first to employ serum media supplemented with trypsin to proliferate the HAstVs in human embryo kidney (HEK) cells in 1981. Other cell lines, such as African green monkey kidney Vero cells (e.g., MA-104), were not permissive for the virus even after first passages in HEK cells(111). In the presence of trypsin, analogous virus adaptation-based investigations in embryonic kidney cells have facilitated the multiplication of bovine (112) and porcine (113) astroviruses.

The capacity to grow MamAstV 1 in colonic cancer cells (CaCo-2) straight from feces, without prior adaptation to cell culture, was a huge breakthrough (114). After 2–3 days of infection, a cytopathic impact is often observed. A study that tested the ability of laboratory strains of HAstVs 1–7 to replicate in a variety of human and simian cell lines, including CaCo-2 and MA-104, found that propagation can be successful in a variety of cell lines (13). Furthermore, whereas adenocarcinoma cell lines appear to be the most widely employed cells for growing wild-type HAstV, human hepatoma cell lines can also be propagated directly from stool specimens. PLC/PRF/5 (115). While virus isolation is a helpful technique for studying astrovirus biology, it is not a suitable diagnostic tool for detecting astrovirus in

diagnostic laboratories because of the lengthy turnaround times and difficulties of isolation.

11.3 Immunodetection and Antigenic Typing

The capacity to propagate astroviruses in experimental animals has facilitated the manufacture of antisera, allowing for serotype characterization (116) and the creation of a radioimmune assay for detection of anti-MamAstV 1 (serotypes HAstV 1–8) antibodies (117). Following that, neutralizing antibodies were measured (118) and astrovirus-specific monoclonal antibodies were produced (119-121).

In a cohort study of patients with gastroenteritis in 1990, the first assessment of an indirect enzyme immunoassay (EIA) that used both a monoclonal antibody directed toward the capsid of MamAstV 1 for capture and a polyclonal antibody for detection was achieved, with a sensitivity of 91 percent and a specificity of 96 percent when compared to IEM (122). The detection approach, which was originally based on a peroxidase-labeled goat antibody, has been modified using biotin-avidin, which has proven to be useful for large epidemiological research and routine fecal sample screening (123).

In addition to their quick biotin-avidin EIA, Moe et al. developed and tested an RNA-probe hybridization. Despite its high sensitivity, the probe assay did not detect any more astrovirus-positive feces than EIA (123).

EIA has been used in a variety of clinical settings. Serotype identification and prevalence studies in the United Kingdom (109, 124) and South America (125), as well as efforts to type material gathered from several continents, have all been published (126), and all helped to improve our understanding of astrovirus epidemiology. Rapid immunochromatography techniques that identify astroviruses and promise high sensitivity and specificity have just become commercially available, however there are currently few studies testing their performance (127). Although EIA tests are far easier to execute than EM and have been shown to be just as effective (105), the introduction of molecular diagnostic techniques has impeded their use and advancement.

11.4 Molecular Diagnostics

11.4.1 Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Quantitative Reverse Transcription PCR (RT-qPCR)

Molecular approaches based on the amplification of viral genomes or transcripts significantly improved detection sensitivity as compared to EM, immunoassays, or virus isolation. In clinical laboratories, RT PCR has become a common method for diagnosing astrovirus infection, with detection levels as low as 10 to 100 genome copies per gram of feces (25, 107), and the capacity to construct type-specific detection systems. However, in terms of amplification efficiency and the ability to detect variant strains, the design of amplification systems, particularly the inherent properties of the primers, is crucial. For example, some MamAstV 1 (serotypes HAstV 1–8) RT-PCR systems target non-coding regions of the virus in a very sensitive and specific manner, while others are designed into conserved motives of the capsid, allowing subsequent typing but with the risk of sub-optimal amplification efficiency (for a complete review including a table of the most commonly used RT-PCR systems, see (128)).

Nucleic acid sequence-based amplification (NASBA) is a non-RT-PCR approach for detecting MamAstV 1 (serotypes HAstV 1–8) that has shown good agreement with RT-PCR-based methods (129). Additional primers have been designed and used to define novel viral populations (130-132) following the discovery of distant HAstV strains, MLB (133, 134) and VA1/HMO-C (135). Aside from human astroviruses, RT-PCR techniques have been developed to detect astroviruses in wild animals (136, 137), livestock (138, 139) or pets (140, 141). Despite the fact that consensus primers may detect a wide range of astroviruses in animal and human strains, there is no universal pan-astrovirus RT-PCR approach.

Parallel to the introduction of PCR primers, the use of real-time PCR (qPCR) in diagnostic settings has improved the detection of astrovirus infections by minimizing false positives, allowing quantification of viral loads, and shortening turnaround times (a positive or negative result is usually available within 24 hours of specimen collection) (142-145). In qPCR, a nucleic acid dye (commonly SYBR green) is utilized, followed by melting curve analysis, or a specifically designed hydrolysis probe connected to a fluorophore (typically Taqman). One-step RT-qPCR methods have also been developed (146, 147). An integrated cell culture/RT-qPCR assay that may identify low quantities of astrovirus after seven days or less incubation has been proposed as a further improvement (148), albeit this approach has largely remained of scholarly curiosity. Despite advances in the ability to precisely quantify viral loads, interpreting very low levels of virus in connection to clinical symptoms, particularly in asymptomatic people, remains difficult (149).

11.4.2 Multiplex RT-PCR for Enteric Pathogens Panels

Multiplex RT-PCR panels, which include astroviruses and other gastrointestinal pathogens, have evolved over time to address the demand for a quick, accurate, and cost-effective diagnosis. Early attempts to detect HAstVs, noroviruses, adenoviruses, sapoviruses, and enteroviruses in stool samples using end-point (150, 151) or qPCR (152) found to be as effective as single-plex PCR. In the case of enteroviruses, examining melting curves allowed for the detection of dual-infection by producing dual peaks, and was at least ten times more sensitive than end-point PCR (152). Since then, several multiplex assays based on various detection formats have been developed (153-161) and employed to diagnose astrovirus infection in individuals (162-170) or animals (171-175). Unexpectedly, the FilmArray Gastrointestinal Panel (BioFire Diagnostics, Salt Lake City, UT, USA) allows for the simultaneous identification of 22 different enteric pathogens directly from stool specimens, with reported sensitivity and specificity of 100 percent and 99.9 percent, respectively, for MamAstV 1 (but not MamAstV 6 and 9), and processing times of around one hour (176).

Several studies have highlighted the benefits of using a comprehensive multiplex PCR panel for the identification of known causative agents, as well as for the determination of pathogens not requested or unable to be tested by conventional tests to streamline the diagnosis of presumptive infectious diarrhea (177, 178). For example, the FilmArray® Gastrointestinal Panel has been used to identify astrovirus infections in diarrheic patients who had previously tested negative for Clostridium difficile and/or rotavirus (179). Another study found 11 cases of astrovirus infection among 245 stool samples from juvenile patients using the Seeplex Diarrhoea ACE Detection multiplex PCR assay (Seegene, Seoul, Korea) in combination with traditional assays (180). Other broad multiplex PCR assays, such as Luminex technology (Austin, TX, USA), have demonstrated a high ability to detect gastrointestinal infections in immunocompromised patients, with assay performance comparable to the tests studied (181).

11.4.3 Medium to High Density Detection Systems: Nanofluidic PCR and Microarrays

It is now possible to run qPCR in parallel using nanoliter capacity chambers, cutting the cost per test, because to advances in miniaturization and the creation of nanofluidic systems. For example, a microfluidic qPCR system based on numerous single-plex TaqMan qPCR assays was able to identify 13 viruses quantitatively with a sensitivity of 2 copies per microliter, including human astroviruses (182). A nanofluidic qPCR technique was used to detect and quantify 19 enteric viruses in a recent study, and the sensitivity was shown to be 1.6 log lower than traditional RT-qPCR and 2.7 log lower than digital RT-PCR for the identification of human astroviruses (183). In both studies, a preamplification step is necessary to increase the amount of target molecules present in nano-volumes. Another method involves parallelizing specialized probe/target nucleotide hybridization on microarrays, which might be utilized to detect multiple diseases in a single test.

In a panel of archival stool samples, the applicability of microarray technology for the detection of enteric pathogens was first tested for astroviruses and noroviruses, and it allowed for the characterization of known genotypes in some cases, though a large number of astroviruses remained untypable (184). A DNA oligonucleotide microarray with specific short capture probes of 17–20 nucleotides was proposed two years later for the identification of all eight HAstV serotypes (2). Another project, known as the Combimatrix custom microarray, used probe sequences that were both conserved and varied to detect human astroviruses, noroviruses, adenoviruses, and rotaviruses, demonstrating the absence of cross-reactivity across the four viruses (185).

In order to increase our understanding of the disease's genesis in people and animals, a more modern format of microarray was designed to detect over 100 virus species connected to gastrointestinal disorders in vertebrates (186). Despite the fact that microarrays are predicated on sequence homology with specified known viruses, a Virochip was utilized to identify an astrovirus in a domestic rabbit with gastroenteritis after standard diagnostic approaches, such as virus isolation, had failed (76). Unfortunately, the complexity and cost of the methods may limit the practical applicability of microarray research.

11.4.4 High Throughput Sequencing (HTS)

Metagenomics, which entails the simultaneous sequencing and subsequent description of all nucleic acid molecules identified in a sample, was made possible with the introduction of HTS. It depicts a collection of disruptive technologies that surpass PCR and other hypothesis-based detection procedures. Deep sequencing, which combines random amplification of microbial genomes or transcripts

with appropriate downstream data mining, can yield more comprehensive taxonomic information than diagnostic PCRs (187). Combining random amplification of microbial genomes or transcripts with proper downstream data mining can also be utilized to uncover new diseases without any prior notion. As a result, the application of HTS in research facilities has permitted a leap ahead in the identification and characterization of astroviruses in a variety of animals, including humans, over the last few years (50, 59, 61, 72, 76, 79, 188-197).

The discovery of partial/complete astrovirus genomes among complex polymicrobial flora provided valuable insight into viral diversity, pathogenesis, and astrovirus strain emergence, despite the fact that the primary goal of many of these studies was not to diagnose astrovirus infection in symptomatic patients. In addition, neurotropic astroviruses have been discovered in humans (198-204), cattle (54-57, 205, 206) and mink (66) using metagenomics to diagnose disorders of unknown origin. Indeed, owing of the high labour and costs associated with HTS, its use for routine microbiological diagnosis is difficult. Although HTS investigations are less expensive overall than other approaches, a large budget per sample is still necessary, especially when only a few samples must be evaluated separately from cohorts.

In the end, the capacity to give correct and timely results allows for major improvements in laboratory organization and bioinformatic process. Although HTS is already a routine method for detecting pathogens, its translation into medical and actionable diagnosis is still in its early stages, and it is now being utilized to treat the most serious and life-threatening infections.

Conflict of Interest

The authors declared no conflicts of interest.

Author Contributions

MRP: writing- original draft preparation, SB contributed to review and editing, AM participated in the provision of data set, JR contributed to the conception of study and supervision. All authors read and approved the final manuscript.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

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