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Assessing the Prevalence of Astroviruses in Water Environments: A Systematic Review and Meta-analysis

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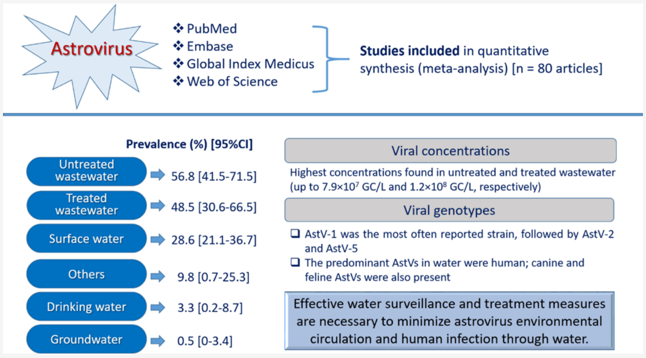
ABSTRACT: Astroviruses (AstVs) are a major cause of gastroenteritis, especially in children. They can be transmitted through various pathways, including environmental contamination via water matrices. This study aimed to investigate the prevalence of AstV in different types of water, such as untreated and treated wastewater, surface water (e.g., rivers, lakes, and seawater), groundwater, drinking water, and other water matrices (e.g., irrigation water, gray water, reservoir water, floodwater, and pig slaughterhouse effluents). The meta-analysis included 80 articles, and the overall prevalence of AstV in water matrices was 36.6% [95% confidence interval (CI) of 29.6−44.0]. The highest prevalence was found in untreated wastewater at 56.8% (95% CI of 41.5−71.5), followed by treated wastewater at 48.5% (95% CI of 30.6−66.5), surface water at 28.6% (95% CI of 21.1−36.7), other matrices at 9.8% (95% CI of 0.7−25.3), drinking water at 3.3% (95% CI of 0.2−8.7), and groundwater at 0.5% (95% CI of 0.0−3.4). The most frequent AstVs detected in water environments were human AstVs, but canine and feline AstVs were also detected. Our findings highlight the importance of water as a potential route for AstV transmission, even in high-income countries. Effective water surveillance and treatment measures are necessary to minimize AstV environmental circulation and human infection through water.



Highest concentrations found in untreated and treated wastewater (up to 7.9×10^7 GC/L and 1.2×10^8 GC/L, respectively)

☐ AstV-1 was the most often reported strain, followed by AstV-2 and AstV-5

☐ The predominant AstVs in water were human; canine and feline AstVs were also present

Effective water surveillance and treatment measures are necessary to minimize astrovirus environmental circulation and human infection through water.

1. INTRODUCTION

Astroviruses (AstV) are single-stranded, positive-sense, non-enveloped RNA viruses with an icosahedral capsid, belonging to the Astroviridae family. In 1975, 28−30 nm particles were observed via electron microscopy in the stools of children suffering from vomiting and mild diarrhea.[1] That same year, Madeley and Cosgrove coined the term "astrovirus" to describe small round viruses with a characteristic star-like appearance, found in the feces of hospitalized infants with gastroenteritis.[2] Since then, AstVs have been detected in a range of animals, including mammals, birds, reptiles, amphibians, fish, lower vertebrates, and invertebrates. AstV research has significantly accelerated with the development of metagenomic surveillance studies. These studies have led to the discovery of novel, highly divergent astroviruses in a variety of animal species, including humans. AstV has been classified by the International Committee on Taxonomy of Viruses (ICTV) into two genera: Mamastrovirus (MAstV), which infects mammalian species, and Avastrovirus (AAstV), which infects avian species. Classification under the genus level has been subjected to revisions, and sequence comparison (at the amino acid level) of the capsid-encoding ORF2 has been used to define viral species. A 2010 ICTV proposal calculated ORF2 mean amino acid distances for MAstVs and AAstVs, defining 19 MAstV and three AAstV species. Since then, several putative novel AstV species have been discovered, but an update of the AstV classification is required.

Human infections are mostly caused by MAstV species 1 (MAstV-1), also termed "classic" human AstVs.[3] Genetically divergent human AstV species, termed "atypical" or "animal-like" strains, of probable zoonotic origin, have been discovered,

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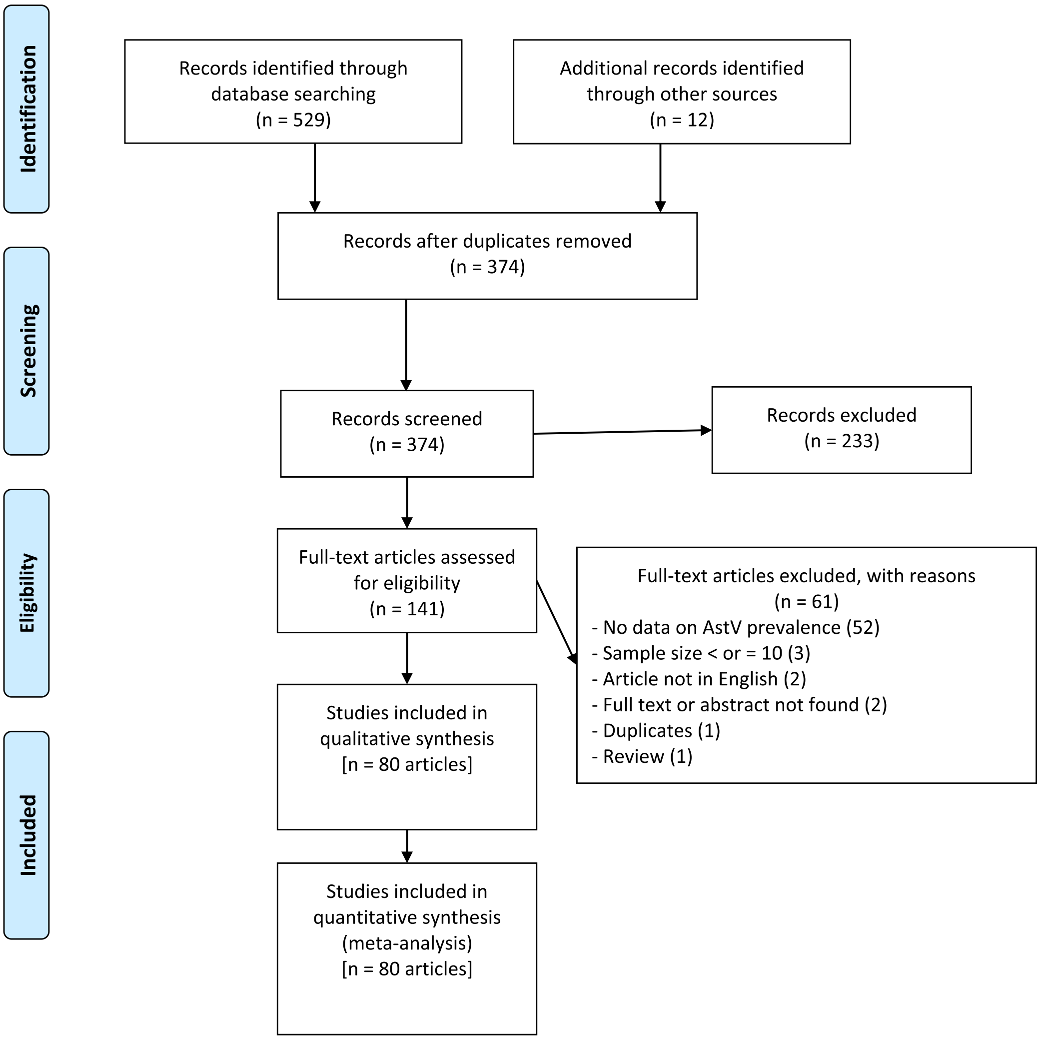

Figure 1. PRISMA diagram depicting the flow of information through the different phases of the systematic review.

including strain Melbourne (MLB) (species MAstV-6),[4] strain Virginia/Human-Mink-Ovine-like (VA/HMO) (species MAstV-8 and MAstV-9),[5] and tentative species MAstV-20.[6]

Astroviruses are a significant cause of disease, particularly in children, and are the second most common cause of gastroenteritis in this age group after rotaviruses,[7] and a leading cause of mortality in children worldwide.[8] Infection is associated with a wide range of symptoms, including vomiting, diarrhea, abdominal pain, fever, and dehydration. In severe cases, hospitalization may be required, especially in young children, the elderly, and immunocompromised individuals. In addition to these viruses causing gastroenteritis, recent studies have identified serious extraintestinal manifestations of AstV infection, such as meningitis and encephalitis.[9] In animals, AstVs have been also been associated with CNS infection[10] and with respiratory disease in deer[11] and hepatitis and nephritis in avian species.[12]

Human astrovirus (HAstV) infections are reported in both developed and developing countries, but a higher incidence is observed in low-income countries. Infections occur throughout the year, but a peak of detection is observed in colder months in temperate regions that decreases drastically in warmer months.[13]

AstVs are primarily transmitted through fecal−oral contact, direct physical contact, or consumption of contaminated food and water.[14] Human AstVs are shed in the feces of infected individuals.[13] A study found that the shedding levels of AstV serotypes 1−4 and 8 varied at viral concentrations ranging from 3.4×10^{8} to 1×10^{13} per gram of feces, with significantly higher titers detected in feces containing serotype 3.[15] Like other gastroenteric viruses, the occurrence of human astrovirus (HAstV) has been routinely reported in sewage and several environmental matrices worldwide. Astroviruses are known to persist in the environment, particularly in water sources, leading to concerns about their transmission and potential for outbreaks. Studies have shown that astroviruses can remain infectious in water for extended periods.[16] The presence of human AstVs in water samples indicates a potential risk of infection through water, highlighting the significance of investigating viruses in water matrices for epidemiological and risk assessment purposes.

Previous systematic reviews of AstV have mainly investigated their epidemiology in patients with gastroenteritis,[17] especially in children. Only one systematic review has examined contamination of food by AstV.[18] Moreover, while one systematic review has explored the rate of decay of AstV in water, there is currently no study that has comprehensively investigated the overall contamination of AstV in different water matrices.[19] Therefore, this study aims to fill this gap in knowledge by providing a comprehensive assessment of the overall prevalence of AstV in water matrices.

2. METHODS

2.1. Protocol and Registration. This systematic review was conducted between October 2022 and April 2023, and its protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO, no. CRD42022332153). The review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Figure 1) standard guidelines.[20]

2.2. Data Sources and Search Strategy. An extensive literature search was conducted in electronic databases, including PubMed, Embase, Global Index Medicus, and Web of Science, up until March 20, 2023, to identify all relevant studies reporting on the prevalence of AstV in various water matrices. A manual search was also conducted in other databases to find any additional papers missed from the online search. The search strategy combined keywords related to AstV, water environments, and surveillance. Table S1 shows the complete description of the search strategy.

2.3. Study Selection. To be included in this systematic review and meta-analysis, studies had to provide data on the prevalence of AstV in water environments. We excluded studies that were systematic reviews, meta-analyses, comment papers, case reports, and case series, research news, had no water matrix, had no extractable data, contained duplicate data, had a sample size of less than or equal to 10, or were not written in English or French. A complete list of exclusion criteria is provided in Table S2.

2.4. Data Extraction and Management. To ensure the quality of data extraction, two reviewers independently screened the titles and abstracts of all studies using the Rayyan platform,[21] and any discrepancies were resolved by a third reviewer. Data from selected studies were extracted using a predesigned Google data abstraction form, which included information such as the first author's name, year of publication, sampling period, and country. The water matrices were categorized into five main groups, namely, untreated wastewater, treated wastewater, surface water, drinking water, and groundwater. Any water matrix that did not fit into these categories was classified as an "other water matrix". Data on the total number of samples analyzed, the number of AstV-positive samples, the type of AstV (human or animal strains), and the viral loads in the water matrix were documented. In cases in which different detection methods or viral target genes were used on the same samples, the highest detection rate was reported. This systematic review also recorded the AstV detection and characterization methods used in each study, including molecular and culture-based methods. To ensure accuracy, all included studies were evaluated by two reviewers.

2.5. Quality Assessment. We used a tool developed by Hoy et al.[22] to assess the risk of bias in the included studies (Table S3). This tool evaluated the risk of bias in the studies by considering nine criteria, including representation of the study's target population, representation of the sample, form of random selection, clear definition of the water matrix, validity and reliability of the assay detection method, mode of data collection, length of the study period, and reporting of numerators and denominators for AstV prevalence. The studies were classified as low risk (7−9), moderate risk (4−6), or high risk (0−3) of bias, allowing us to evaluate their rigor and transparency in detail.

2.6. Data Synthesis and Statistical Analysis. The meta-analysis was conducted using a random-effects model developed by DerSimonian and Laird in 1986 to estimate the pooled prevalence of AstV in water environments.[23] The degree of heterogeneity among the studies was assessed using the Cochrane Q statistical test and reported I^2 values.[24] I^2 values of 25%, 50%, and 75% were used to represent low, moderate, and high heterogeneity, respectively, following the method of Higgins et al.[24] Subgroup analysis was conducted to investigate potential sources of heterogeneity and was performed on the basis of factors such as nation, WHO and UNSD regions, country income level, midyear period, water matrix, and detection method for AstV. The midyear period of the study was defined as the halfway point in the duration of the study. Additionally, to evaluate the potential drivers of variation, publication bias was assessed using funnel plots and Egger's test developed by Egger et al. in 1997.[25] A significant difference was defined as a p value of <0.05. R version 4.1.0 was used to perform all statistical analyses.

3. RESULTS

3.1. Study Selection. A total of 529 entries were initially identified through a database search, and 12 additional records were discovered through other sources (Figure 1). After 167 duplicates had been removed, 374 unique records were screened by title and abstract. Of these, 141 full-text articles were evaluated for eligibility, and 61 were excluded for the reasons listed in Table S2. Ultimately, the screening resulted in the inclusion of 80 articles in both qualitative and quantitative synthesis.

3.2. Overall Study Characteristics. Tables S4 and S5 provide the characteristics of the 80 included studies, published between 1998 and 2023 and conducted over a sampling period that ranged from 1997 to 2021. The studies were conducted in 30 different countries, with China being the most represented (12.5%), followed by Canada and France (8.8% each). In terms of WHO regions, America was the most represented (31.3%), followed by the Western Pacific (27.5%) and Europe (25.0%). For UN regions, the most represented regions were Eastern Asia (21.3%) and South America (18.8%). On the basis of the Sustainable Development Goal (SDG) regions, Europe and Northern America were the most represented (36.3%), followed by Eastern and South-Eastern Asia (28.8%) and Latin America and the Caribbean (20.0%). A majority of the studies were conducted in high-income countries (61.3%).

In terms of the categories of water matrices, untreated wastewater was the most frequently studied (32.8%), followed by surface water (28.4%) and treated wastewater (22.4%). The studies included in this analysis employed two different methods for the detection of AstV in water environments. The most commonly used method was conventional reverse transcription polymerase chain reaction (RT-PCR), which was used in 60.0% of the studies. Real-time RT-PCR was the second most used method with a frequency of 40.0%. Only 38.8% of the studies reported using controls for the concentration, extraction, and amplification of nucleic acids. Among those that did use internal controls, the most frequently used were murine norovirus, adenovirus, bacteriophage MS2, and bacteriophage PP7. A majority of the studies (95%) were classified as having a moderate risk of bias (Table S6).

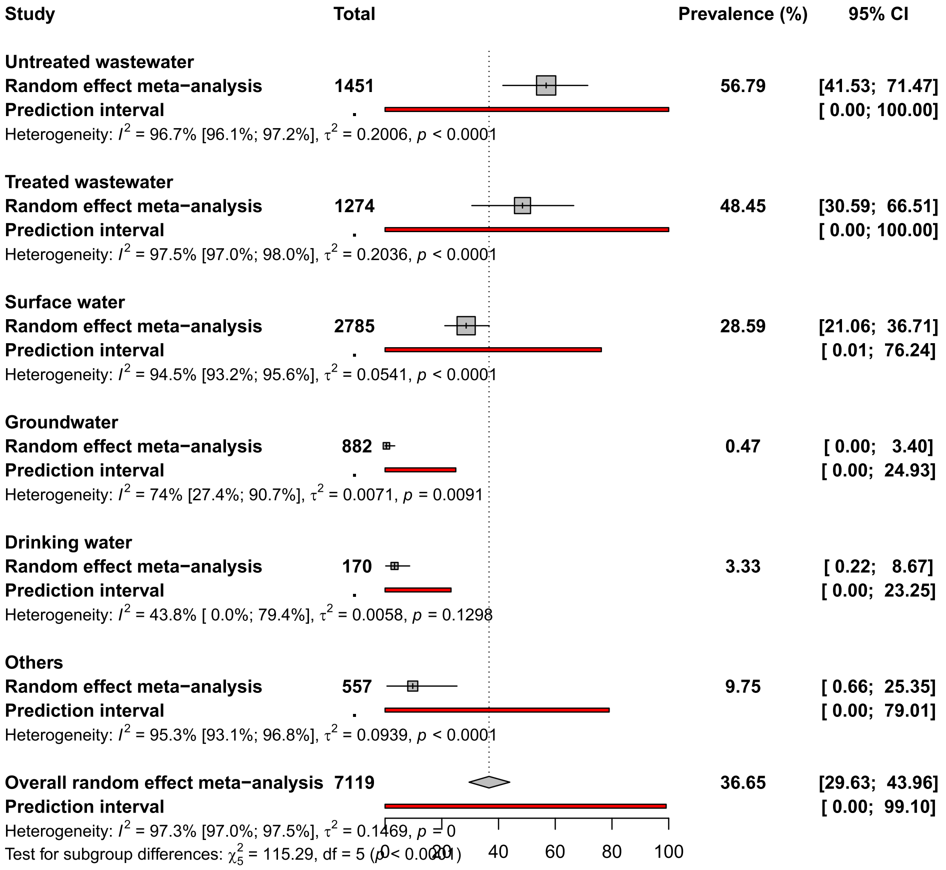

Figure 2. Prevalence observed in the different water matrices.

3.3. Astrovirus Prevalence in Water Matrices. Figure 2, Figure S1, Table 1, and Table S10 show the prevalence of AstV in various water matrices. The overall prevalence of AstV in water was 36.6% [95% confidence interval (CI) of 29.6−44.0]. The prevalence of AstV varied on the basis of the water matrix, with the highest prevalence observed in untreated wastewater at 56.8% (95% CI of 41.5−71.5), followed by treated wastewater at 48.4% (95% CI of 30.6−66.5), surface water at 28.6% (95% CI of 21.1−36.7), other matrices at 9.8% (95% CI of 0.7−25.3), drinking water at 3.3% (95% CI of 0.2−08.7), and groundwater at 0.5% (95% CI of 0.0−3.4).

3.4. Astrovirus Viral Load in Water Matrices. Thirty-seven studies, published between 2005 and 2022, reported the viral load of AstV in water environments (Table S7). The concentrations of AstVs in water environments ranged from 1.8×10^{-1} genome copies (GC)/L to 1.2×10^{8} GC/L (Table S3). The concentrations of AstV in positive samples varied significantly across different types of water environments, with the highest concentrations found in untreated and treated wastewater ($\leq 7.9 \times 10^{7}$ and $\leq 1.2 \times 10^{8}$ GC/L, respectively). Surface water had concentrations ranging from 5.7 to 4.4×10^{6} GC/L, while, in one study, drinking water had a concentration of 2.3×10^{3} GC/L. The concentration of AstV in other types of water samples, on the contrary, ranged from 0.18 to 2.6×10^{3} GC/L.

3.5. Astrovirus Genotyping Results. Twenty-three studies, published between 2003 and 2023, reported the genotyping of MAstV-1 in water environments (Table S8). Type 1 (AstV-1) was the most often reported strain, with 142 samples, followed by AstV-2 (n = 53) and AstV-5 (n = 39). AstV-4 or -8 was reported in 41 samples, whereas AstV-3 and AstV-6−8 were found in 17, 8, 13, and 17 samples, respectively. MLB1-like and VA-like AstV strains were reported with a variable number of samples. In addition, five putative recombinants are also described. Finally, animal AstVs, such as feline, canine, porcine, and rodent AstVs, were also identified, although the frequency of their occurrence was not always available because they were detected by NGS.

3.6. Detection of Infectious Astroviruses by Cell Culture. Five studies published between 1998 and 2007 employed culture-based approaches for isolating AstV, as shown in Table S9. The primary cell lines utilized were CaCo-2 and PLC/PRF/5. Of the 139 cell cultures tested, 20 tested positive for infectious AstV, with the highest number of positive tests observed in the PLC/PRF/5 and CaCo-2 cell lines. Surface water was the most frequently tested water matrix, with 107 of the total of 139 tests conducted, followed by untreated wastewater with 30 tests and drinking water with two tests. Although no positive tests were detected in drinking water, positive tests were identified in eight of 30 untreated wastewater tests and 12 of 107 surface water tests.

3.7. Heterogeneity and Publication Bias. The levels of heterogeneity and publication bias are presented in Table 1 and Figure S2. High levels of heterogeneity ($H > 1$ and $I^2 > 75\%$) were observed in untreated and treated wastewater, surface water, and other matrices, while low to moderate heterogeneities were observed in drinking water ($I^2 < 50\%$) and groundwater ($I^2 < 75\%$) studies. The estimation of prevalence data in several water matrix groups was associated with the presence of publication bias ($p < 0.05$ for Egger's test). A funnel plot (Figure S2) confirmed the publication bias results obtained by Egger's test.

Table 1. Summary of the Results of Global Meta-analysis of the Prevalence of Astrovirus in Different Water Matrices Divided by the Risk of Bias and Process Control

water matrix	prevalence (%) (95% CI)	95% prediction interval	no. of studies	no. of samples	*H*[b] (95% CI)	I^2[c] (95% CI)	*p* heterogeneity
untreated wastewater							
overall	56.8 (41.5–71.5)	0–100	38	1451	5.5 (5.1–6)	96.7 (96.1–97.2)	<0.001
low risk of bias	44.3 (28–61.3)	NA[a]	2	36	1	0	0.409
process controlled	47.6 (20.4–75.5)	0–100	11	365	5.2 (4.4–6.1)	96.3 (94.7–97.3)	<0.001
treated wastewater							
overall	48.5 (30.6–66.5)	0–100	26	1274	6.4 (5.8–7)	97.5 (97–98)	<0.001
process controlled	49.1 (25.6–72.7)	0–100	14	564	5.5 (4.8–6.4)	96.7 (95.6–97.6)	<0.001
surface water							
overall	28.6 (21.1–36.7)	0–76.2	33	2785	4.3 (3.8–4.7)	94.5 (93.2–95.6)	<0.001
low risk of bias	47.9 (38–58)	NA[a]	1	96	NA[a]	NA[a]	1
process controlled	27.7 (15.6–41.6)	0–84.4	14	1700	5.4 (4.7–6.3)	96.6 (95.4–97.5)	<0.001
drinking water							
overall	3.3 (0.2–8.7)	0–23.2	5	170	1.3 (1–2.2)	43.8 (0–79.4)	0.130
process controlled	3 (0–12.7)	0–100	3	102	1.7 (1–3.1)	64.6 (0–89.9)	0.059
groundwater							
overall	0.5 [0–3.4]	0–24.9	4	882	2 (1.2–3.3)	74 (27.4–90.7)	0.009
process controlled	1.4 [0–5.9]	NA[a]	1	72	NA[a]	NA[a]	1
others							
overall	9.8 (0.7–25.3)	0–79	10	557	4.6 (3.8–5.6)	95.3 (93.1–96.8)	<0.001
low risk of bias	11.2 (5.1–19.2)	NA[a]	1	80	NA[a]	NA[a]	1
process controlled	7.3 (0–22.7)	0–74.2	5	166	2.3 (1.5–3.5)	80.3 (53.8–91.6)	<0.001

[a] Not applicable. [b] *H* is a measure of the extent of heterogeneity; a value of >1 indicates a potential heterogeneity of the prevalence of astrovirus. [c] I^2 describes the proportion of total variation in the prevalence of astrovirus that is due to heterogeneity; a value of >50% indicates the presence of heterogeneity.

3.8. Subgroup Analyses.

The prevalence of AstV in water environments significantly varied among different countries, UN regions, midyears, and water matrices. No significant differences were noted among WHO regions, SDG regions, income levels, or AstV detection methods. Sweden had the highest prevalence of AstV in water environments (63.4%; 95% CI of 15.7–99.1), followed by China (60.8%; 95% CI of 34.7–84.1) and Singapore (58.8%; 95% CI of 33.0–82.4). For the UN regions, the prevalence was higher in Northern Europe (66.5%; 95% CI of 49.2–81.9), followed by Southern Africa (50.4%; 95% CI of 01.1–99.1) and Eastern Asia (46.2%; 95% CI of 26.5–66.6). The prevalence rates in high-income countries (39.8%) were relatively higher than those in lower-to middle-income countries (24.3%). According to the midyear, the highest prevalence was observed in 2019 (93.3%; 95% CI of 61.2–100), followed by 2014 (70.6%; 95% CI of 43.2–91.9) and 2015 (47.3%; 95% CI of 12.3–83.8).

4. DISCUSSION

AstVs are a common cause of gastroenteric infection in children and have been associated with central nervous system infections, particularly in immunocompromised individuals,[26] highlighting their relevance for public health. Astroviruses can be transmitted through various routes, including foodborne and waterborne. In particular, transmission can occur through consumption of contaminated food, direct exposure to polluted water, or participation in recreational activities.[27] To prevent environmental transmission, monitoring of contamination levels in different aquatic environments and other environmental matrices is essential.

Our study aimed to provide a comprehensive analysis of the global prevalence of AstVs in various water sources. We conducted a systematic review of 80 articles published between 1998 to 2023. Our findings revealed a high overall prevalence of AstV in water, at 36.6%, which is significantly higher than the levels of other waterborne viruses such as hepatitis A (16.7%) and E (9.8%).[28] The prevalence of AstVs varies significantly depending on the nature of the water matrix. The highest prevalence was found in untreated wastewater, at 56.8%, while treated wastewater had a prevalence of 48.5%. This indicates that water treatment partially eliminates AstV, although contamination remains significant in treated wastewater. Astrovirus is known to be resistant to many physical and chemical stimuli, affecting its presence in wastewater and spread through the fecal–oral pathway.[29] However, treatments applied to drinking water seem to be more effective against AstV, as noted by the low prevalence (0.3%) in this water matrix. Untreated wastewater had a high concentration of AstV ($\leq 7.9 \times 10^7$ GC/L), while drinking water had a reported concentration 4 log lower (2.3×10^3 GC/L), indicating the effectiveness of drinking water treatments.

Our study also found a significant prevalence of AstVs in surface water (28.6%). This contamination is likely due to the shedding of high levels of AstV in the feces and vomit of infected individuals, which is then transported into surface

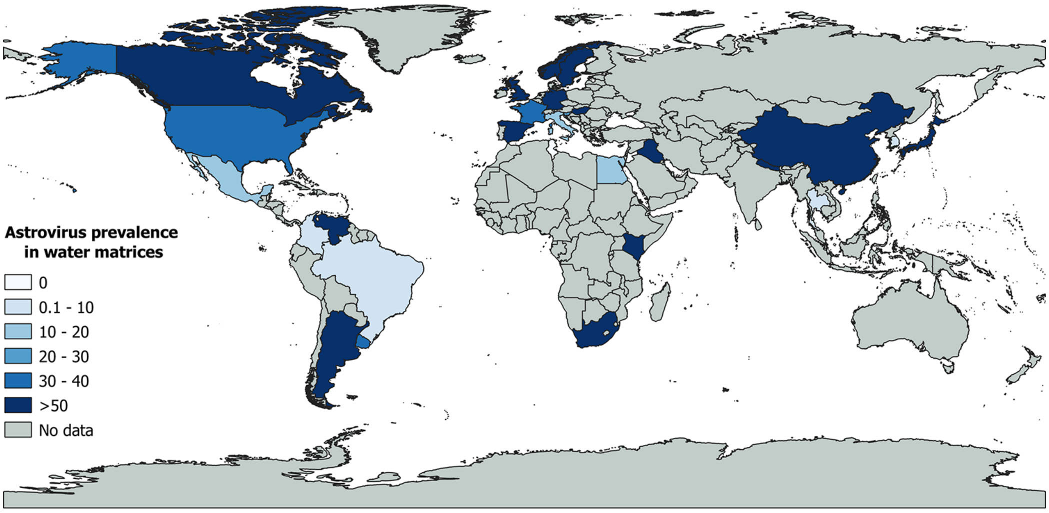

Figure 3. Astrovirus prevalence in water matrices worldwide.

water through the discharge of treated and untreated wastewaters.[30] Groundwater, on the contrary, had the lowest prevalence of AstV (0.5%). This could be attributed to the natural filtration capacities of underground settings, which makes groundwater less likely to be contaminated with pathogens.[31]

With regard to the prevalence of AstV in different countries worldwide (Figure 3), high-income Sweden had the highest prevalence of AstV (63.4%) and Northern Europe had the highest prevalence among UN regions (66.5%). Similarly, high-income countries had the highest prevalence of AstV (39.8%). The high prevalence of AstV in high-income countries may be attributed to more effective detection technologies and higher levels of testing. According to genotyping results, AstV-1 is the most prevalent MAstV-1 type found in different water matrices. Additionally, type 8 of MAstV-1 is also detected frequently in water. In addition to "classic" AstVs, MLB- and VA-like AstVs have also been identified in water. MLB1 was the first novel human astrovirus discovered by viral metagenomics, found to be genetically highly divergent from the eight serotypes of classic human astroviruses. It was initially identified in a stool sample from a child with diarrhea in Melbourne, Australia.[4] Shortly thereafter, the same group reported VA1 AstV, which was discovered during an outbreak of gastroenteritis in a child care center in Virginia, USA.[5] Using conserved sequence primers, VA-like and MLB-like AstVs (VA2, VA3, and MLB2) were also reported from patients in India and the USA.[32] In recent years, the discovery of novel astroviruses has improved our understanding of the diversity within the viral family. These novel astroviruses have nucleotide sequences that differ from those of classical astroviruses (HAstV-1−8), presenting a unique challenge for their detection in environmental samples. Many of the studies included in this review used conventional PCR or real-time PCR assays to detect astroviruses. Notably, the effectiveness of these assays in detecting novel astrovirus strains can vary, depending on the primer sets used. This raises the question of whether some studies may have missed the presence of novel astrovirus strains in their water samples. The emergence of new astrovirus strains and the potential differences in their epidemiology compared with classical astroviruses warrant continued review and methodological adaptation in the field of astrovirus detection. Our study also identified animal strains of AstV in water matrices, specifically feline, canine, porcine, and rodent AstV. This highlights the possibility of animal contamination of water sources for human use and the possible exposure to viruses with zoonotic potential.

Our systematic review has some limitations. First, the data were highly heterogeneous ($I^2 > 75\%$), particularly for the most contaminated water matrices, with only 6.3% of the studies conducted in lower- to middle-income economies where hygiene, sanitation, and water treatment are inadequate, and access to safe drinking water is limited. Considering the high prevalences observed in high-income economies, we can hypothesize that the prevalence in other-income economies would be even higher if the same detection methods were used.

The methodological issue is particularly relevant given the wide range of methods used in the different studies in terms of sample collection approaches, concentration methods used, and molecular identification techniques employed. Different sampling methods were used in the studies, including grab samples, composite samples, and a mixture of grab/composite samples. Furthermore, there was considerable variation in the concentration (primary and secondary) methods used. These included techniques such as polyethylene glycol precipitation, ultrafiltration, electronegative membrane filtration, ultracentrifugation, aluminum hydroxide adsorption−precipitation, aluminum chloride precipitation, skimmed milk flocculation, and others. It is well-known that different concentration methods can provide different recoveries, and this variability hampers a direct comparison of results. The diversity also extends to the methods used for astrovirus identification. Some studies relied on sequencing-based analysis, while others used RT-qPCR/RT-dPCR-based approaches. All of these factors contribute to the inherent variability in the reported results and should be carefully considered when findings are interpreted across studies.

The results of this review show that only 5% of the studies had a low risk of bias, indicating that factors that affect their validity or reliability could have influenced the results. There was also considerable publication bias, suggesting that the selection of studies may have influenced our conclusions.

5. CONCLUSION

Our study highlights a significant overall prevalence of AstV in various water matrices, particularly in treated and untreated wastewater and surface water. Although drinking water is only slightly contaminated, it is still a source of contamination. As human AstV strains are the most common in water, policy and research should focus on preventive measures to mitigate the spread of AstV through water matrices.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.3c00415.

> Search strategy, reasons for the exclusion of eligible studies, items for risk of bias assessment, characteristics of included studies, individual study characteristics, risk of bias assessment details, characteristics of studies describing viral loads, description of viral types in studies, characteristics of studies describing astrovirus isolation in culture, detailed results of meta-analysis for the prevalence of astrovirus in various water matrices, and a global prevalence estimate figure and a funnel chart illustrating the publication distribution of astrovirus prevalence in water matrices (PDF)

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Notes

The authors declare no competing financial interest.

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