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Water Research X

A systematic review of influenza virus in water environments across human, poultry, and wild bird habitats

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ABSTRACT

Influenza, a highly contagious acute respiratory disease, remains a major global health concern. This study aimed to comprehensively assess the prevalence of influenza virus in different aquatic environments.

Using 43 articles from four databases, we thoroughly examined water matrices from wastewater treatment plants (WTPs) and other human environments, as well as poultry habitats and areas frequented by migratory wild birds.

In WTP influents (10 studies), positivity rates for influenza A ranged from 0.0 % to 97.6 %. For influenza B (8 studies), most studies reported no positivity, except for three studies reporting detection in 0.8 %, 5.6 %, and 46.9 % of samples. Within poultry habitats (13 studies), the prevalence of influenza A ranged from 4.3 % to 76.4 %, while in environments frequented by migratory wild birds (11 studies), it ranged from 0.4 % to 69.8 %. Geographically, the studies were distributed as follows: 39.5 % from the Americas, 18.6 % from Europe, 2.3 % from South-East Asia and 39.5 % from the Western Pacific.

Several influenza A subtypes were found in water matrices, including avian influenza (H3N6, H3N8, H4N1, H4N2, H4N6, H4N8, H5N1, H5N8, H6N2, H6N6, H7N9, H0N8, and H11N9) and seasonal human influenza (H1N1 and H3N2). The existing literature indicates a crucial requirement for more extensive future research on this topic. Specifically, it emphasizes the need for method harmonization and delves into areas deserving of in-depth research, such as water matrices pertaining to pig farming and prevalence studies in low-income countries.

Introduction

Seasonal influenza is a respiratory infection caused by influenza viruses circulating across all regions of the globe. There are four different types of influenza viruses: types A, B, C, and D (WHO 2023). Of these, influenza A and B viruses are responsible for between 3 and 5 million cases of severe illness and 290,000 to 650,000 respiratory-related deaths worldwide annually (Iuliano et al., 2018). Currently, the human

population is mainly affected by the A(H1N1)pdm09 and A(H3N2) subtypes of influenza A viruses (Uyeki et al., 2022; World Health Organization 2022). On the other hand, influenza B viruses circulating among humans are divided into two distinct lineages, defined as B/Victoria/2/87 and B/Yamagata/16/88, but since April 2020, B/Yamagata viruses have not been detected worldwide (Paget et al., 2022). Influenza B virus infections account for 17–40 % of all annual influenza cases according to surveillance data for years 2017–2022 (Caini et al., 2019; Paget et al., 2022). Influenza can cause a significant number of direct and indirect deaths. The 1918 Spanish influenza epidemic killed an estimated 50 to 100 million people globally (Taubenberger et al., 2019). The Asian influenza epidemic of 1957, the Hong Kong influenza epidemic of 1968, and the 2009 swine influenza epidemic are examples of events that have had a significant impact on global health.

Zoonotic transmission of influenza viruses is a major concern because it can lead to the emergence of new variants that can have a significant impact on human health (Lyons and Lauring 2018; Shao et al., 2017). Zoonotic infections with influenza A viruses, including subtypes such as avian influenza A viruses (H3N8, H5N1, H5N6, H7N9, H7N7, and H9N2), and swine influenza A viruses (H1N1, H1N2, and H3N2) are well documented (WHO 2018) through direct contact with infected animals or contaminated environments. However, these viruses have not yet developed the capacity for sustained transmission between humans.

The main hosts of influenza A are birds, with aquatic birds, in particular, serving as natural reservoirs for the virus (Blagodatski et al., 2021; Shriner and Root 2020). Avian influenza viruses are known to infect and spread primarily among wild migratory waterfowl and domestic poultry, but there is scientific evidence that some avian influenza viruses can also infect and spread to other animals, such as mammals that eat infected birds or poultry, or come into contact with sick or dead infected birds, e.g., wild mammals (Floyd et al., 2021), marine mammals (Leguia et al., 2023) farm mammals (Stadejek et al., 2023), stray or domestic animals (Lee et al., 2008; Song et al., 2008), but also zoo animals (Amonsin et al., 2006; Nofs et al., 2009). All known influenza A virus subtypes have been found in birds, with the exception of subtypes H17N10 and H18N11, which have only been found in bats (CDC 2023). Historically, waterfowl have been the predominant group of species acting as reservoirs for the virus. However, solid evidence show that colony-breeding seabirds have taken an increasing role on this, with an extension of the period of high virus circulation to include the summer season, as opposed to the usual autumn and winter months (Adlhoch et al., 2023). Interestingly, these avian hosts often carry the virus without symptoms, shed it in their secretions and act as hidden reservoirs for the pathogen (Blagodatski et al., 2021; Shriner and Root 2020). In Europe, the currently circulating H5 avian influenza viruses of clade 2.3.4.4b have been classified as low risk for the general population and low to moderate risk for individuals with frequent occupational or recreational contact with infected birds (Adlhoch et al., 2023).

Pigs play a crucial role in the transmission dynamics of influenza viruses (Ma 2020). These animals can be infected with both human and avian strains because they have cellular receptors that can bind to both types of virus. This characteristic makes pigs a "mixing vessel" in which viruses of different origins can mix and rearrange genetic material, giving rise to new strains of influenza. This is exactly what happened with the H1N1 virus responsible for the 2009 swine flu epidemic.

A key aspect of the influenza transmission cycle is the role of the aquatic environment as a potential reservoir for influenza viruses. Bodies of water such as lakes and ponds can harbour these viruses for extended periods of time. Direct transmission between waterfowl can occur via the respiratory route. However, research has shown that indirect transmission via virus-contaminated water sources is also possible (Markwell and Shortridge 1982; Roche et al., 2009; Rohani et al., 2009).

Previous reviews have addressed the presence of influenza viruses in aquatic water ecosystems. These include the 2016 systematic review and quantitative meta-analysis conducted by Dalziel et al., which specifically examined the environmental variables influencing the degradation of low pathogenic avian influenza virus (LPAIV) in water (Dalziel et al., 2016). In addition, an earlier Dutch review from 1987 examined the epidemiology and ecology of influenza viruses, particularly in relation to waterfowl. This work highlighted the central role of waterfowl, such as mallards, in the maintenance, spread, and possible reassortment of different subtypes that affect both avian species and humans (van Tongeren and Voous 1987). A recent systematic review and meta-analysis examined the persistence of enveloped viruses, including the influenza virus, in environmental waters and untreated wastewater (Silverman and Boehm 2021). However, a complete overview of the presence of influenza viruses in different water environments is currently lacking. To fill this gap, this study comprehensively investigated the occurrence of influenza virus in different water environments.

Methods

Eligibility criteria

This systematic review, conducted in accordance with the PRISMA (2020) guidelines (Page et al., 2021), aimed to collect and analyse the available literature on the presence of the influenza virus in different water environments, including wastewater from treatment plants (WTP), poultry farms, and wild migratory bird habitats. This included looking at its prevalence, concentrations, different types and subtypes, and methods used to isolate and identify it. To be considered for inclusion, each source had to meet specific criteria. These included addressing the laboratory detection of influenza viruses in water matrices, and being published in either English or French. Conversely, articles were excluded if they lacked information on influenza viruses in water matrices, were categorized as reviews or case reports, or were duplicates.

Search strategy and selection process

The methodology used for this systematic review is detailed in the review protocol, available at https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022332153. The search process took place on 10 February 2023, and was updated on 3 August 2023, across four databases: Medline (Ovid), Embase (Ovid), Global Health (Ovid), and Web of Science (search field = topic) (Table S1). Additional articles were identified through the citation search. Records were processed using EndNote software to remove duplicate entries. These records were then imported into the Rayyan Systematic Review (Ouzzani et al., 2016). Two authors (GRT and SK) performed an initial screening of the records based on their titles and abstracts. For full text screening, several authors collaborated to validate decisions. Articles that met the inclusion criteria after full screening were finally included in the systematic review.

Extraction of data and specific information

At least two independent authors were responsible for data extraction. The information collected included key study attributes such as the primary authors' names, geographical locations (country where environmental samples were collected), settings (WTP, poultry settings, or wild bird habitats), study duration, use of controls for water sample concentration or standard procedures, types of water matrices, types of influenza viruses, and analytical approach used to detect influenza viruses.

In addition, specific details were collected, including virus concentrations (measured in genome copies per litre in water or in copies per gram in settled solids), influenza virus subtypes detected and their respective numbers, culture details (cell lines or embryonated chicken eggs), methods of identification after viral isolation.

To calculate the proportion of positive samples for influenza virus, both the total number of water samples tested and the total number of positive samples were also recorded.

Data analysis

The weighted prevalence of the influenza virus in water matrices was calculated using the R software, with the sum of influenza-positive water samples as the numerator, and the total number of water samples tested as the denominator (Borenstein et al., 2010; Schwarzer 2007). To assess potential publication bias, we used two-tailed p-values obtained from Egger's test (Egger et al., 1997).

Quality assessment

The quality of each article included in the review was evaluated independently by at least two authors using the quality assessment tool for prevalence studies developed by Hoy et al. (Hoy et al., 2012). The criteria for the assessment are listed in Table S2. In cases of disagreement, a consensus was reached by discussion between the investigators.

Results

Search results

A total of 9206 published articles were retrieved from four databases: Medline ($n = 1839$), Embase ($n = 3120$), Global Health ($n = 1481$), and Web of Science ($n = 2766$). After removing 3628 duplicate records, 5578 records were screened, resulting in the exclusion of 5372 records. Subsequently, 206 reports were sought for retrieval, with one not successfully retrieved, leaving 205 reports to be assessed for eligibility. Of these, 169 were excluded for various reasons, including lack of influenza data in water matrices ($n = 129$), language limitations ($n = 32$), being a review ($n = 7$), and duplication ($n = 1$). Seven additional articles were found through the citation search, giving a total of 43 articles that met the inclusion criteria (Fig 1) (Ahmed et al., 2023a; Ahmed et al., 2023b; Ahrens et al., 2023; Ando et al., 2023; Asadi et al., 2023; Bo et al., 2021; Boehm et al., 2023a; Boehm et al., 2023b; Borchardt et al., 2017; de Melo et al. 2023; Dumke et al., 2022; Germeraad et al., 2020; Hayes et al., 2023; He et al., 2017; Heijnen and Medema 2011a; Henaux et al., 2012; Hinshaw et al., 1979; Horm et al., 2012; Horm et al., 2013; Ito et al., 1995; Khalil et al., 2022; Lai and McPhillips 1999; Leung et al., 2007; Li et al., 2022; Markt et al., 2023; Markwell and Shortridge 1982; Mercier et al., 2022; Munoz-Aguayo et al., 2019; Okuya et al., 2015; Okuya et al., 2023; Ornelas-Eusebio et al., 2015; Pawar et al., 2019; Rector et al., 2022; Rothman et al., 2021; Toribio-Avedillo et al., 2023; Vo et al., 2023; Vong et al., 2008; Wolfe et al., 2022; Wolken et al., 2023; Zafeiriadou et al., 2023; Zhang et al., 2011; Zhang et al., 2022; Zhang et al., 2012).

Study characteristics and quality assessment

The characteristics of the 43 included studies showed a remarkable diversity, as shown in Table S3. The studies covered a time period from August 1977 to March 2023. A surge in WBE research since 2022 was evident in the studies. This is largely due to the widespread evidence of the effectiveness of environmental surveillance for SARS-CoV-2, which led many groups to investigate other respiratory viruses.

The included studies covered different countries (Fig. 2), with a significant proportion coming from the United States (25.5 %), Japan (11.6 %), and China (including Hong Kong, Macao, and Taiwan regions, 16.2 %). The distribution of studies by WHO regions, was as follow: 39.5 % from the Americas, 18.6 % from Europe, 2.3 % from South-East Asia, and 39.5 % from the Western Pacific. The majority (81.4 %) of the studies were from high-income countries, with the remainder distributed between upper- and lower-middle-income countries. Tables 1-3 and Fig. 2 show the countries where samples were collected in the included studies.
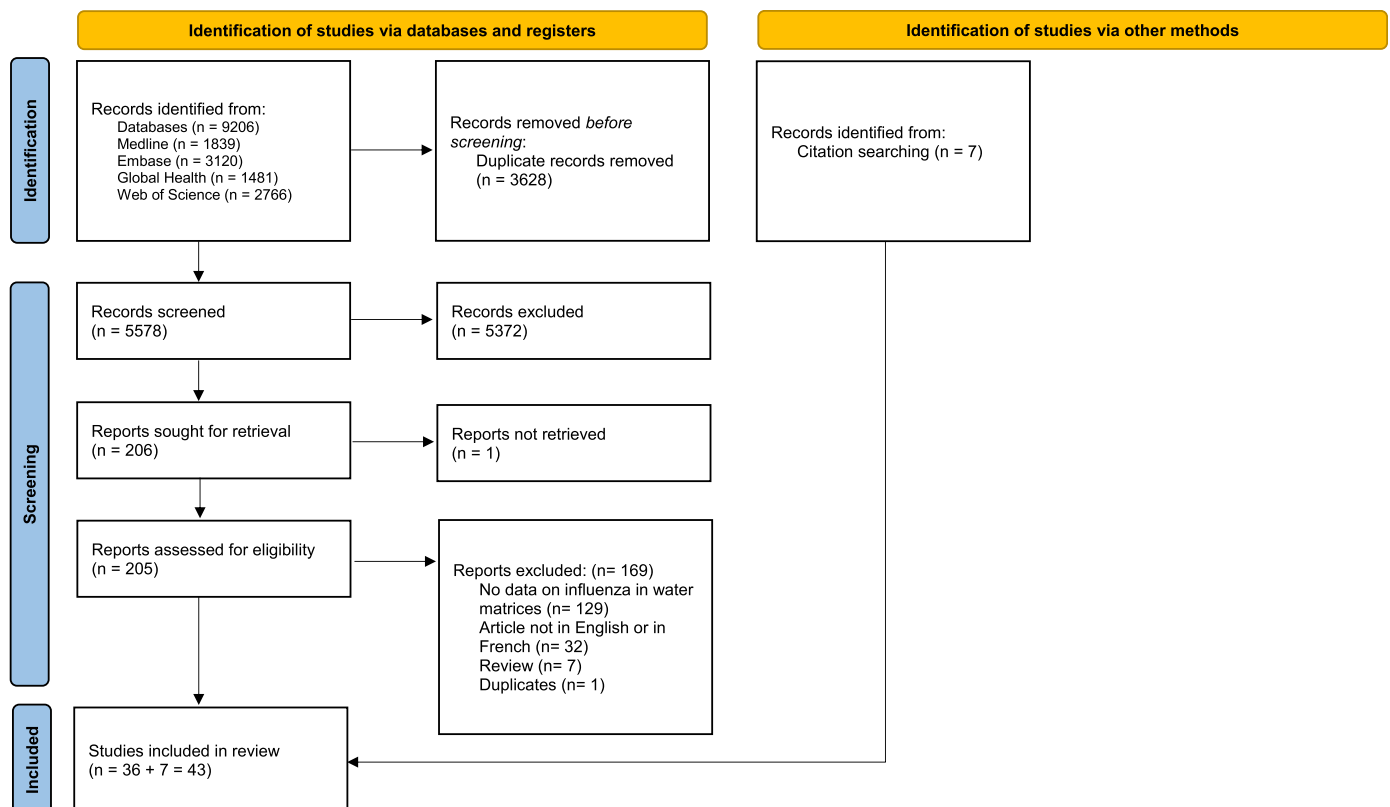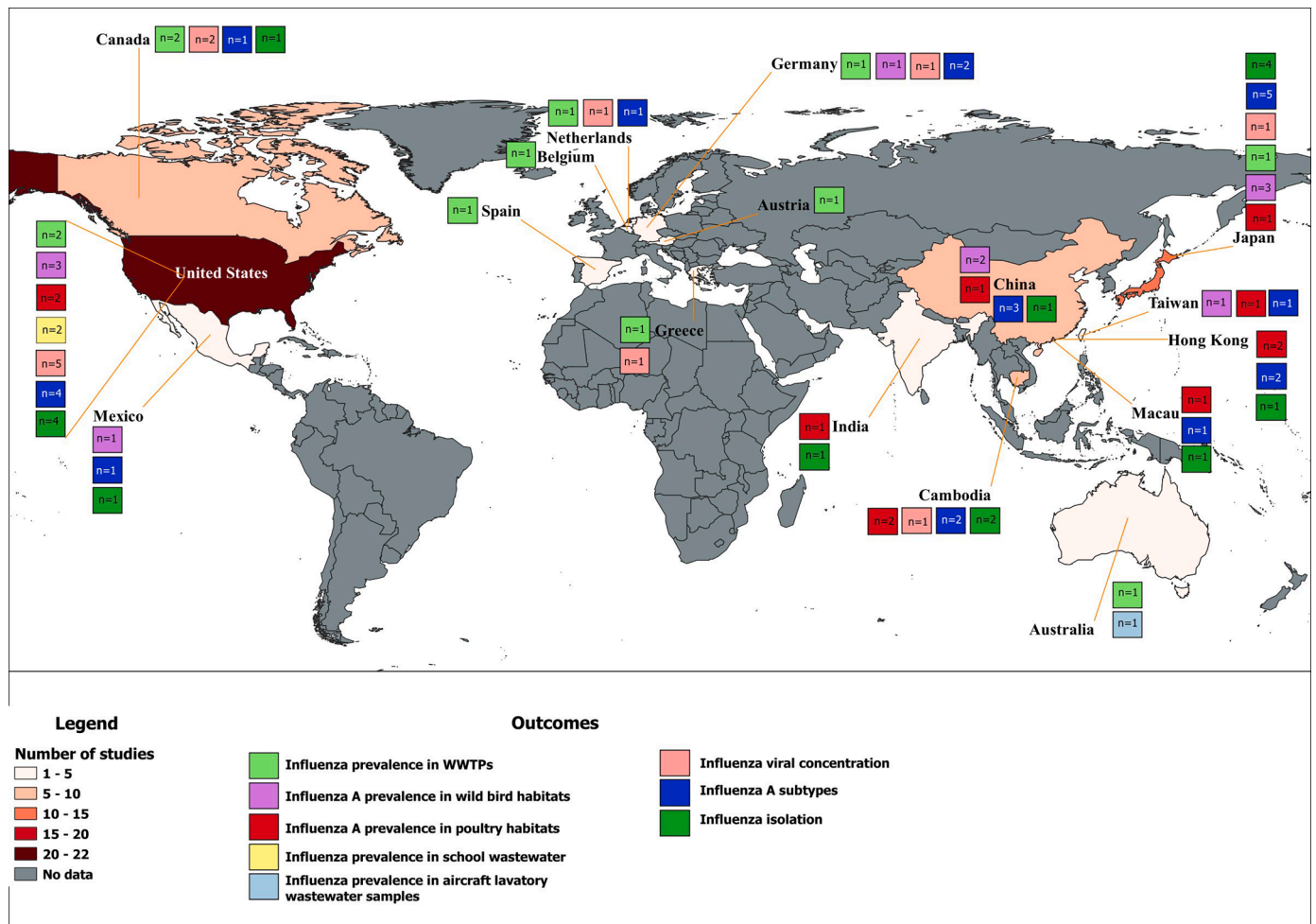

Fig. 1. Study selection.

Fig. 2. Geographical distribution of studies included in the systematic review.

Among the methods used to detect influenza, real-time RT-PCR was used in 69.6 % of cases, while 30.4 % relied on influenza isolation on embryonated chicken eggs or Madin-Darby canine kidney (MDCK) cells, followed by hemagglutination assay, real-time RT-PCR, conventional RT-PCR, or electron microscopy. Fifty-six percent of the studies included in the analysis did not evaluate inhibition or recovery rates from water matrices, and 44 % included internal controls. Almost all the articles (97.6 %) were considered to have a moderate risk of bias (Table S4).

Prevalence of influenza A and B in water matrices from urban wastewater treatment plants and in other human water settings

Of the 15 studies on influenza in wastewater collected at WTP, 12 studies provided prevalence data. Of these, 10 investigated the prevalence of influenza A, and eight focused on influenza B (Tables 1 and S5). Specifically, for Influenza A, the proportion of positive samples ranged from 0.0 % to 97.6 %. For Influenza B, most studies reported no positivity, except for three studies reporting detection in 0.8 %, 5.6 %, and 46.9 % of samples.

Four studies focused on wastewater in specific settings other than wastewater treatment plants. Two of these, examined wastewater samples from aircraft lavatories and sewage from schools. In aircraft samples, Ahmed and coworkers found that 4 out of 24 samples tested positive for influenza A, giving a prevalence of 16.7 % (Ahmed et al., 2023b). In school sewage, Wolken and collaborators found a lower prevalence of influenza A (32/1217 samples tested positive, 2.6 %) (Wolken et al., 2023). No samples tested positive for influenza B. Two other studies conducted in schools detected influenza A RNA but did not

provide prevalence data (Vo et al., 2023; Wolfe et al., 2022).

The prevalence of influenza A and B in water matrices from urban wastewater treatment plants is shown in Table 1.

Prevalence of influenza A in water matrices within poultry environments and in water matrices of migratory wild bird

There were 13 studies on the occurrence of influenza A in poultry environments (Tables 2 and S5). In relation to poultry drinking water (3 studies), the proportion of samples positive for influenza A ranged from 4.2 % (95 % CI 3.3–5.3) to 40.0 % (95 % CI 16.3–67.7). In sewage (5 studies), the virus prevalence ranged from 8.4 % (95 % CI: 7.0–10.3) to 76.4 % (95 % CI: 74.4–78.2). In other water sources (6 studies), such as puddles, wells, and water collected in duck cages, the proportion of influenza A-positive samples ranged from 6.2 % (95 % CI: 0.7–20.8) to 50.0 % (95 % CI: 23.0–76.9).

Eleven studies included data on influenza A prevalence in wild bird habitats (Tables 3 and S5). Influenza A positivity rates varied from 0.4 % (95 % CI: 0.0–2.3) to 69.8 % (95 % CI: 63.7–75.4).

The prevalence of influenza A in water matrices within poultry environments and in water matrices of migratory wild birds is shown in Tables 2 and 3, respectively.

Viral concentration of influenza A and B across diverse water sources

Twelve studies reported data on concentration of influenza virus. Seven studies documented the viral concentration of influenza A in WTPs, with values ranging from $<4.9 \times 10^1$ to 2.5×10^{10} genome copies

Table 1
Prevalence of influenza A and B in water matrices from urban wastewater treatment plants.

Authors	Countries	Study period	Settings	Environmental matrices	Influenza type	Total number	Number of samples with Influenza	Prevalence (%) [95% CI]
(Ahmed et al., 2023a)	Australia	Jan/2022-Jun/2022	Wastewater treatment plants	Untreated wastewater	Influenza A	46	33	71.7 [56.5–84]
(Ahmed et al., 2023a)	Australia	Jan/2022-Jun/2022	Wastewater treatment plants	Untreated wastewater	Influenza B	46	0	0 [0–7.7]
(Ando et al., 2023)	Japan	Oct/2018-Jan/2023	Wastewater treatment plants	Untreated wastewater	Influenza A	296	34	11.5 [8.1–15.7]
(Asadi et al., 2023)	Canada	Sep/2022-Jan/2023	Wastewater treatment plants	Untreated wastewater	Influenza B	155	0	0 [0–2.4]
(Boehm et al., 2023a)	United States of America	Feb/2021-Jun/2022	Wastewater treatment plants	Wastewater settled solids	Influenza A	216	60	27.8 [21.9–34.3]
(Boehm et al., 2023a)	United States of America	Feb/2021-Jun/2022	Wastewater treatment plants	Wastewater settled solids	Influenza B	216	12	5.6 [2.9–9.5]
(Dumke et al., 2022)	Germany	Jan/2022-May/2022	Wastewater treatment plants	Untreated wastewater	Influenza A	273	64	23.4 [18.5–28.9]
(Dumke et al., 2022)	Germany	Jan/2022-May/2022	Wastewater treatment plants	Untreated wastewater	Influenza B	273	128	46.9 [40.8–53]
(Heijnen and Medema 2011a)	Netherlands	Oct/2009-Feb/2010	Wastewater treatment plants	Surface water	Influenza A	10	4	40 [12.2–73.8]
(Heijnen and Medema 2011a)	Netherlands	Oct/2009-Feb/2010	Wastewater treatment plants	Untreated wastewater	Influenza A	10	1	0 [0–30.8]
(Heijnen and Medema 2011a)	Netherlands	Oct/2009-Feb/2010	Wastewater treatment plants	Treated wastewater	Influenza A	10	0	10 [0.3–44.5]
(Markt et al., 2023)	Austria	Sep/2021-Apr/2022	Wastewater treatment plants	Untreated wastewater	Influenza B	538	0	0 [0–0.7]
(Mercier et al., 2022)	Canada	Feb/2022-May/2022	Wastewater treatment plants	Untreated wastewater	Influenza A	131	79	60.3 [51.4–68.7]
(Mercier et al., 2022)	Canada	Feb/2022-May/2022	Wastewater treatment plants	Untreated wastewater	Influenza B	131	0	0 [0–2.8]
(Rector et al., 2022)	Belgium	Dec/2020-Sep/2022	Wastewater treatment plants	Untreated wastewater	Influenza A	89	10	11.2 [5.5–19.7]
(Rector et al., 2022)	Belgium	Dec/2020-Sep/2022	Wastewater treatment plants	Untreated wastewater	Influenza B	89	0	0 [0–4.1]
(Rothman et al., 2021)	United States of America	Aug/2020-Jan/2021	Wastewater treatment plants	Untreated wastewater	Influenza A	94	10	10.6 [5.2–18.7]
(Toribio-Avedillo et al., 2023)	Spain	Sep/2021-Nov/2022	Wastewater treatment plants	Untreated wastewater	Influenza A	122	13	10.7 [5.8–17.5]
(Toribio-Avedillo et al., 2023)	Spain	Sep/2021-Nov/2022	Wastewater treatment plants	Untreated wastewater	Influenza B	122	1	0.8 [0–4.5]
(Zafeiriadou et al., 2023)	Greece	Sep/2022-Mar/2023	Wastewater treatment plants	Untreated wastewater	Influenza A	127	124	97.6 [93.3–99.5]

(g.c.)/L (Table S6) (Ando et al., 2023; Asadi et al., 2023; Boehm et al., 2023a; Dumke et al., 2022; Hayes et al., 2023; Heijnen and Medema 2011a; Vo et al., 2023; Zafeiriadou et al., 2023). For river water, which was investigated in only one study, the detected viral concentration for influenza ranged from 1.6 to 2.8×10^3 g.c./L (Heijnen and Medema 2011a). As regards other human water settings, university wastewater samples contained influenza A concentrations ranging from 2.63×10^4 to 1.27×10^5 copies per gram of settled solids and school wastewater samples had a mean concentration of 3.1×10^1 g.c./L (Wolfe et al., 2022; Wolken et al., 2023). For influenza B, only one study reported viral concentrations in untreated wastewater from a WTP, with concentrations ranging from 7.2×10^2 to 2.1×10^7 g.c./L (Dumke et al., 2022). In two studies conducted in poultry environments, influenza A viral concentration ranged from 0.1×10^1 to 3.3×10^2 g.c./L in groundwater supply wells, lagoons, and ponds on poultry farms (Borchardt et al., 2017). Another study reported influenza A viral concentrations of 1×10^4 g.c./L in both puddles and wells, while in drinking water collected from duck cages, the viral concentration was as high as 2×10^8 g.c./L (Horm et al., 2012).

Influenza A subtypes across wastewater treatment plants, poultry environments, and wild migratory bird environments

Influenza A subtypes were reported in 25 of the 43 studies (Table S7). Within this range, five studies were associated with water matrices from WTPs (Ando et al., 2023; Boehm et al., 2023b; Dumke et al., 2022; Heijnen and Medema 2011a; Vo et al., 2023), ten with poultry

environments (Borchardt et al., 2017; Germeraad et al., 2020; He et al., 2017; Horm et al., 2012; Horm et al., 2013; Leung et al., 2007; Li et al., 2022; Markwell and Shortridge 1982; Vong et al., 2008; Zhang et al., 2012), nine with bird habitats (Ahrens et al., 2023; Hinshaw et al., 1979; Ito et al., 1995; Khalil et al., 2022; Okuya et al., 2015; Okuya et al., 2023; Ornelas-Eusebio et al., 2015; Zhang et al., 2011), and one with both bird and poultry environments (Bo et al., 2021). In water from WTPs, subtypes mainly included seasonal human influenza, such as pandemic influenza A (H1N1) 2009 and H3N2. Distinct influenza A subtypes, including avian influenza (H5N1, H7N9, H10N7, and H9N2) and human seasonal influenza H3N2, were identified in waterbodies associated with poultry. Water samples collected from migratory wild bird habitats showed the presence of specific influenza A subtypes, including avian influenza (H3N6, H3N8, H4N1, H4N2, H4N6, H4N8, H5N1, H5N8, H6N2, H6N6, H7N9, H0N8, and H11N9) and human seasonal influenza (H1N1 and H3N2).

Isolation and identification of influenza A viruses from poultry and wild bird environments

In 17 studies conducted in poultry or wild bird habitats, attempts were made to isolate the virus using culture methods, with successful isolation in all but one case (Borchardt et al., 2017; Hinshaw et al., 1979; Horm et al., 2012; Horm et al., 2013; Ito et al., 1995; Khalil et al., 2022; Lai and McPhillips 1999; Leung et al., 2007; Markwell and Shortridge 1982; Munoz-Aguayo et al., 2019; Okuya et al., 2015; Okuya et al., 2023; Ornelas-Eusebio et al., 2015; Pawar et al., 2019; Vong et al., 2008;

Table 2
Prevalence of influenza A in water matrices within poultry environments.

Authors	Countries	Study period	Settings	Environmental matrices	Influenza type	Total number	Number of samples with Influenza	Prevalence (%) [95% CI]
(Bo et al., 2021)	China, Taiwan Province of China	2014–2018	Habitat of poultry	Sewage	Influenza A	48,412	17,367	35.9 [35.4–36.3]
(Borchardt et al., 2017)	United States of America	2015	Habitat of poultry	Others	Influenza A	27	4	14.8 [4.2–33.7]
(Germeraad et al., 2020)	Netherlands	2017	Habitat of poultry	Others	Influenza A	9	4	44.4 [13.7–78.8]
(He et al., 2017)	China, Hong Kong SAR	Mar/2013-Apr/2015	Habitat of poultry	Drinking water for poultry	Influenza A	1646	70	4.3 [3.3–5.3]
(He et al., 2017)	China, Hong Kong SAR	Mar/2013-Apr/2015	Habitat of poultry	Sewage	Influenza A	1354	114	8.4 [7–10]
(Horm et al., 2012)	Cambodia	Apr/2007-Feb/2010	Habitat of poultry	Others	Influenza A	32	2	6.2 [0.8–20.8]
(Horm et al., 2013)	Cambodia	2011	Habitat of poultry	Others	Influenza A	145	30	20.7 [14.4–28.2]
(Leung et al., 2007)	Japan	Aug/2004-Nov/2006	Habitat of poultry	Drinking water for poultry	Influenza A	2916	207	7.1 [6.2–8.1]
(Li et al., 2022)	China	Jan/2014-Nov/2018	Habitat of poultry	Sewage	Influenza A	1969	1504	76.4 [74.4–78.2]
(Markwell and Shortridge 1982)	China, Hong Kong SAR	Jul/1978-May/1981	Habitat of poultry	Sewage	Influenza A	35	23	65.7 [47.8–80.9]
(Munoz-Aguayo et al., 2019)	United States of America	Not specified	Habitat of poultry	Others	Influenza A	14	7	50 [23–77]
(Pawar et al., 2019)	India	Jan/2015-Nov/2016	Habitat of poultry	Drinking water for poultry	Influenza A	15	6	40 [16.3–67.7]
(Vong et al., 2008)	Cambodia	Feb/2006-Aug/2006	Habitat of poultry	Others	Influenza A	40	11	27.5 [14.6–43.9]
(Zhang et al., 2012)	China, Macao SAR	Not specified	Habitat of poultry	Sewage	Influenza A	48	24	50 [35.2–64.8]

Table 3
Prevalence of influenza A in water matrices of migratory wild bird environments.

Authors	Countries	Study period	Settings	Environmental matrices	Influenza type	Total number	Number of samples with Influenza	Prevalence (%) [95% CI]
(Ahrens et al., 2023)	Germany	Nov/2020-Oct/2020	Habitat of wild birds	Surface water	Influenza A	44	27	61.4 [45.5–75.6]
(Bo et al., 2021)	China, Taiwan Province of China	2014–2018	Habitat of wild birds	Sewage	Influenza A	48,412	17,367	35.9 [35.4–36.3]
(Henaux et al., 2012)	United States of America	Not specified	Habitat of wild birds	Surface water	Influenza A	597	12	2 [1–3.5]
(Ito et al., 1995)	United States of America	1992–1994	Habitat of wild birds	Surface water	Influenza A	102	12	11.8 [6.2–19.6]
(Khalil et al., 2022)	Japan	Nov/2018-Mar/2019	Habitat of wild birds	Surface water	Influenza A	234	44	18.8 [14–24.4]
(Lai and McPhillips 1999)	United States of America	Not specified	Habitat of wild birds	Surface water	Influenza A	33	5	15.2 [5.1–31.9]
(Okuya et al., 2015)	Japan	Nov/2012-Mar/2013	Habitat of wild birds	Surface water	Influenza A	126	6	4.8 [1.8–10.1]
(Okuya et al., 2023)	Japan	Nov/2020-Mar/2021	Habitat of wild birds	Surface water	Influenza A	252	176	69.8 [63.8–75.4]
(Ornelas-Eusebio et al., 2015)	Mexico	Oct/2010-Mar/2011	Habitat of wild birds	Surface water	Influenza A	240	1	0.4 [0–2.3]
(Zhang et al., 2011)	China	Oct/2007	Habitat of wild birds	Surface water	Influenza A	95	1	1.1 [0–5.7]
(Zhang et al., 2022)	China	Mar/2007-Dec/2008	Habitat of wild birds	Surface water	Influenza A	963	10	1 [0.5–1.9]

Zhang et al., 2011; Zhang et al., 2022). In contrast, no attempts have been made to culture the virus in studies related to WTPs or other human settings such as schools, universities, or aircraft. Embryonated chicken eggs were the predominant technique used to isolate influenza virus, while MDCK cells were also used in two cases (Table S8). After virus culture, the most common identification method used was the haemagglutination assay. Electron microscopy and molecular methods (such as real-time RT-PCR or conventional RT-PCR) were also used in some studies. In poultry environments, the percentage of positive hemagglutination test results ranged from 0 % to 100 % across studies (Borchardt et al., 2017; Horm et al., 2012; Horm et al., 2013; Leung et al., 2007; Markwell and Shortridge 1982; Munoz-Aguayo et al., 2019;

Pawar et al., 2019). In the context of wild bird habitats, positive proportions ranged from 0.4 % to 69.8 % (Hinshaw et al., 1979; Ito et al., 1995; Khalil et al., 2022; Lai and McPhillips 1999; Okuya et al., 2015; Okuya et al., 2023; Ornelas-Eusebio et al., 2015; Zhang et al., 2011; Zhang et al., 2022).

Publication bias

The Egger test yielded p-values of 0.495, 0.103, and 0.499 for poultry environments, migratory wild bird environments, and WTP, respectively, indicating the absence of significant publication bias.

Discussion

This study aims to address a crucial aspect of the influenza transmission cycle, which is the potential role of the aquatic environments as a reservoir for influenza viruses. Bodies of water such as lakes and ponds can harbor these viruses for extended periods. Currently, there is a lack of comprehensive understanding regarding the occurrence of influenza viruses in various water environments. Therefore, our study aims to investigate thoroughly the extent to which influenza viruses are present in distinct aquatic settings (prevalence, virus concentrations, and detected subtypes), providing essential background information on the circulation dynamics on influenza viruses in such settings.

After an extensive search of bibliographic resources, a total of 43 papers were selected, two of which covered multiple research topics (on poultry and wild bird habitats, and on WTPs and schools, respectively). No studies were found on influenza in water environments associated with pigs. It is therefore imperative that future research addresses this area, with a particular focus on pig housing environments.

The studies cover a very wide time frame, from 1977 to 2023, with the studies on human water environments starting in 2009, but showing a significant increase in the period 2022–2023. The studies on domestic and wild bird habitats date back to 1977, showing that the importance of these hosts and their aquatic environments for influenza viruses has long been recognized.

Within the poultry environments, the prevalence of influenza A (from 4.3 % to 76.4 %) indicates a significant presence of the virus in different water matrices, with a heterogeneous distribution of the virus within this ecosystem. Migratory wild bird habitats also showed a considerable presence of influenza A, with prevalence ranging from 0.4 % to 69.8 %, with relevant regional differences. These geographical variations warrant further investigation.

Specific influenza A subtypes, including avian influenza and human seasonal influenza, were detected in waterbodies associated with poultry and in water samples from migratory wild bird habitats. Depending on the specific subtype, avian influenza can be classified as either highly pathogenic (HPAI) or low pathogenic (LPAI), resulting in different symptoms in infected birds. LPAI is associated with a mild disease that often goes unnoticed or has no observable symptoms. On the other hand, HPAI, which is associated with subtypes (H5 and H7) of type A, cause severe disease in birds, characterized by rapid transmission and high mortality rates in different species. The HPAI H5N1 was identified in three studies in Cambodia in 2007 and 2011 and in China in 2007–2008 in water sampled from both birds and poultry environments. This subtype was first identified in China in 1996 and infected humans in Hong Kong in 1997, resulting in 18 cases and six deaths. Since 2003, this virus has evolved into different phylogenetic lineages and human H5N1 HPAI infections have been reported in several countries. Although human-to-human transmission hasn't been observed, H5N1 HPAI has pandemic potential. Importantly, it has been found in migratory waterfowl, which may have contributed to its spread to new regions.

When examining the prevalence of influenza in wastewater samples collected from WTPs, the proportion of samples positive for influenza A ranged from 0.0 % to 97.6 %. In contrast, influenza B had a lower prevalence, with most studies reporting a zero detection rate, one study from California, USA, reporting 5.6 % and one from Germany reporting 46.9 %. These differences may be related to regional differences in virus circulation, local vaccination efforts, sporadic outbreaks or methodological issues. In terms of the chronology of studies on the occurrence of influenza virus in urban wastewater, the first investigation date back to 2009 (Heijnen and Medema 2011b), when researchers analyzed the presence of both common influenza A and the specific 2009 pandemic H1N1 strain in wastewater and surface water samples in the Netherlands. All other studies related to wastewater-based epidemiology for influenza viruses were published between 2022 and 2023, following the emergence of COVID-19, and the successful implementation of global environmental surveillance worldwide for this and other respiratory viruses (Barcellos et al., 2023; Bhattacharya et al., 2021). Several studies have demonstrated indeed the potential of WBE to monitor and predict human influenza outbreaks using samples collected from wastewater treatment plants. The range of influenza virus concentrations detected in untreated wastewater showed significant variability. Similarly, water sources associated with poultry environments and habitats of migratory wild birds showed a wide range of virus concentrations, with up to 2×10^8 genome copies /L) detected in drinking water collected inside duck cages in Cambodia. These variations may be potentially related to different levels of contamination, local epidemiological conditions, and various other influencing factors, including analytical ones.

In 16 studies carried out in poultry or wild bird habitats, attempts were made to isolate the virus using culture methods, with successful isolation achieved in all but one case, hence indicating the presence of infectious virus in these environments and the possibility of transmission through contaminated water. This has implications for both animals and humans who come into contact with these water sources, particularly in densely populated areas where the risk of exposure is higher. Of particular importance is the work of Zhang et al., which highlights the potential for the transmission of avian influenza (H5N1) between wild waterfowl and poultry, with water bodies close to habitats playing a key role (Zhang et al., 2022).

The persistence of influenza virus in water sources can be influenced by several environmental factors, including temperature, pH, salinity, and water type (Perlas et al., 2023; Ramey et al., 2022). These factors collectively influence the risk of virus transmission through contact with contaminated water environments.

While we initially aimed to conduct a meta-analysis to estimate the prevalence of influenza viruses across various habitats, this approach proved unfeasible due to a paucity of studies and limited data on factors influencing viral variability in different environments. General factors affecting this variability include the sensitivity of detection methods, geographical region, seasonality, sample volume, and the specific type of Influenza virus. In human water treatment plants, the prevalence of the Influenza virus is subject to multiple variables. These include the stage at which water samples are collected during the treatment process (e.g., untreated, partially treated, or fully treated water), the efficacy of treatment methods (such as ozonation, UV irradiation, and filtration) in inactivating and/or removing the virus, the concentration of the virus in incoming wastewater, seasonal fluctuations in virus activity, and factors related to human population density and community hygiene practices. In poultry environments, factors specific to this setting include the density of poultry housing (overcrowding, free-range or caged breeding), vaccination practices, biosecurity measures (like sanitation and visitor control), and the susceptibility of different poultry species to various subtypes of Influenza A virus. Regarding wild bird habitats, known as reservoirs and vectors for influenza viruses, variables include seasonal patterns and bird migration trajectories, bird density at sampling sites, species-specific susceptibility to Influenza A virus subtypes, and environmental conditions such as pH, temperature, and salinity, which influence the virus's survival and persistence in water bodies. These complexities highlight the challenge in synthesizing data across diverse habitats and underscore the need for more targeted research to better understand the environmental dynamics of the Influenza virus. The geographical distribution of the studies included in this review is noteworthy, covering a wide range of countries, with prominent contributions from the United States, Japan, and China. It's worth noting that the majority of the studies were conducted in high-income countries, highlighting the need for more research in middle- and lower-income countries to comprehensively address this public health concern on a global scale.

The predominance of real-time RT-PCR as the primary method for influenza detection, used in 68.2 % of the studies, underlines its effectiveness in research related to influenza in aquatic environments. On the other hand, the use of influenza isolation from embryonated chicken

eggs or MDCK cells followed by various identification methods in 29.2 % of cases demonstrates the diversity of approaches used in influenza studies.

Looking ahead, future research efforts should prioritize the standardization of sampling protocols and methodologies for detection and/or quantification of influenza virus in water environments, to facilitate better comparability between studies, and the systematic inclusion of a minimum set of method quality controls (e.g. recovery and inhibition controls) and information (limit of detection, limit of quantification and analytical repeatability) in environmental virology studies.

The limited number of studies, as evidenced by the 43 identified in this review, underscores the pressing need for more comprehensive data to gain a nuanced understanding of the issue. In the context of the influenza virus, which possesses pandemic potential, it is imperative not to overlook any potential transmission pathways. Therefore, we advocate for a concerted effort to expand the scope and depth of data collection in order to better grasp the dynamics of influenza virus presence in various water environments. A more extensive dataset will not only contribute to a more robust understanding of the ecological aspects of influenza transmission but also play a crucial role in informing preventive strategies to mitigate potential pandemic threats.

Although this study provides valuable insights into the prevalence of influenza viruses in water environments, several limitations should be acknowledged. The paucity of studies in certain regions and water sources, coupled with the limited sample size in certain research efforts, limits the generalisability of the findings. To address these limitations, future research should prioritise larger sample sizes and more extensive geographical coverage.

Conclusion

- This study underscores the worldwide significance of influenza surveillance in aquatic environments, encompassing wastewater, poultry settings, and habitats frequented by migratory wild birds.
- Despite an extensive search, no studies on influenza in water environments associated with pigs were found, underscoring the need for future research in this area.
- The review highlights the need for standardized analytical approaches and further research in under-represented regions and water sources.
- In conclusion, this review offers valuable insights into the prevalence of influenza in aquatic environments and emphasizes the importance of continuous research and surveillance. This will enhance our understanding of disease epidemiology and inform global public health strategies.

CRediT authorship contribution statement

S Kenmoe: Conceptualization, Formal analysis, Supervision, Validation, Writing – original draft, Writing – review & editing. **GR Takuissu:** Data curation, Methodology, Validation, Writing – review & editing. **JT Ebogo-Belobo:** Data curation, Methodology, Writing – review & editing. **C Kengne-Ndé:** Data curation, Writing – review & editing. **DS Mbaga:** Data curation, Methodology, Writing – review & editing. **A Bowo-Ngandji:** Data curation, Methodology, Writing – review & editing. **JL Ondigui Ndzie:** Data curation, Methodology, Writing – review & editing. **R Kenfack-Momo:** Data curation, Methodology, Writing – review & editing. **S Tchatchouang:** Data curation, Methodology, Writing – review & editing. **R Lontuo Fogang:** Data curation, Methodology, Writing – review & editing. **E Zeuko'o Menkem:** Data curation, Methodology, Writing – review & editing. **GI Kame-Ngasse:** Data curation, Methodology, Writing – review & editing. **JN Magoudjou-Pekam:** Data curation, Methodology, Writing – review & editing. **S Puzelli:** Writing – review & editing. **L Lucentini:** Writing – review & editing. **C Veneri:** Writing – review & editing. **P Mancini:** Writing – review & editing. **G Bonanno Ferraro:** Writing – review & editing. **M Iaconelli:** Writing – review & editing. **C Del Giudice:** Writing – review & editing. **D Brandtner:** Writing – review & editing. **E Suffredini:** Validation, Writing – review & editing. **G La Rosa:** Conceptualization, Formal analysis, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

There is no further data to share beyond what is available in the main text and supplementary material.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.wroa.2023.100210.

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