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Review Article

Occurrence of *Vermamoeba vermiformis* in Environment and Keratitis Samples in Iran as a Public Health Concern: A Systematic Review

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Abstract

Background: *Vermamoeba vermiformis* is one of the prevalent Free-living amoebae (FLA) in the environment. In recent years, studies regarding the distribution of *V. vermiformis* have increased, focusing on the importance of this FLA as a human pathogen but its pathogenic potential is still unclear. Moreover, the endosymbiosis relationship between *V. vermiformis* and microorganisms may lead to the development of multi-antibiotic drug resistance pathogens and may increase FLA virulence.

Methods: We aimed to perform a systematic review of the data on the occurrence of *V. vermiformis* in the environment and keratitis samples in Iran. Articles on the occurrence of *V. vermiformis* in environment and keratitis samples in Iran were systematically searched in PubMed, Scopus, and Google Scholar between January 2000 and July 2023. The keywords were carefully selected and accurately used to search for all relevant research articles to the title of the article.

Results: Fourteen studies (n = 14) with a combined total of 436 environmental and clinical samples were included. The overall prevalence of *V. vermiformis* across all studies ranged from 2% to 50%, with the highest detection rates observed in drinking and surface waters.

Conclusion: Our findings suggest a wide distribution of *V. vermiformis* in water and different environmental sources in Iran. More comprehensive studies on the pathogenesis of this genus and the relationship between *V. vermiformis* and its endosymbiosis are research priorities.

Introduction

Free-living amoebae (FLAs), such as *Acanthamoeba*, *Vermamoeba*, *Balamuthia*, and *Naegleria* are widely distributed in nature and are considered ubiquitous (1, 2). *Acanthamoeba* is the most common FLA, classified into 23 genotypes (T1–T23) based on the small subunit of the 18s rRNA gene (3). FLAs are known to cause neurological and ocular complications through the central nervous system (CNS) and *Acanthamoeba* diseases, including *Acanthamoeba* granulomatous amoebic encephalitis (GAE) and keratitis (AK) (3, 4).

In 1997, *V. vermiformis* was identified as one of the FLAs and classified within the *Hartmannella* genus (5, 6). These pathogenic protozoa have two forms in their life cycle: trophozoite and cyst. The trophozoite exhibits an elongated, slug-like morphology with granular material in the cytoplasm, while the cyst form is small and has a two-layered wall. The encystment process typically occurs within 9-10 hours (7, 8).

Among FLAs, *Vermamoeba* and *Acanthamoeba* are the most prevalent genera. *V. vermiformis* has been isolated from various sources and is frequently found in different types of water sources, such as swimming pools, tap water, mineral water, thermal water, as well as in soil, dust, oral cavity, and dental units (1, 5, 8-16). While *V. vermiformis* is commonly reported in environmental samples, it is less frequently detected in clinical samples. Some studies have focused on *V. vermiformis* as an environmental contaminant, and there have been a few reports linking it to cases of keratitis (6, 17). Also, the role of *V. vermiformis* as a pathogenic FLA of humans is still unclear, and its draft genome has been only recently sequenced (18).

This FLA has also been reported to thrive in hospital water, particularly in ICU and CCU units, as well as in contact lenses and the biofilm of an ophthalmology hospital ward (19, 20). This ability to thrive in diverse environments increases the risk of infection, particularly for immunocompromised individuals, and may lead to more severe outcomes. Consequently, the capacity of *V. vermiformis* to persist in various environmental sources makes it a potential public health threat (17, 19, 20). *V. vermiformis* has shown remarkable resilience, as it can survive even in extreme temperatures. It has been isolated from snow samples, and it is capable of colonizing hot water systems (21, 22). Moreover, *V. Vermamoeba* has been implicated in food contamination, as it has been isolated from the gastrointestinal tract and feces of pigs, serving as a potential source for zoonotic food-borne contamination (23). Furthermore, it has also been found in kitchens (24).

Although *V. vermiformis* infections are rare, they have been reported in cases such as ocular ulcers and keratitis, and a symbiosis between *V. vermiformis* and *Acanthamoeba* from keratitis patients (17, 24). Further research is needed to fully understand the potential pathogenicity of *V. vermiformis* and its impact on human health.

V. vermiformis has been reported to harbor important bacterial pathogens such as *Legionella*, *Mycobacterium chelonae*, and various *chlamydia* species, as well as giant viruses like Fausto virus (23,25-29). This endosymbiotic relationship between *V. vermiformis* and these microorganisms may lead to multi-antibiotic drug resistance and increased virulence. Therefore, the bacterial and viral endosymbionts with FLAs may confer to the host, posing a risk, especially for immunocompromised individuals (6). Iran, with its diverse climatic zones, extensive freshwater systems, and varying levels of sanitation infrastructure, provides a unique ecological setting for studying the distribution of free-living amoebae. Several studies from Iran have reported *V. vermiformis* in both natural and hospital environments, highlighting its potential public health relevance (11-17).

Therefore, this systematic review aimed to investigate the occurrence of *V. vermiformis* in environment and keratitis in Iran.

Methods

The Preferred Reporting Items for reviews (PRISMA) guidelines were followed for this systematic review. To determine the occurrence of *V. vermiformis* in environment and keratitis in Iran, a literature search was carried out ranging from 1 June 2000 to 18 July 2023 in PubMed, Scopus, and Google Scholar. The literature was searched by using a combination of keywords as follows: "*Vermamoeba vermiformis*" AND "*Hartmannella*" AND "free-living amoebae OR FLA" AND "keratitis" AND "environment" AND "Iran" in the English language. This was complemented by using the check of references of the published articles to access their related studies.

For an article to be included in this study, it had to be peer-reviewed and available in full text, with its primary objective to isolate and identify *V. vermiformis* is one of the worldwide prevalent Free-living amoebae (FLA) in environments and keratitis in Iran. However, case reports of *V. vermiformis* in environment and keratitis, in Iran were included. A systematic review was performed for all the selected studies. Articles that were published in languages other than English, institutional protocols, other review papers, or other FLAs for the analysis of environment and keratitis in Iran were excluded from the study (Fig. 1).

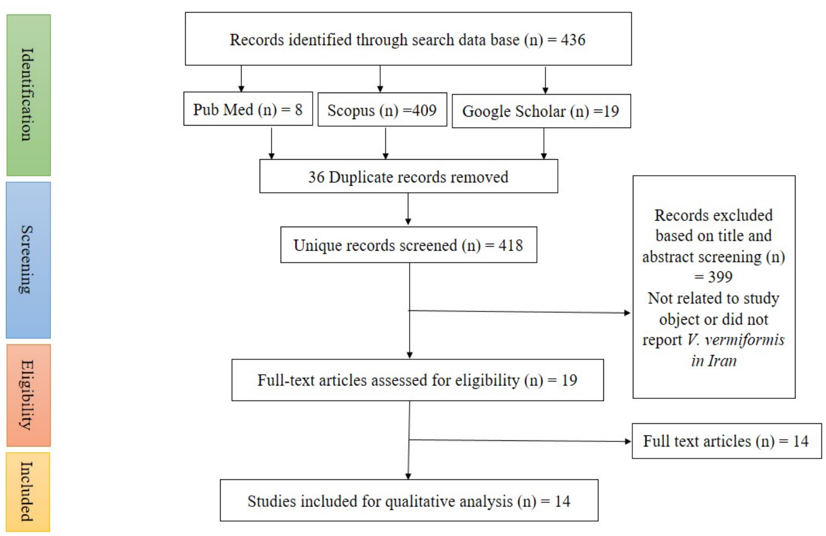

Fig. 1: Preferred Reporting Items for Systematic Reviews (PRISMA) diagram for selection of articles

Results

A total of 436 articles were systematically searched in PubMed, Scopus, and Google Scholar between January 2000 and July 2023. After accounting for duplication, 418 articles were identified for review. Upon examination of titles and abstracts, 399 records were excluded, leaving 19 articles that met the inclusion criteria. Finally, 14 full-text articles were assessed (Table 1).

Table 1: Included studies conducted occurrence of *Vermamoeba vermiformis* in environment and keratitis in Iran since 2000

Source	Rate (%)	City	Year	Reference
Dental-unit water	36.63	Jahrom	2022	(30)
Surface water, Drinking water and Stagnant water	50	Alborz	2022	(31)
Household biofilm	16.7	Tehran	2021	(24)
Recreational Public Soils	23.7	Mazandaran	2021	(32)
Dialysis fluid samples of hydraulic systems in hemodialysis units	5.18	Jahrom	2022	(33)
Mineral springs	12	Guilan	2020	(13)
Hot springs and beaches	2.4	Caspian Sea	2020	(34)
Intensive Care Unit (ICU) and Critical Care Unit (CCU) and Their Surrounding Environments	15	Tehran	2018	(19)
Drinking water and environmental sources	50	Semnan	2017	(9)
Surface and tap water	32.92	Shiraz	2016	(35)
Water	20	Kish	2015	(36)
Recreational water	5	Ahvaz Ilam	2015	(37)
Contact lenses	2	Tehran	2015	(17)

Discussion

Free-living amoebae (FLA) are widely distributed in both natural and artificial habitats (1-9). Initially*, V. vermiformis* was categorized within the *Hartmanella* genus using a microscopic key. However, recent taxonomy places *V. vermiformis* in the class Echinamoebida. Finally, *V. vermiformis* 18s rRNA genome has been sequenced to found different strains (6). Similar to other FLAs, its life cycle has an active stage (trophozoite) and a dormant stage (cyst). *V. vermiformis* using phagocytosis for feeding when attached to surfaces and is cultured using bacteria and yeast in vitro (38).

V. vermiformis provides a habitat for pathogenic bacteria such as *Stenotrophomonas maltophilia*, *Candidatus Rubidus massiliensis*, *Bacillus anthracis*, *Pseudomonas aeruginosa*, *Legionella* spp., and *Mycobacterium chelonae* (26, 39, 40). Wadowsky et al have proposed that *V. vermiformis* could play a significant role as protists in the ecology of *Legionella pneumophila* within water systems (41). Some of these microorganisms are not only human pathogens but also commonly associated with conferring resistance against pathogenic viruses, bacteria, and fungi. The incidence of FLA-related diseases may vary due to differences in the prevalence of risk factors and the sensitivity of health surveillance strategies (42). Recent studies indicate that *V. vermiformis* is frequently detected in dust, soil, air, and water sources, including cases of keratitis (9, 17, 19, 20, 23, 31, 36, 37, 43).

V. vermiformis isolates from water

It has been consistently isolated worldwide from various water sources, including tap and bottled water, rivers, seawater, beaches, swimming pools, springs, and thermal waters. Additionally, instances of *V. vermiformis* presence have been documented in snow water. (6, 9, 12, 18, 44). Until now, numerous strains of *Vermamoeba* have been documented across different regions of Iran. Niyyati et al detected *Vermamoeba* in tap water and recreational water in the southwestern region and Kish Island. They reported a high occurrence of potentially FLA in both tap water and recreational water (36). Furthermore, they noted that all recreational water samples showed FLA contamination, suggesting that inadequate filtration might be responsible for waterborne contamination (36, 37). Similarly, Javanmard et al reported the presence of *V. vermiformis* in drinking, surface, and stagnant water sources in the Alborz province. The findings underscored a notable connection between the presence of FLAs, particularly in drinking water, and heightened human activity in places such as sports clubs, hospitals, and eye clinics (31). An investigation also documented the presence of *V. vermiformis* in drinking water, notably with a significant prevalence observed in Semnan city (9). Similarly, another study identified *V. vermiformis* in a swimming pool situated in Shiraz city (35). Furthermore, *V. vermiformis* was isolated from both hot spring mineral water in Guilan and the Caspian Sea in Mazandaran (34, 35). Additionally, reports from Thailand indicated the presence of *V. vermiformis* in tissue samples of freshwater fish (2). Moreover, in samples of biofilms and drinking water, the abundance of *V. vermiformis* exceeded that of *A. castellanni* (45). This outcome suggests the potential significance of enhancing water filtration techniques by using smaller filters.

V. vermiformis isolates from environment

Vermamoeba has been discovered in various settings, including towers, hospitals, household sewages, industrial composts, dust, dental units, soil and bioaerosols, and in humans (40, 46). Specifically in relation to humans, it has been identified in HIV/AIDS patients and dialysis fluid samples within hydraulic systems of hemodialysis units (33, 43). Numerous studies have reported *V. vermiformis* isolations from diverse environmental sources, such as farmland soils, recreational areas, and public spaces (9, 12, 32). The presence of *V. vermiformis* in soil can pose a significant risk to both residents (32). Niyyati et al revealed the presence of *V. vermiformis* in Critical Care Units (CCU) and Intensive Care Units (ICU). Particularly concerning is the identification of *V. vermiformis* in hospital environments. This finding emphasizes that the presence of FLAs in both environmental and clinical samples from hospitals could directly threaten the health status of patients, particularly in immunosuppressed patients. Specifically, future studies should adopt multicenter surveillance programs across different provinces of Iran to evaluate the prevalence of FLAs in various hospital units, including ICUs, CCUs, dialysis centers, and ophthalmology wards. These studies should combine culture-based methods with molecular identification techniques (such as PCR and sequencing of 18S rRNA or mitochondrial genes) to ensure accurate detection and genotyping of *V. vermiformis* and related amoebae (18).

We also recommend establishing collaborative research networks involving microbiologists, ophthalmologists, environmental health specialists, and infection control teams to systematically monitor potential contamination routes (e.g., tap water, humidifiers, air-conditioning systems, and medical instruments). Developing a national database for reporting FLA contamination in hospital environments would improve data sharing and early detection of infection clusters. Furthermore, prospective studies focusing on immunocompromised or transplant patients could clarify the clinical significance of *V. vermiformis* colonization and its interaction with bacterial endosymbionts that may contribute to opportunistic infections. Collaborative projects with public health authorities could also facilitate the creation of standardized disinfection and water management guidelines specific to FLA control in hospital settings. These research efforts would not only fill current data gaps but also strengthen the evidence base for preventing FLA-related infections in healthcare facilities in Iran and similar endemic regions. The occurrence of FLA cysts within hospital environments thus presents a serious health concern (19). Furthermore, these amoebas have the capability to serve as carriers and transmitters of pathogenic bacteria (47). Cysts of FLAs, including *Acanthamoeba* spp., exhibit remarkable resistance to disinfectants and are capable of enduring challenging environmental conditions (48). There is limited data regarding the presence of FLA in both hospitalized patients and their clinical setting environment. In fact, Iran has seen only a handful of studies investigating the prevalence of *Acanthamoeba* spp. among specific patient groups, such as cancer patients, heart transplant recipients, and immunosuppressed patients (1, 49). Similar investigations have been conducted globally to assess the contamination of hospitals from various sources by FLAs (50, 51). Finally, increase awareness of hospital staff about FLAs, improvement of disinfection methods and using efficient disinfectants in hospital, particularly in wards which immunodeficient or immunosuppressed patients hospitalized is necessary (Fig. 2).

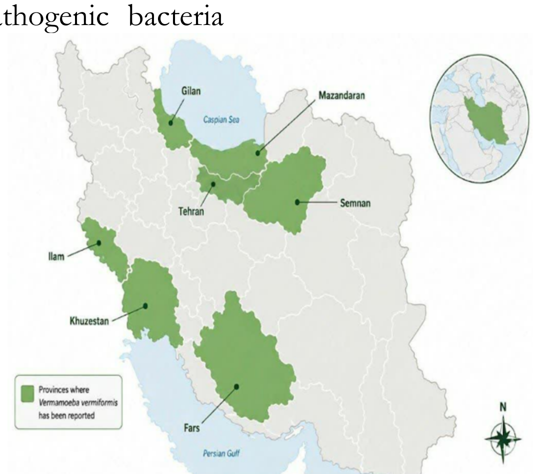

Fig. 2: Occurrence of *V. vermiformis* in different regions of Iran

V. vermiformis isolates from AK

Acanthamoeba keratitis (AK) is a painful and severe corneal disease the potential to result in blindness (52). The symptoms and clinical manifestations of AK encompass intense pain, excessive tearing, sensitivity to light (photophobia), inflammation, corneal abrasions and cloudiness, even corneal perforation, and vision loss (53). In contrast, AK can also manifest in immunocompetent individuals due to inadequate lens care practices or following corneal trauma (53). The role of FLAs in causing AK still requires further clarification regarding their pathogenicity. Over recent years, the prevalence of AK has witnessed a rapid increase, driven by heightened awareness of this infection as a significant threat to human health (54). Although *V. vermiformis* infections are relatively rare, evidence suggests that it may play a role in ocular diseases such as *Acanthamoeba* keratitis, either alone or in mixed infections (54,55). *V. vermiformis* can adhere to and invade corneal epithelial cells, potentially causing cytopathic effects like those observed with *Acanthamoeba* species. Moreover, co-infections with *Acanthamoeba* may enhance the pathogenic potential of both organisms. Endosymbiotic relationships with bacteria and viruses within *V. vermiformis* may further increase virulence and resistance, creating a complex microenvironment that could exacerbate corneal damage. Understanding these mechanisms is essential for clarifying the pathogenic contribution of *V. vermiformis* in AK and for developing more effective diagnostic and therapeutic strategies (27,26-29).

Several effective methods for isolating FLAs in cases of keratitis have been demonstrated. These include corneal scraping, corneal biopsies, and corneal smears, alongside the collection of contact lenses and related paraphernalia such as lens cases and cleaning solutions. For the detection of FLAs in patients with keratitis, it is recommended to use molecular testing methods, such as conventional Polymerase Chain Reaction (PCR) (55, 56). Hajialilo et al reported the presence of *V. vermiformis* in one culture containing mixed amoebae, including *Acanthamoeba* and *Vermamoeba*, and another culture showing the exclusive presence of *Vermamoeba*. Certain strains of *Acanthamoeba* (T2, T3, T4, T11), *Vahlkampfia*, and *V. vermiformis* have been implicated as potential causes of amoebic keratitis in Iran (17). In recent years, aside from *Acanthamoeba*, other FLAs have also been reported in cases of keratitis (57). Lasjerdi et al found *V. vermiformis* in ophthalmology (20). A previous study demonstrated that *V. vermiformis* isolated from a human keratitis case could induce a cytopathic effect on keratocytes in vitro as observed with *Acanthamoeba*. However, as of now, no confirmed instances of deep-seated infections resulting from *V. vermiformis* have been documented in the literature (58,59). The pathogenic potential of *V. vermiformis* is thought to be related to several biological factors, including its ability to form resilient cysts that withstand adverse environmental conditions, its capacity to adhere to and invade corneal epithelial cells, and its role as a "Trojan horse" for harboring pathogenic microorganisms such as *Legionella, Mycobacterium, and Chlamydia*-like bacteria. These endosymbiotic relationships can enhance both the survival and virulence of the amoeba, thereby contributing to ocular infections such as *Acanthamoeba* keratitis (26,29-31).

V. vermiformis isolates from worldwide

Vermamoeba has been discovered in various settings worldwide, including water sources, tap water, hospital water systems, hospital air conditioning units, snow, soil, fish, strawberries, and vegetables (Table 2). Pangnier et al highlighted the presence of *V. vermiformis* in hospital water in France as a significant concern for immunocompromised patients (59). Previous studies have consistently indicated elevated levels of protozoa in hospital water, with prevalence ranging from 50% to nearly 100% in certain locations (60).

Numerous other studies have also reported the presence of *V. vermiformis* in various water sources (Table 2) (Fig. 3). For instance, *V. vermiformis* was first identified in the water resources of the island of Lanzarote (61). Moreover, isolates collected in this study were found in recreational areas with high human activity, underscoring the importance of raising awareness (62). Filippo et al, detected *V. Vermiformis* in natural thermal waters in Italy (63). Furthermore, studies from Brazil, Egypt, Korea, Italy, and Turkey have also reported the presence of *V. Vermiformis* (64-69). Additionally, reports from Philippines indicated the presence of *V. vermiformis* in the intestinal contents of freshwater fish (2). Interestingly, this FLA has been detected in vegetables and strawberries in Spain, suggesting that consumers may be exposed to protozoan parasites by consuming organic produce.

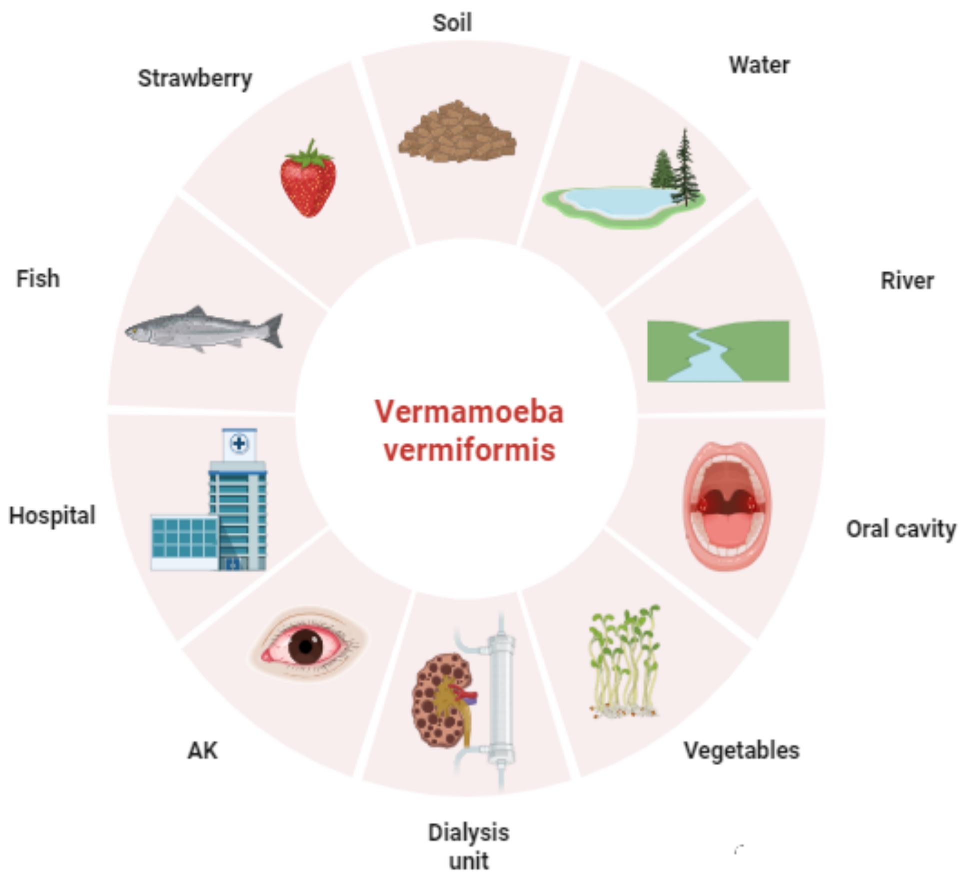

Fig. 3: Sources of *V. vermiformis* presence in the world

These findings underscore the importance of assessing the risk of foodborne protozoan parasites present in organic leafy greens and strawberries available in local markets (70). Reyes-Batlle et al reported a 3% prevalence of *V. vermiformis* in irrigation waters and soils of an insular arid agroecosystem, indicating the ability of FLAs to withstand harsh environmental conditions (71). Another study demonstrated the isolation of *V. vermiformis* from hospital air conditioning units, raising concerns about the risk of *Vermamoeba* infection in healthcare settings. Additionally, these amoebas could serve as hosts for fungi, bacteria, and viruses, indirectly facilitating the transmission of these pathogens. To mitigate these risks, regular cleaning of air conditioner filters and grilles using effective disinfectants is essential to reduce the prevalence of FLAs (72).

Table 2: Occurrence of *V. vermiformis* in the world

source	Rate (%)	City	Year	Reference
hospital water	11.6	France	2006	60
Snow mount		Spain	2015	21
Soil	20.8	Spain	2016	62
Water	10	Spain	2016	22
Freshwater fish	49	Philippines	2017	2
Water	55	Italy	2019	63
Water	25	Brazil	2023	64
Drinking water	9.6	Egypt	2021	65
Oreochromis sp		Malesia	2023	74
Water	45.6	Italy	2015	66
Soil	8.6	Santiago	2021	75
Water		Korea	2016	67
Tap water		Turkey	2018	68
Well water	50	Turkey	2020	69
Hospital air condition	41.6	Turkey	2017	72
Vegetables and strawberry	17.3	Spain	2023	70
irrigation waters and soils	53.8	Spain	2021	71
Domestic and hospital water	55	Australia	2022	73

Conclusion

Considering the prevalence of *V. vermiformis* in water sources especially drinking water, environment and cases of keratitis is potential to harbor pathogenic microorganisms, and it is highly vital to provide adequate disinfection and enhancing water filtration in all drinking water distribution systems. Recent studies have demonstrated that chlorine dioxide, hydrogen peroxide vapor, and peracetic acid are effective against *V. vermiformis* cysts and trophozoites when applied at appropriate concentrations and contact times. Thermal disinfection (e.g., maintaining hot water systems above 60 °C) and routine biofilm removal in water storage tanks and pipelines are also recommended strategies to minimize amoebic persistence.

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Conflict of interests

The authors declare that there is no conflict of interest.

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