**Vermamoeba vermiformis**—Global Trend and Future Perspective

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Free living amoebas (FLAs) are considered widespread in nature and therefore called ubiquitous (Lares-Villa et al., 2010); its spectrum of habitation extends from aquatic reservoir to terrestrial places (Scheikl et al., 2014). Of the organisms considered as free living amoebae; members of the genera *Acanthamoeba*, *Naegleria*, *Hartmannella*, and *Balamuthia* are known to infect humans (World Health Organization) and are considered medically important due to the fatal disease observed in their hosts. These organisms are considered free living in nature but are considered parasitic once they enter the hosts.

Previously known as *Hartmannella vermiformis*, *Vermamoeba vermiformis* has been separated to a group under the order *Echinamoebida* as it is different from all other *Hartmannella* spp. in that it is worm-shaped and not clavate. Various sequences in GenBank isolated from environmental sequences points to multiple lineage (Smirnov et al., 2011). Page 1997 and Smirnov et al. (2011) provide morphologic descriptions which are key and invaluable in the identification of *V. vermiformis* coupled with the usual culture and molecular results. Widely dispersed in an array of environmental niches, viable *V. vermiformis* has been isolated from springs with recorded temperatures of 40°C and snow samples of below 0°C at altitudes of greater than 2700 m above sea level (Reyes-Batlle et al., 2015). Hospital environments have also shown to be positive for *Vermamoeba* isolates that are thermotolerant up to 46.5°C–55.7°C.

It should be noted that in the identification of amoeba, species may be diverse even in a small area. It has been suggested that there is no correlation between geographical origin of the strain and their relatedness. Multiview approach as it is mentioned above remains the best practice for identification. Trophozoites should be studied for characteristic morphology, locomotion, nuclear characteristics, and cyst structure. Molecular identification is the best method for definitive identification of the target organisms up to the genetic level and is not hampered by polymorphisms that may be encountered in microscopic observation (Walochink et al., 2002). Microscopic observation is essential so as to provide phenotypic characteristics that can be coupled to genetic sequences that will be deposited in databases (De Jonckheere et al., 2012).

Trophozoites length is 22.5–42.5 and 2.5–5.0 μm in width, are elongated, cylindrical, limax or slug-like in motility by the aid of a single pseudopodia as shown in Figs. 1 and 2; in some cases, extensively branching with 4–5 pseudopodia as observed in samples isolated from water, an example of which can be seen in Fig. 3 with the trophozoite extending lateral projections. Average speed of locomotion has been measured at 36.4 μm/min. Diameter of nucleus is 2–3 μm (Dykova et al., 1997). Transmission electron microscopy (TEM) investigations of *V. vermiformis* culture stages have demonstrated 6–8 μm trophozoites and scanning electron microscopy (SEM) has shown approximately 6 μm cystic stages (Fouque et al., 2012).

*V. vermiformis* for effective grazing of prey bacteria requires that the bacterial prey be attached to the surface of the free-living amoeba (FLA) as it is known to be a surface feeder. Laboratory models have demonstrated that mere suspension of FLA surface feeder types with prey bacteria is insufficient to initiate feeding. Rather, it promotes starvation response, where encystment is observed in the case of *Acanthamoeba castellanii* and the formation of round bodies in *Vermamoeba*. Further, initiation of encystment in a single cell of *A. castellanii* promotes a cascade of encystment on the surrounding cells as well which suggests cell to cell signaling. Encysted cells transform to trophic stages once introduced to a surface with bacterial prey (Pickup et al., 2007).

Cyst diameters are recorded at 6–9.5 μm (Dykova et al., 1997). Cyst wall has two layers (Fouque et al., 2015a). Studies are rare relative to *Vermamoeba* wall composition. Cyst walls are described as being composed of proteins and low density of glucose
polymer making it unsuitable to be stained by cellulose-specific markers. The bilayer cyst wall contains an endocyst 140 nm thick and an ectocyst measuring the same, which is composed of layers of filamentous material (Fouque et al., 2012). These structures points out to the resistance of this free-living-amoebe against disinfection particularly when it is in the cystic stage. In addition, studies relative to the ultrastructure and signaling pathways, which direct encystment and excystment are lacking; bridging this gap will enhance our knowledge on the inner workings of FLAs and may offer a better understanding in the development of appropriate and effective interventions in as far as disinfection and prevention of spread is concerned as well as the possible interactions in terms

Fig. 1. Micrograph of isolated Vermamoeba vermiformis (MF716853). (A) Trophozoite with extension of cytoplasmic pseudopodia (black arrow) and (B) cystic stage.

Fig. 2 Vermamoeba trophozoite isolated from underground water.
of hazard or benefit when introduced to a suitable host. Mature cysts exhibit a natural fluorescence when subjected to wavelengths of 488 nm because of the presence of natural flavins and nicotinamide compounds from without and within the cyst structures (Dillon et al., 2014a). Some mature cyst may demonstrate binucleation (Cabello-Vilchez et al., 2014) and excystment is mediated by the organization of large vesicles proposed to contain hydrolases close to the cyst wall to aid in the transition. Encystation and excystation remains an area of Vermamoeba concern with gaps in research as this FLA is unique compared to Acanthamoeba and Naegleria (Fouque et al., 2015a). An immature cyst has a single nucleus with a single central nucleolus (Dykova et al., 1997). Chromatin bodies between the nucleolus and nuclear membrane have been noted in all stages especially during encystation (Dykova et al., 1997).

Studies have shown that 100% encystment takes about 9 h in 25°C–37°C under alkaline pH (8–9) in moderate osmotic pressure (0.1 mol L⁻¹ KCl). Further, encystment rate is directly proportional to cell concentration. It should be noted that variations of results were observed between environmental samples and reference strains (ATCC 50237) (Trouilhe et al., 2014). Viable strains of Vermamoeba have been isolated from filtered hot springs with recorded temperatures of 41°C–53°C and pH of 4.90–7.0. This is an evidence that filtration alone is insufficient for decontamination of water samples with this FLA (Solgi et al., 2012). Hence, improved methods of filtration and other disinfection methodologies appropriate to eliminate even cyst forms are of importance.

**Epidemiology**

Large concentrations of research and collaboration in the study of Vermamoeba are found in Europe and South East Asia and viable trophic and cystic forms have been isolated from a wide array of environmental conditions as well as in a variety of inanimate surfaces and living tissue. Fig. 4 maps and Fig. 5 outlines that Vermamoeba has been isolated from various sources such as natural water sources (Armand et al., 2016; Asiri et al., 1990; Hsu et al., 2011; Mears et al., 2016; Nazar et al., 2012; Tsvetkova et al., 2004; Park, 2016), soil (Cateau et al., 2014a; De Jonckheere et al., 2012), snow (Reyes-Batlle et al., 2015) and even in industrial compost heap, bio aerosols (Conza et al., 2013), and cooling towers (Scheikl et al., 2014). Hospital (Cateau et al., 2009, 2014b; Fields et al., 1990; Lasjerdi et al., 2015; Nahapetian et al., 1991; Neri et al., 2015; Henning et al., 2007; Ozcelik et al., 2017; Rohr et al., 1998; Sanden et al., 1992) and household samples (Gaëlle Reteno et al., 2015; Lu et al., 2017) have also been identified and are of concern relative to immunocompromised individuals. Mammals, birds and humans have also contributed to the isolation of this ubiquitous FLA. Human samples contaminated with Vermamoeba are rare and have only been reported so far in published articles with institutions originating from Costa Rica (Cabello-Vilchez et al., 2014), Scotland (Cabello-Vilchez et al., 2014), Spain (Cabello-Vilchez et al., 2014; Lorenzo-Morales et al., 2007), France (Bouchoucha et al., 2016), Iran (Abedkhojaste et al., 2013; Hajialiloo et al., 2015), Japan (Inoue et al., 1998), and Peru (Cabello-Vilchez et al., 2014). Interestingly, Vermamoeba has even been isolated from bottled water (Montalbano Di Filippo et al., 2015).

Isolation of Vermamoeba from a variety of natural and man-made water sources highlights the main source of spread of this FLA to other surfaces increasing the potential for infection. Vermamoeba demonstrates survival in cold environments and high altitudes like snow collected from mountains to thermotolerant activities of samples recovered from hot springs. Tap (Delafont et al., 2015; Loret and Greub, 2010; Niyiyati et al., 2015; Lal et al., 2015), and tank water isolates (Dobrowsky et al., 2016; Qin et al., 2017; Wadowsky et al., 1991) are important concerns as this is indicative of both the level and methodology of sanitation employed by the water treatment facility (Smimov and Michel, 1999; Thomas et al., 2008; Walochink et al., 2002) as well as the resistance to disinfection exhibited by this FLA. Its isolation in biofilms as well as the endosymbiotic relationship of bacteria, fungi, viruses and virions is of interest in terms of their interactions in biofilms with the amoeba cell.
Vermamoeba isolation from animals (Kadlec, 1978; Milanez et al., 2017; Chavatte et al., 2016; Mulec et al., 2016; Dyková and Tyml, 2016; Dykova et al., 2005) provides substantial knowledge in the biological pathway of spread of this organism through animate vectors. The explanation of how this FLA behaves inside its animal host may significantly contribute to literatures of its effective control and subsequently provide more models of infectious process to be observed and compared relatively to human pathogenesis. As human isolates of Vermamoeba have been documented (Inoue et al., 1998; Montalbano Di Filippo et al., 2015) but no clear proof of its exclusivity as the sole causative agent of the infectious or inflammatory process are put forth for the direct role of Vermamoeba as a mere hitchhiker, irritant, infectious agent or biological niche for increased virulence of endosymbionts remains elusive.

Pathogenesis

Vermamoeba isolated from Tilapia kidney tissues have been demonstrated to cause pathogenesis when introduced to other tilapias, gold fishes and carps (Dyková and Tyml, 2016). Highest incidence of proliferation of Vermamoeba in these animals models were observed in the liver, spleen pancreas and mesenteries in the form of granulomas. Carps were found to be most susceptible but mice models for pathogenicity studies failed to provide conclusive results (Dykova et al., 1997). Efforts to test Legionellosis via Vermamoeba vector utilized the inoculation of murine models. In vivo results show that inhalation of Vermamoeba with Legionella pneumophila endosymbiont can potentially cause pulmonary disease (Brieland et al., 1997a). Cell models for cytopathic effects of Vermamoeba have been conducted on keratocyte monolayers. Clinical isolates were tested and after 24 h of incubation with cell cultures, and showed cellular damage. Further, cytopathic products from Vermamoeba also induced cytopathic effects even without cellular contact. These observations are similar with those observed in studies of A. castellanii—the causative agent of Acanthamoeba keratitis (Kinnear, 2003).

Vermamoeba has also been discovered from actual keratitis cases (Balczun and Scheid, 2017) as well as simultaneous isolation of both Vermamoeba and Acanthamoeba in a case of severe amoebic keratitis. The patient’s corneal scrapings and contact lens solution were both positive for Acanthamoeba and Vermamoeba cyst and trophozoites (Lorenzo-Morales et al., 2007; Hajialilo et al., 2015; Dyková and Tyml, 2016). Taking into consideration this particular case, it can be hypothesized that endosymbionts present in the
isolated FLAs may also play a role in increasing the gravity of the infection and can also influence the patient’s response to drug treatment or other types of intervention.

Contact lens solutions from patients suffering from keratitis have been found positive for *Vermamoeba* isolates and bacteria such as *Enterobacter cloacae, Stenotrophomonas maltophilia, Xanthobacter flavus, Pseudomonas aeruginosa,* and *Mycobacterium chelonae* (Bouchoucha et al., 2016). This manifestation of a variety of bacterial cocultures may either be reflective of environmental or nosocomial contaminants or poor hygiene the patient was employing relative to contact lens care leading to keratitis.

At present, there is only one published case of an exclusive isolation of *Vermamoeba* from a patient’s contact lens solution and storage case. The patient manifested with eye pain, redness, blurred vision, photosensitivity, tearing and a sensation of a foreign body in her eye. The infection was poorly responsive to anti-Acanthamoeba drugs but was resolved with Polyhexamethylene biguanide (PHMB) 0.02% treatment for 2 weeks (Abedkhojaste et al., 2013). This documented case of a single-species-amoebla isolate helps in understanding the pathogenicity that *V. vermiformis* can confer to a susceptible mammalian host and further identification of similar cases are necessary to establish the spectrum of conditions that may be observed in immunocompetent and in immunocompromised individuals.

Hospital ward dust and biofilms have also been shown to harbor *Vermamoeba* in the same way that medical instruments and slit lamps have been positive for *Acantthamoeba* T4 genotype. Other isolates were *Vahlkampfia, Naegleria,* and *Echinamoeba* (Lasjerdi et al., 2015). Nasal swabs from an HIV patient demonstrated positivity for *Vermamoeba* with an endosymbiont of pathogenic *M. chelonae* (Cabello-Vilchez et al., 2014). What is interesting about this report aside from the discovery of yet another endosymbiont of *Vermamoeba* is that it shows how this FLA can thrive in the nasal mucosa of a mammalian host which leads us to hypothesize that *V. vermiformis* can confer to a susceptible mammalian host and further identification of similar cases are necessary to establish the spectrum of conditions that may be observed in immunocompetent and in immunocompromised individuals.

Fig. 5  *Vermamoeba* Research Mapping (VRM) according to sampling sources.

Endosymbionts

Various studies and models have shown how *V. vermiformis* can act as a “Trojan Horse” for pathogenic strains of bacteria the likes of *Bacillus anthracis, P. aeruginosa, Legionella spp., Neochlamydia hartmannellae, Waddlia* and other *Chlamydia*-like endosymbionts. This provision of intracellular niche for growth and replication is not limited to bacteria but are also extended to fungal species and viruses (Balczun and Scheid, 2017). Surprisingly, there is no such documentation on published articles yet relatively to preying of *Vermamoeba* on other FLAs (and FLA stages thereof).
Identification of endosymbionts of FLA in particular endosymbionts of Vermamoeba is valuable not only in the perspective of the microbial, fungal, or viral tenacity but in the possibilities of these amoeba-resisting-organisms potential for pathogenicity. Supporting to this, it is of great curiosity to establish if their relationship with a suitable amoebic host influences acquisition of virulence factors and the forces this relationship exert on each organism’s survival, spread, development and evolution (Scheid, 2014).

A variety of factors have been suggested to influence the predator-prey interaction between FLA and bacteria. Amoeba ingestion of bacteria can be inhibited by toxic pigments, outer membrane structures and other virulence factors unique for bacteria (Weekers et al., 1993). Furthermore, environmental strains and clinical isolates have been shown to behave differently compared to their laboratory control counterparts (Wadowsky et al., 1991). Hence, the importance of thoroughly understanding the predator-prey relationship not only in the research perspective of endosymbiosis but also on its effects to the environment like the release of nitrogen from soil and possibly water environments as a by-product of the digestion of bacteria and other microbes by amoeba.

A cyst-forming FLA is a potential host for endosymbionts and offers a barrier of protection against adverse conditions such as temperatures in extreme, Ultra-violet radiation, ozone, chloride dioxide, monochloramine and heavy metals like copper and silver (Muchesa et al., 2017). It is clearly demonstrated in several studies that low disinfectant concentrations have limited activity on FLA and allows for the formation of biofilms that in turn increases amoeba and bacteria persistence. The passage of endosymbionts in amoebal cells has potential in the alteration of virulence or antibiotic resistance which in turn may play a significant role in the bacterial survival within an infected host’s macrophages (Muchesa et al., 2017). FLA being hosts to bacteria provides a “biological gym” for pathogens to potentially enhance their disease causing potentials. Further, antibiotic resistant bacteria can exchange genetic materials with other intracellular pathogens in developing novel virulence traits for enhanced survival within macrophages (Rubenina et al., 2017). Although Vermamoeba as a causative agent of a disease state is still debatable, its viable isolation in human diseased tissues suggests a role in the inflammatory process (Muchesa et al., 2017). Hence, the importance of contributing to the growing body of research performed on Vermamoeba and a wider perspective of its implication not only to the environment but also to mammalian and nonmammalian host is necessary.

Bacteria

Modeling pathogenicity studies for V. vermiformis has found it useful in utilizing axenic cultures of Strain CDC 19 deposited as American Type Culture Collection (ATCC 50237). It is particularly useful for protozoan-bacterial interaction research (Fields et al., 1990). Evidence points that a 170-kD Gal/GalNAc is one lectin receptor of V. vermiformis exploited by L. pneumophila for its attachment and invasion and that other proteins and time-dependent tyrosine dephosphorylation are elements also involved in the process (Venkataraman et al., 1997). Legionella spp. has been observed to survive in encysted cells (Armand et al., 2016) and cyst walls as well (Greub and Raoult, 2003), fluorescent in situ hybridization (FISH) can be utilized in the intracellular identification of this bacteria within FLAs (Kuiper et al., 2004). It has been demonstrated that virulence of this bacteria is essential for the optimal intrapulmonary growth in mice models and supports the hypothesis that Vermamoeba potentially enhances bacterial growth and multiplication (Brieland et al., 1997b) and that temperature also plays a role in the endosymbionts replication within FLAs (Buse et al., 2017). Further, infection of mice host with Vermamoeba has been demonstrated to alter immune response which may be detrimental to the resolution of infections that may be caused by its corresponding endosymbiont which in turn may lead to more complicated inflammatory conditions. It has been demonstrated that cycloheximide interferes with the uptake of L. pneumophila (Abu Kwaik et al., 1994).

V. vermiformis has also been demonstrated to harbor S. maltophilia, an opportunistic pathogen isolated in hospital water systems. S. maltophilia has also been recovered from amoebal structures after 28 days exposure to harsh conditions demonstrating the role of V. vermiformis play in the survival, growth, and spread of this pathogen (Cateau et al., 2014b). In vitro models have shown that P. aeruginosa forms microcolonies restrictive of V. vermiformis grazing and that Escherichia coli and Staphylococcus aureus are favored as prey. However, clinical and environmental strains of the said bacteria behave differently when coinoculated with Vermamoeba (Rubenina et al., 2017; Cateau et al., 2008; Chavatte et al., 2014).

N. hartmannellae has a Chlamydia-like life cycle within cells of V. vermiformis. But unlike Chlamydia spp. does not reside inside vacuoles (Horn et al., 2000; Bou Khalil et al., 2016). Vermamoeba harboring this organism grew faster compared to counterparts without the same endosymbionts and suggests possible upregulation of biological processes in the presence of a suitable intracellular resident. Conversely, downregulation of biological processes has also been observed in cocultures of Acanthamoeba spp. and Parachlamydia-related endocytobiont making these interplay of reactions an interesting area for research (Collingro et al., 2004). Relative to this alteration of biochemical processes, Vermamoeba infected with these bacteria have a lost activity for encystation compared to noninfected counterparts that may be significant in either prolonging the life span or making the FLA more susceptible to harsh conditions. This is another area of interest as the same interactions are not observed in Legionella infected Vermamoeba (I-Henning et al., 2007). Lastly, very interesting is the endosymbiotic relationships shared between organisms and how they ultimately affect these organisms behavior in general and its protein expression in particular such as in the case of V. vermiformis harboring intracellular Chlamydia-like bacteria of N. hartmannellae, which in turn is infected with viruses and acts not only as an endosymbiont but as a bacteriophage endosymbiont of this FLA (Schmid et al., 2001; Michel et al., 2001).

Francisella novicida has been modeled in vitro in place of Francisella tularensis to demonstrate Francisella and Vermamoeba interactions. Results show that F. novicida replicates rapidly in Vermamoeba hosts compared to A. castellanii. F. novicida persists
and replicates inside nonacidic vacuoles of the amoebal host compared to being in the cytosol when in mammalian cells which is demonstrative of survival advantages of endosymbionts from exposure to harsh environments. IgIC protein is suspected of being essential for intravacuolar proliferation and interference with phagolysosomal fusion (Santic et al., 2011). It has been demonstrated in this model that *V. vermiformis* infected with *F. novicida* behaved the same as those infected with *Chlamydia*-like bacteria wherein their encystation was delayed and trophic stages were encouraged to persist.

Nasal swabs from HIV patients in Lima, Peru have been positive for *V. vermiformis* along with *M. chelonae* and encourages further studies in concepts of survival and propagation in the environment and host (Cabello-Vilchez et al., 2014). *Mycobacterium bovis*, the agent of Bovine tuberculosis survives within encysted cells of *Vermamoeba* for over 60 days which induces the development of pulmonary tuberculosis when inoculated in Balb/c mice, proof of bacterial viability within the amoebal cell in extended periods of time contributory to potential transmission of pathogenic organisms and causation of disease states to susceptible hosts (Sanchez-Hidalgo et al., 2017).

**Fungus**

Cocultures of fungi and amoebal supernatant has shown that *V. vermiformis* has a positive influence in *Aspergillus fumigatus* filamentation and may contribute to the severity of deep-seated infections with the organism (Maisonneuve et al., 2016). *Candida* yeast cells can also be internalized by *Vermamoeba* cells and are important considerations relative to immunocompromised host systems (Barbot et al., 2012). *Exophiala dermatitidis* and *Fusarium oxysporum* has been demonstrated to show increased growth potential in the presence of *Vermamoeba* trophozoites without observable detrimental effects to the FLA (Cateau et al., 2009, 2014a).

**Viruses**

*Faustovirus* is a new viral lineage discovered from *Vermamoeba* in the same manner as other giant viruses have been isolated from *Acanthamoeba*. These findings contribute to the knowledge *Vermamoeba* may be playing as viral protein factories within potential mammalian and nonmammalian systems (Gaëlle Reteno et al., 2015). In addition, some viruses may also act as virophages or virions which have been isolated from viral factories found within *Acanthamoeba* cells. These virions exploit the “host virus” machinery for replication similar to the manner of a bacteriophage (Bekliz et al., 2016). It is in this light that study of *Vermamoeba* and its endosymbionts are still further growing in that these research should encompass not only bacteria, fungi and viruses but virophages as well. At present, identification of virophages has been more dependent on metagenomics than culture methodologies.

**Isolation and Identification of *Vermamoeba***

**Sample Collection and Processing of Samples Potentially Containing *Vermamoeba***

*Vermamoeba* sp. has been successfully isolated from environmental samples through the analysis of subsurface water (Solgi et al., 2012), tap water samples (Wang et al., 2012), and hospital water systems (Maisonneuve et al., 2016). Although an established collection method (Method 1623) of 10 L of water sample for processing from the US Environmental Protection Agency (EPA) exists to guide researchers, there are a number of modifications that have been used in different studies in terms of the amount of water sample collected from sources ranging from 100 mL (Scheikl et al., 2014) to 1 L (Montalbano Di Filippo et al., 2015). It is important to note, however, that the volume of water sample collected at one time, though ample, may not necessarily mean successful isolation of *Vermamoeba* or FLAs of interest and that isolation rates may be increased by increasing the number of samples taken in terms of a wider and broader distribution of sampling areas and sampling frequencies from time of the day, days in a week and consideration of weather and seasonal conditions.

Generally, there are two phases of processing collected samples namely: concentration (filtration Solgi et al., 2012; Montalbano Di Filippo et al., 2015; Niyiyati et al., 2015 and ultracentrifugation Milanez et al., 2017) and cultivation (Pickup et al., 2007; Ozcelik et al., 2017; Abedkhojaste et al., 2013; Dykova et al., 2005; Fouque et al., 2015b; Scheikl et al., 2016) of resulting pellets after concentrating the samples. Cultivation protocols for the isolation of *Vermamoeba* sp. is relatively almost the same as compared with other FLAs which are cultured in Non nutrient agar lawned with either heat killed (Dykova et al., 1997; Cabello-Vilchez et al., 2014) or live colonies (Pickup et al., 2007) of *E. coli* which may serve as food source for the growing trophozoites in the media.

**Microscopic and Molecular Protocols for Identification of *Vermamoeba***

Identification of *Vermamoeba* sp. cystic stages and trophozoite forms may pose a challenge to researchers due to the fact that cystic stages may have morphological similarities to other cystic forms of FLAs. Although a morphologic key devised by Page (Solgi et al., 2012; Dykova et al., 2005) is available as reference to identify one FLA to another through the use of either a regular light microscope (Montalbano Di Filippo et al., 2015; Dykova et al., 2005) it is still highly recommended to use alternative methods such as transmission electron microscopy (Dykova et al., 1997) for morphologic analysis from cultures or the use of in situ hybridization (Dykova et al., 2005) using probes from samples obtained from lesions of patients infected.
Due to these challenges of identifying *Vermamoeba* spp. through microscopic methods, it is highly encouraged to utilize molecular techniques in the exact identification of this FLA. Current trends in extracting the DNA of this amoeba includes the use of phenol chloroform (Abedkhojaste et al., 2013; Hajialilo et al., 2015), available DNA extraction kits and Chelex resin (Solgi et al., 2012). More so, a number of specific primer kits are available for use which targeting the 18S rRNA of the protozoan, nonspecific universal primers may also be used for the direct identification of the organism provided that DNA sequencing will be performed.

**Control and Disinfection of Vermamoeba**

Relative to control of *Vermamoeba* in water samples, it has been shown that both cyst and trophozoites were susceptible to concentrations of chlorine ranging from 2.0–4.0 ppm. Concentrations above 10.0 ppm was shown to be lethal to *Vermamoeba* cocultured with *L. pneumophila* as it remained viable in concentrations of 4.0 ppm and further exposure to 60°C made all trophic states of the FLA nonviable (Donlan et al., 2005; Dupuy et al., 2016; Kuchta et al., 1993). In vitro studies have demonstrated that German drinking water regulation (10 + 100 µg/L Ag + Cu) s insufficient to inactivate *Vermamoeba* relative to 1:10 silver/copper combination (Rohr et al., 2000). Presence of biofilms in water systems including premise plumbing requires special attention as it increases survival of organisms in its community as FLAs can re-colonize waters rapidly in as early as 24 h after disinfection (Wang et al., 2012; Scheidt et al., 2016).

Heating and regular treatment of water with sodium hypochlorite, sodium bromide or isothiazolinones was not sufficient to significantly reduce occurrences of *Acanthamoeba* and *Vermamoeba* in water systems (Scheidt et al., 2014). Studies on reference and environmental strains of *Vermamoeba* show that cysts only became fully inactivated by the following intervention: 15 mg/L of chlorine for 10 min; 60°C for 30 min; and 0.5 g/L equivalent H₂O₂ of PAA mixed with H₂O₂ for 30 min; this methodology is in accordance to the French Circular relative to Legionella risk prevention in health facilities. In addition, subtilisin (0.625 U/mL for 24 h) has also shown promise in cyst inactivation (Fouque et al., 2015b). Modeling studies on dental unit water lines were also not successful in demonstrating susceptibility of *Vermamoeba* to commercial disinfectants (Costa et al., 2016).

Studies conducted on river supplied water treatment facility recorded the presence of *Vermamoeba* even in the ozonated, sand-filtered and carbon-filtered water samples. In addition, several species of FLAs were present in the different stages of water treatment along with the presence of free bacteria and endosymbions as well (Thomas et al., 2008). More interesting is that there was a higher diversity of FLA species isolated from the treatment process compared to the raw river water which suggests FLA colonization of the treatment facility. Amoeba infected with Amoeba-resisting-bacteria were mostly recovered from biofilm samples which suggests that biofilm environments facilitate effective interaction with and uptake of bacteria by FLAs. This study is evidence that even a well-operated treatment plant will not be an absolute barrier to contain these microbes and that continuous study is needed to further improve water treatment methodologies from a wide variety of microorganisms.

Solar pasteurization has been shown to reduce gene copies of *Vermamoeba* and *Naegleria* spp. in high temperatures of 68°C–93°C and 74°C–93°C respectively and that *Acanthamoeba* spp. is more resistant to heat compared to *Vermamoeba* (Dobrowsky et al., 2016).

There is no established intervention for *Vermamoeba* found in human hosts. *Acanthamoeba* trophozoites on the other hand are resolved by miconazole nitrate and natamycin while cystic stages are only sensitive to natamycin in as far as in vitro tests are concerned. Records show however that patients do not readily respond to these antiamoebic drugs (Inoue et al., 1998).

**In Vitro and In Vivo Models**

In vitro models for *Vermamoeba* and endosymbiont susceptibility have been modeled using dental unit water line systems (DIUWL). This method is also useful in studying polymicrobial biofilms of bacteria, fungi, other FLAs and viruses or virions of interest from within and without the FLAs and bacteria (Dillon et al., 2014a,b; Donlan et al., 2005; Costa et al., 2016). Biofilm models are preferred when observing *L. pneumophila* interaction and survival within *Vermamoeba* cells in water systems (Buse et al., 2017). Spiking models are recommended in developing protocols for FLA recovery (Chavatte et al., 2014) and FLA starvation responses can be studied using suspended cells and agar systems with and without bacterial prey (Pickup et al., 2007) in addition, time lapse video, light and scanning electron microscopy are useful tools in cell culture studies like keratocyte-amoeba cocultured observations (Kinnear, 2003) as well as in other studies where the focus is image analysis at set time intervals. In vivo studies have exploited the availability of mice for murine models in demonstrating replication of *L. pneumophila* inside a host system (Brieland et al., 1997a) and A/J mice models of intratracheal inoculation have been successful in supporting hypotheses for intrapulmonary infection with *Legionella* strains living as endosymbions within *Vermamoeba* (Brieland et al., 1997b). Giant viruses from arthropods can be isolated using *V. vermiformis* as described by Pagnier et al. (2015) with modifications that Vancomycin 10 µg/mL, Ciprofloxacin 20 µg/mL, Imipenem/Cilastatin 10 µg/mL, Doxycycline 20 µg/mL, and Voriconazole 20 µg/mL were added to the amoebal suspensions to prevent bacterial and fungal contamination (Temmam et al., 2015).
Vermamoeba being isolated in the environment is still a progressively growing body of research. Broader perspectives of its role in environmental ecology and possible direct role in the pathogenesis among nonmammalian and mammalian hosts are still being discovered, hence, the development of more efficient recovery and isolation techniques from environmental and biological samples for culture and molecular identification are necessary. Studies in the area of its viable recovery in biological hosts is still in its infancy discovered, hence, the development of more efficient recovery and isolation techniques from environmental and biological samples of endosymbionts as well as furnishing a fortified cellular stronghold for environmental and chemical inclemencies further opens up gaps to be filled by the scientific community. Vermamoeba submits a plethora of wonder for researchers to ponder upon in the perspectives of this FLA as being ubiquitous in the environment ready to be exploited by potential endosymbionts but more so as how this seemingly harmless FLA can be a reliable host for the most drug-resistant bacteria as well as being a possible virulence and evolutionary cohort of a wide selection of prokaryotes, viruses, and viroids.

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