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REVIEW ARTICLE

Interaction between zoonotic bacteria and free living amoebas. A new angle of an epidemiological polyhedron of public health importance?

Interacción entre bacterias zoonóticas y amebas de vida libre: ¿un nuevo ángulo de un poliedro epidemiológico de importancia en salud pública?

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RESUMEN

Desde hace varios años, diversos estudios se han abocado a estudiar la endosimbiosis entre especies bacterianas y amebas de vida libre, pero los eventos que conlleva esta interacción en relación con los mecanismos de ingreso y escape bacterianos no están del todo claros. Las amebas de vida libre, especialmente *Acanthamoeba castellanii*, son consideradas importantes depredadores bacterianos, por lo que tienen un significativo rol ambiental en el control de las comunidades microbianas. Sin embargo, diversas bacterias son capaces de evitar la digestión por parte del protozoo y beneficiarse de la relación con este. *A. castellanii* es un microorganismo ubicuo que se encuentra en ambientes acuáticos y terrestres. Estos nichos, particularmente los ambientes húmedos, los comparte con diferentes géneros bacterianos, entre los que se encuentran patógenos para el ser humano, muchos de ellos de carácter zoonótico, los cuales pueden llegar a establecer una relación endosimbiótica con la ameba, pudiendo sobrevivir en las vacuolas del protozoo desde días hasta meses. La literatura científica describe una gran cantidad de especies bacterianas que interactúan con *A. castellanii*. El objetivo de esta revisión es describir aspectos relevantes de las interacciones establecidas entre *A. castellanii* y diferentes géneros bacterianos, la mayoría de ellos de carácter zoonótico, y su importancia para la salud pública.

Palabras clave: amebas, *Acanthamoeba,* endosimbiosis, bacterias zoonóticas.

SUMMARY

Since many years ago, several studies reported the endosymbiosis between bacteria species and free living amoebas. However, the mechanisms involved in the bacteria penetration and release from the amoeba are not clear. The free living amoebas especially *Acanthamoeba castellanii* are considered important bacteria predators, for that reason they have a significant role in the control of microbial populations in particular environments. However, some bacteria are capable to avoid the digestion from the amoeba and take advantage of this intimate relationship. *A. castellanii* is an ubiquitous organism present in aquatic and soil environments. Particularly in humid environments they are found sharing with different bacteria species, including those pathogen for humans transmitted by animals. The interaction between the bacteria and the amoebas may result in a close endosymbiotic relationship that allows the bacteria to survive inside the vacuoles of the protozoa for days or months. The purpose of this review is to describe the relevant aspects of the interaction between *A. castellanii* and different bacteria species, mostly those with relevance in public health and related with zoonosis.

Key words: amoebas, *Acanthamoeba*, endosymbiosis, zoonotic bacteria.

INTRODUCTION

During the last decades there has been an increasing interest among microbiologists, epidemiologists, public health specialists and clinicians on the free living amoebas (FLA), because of their participation in the ophthalmological process and their role as potential reservoirs and vectors of pathogenic microorganisms for animals and human beings (Kahn 2006, Oddó 2006, Wagner *et al* 2006, Thomas *et al* 2010, Anacarso *et al* 2012, Khan and Siddiqui 2014, Scheid 2014).

Virus, bacteria and fungi have been described as beneficiaries of the interaction with FLA, specially with *A. castellanii*. Of particular interest in these interactions are the human pathogens and those pathogens associated with animals that are capable to survive and or replicate inside the amoebas. This intra-amoeba stage has been recognised as an endosymbiosis stage, and recently Scheid (2014) proposed the term endocytobionts to refer to those microorganisms capable to survive inside the amoebas. There is a general consensus that survival is one of the fundamental benefits that the protozoa provide to the prokaryote organisms. However, from the epidemiological and public health perspective, there are other aspects that are important to consider such as the resistance to the amoebic depredation and the bacterial protection inside

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FLA to chemical treatments, in addition to prokaryotes proliferation in a protective shield inside the amoeba (Barker and Brown 1994, Pagnier *et al* 2008, Thomas *et al* 2010, Sandstrom *et al* 2011, Khan and Siddiqui 2014, Scheid 2014).

The purpose of this review is to provide the scientific community with up to date information of the interactions described between FLA and some zoonotic bacteria and the relevance of these interactions in the epidemiology and their impact on public health.

GENERAL CHARACTERISTICS OF GENUS *Acanthamoeba*

The genus *Acanthamoeba* includes a large group of FLA that are widely distributed in nature. Taxonomically, the group belongs to the kingdom Protozoa, division Sarcomastigophora, class Lobosea, order Amoebida, family Acanthamoebidae. Currently there are more than 24 known species but only some of them are capable to produce infections in animals and humans. In this group are included *A. castellanii*, *A. culbertsoni*, *A. polyphaga, A. rhysodes, A. lenticulata* and *A. astroyxis* (Stothard *et al* 1998, Visvesvara *et al* 2007, Khan 2006).

The FLA have universal distribution and they have been isolated from soil, air conditioning equipment, contact lens, fresh natural and treated water, sea water, public pools, residual waters, dental units and hospital environment and supplies, as well as from cell cultures. The amoebas have also been isolated from plants, animals, and from nasopharyngeal swabs of humans apparently healthy, and from patients with immunodeficiency (De Jonckheere 1991, Geisen *et al* 2014, Scheid 2014).

The life cycle of the different species of *Acanthamoeba* showed a vegetative form or trophozoite, mainly when the protozoa is in a humid environment and in the presence of nutrients. However, the protozoa developed a cyst when subjected to extreme conditions such as dry and low in nutrients. The trophozoite form is irregular and presents multiple retractile pseudopodia and thorn-like filamentous called acanthopodia (Oddó 2006, Gallego 2007). In the case of *A. castellanii*, the trophozoite size varies between 13 to 22.5 µm, they have a granular cytoplasm limited by a narrow ectoplasmic zone that produces numerous phyllopodia of acicular aspect and acanthopodia. The acanthopodia are retractile cytoplasmic extensions specialised in catching nutrients, adherence and motility. This form is the predominant form of the amoeba under ideal growth conditions that include an environment with abundant nutrients, neutral pH, temperature of 30 °C and osmolality between 50 - 80 mOsmol (Khan 2006, Gallego 2007, Visvesvara *et al* 2007). The FLA are capable to multiply in trophozoite stage by binary fusion with the presence of polar centrioles either endo or extra-nuclear and disappearance of the nucleolus during the cariokinetic process (Gallego 2007).

On the other hand, the cysts of *A. castellanni* present a characteristic start shape and vary in size between 9 and 12 µm. Its composition is 33% protein, 4 to 6% lipids, 35% carbohydrates (mainly cellulose), 5% inorganic matter and 20% of unidentified material. The cyst presents two walls clearly identifiable under the microscope. The external wall or exocyst has a smooth surface and its composition is mainly protein, polysaccharides and lipids. In contrast, the internal wall or endocyst is polygonal and formed by carbohydrate, including cellulose. Both walls are normally separated by a space except in some points where they both create pores named ostioles. The main function of ostioles is to monitor and detect the changes in the environment. Each ostiole has a gateway named opercule, it is throughout one of those opercules that emerging of the amoeba occurs during the transition from the cyst to the vegetative form (Pussard and Pons 1977, Khan 2006, Gallego 2007, Visvesvara *et al* 2007).

A. castellanii is the etiological agent responsible of serious cases of ocular keratitis, meningoencephalitis and systemic diseases associated to immunocompromised hosts. Therefore, it is considered an important opportunistic parasite in humans (Kahn 2006, Oddó 2006). The pathogenesis of *A. castellanii* occurs when the amoeba is in its trophozoite stage and the first step is the adherence to the host cells. The adherence is mediated by a mannose-binding protein that is expressed in the surface of the amoeba (Visvesvara *et al* 2007).

After their discover in 1930, these amoebas were ignored for the following 30 years until the end of the 1950's when its pathogenic potential was demonstrated in cell cultures and in laboratory animals (Jahnes *et al* 1957, Culbertson *et al* 1959, Stothard *et al* 1998, Khan 2006, Khan 2009). Currently is well known that *A. castellanii* and other species of the same genus have the capability of living as free organisms and as parasite when they occasionally invade a susceptible host. In humans, the diseases more frequently associated with these organisms are skin infections, granulomatous encephalitis, and keratitis. Keratitis has shown an exponential increase as result of the number of healthy individuals using contact lenses. On the other hand, granulomatous encephalitis and the skin infections have a considerable increase associated with increment in the number of immunocompromised patients (Ma *et al* 1990, Marciano-Cabral *et al* 2000, Marciano-Cabral y Cabral 2003, Khan 2006, Oddó 2006).

Acanthamoeba AS HOST OF BACTERIA

Acanthamoeba species and especially *A. castellanii*, have an important role in the ecology of multiple ecosystems due to their participation in the recycling of nutrients in aqueous environments. Since many years ago, the study of the relationships between amoeba and bacteria has had some interest, however, the results related to this association have not been confirmed (Nguyen 2011). Despite

of this, it has been suggested that the interaction between *Acanthamoeba* and bacteria may occur in a similar manner to the interaction between prokaryotes and macrophages (Kwaik *et al* 1998, Cosson and Soldati, 1998). The capability of *Acanthamoeba* as host for bacteria is important because the majority of the prokaryotes that parasite or interact with these amoebas are pathogens or potential pathogens for humans (Anacarso *et al* 2012, Khan and Siddiqui 2014, Scheid 2014).

Some bacteria species have the capability to evade the digestion in the vacuoles of the protozoa, survive and replicate using the amoeba as host. Also, the bacteria can utilize the protozoa as vehicle for carrier and dissemination, as a protection and as an ideal incubator-like replication environment (Michel *et al* 2005, Berk *et al* 2008, Thomas *et al* 2010, Anacarso *et al* 2012, Khan and Siddiqui 2014, Scheid 2014).

Thomas *et al* (2010) made a complete review of the existing studies to create a list of the pathogenic bacteria that interact with protozoa. This list was compared with 539 bacterial species that are described in a list of microorganisms that are responsible of disease in human and animals know as CCL 3 Universe List (Candidate Contaminant List Microbes: Identifying the Universe) created by the United States - Environmental Protection Agency[1]. Of the 539 species, 102 can survive in contact with several species of amoebas. Of those 102 species, 39% are capable of survive inside the amoeba and 30% can do that in more than one amoeba specie. In addition, 31 of those bacteria are capable to replicate in more than one amoeba specie. However, these data are underestimated because most of the studies were performed using only two amoeba species: *A. polyphaga* and *A. castellanii*. This fact represents a relevant limitation due to the restriction in the number of amoeba species that were used to assess the interaction of the bacteria with other species of FLA (Thomas *et al* 2010).

Among the human pathogenic bacteria that can establish endosimbiosis with amoebas of the genus *Acanthamoeba* are included *Legionella pneumophila*, the ethiological agent of legionellosis (Rowbotham 1980), *Escherichia coli* O157:H7, the enteric pathogen responsible of entero-hemorrhagic diarrhea (Barker *et al* 1999), *Coxiella burnetii*, etiological agent of Q fever (La Scola and Raoult 2001), *Pseudomonas aeruginosa* etiological agent of keratitis (Michel *et al* 1995); the etiological agent of cholera, *Vibrio cholerae* (Thom *et al* 1992), *Simkania negevensis*, responsible of pneumonia (Kahane *et al* 2001), *Listeria monocytogenes*, etiological agent of listeriosis (Ly and Muller 1990); *Mycobacterium avium*, associated with respiratory disease (Krishna-Prasad and Gupta 1978, Steinert *et al* 1998) and *Salmonella* Typhimurium, associated with gastroenteritis (Gaze *et al* 2003). It has been reported that those pathogens are capable to survive and replicate inside the amoeba. This ability allow the bacteria to replicate in enough number to produce disease and also allow them successfully evade the host defenses and antimicrobial therapies (Thomas *et al* 2010). In addition, some bacteria species that require special conditions for its culture can also survive and replicate inside the amoeba. Among this group of bacteria that requires special conditions of microaerobic conditions are included *Campylobacter* spp. and *Helicobacter pylori* (Winiecka-Krusnell *et al* 2002, Axelsson-Olsson *et al* 2005, Axelsson-Olsson *et al* 2007) or bacteria that require anaerobic conditions such as *Clostridium frigidicarnis* (Pagnier *et al* 2008), *Prevotella intermedia* and *Porphyromonas gingivalis* (Wagner *et al* 2006).

Some authors have demonstrated that some bacteria species are capable to survive in the protozoa even during the cyst stage. This property ensures the bacteria a high degree of resistance in unfavorable conditions and overcome chemical disinfection (Gallego 2007, Khan 2006, Thomas *et al* 2010). In addition, this ability is extremely relevant in public health since the chemical disinfection is not capable of inactivate these pathogens when inside the amoeba. Among the prokaryotes that are able to survive inside the amoeba meanwhile it is in the cyst stage are included *V. cholerae* (Thom *et al* 1992), some species of *Mycobacterium* (Adekambi *et al* 2004, Thomas and McDonnell, 2007), *L. pneumophila* (Kilvington and Price, 1990), *Francisella tularensis* (Abd *et al* 2003, El-Etr *et al* 2009) and preliminary studies have indicated this same ability in *Arcobacter butzleri* (Fernández *et al* 2012). In addition, some authors have reported horizontal gene transfer between bacteria inside the protozoa's vacuoles (McCuddin *et al* 2006). This potential genetic transfer has great relevance in the transmission of genes associated with multidrug resistance using the amoeba as the facilitator in the transmission of these genes since the protozoa is the reservoir for several bacteria species.

It is important to mention that currently the term reservoir is applicable to the amoebas that contain bacteria as endosymbiont that have the capability to replicate and survive inside them. In contrast, the term "Troyan horse" or endocytobiont is applicable to the amoebas that contain as endocytobiont bacteria that are only capable to survive without replication inside the amoeba (Barker and Brown 1994, Greub and Raoult 2004, Khan 2006, Scheid 2014).

ZOONOTIC *Salmonella* SPECIES AND *Acanthamoeba*

Salmonella genus includes more than 2500 serovars or seroptypes, and many of them are relevant in zoonosis. The taxonomy of *Salmonella* is difficult and was modified (Porwollik *et al* 2004) grouping all the serovars in two species: *S. enterica* and *S. bongori*. The serovars with potential zoonosis relevance are included in the species *S. enterica* that includes six subspecies, one of them being the subspecie (subsp) *enterica* which groups all

[1] www.epa.gov

the zoonotic serovars such as *S. enterica* subsp. *enterica* serovar Typhimurium (former *S. typhimurium*) that for historical reason is mentioned as *Salmonella typhimurium* or *S. typhimurium*.

Gaze *et al* (2003) studied the interaction between *S. typhimurium* and *A. polyphaga*, determining that the amoeba is capable of incorporate the bacteria and the bacteria is localised in large quantities in contractile vacuoles during their early phases of logarithmic growth as confirmed by molecular markers of growth. This characteristic allows the bacteria reach 100 to 200 CFU per vacuole by the 4th day of co-culture. From this study, it was deduced that *S. typhimurium* is capable of establish endosimbiosis and replicate inside the amoeba therefore the protozoa is now considered an environmental reservoir for this bacteria.

Other studies have also demonstrated that *S. typhimurium* and *S. dublin* are capable to survive as endosymbiont of *A. polyphaga* and *A. rhysodes* (Tezcan-Merdol *et al* 2004) and later Bleasdale *et al* (2009) demonstrated that the pathogenicity island 2 of *Salmonella* that encode for a type III secretion system, is upregulated during the infection in *A. polyphaga* by *S. typhimurium* and apparently is essential for the intra-amoeba survival of this bacteria. This phenomenon suggests that some of the bacteria properties originally considered as virulence factors, which promote the bacteria pathogenesis in animals and humans, might evolved into other function more relevant in microbial ecology.

It has been demonstrated that the survival of *S. typhimurium* inside bovine rumen protozoa such as *Eudiplodinium*, *Metadinium*, *Polyplastron*, *Isotricha*, *Entodinium*, *Ophryoscole* and *Diplodinium*, is a factor that increase its virulence becoming more invasive in cell cultures *in vitro* than in models *in vivo*. This hyper-virulence mediated by protozoa was also observed in *S. agona* and *S. infantis* (Bearing *et al* 2005), this observation may indicate that the endosymbiont stage in FLA can contribute to increase the virulence of different serovar of *Salmonella*. In fact, in *S. enerica* serovar Typhi, the etiological agent of typhoid fever and exclusively pathogen of humans, it has been demonstrated that its interaction with *A. castellanii* increase their persistence in the environment and also improve their ability to survive in the human gut (Douesnard-Malo and Daigle 2011).

Legionella AND *Acanthamoeba*

Legionella pneumophila was decribed in the 1980 as the etiological agent of Legionaries' disease, a respiratory syndrome of high lethality (Fraser *et al* 1977). Although until now there are no data that indicate the existence of known animal reservoirs or that the bacteria affects animals, it was included in this review because *L. pneumophila* is an environmental bacteria wildly present in nature that is capable of parasite different species of protozoa. This possibility has major epidemiological interest because *Acanthamoeba* is a natural host for *Legionella* (Bruggemann *et al* 2006) but, in addition, this bacterium can replicate inside other amoebas such as *A. castellanii, Naegleria sp.* and *Hartmannella sp.* (Cianciotto and Fields 1992, Koubar et al 2011, States *et al* 2013) as well as inside some environmental ciliated protozoa (Koubar *et al* 2011). Some authors have suggested that this protozoa may contribute, under selective pressure, to the virulence characteristics of the bacteria interacting with human cells since *Legionella* has the capability of evade the degradation of the phagolysosome in the amoeba (Bruggemann *et al* 2006, Koubar et al 2011). Since *Legionella* is capable of replicate inside the protozoa is safe to consider *Acanthamoeba* as an important reservoir for *L. pneumophila* (Rowbotham 1980, Kwaik *et al* 1998, Koubar et al 2011). Their survival and replication capacities in both human macrophages and *A. castellanii* is due to the inhibition of the fusion of the phagosome with the lysosome, a process that it is regulated by the genes *dot/icm* of *L. pneumophila* (Gao *et al* 1997).

The relationship among *Legionella* and free living amoeba must be considered as an important factor incident in their ecology and epidemiology. There are several studies providing sufficient evidence that these protozoan are important determinants for survival, permanence and multiplication of *Legionella* in drinking water systems, representing a public health problem (States *et al* 2013). On the other hand, like numerous bacteria, *L. pneumophila* can acquire a viable but not culturable (VBNC) state under unfavorable environmental conditions. In this state, it is unable to replicate in standard medium but remain alive and able to synthesize some virulence proteins. However, resuscitation assays with *A. castellanii* were unsuccessful; suggesting that the presence of virulence factors in VBNC is insufficient to revert to their normal bacillary form (Alleron *et al* 2013). These last two aspects must be considered by organizations and sanitary authorities that are responsible for providing public health protection and safe drinking water.

Listeria monocytogenes AND *Acanthamoeba*

Listeria monocytogenes is a zoonotic bacteria that is recognized as facultative intracellular pathogen whose transmission to humans occurs via contact with animals, neonatal dissemination and the ingestion of contaminated food, mainly of animal origin (Ramaswamy *et al* 2007). This foodborne opportunistic pathogen is capable to switch from an environmental saprophyte to a potentially fatal human pathogen. Thus, the interaction of *L. monocytogenes* with environmental protozoa in natural and man-made ecosystems may have significant implications for food safety and public health (Schuppler 2014), especially because *Listeria* is known for their biocenotic connections with a wide range of hydrobionts, warm-blooded animals, and even plants (Pushkareva *et al* 2010).

Several reports in 1990 described the relation between *Listeria* and *Acanthamoeba,* suggesting that this bacterium may be ingested by the amoeba but it is not digested and can replicate intracellularly (Ly and Müller 1990). However, recent studies have reported that *Listeria* is not capable of survive (Akya *et al* 2009) or replicate inside the amoeba (Akya *et al* 2010). *L. monocytogenes* is incapable of persisting inside *A. polyphaga* and *A. lenticulata* but it is possible to observe large bacterial aggregates on the surface of the protozoa that are the result of the bacteria immobilization combined with their attaching to filamentous material, probably of amoebic origin. The immobilization and formation of bacterial aggregates appears to be a strategy of *Acanthamoeba* to catch and feed with motile bacteria (Doyscher *et al* 2013). The contradictions of different studies have created a need for new research in relation to the interactions of *L. monocytogenes*-FLA, in particular because Zhou *et al* (2007) demonstrated that *L. monocytogenes* in coculture with *A. castellanii* did not induce to kill the bacteria. However, Anacarso *et al* (2012) demonstrated that *L. monocytogenes* in association with FLA was not detectable by time 0 up to 48 h of co-culture, remaining viable and showing the ability to intracellularly multiply by > 4 log cycle up to 72 h, after a 48 h initial eclipse phase similar to that observed in *S*. Enteritidis. On the other hand, Pushkareva *et al* (2010) reported that *L. monocytogenes* can be active phagocyted by *Tetrahymenae pyriformis* and included in food vacuoles, where a gradual destruction of bacterial cells was observed. However, some *Listeria* cells proved to be resistant to digestion, allowing the maintenance of the bacterial population associated with this ciliate protozoon.

Since many food-borne bacteria, including *L. monocytogenes*, are able to interact with protozoa and taking into account that protozoa have been found in food-processing areas and in food industry environments, FLA and other protozoa could have a role in the contamination of food by this foodborne pathogen.

Yersinia AND *Acanthamoeba*

Yersinia enterocolitica is another zoonotic bacterium that has clinical relevance, since is capable to produce enteric disease in humans and is transmitted via water and contaminated food (EFSA 2013, Schieman 2013). The infection is mainly observed in newborn babies and in pre-school age children and is characterize for acute enteritis with fever and diarrhea. In adolescent and adults the disease presentation is mainly a pseudo-appendicitis with sequela of arthritis and erythema nodosum but, in some cases, with septicemia (Nesbakken 2013). *Y. enterocolitica* and *A. castellanii* shared ecological niches such as humid environments, vegetables, material of domestic use (Falcão *et al* 2004) and some anthropogenic environments like domestic refrigerators (Vaerewijck *et al* 2010) and dishcloths (Chavette *et al* 2014). Recently, it was demonstrated that *A. castellani* increase the survival of *Y. enterocolitica* at 25 °C under certain conditions of availability of nutrients and a 37 °C in environments with lack of nutritional elements (Lambrecht *et al* 2013).

Yersinia is capable to evade digestion by *Acanthamoeba*, in fact is the amoeba that provides the conditions to survive and replicate in environments that are not optimal for the bacteria, this mean at a temperature of 37 °C that is above the optimal for the bacteria. In addition, *Yersinia* can survive in co-culture with *A. castellanii* at least for 14 days without decrease in the number of viable bacteria (Lambrecht 2013). These observations confirm that the bacteria can survive in presence of the protozoa whether is inside *A. castellanii* or in the extracellular environment (Anacarso *et al* 2012). The interaction between *Acanthamoeba* and *Yersinia* is considered a relationship that facilitates the replication and survival of the bacteria without affecting the amoeba when both are in an environment with availability of nutrients (Greub and Raoult 2004, Siddiqui and Khan 2012). Similar observations were made by Pushkareva *el al* (2010) on the dynamics of *Yersinia* interacting with *Tetrahymenae pyriformis* where *Y. enterocolitica* survives for more than two months in association with this protozoon at 25 °C and 4 °C. The same authors (Pushkareva *el al* 2010) simulated experimentally the migration of *Y. pseudotuberculosis* along trophic chains from the lowest to the highest level. There is no information on *Y. enterocolitica* and this kind of migration. However, considering their long term survival in aquatic organisms it seems to be necessary to clarify this point because it could provide a better understanding about the routes of circulation of *Y. enterocolitica* in natural ecosystems.

When *Yersinia* is internalised, *A. castellanii* works as protective shield including against chemical agents such as chlorine (King *et al* 1988). This advantage is of great relevance in public health because chlorine is one of the most common components used in the disinfection of surfaces, in particular in the food industry, where *Yersinia* could be introduced through animal meat (Vanantwerpen *et al* 2015). The incidence data on human *Yersinia* infection in USA for 2013 was 0.36, showing 7% increase (CDC 2014).

Campylobacter AND *Acanthamoeba*

The species of *Campylobacter* genus are Gram negative, non-spore forming rods. One of main features is their shape as comma or s that apparently represents an adaptation to the environment in the intestinal mucosa, which allows or facilitates its motility in viscous liquids or environments. *Campylobacter* is not capable to use sugars and obtain its energy from peptide or intermediary products of the tricarboxilic acid cycle, not derived of carbohydrates. The microorganisms are widely distributed in nature. They are regular commensals in the intestinal tract of different species of blood-warm animals (Fernández *et al* 2007). Some

Campylobacter species are recognized as important enteric pathogens for humans in whom produces inflammatory diarrhea with blood and mucus and faecal leukocytes or a watery diarrhea similar to *E. coli* LT infection (Debruyne *et al* 2008, Fernández 2008). Some species, such as *C. jejuni* can be isolated in environmental samples such as water bodies or places with constant humidity (Hänninen *et al* 2003, Fernández *et al* 2003), these niches can be shared with *Acanthamoeba* (Nguyen 2011).

It has been demonstrated that *C. jejuni* can survive at least for 10 days as endosymbiont in *A. castellanii* (Villanueva 2005). Other studies also have demonstrated that this bacteria can resist the digestion of the amoeba (Axelsson-Olsson *et al* 2005, Snelling *et al* 2005). In addition, the bacteria can invade, survive and replicate inside amoebas of FLA (Axelsson-Olsson *et al* 2007). In the case of *A. polyphaga* it has been demonstrated that *Campylobacter* is capable of replicate in aerobic co-culture at 37 °C and survive there for more than two months. However, the cellular and molecular mechanisms that allow this are unknown (Olofsson *et al* 2013). Moreover, it has been demonstrated that the co-culture of *C. jejuni* with *A. castellanii* results in a delay in the loss of viability and an increment in the survival of *Campylobacter* (Baré *et al* 2010). In addition, the experimental transmission of *C. jejuni* in endosimbiosis with *A. castellanii* to specific pathogens free (SPF) chickens (González 2008), as well as Broiler chickens (Snelling *et al* 2008, Flores-Martin 2009), is possible.

Since *Acanthamoeba* increase the persistence of *C. jejuni* and this bacterium can be transmitted experimentally as endosymbiont to SPF chicken and broiler chickens, the presence of FLA in the environment of poultry houses may have relevant implications for the ecology and epidemiology of this zoonotic pathogen, universally recognized as pathogen transmitted by food, especially by food derived from poultry.

Arcobacter AND *Acanthamoeba*

The species of this genus are Gram negative, non sporulated rods with monotrichous or amphytrichous flagella. The bacteria has a curve, helicoid or in a form of italic S morphology, it is aero-tolerant with a chemoorganotrophic metabolism, and most of the source of energy comes from amino-acids o intermediary products of the tricarboxilic acid cycle, since this microorganism is not capable of using carbohydrates for fermentation or oxidation (Debruyne *et al* 2008). Currently, the relevance of this genus is because some of their species, particularly *A. butzleri*, are considered emergent enteropathogens and potential zoonotic agents (Cardoen *et al* 2009, ICMSF 2002, Ho *et al* 2006 Snelling *et al* 2006). In humans, *Arcobacter* produces gastrointestinal disease with persistent watery diarrhea that can lead to complications, with septicemia and peritonitis On *et al* 1995, Lau *et al* 2002, Fernández *et al* 2004, Collado and Figueras 2011). In animals, *Arcobacter* is isolated frequently from the intestinal tract of different animal species and causing diseases in some of them. The most severe clinical presentations in bovine and porcine are abortion, mastitis and diarrhea (Ho *et al* 2006, Collado and Figueras 2011). In addition, reports indicate the presence of these bacteria in intestinal samples of healthy animals, mainly wild birds and from farm, suggesting that these animals may be a reservoir of the bacteria (Lehner *et al* 2005, Fernández *et al* 2007, Houf 2010, Collado and Figueras 2011).

Members of *Arcobacter* genus are considered an atypical group among the ε-proteobacteria due to the great diversity in habitat and hosts where they can be isolated, mainly in humid environments, where they interact with FLA (Debruyne *et al* 2008). The wild range in the distribution of this bacteria and FLA facilitate the interaction between these two organisms and underline the existence of possible protozoa reservoirs present in nature, which may be part of alternative cycles of life that include a facultative stage of the bacteria inside the FLA. This modality represents a new alternative in the epidemiology of *Arcobacter* genus (Fernández *et al* 2012, Medina *et al* 2014).

Currently it has been described that under laboratory conditions *A. butzleri* can establish an endosymbiotic relation with *A. castellanii* surviving inside the amoeba more than 30 days. During this time it is possible to confirm bacteria replication. For this reason, *A. butzleri* may be an endosymbiont and the amoeba can act as "Troyan horse" and contribute to the increment of virulence and to the environmental dissemination of *A. butzleri* (Fernández *et al* 2012, Flores-Martin 2013, Medina *et al* 2014).

Molecular studies describing the endosymbiotic interaction between these organisms indicate that the recognition of the bacteria by *A. castellanii* included the participation of protein bound to galactose, mannose and glucose, present in the outer membrane of the amoeba and the amoeba recognized these sugar residues that are part of the glycoproteins and lipopolysaccharides present in the external membrane of *A. butzleri*. The recognition of these structures allowed the bound of both organisms that trigger the subsequent internalization of the bacteria. This process is essential for the establishment of endosymbiosis (Medina *et al* 2014).

It has been also described that during the internalization of the bacteria, *A. castellanii* requires the formation of actine filamentous and the participation of the transduction pathways mediate by P13K and RhoA in which the tyrosine kinase activity is fundamental. In addition, using transmission electronic microscopy and inhibition assays of the phagolysosome fusion, it has been possible estimate that the survival of *A. butzleri* as endosymbiont of *A. castellanii* may be related with the capability of the bacteria to keep alive in the vacuole not fused with the lysosomes or delays the fusion of these organelles (Medina *et al* 2014).

Mycobacterium AND *Acanthamoeba*

The genus *Mycobacterium* represents a group of aerobic, slow-growing, non sporulated, acid-alcohol resistant rods, with abundant cytoplasmic granules. *Mycobacterium tuberculosis* and *M. leprae* are the etiological agents representative of the genus traditionally considered the more important for humans (Brennan 1998). However, with the AIDS pandemic there are several other species, recognized as opportunistic pathogens, such as *M. avium* and other species of the *Mycobacterium* genus (Young *et al* 1986, Shafer and Sierra 1992).

The internalization of *Mycobacterium* in free living protozoa could be a rare event in normal conditions. Recent studies suggested that in the case of *M. bovis*, the amoebas may contribute to the inactivation of the prokaryote instead they represent a potential environmental reservoir (Mardare *et al* 2013). Moreover, *M. avium* is an opportunistic pathogen that can be isolated from different animal species in land and watery environments (Falkinham 1996, White *et al* 2010, Salgado *et al* 2011). This bacterium can access *A. castellanii* and establish an endosymbiotic association with capability of intracellular replication between 30 and 37 °C (Cirillo *et al* 1997, Iovieno *et al* 2010, White *et al* 2010). Some studies suggest that probably *Acanthamoeba* is essential for the virulence of *M. avium* in patients with AIDS and as protective element against the antimicrobial agents (Miltner and Bermudez 2000).

Furthermore, long time symbiotic interactions between *A. lugdunensis* and a mycobacteria species related to *M. avium* and *M. intracellulare* were described recently, suggesting that FLA could be a stable environmental reservoir for some mycobacteria (Thomas *et al* 2010).

Because of the similarities between mechanisms allowing microorganisms to escape phagocytosis and/or digestion by FLA and the mechanisms allowing these same microorganisms to escape phagocytosis and/or digestion by macrophages, FLA have been proposed as a tool to recover potentially new pathogenic species from various environments (Drancourt 2014). *Mycobacterium massiliense* was isolated from the sputum and bronchoalveolar fluid of a patient with hemoptoic pneumonia by plating on axenic media and amoebal coculture with *A. polyphaga* (Adekambi *et al* 2004). The same could be applied for some opportunistic non-tuberculous mycobacteria (NTM) associated to freshwater, like *M. fortuitum*, *M. gordonae*, and *M. kansasii*, that resist amoeba digestion and might contribute to the health burden through wound and soft tissue infections (Delafont *et al* 2014, Drancourt 2014, Ashbolt 2015). Being these opportunistic NTM associated to drinking-water pipe biofilms, further studies are necessary to elucidate, from a public health point of view, the epidemiological importance of FLA and their relationships with this and other pathogenic water-borne groups of bacteria.

CONCLUSIONS AND RELEVANCE OF THE INTERACTIONS BETWEEN ZOONOTIC BACTERIA AND *Acanthamoeba*

There are several studies indicating the relevance of the relationships bacteria-amoeba and how amoebas may play a role as "Trojan horses" for the prokaryotes, even in those cases when the amoeba is in the cyst stage, favoring the proliferation of bacteria in hostile environments, allowing its transporting including its cooperation with their pathogenicity since these protozoa are highly resistant to disinfecting agents.

Despite that the mechanisms of coming in and out of the amoeba are not completely clear, is known that it occurs in nature and that these processes may last days or months. The fact that *Acanthamoeba* is an ubiquitous protozoa that can be found in practically all environments that preserve the humidity, including vent systems, contact lenses and public swimming pools, is very relevant making necessary to know the mechanisms how these interactions occur. Apart from being an incubator and transportation vehicle for some bacteria, the protozoa by itself can be a pathogen for humans.

The intracellular growth of the prokaryotes has been associated with improving their survival in the environment and increase in resistance against antibiotics. The advantage of utilizing FLA during *in vitro* studies is that the research on virulence and pathogenesis of the internalized bacteria can be done in non-mammalian cells as a model based in the nature reality. In comparison to cell cultures, the amoebas are easy to work in experimental models. On the other hand, bacteria are also easy to work and can be genetically manipulated. This should allow the possibility to use mutants to study and analyze possible bacteria-host interactions. Therefore, the utilization of this amoebic model may allow a better understanding of the interactions between prokaryote and eukaryote cells to clarify epidemiological aspects and to help in the development of new therapeutic agents and to better recognize and treat infections (Sandstrom *et al* 2011).

Finally, from the epidemiological and public health point of views, when the interaction amoeba-bacteria yield a more survival of the prokaryote and in many occasions in a major environmental persistence, that may allow to explain the transmissibility and endimicity of many of those bacteria.

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