Listeria monocytogenes symptoms and prevention

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A 1981 fatal outbreak from the consumption of contaminated coleslaw in Canada (Schlech et al. 1983), together with epidemics in the following years that were associated with dairy foods such as pasteurized milk (Fleming et al. 1985) and Mexican-style cheese (James 1985), highlighted major concerns about the survival and growth capabilities of the pathogen L. monocytogenes in contaminated foods especially during storage at refrigeration temperatures. Since these epidemics, L. monocytogenes has been widely acknowledged both as an important hazard in food industries, in addition to its significance as a medically significant pathogen.

L. monocytogenes is a Gram-positive, intracellular bacterial pathogen, facultatively anaerobic, rod-shaped (0. 4 – 0. 5 µm in diameter and 0. 5 – 2 µm in length) and non-spore-forming. Biochemical analysis demonstrated that L. monocytogenes are oxidase-negative, catalase-positive and hydrolyse esculin, are methyl red and Vogus-Proskauer positive, do not hydrolyse urea and is incapable of producing H2S or indole (Feresu and Jones 1988). Typically Listeria spp. have a low % [G+C] content (< 50%).

As one of the six species of the genus Listeria, L. monocytogenes belongs to a genus of the Corynebacteriaceae family that comprises of L. monocytogenes, L. grayi, L. innocua, L. ivanovii, L. seeligeri, and L. welshimeri. While L. monocytogenes is pathogenic to both humans and animals, L. ivanovii is only pathogenic to animals, mainly sheep and cattle. The rest of the Listeria species are known not to cause any disease (Cossart 2007).

L. monocytogenes shows resistance to extreme environmental conditions. For example, high concentrations of salt (up to 10% NaCl), a wide pH range (4. 5 – 9) as well as low temperatures (Vazquez-Boland et al. 2001). From a clinical perspective, L. monocytogenes is also able to colonize and persist in the gallbladder, which has a high pH content (Begley 2009). This finding suggests the occurrence of both long-term and chronic infections, as well as the ability of the bacterium to survive within the microenvironments of the gastrointestinal tract. Though the optimum growth temperature range for L. monocytogenes growth is between 30°C – 37°C, not only does growth has been shown to occur between 3°C – 45°C (Junttila 1988), the bacteria has been demonstrated to grow at temperatures between 1°C – 4°C (Farber 1991). This adaptability of L. monocytogenes to a variety of temperatures suits it for survival and growth in either processed or refrigerated food items.

Though L. monocytogenes is ubiquitous in the environment, this bacterium is most commonly found in decaying vegetation and also soil (Weis 1975). Following ingestion of L. monocytogenes by a susceptible person, the bacteria can make the transition from being a saprophyte to a parasite that promotes the survival and replication of the bacterium within host cells (Freitag 2006). L. monocytogenes has been demonstrated to be capable colonizing a variety of inert surfaces, as well as able to form biofilms on food-processing surfaces (Roberts and Wiedmann 2003). The presence of L. monocytogenes in numerous environments such as farms, soil, water, silage produced from contaminated grasses and also food processing facilities, indicates that there L. monocytogenes has many opportunities to contaminate the food production process (Cossart and Bierne 2001).

Listeriosis

Though human listeriosis outbreaks following ingestion of L. monocytogenes contaminated food items have been reported previously, only recently the bacterium has been recognized as a major cause of human infections (Lecuit 2007). This is due to increased numbers of susceptible, immuno-compromised individuals, the increase in large-scale agro-industrial plant development, as well as an increased reliance on refrigerated food items.

Fresh vegetables are an example of origins of contaminants; it can be contaminated from the soil or from manure that were originally used as fertilizers and food. Animals may also carry the bacterium asymptomatically and contaminate foods of animal origin. Other examples of food items that are most linked to listeriosis outbreaks include ready-to-eat meats, undercooked meats, cold cuts, pâté (McLauchlin et al. 1991), salads, dairy products, especially soft cheeses (Linnan et al. 1988) and milk that is either inadequately pasteurized or contaminated post-pasteurization (Fleming et al. 1985, Jackson et al. 2011).

Listeriosis in Humans

A previous study has demonstrated that asymptomatic carriage of L. monocytogenes does occur within the intestinal tract of 5% or more of healthy humans (Grif et al. 2003). However extremely rare infections also occur in healthy adults and young children, where there is increasing evidence in recent years to prove that listeriosis may also occur in healthy individuals within just 24 h post ingestion of highly contaminated food (Swaminathan and Gerner-Smidt 2007).

Those most at risk from listeriosis are generally immuno-compromised individuals such as diabetics, AIDS patients, those with renal failure, organ transplant patients, cancer patients and also elderly adults. Diarrhoea is usually an early symptom of the infection. Advanced symptoms in these susceptible individuals include septicemia or meningoencephalitis. The main clinical features of L. monocytogenes meningitis are abnormal movements, seizures, as well as alteration of consciousness. L. monocytogenes meningitis cases have been shown to have the highest mortality rate (22%) in comparison to all types of bacterial meningitis (Lecuit 2001). Certain patients are known to experience rare and localized infections, for example due to direct inoculation of the bacterium (Schlech 2000, Lecuit 2001).

Others at high risk of the disease also include pregnant women, foetus and also newborns (Vazquez-Boland et al. 2001). Due to the fact that pregnant women have a naturally depressed, cell-mediated immune system (Weinberg 1984), pregnant women is more likely to acquire listeriosis upon post consumption of contaminated food compared to other individuals. By gaining access to the maternal circulation, L. monocytogenes colonizes the placenta to induce placentitis, infecting the defenseless foetus. As opposed to maternal illness, the severity of infection within foetal and neonatal is much higher. The common fatal symptoms include pre-term labour, amnionitis, spontaneous abortion, stillbirth as well as early onset sepsis (Vazquez-Boland et al. 2001).

The occurrence of fatality due to listeriosis is continuing to decrease in industrialized countries in recent years. This has been attributed to the

stricter implementation of food quality and safety (Allerberger and Wagner 2010).

Listeriosis in Animals

Various species of animals can be infected with L. monocytogenes, but clinical disease is rare. The bacterium can also live within the intestines of healthy animals without causing any infections. Though most cases of animal listeriosis are generally seen in ruminants, this disease can also occur in poultry and other birds, pigs, dogs, cats, domestic and wild rabbits, and many other small mammals. Infected ruminants have been shown to experience encephalitis, septicemia, and even abortions (Schoder et al. 2003). The course of disease in sheep and goats is more rapid and death may occur 24 – 48 h upon the onset of symptoms. In cattle however, the course of disease is less acute.

Many L. monocytogenes-infected animals excrete the bacterium in faeces and milk. This is a common source of animal to animal spread of infection. Grass silage is presumed to be the source of infection, as it can be contaminated with large numbers of L. monocytogenes. This is because the low pH of silage can enhance the growth of L monocytogenes cells (Pham 2006). Besides silage, the bacterium has also been isolated from other sources such as water troughs, manure, soil and animal feeds. L. monocytogenes infection may also cause mastitis in cattle and sheep (Wagner et al. 2005).

In ruminants such as sheep, infections that lead to lesions in the brain stem, result in characteristic clinical symptoms (Rebhun and deLahunta 1982).

Typical symptoms of listeriosis in ruminants include turning or twisting of the head to one side and walking in circles, drooping of the eyelid and ear caused by paralysis of the unilateral facial nerve. The infected ruminant may also drool saliva as a result of partial pharyngeal paralysis (Rebhun and deLahunta 1982).

Animals that excrete L. monocytogenes cells in faeces have been suggested as the primary cause of entry of this pathogen into food-processing plants (Schonberg and Gerigk 1991). The growth and multiplication of L. monocytogenes cells is usually promoted by not only the high humidity, but also the nutrient rich waste present within certain food production plants. Hence, it is not surprising that animal listeriosis does pose a serious contamination risk for the food industry in general.

Pathophysiology of L. monocytogenes

L. monocytogenes is usually ingested with contaminated food (Error: Reference source not found). In immuno-compromised individuals, L. monocytogenes invades the epithelial cells of the intestines and spreads to other parts of the body by cell-to-cell spread. L. monocytogenes secretes invasins (InlA + InlB) to enable it to penetrate cells of the intestinal epithelial lining (Gaillard et al. 1987, Mengaud 1996). L. monocytogenes cells that cross the intestinal epithelial barrier are then carried by the lymph or blood to the mesenteric lymph nodes, the spleen, and also the liver (Marco 1992, Pron 1998). Entry into the host’s monocytes, macrophages, or polymorphonuclear leukocytes promotes growth of L. monocytogenes, and the infection becomes blood-borne (septicemic) dissemination.

L. monocytogenes then enters the liver after the intestinal translocation and carriage by the bloodstream (Marco 1992, Dramsi 1998). Hepatocytes are generally the main site of L. monocytogenes multiplication within the liver (Vazquez-Boland et al. 2001). When there is an inadequate immune response by the host, L. monocytogenes usually multiplies unlimitedly within the liver parenchyma, possibly releasing the bacteria into blood to cause bacteremia. Via the bloodstream, L. monocytogenes cells will reach the brain in order to cross the blood-brain barrier (Kirk 1993). High levels of L. monocytogenes cells in the brain accompanied by bacteremia will generally result in meningoencephalitis (Tunkel 1993, Tuomanen 1996).

In pregnant women, L. monocytogenes usually gains access to the foetus by entering the endothelial layer of the placental barrier (Gray 1966). The bacterial cells will reach the bloodstream of the foetus by firstly colonizing the trophoblast layer. The bacteria then will reach the bloodstream of the foetus by translocating across the endothelial barrier. This will usually result in infection and the possible subsequent death of the foetus within the uterus, or occasionally even the premature birth of a severely-infected neonate (Vazquez-Boland et al. 2001).

Virulence Factors of L. monocytogenes

A wide array of virulence factors is wielded by L. monocytogenes to assist the bacterium to interact and manipulate the host cells. Virulence genes of L. monocytogenes are known to be optimally expressed at 37°C, but expressions almost does not occur at 30°C (Freitag et al. 2009). The key transcriptional activator of L. monocytogenes virulence factor genes, known as PrfA, is also known to be thermo-regulated. PrfA is usually activated upon

the ingestion of L. monocytogenes contaminated foods (Error: Reference source not found). PrfA is also known to regulate a variety of the bacterium's virulence genes (Camejo et al. 2011, Stavru et al. 2011) as well as other core genome genes.

Internalins (InlA and InlB), which are L. monocytogenes surface proteins, have been previously shown to involve in the invading of the host cells (Seveau et al. 2007). InlA is known to bind E-cadherin, which is the host cell's adhesion molecule, whereas InlB binds to Met, which is the hepatocyte growth factor (HGF) receptor. By the binding of internalin proteins to E-cadherin and Met, L. monocytogenes cells are able to gain entry into the host cells; this is done by taking advantage of the endocytic machinery of the host cells (Pizarro-Cerda and Cossart 2006).

Once internalized within the host cell, L. monocytogenes mediates escape from membrane-bound vacuoles through the secretion of Listeriolysin O (a pore-forming haemolysin) (Gaillard et al. 1987), as well as two phospholipases: phosphatidylinositol (PI) phospholipase (PLC-A) (Camilli et al. 1993) and phosphatidylcholine (PC) phospholipase C (PLC-B) (Grundling et al. 2003). Together, these proteins assist in breaking down the host phagosome that contains L. monocytogenes cells. This is done to allow the bacterium to escape into the host cytosol (Kathariou et al. 1987, Camilli et al. 1991, Mengaud et al. 1991, Vazquez-Boland et al. 1992, Schnupf and Portnoy 2007, Scortti et al. 2007). Upon entering the host cell's cytosol, L. monocytogenes cells begin to replicate (O'Riordan et al. 2003, Joseph and Goebel 2007), and then with the assistance of actin polymerization mediated cell-cell spread , the bacterium moves through the host cell for the purpose

of migrating into the neighbouring host cells. The actin polymerization mediated cell-cell spread process is directed by ActA. The ActA protein binds and activates Arp2/3, which is a seven-protein host complex. Arp2/3 has been shown to induce actin polymerization as well as generate actin filaments (Pizarro-Cerda and Cossart 2006). Upon entry into the adjacent host cell, L. monocytogenes cells secretes both Listeriolysin O and also PC-PLC to assist the bacteria in escaping from the double-membrane secondary vacuoles, known as listeriopods, which were formed as a result of cell-to-cell spread (Freitag et al. 2009).

Invasion of Mammalian Cells by L. monocytogenes

L. monocytogenes has evolved a number of strategic methods to evade or resist killing by the innate immune response of mammalian phagocytic cells that are usually known to phagocytose and degrade most pathogens that invade the host cells (Ryter and De Chastellier 1983). The bacterium is able to multiply in a variety of mammalian cell types such as professional phagocytic cells, for example, J774 macrophage-like cells (Portnoy et al. 1992), as well as non-professional phagocytes such as epithelial cells (Rácz et al. 1972), endothelial cells (Drevets et al. 1995) and hepatocytes (Conlan and North 1992). Marco et al. (1992) previously demonstrated that in mice that were infected with L. monocytogenes cells, the bacterium first infected the macrophage cells, followed by infection of the hepatocytes in the liver. L. monocytogenes has also been shown in a separate study to be able to efficiently invade hepatocytes in vitro (Wood et al. 1993).

Within mammalian host cells, L. monocytogenes is internalized within membrane-bound phagosomes upon adhering to host cells. The bacterium

then escapes into the host cytosol from the phagosome by disrupting the phagosomal membrane. Within the host cytosol, L. monocytogenes grows and multiplies, and then proceeds to infect neighbouring host cells (Freitag et al. 2009). Gaillard et al. (1987) showed L. monocytogenes was able to initiate entry into human colon carcinoma cell line Caco-2, and multiply within the host cytosol. That same study also provided evidence to show that L. monocytogenes was able to induce phagocytosis by Caco-2 cells. Francis and Thomas (1996) demonstrated recovery of a higher numbers of L. monocytogenes cells of hemolytic strains from both HeLa and Caco-2 cell lines, in comparison to non-hemolytic strains. Furthermore, the extensive morphological changes that the host cells exhibited not only included loss of confluence and host cell lysis, but also the presence of very high counts of L. monocytogenes cells within the host cells were detected (Francis and Thomas 1996).

Is there an Environmental Reservoir for L. monocytogenes?

Although L. monocytogenes causes severe disease in human and animal hosts, unlike other Gram-negative intracellular pathogens, this pathogen has no recognized animal reservoir. Several studies have suggested that as a result of interaction with soil-borne organisms such as protozoa, a number of intracellular pathogens are able to maintain its virulence genes (Barker and Brown 1994, Adiba et al. 2010, Lamrabet et al. 2012). For example, Salmonella spp. interacts with protozoans such as amoebae and the ciliate Tetrahymena pyriformis (Tezcan-Merdol et al. 2004, Brandl et al. 2005). Miltner and Bermudez (2000) have suggested the possible role of Acanthamoeba castellanii as an environmental host for the pathogenic

Mycobacterium avium. Furthermore, protozoan cells that were harbouring Legionella pneumophila were identified as the cause of a fatal outbreak of Legionnaires' disease outbreak during a convention in Philadelphia in 1976.

While Acanthamoeba spp. is known to harbour a number of bacterial pathogens, L. monocytogenes has been recently demonstrated to be phagocytosed and rapidly degraded by the host amoeba within just 2 h of ingestion (Akya et al. 2010). In view of this result, the question arises whether protozoa could act as a potential reservoir for L. monocytogenes.

Interactions between Bacteria and Protozoa

Protozoa are unicellular eukaryotic microorganisms that are ubiquitously present in diverse habitats. They feed heterotrophically and are generally recognised as the major consumers of bacteria in the environment. Protozoan cells can be present either singly or as colonies of cells (eg. Volvox spp.), may swim freely (Paramecium spp.), or are parasitic for other animals (eg. Trypanosoma spp.).

Briefly, there are three main groups of bacterivorous protozoa: amoebae, ciliates and flagellates. Amoebae are phagotrophic protozoa that feed on algae, bacteria, plant cells, and smaller protozoans. Amoebae move by changing cell shape, forming pseudopods (temporary foot-like structures) with diverse morphologies. Ciliates can be found almost everywhere there is water, such as lakes, rivers, oceans and soil. They are characterized by large numbers of hair-like organelles (called cilia) that are involved in movement of the cells, chemotaxis, as well as predation of bacteria in the form of filter-feeding (Fenchel 1987). Flagellates have whip-like appendages (called

flagella) for the main purposes of locomotion as well as to direct food particles or cells into its mouth-like opening.

Bacterial mortality in the environment is suggested to be mainly a result of feeding of bacteria by protozoans (Pernthaler 2005). A majority of protozoa feed by phagocytosis, a process by which they engulf bacteria and digest them within a food vacuole. Briefly, once the bacterial prey is captured, it is packaged into a food vacuole. Once inside the protozoan food vacuole, the process of digestion commences (Fenchel 1987). This is carried out through the release of host proteases and lysozymes into the food vacuole in order to break down the bacteria within the food vacuole. This will supply the protozoa with energy and nutrients for its growth. The acidic environment within the food vacuoles assists the protozoa in disabling the bacterial prey for digestion. The products of the digestion process are then released into the cytoplasm. However, previous studies have clearly demonstrated that not all bacteria are digested as food source. Some types of bacteria survive within the protozoa in order to persist and utilize those protozoan cells as a host.

The major outcomes in a bacteria-protozoa interaction (Error: Reference source not found) include:

Upon phagocytosis, the bacteria multiplies to high numbers within the vacuoles, resulting in massive enlargement of these vacuoles that will eventually cause lysis of the host, releasing free bacteria into the extracellular environment, e. g. Legionella pneumophila (Rowbotham 1983).

The same process as (1), except that following lysis of host, free bacteria is released alongside intact vacuoles containing infectious bacteria, e. g. L. pneumophila (Rowbotham 1983).

Ingested bacteria multiply within the host but not able to cause lysis of the host, e. g. Coxiella burnetti (La Scola 2001).

Ingested bacteria survive within encysted protozoa, e. g. L. pneumophila (Rowbotham 1983).

A number of important studies have previously shown that pathogenic bacteria that are able to survive within protozoans can be protected from external stresses such as chemical disinfectants and antibiotics (King 1988, Berk et al. 1998, Brandl et al. 2005, Bichai et al. 2008). It is likely that the ability of a number of intracellular bacterial pathogens to resist killing by its host protozoan cells may have resulted in their evolution as pathogens of the mammalian kingdom. Indeed it is possible that protozoan cells are the link between bacteria that inhabits the environment and the bacteria that cause diseases in mammals such as humans.

Protozoa as Model Organisms for Study of Pathogenesis

Protozoan cells have been previously utilized as model organisms for studies in various fields such as evolution and ecology (Friman et al. 2008), population and community biology (Holyoak 2005), the role of organelles (Smith et al. 2007) as well as toxicity studies (Stefanidou et al. 2008). The use of protozoa in the study of host-pathogen interactions has its advantages and has increasingly become more common in recent years, most importantly in infectious diseases studies. Infection studies generally utilized

mammalian species such as mice and sometimes even humans as the host systems. By using the mammals as the host system, the analysis is not only expensive, time consuming and subject to extensive ethical review, it is also technically challenging and complex. In contrast, similar studies in protozoans are more convenient, quicker and also cost-effective (Montagnes et al. 2012).

As model systems, protozoan cells can also help in understanding better the mechanisms of mammalian infectious disease (Montagnes et al. 2012). Intracellular bacterial pathogens have been previously shown to escape the phagolysosomes of protozoa and mammalian phagocytic cells by utilizing similar mechanisms. Hence, protozoa are useful models for studying the pathogenesis of opportunistic, human pathogens. In terms of evolution, single-celled organisms such as protozoans are older than multi-celled organisms such as mammals. Hence the possibility that several mammalian pathogenic bacteria would have evolved from intracellular pathogens within protozoan cells, cannot be ruled out (Montagnes et al. 2012). For example, the interactions of L. pneumophila-mammalian cells and L. pneumophila-protozoa sharing a number of phenotypical and molecular similarities between them demonstrate this fact (Barker and Brown 1994, Fields 1996).

It is now clear that protozoan-bacterial pathogen interactions play an important role in transmission of human disease. This was especially evident when protozoa harbouring L. pneumophila were identified as the cause of a Legionnaires' disease outbreak during an American Legion convention in Philadelphia in 1976. A total of 34 fatalities out of 221 cases were reported during that outbreak. Therefore, it is inevitable that studies on bacteria-

protozoa interactions can provide crucial steps into possible prevention of infectious diseases.

The amoeba Dictyostelium discoideum is an example of a useful model in the study of human pathogens, including L. pneumophila (Solomon et al. 2000), Neisseria meningitidis (Colucci et al. 2008) as well as Salmonella enterica serovar Typhimurium (Annesley and Fisher 2009). Other protozoan models commonly studied include the ciliate Tetrahymena spp. (Friman et al. 2008), the marine flagellate Oxyrrhis spp. (Montagnes 2011), and also the choanoflagellate Monosiga spp. (Behringer 2009). There are a number of other advantages with using protozoa as a model system, including the ease with the protozoans can grown in large amounts as well as the simple storage and maintenance techniques. In general, protozoa cultures can be maintained on simple, inexpensive media, such as bacterial suspensions. Protozoa cultures may be easily isolated from a variety of natural and artificial environments. Protozoan cells can also be stored as stock cultures over a long period of time, for example suspending concentrated suspensions of protozoans in DMSO and storing it in -20oC. These stock cultures can be revived with only a little effort.

Interactions between Pathogenic Bacteria and Protozoa

Intracellular bacterial pathogens of humans that parasitize protozoans exist within a privileged environment, protected from external stresses. Thus bacteria-protozoa interactions are likely to have important ecological as well as public health consequences.

The association of pathogens and protozoa may have contributed to the survival and persistence of bacterial pathogens in various natural and artificial environments. Encapsulation of bacterial pathogens within protozoan cells and provides a protective effect against environmental stress, such as predation, starvation, disinfectants and high temperatures. A number of pathogens can survive for extended periods of time within cysts of protozoan cells, and cannot be detected by methodologies based in culture and cannot be killed by the normal anti-bacterial methods and other adverse environmental conditions (Greub and Raoult 2004). A number of studies have shown that following internalization within protozoans, the pathogens have increased virulence and demonstrate increase pathogenicity following infection of mammalian cells (Rasmussen et al. 2005, Steinberg and Levin 2007, Adiba et al. 2010). The definite reasons for this occurrence remain to be explained.

Interactions between Gram- Negative Pathogens and Protozoa

Legionella pneumophila

This bacterium is the causative agent of Legionnaires' disease. It has long been established that it can grow within mammalian cells such as macrophages, monocytes and epithelial cells (Horwitz 1983). Ever since it was demonstrated that L. pneumophila can infect Acanthamoebae spp. (Rowbotham 1980), the interaction between L. pneumophila and protozoa, especially amoeba, has been widely researched at both the cellular and molecular levels. Since then, L. pneumophila has been studied as a model organism for bacteria-protozoa interaction studies and their role in

pathogenesis to mammalian cells. The protozoans Acanthamoeba spp., Balamuthia mandrillaris (Shadrach 2005), Dictyostelium discoideum (Solomon et al. 2000) and Hartmannella vermiformis (Abu Kwaik 1996) have been most commonly utilized in in vitro experiments with L. pneumophila.

Acanthamoebae spp. has been previously shown to protect L. pneumophila against external stresses, such as heat and biocides (Harb et al. 2000). Furthermore, the ability of L. pneumophila to survive and multiply within Acanthamoebae spp. signifies the possible role of Acanthamoebae spp. in transmission of L. pneumophila. Although intra-amoebic replication of L. pneumophila can result in the lysis of host Acanthamoeba spp., a study has demonstrated that Acanthamoeba spp. are capable of allowing the intra-amoebic survival of L. pneumophila for prolonged periods of even up to several months, without experiencing cell lysis (Winiecka-Krusnell and Linder 1999). The outcome of that study suggests that the host amoeba and bacteria have adapted very well with each other resulting in the ability of both microorganisms to survive for longer than usual periods in the environment.

Interestingly, Berk et al. (1998) showed that when Acanthamoebae spp. was co-cultured with L. pneumophila, the amoeba secreted faecal pellets encapsulating viable bacterial cells. L. pneumophila within these faecal pellets appeared to be resistant to treatment with cooling tower biocidal agents. An earlier study demonstrated that L. pneumophila cells within cysts of Acanthamoebae spp. are able to survive treatment with high concentrations of free chlorine (Kilvington and Price 1990). Both studies implicate the amoeba in the dissemination of L. pneumophila.

Similarities between the interactions of L. pneumophila-amoeba and L. pneumophila-mammalian cell interactions have been noted previously. This includes the uptake of L. pneumophila by a coiling phagocytosis followed by the presence of the bacteria within host phagosomes. L. pneumophila-containing phagosomes are generally surrounded by host organelles such as mitochondria, vesicles, and rough endoplasmic reticulum (Bozue and Johnson 1996, Gao et al. 1997, Abu Kwaik et al. 1998, Hilbi 2001). Further, it has been demonstrated that in amoeba and mammalian hosts, the expression of virulence factors by L. pneumophila were responsible for the inhibition of phagosome-lysosome fusion within the host (Bozue and Johnson 1996). These similarities have been taken to suggest that amoeba-derived L. pneumophila are primed to cause infection within mammalian cells.

Similarities aside, there are a number of important differences in the interaction of L. pneumophila with mammalian and protozoan hosts systems. Firstly, L. pneumophila does not require host protein synthesis in macrophages, but it does in H. vermiformis. Secondly, the bacterium is capable of inducing apoptosis in mammalian cells, but do not do so in A. polyphaga (Harb et al. 2000). By using mutant strains of L. pneumophila that are defective for cytotoxicity, intracellular survival, and replication in both macrophage-like cells as well as A. polyphaga, evidence provided by Gao et al. (1997) demonstrated that while these mutants were not able to attach to A. polyphaga, attachment to the macrophages-like cells was only slightly impaired. By utilizing different mechanisms in order to infect different hosts, this implies that L. pneumophila has evolved these mechanisms to widen its

choice of hosts so that the bacterium is able to survive in a variety of environments.

Garcia et al. (2007) reported that A. polyphaga protected intra-amoebic L. pneumophila from killing by sodium hypochlorite (NaOCl). The resuscitation of viable but non-culturable (VBNC) L. pneumophila in NaOCl-treated water occurred with the assistance of A. polyphaga hosts. Besides that, the encystation of A. polyphaga was shown to be prevented by the presence of intra-amoebic L. pneumophila (Ohkuma 1978).

Escherichia coli

E. coli, mostly pathogenic strains such as O157: H7 and K1 have been widely studied for their interaction with protozoa. One of the earliest works on E. coli-protozoa interaction showed that the non-pathogenic E. coli B/r was predated upon by the ciliate Colpoda steinii (Drake and Tsuchiya 1976). A separate study by Gourabathini et al. (2008) showed that C. steinii viable counts increased following feeding on the pathogenic E. coli O157: H7, indicating the ciliate utilized this pathogen as a food source to grow. This is a unique finding, as other similar studies have demonstrated the ability of pathogenic strains of E. coli to survive within other types of protozoa such as Acanthamoeba spp. and the ciliate T. pyriformis. This observation could either be an indication that the pathogenic E. coli O157: H7 do not have the ability to escape killing by C. steinii, or that Acanthamoeba spp. and T. pyriformis do not have a killing mechanism in place for E. coli O157: H7. Schlimme et al. (1995) demonstrated that E. coli K12 resisted digestion by Tetrahymena spp. and were secreted from Tetrahymena spp. as viable cells within faecal pellets. Barker et al. (1999) demonstrated that E. coli O157 was

able to survive and multiply within A. polyphaga, following a co-culture period of up to 35 days. Another study showed that E. coli cells that were present within the drinking water reservoir isolate ciliate Cyclidium sp. demonstrated high resistance