Unutarstanični život bakterija Francisella i Legionella u amebama

Ožanič, Mateja; Marečić, Valentina; Gobin, Ivana; Šantić, Marina

Source / Izvornik: **Medicina Fluminensis : Medicina Fluminensis, 2016, 52, 49 - 54**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:184:926214

Intracellular life of *Francisella* and *Legionella* within amoebae cells

Unutarstanični život bakterija *Francisella* i *Legionella* u amebama

Mateja Ožanič, Valentina Marečić, Ivana Gobin, Marina Šantić*

Department of Microbiology and Parasitology, Faculty of Medicine, University of Rijeka, Rijeka

Abstract. free-living amoebae are present in the nature, feeding mainly with bacteria, fungi, and algae. Some microorganisms have evolved different mechanisms to resist the digestion by amoebae and they are called "amoeba-resistant microorganisms". Some of the important human bacterial pathogens belong to this category including *Cryptococcus neoformans, Chlamydophila pneumoniae*, *Mycobacterium avium*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Legionella* spp., and *Francisella tularensis*. *Francisella* and *Legionella* are gram negative facultative intracellular bacteria. Although the diseases they cause are completely different, they share some of the unique features in intracellular lifestyle within amoeba cells.

Key words: amoeba; *Francisella*; *Legionella*

Sažetak. Slobodno-živuće amebe prisutne su u prirodi, a hrane se uglavnom bakterijama, gljivama i algama. Neki mikroorganizmi razvili su različite mehanizme kojima izbjegavaju razgradnju unutar stanica ameba te se nazivaju „ameba-otporni mikroorganizmi". Bakterije koje pripadaju toj skupini uključuju *Cryptococcus neoformans, Chlamydophila pneumoniae*, *Mycobacterium avium*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Legionella* spp. i *Francisella tularensis*. *Francisella* i *Legionella* su gram negativne fakultativno unutarstanične bakterije. Iako dovode do potpuno drugačijih oblika bolesti, te bakterije pokazuju sličan životni ciklus unutar stanica ameba.

Ključne riječi: ameba; *Francisella*; *Legionella*

*Corresponding author:
Prof. dr. sc. Marina Šantić
University of Rijeka, Faculty of Medicine
Braće Branchetta 20, 51 000 Rijeka
e-mail: marina.santic@medri.uniri.hr

http://hrcak.srce.hr/medicina

INTRODUCTION

Nonpathogenic bacteria are taken up by the cells into vacuoles or phagosomes that are processed into endocytic pathway during which the vacuoles matures into the lysosomes where the bacterium is degraded. Phagocytosis and degradation of bacteria within the phagolysosomes is our first line of defense against microorganisms. To avoid this killing within phagocytic cells intracellular pathogens have evolved different strategies to survive and evade phagosome lysosome fusion: (i) escape from the phagosome into the cytoplasm such as the case for *Listeria* and *Shigella*[1,2]; (ii) adapt to the acidic harsh environment within the phagolysosomes, such as the case of *Coxiella*[3]; and (iii) the more common strategy is to modulate biogenesis of the phagosomes into niches permissive for intracellular replication, such as the case of *Mycobacterium, Chlamydia, Brucella, Salmonella* and *Legionella*[4-8]. Understanding the mechanisms by which pathogens explore the vesicle trafficking in different host is extremely important for understanding the ability of certain microbe to cause disease and is essential for designing novel strategies for prevention and treatment.

Most of the bacteria use similar mechanisms to adapt to different niches such as the case for *Legionella.* The life cycle of *Legionella* in amoebae and macrophages is very similar. Why *Francisella* uses different cycle in these pahagocitic cells?

Free-living amoebae are ubiquitous in natural sources such as soil, freshwater and dust, providing multiple opportunities for contact with humans[9-11]. They are also frequently isolated from anthropogenic ecosystems such as tap water, air conditioning units, cooling towers, jacuzzi tubs and hydrotherapy pools in hospitals, feeding on the microbial biofilm present in those systems[11-14]. Free-living amoebae such as *Acanthamoeba* and *Hartmannella* have been found in large variety of natural and anthropogenic habitats. Free-living amoebae feed mainly on bacteria, fungi, and algae by phagocytosis. In contrast, some bacteria can act like real amoeba pathogens able to lyse the amoeba cells before or after completing an intra-amoeba replication cycle. In addition, some microorganisms have shown the ability to survive the uptake by free-living amoeba and prevent intracellular destruction. These "amoeba-resistant microorganisms" include bacteria, viruses, and fungi. Bacteria are considered as symbionts when they manage to live in association with amoeba for a specific period of their lifetime[15,16]. Amoeba can serve as hosts for a large number of pathogenic bacteria, including *Francisella tularensis*, *Legionella pneumophila*, *Coxiella burnetii*, *Listeria monocytogenes*, many *Mycobacterium spp*., *Chlamydia* related bacteria, *Escherichia coli* serotype O157[11,17-23]. Taken together, both amoebal symbionts and amoebal pathogens may use amoeba as their replicative niche[24]. Amoeba play an important role for transmission of human pathogen bacteria to humans and they could be described like ''Trojan horse'' in the world of microbes[13,25,26]. Understanding the intracellular lifestyle of bacteria within amoeba cells is important to understanding of bacterial ecology and transmission to humans.

The intracellular life of *Francisella* and *Legionella* within amoebae cells will be discussed in this paper.

INTRACELLULAR LIFE OF *FRANCISELLA TULARENSIS*

F. tularensis is a gram negative, highly infectious, facultative intracellular bacterium that causes fulminating disease tularemia. The genus *Francisella* includes four organisms: *F. tularensis* subsp. *tularensis* (type A), *F. tularensis* subsp. *holarctica* (type B), *F. tularensis* subsp. *mediasiatica* and *Francisella novicida*. The ability of *F. tularensis* to envade and proliferate within cells was shown to be of great relevance for the development of tularemia[27-30]. *F. novicida* is able to survive and replicate within various cell types, macrophages, dendritic cells, epithelial cells, including amoebae cells[31-35]. *F. tularensis* subsp. *holarctica* and *F. novicida* have a strong association with fresh water environments, free living amoeba and biofilms[36,37]. It is assumed that the bacterium survives in such waters by different

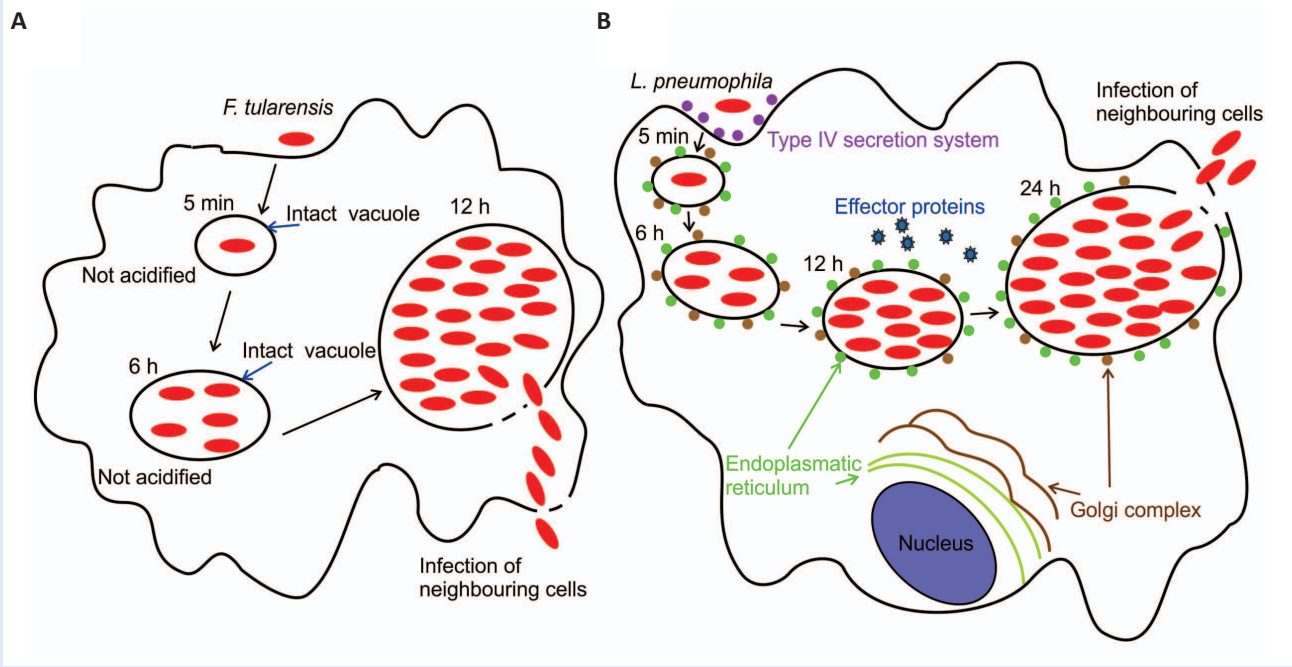

Figure 1. Intracellular life style of *F. tularensis* and *L. pneumophila* within amoebae cells. After phagocytosis by amoeba, bacteria are localized in intact vacuoles. Bacteria reside in the vacuoles and replicates. Purple circle – Type IV secretion system, Green circle – Endoplasmatic reticulum, Brown circle – Golgi complex, Blue star – effector protein.

models; i) persists in open water in a viable but not cultivable state in which bacteria are not infectious[38]; ii) lives in biofilm which is considered as an important niche for environmental survival and persistence of *Francisella*[39]; and iii) associated with protozoa.

Previous *in vitro* studies showed that *F. tularensis subsp. holarctica, tularensis,* and *F. novicida* are able to enter and multiply within *A. castellanii*[20,33,35,40-42], *H. vermiformis*[35] and *Dictyostelium discoideum* (unpublished data) cells. Once inside the cells the bacterium resides and replicates within membrane-bound vacuoles known as *Francisella*-containing vacuoles[35] (FCVs) (Figure 1A). In mammalian cells bacteria escape from *Francisella*-containing vacuoles (FCVs) and proliferate in cytosol, which is the major difference between these two host cells. In addition, imaging studies using the lysosomotropic agent LysoTracker Red DND-99, which concentrates in acidified vesicles and compartments, have shown that FCVs did not acquire this dye at any time point during the infection of amoebae with *F. tularensis* subsp. *novicida*[43]. It has also been shown that *F. novicida* blocks lysosomal fusion within *A. castellanii*[33]. In contrast, in mammalian cells the transient acidification of the FCV is essential for subsequent bacterial escape and replication of *Francisella* in the macrophage cytosol[44-47]. Therefore, *Francisella* escapes from acidified vacuoles in human and arthropod-derived cells, but replicates within non-acidified FCV in *H. vermiformis*[35].

The ability of *Francisella* to survive and replicate inside the protozoa cell seems to be of great importance in sustaining the life cycle of *Francisella* in aquatic environment.

INTRACELLULAR LIFE OF *LEGIONELLA PNEUMOPHILA*

L. pneumophila is an intracellular gram-negative bacterium, ubiquitous in the aquatic environment and important causative agent of community-acquired and nosocomial bacterial pneumonia. At present, 52 characterized species belongs to genus *Legionella* of which more than a half has been implicated in human disease[48]. However, *L. pneumophila* accounts for over 90% of legionellosis. Two distinct syndromes of the disease are known in the infection caused by *L. pneumophila;* Legionnaires' disease, a severe, acute pneumonia and a self-limiting flu-like infection termed Pontiac fever.

The bacterium enters the human body via inhalation of aerosol droplets. Once in the lungs, *L. pneumophila* invades and replicate mainly in alveolar macrophages[49,50]. The pathogenesis of legionellosis depends also on prior adaptation of *L. pneumophila* in the natural water environment. In freshwater, *Legionella* survive and replicate within free-living protozoa including ciliates *Tetrahymena* and *Cyclidium spp.* as well as amoeba species belonging to *Acanthamoeba, Hartmanella, Valkampfia, Naegleria and Dictyostelium.* In contrast to protozoa macrophages are not „natural" host cells for *Legionella*, although they utilize the same mechanism to avoid the degradation by these cells.

Free-living amoeba could serve as a good model in studying environmental existence and adaptation of *Francisella*.

Upon phagocytosis by protozoa *Legionella* utilizes the Icm/Dot type IV secretion system (T4SS) which plays important role in hijacking the normal phagocyte-lysosome pathway[17,51]. The bacterium resides in the *Legionella*-containing vacuole (LCV) and avoids the degradation by the lysosomes[52]. Within a few minutes, mitochondria and endoplasmic reticulum (ER)-derived vesicle are recruited to the LCV which becomes remodeled into an ER-derived compartment. The bacterium replicate extensively, disrupts phagosomal membrane escapes into the host cell cytosol, lyses and exit the cells to get ready for the new infection cycle[53].

Free-living protozoa represent valuable experimental model to study *Legionella* ecology and pathogenesis, as its natural hosts and as a paradigm for infection of human macrophages.

CONCLUSION

F. tularensis has shown a different lifestyle within macrophages and protozoa cells. Within macrophages *F. tularensis* escape from FCV and replicate in the cytosol, while in *H. vermiformis* bacteria replicate in intact vacuoles. However, the molecular and cellular aspects of infection by *L. pneumophila* in both protozoa and mammalian phagocytes are similar. Once engulfed by phagocytic cell, bacteria replicate extensively within phagosome. It is followed by lyses of host cell and proliferation of bacteria into the environment.

F. tularensis and *L. pneumophila* shares very similar life style in protozoa cells. It is likely that association of these bacteria with protozoa is a major factor in the continuous persistence of bacteria in the environment, as well as transmission of bacteria to humans. In addition, the human's cells seem to be the dead-end from the bacterial point of view, as no human-to-human transmission has been reported for *Francisella* or *Legionella.*

Conflicts of interest statement: The authors report no conflicts of interest.

REFERENCES

1. Molloy S. Bacterial pathogenicity: A competent escape for Listeria. Nat rev 2012;10:670.
2. Senerovic L, Tsunoda SP, Goosmann C, Brinkmann V, Zychlinsky A, Meissner F et al. Spontaneous formation of IpaB ion channels in host cell membranes reveals how Shigella induces pyroptosis in macrophages. Cell Death Dis 2012;3:e384.
3. Howe D, Shannon JG, Winfree S, Dorward DW, Heinzen RA. Coxiella burnetii phase I and II variants replicate with similar kinetics in degradative phagolysosome-like compartments of human macrophages. Infect Immun 2010;78:3465-74.
4. Isaac DT, Isberg R. Master manipulators: an update on Legionella pneumophila Icm/Dot translocated substrates and their host targets. Future Microbiol 2014;9:343-59.
5. Krieger V, Liebl D, Zhang Y, Rajashekar R, Chlanda P, Giesker K et al. Reorganization of the endosomal system in Salmonella-infected cells: the ultrastructure of Salmonella-induced tubular compartments. PLoS pathog 2014;10:e1004374.
6. Myeni S, Child R, Ng TW, Kupko JJ 3rd, Wehrly TD, Porcella SF et al. Brucella modulates secretory trafficking via multiple type IV secretion effector proteins. PLoS pathog 2013;9:e1003556.
7. Scidmore MA. Recent advances in Chlamydia subversion of host cytoskeletal and membrane trafficking pathways. Microbes Infect 2011;13:527-35.
8. Sullivan JT, Young EF, McCann JR, Braunstein M. The Mycobacterium tuberculosis SecA2 system subverts phagosome maturation to promote growth in macrophages. Infect Immun 2012;80:996-1006.
9. Schuster FL, Visvesvara GS. Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. Int J Parasitol 2004;34:1001-27.
10. Valster RM, Wullings BA, van den Berg R, van der Kooij D. Relationships between free-living protozoa, cultivable Legionella spp., and water quality characteristics in

three drinking water supplies in the Caribbean. Appl Environ Microbiol 2011;77:7321-8.

11. Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: Acanthamoeba spp., Balamuthia mandrillaris, Naegleria fowleri, and Sappinia diploidea. FEMS Immunol Med Microbiol 2007;50:1-26.
12. Coskun KA, Ozcelik S, Tutar L, Elaldi N, Tutar Y. Isolation and identification of free-living amoebae from tap water in Sivas, Turkey. Biomed Res Int 2013;1:675145.
13. Evstigneeva A, Raoult D, Karpachevskiy L, La Scola B. Amoeba co-culture of soil specimens recovered 33 different bacteria, including four new species and Streptococcus pneumoniae. Microbiology 2009;155:657-64.
14. Martinez AJ, Visvesvara GS. Free-living, amphizoic and opportunistic amebas. Brain pathol 1997;7:583-98.
15. Clemens DL, Lee BY, Horwitz MA. Francisella tularensis enters macrophages via a novel process involving pseudopod loops. Infect immun 2005;73:5892-902.
16. Fritsche TR, Gautom RK, Seyedirashti S, Bergeron DL, Lindquist TD. Occurrence of bacterial endosymbionts in Acanthamoeba spp. isolated from corneal and environmental specimens and contact lenses. J Clin Microbiol 1993;31:1122-6.
17. Abu Kwaik Y, Gao LY, Stone BJ, Venkataraman C, Harb OS. Invasion of protozoa by Legionella pneumophila and its role in bacterial ecology and pathogenesis. Appl Environ Microbiol 1998;64:3127-33.
18. Amann R, Springer N, Schonhuber W, Ludwig W, Schmid EN, Müller KD et al. Obligate intracellular bacterial parasites of acanthamoebae related to Chlamydia spp. Appl Environ Microbiol 1997;63:115-21.
19. Berger P, Papazian L, Drancourt M, La Scola B, Auffray JP, Raoult D. Ameba-associated microorganisms and diagnosis of nosocomial pneumonia. Emerg Infect Dis 2006;12:248-55.
20. Greub G, Raoult D. Microorganisms resistant to free-living amoebae. Clin Microbiol Rev 2004;17:413-33.
21. La Scola B, Raoult D. Survival of Coxiella burnetii within free-living amoeba Acanthamoeba castellanii. Clin Microbiol Infect. 2001;7:75-9.
22. Ly TM, Muller HE. Ingested Listeria monocytogenes survive and multiply in protozoa. J Med Microbiol 1990; 33:51-4.
23. Steinert M, Birkness K, White E, Fields B, Quinn F. Mycobacterium avium bacilli grow saprozoically in coculture with Acanthamoeba polyphaga and survive within cyst walls. Appl Environ Microbiol 1998;64:2256-61.
24. Taylor M, Mediannikov O, Raoult D, Greub G. Endosymbiotic bacteria associated with nematodes, ticks and amoebae. FEMS Immunol Med Microbiol 2011;64:21-31.
25. Barker J, Brown MR. Trojan horses of the microbial world: protozoa and the survival of bacterial pathogens in the environment. Microbiology 1994;140:1253-9.
26. Molmeret M, Horn M, Wagner M, Santic M, Abu Kwaik Y. Amoebae as training grounds for intracellular bacterial pathogens. Appl Environ Microbiol 2005;71:20-8.
27. Chong A, Celli J. The francisella intracellular life cycle: toward molecular mechanisms of intracellular survival and proliferation. Front Microbiol 2010;1:138.
28. Conlan JW, Zhao X, Harris G, Shen H, Bolanowski M, Rietz C et al. Molecular immunology of experimental primary tularemia in mice infected by respiratory or intradermal routes with type A Francisella tularensis. Mol Immunol 2008;45:2962-9.
29. Forsman M, Sandstrom G, Sjostedt A. Analysis of 16S ribosomal DNA sequences of Francisella strains and utilization for determination of the phylogeny of the genus and for identification of strains by PCR. Int J Syst Bacteriol 1994;44:38-46.
30. Oyston PC, Sjostedt A, Titball RW. Tularaemia: bioterrorism defence renews interest in Francisella tularensis. Nat rev 2004;2:967-78.
31. Bolger CE, Forestal CA, Italo JK, Benach JL, Furie MB. The live vaccine strain of Francisella tularensis replicates in human and murine macrophages but induces only the human cells to secrete proinflammatory cytokines. J Leukoc Biol 2005;77:893-7.
32. Bosio CM, Dow SW. Francisella tularensis induces aberrant activation of pulmonary dendritic cells. J Immunol. 2005;175:6792-801.
33. El-Etr SH, Margolis JJ, Monack D, Robison RA, Cohen M, Moore E et al. Francisella tularensis type A strains cause the rapid encystment of Acanthamoeba castellanii and survive in amoebal cysts for three weeks postinfection. Appl Environ Microbiol 2009;75:7488-500.
34. Hall JD, Craven RR, Fuller JR, Pickles RJ, Kawula TH. Francisella tularensis replicates within alveolar type II epithelial cells in vitro and in vivo following inhalation. Infect immun 2007;75:1034-9.
35. Santic M, Ozanic M, Semic V, Pavokovic G, Mrvcic V, Kwaik YA. Intra-Vacuolar Proliferation of F. Novicida within H. Vermiformis. Front microbiol 2011;2:78.
36. Broman T, Thelaus J, Andersson AC, Bäckman S, Wikström P, Larsson E et al. Molecular Detection of Persistent Francisella tularensis Subspecies holarctica in Natural Waters. Int J Microbiol 2010;1:851946.
37. Willke A, Meric M, Grunow R, Sayan M, Finke EJ, Splettstösser W et al. An outbreak of oropharyngeal tularaemia linked to natural spring water. J Med Microbiol 2009;58:112-6.
38. Forsman M, Henningson EW, Larsson E, Johansson T, Sandstrom G. Francisella tularensis does not manifest virulence in viable but non-culturable state. FEMS Microbiol Ecol 2000;31:217-24.
39. Mahajan UV, Gravgaard J, Turnbull M, Jacobs DB, McNealy TL. Larval exposure to Francisella tularensis LVS affects fitness of the mosquito Culex quinquefasciatus. FEMS Microbiol Ecol 2011;78:520-30.
40. Abd H, Johansson T, Golovliov I, Sandstrom G, Forsman M. Survival and growth of Francisella tularensis in Acanthamoeba castellanii. Appl Environ Microbiol 2003;69:600-6.
41. Hazlett KR, Caldon SD, McArthur DG, Cirillo KA, Kirimanjeswara GS, Magguilli ML et al. Adaptation of Francisella tularensis to the mammalian environment is governed by cues which can be mimicked in vitro. Infect immun 2008;76:4479-88.
42. Lauriano CM, Barker JR, Yoon SS, Nano FE, Arulanandam BP, Hassett DJ et al. MglA regulates transcription of virulence factors necessary for Francisella tularensis intraamoebae and intramacrophage survival. Proceedings of the Issues Sci Technol 2004;101:4246-9.
43. Clemens DL, Lee BY, Horwitz MA. Francisella tularensis phagosomal escape does not require acidification of the phagosome. Infectt immun 2009;77:1757-73.

44. Asare R, Kwaik YA. Exploitation of host cell biology and evasion of immunity by francisella tularensis. Front microbiol 2011;1:145.

45. Chong A, Wehrly TD, Nair V, Fischer ER, Barker JR, Klose KE et al. The early phagosomal stage of Francisella tularensis determines optimal phagosomal escape and Francisella pathogenicity island protein expression. Infecti immun 2008;76:5488-99.

46. Santic M, Molmeret M, Abu Kwaik Y. Modulation of biogenesis of the Francisella tularensis subsp. novicida-containing phagosome in quiescent human macrophages and its maturation into a phagolysosome upon activation by IFN-gamma. Cell Microbiol 2005;7:957-67.

47. Wehrly TD, Chong A, Virtaneva K, Sturdevant DE, Child R, Edwards JA et al. Intracellular biology and virulence determinants of Francisella tularensis revealed by transcriptional profiling inside macrophages. Cell Microbiol 2009;11:1128-50.

48. Newton HJ, Ang DK, van Driel IR, Hartland EL. Molecular pathogenesis of infections caused by Legionella pneumophila. Clin Microbiol Rev 2010;23:274-98.

49. Cirillo JD, Falkow S, Tompkins LS. Growth of Legionella pneumophila in Acanthamoeba castellanii enhances invasion. Infect immun 1994;62:3254-61.

50. Brieland JK, Fantone JC, Remick DG, LeGendre M, McClain M, Engleberg NC. The role of Legionella pneumophila-infected Hartmannella vermiformis as an infectious particle in a murine model of Legionnaire's disease. Infect immun. 1997;65:5330-3.

51. Gao LY, Harb OS, Abu Kwaik Y. Utilization of similar mechanisms by Legionella pneumophila to parasitize two evolutionarily distant host cells, mammalian macrophages and protozoa. Infect immun 1997;65:4738-46.

52. Isberg RR, O'Connor TJ, Heidtman M. The Legionella pneumophila replication vacuole: making a cosy niche inside host cells. Nat rev 2009;7:13-24.

53. Molmeret M, Bitar DM, Han L, Kwaik YA. Disruption of the phagosomal membrane and egress of Legionella pneumophila into the cytoplasm during the last stages of intracellular infection of macrophages and Acanthamoeba polyphaga. Infect immun 2004;7:4040-51.