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Persistence of Categories A and B Select Agents in Environmental Matrices

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List of Acronyms and Abbreviations

ATCC®	American Type Culture Collection (ATCC) [Manassas, VA]
BHI	brain heart infusion
CDC	Centers for Disease Control and Prevention
CFU	colony forming units
CHAB	cystine heart agar blood
gfp	green fluorescent protein
HEPA	high-efficiency particulate air
LCV	large-cell variants
LVS	live vaccine strain
mS	millisiemens
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PFU	plaque forming units
ppm	parts per million
RH	relative humidity
SCV	small-cell variants
SDC	small dense cells
SERRA	Support for Environmental Rapid Risk Assessment
T_4	time needed to decrease the viral load by 4 $\log_{10}$
T_{99}	time required for the initial titer to decrease by 99%
µL	microliter
US EPA	U.S. Environmental Protection Agency
UV	ultraviolet
µW	microwatt
VEE	Venezuelan equine encephalitis (or encephalomyelitis)
VBNC	viable but nonculturable

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Executive Summary

Persistence is the ability of an organism to remain viable (e.g., to remain alive or in the case of viruses to remain infective) over time under a given environmental condition and medium. Understanding a bioterrorism agent's ability to persist within the environment will help properly assess and respond to the agent's release. The purpose of this study was to summarize persistence data generated since the literature review conducted by Sinclair et al. (2008)[1] as well as review the literature for persistence data on agents not addressed by Sinclair et al. (2008). These data are critically important for at least two reasons; 1) to allow informed emergency response and remediation decisions following a contamination incident, and 2) to identify gaps in the current state of the science, and to focus research toward closing these identified gaps. The scope of this literature review generally focused on agents that were determined to be viable by culturing on artificial media and/or infecting tissue cultures. In some cases evidence of an agent entering a viable but nonculturable state was discussed. Relevant English language literature was identified primarily by searching each agents name and the terms "persistence", "recoverable", "survival", "survivability", and "viability" using Google Scholar. Table ES-1 identifies the agents covered in this review, identifies the number of studies reviewed by agent if addressed by Sinclair et al. (2008), and lists the number of studies (excluding Sinclair et al. [2008] and works cited therein) presenting original quantitative persistence data that were summarized in this report by environmental media (i.e., aerosol, fomite, soil, and water). A total of 94 sources were reviewed for this report including ecologically-based studies and literature reviews (not shown on Table ES-1) and excluding Sinclair et al. (2008) and works cited therein.

Table ES-1. Agents Investigated and Number of Persistence Studies Identified

Agent Reviewed	Number of Studies Included in the Review by Sinclair et al. (2008)*	Number of Studies Included in this Review†			
		Aerosol	Fomite	Soil	Water
Bacillus anthracis (vegetative cells only)	2	0	3	0	0
Brucella species (e.g., *suis, melitensis, abortus*, etc.)	Not reviewed	0	3	1	2
Burkholderia mallei	Not reviewed	0	0	0	3
Burkholderia pseudomallei	Not reviewed	0	1	5	7
Coxiella burnetii	Not reviewed	0	0	1	0
Francisella tularensis	6	1	2	0	6
Viral encephalitis and hemorrhagic fever agents	9	2	4	0	2
Yersinia pestis	6	0	1	3	4

Source: Sinclair, 2008, Applied and Environmental Microbiology 74(3):555-563

* The number of studies cited by Sinclair et al. (2008) presenting quantitative persistence data.

† The number of studies (excluding Sinclair et al. [2008] and works cited therein) presenting original quantitative persistence data; ecologically-based studies and literature review data are included in this review but not reflected in this table.

[1] Sinclair, R. et al., 2008. Applied and Environmental Microbiology 74(3):555-563.

The summary that follows provides a quick indication of the durations that the agents could persist in the four types of environmental media, although "fomite" (i.e., surfaces or materials likely to carry infection) represents many different kinds of materials/surfaces. Some data documented survival of a given duration, but did not document survival until extinction. So, actual persistence could exceed the persistence documented in the literature. Data for survival of the bacterial agents listed in Table ES-1 for an aerosol medium were scant; *F. tularensis* survived 5 days and *Y. pestis* survived 57 minutes. The data for bacterial agent survival on fomites were more complete, including *B. anthr*acis (vegetative cells only), *Brucella* species, *Burkholderia mallei*, *Burkholderia pseudomallei*, and *Y. pestis*. Literature values ranged from 3 days (*B. pseudomallei*) to 3 months (*B. mallei*). For survival in soil, data were available for *Brucella* species, *B. pseudomallei*, *C. burnetii,* and *Y. pestis*. Literature values ranged from 20 days (*C. burnetii*) to 30 months (*B. pseudomallei*). For survival in water, we found survival data for bacterial agents *B. anthracis*, *Brucella* species, *B. mallei*, *B. pseudomallei*, *F. tularensis*, and *Y. pestis*. Literature values ranged from 6 days (*B. anthracis*, vegetative cells only) to 16 years (*B. pseudomallei)*. For viral encephalitis and hemorrhagic fever agents, persistence was documented for aerosol medium at 120 days, for fomites at 5 days, and for water at 69 days.

Persistence durations are likely affected by the agent species and strain evaluated, the agent preparation/application methods, media specific differences (e.g., water/soil chemistry, porous or nonporous fomite surface types, etc.), and the methods used to recover and quantify the agent. The environmental conditions (e.g., temperature, relative humidity) during the persistence testing are also major drivers with regard to agent survivability. Sunlight and elevated temperatures were often reported to shorten the persistence duration of several agents including *B. mallei*, *Brucella* species, and viral encephalitis and hemorrhagic fever agents. Numerous studies indicate that other agents (e.g., *B. pseudomallei* and *Y. pestis*) seem to prefer warmer conditions. Moist conditions were found to improve *B. abortus*, *B. mallei*, and *B. pseudomallei* persistence compared to more dry environments.

Following the release of an agent, other organisms/bacteria may adversely affect the persistence of the agent or enhance the agent's survival in the environment. For example, in recent studies the soil bacterium, *Burkholderia multivorans*, appeared to inhibit *B. pseudomallei* growth, and the bacterium, *Hylemonella gracilis*, eventually dominated a filtered river water sample, apparently by inhibiting the long-term survival of *Y. pestis*. However, other recent studies have shown that amoebae (e.g., *Acanthamoeba castellanii*) may provide a niche for the environmental survival of *B. anthracis*, *B. pseudomallei*, *C. burnetii*, and *F. tularensis*. A 2006 study indicated that the rhizosphere may enhance the proliferation of vegetative *B. anthracis* cells. Some agents may form biofilms that enhance environmental survival, including *B. abortus* and *F. tularensis*. In addition to determining persistence durations under different conditions, the ecological factors contributing to the potential establishment of an agent in the environment are also important to understand.

This report highlights broad gaps in persistence data. For example, no persistence data for aerosols were identified for *B. anthracis* (vegetative cells), *Brucella* species, *B. mallei*, *B. pseudomallei*, or *C. burnetii*. Persistence data in soil were lacking for *B. anthracis* (vegetative cells), *B. mallei*, *F. tularensis*, and the viral encephalitis and hemorrhagic fever agents. Likewise, no persistence data for fomites and water were identified for *C. burnetii*.

1. Introduction

The Centers for Disease Control and Prevention (CDC) categorizes bioterrorism agents based on their threat to national security. For example, Category A agents are "easily disseminated or transmitted from person to person" and cause high rates of mortality, while Category B agents are "moderately easy to disseminate" and cause illness with low mortality (CDC, 2013). Persistence is the ability of an organism to remain viable (i.e., to remain alive or in the case of viruses to remain infective) over time under a given environmental condition. Sinclair et al. (2008) conducted a literature review on the persistence of Category A agents in the environment to help assess risk and guide response actions. As described by Sinclair et al. (2008), viable agent persistence "will affect decontamination, infection rates, and encompassing geographic areas. Therefore, knowledge of microbial ecology and defensive public preparation are important factors in limiting bioterrorism-related morbidity and mortality." Unfortunately, limited Category A persistence data (e.g., agent die-off rates following a release) were available at the time of Sinclair's review in 2008.

2. Purpose

The purpose of this study was to determine the state of scientific knowledge about the persistence of CDC-listed Category A agents since the Sinclair et al. (2008) article was published and to several Category B agents that were not addressed by Sinclair et al. (2008). These data will allow informed emergency response and remediation decisions following a contamination incident, and will identify gaps in the current state of the science so that research can be conducted to close these gaps. The agents that are addressed in this review are shown in Table 1. While data from attenuated or vaccine strains of agent were used in this review, only limited surrogate (e.g., same genus but different species than the target agent) data were considered. With regards to persistence of *Bacillus anthracis*, only the vegetative (not spores) form of the organism was considered, as the spore-form is known to persist for decades or more. In general, much of the persistence work published recently has focused on using the actual agents rather than surrogates, possibly to reduce the uncertainty of extrapolating results from one species to another. The scope of this study generally focused on agents that were viable as determined by culturing on artificial media and/or infecting tissue cultures. In some cases evidence of an agent entering a viable but nonculturable (VBNC) state is discussed. The ability of an agent to subsequently cause infection or induce human disease following release into the environment is beyond the scope of this review. Data from Sinclair et al. (2008) were summarized briefly within this document as applicable.

Table 1. Agents Investigated

Agent	Disease	CDC Category	Organism Type	Reviewed by Sinclair et al. (2008)?
*Bacillus anthracis**	Anthrax	A	Bacteria	Yes
Brucella species (e.g., *suis, melitensis, abortus*, etc.)	Brucellosis	B	Bacteria	No
Burkholderia mallei	Glanders	B	Bacteria	No
Burkholderia pseudomallei	Melioidosis	B	Bacteria	No
Coxiella burnetii	Q fever	B	Bacteria	No
Francisella tularensis	Tularemia	A	Bacteria	Yes
Viral encephalitis and hemorrhagic fever agents	Encephalitis and hemorrhagic fever	A and B	Virus	Yes
Yersinia pestis	Plague	A	Bacteria	Yes

* This review focused on vegetative *B. anthracis* only.

3. Methods

Data for the agents targeted in this literature review (Table 1) were considered from unclassified reports, peer-reviewed journal articles, and published books. The review focused on the environmental persistence of the agents associated with aerosols, fomites (i.e., surfaces or materials likely to carry contamination), soil, and water. The media of interest play potentially important roles in the fate and transport of the agents, as well as potential media to which humans could be easily exposed. For example, humans could be exposed to agents via the inhalation of contaminated air, physical contact with contaminated fomites or soil (or the inhalation or reaerosolized agents from fomites and soil), and ingestion of contaminated water. The search was limited to articles published in English, but there was no restriction on geographic location. The literature search for Category A agents previously reviewed by Sinclair et al. (2008) generally focused on publications from 2007 to 2013. However, relevant articles identified from earlier time periods were also included in this review if not previously included in the review by Sinclair et al. (2008). The literature search for Category B agents, which were not reviewed by Sinclair et al. (2008), was more open to publications from all time periods. Books were limited to those published or revised in the last five years. The primary search engine used was Google Scholar. Search terms included the agent name: *anthracis, brucella, mallei, pseudomallei, burnetii, tularensis*, viral hemorrhagic fever, filovirus, arenavirus, ebola + marburg, lassa + machupo, viral encephalitis, or *pestis.* The search terms were the agent name plus "persistence", agent name plus "recoverable", agent name plus "survival" and "survivability", and agent name plus "viability", for example:

- *anthracis* + persistence
- *anthracis* + recoverable
- *anthracis* + survival + survivability
- *anthracis* + viability

In general, the first 100 returns (sorted by relevance) for each search term were reviewed for potentially applicable data. Additional search terms were used in conjunction with the agent name plus persistence approach as a check to ensure important variables affecting the agent's persistence were captured including: temperature, relative humidity (RH), surface type, agent preparation, associated environmental matrix, time, and ultraviolet (UV) light or solar radiation. Fewer returns (approximately 20 to 40) were reviewed for the additional searches.

In addition, the U.S. Environmental Protection Agency's (US EPA) Support for Environmental Rapid Risk Assessment (SERRA) database was used to search literature on persistence for *B. anthracis*, *Brucella* species, *B. mallei*, *B. pseudomallei*, *F. tularensis*, and *Y. pestis*. Data from the "Fate and Persistence" node of SERRA was used as applicable. The PubMed database was occasionally used in the literature review as a check against articles being identified with Google Scholar. As part of the literature review, potentially useful references in the bibliographies of the obtained documents were reviewed for relevance and obtained as applicable.

This report was generated using references (secondary data) that could not be evaluated for accuracy, precision, representativeness, completeness, or comparability and therefore no assurance can be made that the data extracted from these publications meet the stringent quality assurance requirements of the US EPA. However, the sources of secondary data were limited to peer-reviewed documents. While there are no formal quality requirements (professional judgment was used), each article was assessed qualitatively according to the document evaluation categories and data quality indicators shown in Table 2. These data quality indicators are the same as those used for documents included in US EPA's SERRA database. The articles cited within this report have generally been incorporated into the SERRA database, including completed "document criteria reports" that summarize the information presented in Table 2 for each article. The SERRA database is available at https://serra.cbiac.org/serra/ and requires registration for an account prior to use.

Table 2. Document Evaluation Categories and Data Quality Indicators

Document Evaluation Category	Data Quality Indicators
Literature/Scientific Content Level:	**A. Primary research publication** **B. Scientific/technical assessment** **C. Topical/literature review** **D. Opinion article**
Statistical Analysis:	**A. Statistical findings mentioned or presented (summary)** **B. Methodology, raw data and statistical findings presented (comprehensive)** **C. Statistics not indicated** **D. Not applicable**
Technology/Methods Used:	**A. All novel technology/methods**

	B. Some novel technology/methods, some established technology/methods
	C. All established technology/methods
	D. Origin of technology/methods unknown
	E. Not applicable
Experimental Methods:	A. Included with satisfactory detail
	B. Not included
	C. Incomplete/missing important information
	D. Not applicable
Model Organism(s) Used:	A. No surrogate agent used (original agent used for all studies)
	B. Surrogate agent used for some studies
	C. Surrogate agent used for all studies
	D. Not applicable
Controls:	A. Controls used for all studies
	B. Controls used for some studies but not others
	C. No controls used for any of the studies
	D. Controls not indicated/described
	E. Not applicable

4. *Bacillus anthracis* Vegetative Cell Persistence

B. anthracis is a member of the *Bacillus cereus* group, which is commonly found within soils. However, unlike the other members of the *B. cereus* group, *B. anthracis* can cause three different types of illness in humans: cutaneous, gastrointestinal, or inhalational anthrax. Robert Koch first described the etiology of anthrax in 1876 (Koch, 1876). Hudson et al. (2008) has described the history of significant work which has been devoted to the organism in the years since by Koch, Louis Pasteur, and others, as well as the use of *B. anthracis* as a bioweapon. When vegetative *B. anthracis* cells are exposed to unfavorable growth conditions, such as contact with air, they rapidly sporulate to form persistent spores (Hudson et al., 2008). Due to their known prolonged persistence in the soil (Kracalik et al., 2013), *B. anthracis* spores have been the focus of many studies, while a lesser amount of work has focused on the vegetative form of the bacterium. This review focuses only on studies conducted with vegetative cells of *B. anthracis*. A separate document focusing on the persistence of *B. anthracis* vegetative cells and spores in soil is in process.

4.1. *B. anthracis* Vegetative Cells on Fomites

The persistence of vegetative *B. anthracis* cells is considered to be significantly less than spores on environmental surfaces because of their nutrient demands, fragile nature, and inability of compete with other microorganisms (Hugh-Jones and Blackburn, 2009). Therefore, fewer studies have devoted their focus to vegetative work. This review found only three studies that dealt with the persistence of vegetative *B. anthracis* cells or surrogate *Bacillus* species on different fomites; two of which focused on the ability of the bacteria to form biofilms within nutrient broth.

Galeano et al. (2003) found that when high concentrations of *B. anthracis* Sterne vegetative cells, approximately 10^6 to 10^7 colony forming units (CFU) per milliliter (mL), were placed on stainless steel coupons and held at 25 °C and 80% RH to prevent desiccation, the vegetative cell counts increased over 24 hours. The study also tested antimicrobial-coated stainless steel coupons and found that culturable vegetative cells began to decrease after only 2 hours of incubation on the treated coupons and were inactivated by three orders of magnitude by 24 hours (Galeano et al., 2003). Unfortunately, the study only quantified cell viability up to 24 hours, and thus does not increase our understanding of persistence on stainless steel over greater temporal scales (i.e., >1 day).

The capability of *B. anthracis* to form biofilms might have a profound impact on its growth and persistence on various surfaces. Due to their structure, biofilms are known to be relatively antibiotic resistant (Lee et al., 2007). Therefore, biofilm microenvironments might create a more suitable environment for vegetative *B. anthracis* to flourish. Auger et al. (2009) tested multiple strains of the *B. anthracis* surrogates *Bacillus thuringiensis* and *B. cereus* for their ability to form biofilms on polyvinyl chloride microtiter plates containing lysogeny broth with bactopeptone at 30°C. After 72 hours of incubation, 47% of the *B. thuringiensis* and 37.5% to 40% of the nonclinical and diarrheal *B. cereus* strains formed biofilms (Auger et al., 2009). In another study, *B. anthracis* Sterne cells began to form a biofilm on glass microtiter plates within 8 hours when brain heart infusion (BHI) broth was held under static conditions, and a mature biofilm was found after 7 days of incubation. In contrast, when the BHI broth was laminarly flowed within a three-channel flow cell, 24 hours were required for microcolony formation, yet a mature biofilm was again noted after 7 days (Lee et al., 2007). As these studies show, *B. anthracis* has the capacity to form biofilms under select conditions, and it is possible that the biofilms may aid in its persistence within the environment.

As mentioned previously, the ability of *B. anthracis* to form biofilms could have a profound impact on environmental persistence and proliferation. Biofilm formation protects *B. anthracis* cultures through a variety of mechanisms: the multi-layer structure of biofilms serves to protect the inner cells from potentially hazardous conditions. Biofilms are sloughed off in clumps thereby creating a mechanism for transportation with embedded protective capabilities. Finally, biofilm formation can create areas of nutrient limitation, thereby triggering spore formation for the *B. anthracis* cells (Lee et al., 2007). However, Lee et al. (2007) determined that the environmental conditions during which the biofilm is formed impacts the ratio of spores to vegetative cells founds within a mature biofilm. When biofilms were supplemented with 5% carbon dioxide (CO_2) during culture, the vegetative cell concentration was greater than 88% as compared to less than 50% under normal atmospheric conditions (no

supplemented CO_2) (Lee et al., 2007). The work by Lee et al. (2007), as well as Galeano et al. (2003) and Auger et al. (2009), is summarized in Table 3.

For Table 3, and the other persistence tables presented in this report, the longest persistence duration is shown for each applicable agent, material, and environmental condition. To better describe the upper range of persistence, a later column "shortest duration without persistence" is also included. Actual persistence will likely fall between the durations presented in these columns. For example, Galeano et al. (2003) tested the persistence of *Bacillus* species at 0, 2, 6, and 24 hours. When testing was conducted with stainless steel with an antimicrobial coating, viable cells were recovered at 6 hours but not at 24 hours, so under the tested conditions the bacteria survive between 6 and 24 hours. When testing was conducted with stainless steel, persistence occurred at the longest duration tested (i.e., a duration was not tested that failed to recover viable bacteria), and the actual persistence duration could be longer than 24 hours, which is reflected on Table 3.

Table 3. *Bacillus* Species (Vegetative Cells) Persistence on Fomites

***Bacillus* species**	**Material**	**Environmental Condition**	**Longest Duration with Persistence**	**Shortest Duration without Persistence**	**Study**
B. cereus T, *B. subtilis* 168, and *B. anthracis* Sterne	Stainless steel	25°C, 80% RH	24 hours*	--	Galeano et al. (2003)
	Stainless steel with an antimicrobial coating	25°C, 80% RH	6 hours	24 hours	
B. anthracis Sterne	Polystyrene and glass	Biofilm growth in static BHI broth, 37°C, 5% CO_2	7 days*	--	Lee et al. (2007)
	Glass	Biofilm growth in laminar flowing BHI broth, 37°C	7 days*	--	
B. thuringiensis and *B. cereus*	Polyvinyl chloride	Biofilm growth in lysogeny broth with bactopeptone, 30°C	3 days*	--	Auger et al. (2009)

-- Not tested/not reported.

* The longest duration tested (i.e., the actual persistence duration could be longer).

4.2. *B. anthracis* Vegetative Cells in Soil

Sinclair et al. (2008) noted that the life cycle of *B. anthracis* includes a soil dwelling stage and that viable spores can be recovered after several years (e.g., 40 years based on the work by Manchee et al. [1994]). However, other than a cursory mention that germination and multiplication may occur within the soil dwelling stage, no mention as to how, and under what conditions *B. anthracis* spores germinate within soil were highlighted within the Sinclair et al. (2008) review. The concept that spores germinate and propagate within soil is not new. For example, West and Burges (1985) spiked spores of *B. thuringiensis* and *B. cereus* into sandy silt loam soils supplemented with grass clippings or chicken manure. Their results showed that *B. thuringiensis* spores germinated and propagated within the grass-supplemented soil, but *B. cereus* did not. After 24 days, the *B. thuringiensis* counts came to a plateau and remained at 22 times the spore inoculum level for the duration of the experiment, while *B. cereus* had an initial germination spike then declined to 0.11 times the inoculum. *B. thuringiensis* and *B. cereus* spores germinated within the manure-supplemented soil; however, after the initial burst of activity the viability counts decreased to 0.22 times the inoculum value for *B. thuringiensis* and to 0.098 times the inoculum for *B. cereus* (West and Burges, 1985). These results indicate that though spores may germinate when an abundance of fresh nutrients are available within supplemented soils, the germinated cells do not readily persist in the manure-soil environment (West and Burges, 1985). Others works have suggested that *B. thuringiensis* can proliferate within vegetation. Tilquin et al. (2008) found that *B. thuringiensis* subspecies *israelensis* spores, originally sprayed in French lands as a means of mosquito control, were found in high concentrations within leaf matter (3×10^5 spores per gram [g]). The authors suggest that the high concentrations of *B. thuringiensis* found within the decaying leaf litter may be because the leaf litter is a specific microenvironment in which the organism can persistence and grow. Specifically the work targeted two key elements to the leaf litter microenvironment that potentially contributed to the seen increase in spore counts– low oxygen levels and low decomposition rates (Tilquin et al., 2008).

While these studies did not utilize vegetative *B. anthracis,* their work is important because both *B. thuringiensis* and *B. cereus* spores are routinely used as surrogates for *B. anthracis* spores. A limited number of studies have also focused on the ability of *B. anthracis* to propagate within soil environments. In one such work, *B. anthracis* spores were found to germinate and propagate within the rhizosphere of a common pasture grass (Saile and Koehler, 2006). The authors proposed that the plant roots enhanced the germination and proliferation of vegetative cells, as nearly 50% of the inoculated spores germinated in the presence of plant roots, where as little to no spores germinated in the absence of the grass (Saile and Koehler, 2006). The study also found evidence of the plasmid pBC16, which confers tetracycline resistance, being transferred between two strains of *B. anthracis* within their model rhizosphere system. This finding is significant as it provides strong evidence of metabolically active *B. anthracis* cells within the plant-soil environment (Saile and Koehler, 2006).

A series of studies have focused on the interaction between worms (nematodes or earthworms) and *Bacillus* species in both their vegetative and spore forms. Laaberki and Dworkin (2008) fed *B. anthracis* Sterne in Nematode Minimal Media agar (spores) or Nematode Growth Medium plates (vegetative cells) to laboratory-controlled nematodes. They found that the digestive track of the worm killed the consumed vegetative cells, but spores were able to pass unaffected through their system into their

feces. This conclusion is supported by work conducted by Hendriksen and Hansen (2002). However, Hendriksen and Hansen (2002) were able to further deduce that *B. thuringiensis* spores consumed with soil germinated within the gut of the earthworm prior to resporulation and defecation. Furthermore, the study showed that *B. thuringiensis* was able to germinate within the hindgut of three of the four tested earthworm species, indicating that this is not a species limited occurrence, but rather a widely distributed condition (Hendriksen and Hansen, 2002). More recently, the interaction between earthworms and *B. anthracis* has been shown to be dependent upon the presence of various bacteriophages (viruses that infect bacteria). The bacteriophages appear to induce various phenotypic changes to the *B. anthracis* vegetative cells that alter their capacity "to sporulate, produce exopolysaccharide, form biofilms, and survive long-term in soil" (Schuch and Fischetti, 2009). Multiple studies have shown that only bacteriophage-infected *B. anthracis* are capable of infecting the intestinal tract of earthworms, and that the bacteriophage present are variable (Schuch and Fischetti, 2009; Schuch et al., 2010). Schuch et al. (2010) showed that one bacteriophage, Wip1, was present in high numbers for three years within a Pennsylvania forest floor soil before the population was dramatically replaced by a second, Wip4, bacteriophage for the next three years of the study. This demonstrates that even the bacteriophage population within environmental *B. anthracis*-like isolates is variable due to any number of environmental factors potentially including the composition of the soil that they are ingesting.

Another point of interest is the comparison of the earthworm lifestyle to what is known regarding the *B. anthracis* spore lifecycle within soils. Both earthworms and *B. anthracis* spores prefer alkaline soils with high calcium levels and rich in organic matter (Hugh-Jones and Blackburn, 2009; Schuch et al., 2010). Anthrax events also seem to occur more commonly after seasonal flooding events when earthworms retreat to the soil surface to escape the water-saturated ground (Schuch et al., 2010). The combination of lifestyle patterns, hindgut colonization, and anthrax occurrence patterns points to a significant relationship between anthrax occurrence and earthworms. While direct sampling at enzootic areas would be required to definitively determine a correlation between earthworms and anthrax outbreaks, their overlapping lifestyles suggests that *B. anthracis* spores are carried upward to the soil surface and potentially onto vegetation via colonized earthworm digestive systems.

4.3. *B. anthracis* Vegetative Cells in Water

Sinclair et al. (2008) presented data from Busson (1911) and Mitscherlich and Marth (1984) indicating that vegetative *B. anthracis* persists for 72 hours to 6 days in water. Since Sinclair et al. (2008), only one study was identified that looked at environmental waters. Dey et al. (2012) utilized a low nutrient creek water to assess the interaction of *B. anthracis* with *Acanthamoeba castellanii*, a common soil amoeba. The work clearly showed a pathway where ingested *B. anthracis* spores germinate within the amoeba and replicate to the point of lysing the amoeba host. Upon lysis, the vegetative cells then sporulated in the low nutrient creek water. Virulent *B. anthracis* contains two plasmids, pX01 and pX02. Both plasmids must be present for virulence; pX01codes for the exotoxin and pX02 codes for encapsulation. Interestingly, a pX01 plasmid dependence was noted for spore germination within the amoeba. The

researchers hypothesized that this germination dependence may explain why pX02 is lacking in multiple natural *B. anthracis* strains – it is not required for proliferation (Dey et al., 2012).

4.4. *B. anthracis* Vegetative Cell Persistence Gaps

Within this literature search, no specific data on the persistence of aerosolized *B. anthracis* vegetative cells were found. Furthermore, little data were found regarding their persistence on various surfaces. There seems to be some indication that cells can proliferate for a short period of time (e.g. 24 hours; Galeano et al., 2003); however, to date the open literature does not provide data on extended periods of incubation, or a full understanding of when and where proliferation occurs. In addition, testing of vegetative cells on fomites was not conducted at environmental conditions <25°C, <80% RH, or with simulated sunlight. Sagripanti et al. (2007) conducted a systematic decontamination analysis of multiple *Bacillus* species spores on various materials of interest. US EPA (2010a) has also conducted a study looking at the persistence of *B. anthracis* spores on wood, glass, concrete, and soil in simulated sunlight. Future work utilizing similar experimental designs as either Sagripanti et al. (2007) or US EPA (2010a) with vegetative cells would shed significant light on the overall persistence of *B. anthracis* cells. Studies have also shown that *B. anthracis* is capable of forming biofilms on glass and polyvinyl chloride surfaces when covered with nutrient media, however the required conditions and the overall persistence of the formed biofilms have yet to be explored (Auger et al., 2009; Lee et al., 2007).

The interaction between soil and water species (e.g., amoebae, bacteriophages, earthworms, and grasses) with *B. anthracis* have been brought to the forefront in recent studies (Dey et al., 2012; Hendriksen and Hansen, 2002; Laaberki and Dworkin, 2008; Saile and Koehler, 2006; Schuch and Fischetti, 2009; Schuch et al., 2010). However, there remain gaps within the overall understanding of how the various species influence *B. anthracis* proliferation within soil or water. The studies included herein have shown that *B. anthracis* can colonize the hindgut of earthworms and infect amoeba, however questions remain on if this colonization actually increases spore numbers within the surrounding environment. There also remains questions regarding the virulence of the *B. anthracis* spores if/when they do persist. Dey et al. (2012) found that only the pX01 plasmid was required for spore germination within amoeba, and may account for why pX02 is often lacking in environmental strains. However, it is unknown if *B. anthracis* can regain virulence with the uptake of the pX02 plasmid in the environment. A clear linkage between the various soil or waterborne species and the known *B. anthracis* geographic distribution patterns of *B. anthracis* as presented by Blackburn et al. (2007) and Griffin et al. (2009) could strengthen the epidemiological connection between soils and anthrax incidence.

5. *Brucella* species Persistence

There are several closely related species of *Brucella* that are each associated with a different host. For example, *Brucella melitensis* is typically associated with goats, *Brucella suis* is associated with swine, and *Brucella abortus* is often associated with cattle (Franz et al., 1997). All three of these *Brucella* species are

capable of infecting humans (Franz et al., 1997), and are CDC-listed agents. Accordingly, persistence data were gathered for these three *Brucella* species. Persistence data were not identified for other species of *Brucella*.

5.1. *Brucella* species on Fomites

Three studies were identified that evaluated the persistence of *B. suis* on fomites, which are summarized in the following paragraphs and Table 4. These studies were based on the inoculation of 10^7 to 10^8 CFU of *B. suis* onto the material coupons. The material coupons were sterilized by autoclaving or gamma irradiation prior to inoculation with *B. suis*. Fomite persistence studies evaluating *Brucella* species other than *B. suis* were not identified.

Table 4. *B. suis* (ATCC 23444) Persistence on Fomites

Material	Environmental Condition	Longest Duration with Persistence	Shortest Duration without Persistence	Study
Aluminum	22°C, 35% RH	7 days*	--	Ryan (2010)
Carpet	22°C, 35% RH	7 days*	--	
Keyboard keys	22°C, 45% RH	7 days*	--	
Painted joint tape	22°C, 45% RH	4 hours	8 hours	
Aluminum	22°C, 40% RH	28 days*	--	US EPA (2010b)
	22°C, 50% RH, simulated sunlight	7 days	10 days	
	5°C, 10% RH	28 days*	--	
	5°C, 60% RH, simulated sunlight	5 days	5 days	
Concrete	22°C, 40% RH	<7 days	7 days	
	22°C, 50% RH, simulated sunlight	<1 day	1 day	
	5°C, 50% RH	7 days	14 days	
	5°C, 50% RH, simulated sunlight	<1 day	1 day	
Glass	22°C, 40% RH	28 days*	--	
	22°C, 50% RH, simulated sunlight	1 day	2 days	
	5°C, 10% RH	28 days*	--	
	5°C, 50% RH, simulated sunlight	2 days	2 days	
Soil	22°C, 40% RH	28 days*	--	
	22°C, 50% RH, simulated sunlight	14 days*	--	
	5°C, 10% RH	28 days*	--	
	5°C, 60% RH, simulated sunlight	14 days*	--	
Wood	22°C, 40% RH	<21 days	21 days	
	5°C, 10% RH	28 days*	--	
Aluminum	22°C, 40% RH	56 days*	--	Calfee and Wendling (2012)
	5°C, 30% RH	56 days*	--	
Glass	22°C, 40% RH	56 days*	--	
	5°C, 30% RH	56 days*	--	
Wood	22°C, 40% RH	42 days	56 days	
	5°C, 30% RH	56 days*	--	

-- Not tested/not reported.
* The longest duration tested (i.e., the actual persistence duration could be longer).

The US EPA conducted persistence testing with *B. suis* following inoculation onto aluminum, carpet, keyboard keys, and painted joint tape (Ryan, 2010). Briefly, material coupons were inoculated with 1 x 10^7 viable *B. suis* in 100 microliter (µL) aliquots of stock suspension (BHI broth). The inoculated coupons were held at approximately 35% RH or 45% RH and 22°C. At various time points, the coupons were placed in phosphate buffered saline (PBS) and agitated on an orbital shaker to extract the *B. suis* from the materials. Aliquots of the undiluted extract and associated serial dilutions were spread plated onto BHI agar and incubated for up to 72 hours at 37°C. *B. suis* was found to persist on aluminum, carpet, and keyboard keys for at least 7 days (the longest duration tested). On painted joint tape (surrogate for painted wallboard), *B. suis* was recovered after 4 hours but not after 8 hours.

Similar persistence testing with *B. suis* was conducted by US EPA (2010b) using surfaces of aluminum, concrete, glass, soil, and, on a limited basis, wood. Testing was conducted at two temperatures (approximately 5°C and 22°C) with and without exposure to UV radiation intended to mimic sunlight. The tests were conducted under ambient/uncontrolled RH that generally averaged from 40% to 60% RH, although the long term testing at 5°C without UV averaged about 10% RH. In the absence of UV, *B. suis* persisted for at least 28 days on aluminum, glass, and soil at 5°C and 22°C, for 7 days on concrete at 5°C (although persistence on concrete was <7 days at 22°C), and for 28 days on wood at 5°C (although persistence on wood was <21 days at 22°C). Persistence was decreased in the presence of UV. *B. suis* was recovered from aluminum after 5 days at 5°C and after 7 days at 22°C, from glass after 2 days at 5°C and after 1 day at 22°C, from soil after 14 days at 5°C and 22°C when exposed to UV. *B. suis* persisted <1 day on concrete when exposed to UV at 5°C and 22°C. *B. suis* persistence on wood was not tested under simulated sunlight. *B. suis* appeared to generally persist better at 5°C than 22°C (US EPA, 2010b).

Even longer term *B. suis* persistence testing (56 days) was reported by Calfee and Wendling (2012) on glass, aluminum, and wood. Testing was conducted at two environmental conditions: 22°C and 40% RH, and 5°C and 30% RH. *B. suis* persisted on aluminum and glass under both environmental conditions for at least 56 days. *B. suis* also persisted on wood for 56 days at 5°C and persisted for approximately 42 days at 22°C. *B. suis* persistence was higher at 5°C than 22°C (Calfee and Wendling, 2012).

5.2. *Brucella* species in Soil

Brucella species can persist for several weeks in soil (Franz et al., 1997; Charters, 1980) and in dust (Franz et al., 1997). Nicoletti (1980) briefly summarized the persistence results of *B. abortus* in soil. The cited data were either incomplete or from foreign language publications. As a result, the details of the persistence studies summarized by Nicoletti (1980) were limited. Nevertheless, *B. abortus* appeared to survive better in moist soil than dry soil, as the bacterium persisted 66 days in wet soil, persisted 48 to 73 days in soil at 90% humidity, and persisted <4 days in dried soil (Nicoletti, 1980). As shown in Table 4 for fomites, *B. suis* can persist at least 28 days (the longest duration tested) in soil held at 5°C or 22°C (US EPA, 2010b).

Jones et al. (2010) noted that sunlight and elevated temperatures impact *B. abortus* survival, such that persistence in the environment is expected to be a few weeks in Yellowstone National Park during the

summer. More specifically, Aune et al. (2012) found that *B. abortus* can persist on fetal tissues, soil, or vegetation in the Greater Yellowstone Area for several weeks depending upon the month, temperature, and sunlight. For example, the persistence of *B. abortus* applied to fetal tissues (bovine and bison) held in shaded areas was measured twice weekly. Fetuses deployed in February, March, April, and May had *B. abortus* persistence of 81, 77, 69, and 25 days, respectively (Aune et al., 2012). Soil and vegetation sampling at naturally occurring *B. abortus* contaminated sites associated with bison births or abortions was also conducted weekly. *B. abortus* persisted 10 to 43 days at sites identified in April and persisted for 7 to 26 days at sites identified in May (Aune et al., 2012).

5.3. *Brucella* species in Water

Franz et al. (1997) reported that *Brucella* species can survive for several weeks in water, but the bacteria are sensitive to heat. (Franz et al. [1997] did not specify the temperatures associated with heat sensitivity.) A literature review by Nicoletti (1980) found data indicating that *B. abortus* in water survived >57 days at 8°C, 77 days at room temperature, and <1 days at 37°C. Many of the specific details surrounding the persistence of *B. abortus* in the environment were not summarized by Nicoletti (1980). Falenski et al. (2011) inoculated mineral water (held at 20°C) with *B. abortus* (strain 1119-3) at 5×10^7 CFU mL^{-1}; *Brucella* colonies were detected after 60 days but not after 63 days.

Gilbert and Rose (2012) used autoclaved, dechlorinated municipal water inoculated with 10^6 CFU mL^{-1} *B. melitensis* or *B. suis* to assess persistence at 5°C and 25°C. Both *Brucella* species were culturable between 1-2 days at 25°C and between 7-9 days at 5°C. In addition to culturing, viability was assessed by measuring metabolic (i.e., esterase) activity. Based on esterase production, both *Brucella* species remained viable for 30 days (the longest study duration) at 5°C and 25°C, suggesting that the bacteria entered a VBNC state. The persistence data from Nicoletti (1980), Falenski et al. (2011), and Gilbert and Rose (2012) are summarized in Table 5.

Table 5. *Brucella* Species Persistence in Water

Brucella species	Environmental Condition	Longest Duration with Persistence	Shortest Duration without Persistence	Study
B. abortus	Room temperature	77 days*	--	Nicoletti (1980)
	Lake water, 8°C, pH 6.5	>57 days*	--	
	Lake water, 37°C, pH 7.5	<1 day*	--	
B. abortus (1119-3)	Mineral water, 20°C	60 days	63 days	Falenski et al. (2011)
B. melitensis (ATCC 23456)	Autoclaved, dechlorinated municipal water, 5°C	7 days	9 days	Gilbert and Rose (2012)
	Autoclaved, dechlorinated municipal water, 25°C	1 day	2 days	
B. suis (EAM 562)	Autoclaved, dechlorinated municipal water, 5°C	7 days	9 days	
	Autoclaved, dechlorinated municipal water, 25°C	1 day	2 days	

-- Not tested/not reported.

* Data obtained from a literature review, the shortest duration without persistence was not reported.

5.4. *Brucella* species Persistence Gaps

Specific data on the survival of *Brucella* species in aerosols were not identified. However, *Brucella* species appears to be adversely affected by warmer temperatures and exposure to sunlight (e.g., Jones et al., 2010). On fomites, *Brucella* species persistence varied by surface type (e.g., Ryan, 2010). It is uncertain if material-specific interactions adversely affected recovery from some surfaces (e.g., painted joint tape and concrete). Recent evidence indicates that *Brucella* species may enter a VBNC state (e.g., Gilbert and Rose, 2012). The persistence of *B. melitensis* and *B. suis* in water (<9 days at 5°C and 25°C) reported by Gilbert and Rose (2012) was considerably lower than the persistence of *B. abortus* in water (>57 days at 8°C and 20°C) reported by Nicoletti (1980) and Falenski et al. (2011), respectively. It is uncertain if the difference is attributed to species-level differences or the study parameters. Under certain conditions (e.g., nutritionally deficient, low oxygen) *B. abortus* was found to aggregate and produce biofilms, which appear to enhance the bacteria's tolerance to desiccation (Almirón et al., 2013). Additional research is needed on the mechanisms that contribute to the environmental persistence of *Brucella* species including species-specific data. For example, for fomites, only persistence data associated with *B. suis* were identified.

6. *Burkholderia mallei* Persistence

B. mallei is primarily associated with horses and is not expected to survive outside its host for long durations (Gilad et al., 2007; Dvorak and Spickler, 2008). Dvorak and Spickler (2008) reviewed data indicating that *B. mallei* might survive in wet, humid, or dark conditions for 3 to 5 weeks, but survival is reduced in the presence of sunlight. Specific data relative to the limited survival of *B. mallei* outside the host including the environmental media or surface types associated with persistence were not provided.

6.1. *B. mallei* on Fomites

Specific studies designed to generate persistence data of *B. mallei* on fomites were not identified. However, Malik et al. (2012) stated that *B. mallei* "has an affinity for warm and moist conditions and may survive for up to 3 months in stable bedding, manure, feed and water troughs (particularly if heated), wastewater and equine transporters (saddler and harness equipment)". Specific data supporting these statements were not provided by Malik et al. (2012), but the statement seems to support the environmental conditions more suitable for *B. mallei* persistence noted by Dvorak and Spickler (2008).

6.2. *B. mallei* in Water

Three studies were identified investigating the persistence of *B. mallei* in water (Table 6). Miller et al. (1948) reported that *B. mallei* inoculated into tap water survived 4 weeks at room temperature, but *B. mallei* was not recovered after 5 weeks. Moore et al. (2008) inoculated sterile distilled, deionized water with *B. mallei* (approximately 10^6 CFU mL^{-1}) and found that the bacterium was culturable after approximately 27 days but not culturable after 31 days (this persistence duration was estimated from Figure 1 of Moore et al. [2008]). Gilbert and Rose (2012) used autoclaved, dechlorinated municipal water inoculated with 10^6 CFU mL^{-1} *B. mallei* to assess persistence at 5°C and 25°C. *B. mallei* was found to be viable by culturing for only for 1 to 2 days when held at 25°C or 5°C. In addition to culturing, viability was assessed by measuring metabolic (i.e., esterase) activity. Based on esterase production, *B. mallei* remained viable for 30 days (the longest study duration) at 5°C and 25°C, suggesting that the bacterium remained alive but was unable to be recovered by culturing on an artificial medium.

Table 6. *B. mallei* Persistence in Water

B. mallei Isolate	Environmental Condition	Longest Duration with Persistence	Shortest Duration without Persistence	Study
Not specified	Tap water, room temperature	4 weeks	5 weeks	Miller et al. (1948)
ATCC 23344	Sterile distilled, deionized water	27 days	31 days	Moore et al. (2008)
M-13	Autoclaved, dechlorinated municipal water, 5°C	1 day	2 days	Gilbert and Rose (2012)
	Autoclaved, dechlorinated municipal water, 25°C	1 day	2 days	

6.3. *B. mallei* Persistence Gaps

With the exception of survival data in water, relatively little persistence data were identified for *B. mallei*. Specific data on the survival of *B. mallei* in aerosols or on soil were not identified. The information on *B. mallei* persistence on fomites was not supported with specific data or laboratory controlled studies. Although three persistence studies were identified with *B. mallei* in water, the results were somewhat conflicting. Gilbert and Rose (2012) observed considerably shorter durations of culturability than Miller et al. (1948) or Moore et al. (2008). It is unknown what caused such different persistence results or why *B. mallei* entered a VBNC state during the Gilbert and Rose (2012) study.

7. *Burkholderia pseudomallei* Persistence

7.1. *B. pseudomallei* on Fomites

One study focused on the persistence of *B. pseudomallei* on fomites. Shams et al. (2007) investigated the survival of *B. pseudomallei* (suspended in Butterfield buffer or BHI broth) applied to glass, stainless steel, paper, and polyethylene. Testing was conducted with two *B. pseudomallei* isolates (ATCC 11668 and 23343). Test temperatures and RH were not specified in Shams et al. (2007). Viability was assessed using culture methods and a solid-phase cytometer to detect esterase activity. When analyzed by culture and applied in Butterfield buffer, both isolates of *B. pseudomallei* persisted between 6 and 24 hours on all materials, except isolate ATCC 11668, which persisted between 24 hours and 3 days on paper (Table 7). When analyzed by culture and applied in BHI broth, both isolates of *B. pseudomallei* persisted between 24 hours and 3 days on glass and persisted between 3 and 7 days on paper (Table 7). When applied to polyethylene in BHI broth, the ATCC 11668 isolate persisted between 24 hours and 3 days, and the ATCC 23343 isolate persisted between 3 and 7 days (Table 7). When applied to stainless steel in BHI broth, the ATCC 11668 isolate persisted between 3 and 7 days, and the ATCC 23343 isolate persisted between 6 and 24 hours (Table 7). When assessed by esterase activity (not shown in Table 7), *B. pseudomallei* generally persisted at least 14 days when applied in Butterfield buffer and persisted at least 35 days when applied in BHI broth. The measure of esterase activity detected longer persistence, possibly because the bacteria entered a VBNC state or because the cells were injured. Persistence appeared to increase when *B. pseudomallei* was suspended in BHI broth, which is a more complex medium with slower drying than Butterfield buffer.

Table 7. *B. pseudomallei* Persistence on Fomites from Shams et al. (2007)

B. pseudomallei Isolate	Material	Application Suspension	Longest Duration with Persistence	Shortest Duration without Persistence
ATCC 11668	Glass	Butterfield buffer	6 hours	24 hours
ATCC 23343		Butterfield buffer	6 hours	24 hours
ATCC 11668		BHI broth	24 hours	3 days
ATCC 23343		BHI broth	24 hours	3 days
ATCC 11668	Paper	Butterfield buffer	24 hours	3 days
ATCC 23343		Butterfield buffer	6 hours	24 hours
ATCC 11668		BHI broth	3 days	7 days
ATCC 23343		BHI broth	3 days	7 days
ATCC 11668	Polyethylene	Butterfield buffer	6 hours	24 hours
ATCC 23343		Butterfield buffer	6 hours	24 hours
ATCC 11668		BHI broth	24 hours	3 days
ATCC 23343		BHI broth	3 days	7 days
ATCC 11668	Stainless steel	Butterfield buffer	6 hours	24 hours
ATCC 23343		Butterfield buffer	6 hours	24 hours
ATCC 11668		BHI broth	3 days	7 days
ATCC 23343		BHI broth	6 hours	24 hours

7.2. *B. pseudomallei* in Soil

As reviewed by Dance (2000), *B. pseudomallei* occurs in tropical and sub-tropical climates and is associated with decaying organic matter in the environment. While *B. pseudomallei* is infrequently found in surface soil (the organism may be adversely affected by sunlight), subsurface samples (e.g., at a 25-60 centimeter [cm] depth) are more likely to yield *B. pseudomallei* (Dance, 2000). *B. pseudomallei* is a facultative anaerobe (Reckseidler et al., 2001) and can grow in anoxic environments (Dance, 2000). Laboratory studies have demonstrated long survival durations in soil at room temperature, although some *B. pseudomallei* strains survive months in the laboratory at 5°C (Dance, 2000). *B. pseudomallei* has been recovered from soil with a wide range in pH, but the bacterium may prefer slightly acidic conditions (Dance, 2000). More detailed data on the persistence of *B. pseudomallei* in soil are provided in the following paragraphs and Table 8.

Table 8. *B. pseudomallei* Persistence in Soil

B. pseudomallei Isolate	Environmental Condition	Longest Duration with Persistence	Shortest Duration without Persistence	Study
9 isolates (unspecified) from soil collected from a sheep paddock in Australia	Initially moist soil stored in plastic bags on a shaded shelf in a laboratory at ambient temperature (13°C to 33°C)	30 months (other soil samples tested had shorter durations)	36 months (other soil samples tested had shorter durations)	Thomas and Forbes-Faulkner (1981)
8 isolates (H35, H43, H47, G106, G123, G139, Gd154, and Gd173) from water and soil in China (Hainan, Guangxi, and Guangdong)	0% water content at room temperature	30 days†	--	Tong et al. (1996)
	5% water content at room temperature	40 days†	--	
	10% water content at room temperature	70 days†	--	
	20% water content at room temperature	439 days†	--	
	40% water content at room temperature	726 days*	--	
	80% water content at room temperature	726 days*	--	
12 isolates (1 through 12) from 12 melioidosis patients in Taiwan	5% water content	16 days	20 days	Chen et al. (2003)
	10% water content	50 days	70 days	
	15% water content	150 days*	--	
	20% water content	150 days*	--	
Isolate (unspecified) from soil collected near an ephemeral creek in Australia	Soil dried (<0.1% moisture), after 104 days the moisture was adjusted to 15%	113 days*	--	Larsen et al. (2013)
Isolate (unspecified) from soil collected near an ephemeral creek in Australia	Soil intermittently wetted (9.9% moisture), after 104 days the moisture was adjusted to 15%	113 days*	--	
KN07	Sterile soil inoculated with *B. multivorans*, *B. pseudomallei*, and other soil bacteria	<10 days	10 days	Lin et al. (2011)

-- Not tested/not reported.

* The longest duration tested (i.e., the actual persistence duration could be longer).

† Although apparently tested, the shortest duration without persistence was not reported.

As described by Thomas and Forbes-Faulkner (1981), Australian soil samples were collected from a-fenced area with sheep and then stored in plastic bags on a shaded shelf in a laboratory at ambient temperature (13°C to 33°C). Attempts at *B. pseudomallei* isolation by culture were made at 6-month intervals. *B. pseudomallei* was found to survive up to 30 months in soil (described as a moist medium clay originally collected from a soil depth of 40-45 cm).

B. pseudomallei appears to benefit from moist soil, but may persist in soil that gradually dries. Tong et al. (1996) reported survival times of 40, 70, 439, and at least 726 days in soil with water contents of 5%, 10%, 20%, and 40%, respectively. Chen et al. (2003) similarly observed that *B. pseudomallei* survived approximately 16 to 20 days in soil with a water content of 5%, approximately 50 to 70 days in soil with 10% water, and at least 150 days in soil with 15% to 20% water. Larsen et al. (2013) inoculated *B. pseudomallei* into sterile soil to study the influence of soil moisture on survival. *B. pseudomallei* was not recovered following inoculation into dry soil. However, *B. pseudomallei* persisted in initially moist soil (approximately 10% moisture) that underwent drying for at least 91 days and survived 113 days (the longest duration tested) when intermittently irrigated with sterile distilled water. Larsen et al. (2013) concluded that dry endemic soil may act as a reservoir for *B. pseudomallei* during the dry season.

Based on testing at 4°C, 22°C, 25°C, 30°C, 37°C, 42°C, and 45°C, Chen et al. (2003) reported that 37°C to 42°C were optimal temperatures for *B. pseudomallei* growth in soil, although some strains were able to grow at 4°C. Chen et al. (2003) also reported that the optimal soil pH was 6.5 to 7.5 (based on testing at pH values of 3.5, 4, 4.5, 5.5, 6.5, 7.5, 8, and 8.5), although 2 of 12 strains survived at pH 4 in soil. Although not specifically tested in soil, Tong et al. (1996) reported optimal survival conditions for *B. pseudomallei* to be temperatures of 24°C to 32°C (based on testing at 0°C, 8°C, 16°C, 24°C, 32°C, 40°C, and 48°C) and pH ranging from 5 to 8 (based on testing at pH values of 2, 3, 4, 5-8, 9, and 10). In normal saline (the salt content was undefined), eight strains of *B. pseudomallei* averaged 18 days survival at 0°C and averaged 28 days survival at 40°C (Tong et al., 1996). Tong et al. (1996) also reported that *B. pseudomallei* were killed by radiation from an UV lamp (465 microwatt [µW]/square centimeter [cm^2] for 7.75 minutes), but noted that the radiation associated with natural sunlight can be absorbed by other materials thereby limiting sunlight's killing effect.

Studies have been conducted in Thailand to determine soil properties associated with the presence of *B. pseudomallei*. Palasatien et al. (2008) found that *B. pseudomallei* was primarily associated with sandy soil, at a depth of 30 cm, pH of 5.0 to 6.0, >10% moisture, and elevated total nitrogen and chemical oxygen demand relative to soils without recoverable *B. pseudomallei* (Palasatien et al., 2008). Palasatien et al. (2008) noted that under conditions of low water content, organic matter in the soil may enable *B. pseudomallei* to survive. Suebrasri et al. (2013) evaluated Thailand soil properties associated with *B. pseudomallei* during the rainy season and dry season. A higher prevalence of *B. pseudomallei* positive locations (22%) occurred during the rainy season compared with 6% positive locations during the dry season. During the rainy season, three soil properties were identified as being different between positive and negative sites. Locations with *B. pseudomallei* had a mean soil pH of 6.05, a percentage of water holding capacity of 31.92%, and an iron concentration of 16.33 milligram per kilogram (mg kg^{-1}). Locations negative for *B. pseudomallei* had a mean soil pH of 5.51, a percentage of water holding capacity of 40.17%, and an iron concentration of 109.86 mg kg^{-1}. The percentage of water holding

capacity is a function of soil composition with sandy soils having a lower water holding capacity than soils comprised of silt and clay. During the dry season only the manganese content was identified as being different between *B. pseudomallei* positive sites (138.46 mg kg^{-1}) and negative sites (46.88 mg kg^{-1}) (Suebrasri et al., 2013). The authors discussed the possibility that *B. pseudomallei* entered a VBNC state to survive the dry season (Suebrasri et al., 2013). Despite the statistical differences between some of the physiochemical properties of soil at *B. pseudomallei* positive and negative sites, scatter plots shown by Suebrasri et al. (2013) (but not shown in this report) demonstrate nearly complete overlap between the results at positive and negative sites. Additional research is needed to understand the influence of soil characteristics on the persistence of *B. pseudomallei.*

Based on soil samples (collected at a depth of 30 cm) from northern Australia, Kaestli et al. (2009) found that the environmental parameters associated with the presence of *B. pseudomallei* differed depending upon undisturbed and manipulated (e.g., farmed) areas. In undisturbed areas, *B. pseudomallei* was associated with closeness to streams, moist soil, grassy areas, and areas associated with native animals (e.g., wallabies). In manipulated areas, *B. pseudomallei* was associated with the presence of livestock, clay loam soil (i.e., a clay, silt, and sand mixture), red brown (an indication of oxidized iron) clay soil, and lower (5.5) soil pH. Kaestli et al. (2009) also noted that *B. pseudomallei* was recovered from dry soil in manipulated areas suggesting that other factors, besides water, support *B. pseudomallei* growth. Later research by Kaestli et al. (2012) found that *B. pseudomallei* was associated with the rhizosphere, roots, and above ground parts of various grasses, especially non-native grasses introduced for grazing animals.

Lin et al. (2011) demonstrated that other soil bacteria (e.g., *Burkholderia multivorans*) could inhibit *B. pseudomallei* growth. The authors inoculated sterile soil with *B. multivorans*, *B. pseudomallei*, and other soil bacteria. Cultures resembling *B. multivorans* were observed 10 days after inoculation, but *B. pseudomallei* was not observed. Polymerase chain reaction (PCR) techniques were able to detect *B. pseudomallei* DNA 20 days after inoculation but not after 30 days.

As reviewed by Inglis and Sagripanti (2006), *B. pseudomallei* can form biofilms and survive in amoeba cysts and fungi, which may be important aspects of *B. pseudomallei* in the environment. For example, Levy et al. (2009) detected *B. pseudomallei* genetic material in fungal spores collected from soil samples. The soil samples were not culture positive for *B. pseudomallei*. Levy et al. (2009) speculated that the association of the bacteria with fungal spores might contribute to *B. pseudomallei* persistence and dispersal in the environment.

7.3. *B. pseudomallei* in Water

Several studies have documented *B. pseudomallei* survival in distilled and environmental waters (Table 9). Moore et al. (2008) reported that *B. pseudomallei* survived in sterile distilled, deionized water for at least 200 days. Wuthiekanun et al. (1995) reported that *B. pseudomallei* persisted at least 3 years in sterile distilled water without any additional nutrients; the ambient temperature varied from 25°C to 40°C. Pumpuang et al. (2011) noted that *B. pseudomallei* persisted for 16 years in distilled water held at 25°C.

Table 9. *B. pseudomallei* Persistence in Water

B. pseudomallei Isolate	Environmental Condition	Longest Duration with Persistence	Shortest Duration without Persistence	Study
1026b	Sterile distilled, deionized water	200 days*	--	Moore et al. (2008)
Soil isolate (unspecified)	Sterile distilled water, ambient temperature 25°C to 40°C	3 years*	--	Wuthiekanun et al. (1995)
207a	Distilled water, 25°C	16 years*	--	Pumpuang et al. (2011)
NCTC 10276, NCTC 13177, and BCC 11	Filter-sterilized rain water	28 days*	--	Robertson et al. (2010)
	Sterile distilled water, 40°C	28 days*	--	
	Sterile distilled water, 20°C	28 days*	--	
	Sterile distilled water, 2°C	28 days*	--	
	Sterile distilled water, pH 7	28 days*	--	
	Sterile distilled water, pH 6	28 days*	--	
	Sterile distilled water, pH 5	28 days*	--	
	Sterile distilled water, pH 4	28 days*	--	
NCTC 10276	Sterile distilled water, pH 3	5 days	7 days	
NCTC 13177	Sterile distilled water, pH 3	7 days	14 days	
BCC 11	Sterile distilled water, pH 3	2 days	5 days	
NCTC 13177	Water with an artificial sea salt concentration of 0.004%	28 days*	--	
	Water with an artificial sea salt concentration of 0.04%	28 days*	--	
	Water with an artificial sea salt concentration of 0.4%	28 days*	--	

B. pseudomallei Isolate	Environmental Condition	Longest Duration with Persistence	Shortest Duration without Persistence	Study
12 isolates (1 through 12) from melioidosis patients in Taiwan	Water with an artificial sea salt concentration of 4%	28 days*	--	Chen et al. (2003)
	Water with an artificial sea salt concentration of 40%	1 day	2 days	
	Autoclaved pond water	150 days*	--	
	Autoclaved river water	150 days*	--	
	Autoclaved estuary water	20 days	36 days	
	Autoclaved sea water	8 days	12 days	
ATCC 23343	Autoclaved, dechlorinated municipal water, 5°C	1 day	2 days	Gilbert and Rose (2012)
	Autoclaved, dechlorinated municipal water, 25°C	30 days*	--	
NCTC 13177	Artificial sea water exposed to sunlight	60 minutes	90 minutes	Sagripanti et al. (2009)
	Sterile distilled water exposed to sunlight	60 minutes	90 minutes	
	Rain water exposed to sunlight	60 minutes	90 minutes	

-- Not tested/not reported.

* The longest duration tested (i.e., the actual persistence duration could be longer).

B. pseudomallei persistence in sterile distilled water, rain water (sterilized by filtration), and artificial sea water was studied for 28 days (Robertson et al., 2010). *B. pseudomallei* survived 28 days in distilled water and rain water. More specifically, *B. pseudomallei* survived 28 days in distilled water at 40°C, 20°C, and 2°C. *B. pseudomallei* also survived 28 days in distilled water at pH levels of 7, 6, 5, and 4. In distilled water at a pH level of 3, *B. pseudomallei* persistence varied by isolate (i.e., 5 to 7 days for NCTC 10276, 7 to 14 days for NCTC 13177, and 2 to 5 days for BCC 11), although epifluorescent microscopy identified a few viable cells after 28 days. *B. pseudomallei* survived 28 days in water with artificial sea salt concentrations of 0.004% to 4%; however, at a 40% artificial sea salt concentration, *B. pseudomallei* only persisted 1 to 2 days. The various sea salt concentrations were prepared using an artificial seawater base that was reconstituted with demineralized hypo-osmolar water and then autoclaved (Robertson et al., 2010).

Chen et al. (2003) observed long term *B. pseudomallei* survival (150 days, the longest duration tested) in water collected from ponds and rivers, but much shorter persistence occurred in estuarine water (persisting approximately 20 days but <36 days) and seawater (persisting approximately 8 days but <12 days).

Gilbert and Rose (2012) used autoclaved, dechlorinated municipal water inoculated with *B. pseudomallei* to assess persistence at 5°C and 25°C. *B. pseudomallei* was culturable for 30 days (the longest duration tested) at 25°C and was culturable between 1-2 days at 5°C. Metabolic (i.e., esterase) activity was also measured using solid-phase cytometry to determine viability. Based on esterase production, *B. pseudomallei* remained viable for 30 days at 25°C and 5°C. These results suggest that *B. pseudomallei* entered a VBNC state at 5°C (Gilbert and Rose, 2012).

A study of unchlorinated groundwater (rural water supplies) in northern Australia, found that positive *B. pseudomallei* water samples were primarily associated with acidic water (e.g., pH of 6.3 to 6.8), low water hardness (e.g., 25 to 100 mg per liter [L^{-1}]), low salinity (e.g., 0.02 to 0.07 millisiemens [mS] cm^{-1}), and higher iron levels (e.g., 2 to 4 mg L^{-1}) than samples negative for *B. pseudomallei* (Draper et al., 2010).

Sagripanti et al. (2009) studied the survival of *B. pseudomallei* in water (sterile distilled water, artificial seawater, and rainwater) exposed to sunlight at Perth, Australia via UV transparent dishes held on ice. In all three water types, the bacteria survived 60 minutes, but the bacteria were not culturable at 90 minutes. Sagripanti et al. (2009) also investigated the impact of sunlight on *B. pseudomallei* internalized by amoeba, which resulted in similar *B. pseudomallei* persistence as when exposed in water only (i.e., no apparent protection from sunlight was provided to *B. pseudomallei* internalized by amoeba).

7.4. *B. pseudomallei* Persistence Gaps

B. pseudomallei can be transported by aerosols (Sprague and Neubauer, 2004), and *B. pseudomallei* has been isolated from aerator spray associated with a water treatment plant (Inglis et al., 2000). However, specific data on the survival durations of *B. pseudomallei* in aerosols were not identified. Only one study (Shams et al., 2007) was identified that investigated the persistence of *B. pseudomallei* on fomites. The

influence of different environmental conditions (temperature and humidity) was not investigated as part of the fomite persistence study. In general, the measured persistence of *B. pseudomallei* may be affected by strain (e.g., Tong et al., 1996), physiologic variability associated with gene regulation (e.g., Larsen et al., 2013), inoculation suspension (e.g., Shams et al., 2007), and method to assess survival (e.g., Shams et al., 2007).

With regard to *B. pseudomallei* persistence in soil and water, Dance (2000) noted that environmental factors dictating the occurrence of *B. pseudomallei* in the environment are not well established. Inglis and Sagripanti (2006) similarly noted that although *B. pseudomallei* is associated with water and soil, it is not uniformly dispersed in the environment and "data on its preferred microhabitat are lacking". Moore et al. (2008) reported that the molecular survival mechanisms for *B. pseudomallei* in water are not understood. Moore et al. (2008) noted several factors that enable the environmental persistence of *B. pseudomallei* including "a versatile metabolic capacity which allows the use of a variety of carbon sources for growth, the ability to live inside other microorganisms including protozoa and fungi and the ability to tolerate a wide range of environmental conditions including a variety of soils and water environments".

8. *Coxiella burnetii* Persistence

The bacterium *C. burnetii* is an obligate intracellular organism that develops spore-like forms resistant to environmental stressors (Arricau-Bouvery and Rodolakis, 2005). The bacterium may exist as "large-cell variants (LCV), small-cell variants (SCV), and small dense cells (SDC)" physiological forms, with SCV and SDC being the environmentally persistent forms (Arricau-Bouvery and Rodolakis, 2005). *C. burnetii* is not known to be overly affected by high or low temperatures, drought, or humidity levels (Aitken et al., 1987). The stability of SCV can theoretically result in environmental persistence for years after being shed from infected animals (Kersh et al., 2013). *C. burnetii* can invade a variety of hosts including amoebae, ticks, birds, and mammals that likely allow the bacterium to be disseminated throughout the environment (Arricau-Bouvery and Rodolakis, 2005).

8.1. *C. burnetii* in Aerosols

"The environmental survival of *C. burnetii* allows it to be transported by wind far away from its original source" (Arricau-Bouvery and Rodolakis, 2005). Welsh et al. (1958) inoculated pregnant sheep with *C. burnetii* and then sampled the air of the sheep's pens for the bacterium. *C. burnetii* was found in the air 9 to 14 days (the longest duration sampled) after parturition. Welsh et al. (1958) acknowledged that some cross-contamination in air samples likely occurred even though the sheep were housed in individual cubicles. In addition, ongoing sources of contamination (e.g., infected sheep feces) might have contributed to the air contamination. That is, detection of viable *C. burnetii* in individual cubicles may have been due to cross contamination or may have been caused by ongoing sources of contamination – infected sheep feces, rather than actual persistence of the bacterium after parturition (Welsh et al., 1958). Kersh et al. (2013) detected *C. burnetii* DNA in the air of a goat farm, which was associated with a

Q fever outbreak one year earlier. Quantitative estimates of persistence from such data are difficult to make given the potential for re-introduction of the bacteria into the sampled areas.

8.2. *C. burnetii* on Fomites

Welsh et al. (1958) indicated that "epidemiological evidence exists that *C. burnetii* may be carried over considerable distances on inanimate objects, such as clothing, wool, hair, straw, packing materials, and dust." The authors did not document the durations or environmental condition that *C. burnetii* may remain viable. Kersh et al. (2013) detected *C. burnetii* DNA in high-efficiency particulate air (HEPA) vacuum samples from the floors and furniture of a farmhouse associated with a Q fever outbreak one year earlier. During a Q fever outbreak in 2008, de Bruin et al. (2011) used quantitative PCR to detect *C. burnetii* DNA at affected farms. Interestingly, more copies of the target DNA sequence were detected from dusty environmental surfaces than from veterinary samples. As concluded by de Bruin et al. (2011), the sampling of surfaces that have accumulated dust reflect the occurrence of *C. burnetii* over a relatively long time period, while the veterinary samples provide an indication of *C. burnetii* shedding at that moment only. The authors acknowledged that the quantitative PCR does not give an indication of the bacterium's viability. *C. burnetii* is a difficult organism to culture given its "virulence and complicated growth requirements" (de Bruin et al., 2011).

8.3. *C. burnetii* in Soil

Evstigneeva et al. (2007) inoculated *C. burnetii* into various peat and loamy soils, which were then held at 20°C, 4°C, and -20°C. The bacterium's survival was determined by inoculation of soil liquids into mice and monitoring the immunofluorescence of the mice spleens. Results were based on the number of *C. burnetii* in the field of vision and the brightness of their fluorescence. *C. burnetii* survived for 20 days (the longest duration tested) in all soil types and temperatures (Evstigneeva et al., 2007). Properties of the soils studied at the three temperatures included: hygroscopic moisture ranged from 1.14% to 7.04%, pH ranged from 6.10 to 7.75, organic carbon ranged from 1.28% to 14.26%, and the particle-size composition for <0.01 millimeter (mm) particles ranged from 29.6% to 40.9% and for <0.001 mm particles ranged from 10.3% to 23.2% (Evstigneeva et al., 2007).

Welsh et al. (1959) periodically sampled soil from ranches with *C. burnetii* infected sheep. Soil was collected from lamb birthing areas from one lambing season until the next lambing season. *C. burnetii* was present in the soil for up to 150 days, although it is possible that reinfection occurred at the sample locations (Welsh et al., 1959). Kersh et al. (2013) detected *C. burnetii* DNA in the soil of a goat farm, which was associated with a Q fever outbreak one year earlier.

La Scola and Raoult (2001) found that *C. burnetii* was able to survive within amoeba (*A. castellanii*) in a laboratory study. Maurin and Raoult (1999) reported that *C. burnetii* inside amoeba survived for 6 weeks. La Scola and Raoult (2001) concluded that soil amoeba could provide an intracellular niche for the survival of *C. burnetii* in a spore-like form.

8.4. *C. burnetii* Persistence Gaps

Data on the persistence of *C. burnetii* in water were not specifically identified. Much of the persistence data of *C. burnetii* (in aerosols, on fomites, and some soil studies) were based on environmental sampling associated with Q fever outbreaks. These studies often used PCR techniques that did not assess the organism viability nor were the studies conducted in controlled laboratory settings, so recontamination could have occurred. Arricau-Bouvery and Rodolakis (2005) noted that "the physiological forms of [*C. burnetii*] excreted in milk, feces or placentas are unknown. The form excreted by the host and the hypothetical possibility that LCV could convert to SCV in the environment are crucial information for implementing adequate strategies for the disinfection of feces or parturition products that could directly contaminate humans or the environment."

9. *Francisella tularensis* Persistence

F. tularensis (including the *tularensis*, *holarctica*, and *novicida* subspecies) is the causative agent of tularemia. Based on the review by Hazlett and Cirillo (2009), the bacteria are capable of surviving in diverse environments and can infect mammals, arthropods, and protozoans. For example, *F. tularensis* subspecies *tularensis* is found in rabbits and rodents of North America with transmission occurring via ticks and bloodsucking flies (e.g., horseflies). The *tularensis* subspecies has not traditionally been known to be associated with an aquatic reservoir. However, *F. tularensis* subspecies *holarctica*, which is found more widely in the northern hemisphere, is associated with water-borne disease and is transmitted by mosquitoes, ticks, and biting flies (Hazlett and Cirillo, 2009). *F. tularensis* subspecies *novicida*, sometimes referred to as *F. novicida*, is generally less virulent than other *F. tularensis* subspecies and "is associated with water-borne transmission" (Durham-Colleran et al., 2010). "The endemic nature of *Francisella* across the northern hemisphere suggests environmental persistence most probably utilizing numerous mechanisms" (Mahajan et al., 2011).

A study was conducted on the distribution of dog ticks (*Dermacentor variabilis*) infected with *F. tularensis* subspecies *tularensis*, which is believed to be responsible for the sustained outbreak of tularemia on the island of Martha's Vineyard, Massachusetts (Goethert and Telford, 2009). A small niche (approximately 290 meters in diameter) was identified where *F. tularensis* is perpetuated and genetic diversity is generated, although the authors noted that there was nothing obvious in the habitat to enhance persistence or transmission (Goethert and Telford, 2009). In discussing pneumonic tularemia on Martha's Vineyard in 1978 and 2000, Feldman et al. (2001) noted that the patients were likely exposed on the southern coast where closeness to brackish ponds and ocean-related precipitation might contribute to *F. tularensis* persistence. Interestingly, the *F. tularensis*-infected ticks analyzed by Goethert and Telford (2009) were collected from the southern coast near Squibnocket. Later work by Berrada and Telford (2011) suggested that *F. tularensis* subspecies *tularensis* persists on Martha's Vineyard because of sulfur/salt-influenced water or soil that is conducive to *F. tularensis* growth.

Nakazawa et al. (2010) modeled the ecological niche of *F. tularensis* in the United States at the subspecies (*tularensis* and *holarctica*) and clade (A1, A2, A1a, A1b of the *tularensis* subspecies) level.

Coarse-scale (e.g., county-level) ecological parameters were used to identify potentially distinct niches. Variables in the model included measures of days with frost, temperature, precipitation, solar radiation, and topographical characteristics. The model predicted that the *tularensis* and *holarctica* subspecies distributions actually overlap much of the country except *tularensis* is predicted to be absent from some northern/northeastern states and *holarctica* is predicted to be absent from five southern states. At the A1 and A2 clade level, predicted distributions were dramatically different with A1 occurring in the central to southeastern states and A2 occurring in the western states. Relative to A1 strains, A2 strains were predicted at higher elevations, with less precipitation, lower temperatures, and more days with frost. Differences in the predicted distributions of clades A1a and A1b were not apparent.

The above mentioned studies indicate that *F. tularensis* does persist in the environment, but specific reservoirs and the role of hosts remain elusive. More specific research is summarized below that investigated the persistence of *F. tularensis* in various environmental media.

9.1. F. tularensis in Aerosols

Sinclair et al. (2008) summarized three studies (Cox, 1971; Cox and Goldberg, 1972; Ehrlich and Miller, 1973) from the 1970s on the persistence of *F. tularensis* aerosols and estimated times required for the initial titer to decrease by 99% (T_{99} values). Based on the 24 T_{99} values (e.g., 0.48 hours [29 minutes following wet dissemination at 50% RH] to 131 hours [5 days following wet dissemination at 90% RH]) estimated by Sinclair et al. (2008), aerosolized *F. tularensis* is expected to possibly persist for several minutes to a few days. However, other than the T_{99} value of 131 hours, all T_{99} values were ≤7.93 hours indicating that *F. tularensis* is most likely to persist <1 day in an aerosol. Sinclair et al. (2008) did not comment specifically on the uniqueness of the T_{99} value of 131 hours, which was estimated from relatively short study durations (e.g., aerosol ages of 15 minutes by Cox [1971] and Cox and Goldberg [1972]) as were many of the other T_{99} values. The oldest aerosol age for which *F. tularensis* data were evaluated by Sinclair et al. (2008) was a 64-minute study duration reported by Ehrlich and Miller (1973).

One *F. tularensis* persistence article (Hood, 2009) for aerosols that had been published since the review by Sinclair et al. (2008) was identified. Hood (2009) studied the persistence of *F. tularensis* strain Schu S4 in aerosols (particle sizes of <3 micrometers [µm, 3 µm to 6 µm, and >6 µm) held at 9°C to 13°C and 72% to 85% RH, and found persistence after 45 minutes to 60 minutes (the longest durations tested). These tests were conducted in a highly ventilated system such that the exposures were equivalent to open air exposures. Interestingly, the author noted that unidentified constituents in the air (possibly olefins from oil refineries and dense car populations) may have reduced *F. tularensis* viability (i.e., lowered the percent survival). The viability of *F. tularensis* <3 µm particles after 45 minute exposures ranged from 10% to 80% survival depending on the wind direction associated with the air intake during testing. In addition, the viability of *F. tularensis* <3 µm particles was reduced in the ventilated system but viability was not reduced in comparable testing conducted with a non-ventilated system (Hood, 2009).

9.2. *F. tularensis* on Fomites

Persistence testing was conducted with *F. tularensis* live vaccine strain (LVS) following inoculation onto aluminum, carpet, keyboard keys, and painted joint tape (Ryan, 2010). Briefly, material coupons were inoculated with 1 x 10^7 viable *F. tularensis* in 100 µL aliquots of stock suspension (Muller-Hinton broth). The inoculated coupons were held at approximately 35% RH and 23°C. At various time points, the coupons were placed in PBS and agitated on an orbital shaker to extract the *F. tularensis* from the materials. Aliquots of the undiluted extract and associated serial dilutions were spread plated onto chocolate II agar plus ISoVitaleX™ (Becton, Dickinson and Company, Franklin Lakes, NJ) and incubated for up to 72 hours at 37°C. *F. tularensis* was found to persist (although the mean recovery was <1%) on the keyboard key material after 7 days (the longest duration tested).The longest *F. tularensis* persistence (also <1% mean recoveries) on the other materials was 8 hours on aluminum and painted joint tape and 4 hours on carpet.

Faith et al. (2012) reported that *F. tularensis* LVS (100-300 CFU) applied to filter paper and allowed to desiccate survived between 20 and 40 minutes. Only one study (Wilkinson, 1966) with data on *F. tularensis* persistence on fomites was identified by Sinclair et al. (2008). Wilkinson (1966) used aerosol deposition of *F. tularensis* LVS onto stainless steel. At 25°C, *F. tularensis* persisted approximately 15 days at 10% RH, 3 days at 65% RH, and 2 days at 100% RH, as determined via culture. At 37°C *F. tularensis* persistence decreased much more rapidly (e.g., within 16 hours at 55% to 100% RH), although under conditions of 0% RH *F. tularensis* remained viable for 16 days.

As summarized on Table 10, Wilkinson (1966) and Ryan (2010) found that on some materials (i.e., keyboard keys or stainless steel) *F. tularensis* is capable of surviving for several days at 23°C to 25°C. Shorter persistence (<24 hours) was observed at warmer temperatures (37°C) by Wilkinson (1966) or on other materials (aluminum, carpet, and painted joint tape) by Ryan (2010). At 23°C and 35% RH, Ryan (2010) recovered *F. tularensis* LVS after 8 hours on aluminum and painted joint tape (but not after 3 days) and after 4 hours on carpet (but not after 8 hours). Wilkinson (1966) observed increased persistence with decreased humidity including persistence of *F. tularensis* for at least 16 days under hot and dry conditions. Faith et al. (2012), however, found rapid loss of viability within 40 minutes under conditions of desiccation. Faith et al. (2012) used considerably lower inoculums levels (100 to 300 CFU) than Wilkinson (1966) (10^7 CFU), and the bacteria used by Faith et al. (2012) were washed and resuspended in PBS. Wilkinson (1966) used *F. tularensis* aerosolized along with growth media, such that the bacteria were likely coated with an outer layer of non-living material, which could have contributed to the longer persistence.

Table 10. *F. tularensis* LVS Persistence on Fomites

Material	Environmental Condition	Longest Duration with Persistence	Shortest Duration without Persistence	Study
Stainless steel	25°C, 10% RH	15 days*	--	Wilkinson (1966)
	25°C, 65% RH	3 days*	--	
	25°C, 100% RH	2 days*	--	
	37°C, 0% RH	16 days*	--	
	37°C, 55% RH	16 hours*	--	
	37°C, 65% RH	12 hours*	--	
	37°C, 80% RH	12 hours*	--	
	37°C, 100% RH	9 hours*	--	
Aluminum	23°C, 35% RH	8 hours	3 days	Ryan (2010)
Carpet	23°C, 35% RH	4 hours	8 hours	
Keyboard keys	23°C, 35% RH	7 days*	--	
Painted joint tape	23°C, 35% RH	8 hours	3 days	
Filter paper	Desiccated	20 minutes	40 minutes	Faith et al. (2012)

-- Not tested/not reported.

* The longest duration tested (i.e., the actual persistence duration could be longer).

9.3. *F. tularensis* in Water

Water Temperature and Salinity

Two studies were identified that evaluated the survival of *F. tularensis* in water at different temperatures or salinities. One study (Gilbert and Rose, 2012) found no persistence after 1 day in de-chlorinated municipal water at 5°C and 25°C, but persistence of at least 14 days at 8°C. A second study (Berrada and Telford, 2011) found relatively long survival at 21°C in brackish water. The salt and sulfur content of the brackish water may have contributed to the high survival reported by Berrada and Telford) (2011) compared to the municipal water used by Gilbert and Rose (2012). More details on these studies are provided in the following paragraphs.

Gilbert and Rose (2012) used autoclaved, dechlorinated municipal water inoculated with 10^6 CFU mL^{-1} *F. tularensis* LVS and NY98, which was then held at 5°C, 8°C, or 25°C. Both *F. tularensis* LVS and NY98 are *holarctica* subspecies (US EPA, 2012). Viability was assessed by culture on chocolate II agar at 35°C. When held at 8°C, *F. tularensis* LVS was culturable after 14 days (but not after 21 days) and *F. tularensis* NY98 was culturable after 28 days (but not after 30 days). *F. tularensis* LVS and NY98 were not culturable from water held at 5°C or 25°C after 1 day (i.e., the shortest duration tested without persistence). The authors note that *F. tularensis* may have entered into a VBNC state at 5°C and 25°C.

Berrada and Telford (2011) studied the persistence of *F. tularensis* subspecies *tularensis* (SSTR9 10-7), *F. tularensis* LVS, and *F. novicida* U112 in freshwater, brackish-water, and saline (0.85% sodium chloride) when stored at room temperature (21°C). The salinity of the freshwater and brackish-water samples was not reported, but the sodium levels were 10 mg/L and 3,604 mg/L, respectively. Culturability was assessed on cystine heart agar blood (CHAB) supplemented with 8% rabbit blood and antibiotics with incubation at 37°C. Inoculum levels were $>10^7$ CFU mL^{-1}. In freshwater, *F. tularensis* subspecies *tularensis* (SSTR9 10-7) and *F. tularensis* LVS were culturable after 10 days, but not after 14 days, and *F. novicida* U112 was culturable after 7 days, but not after 10 days. Persistence was longer in brackish-water as *F. tularensis* subspecies *tularensis* (SSTR9 10-7) and *F. novicida* U112 were culturable after 28 days, but not after 34 days, and *F. tularensis* LVS was culturable after34 days (the longest duration tested). In saline, *F. tularensis* LVS and *F. novicida* U112 were culturable after 18 days, but not after 21 days, and *F. tularensis* subspecies *tularensis* (SSTR9 10-7) was culturable after 21 days, but not after 28 days. The authors noted that the brackish-water samples contained much higher sulfur residues (168-219 mg mL^{-1}) than the freshwater samples (2-5 mg L^{-1}), which might enhance *F. tularensis* survival. In fact, "sulfur-containing amino acids, cysteine or cystine, are usually required for the cultivation of *F. tularensis*" (Berrada and Telford, 2011). Similarly, 1% sodium chloride was previously reported as enhancing *F. tularensis* culture growth (Berrada and Telford, 2011).

Sinclair et al. (2008) summarized two studies (DeArmon et al., 1962; Forsman et al., 2000) evaluating the persistence of *F. tularensis*. An environmentally relevant study of *F. tularensis* persistence in tap water at 8°C (Forsman et al., 2000) was associated with a T_{99} value of 33.7 days (Sinclair et al., 2008). Forsman et al. (2000) noted that *F. tularensis* in water can enter a VBNC state. The other T_{99} values reported by Sinclair et al. (2008) were less applicable for environmental scenarios as the studies focused on the shelf-life of cultures specifically prepared (e.g., packaged in polyethylene ampoules or freeze-dried) for long-term storage (DeArmon et al., 1962).

Nutrients and Protozoa

Three studies were identified that found associations with high nutrient and/or protozoan levels with *F. tularensis* survival in water. Broman et al. (2011) collected surface water samples (e.g., lakes and rivers) from tularemia endemic areas (i.e., the cities Ljusdal and Örebro) in Sweden. The aquatic systems sampled could be characterized as eutrophic. *F. tularensis* subspecies *holarctica* was detected in water "during three consecutive years, indicating that the bacterium may persist in water for several years" (Broman et al., 2011).

Thelaus et al. (2009) investigated the persistence of *F. tularensis* subspecies *holarctica* in the laboratory with lake water. The lake water originated from Lake Nydala in northern Sweden and was filtered and autoclaved prior to use in the experiment. The lake water was then seeded with 6.0×10^5 *F. tularensis* cells mL^{-1}) under differing nutrient and predation conditions. Water samples (1 mL) were subsequently sampled and bead-beaten in an attempt to release intracellular bacteria, spread on selective modified Thayer-Martin agar, and incubated at 37°C for 4 days. The bacteria persisted 1 day but were unable to be cultured after 5 days in a high-nutrient condition (total nitrogen 3.0 mg L^{-1} and total phosphorus 0.4 mg L^{-1}) with protozoa (*Euglenozoa*, *Cryptomonas*, and *Ochromonas*). Under high nutrient conditions (without protozoa) or under low-nutrient conditions (total nitrogen 0.3 mg L^{-1} and total phosphorus

0.008 mg L^{-1}) with protozoa (*Euglenozoa*, *Cryptomonas, Ochromonas*, *Strombilidium*, *Paramecium*, and *Urothrica*), *F. tularensis* were culturable after 16 days, but not after 26 days. Under low-nutrient conditions without protozoa, *F. tularensis* were culturable after 26 days, but not after 38 days. Interestingly, microscopic counts of *F. tularensis* labeled with green fluorescent protein (*gfp*) were obtained over the entire 38-day experiment without significant effects observed for nutrient levels or predation, although over the first 16 days *Francisella-gfp* abundance increased under the high nutrient conditions with protozoa. *F. tularensis* cells were observed as planktonic as well as inside the protozoa, which was most efficiently determined by detection of *gfp*-produced fluorescence. *F. tularensis* ingested by the protozoa were not rapidly degraded, and *F. tularensis* could escape from the protozoa. The inability to grow on culture plates indicates that the *F. tularensis* cells had entered a VBNC state. The inability to culture *F. tularensis* may be indicative of symbiotic bacteria that obtain proteins from the host cell (e.g., the protozoa) and down-regulate the synthesis of proteins, possibly losing the ability to replicate outside the host. Thelaus et al. (2009) also reported that *F. tularensis* lost its virulence in mice after incubation in the lake water (after 120 days), irrespective of treatment. Apparently, *F. tularensis* can survive and reproduce in water for relatively long periods of time (including waters with high nutrient levels and protozoan predation), although virulence may be lost.

El-Etr et al. (2009) indicated that amoeba may be important with regard to the environmental persistence of *F. tularensis*. The amoeba, *A. castellanii*, was shown to rapidly encyst in response to *F. tularensis* infection, and virulent strains of *F. tularensis* were shown to survive in the cysts for at least 3 weeks post-infection (El-Etr et al., 2009). The authors discuss the possibility that *F. tularensis* in amoeba cysts enables the bacteria to survive drying and other adverse environmental conditions.

Biofilms

The ability of *F. tularensis* strains to form biofilms may be important for its persistence in the environment. *F. tularensis* subspecies *tularensis* strains SchuS4 and FT-10, *F. tularensis* LVS, and *F. tularensis* subspecies *novicida* form biofilms on abiotic surfaces such as glass (Margolis et al., 2010). Margolis et al. (2010) reported persistence durations of at least 7 days for *F. tularensis* subspecies *novicida* on chitin-containing surfaces, at least 5 days for *F. tularensis* subspecies *novicida* under flow conditions, at least 6.3 days for *F. tularensis* LVS and *F. tularensis* subspecies *novicida* under static conditions, and at least 1 day for *F. tularensis* subspecies *tularensis* strains SchuS4 and FT-10 under static conditions (Table 11). Durham-Colleran et al. (2010) demonstrated that *F. tularensis* subspecies *novicida* could produce biofilms (and persist for at least 2 days) at 25°C and 37°C on polystyrene (and on polyvinyl-chloride at 37°C) when grown in tryptic soy broth supplemented with cysteine. Mahajan et al. (2011) also demonstrated that *F. tularensis* LVS can form biofilms in moderately hard water at 37°C and persist for at least 15 days without nutrients or host cells. Durham-Colleran et al. (2010) concluded that "by forming a biofilm in natural ecosystems, the nutritionally fastidious *Francisella* may be able to survive the environmental conditions of mud and waterways. In addition, biofilm formation could be a mechanism *Francisella* utilizes for persistence within the tick arthropod vector."

Table 11. *F. tularensis* Persistence in Water

F. tularensis Subspecies and Strain	Environmental Condition	Longest Duration with Persistence	Shortest Duration without Persistence	Study
holarctica LVS	Autoclaved, dechlorinated municipal water, 5°C	<1 day	1 day	Gilbert and Rose (2012)
holarctica NY98	Autoclaved, dechlorinated municipal water, 5°C	<1 day	1 day	
holarctica LVS	Autoclaved, dechlorinated municipal water, 8°C	14 days	21 days	
holarctica NY98	Autoclaved, dechlorinated municipal water, 8°C	28 days	30 days	
holarctica LVS	Autoclaved, dechlorinated municipal water, 25°C	<1 day	1 day	
holarctica NY98	Autoclaved, dechlorinated municipal water, 25°C	<1 day	1 day	
tularensis SSTR9 10-7	Filter-sterilized freshwater, 21°C	10 days	14 days	Berrada and Telford (2011)
holarctica LVS	Filter-sterilized freshwater, 21°C	10 days	14 days	
novicida U112	Filter-sterilized freshwater, 21°C	7 days	10 days	
tularensis SSTR9 10-7	Filter-sterilized brackish-water, 21°C	28 days	34 days	
holarctica LVS	Filter-sterilized brackish-water, 21°C	34 days*	--	
novicida U112	Filter-sterilized brackish-water, 21°C	28 days	34 days	
tularensis SSTR9 10-7	Filter-sterilized saline, 21°C	21 days	28 days	
holarctica LVS	Filter-sterilized saline, 21°C	18 days	21 days	
novicida U112	Filter-sterilized saline, 21°C	18 days	21 days	
holarctica LVS	T_{99} value in tap water, 8°C	33.7 days†	--	Sinclair et al. (2008)
holarctica	Lake water with bacteria predators (protozoa) and high nutrient conditions	1 day	5 days	Thelaus et al. (2009)
holarctica	Lake water with bacteria predators (protozoa) and low nutrient conditions	16 days	26 days	
holarctica	Lake water without bacteria predators and high nutrient conditions	16 days	26 days	

F. tularensis Subspecies and Strain	Environmental Condition	Longest Duration with Persistence	Shortest Duration without Persistence	Study
holarctica	Lake water without bacteria predators and low nutrient conditions	26 days	38 days	
novicida	Biofilm growth on chitin-containing surfaces, in Chamberlain's defined medium without glucose, 30°C	7 days*	--	Margolis et al. (2010)
novicida	Biofilm growth under flow conditions (0.1 mL minute^{-1}), 20°C to 22°C	5 days*	--	
novicida and *holarctica* LVS	Biofilm growth under static conditions, 26°C and 37°C	6.3 days*	--	
tularensis SchuS4 and FT-10	Biofilm growth under static conditions, 37°C	1 day*	--	
novicida	Biofilm growth on polyvinyl-chloride in tryptic soy broth supplemented with cysteine, 37°C	2 days*	--	Durham-Colleran et al. (2010)
novicida	Biofilm growth on polystyrene in tryptic soy broth supplemented with cysteine, 25°C and 37°C	2 days*	--	
holarctica LVS	Biofilm growth in moderately hard water, 37°C	15 days*	--	Mahajan et al. (2011)

-- Not tested/not reported.

* The longest duration tested (i.e., the actual persistence duration could be longer).

† Data obtained from a literature review, the shortest duration without persistence was not reported.

The ability of *F. tularensis* species to adhere to chitin-containing surfaces was investigated by incubating *F. tularensis* subspecies *novicida* with crab shell (Margolis et al., 2010). After 1 hour at 30°C, bacteria were observed adhered to the shell surface. After 1 week, bacterial communities were present consisting of individual bacteria surrounded by a matrix of extracellular polymeric substance. The observed community structure suggests that *F. tularensis* subspecies *novicida* can attach to and proliferate as biofilms on chitin, an environmentally relevant surface including copepod and zooplankton shells and exoskeletons of arthropods. The authors also note that *F. tularensis* subspecies *novicida* likely uses chitinases for the hydrolysis of chitin providing a local nutrient source for *F. tularensis* subspecies *novicida* persistence and growth. Data on the persistence of *F. tularensis* in water are summarized in Table 11.

9.4. *F. tularensis* Persistence Gaps

This review of the literature published since Sinclair et al. (2008) did identify additional studies focused on the persistence of *F. tularensis* on in aerosols, on fomites, and in water, but no more recent studies were identified assessing persistence in soil, or studying the influence of solar radiation (UV light) on *F. tularensis* persistence.

Many of the studies reviewed attempted to characterize the environmental reservoir of *F. tularensis*. *F. tularensis* does exist in some aquatic and soil environments, and evidence is accumulating that the natural reservoirs may include protozoa and/or biofilms. The mechanism by which *F. tularensis* persists or establishes an environmental reservoir following a release is unknown, but bacterial survival may be associated with high nutrient content, brackish/saline environments, protozoan predation, biofilms, and relatively high sulfur or cystine content.

10. Viral Encephalitis and Hemorrhagic Fever Agents Persistence

Category A viral hemorrhagic fever agents include filoviruses (e.g., Ebola and Marburg) and arenaviruses (e.g., Lassa and Machupo) (CDC, 2013). The Sinclair et al. (2008) review identified persistence data on the Marburg virus and Lassa virus. Sinclair et al. (2008) also identified data for flaviviruses (Japanese encephalitis virus, St. Louis encephalitis virus, and yellow fever virus), bunyaviruses (hantavirus and Crimean-Congo virus), and alphaviruses (Venezuelan equine encephalitis [or encephalomyelitis] [VEE] virus). Alphaviruses (e.g., VEE) causing viral encephalitis is an example of a Category B agent, and hantavirus is an example of a Category C agent (an emerging infectious disease) (CDC, 2013).

10.1. Viral Encephalitis and Hemorrhagic Fever Agents in Aerosols

In aerosols, Sinclair et al. (2008) reported that the Marburg virus was not persistent in air (based on data from Belanov et al., 1996). Sinclair et al. (2008) estimated T_{99} values of 1.42 to 3.58 hours for the Lassa virus, 5.97 to 11.2 hours for the Japanese encephalitis virus, 2.32 to 291 hours [12.1 days] for the VEE

virus, and 9.58 to 2,875 hours [119.8 days] for the St. Louis encephalitis virus. Although T_{99} values were not estimated, hantavirus is reportedly stable in air (Schmaljohn and Hjelle, 1997 as cited in Sinclair et al., 2008). Based on data presented by Sinclair et al. (2008), the Japanese encephalitis virus and VEE virus appeared to have inverse relationships with RH. The highest T_{99} values for the St. Louis encephalitis virus (2,875 hours and 51.1 hours were associated with the lowest RH levels tested (23% and 35%, respectively). The other T_{99} values for the St. Louis encephalitis virus ranged from 9.28 to 25.4 hours over RH levels of 46% to 80% without any apparent trends.

Piercy et al. (2010) studied the persistence of Ebola and Marburg viruses in aerosols held at approximately 50% RH and 22°C. The viruses could be detected (i.e., infect cell cultures) after 90 minutes (the longest duration tested) in the aerosol. Smither et al. (2011) used spiders' webs to capture aerosolized Ebola and Marburg viruses as an alternative method of assessing persistence. Smither et al. (2011) reported that both viruses survived 60 minutes (the longest duration tested) when held at approximately 50% RH and 22°C, similar to the results obtained using the more dynamic aerosol system of Piercy et al. (2010). The available persistence data identified for viral encephalitis and hemorrhagic fever agents in aerosols is also summarized in Table 12.

Table 12. Viral Encephalitis and Hemorrhagic Fever Agents Persistence in Aerosols

Virus	Environmental Condition	Longest Duration with Persistence	Shortest Duration without Persistence	Study
St. Louis encephalitis virus	21°C, 23% RH	119.8 days† (T_{99} value)	--	Sinclair et al. (2008)
VEE virus	9.0°C, 19% RH	12.1 days† (T_{99} value)	--	
Lassa virus	32°C, 80% RH	3.58 hours† (T_{99} value)	--	
Japanese encephalitis virus	24°C, 30% RH	11.2 hours† (T_{99} value)	--	
Ebola virus	22°C, 50% RH	1.5 hours*	--	Piercy et al. (2010)
Marburg virus	22°C, 50% RH	1.5 hours*	--	
Ebola virus	22°C, 50% RH	1 hour*	--	Smither et al. (2011)
Marburg virus	22°C, 50% RH	1 hours*	--	

-- Not tested/not reported.

* The longest duration tested (i.e., the actual persistence duration could be longer).

† Data obtained from a literature review, the shortest duration without persistence was not reported.

10.2. Viral Encephalitis and Hemorrhagic Fever Agents on Fomites

Sinclair et al. (2008) found data (from Belanov et al., 1996) indicating that the Marburg virus was stable in dried blood for 4 to 5 days. Bunyaviruses (Crimean-Congo virus and hantavirus, which can cause hemorrhagic fever) were estimated to have T_{99} values ranging from 2.16 to 2.91 hours, respectively on aluminum (Sinclair et al., 2008, based on research conducted by Hardestam et al., 2007). The persistence data identified for viral encephalitis and hemorrhagic fever agents on fomites is summarized in the following text and Table 13.

Fogarty et al. (2008) reported that the half-lives for henipaviruses (e.g., Hendra virus and Nipah virus, which can cause encephalitis) on fruit (mango) flesh from 12 minutes to 30.3 hours, depending on temperature (generally longer persistence at 22°C than 37°C) and fruit pH (longer persistence at pH 5 than pH 4.5 or 3.5). Desiccation of the viruses within a polystyrene dish resulted in Hendra virus and Nipah virus survival for <15 minutes when stored at 37°C, Hendra virus survival for 30 minutes (but not 1 hour) when stored at 22°C, and Nipah virus survival for 1 hour (the longest duration tested) when stored at 22°C (Fogarty et al., 2008).

Table 13. Viral Encephalitis and Hemorrhagic Fever Agents Persistence on Fomites

Virus	Material	Environmental Condition	Longest Duration with Persistence	Shortest Duration without Persistence	Study
Hantavirus	Aluminum	20°C	2.91 hours† (T_{99} value)	--	Sinclair et al. (2008)
Crimean-Congo virus	Aluminum	20°C	2.16 hours† (T_{99} value)	--	
Marburg virus	Blood	Dried	5 days†	--	
Hendra virus	Polystyrene	Desiccated, 22°C	30 minutes	1 hour	Fogarty et al. (2008)
Hendra virus	Polystyrene	Desiccated, 37°C	5 minutes	15 minutes	
Nipah virus	Polystyrene	Desiccated, 22°C	1 hour*	--	
Nipah virus	Polystyrene	Desiccated, 37°C	5 minutes	15 minutes	
Hendra virus	Mango flesh	22°C, pH 3.5	18 minutes (half-life)	--	
Hendra virus	Mango flesh	22°C, pH 4.5	3.5 hours (half-life)	--	
Hendra virus	Mango flesh	22°C, pH 5	22.4 hours (half-life)	--	
Hendra virus	Mango flesh	37°C, pH 3.5	24 minutes (half-life)	--	
Hendra virus	Mango flesh	37°C, pH 4.5	30 minutes (half-life)	--	
Hendra virus	Mango flesh	37°C, pH 5	5.9 hours (half-life)	--	
Nipah virus	Mango flesh	22°C, pH 3.5	1.4 hours (half-life)	--	

Virus	Material	Environmental Condition	Longest Duration with Persistence	Shortest Duration without Persistence	Study
Nipah virus	Mango flesh	22°C, pH 4.5	5.5 hours (half-life)	--	
Nipah virus	Mango flesh	22°C, pH 5	30.3 hours (half-life)	--	
Nipah virus	Mango flesh	37°C, pH 3.5	12 minutes (half-life)	--	
Nipah virus	Mango flesh	37°C, pH 4.5	30 minutes (half-life)	--	
Nipah virus	Mango flesh	37°C, pH 5	2.2 hours (half-life)	--	
Ebola virus	Polyvinyl chloride	4°C, 55% RH, applied in guinea pig serum and tissue culture medium	14 days	26 days	Piercy et al. (2010)
Ebola virus	Polyvinyl chloride	Room temperature, 55% RH, applied in guinea pig serum and tissue culture medium	<2 days	2 days	
Ebola virus	Glass	4°C, 55% RH, applied in guinea pig serum	14 days	26 days	
Ebola virus	Glass	4°C, 55% RH, applied in tissue culture medium	50 days*	--	
Ebola virus	Glass	Room temperature, 55% RH, applied in guinea pig serum and tissue culture medium	<2 days	2 days	
Ebola virus	Stainless steel	4°C, 55% RH, applied in guinea pig serum and tissue culture medium	<2 days	2 days	
Ebola virus	Stainless steel	Room temperature, 55% RH, applied in guinea pig serum and tissue culture medium	<2 days	2 days	
Marburg virus	Polyvinyl chloride	4°C, 55% RH, applied in guinea pig serum	14 days	26 days	
Marburg virus	Polyvinyl chloride	4°C, 55% RH, applied in tissue culture medium	26 days	50 days	

Virus	Material	Environmental Condition	Longest Duration with Persistence	Shortest Duration without Persistence	Study
Marburg virus	Polyvinyl chloride	Room temperature, 55% RH, applied in guinea pig serum and tissue culture medium	<2 days	2 days	
Marburg virus	Glass	4°C, 55% RH, applied in guinea pig serum	14 days	26 days	
Marburg virus	Glass	4°C, 55% RH, applied in tissue culture medium	26 days	50 days	
Marburg virus	Glass	Room temperature, 55% RH, applied in guinea pig serum and tissue culture medium	<2 days	2 days	
Marburg virus	Stainless steel	4°C, 55% RH, applied in guinea pig serum and tissue culture medium	<2 days	2 days	
Marburg virus	Stainless steel	Room temperature, 55% RH, applied in guinea pig serum and tissue culture medium	<2 days	2 days	
VEE virus	Rubber	20°C to 25°C, 30% to 40% RH	7 days*	--	Sagripanti et al. (2010)
VEE virus	Painted aluminum	20°C to 25°C, 30% to 40% RH	7 days*	--	
VEE virus	Glass	20°C to 25°C, 30% to 40% RH	11.4 days‡	--	
Lassa virus	Glass	20°C to 25°C, 30% to 40% RH	9.7 days‡	--	
Ebola virus	Glass	20°C to 25°C, 30% to 40% RH	5.9 days‡	--	
Rift Valley fever virus	Blood spotted on filter paper	Stored dry at room temperature	2 days	3 days	Näslund et al. (2011)

-- Not tested/not reported.

* The longest duration tested (i.e., the actual persistence duration could be longer).

† Data obtained from a literature review, the shortest duration without persistence was not reported.

‡ The longest duration with persistence was based on the time needed to decrease the viral load by 4 $\log_{10}$.

Piercy et al. (2010) studied the persistence of Ebola and Marburg viruses on plastic (polyvinyl chloride), stainless steel, and glass. The viruses were applied in liquid suspensions (tissue culture medium or guinea pig serum) and allowed to dry for 30 minutes. The inoculated materials were held at 4°C or room temperature at 55% RH. Neither virus was recovered from any surface held at room temperature nor from stainless steel at 4°C (the shortest duration tested in each instance was 2 days). At 4°C, the Ebola and Marburg viruses survived more than 14 days on plastic and glass. In fact, Marburg virus survived 26 days on glass and plastic, and Ebola virus persisted 50 days on glass (the longest duration tested) (Piercy et al., 2010).

Sagripanti et al. (2010) deposited 3 x 10^6 plaque forming units (PFU) of VEE virus on glass, rubber, and painted aluminum and held the inoculated materials in the dark at 20°C to 25°C and 30% to 40% RH. The virus survived for 7 days (the longest duration tested) on each material. Sagripanti et al. (2010) conducted similar testing with VEE virus, Lassa virus, and Ebola virus inoculated onto glass (held at 20°C to 25°C, 30% to 40% RH, and shielded from light). The time needed to decrease the viral load by 4 $\log_{10}$ (T_4) was 11.4 days for VEE virus, 9.7 days for Lassa virus, and 5.9 days for Ebola virus (Sagripanti et al., 2010).

Näslund et al. (2011) spotted blood (containing 4,800 or 120,000 PFU of Rift Valley fever virus) onto Nobuto filter papers. The filter papers were stored at room temperature. At various time points the filter papers were eluted with RNase-free water and the supernatants were added to Vero cells. The Vero cells were infected from extracts of the filter papers inoculated with 120,000 PFU of Rift Valley fever virus after 2 days of storage but not after 3 days. Testing associated with Rift Valley fever virus at 4,800 PFU also demonstrated infected Vero cells (i.e., virus persistence) after 1 day of storage but not after 2 days (Näslund et al., 2011).

10.3. Viral Encephalitis and Hemorrhagic Fever Agents in Water

Sinclair et al. (2008) estimated T_{99} values of 96 hours (4 days) for the vaccine strain of the yellow fever virus (a hemorrhagic fever agent), T_{99} values of 165 to 710 hours (29.6 days) for the adenovirus, which can cause encephalitis, and T_{99} values of 30 hours to 69.4 days for the hantavirus, which can cause hemorrhagic fever. For hantavirus, the longest T_{99} values (69.4, 50, and 31.4 days) were associated with tests conducted at 4°C; the next highest T_{99} value was 13 days at 23°C (based on studies from Hardestam et al., 2007 and Kallio et al., 2006). Sinclair et al. (2008) also provided data for Sicilian virus that indicated an even longer T_{99} value of 325 days at 4°C (based on research from Hardestam et al., 2007). Since the Sicilian virus is known to induce fever but not encephalitis or hemorrhagic fever, these data are not further included in this report. Two additional studies were identified that assessed virus persistence in water as summarized in the following paragraphs.

Fitzgibbon and Sagripanti (2008) tested the persistence of VEE virus at approximately 2.5 x 10^6 PFU mL^{-1} in distilled-deionized (unchlorinated) water and tap water with a chlorine level of 4 to 5 parts per million (ppm). Testing was conducted at 21°C, and the distilled-deionized water was also tested at 4°C and 30°C. VEE virus persisted 21 days (the longest duration tested) in distilled-deionized water held at 4°C, 21°C,

and 30°C; log reductions in virus titer were 0.18 at 4°C, 2.59 at 21°C, and 2.94 at 30°C. In tap water at 21°C, VEE virus persisted for 17 days but became non-detectable within 21 days (Fitzgibbon and Sagripanti, 2008).

Wade et al. (2010) found that VEE virus at 1 x 10^6 PFU mL^{-1} was inactivated within 1 hour in tap water at 21°C with 1 mg L^{-1} free available chlorine or 2 mg L^{-1} total bromine. In the absence of the chlorine and bromine, the VEE virus remained infective in tap water for 4 hours (the longest duration tested) with little reduction in the initial titer level (Wade et al., 2010). The available persistence data identified for viral encephalitis and hemorrhagic fever agents in water is also summarized in Table 14.

Table 14. Viral Encephalitis and Hemorrhagic Fever Agents Persistence in Water

Virus	Environmental Condition	Longest Duration with Persistence	Shortest Duration without Persistence	Study
Hantavirus	4°C	69.4 days† (T_{99} value)	--	Sinclair et al. (2008)
Adenovirus	6°C	29.6 days† (T_{99} value)	--	
Yellow fever virus	37°C	4 days† (T_{99} value)	--	
VEE virus	Distilled-deionized (unchlorinated) water, 4°C, 21°C, and 30°C	21 days*	--	Fitzgibbon and Sagripanti (2008)
VEE virus	Tap water (4 to 5 ppm chlorine), 21°C	17 days	21 days	
VEE virus	Tap water (1 mg L^{-1} free available chlorine or 2 mg L^{-1} total bromine), 21°C	<60 minutes	60 minutes	Wade et al. (2010)
VEE virus	Tap water (without chlorine or bromine), 21°C	4 hours*	--	

-- Not tested/not reported.

* The longest duration tested (i.e., the actual persistence duration could be longer).

† Data obtained from a literature review, the shortest duration without persistence was not reported.

10.4. Viral Encephalitis and Hemorrhagic Fever Agents Persistence Gaps

No data were identified with regard to the persistence of viral encephalitis or hemorrhagic fever agents in soil. Persistence data were only identified for viral hemorrhagic fever agents in aerosols and on fomites. The new aerosol persistence data for the Ebola and Marburg viruses, identified since Sinclair et al. (2008), was limited to testing at one environmental condition (approximately 50% RH and 22°C; Piercy et al., 2010; Smither et al., 2011). Piercy et al. (2010) noted that the assessment of virus persistence in aerosols is affected by RH, the suspension and approach used to aerosolize the virus, and methods used to enumerate the virus. Although all tested viruses persisted 90 minutes in aerosols, Piercy et al. (2010) reported differences in decay rates by virus. Zaire Ebola virus and Lake Victoria Marburg virus had similar decay rates, but the decay rate for the Reston Ebola virus was significantly lower than for the Zaire Ebola virus and Lake Victoria Marburg virus (Piercy et al., 2010). On fomites, Ebola and Marburg viruses were affected by material type (less persistent on stainless steel than plastic and glass) and temperature (less persistent at room temperature than at 4°C) (Piercy et al., 2010). None of the studies identified for viral encephalitis and hemorrhagic fever agents assessed the impact of sunlight on persistence. Persistence data in water (since Sinclair et al., 2008) were limited to research with VEE virus.

11. *Yersinia pestis* Persistence

Plague in humans typically results from *Y. pestis* infected fleas transmitting the bacterium from rodents to humans. Human plague is generally associated with geographic areas having *Y. pestis* circulating in the rodent population. Soil may serve as a reservoir for *Y. pestis*, which might explain prolonged periods where the bacterium is not found in host or vector populations (Drancourt et al., 2006). The persistence of *Y. pestis* in the environment (e.g., soil) might lead to animals becoming infected (e.g., burrowing rodents), which could lead to a new transmission cycle in rodents and fleas (Drancourt et al., 2006). As reported by Pawlowski et al. (2011a), "a growing body of evidence suggests that *Y. pestis* can survive without a host for extended periods under certain environmental conditions while, in many cases, retaining infectivity."

11.1. *Y. pestis* in Aerosols

Sinclair et al. (2008) reviewed one study (Won and Ross, 1966) investigating the persistence of *Y. pestis* as an aerosol. The estimated T_{99} values were less than 1 hour at 26°C, including 34 minutes at 87% RH, 57 minutes at 50% RH, and 45 minutes at 20% RH. The lowest T_{99} value occurred at 87% RH, and Sinclair et al. (2008) noted that organism survival decreased rapidly as RH increased above 50%. Won and Ross (1966) used both 1% peptone and heart infusion broth as the diluents in the assay. At 20% to 50% RH, *Y. pestis* had exponential decay with both diluents. However, at 65% and 87% RH viability of the aerosolized *Y. pestis* was adversely affected with 1% peptone (no viability detected after 20 minutes),

but viability was detected after 90 minutes using heart infusion broth. No more recent data of the persistence of *Y. pestis* in aerosols were identified with this review.

11.2. *Y. pestis* on Fomites

Sinclair et al. (2008) reviewed two studies (Wilkinson, 1966; Rose et al., 2003) investigating the persistence of *Y. pestis* on fomites. The estimated T_{99} values ranged from 7 minutes (on stainless steel at 30°C and 52% RH) to 47.2 hours (on paper at 18°C to 22°C and 55% RH). Most of the testing was conducted at 18°C to 22°C and 55% RH (Rose et al., 2003), and under these conditions *Y. pestis* survived longer (higher T_{99} values) on paper than stainless steel, polyethylene, or glass (Sinclair et al., 2008).

Persistence testing was conducted with *Y. pestis* following inoculation onto aluminum, carpet, keyboard keys, and painted joint tape (Ryan, 2010). Material coupons were inoculated with 2.9×10^7 viable *Y. pestis* in 100 µL aliquots of stock suspension (trypticase soy broth). The inoculated coupons were held at approximately 50% RH and 20°C. At various time points, the coupons were placed in PBS and agitated on an orbital shaker to extract the *Y. pestis* from the materials. Aliquots of the undiluted extract and associated serial dilutions were spread plated onto tryptic soy agar and incubated for up to 72 hours at 37°C. *Y. pestis* was found to persist (although the mean recovery was <1%) on aluminum and painted joint tape for 7 days (the longest duration tested).The longest *Y. pestis* persistence (also <1% mean recoveries) on the other materials was 3 days on the keyboard keys and 8 hours on carpet.

11.3. *Y. pestis* in Soil

Three sources reviewed in Sinclair et al. (2008) (Breneva et al., 2005; Breneva et al., 2006; Mitscherlich and Marth, 1984) reported that *Y. pestis* persisted for 3.5 months in soil at room temperature and persisted for >10 months in soil at colder temperatures (i.e., 4°C to 8°C). Drancourt et al. (2006) demonstrated the persistence of *Y. pestis* in autoclaved, hydrated sand for at least 6 months. (Additional details describing the test conditions were not provided by Drancourt et al. [2006].) In a natural setting, Eisen et al. (2008) found that *Y. pestis* could persist at least 24 days in Arizona soil during late October in an area with limited exposure to sunlight (the intensity/duration of exposure to sunlight was not reported).

Ayyadurai et al. (2008) inoculated sterile soil with *Y. pestis* at 10^6 CFU g^{-1}, which was held at 18.7°C to 24°C (not exposed to sunlight). *Y. pestis* was able to be identified from soil cultures for 30 weeks, but thereafter contamination with *Pseudomonas* species of bacteria interfered with the culturing of *Y. pestis*. However, *Y. pestis* was found to be viable and virulent for up to 40 weeks (the longest duration tested) based on inoculations of soil extracts into mice. The mice died within 72 hours post-infection and the blood cultures were positive for *Y. pestis* (Ayyadurai et al., 2008).

A review article by Eisen and Gage (2009) noted that several studies have shown persistence of *Y. pestis* in soil. Many of the specific details of these tests were not provided (exact durations of persistence and

specific environmental conditions tested), and many of the source documents were not in English. Eisen and Gage (2009) also noted that other researchers have suggested that *Y. pestis* survives in soil inside protozoa, within a biofilm, or as a latent form, rather than as a free-living, metabolically active bacterium. Persistence data for *Y. pestis* in soil is summarized in Table 15.

Table 15. *Y. pestis* Persistence in Soil

Y. pestis Isolate	Environmental Condition	Longest Duration with Persistence	Shortest Duration without Persistence	Study
--	4°C to 8°C	10 months*	--	Sinclair et al. (2008)
--	Room temperature	3.5 months†	--	
--	Autoclaved, hydrated sand	6 months*	--	Drancourt et al. (2006)
Isolate (unspecified) from soil in Arizona	Arizona soil during late October in an area with limited exposure to sunlight (UV light)	24 days*	--	Eisen et al. (2008)
6/69M	18.7°C to 24°C	40 weeks*	--	Ayyadurai et al. (2008)

-- Not tested/not reported.

* The longest duration tested (i.e., the actual persistence duration could be longer).

† Data obtained from a literature review, the shortest duration without persistence was not reported.

11.4. *Y. pestis* in Water

From the Sinclair et al. (2008) review of Mitscherlich and Marth (1984), *Y. pestis* is able to survive in tap and well water for 16 days. Sinclair et al. (2008) also reported that *Y. pestis* may enter into a VBNC state in water as well as possibly being able to survive inside amoeba. Four additional studies on the persistence of *Y. pestis* in water were identified that are summarized in the following paragraphs.

Pawlowski et al. (2011a) inoculated *Y. pestis* (at approximately 10^6 to 10^7 CFU mL^{-1}) into various types of water held at 4°C. The bacterium remained culturable for 28 days (the longest duration tested) in sterilized river water with nearly 10^7 CFU mL^{-1} recovered, artificial sea water with 10^5 CFU mL^{-1} recovered, and non-autoclaved tap water with approximately 10^1 CFU mL^{-1} recovered. *Y. pestis* remained culturable for only 14 to 21 days in autoclaved tap water, and Pawlowski et al. (2011a) confirmed that the bacterium had entered a VBNC state.

Pawlowski et al. (2011b) inoculated *Y. pestis* at 5 x 10^6 CFU mL^{-1} into filtered or autoclaved river water. *Y. pestis* was culturable from the autoclaved river water for >3 years, while the bacterium persisted for 200 days in the filtered river water it was no longer culturable within 265 days in the filtered river water. Pawlowski et al. (2011b) noted that the bacterium *Hylemonella gracilis* actually dominated the filtered river water, apparently inhibiting the long-term survival of *Y. pestis*.

Torosian et al. (2009) evaluated the persistence of several strains of *Y. pestis* in sterilized bottled spring water held at 26°C. Approximately 2 x 10^4 CFU mL^{-1} of *Y. pestis* was inoculated into the water and the bacteria remained culturable for at least 74 days with some strains persisting for at least 2 years (the longest duration tested). The persistence testing was initiated with seed stock generated directly from frozen stock culture or from agar slants. More specifically, the following *Y. pestis* strains (generated from frozen culture): CO92, ZE94, EV76 Bru, JBH, UNH 1B, KIM 10, O19 Tn5, CSH23, K25 Icr, and O19 Ca^{-6} had persistent durations of 94 to 683 days. Other *Y. pestis* strains (UNH 1A, K25 pgm, K25 pst, 5.5, EV76 51F RP, and A1122) generated from frozen culture had persistent durations of at least 2 years. The following *Y. pestis* strains (generated from agar slants) persisted for 74 to 221 days: Harbin, Nepal, UNH 1A, UNH 1B, ZE94, CO92, PB6, PB6 DP, Pexu, K25 Icr, O19 Ca^{-6}, and K25 pst. Torosian et al. (2009) noted that unlike strains originating from frozen stock, none of the strains started from agar slants persisted for 2 years. The authors discussed the possibility that the frozen cells expressed an adaptive tolerance that may have affected persistence. The source of the seed stock used by Torosian et al. (2009) is noted within the "*Y. pestis* strain" column of Table 16. None of the other *Y. pestis* persistence studies in water reported using cultures initiated directly from the frozen state.

Gilbert and Rose (2012) used autoclaved, dechlorinated municipal water inoculated with 10^6 CFU mL^{-1} *Y. pestis* to assess persistence at 5°C and 25°C. *Y. pestis* was culturable for 14 days when held at 25°C and culturable for 1 day when held at 5°C (Table 16). In addition to culturing, viability was assessed by measuring metabolic (i.e., esterase) activity. Based on esterase production, *Y. pestis* remained viable for 30 days (the longest study duration) at 5°C and 25°C, suggesting that the bacterium entered a VBNC state (persistence based on metabolic activity is not presented in Table 16). Data on the persistence of *Y. pestis* in water (i.e., via culturability) are summarized in Table 16.

Table 16. *Y. pestis* Persistence in Water

Y. pestis Strain	Environmental Condition	Longest Duration with Persistence	Shortest Duration without Persistence	Study
--	Tap water and well water	16 days†	--	Sinclair et al. (2008)
Harbin 35	Sterilized (filtered and autoclaved) river water, 4°C	28 days*	--	Pawlowski et al. (2011a)
Harbin 35	Artificial sea water, 4°C	28 days*	--	
Harbin 35	Non-autoclaved tap water, 4°C	28 days*	--	
Harbin 35	Autoclaved tap water, 4°C	14 days	21 days	
A1122	Filtered river water	200 days	265 days	Pawlowski et al. (2011b)
A1122	Autoclaved river water	3 years*	--	
CO92, ZE94, EV76 Bru, JBH, UNH 1B, KIM 10, O19 Tn5, CSH23, K25 lcr, and O19 Ca^{-6} (generated from frozen culture)	Sterilized bottled spring water, 26°C	94 days to 683 days	--	Torosian et al. (2009)
UNH 1A, K25 pgm, K25 pst, 5.5, EV76 51F RP, and A1122 (generated from frozen culture)	Sterilized bottled spring water, 26°C	2 years*	--	
Harbin, Nepal, UNH 1A, UNH 1B, ZE94, CO92, PB6, PB6 DP, Pexu, K25 lcr, O19 Ca^{-6}, and K25 pst (generated from agar slants)	Sterilized bottled spring water, 26°C	74 days to 221 days	--	
A1122 and AZ 94-0666	Autoclaved, dechlorinated municipal water, 5°C	1 day	2 days	Gilbert and Rose (2012)
A1122 and AZ 94-0666	Autoclaved, dechlorinated municipal water, 25°C	14 days	21 days	

-- Not tested/not reported.

* The longest duration tested (i.e., the actual persistence duration could be longer).

† Data obtained from a literature review, the shortest duration without persistence was not reported.

11.5. *Y. pestis* Persistence Gaps

Relatively little data were identified on the persistence of *Y. pestis* in aerosols and fomites, and few studies were found focusing on the impact of various environmental parameters (e.g., humidity, sunlight, and temperature) on persistence. Most persistence data on *Y. pestis* were associated with survivability in water, including testing at different temperatures. Interestingly, *Y. pestis* in water ranged from days to years with trends between environmental condition and persistence not apparent. Gilbert and Rose (2012) noted that some of the variability associated with *Y. pestis* persistence in water might be attributed to differences in water chemistry, organism strain or growth phase, growth media for culturing and recovery, and inoculum levels.

12. Summary

The range of persistence durations (shortest and longest) identified by this review are summarized by agent and medium in Table 17. The table also includes the environmental condition associated with each value and identifies agent/medium combinations lacking persistence data. For agents with persistence data available on fomites, soil, or water, there are environmental conditions conducive to persistence for multiple days. In fact, several agent/medium combinations are associated with persistence of a month (or more) in duration including *Brucella* species on fomites, soil, and water; *B. mallei* on fomites and water; *B. pseudomallei* in soil and water; *F. tularensis* in water; viral encephalitis and hemorrhagic fever agents on fomites and water; and *Y. pestis* in soil and water. Although *C. burnetii* can develop a resistant spore-like form, very limited environmental persistence data was identified. Persistence data were less readily available, and much of the aerosol data summarized in Table 17 were obtained from Sinclair et al. (2008).

Factors that affect persistence are discussed in the following paragraphs by medium (and agent) as available. Generally, persistence may be affected by the various environmental media and factors (e.g., temperature, RH, and sunlight), subspecies and strains, preparation and application methods, and nutrient conditions. Persistence may be increased by the presence of organisms that serve as hosts or to which the biological agents have symbiotic relationships (e.g., possibly amoeba, earthworms), or persistence may decrease based on the present of competing and/or predatory organisms in the environment. Analytical methods may also affect comparison or interpretation of persistence results across studies (e.g., different culture media, incubation temperatures and times may be employed). Similarly, the estimates of persistence may differ depending on the technique used (molecular, culturing, counting, etc.) and the relative occurrence of VBNC bacteria associated with the various techniques.

<u>Aerosol</u>

Persistence data in aerosols were lacking for *B. anthracis*, *Brucella* species, *B. mallei*, *B. pseudomallei*, and *C. burnetii*. Persistence research was conducted with Ebola and Marburg viruses in aerosols, although only one environmental condition (50-55% RH and 22°C) was tested. Other viral encephalitis or hemorrhagic fever agents may behave differently than the viruses tested, especially under different

environmental conditions. As reported by Sinclair et al. (2008) some viruses (i.e., Japanese encephalitis virus and VEE virus) appeared to have an inverse relationship with RH. For *Y. pestis*, Sinclair et al. (2008) noted that in aerosols the bacterium persistence decreased rapidly as RH increased above 50%, although the decrease was much less rapid when heart infusion broth was used as the diluent rather than 1% peptone (Won and Ross, 1966). Based on work with aerosolized *F. tularensis*, Hood (2009) reported that unidentified constituents in outdoor air (possibly olefins) may reduce the persistence of non-spore forming microorganisms.

Fomite

Some persistence data on fomites were available for all agents except *C. burnetii*. The persistence statements identified for *B. mallei* on fomites were likely made based on observation in the field rather than controlled laboratory studies. Nevertheless, it appears that *B. mallei* persistence is greatest under warm humid or moist conditions (Dvorak and Spickler, 2008).

Table 17. Summary of Agent Persistence in the Environment

Agent	Medium	Conditions with Shortest Duration Reported			Conditions with Longest Duration Reported		
		Persistence Duration	Associated Environmental Condition	Reference	Persistence Duration	Associated Environmental Condition	Reference
*Bacillus anthracis**	Aerosol		--			--	
	Fomite	6 hours	25°C, 80% RH on stainless steel coated with silver and zinc zeolite paint	Galeano et al. (2003)	7 days†	37°C on polystyrene and glass as a biofilm in BHI broth	Lee et al. (2007)
	Soil		--			--	
	Water	3 days	Distilled water	Sinclair et al. (2008)	6 days	Water	Sinclair et al. (2008)
Brucella species (e.g., *suis, melitensis, abortus*, etc.)	Aerosol		--			--	
	Fomite	4 hours	22°C, 45% RH on painted joint tape	Ryan (2010)	56 days†	22°C, 40% RH on aluminum and glass; and 5°C, 30% RH on aluminum, glass, and wood	Calfee and Wendling (2012)
	Soil	<4 days	Dried soil	Nicoletti (1980)	43 days	Bison partition sites in Greater Yellowstone, identified in April	Aune et al. (2012)
	Water	<1 day	37°C	Nicoletti (1980)	77 days	Room temperature	Nicoletti (1980)
Burkholderia mallei	Aerosol		--			--	
	Fomite	3 weeks	Environmental survival (specific fomites not identified) in wet, humid, or dark conditions	Dvorak and Spickler (2008)	3 months	On stable bedding, troughs, and harness equipment	Malik et al. (2012)
	Soil		--			--	
	Water	1 day	Dechlorinated municipal water, 5°C and 25°C	Gilbert and Rose (2012)	28 days	Tap water, room temperature	Miller et al. (1948)

Agent	Medium	Conditions with Shortest Duration Reported			Conditions with Longest Duration Reported		
		Persistence Duration	Associated Environmental Condition	Reference	Persistence Duration	Associated Environmental Condition	Reference
Burkholderia pseudomallei	Aerosol		--			--	
	Fomite	6 hours	Applied in Butterfield buffer to glass, paper, polyethylene, and stainless steel; and applied in BHI broth to stainless steel	Shams et al. (2007)	3 days	Applied in BHI broth to paper, polyethylene, and stainless steel	Shams et al. (2007)
	Soil	<10 days	Soil inoculated with antagonistic bacteria (e.g., *B. multivorans*)	Lin et al. (2011)	30 months	Soil stored in plastic bags at ambient temperature (13°C to 33°C).	Thomas and Forbes-Faulkner (1981)
	Water	60 minutes	Water exposed to sunlight	Sagripanti et al. (2009)	16 years†	Distilled water, 25°C	Pumpuang et al. (2011)
Coxiella burnetii	Aerosol		--			--	
	Fomite		--			--	
	Soil	20 days†	20°C, 4°C, and -20°C	Evstigneeva et al. (2007)	20 days†	20°C, 4°C, and -20°C	Evstigneeva et al. (2007)
	Water		--			--	
Francisella tularensis	Aerosol	29 minutes (T_{99} value)	50% RH, wet dissemination	Sinclair et al. (2008); Cox (1971); Cox and Goldberg (1972)	5 days	90% RH, wet dissemination	Sinclair et al. (2008); Cox (1971); Cox and Goldberg (1972)
	Fomite	20 minutes	Desiccated on filter paper	Faith et al. (2012)	16 days†	37°C, 0% RH on stainless steel	Wilkinson (1966)
	Soil	--				--	
	Water	<1 day	Dechlorinated municipal water, 5°C and 25°C	Gilbert and Rose (2012)	34 days†	Brackish-water, 21°C	Berrada and Telford (2011)

Agent	Medium	Conditions with Shortest Duration Reported			Conditions with Longest Duration Reported		
		Persistence Duration	Associated Environmental Condition	Reference	Persistence Duration	Associated Environmental Condition	Reference
Viral encephalitis and hemorrhagic fever agents	Aerosol	1 hour†	22°C, 50% RH	Smither et al. (2011)	120 days (T_{99} value)	21°C, 23% RH	Sinclair et al. (2008)
	Fomite	5 minutes	Desiccated, 37°C	Fogarty et al. (2008)	50 days†	4°C, 55% RH in tissue culture medium on glass	Piercy et al. (2010)
	Soil		--			--	
	Water	<60 minutes	Tap water (1 mg L^{-1} free available chlorine or 2 mg L^{-1} total bromine), 21°C	Wade et al. (2010)	69 days (T_{99} value)	4°C	Sinclair et al. (2008)
Yersinia pestis	Aerosol	34 minutes (T_{99} value)	26°C, 87% RH	Sinclair et al. (2008)	57 minutes (T_{99} value)	26°C, 50% RH	Sinclair et al. (2008)
	Fomite	7 minutes (T_{99} value)	30°C, 52% RH on metal (stainless steel)	Sinclair et al. (2008); Wilkinson (1966)	7 days†	20°C, 50% RH on aluminum and painted joint tape	Ryan (2010)
	Soil	24 days†	Arizona soil during late October in an area with limited exposure to UV light	Eisen et al. (2008)	10 months†	4°C to 8°C	Sinclair et al. (2008)
	Water	1 day	Dechlorinated municipal water, 5°C	Gilbert and Rose (2012	3 years†	Autoclaved river water	Pawlowski et al. (2011b)

-- Not tested/not reported.

* This review focused on vegetative *B. anthracis* only.

† The longest duration tested (i.e., the actual persistence duration could be longer).

One of the unique aspects of persistence testing with fomites is the influence of the material type, which affects persistence and recovery of agent from the material. Most of the testing with fomites was conducted with relatively smooth non-porous surfaces (e.g., aluminum, glass, keyboard keys, plastic, polyvinyl chloride, stainless steel). Limited testing was conducted on concrete and wood (with *B. suis*) and on carpet, painted joint tape, and filter paper (with *F. tularensis*). Differences between fomite materials and persistence were noted for *Y. pestis*. For example, *Y. pestis* persisted longer on paper than stainless steel, polyethylene, or glass (Sinclair et al., 2008). *Y. pestis* persistence was shortest on carpet, when compared with aluminum, painted joint tape, and keyboard keys (Ryan, 2010).

Shams et al. (2007) found that the persistence durations for *B. pseudomallei* on fomites differed depending upon the type of suspension used for application. Longer persistence was observed when *B. pseudomallei* was applied in BHI broth rather than Butterfield buffer. Comparisons between the *F. tularensis* fomite persistence work by Wilkinson (1966) and Faith et al. (2012) also indicated that results may be affected by inoculum levels and inoculation approaches.

The persistence work with *B. anthracis* on fomites was conducted at 80% RH for ≤24 hours to avoid desiccation or within nutrient broth to access the bacteria's ability to form biofilms. The ability of vegetative *B. anthracis* to persist of surfaces after drying is unknown. The influence of cold temperatures is also unknown for *B. anthracis* on fomites as all tests were conducted at ≥25°C. The persistence work with *B. suis* on fomites (e.g., Ryan, 2010; US EPA, 2010b) was one of the few agents that was investigated on multiple materials, at a warm (22°C) and cool (5°C) temperatures, and with and without simulated sunlight. Some materials (e.g., painted joint tape and concrete) appeared to adversely affect survival, as did the warmer temperatures and simulated sunlight. For *F. tularensis*, Wilkinson (1966) generally found better survival at 25°C than 37°C, and better survival under low RH (0-10%) than elevated RH (55-100%). Ebola and Marburg viruses survived better on glass and plastic at 4°C than at room temperature (Piercy et al., 2010). Similarly, henipaviruses persisted on mango flesh and polystyrene longer at 22°C than 37°C (Fogarty et al., 2008).

<u>Soil</u>

Persistence data in soil were identified for *Brucella* species, *B. pseudomallei*, *C. burnetii*, and *Y. pestis*. For *Brucella* species in soil, environmental conditions such as sunlight, elevated temperatures, and dry soil adversely affected persistence (Nicoletti, 1980; Jones et al., 2010). *B. pseudomallei* is associated with decaying organic matter and seems to survive well in moist subsurface soil at warm temperatures (e.g., 24°C to 42°C), although dry conditions and colder temperatures can be tolerated (Tong et al., 1996; Larsen et al., 2013; Chen et al., 2003). *C. burnetii* was found to survive 20 days (the longest duration tested) in soil held at 20°C, 4°C, and -20°C (Evstigneeva et al., 2007).

The importance of the soil environment as a reservoir or ecological niche for several agents is being investigated for several agents including *B. anthracis*, *B. pseudomallei*, *C. burnetii*, and *Y. pestis*. The rhizosphere of grasses may be important for the environmental survival of *B. anthracis* vegetative cells and spores (Saile and Koehler, 2006) and *B. pseudomallei* (Kaestli et al., 2012). Soil amoeba might provide an intracellular niche for *C. burnetii* (La Scola and Raoult, 2001) and *Y. pestis* (Eisen and Gage, 2009). Bacteriophages and worms may also have an important role in the environmental survival of *B.*

anthracis vegetative cells within soil (Schuch and Fischetti, 2009). The potential association with *B. anthracis* and earthworms is interesting as both prefer alkaline soils with high calcium and organic matter content (Hugh-Jones and Blackburn, 2009; Schuch et al., 2010). Some soil bacteria might inhibit the growth of other agents. For example, *B. multivorans* has been shown to inhibit *B. pseudomallei* growth (Lin et al., 2011). The interactions of a released agent with other microorganism might enhance or inhibit the agent's survival and/or establishment of a viable population in the environment. Such dynamics require additional research.

Water

Persistence in water may be affected by sunlight, temperature, nutrients, and salinity. Sunlight has been demonstrated to inhibit the persistence of *B. pseudomallei* in water (Sagripanti et al., 2009). Gilbert and Rose (2012) found that *B. pseudomallei* and *Y. pestis* were culturable longer at 25°C than at 5°C. Other researchers have found that *B. pseudomallei* can survive a wide range of environmental conditions (including temperature, pH, and salinity) in water (Robertson et al., 2010). Interestingly many of the studies with *B. pseudomallei* were conducted with sterilized water samples. It is uncertain how *B. pseudomallei* would survive in natural water and the unknown interactions with other environmental microorganisms. Based on the data presented by Nicoletti (1980), *Brucella* species persistence appears to be shortened by elevated temperatures. For *F. tularensis*, Berrada and Telford (2011) indicated that the salt and sulfur content of brackish water may enhance the bacterium's persistence.

The phenomena of bacteria entering a VBNC state was noted for several agents including *Brucella* species, *B. mallei* , *B. pseudomallei*, *F. tularensis* , and *Y. pestis* (Gilbert and Rose, 2012). The importance of VBNC agents is an area in need of additional research. In addition, some agents appear to survive and benefit from being ingested by amoeba, including *B. anthracis* (Dey et al., 2012), *B. pseudomallei* (Sagripanti et al., 2009), *F. tularensis* (El-Etr et al., 2009). Some bacteria including *F. tularensis* (Mahajan et al., 2011)also have the ability to form biofilms that may be important for survival in water.

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