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MINIREVIEWS

Persistence of Category A Select Agents in the Environment[▽]

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The intentional use of biological agents as weapons could result in deaths in numbers comparable to those expected from the use of nuclear weapons. It is one of the most significant terrorism threats (16, 82) and has the potential to catalyze a general breakdown of society through a loss of human lives, food, livestock, agriculture, and economy. This form of warfare has been a threat for centuries and predates scientific understanding of microorganisms or disease (44). The biological agents used for warfare are easily produced and dispersed, have a delayed onset, cause high rates of morbidity and mortality, and present unique challenges in diagnosis, detection, and treatment (16). Biological agents are diverse and can be deployed to contaminate various environmental media including air, water, food, soil, and fomites.

The well-known global history of military biowarfare and terrorism (16, 42–44, 85) has prompted many governments to prioritize response plans for the event of a biological agent release in a bioterrorism attack (5, 74). An important component to any response plan is an understanding of the survival rate or viability of the biological agent in the surrounding environment (82). Bioterrorism events such as the "anthrax letter" attacks of 2001 (25) have highlighted that the survival and persistence of a biological agent have a significant impact on the microbial hazard and its subsequent effects. Microbial decay and injury 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. The Centers for Disease Control (CDC) prioritizes potential biological terrorism agents as category A if they require intensive public preparedness efforts due to the potential for mass causalities, public fear, and civil disruption (76). These category A select agents are variola major virus (smallpox), *Bacillus anthracis* (anthrax), *Yersinia pestis* (plague), *Clostridium botulinum* toxin (botulism), *Francisella tularensis* (tularemia), and the viral hemorrhagic fever agents *Arenaviridae* (Lassa fever, Junin-Argentine hemorrhagic fever, and Venezuelan hemorrhagic fever), *Bunyaviridae* (hantavirus), *Filoviridae* (Ebola hemorrhagic fever and Marburg hemorrhagic fever), and *Flaviviridae* (St. Louis encephalitis and Japanese B encephalitis).

In an intentional release, exposure may occur by routes in which the bioagent is not transmitted in nature. The potential for transmission is a function of transport and persistence in the environment, with the transport probability based upon both predicted entry ports and other portals not usually considered significant or lacking in nature. Information on the environmental persistence of these agents is limited but essential for estimating where the greatest environmental exposure may occur through a risk assessment framework.

The purpose of this review is to assess the current information on the persistence of select agents on the CDC category A agent list in the environment and its implications in a terrorism response.

ROUTES OF EXPOSURE

The release of vertebrate pathogens may occur intentionally, by accident, or by natural release in the bodily fluids of those infected. All category A agents can be expected to be released in the bodily fluids of infected persons or animals (Table 1). The concentration of these agents in these materials can be significant (Table 2). Most of the agents in nature are primarily transmitted by insect vectors or through animal contact or material contaminated by infected animals. The demonstrated or suspected natural routes of transmission are shown in Table 3. For the bacteria in particular, multiple routes of transmission are possible, although some may play a minor role in nature. The agents' persistence characteristics in aerosols, fomites, and water are detailed in Tables 4, 5, and 6, respectively.

PERSISTENCE IN THE ENVIRONMENT

The maintenance of infectivity by any pathogen outside the host is dependent on a number of factors including temperature, relative humidity, desiccation, and UV light. Temperature has the greatest effect, since the rates of most chemical and physical processes are dependent upon it. Temperature is also the most useful parameter for modeling microbial decay rates for microbes that cannot replicate in the environment. Relative humidity (and desiccation) is also a significant factor for survival in air and on fomites (9).

For comparative purposes and to assess long-term exposure

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TABLE 1. Occurrence of category A select agents in bodily fluids and sewage[a]

Agent	Occurrence in:				Comment	Reference
	Urine	Feces	Saliva	Sewage		
Bacillus anthracis	?	Yes	?	Yes	?	70
Brucella abortus	Yes	?	Yes	?		28
Yersinia pestis	?	?	?	Yes		51
Variola major virus (smallpox)	Yes	?	Yes	?	Dermal scrapes	77
Francisella tularensis	Antigen	Yes	?	?	Animals	86
Lassa fever virus	Yes	?	?	?		
Hantavirus	Yes	Yes	Yes	?	Urine, human feces, animal	54
Dengue virus	Yes	?	?	?	Animals	57
Marburg virus	Yes	Yes	?	?	Animals	18

[a] ?, no data or unknown.

risks, we calculated inactivation coefficients (K_i) with the following calculation for the titers per unit volume (ml, g, or cubic meter of air): [$\log_{10}$ reduction (initial titer − final titer/volume or weight]/total hours of viability (9). Inactivation coefficients were assumed to be linear functions and were not used to calculate T_{90} and T_{99} values, which are the times required for the initial titer to decrease by 90% (T_{90}) and 99% (T_{99}); these values were calculated using the survival curve, which is typically not linear. Therefore, T_{90} and T_{99} values usually underestimate viral survival compared to inactivation coefficients (K_i values). We estimated these from published data tables and/or figures if available.

Aerosols. Research on category A pathogen survival in aerosols is limited, but it is known that most biological agents, with some exceptions, face decay once exposed to air due mainly to freezing, dehydration, and UV exposure during the daylight as well as to many other contributing environmental factors (45, 50). In general, vegetative forms are much more susceptible to open-air conditions than bacterial spores (Tables 4 and 7) (36, 73, 80).

B. anthracis in its vegetative form usually will not survive for long outside of a host and will form spores when exposed to oxygen (26, 88). Early studies on anthrax spores demonstrated very long survival times (Table 7). One limited study examined the survival of anthrax spores in outdoor air and showed that *B. anthracis* spores retained complete viability when exposed for several hours during the nighttime (61). This finding is expected, as spores are highly resistant structures and are at least 10-fold more resistant at night than during the day (82). Additional studies examined air for *B. anthracis* spores and reported viable DNA over several U.S. cites and in the upper atmosphere (15, 33, 60). Other studies of survivability in water and on fomites suggest that the aerosol survival of *B. anthracis* spores could be long (24). Many other modeling studies, animal dose-response models, epidemiological studies, and studies with surrogate organisms (11, 12, 30, 72, 90) that reveal qualitative information related to *B. anthracis* survival in air are available, but experimental evidence is lacking or is classified.

Y. pestis exhibited a constant decay rate when it was aerosolized at an rH of 50% in heart infusion broth (94). An abrupt loss of viable organisms occurs when rH rises above 50%, especially to 85%. *F. tularensis* responded similarly by having the lowest survival at a high rH. Additional studies found that the effect of temperature on survival is linear when the rH is above 85%.

Many of the studies on viral survival exhibited an initial log die-off within the first few minutes of aerosolization. Vaccinia virus behaved in this manner, but ultimate survival was determined by rH and temperature. The T_{99} was about 9 h even under the most challenging rH and temperature (40, 41). Because the Lassa fever virus can be present in pharyngeal secretions and urine for up to 3 to 4 weeks after a patient's clinical signs have subsided (65), there is a long window in which the infective virions can become airborne. An aerosol study found that 75% of the virus was infective after 4 min at 24°C (81). The Ebola and Marburg filoviruses are not typically transmitted by aerosols, but laboratory studies with monkeys demonstrate that the disease could potentially be transmitted through respirable particles (46, 47, 55). Epidemiological investigations have found that most human cases occurred due to direct contact with blood, secretions, or tissues of infected patients or nonhuman primates (6, 10, 53). The survival of Japanese B encephalitis virus was shown to be inversely related

TABLE 2. Concentration and duration of agent release

Agent	Fluid	Concn (ml or g)	Duration	Reference
Variola major virus	Urine	10^2–10^5	19 days	77
Hantavirus	Saliva	10^2	14 days	54
Marburg virus	Feces, urine, and salvia[a]	$10^{2.3}$–$10^{3.3}$	Throughout the illness	18
Flaviviruses	Urine[a]	6–10^3	10 days	57

[a] Fluid in animals.

TABLE 3. Vehicles for the transmission of category A agents in nature

Agent	Transmission in[a]:			
	Air	Water/liquids	Soil	Fomites
Bacillus anthracis	X		X	X
Yellow fever virus (*Flaviviridae*)				
Francisella tularensis	X	X	X	X
Yersinia pestis	X			
Hantavirus (*Bunyaviridae*)	X		X	X
Smallpox virus	X			X
Ebola and Marburg viruses (*Filoviridae*)				X
Lassa virus (*Arenaviridae*)				X

[a] "X" indicates transmission.

TABLE 4. Survival of category A biological agents as aerosols

Disease and agent (exptl conditions, suspending medium)	Initial titer	Temp (°C)	rH (%)	T_{90} (h)	T_{99} (h)	K_i	Reference
Anthrax							
Bacillus anthracis (multiple hours in night air)						Complete recovery	62
Bacillus anthracis						4.64×10^{-7} HPAC[e]	82
Tularemia							
Francisella tularensis SCHU S5 (PBS[b])	1.5×10^{11} CFU/ml	−40	Ambient	1.02	2.05	0.97	27
		−29	Ambient	3.97	7.93	0.25	
		−7	Ambient	2.68	5.35	0.37	
		24	85	1.80	3.60	0.55	
		29	85	1.10	2.21	0.90	
		35	85	0.48	0.96	2.08	
Francisella tularensis LVS (culture medium with a wet dissemination)	3.0×10^{10} CFU/ml	90		65.6[a]	131[a]	0.03	20, 21
		80		1.91[a]	3.82[a]	1.20	
		70		1.10[a]	2.20[a]	2.09	
		60		0.28[a]	0.57[a]	8.00	
		50		0.24	0.48[a]	9.59	
		40		0.24	0.49[a]	9.39	
		30		0.25	0.50[a]	9.20	
		20		0.58[a]	1.16[a]	3.97	
		0		0.58[a]	1.17[a]	3.95	
Francisella tularensis LVS (freeze-dried in peptone broth with a dry dissemination)	3.0×10^{10} CFU/ml	90		0.37[a]	0.74[a]	6.15	20, 21
		80		0.25	0.50[a]	9.20	
		70		0.29[a]	0.58[a]	7.83	
		60		0.34[a]	0.68[a]	6.71	
		50		0.37[a]	0.75[a]	6.09	
		40		0.55[a]	1.10[a]	4.18	
		30		0.57[a]	1.15[a]	4.00	
		20		1.76[a]	3.51[a]	1.31	
		0		1.91[a]	3.82[a]	1.20	
Plague							
Yersinia pestis A-1122 (HIB[c] and/or 1% peptone)	2.0×10^{9} cells/ml	26	87	0.29	0.57	3.49	94
		26	50	0.48	0.95	2.10	
		26	20	0.38	0.75	2.66	
Smallpox							
Vaccinia virus (HIB)	Not listed	10.5–11.5	20	275[a]	551[a]	0.00	40
		10.5–11.6	50	123[a]	246[a]	0.00	
		10.5–11.7	80	47.2[a]	94.4[a]	0.02	
		21.0–23.0	20	30.7[a]	61.4[a]	0.03	
		21.0–23.1	50	26.5[a]	53.0[a]	0.03	
		21.0–23.2	80	7.70	15.4	0.13	
		31.0–33.5	20	30.9[a]	61.9[a]	0.03	
		31.0–33.6	50	8.63	17.3	0.11	
Vaccinia virus (Mcllvaine buffer with 1% horse serum)	Not listed	22	20	55.4[a]	111[a]	0.01	41
		10	50	17.1	34.2[a]	0.05	
		10	80	10.8	21.6	0.09	
HF[d]							
Arenaviridae Venezuelan equine encephalomyelitis virus (HIB)	1.0×10^{10} mouse IP LD_{50}/ml	9.0–9.5	19	146	291	0.00	40
		9.0–9.5	48	36.8	73.7	0.02	
		9.0–9.5	86	19.5	39.0	0.05	
		21–23	19–23	21.9	43.8	0.04	
		21–23	50	9.53	19.1	0.10	
		21–23	81–86	8.06	16.1	0.12	
		20.5–23.5	19	11.0	21.9	0.09	
		20.5–23.5	48	4.38	8.76	0.22	
		20.5–23.5	81–85	1.16	2.32	0.86	
Arenaviridae Lassa virus Josiah strain (Eagle's essential medium with Earle's salts and fetal bovine serum)	6.3×10^{6} PFU/ml	24	30	0.89	1.79[a]	1.12	81
		24	55	0.92	1.85[a]	1.08	
		24	80	0.70	1.42[a]	1.41	
		32	30	1.47[a]	2.94[a]	0.68	
		32	55	1.67[a]	3.33[a]	0.60	
		32	80	1.79[a]	3.58[a]	0.55	
		38	30	1.68[a]	3.36[a]	0.59	
Flaviviridae Japanese encephalitis virus Peking strain (HIB)		24	30	5.59[a]	11.2[a]	0.17	52
		24	55	4.08[a]	8.15[a]	0.24	
		24	80	2.99[a]	5.97[a]	0.33	
Flaviviridae St. Louis encephalitis Parton strain (bovine albumin, borate, saline)	3.1×10^{8} $TCID_{50}$/ml		80	7.29	14.6	0.13	71
			80	6.14	12.3	0.16	
			60	11.5	23.0	0.08	
			61	4.79	9.58	0.20	
			46	12.7	25.4[a]	0.07	
			46	10.7	21.5	0.09	
			35	25.6[a]	51.1[a]	0.03	
			23	1,438	2,875	0.001	
Filoviridae Marburg virus (found not to be stable in air)						3	7

[a] Estimated T_{90} and T_{99} are beyond the time measured in the study.
[b] PBS, phosphate buffer solution.
[c] HIB, heart infusion broth.
[d] HF, hemorrhagic fever.
[e] HPAC, the Defense Threat Reduction Agency's hazard prediction and assessment capability.

TABLE 5. Survival of category A biological agents on fomites

Disease and agent (exptl conditions, suspending medium [titer quantification])[a]	Initial titer	Fomite	Temp (°C)	rH (%)	T_{90}[b]	T_{99}	K_i	Reference
Tularemia								
Francisella tularensis (LVS in HIB [CFU/surface])	1.7×10^7	Metal	25	100	7.70	15.4	0.13	91
	1.0×10^7			65	15.1	30.2	0.07	
	7.0×10^6			10	87.6	175	0.01	
	3.5×10^6		37	100	2.22	4.43	0.46	
	4.0×10^6			80	2.60	5.21	0.38	
	2.3×10^6			65	2.68	5.37	0.37	
	3.1×10^6			55	3.98	7.96	0.25	
Plague								
Yersinia pestis A1122 (HIB with 1% peptone [CFU/surface])	1.2×10^6	Metal	11	30	22.4	44.7	0.04	91
	3.0×10^6		100	30	4.82	9.63	0.20	
	3.0×10^6		52	30	0.06	0.12	16.9	
	2.1×10^6		52	22	1.44	2.88	0.69	
Yersinia pestis A1122 (PB [CFU/surface])	1.5×10^6	Stainless steel	18–22	55	1.01	2.02	0.98	75
		Polyethylene			4.58	9.16	0.21	
		Glass			0.89	1.77	1.13	
		Paper			13.0	26.1	0.07	
Yersinia pestis Harbin (PB and HIB [CFU/surface])	2.8×10^6 in PB	Stainless steel	18–22	55	0.81	1.62	1.24	75
		Polyethylene			1.10	2.20	0.91	
		Glass			1.17	2.35	0.85	
		Paper			3.87	7.75	0.25	
	6.1×10^6 in HIB	Stainless steel	18–22	55	16.8	33.6	0.06	
		Polyethylene			15.0	30.1	0.06	
		Glass			13.6	27.2	0.07	
		Paper			23.6	47.2	0.04	
Smallpox								
Variola minor (from scabs of infected individuals)		Scabs in envelopes stored for up to 13 yr	15–30	35–98	1,491 days	2,983 days	2.79×10^{-5}	93
Vaccinia virus (minimal essential medium plus cell culture medium)	1×10^7 $CCID_{50}$[c]/slide	Glass slides	25	96	155	311	6.43×10^{-3}	58
			25	55	101	201	9.95×10^{-3}	
			25	7	156	312	6.41×10^{-3}	
			37	93	183	367	5.45×10^{-3}	
			37	55	145	291	6.87×10^{-3}	
			37	3	160	320	6.25×10^{-3}	
HF								
Bunyaviridae hantavirus 76-118 (minimal essential medium [PFU/ml])	3.5×10^6	Aluminum discs	20	?	1.45	2.91	0.68	39
Sicilian virus Sabin (minimal essential medium [PFU/ml])	8.2×10^6	Aluminum discs	20	?	1.41	2.82[d]	0.70	39
Crimean-Congo virus (minimal essential medium [PFU/ml])	3.5×10^6	Aluminum discs	20	?	1.08	2.16[d]	0.92	39
Filovirus Marburg		Dried blood					Stable 4–5 days	7

[a] PB, phosphate buffer solution; HIB, heart infusion broth; HF, hemorrhagic fever.
[b] Unless otherwise indicated, T values are in hours.
[c] $CCID_{50}$, 50% cell culture infective dose.
[d] The estimated T_{99} is beyond the time measured in the study.

to rH (52), with longer survival times at lower levels. A study on the St. Louis encephalitis virus, another major flavivirus, found different results; no loss of titer was associated with differences in rH (71).

Limited studies have verified the stability of hantavirus in air, while epidemiological studies have characterized disease transmission to humans as through wounds or inhalation of aerosolized rodent excreta (78).

Fomites. Studies detailing the survival of category A agents on fomites describe surface characteristics, rH, and temperature as major contributors to viability (Table 5) (9). Stainless steel, polyethylene, glass, and paper were assessed for *Y. pestis* survivability. It was found that these pneumonic plague bacteria remained viable much longer on paper, potentially due to surface roughness and hydrophobicity (75). The smallpox virus was less sensitive to differences in surface and environment; it remained viable for up to 2 weeks on fomites with various rH values, temperatures, and surface textures (58). Three *Bunyaviridae* hemorrhagic fever viruses, Hantaan virus, Sicilian virus, and Crimean-Congo virus, showed various survival times on aluminum discs, with the Sicilian fever virus exhibiting a T_{99} of up to 2.2 h (39).

Knowledge of survival of *B. anthracis* on fomites is fairly limited. Several studies have revived spores dried on filter paper after 35 and 41 years (67). Another study recovered anthrax spores from canvas after 22 years (37).

Water. Most of the research on pathogen survival in water has been on waterborne pathogens, i.e., those transmitted by

TABLE 6. Survival of category A biological agents in water

Disease and agent (exptl conditions, suspending medium)	Initial titer	Temp (°C)	T_{90}	T_{99}	K_i	Reference
Anthrax						
Bacillus anthracis (freeze-dried in glass bottles, heated in glycol baths, and then suspended in distilled H_2O)	Not listed	100	3.69	7.38[a]	0.27	23, 24
		90	13.2	26.4	0.07	
		80	62.5	125[a]	0.01	
		3		146 yr[a]		
Bacillus anthracis Pasteur (pH 7 buffer)	Not listed	70	1.94	3.88	0.52	66
		80	0.14	0.28	7.06	
		90	0.01	0.03	69.8	
Bacillus anthracis Pasteur (milk)		70	3.42	6.84	0.29	
		80	0.26	0.52	3.82	
		90	0.02	0.03	60.0	
Bacillus anthracis Pasteur (pH 4.5 buffer)		70	0.153	0.307	6.52	
		80	0.032	0.063	31.6	
		90	0.014	0.028	70.6	
Bacillus anthracis Pasteur (orange juice)		70	0.155	0.310	6.45	
		80	0.050	0.100	20.0	
		90	0.011	0.023	88.2	
Bacillus anthracis Vollum (pH 7 buffer)	Not listed	70	3.78	7.56	0.26	66
		80	0.498	0.997	2.01	
		90	0.082	0.163	12.2	
Bacillus anthracis Vollum (milk)		70	3.30	6.60	0.30	
		80	0.405	0.810	2.47	
		90	0.112	0.223	8.96	
Bacillus anthracis Vollum (pH 4.5 buffer)		70	1.66	3.32	0.60	
		80	0.133	0.267	7.50	
		90	0.027	0.053	37.5	
Bacillus anthracis Vollum (orange juice)		70	1.36	2.72	0.73	
		80	0.127	0.253	7.89	
		90	0.033	0.067	30.0	
Tularemia						
Francisella tularensis (tap water)	1×10^6/ml	8	28.7 days[a]	33.7 days[a]	3.9×10^{-3}	32
Francisella tularensis (cell culture medium with wet dissemination)	3.7×10^{10}	37	58.0	116	1.73×10^{-2}	24
		26	84.8	170	1.18×10^{-2}	
		15	972	1,944	1.03×10^{-3}	
		3	3,317	6,633	3.02×10^{-4}	
		0	2,086	4,171	4.79×10^{-4}	
Francisella tularensis (freeze-dried in peptone broth with dry dissemination)	Not listed	37	162	325	6.16×10^{-3}	24
		27	642	1,284	1.56×10^{-3}	
		15	3,924	7,848	2.55×10^{-4}	
		3	26,154	52,309	3.82×10^{-5}	
		−18	56,552	113,104	1.77×10^{-5}	
Smallpox						
Vaccinia virus (storm water)	Not listed	4.5	29.8 days[a]	59.5 days[a]	1.40×10^{-3}	29
		19–23	72[a]	144[a]	1.39×10^{-2}	
Vaccinia virus (storm water with fetal calf serum)		4.5	29.8 days[a]	59.5 days[a]	1.40×10^{-3}	29
		19–23	5 days	13.9 days[a]	3.0×10^{-3}	
Vaccinia virus (storm water and soil)		4.5	5 days	10 days[a]	8.0×10^{-3}	29
		19–23	24	29	3.47×10^{-2}	
Vaccinia virus (tap water)	10^4–10^5/ml	9	110 to 150 days	170 to >200 days	1×10^{-3}–2.8×10^{-3}	60
		15	No data	200 days	4.0×10^{-4}	
Vaccinia virus (river water/ seawater)	10^4–10^5/ml	9	110–120 days	>200 days	1×10^{-3}–3.4×10^{-3}	60
		15	110–160 days	190 to > 200 days	1×10^{-3}–2.6×10^{-3}	
Vaccinia virus (seawater)	4×10^5	11.5	281	562	3.56×10^{-3}	8
Vaccinia virus (PBS[b])	1×10^6	11.5	320	640	3.13×10^{-3}	8
Hemorrhagic fever						
Flaviviridae yellow fever virus vaccine strain (0.9% saline)	1.8×10^6	37	39	96.0	1.40×10^{-2}	2
Adenovirus (seawater)	1.2×10^4	6	355	710	2.82×10^{-3}	8
Adenovirus (PBS[b])	1×10^4	11.5	82.6	165	1.21×10^{-2}	8
Bunyaviridae hantavirus (cell-free medium)	3.5×10^6/ml	4	30 days	50 days	1.70×10^{-3}	39
		20	54	96	2.50×10^{-2}	
		37	24	48	3.47×10^{-2}	
Bunyaviridae hantavirus	6.9×10^5/ml	23	6.5 days[a]	13 days[a]	6.40×10^{-3}	49
		4	34.7 days[a]	69.4 days[a]	1.20×10^{-3}	
		23	15[a]	30[a]	6.50×10^{-2}	
		4	15.7 days[a]	31.4 days[a]	2.60×10^{-3}	
Bunyaviridae Sicilian virus (cell-free medium)	8.2×10^4/ml	4	150 days	325 days	1.00×10^{-4}	39
		20	16 days	32 days	2.60×10^{-3}	
		37	6 days	10.7 days	1.19×10^{-2}	

[a] Estimated T_{90} and T_{99} are beyond the time measured in the study and/or extrapolated from a survival curve at high temperature. Unless otherwise indicated, T values are in hours.

[b] PBS, phosphate buffer solution.

TABLE 7. Nonkinetic studies on survival of *Bacillus anthracis* (virulent strains) in the environment

Medium	Exptl condition	Survival	Reference
Aerosol	Silk string	4 yr	17
	Silk string	8–10 yr	17
	Silk string	12 yr	17
	Silk string	17 yr	17
	Silk string	71 yr	64
	Open air	No death overnight	61
Water	Pond water	2 yr	64
	Milk	10 yr	64
	Sewage	16 mo	79
	Distilled water	20 mo	79
	Seawater	20 mo	79
	Pond water	18 yr	63
Fomite	Paper filter	35 yr	67
	Paper filter	41 yr	67
	Dry spores from desiccated culture	17 yr	17
	Dry spores from desiccated culture	18 yr	84
	Dry spores, canvas	22 yr for no growth	37
	Dry spores, canvas	3 mo 50% decrease	37
Soil	Moist soil	33 mo	79
	Dry soil	33 mo	79
	Soil, cadaver	15 yr	37
	Gravel, cadaver	20 yr	37
	Soil, cadaver	12 yr	37
	Sealed soil	60–68 yr	56
	Gruinard Island soil	40 yr	59
	Sealed soil	60 yr	92

the fecal oral route (Tables 6 and 7). Humans and animals are the natural hosts of these pathogens, which normally cannot replicate in the environment. Of all the category A select agents, only *B. anthracis* and *F. tularensis* are capable of replication in the environment. However, all of the agents may be excreted in the feces and urine; thus, they are likely to end up in sewage systems or in water during recreational (34) activities. This appears to be the least studied area on environmental survival of category A select agents.

Waterborne outbreaks of *F. tularensis* have been documented (38, 68, 87). *F. tularensis* type A has been isolated from natural waters and mud contaminated by muskrats and beavers (69), and the organisms may be capable of multiplication in these environments (35, 69). *F. tularensis* type B has also been isolated from surface waters, including drinking water supplies (22, 83). An additional study found that the vaccine strain could persist for at least 40 days at 8°C in tap water (32). However, the organisms entered a viable but nonculturable (VBNC) stage, and 65% of the original inoculum remained viable after 140 days. The VBNC organisms were not capable of causing tularemia in mice. The organism is also known to replicate intracellularly in protozoa similarly to the water-based pathogen *Legionella*, and this could act to serve as a reservoir in aquatic environments or at least prolong its persistence (1).

Y. pestis is also capable of transmission by inhalation of aerosol (48). It has been reported to enter the VBNC state when added to deionized water at 28 and 37°C (4) and may be capable of persisting in cysts of amoebas (3). It was reported to survive 16 days in tap water and well water (64) and has been detected in sewage (31).

Early studies show that vegetative *B. anthracis* dies after only 72 h in distilled water (17) or has a maximum survival of 6 days in water (64). There is a controversy as to the life cycle of *B. anthracis* in water (26, 63, 88, 89). Contrarily, *B. anthracis* spores can survive for a much longer time in water. Some suggest that because large herbivores can become infected through ingestion of spores at drying water holes in Africa, it is possible that humans could also contract the illness via water (19). Unfortunately, most information describing the longevity of spores in water is from the early 1900s and lacks detailed descriptions of procedures or lacks data on initial and final concentrations. Recent data extrapolation calculates that *B. anthracis* spores will survive for 620 years at room temperature; however, this assumes a linear relation to survival at decreasing temperatures (23, 24). There are also data on spore survival in water for species taxonomically close to *B. anthracis*, and this information can be used to extrapolate the behavior of *B. anthracis* (64, 80); however, actual experimental evidence using *B. anthracis* is very limited. Table 7 shows some of the limited data available on *B. anthracis* survival.

Hantavirus is excreted in the urine and saliva of rodents and may contaminate water, although waterborne transmission is unproven. A recent study investigated the survival of hantavirus and related arthropod-borne members of the *Bunyaviridae* family in cell culture media at various temperatures (39). A 99.9% decrease in virus titer at 20°C required about 20 days. The most stable of the viruses studied was the Sicilian virus, carried by the sand fly; this virus showed little inactivation after 10 days at 20°C.

Data on the stability of smallpox virus come largely from studies of scabs, vesicles, and bodily fluids (29, 93). Most of the useful studies have been conducted with vaccinia virus, a genetically related virus in the smallpox vaccine. The genus *Orthopoxvirus* is a very stable group of viruses. Little is known about the stability of the arenaviruses and flaviviruses in water or other liquids because they are not known to be naturally transmitted by this route. The flavivirus yellow fever virus, when reconstituted from a vaccine, was able to survive for several days at 37°C (2).

Soil. *Bacillus* species spores have been found and revived from sediments perhaps as old as 1,000 years and some have claimed from Paleozoic salt beds (80). Viable spores of *B. anthracis* have been found after 40 years on Gruinard Island (59), and Lewis reported on recovered viable spores from sealed soil samples stored for 60 and 68 years (56). As soil is part of the organism's ecological life cycle, it is expected that spores persist in particular types of soil for many years, where they may germinate and multiply (26, 88, 89). Table 7 shows that some spores can remain viable in soil for many decades.

Soil moisture and organic matter content are important to the survival of *Y. pestis*. In soil, *Y. pestis* may multiply under favorable soil conditions and will survive for more than 10 months in soil at 4 to 8°C and for 3.5 months at room temperature (13, 14, 64).

DISCUSSION

Exposure is critical to estimating the risk posed by pathogens capable of environmental transmission and creates the greatest amount of uncertainty in estimating the risk of infection to exposed populations (34). Models can be developed to predict exposure after the release of a pathogen in the environment, but die-off or decay rates are critical in this estimation. To have the greatest utility, die-off rates (K_i) need to be as quantitative as possible. This review reveals that information appears to be very limited for select agents in category A. Because no investigators have appeared to use similar methods, data comparison between groups of organisms is of limited value. The development of standardized testing methods and conditions for assessing survival would be of the most value. Consideration must also be given to the usual multiphasic die-off rates, or otherwise they may be underestimated. For example, die-off of organisms is usually most rapid during aerosolization or drying on fomites.

With the limited database, it is difficult to make generalizations; however, it appears overall that the greatest stability of the select agents was seen for liquid environments (Table 6) and the least for aerosols. Desiccation during aerosolization and drying on fomites were major factors contributing to the steep initial die-off trends for fomite and aerosol environments. The viruses were generally more stable in aerosols than were the other agents, except for the spores of *B. anthracis*. *F. tularensis* was the most stable non-spore-forming organism in water, reflecting its potential to grow in this environment. Vaccinia virus and *B. anthracis* were the most environmentally stable agents overall.

The available data would suggest that the greater long-term exposure to agents once released into the environment would be from water or fomites. In these media, agents will persist longer than in aerosols and present the greater hazard. Also, aerosols are usually only a transitory medium for these agents in nature, as the organisms quickly settle out. The potential for resuspension into the aerosol state is largely governed by the potential for survival on a fomite or in a liquid that may be aerosolized. Overall, further survival studies need to be conducted on all category A select agents within the context of human exposure to liquid, soil, fomites, and aerosols.

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