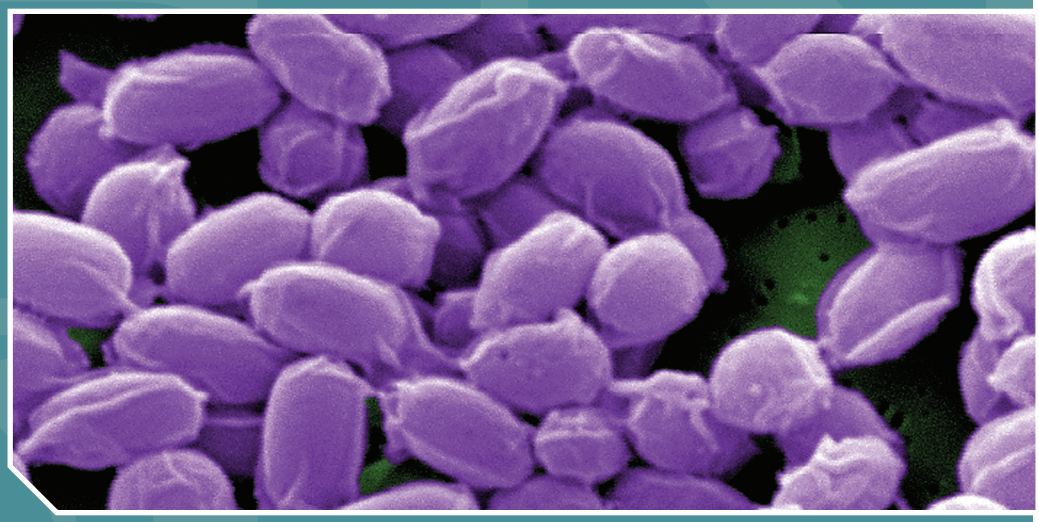
EPA 600/R-15/066 | March 2015 | www.epa.gov/research

United States
Environmental Protection
Agency

Distinguishing Intentional Releases from Natural Occurrences and Unintentional Releases of *Bacillus anthracis:*

LITERATURE SEARCH AND ANALYSIS



Office of Research and Development
National Homeland Security Research Center

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The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development, funded and managed this literature review in collaboration with the Defense Threat Reduction Agency and the Department of Homeland Security under the Battelle/Chemical, Biological, Radiological, and Nuclear Defense Information and Analysis Center, Contract No. SP0700-00-D-3180, Delivery Order 0603, and Technical Area Tasks 794 and CB-11-0232. It has been reviewed by the Agency but does not necessarily reflect the Agency's views. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer or otherwise doesn not constitute or imply its endorsement, recommendation, sale, or favoring by the EPA.

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Acronyms and Abbreviations

AFCS	Advanced Facer Cancellar System
AVA	Anthrax vaccine absorbed
BDS	Biohazard Detection System
CDC	Centers for Disease Control and Prevention
CFU	colony forming units
ENM	ecological niche model
EPA	U.S. Environmental Protection Agency
FBI	Federal Bureau of Investigation
g	gram
mL	milliliter
MLVA	Multilocus variable tandem repeat analysis
NHSRC	National Homeland Security Research Center
PCR	polymerase chain reaction
SNR	Single nucleotide repeats
USDA	United States Department of Agriculture
USPS	United States Postal Service
WNA	Western North America

Acknowledgments

The following individuals and organizations are acknowledged for their contributions and/or review of this report:

U.S. Environmental Protection Agency
Charlena Bowling
Deborah McKean
Tonya Nichols
Eric Rhodes
Frank Schaefer, III
Erin Silvestri

Battelle, Contractor for the U.S. Environmental Protection Agency

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Executive Summary

The purpose of this report was to: (1) survey the scientific literature to determine the current state of the science regarding the presence of *Bacillus anthracis* in the environment and outbreaks of anthrax; (2) identify characteristics that would enable a screening of information about outbreaks to rapidly assess whether an intentional release was a likely cause (in United States settings); and (3) identify gaps in risk-related knowledge associated with *B. anthracis* events in the United States. Being able to identify whether an event was natural (e.g., wildlife or livestock exposure to a carcass site), unintentional (e.g., human exposure to a naturally contaminated animal hide), or intentional (e.g., release of *B. anthracis* by an individual or group to deliberately cause harm) could provide insight into the type of response that might be necessary following a contamination event. The screening approach is not intended to be a tool for performing a criminal investigation or for extensive public health assessment, although the information and logic may inform both endeavors.

A review of literature was used to interpret the exposure aspects of the risk of human anthrax associated with the environmental presence of *B. anthracis*. Until about 1950, the source of human anthrax in the United States was primarily from occupational exposures to *B. anthracis* spores from contaminated animal products. Outbreaks of human anthrax in the United States declined significantly during the first half of the 20th century due to improved industrial hygiene, the closing of domestic animal hair processing mills and tanneries removing the risk to U.S. workers, and effective livestock vaccines. Today, in the United States, a majority of anthrax cases remain cutaneous in nature, resulting from handling contaminated animal products or contact with livestock in a narrowing range of enzootic areas.

B. anthracis is widely distributed across the central United States and the Mississippi Delta. Human infection risk, while low, is most likely in enzootic areas of West Texas, North and South Dakota, Minnesota, and Montana. Because of gaps in knowledge of *B. anthracis* lifecycle, dormancy, loss/gain of virulence plasmids, and human susceptibility, the circumstances under which human exposure to *B. anthracis* occurs and results in disease remains uncertain. An outbreak of anthrax or the detection of *Bacillus anthracis* in an unexpected environment can trigger local, state, or federal response, particularly when there is the potential for human exposure. Response to the event and subsequent risk management depends in part on whether the occurrence of *B. anthracis* is natural, unintentional, or intentional.

The screening approach presented here is designed to help the U.S. Environmental Protection Agency rapidly and systematically determine the likelihood that a detection of or exposure to *B. anthracis* is due to an intentional release. A limited number of parameters need to be considered in this screening process. Given ready access to the necessary data, an initial evaluation can be completed quickly, except for identifying the strain of the *B. anthracis*, which requires about 48 to 96 hours using current technologies. Use of the proposed systematic assessment of the likelihood that an event was intentional might help indicate what type of response might be needed.

1 Introduction

The U.S. Environmental Protection Agency (EPA) assists in the federal environmental response to chemical, biological, and radiological releases. EPA is the lead federal agency for the remediation of areas contaminated with these agents. EPA's responsibilities include threat assessment, hazard identification, detection and reduction, environmental monitoring, and planning and implementing site decontamination and remediation activities. Adequate and valid knowledge is key to being prepared to effectively and efficiently execute these responsibilities. The National Homeland Security Research Center (NHSRC), within EPA's Office of Research and Development, provides knowledge to contain and mitigate contamination and to decontaminate indoor and outdoor environments.

B. anthracis, the causative agent for anthrax, has been recognized as an important biothreat agent for various reasons. The potential use of *Bacillus anthracis* as a biological warfare agent has been evaluated by various nations including the United States, and has been found to meet key factors making a biological pathogen or toxin suitable for a large-scale bio-warfare or terrorist attack [1]. As in a chemical attack, only individuals directly exposed to the released agent will be affected because anthrax is not contagious. Unlike contagious biological agents, *B. anthracis* can produce a controlled and targeted impact. In addition, *B. anthracis* spores are stable and can be prepared and stored for long periods without losing viability. Inhalation anthrax, resulting from an aerosolized release of spores, can be deadly. The Amerithrax attacks of 2001, in which spores of *Bacillus anthracis* were sent by mail to targeted individuals, resulted in sickness and death. For these reasons, anthrax attacks are viewed as an important biological threat.

In many areas of the world, including specific areas in the United States, anthrax is endemic. In these areas outbreaks occur in animals and humans. Continued reporting of human anthrax throughout the world, but especially in areas with increasing human incidence [2], coupled with the potential use of *B. anthracis* spores as a biological weapon, leads to a need to understand human infection risk. Human health risk associated with the presence of *B. anthracis* in the environment in the United States, whether as background levels or as residual levels after an act of bioterrorism [3, 4], depends on a variety of environmental and biological factors (e.g., soil conditions or the pathogen's virulence). Section 2 of this report presents a literature review of the baseline human health risk associated with *B. anthracis* in endemic areas. Section 2 also explores the unintentional exposures for people who could have occupational exposure to *B. anthracis* and for people who handle, process, or eat contaminated animal products.

EPA's role in response to a natural or unintentional event compared to an intentional release would be different. Intentional releases are intended to cause deliberate harm and, when an intentional release occurs, a crime scene is created. In a crime scene, additional agencies must respond and different protocols must be employed in processing the scene. Section 3 presents a screening approach to systematically evaluate whether an occurrence, defined in this report as detection of *B. anthracis* in the environment or an outbreak of anthrax in human or animal populations, is the result of a natural outbreak, an unintentional release, or an intentional release. Differences that could be used to distinguish natural and unintentional releases from intentional releases of *B. anthracis* or outbreaks of anthrax were organized into a key that could be used to rapidly (in less than eight hours, given available data) screen for intentional occurrences.

1.1 Purpose

The purpose of this report was threefold: (1) survey the scientific literature on the presence of *B. anthracis* in the environment and outbreaks of anthrax; (2) identify characteristics of an occurrence that would enable a rapid assessment of whether an intentional release was a likely cause (in United States settings); and (3) determine what gaps exist in risk-related knowledge associated with detection of *B. anthracis* in the environment or outbreaks of anthrax in the United States.

1.2 Methods

Information about *B. anthracis* from reports, peer-reviewed journal articles, books, and government publications was collected in this literature review. Relevant journal articles and peer-reviewed reports were initially identified using PubMed and Google Scholar. Additional articles were also added to the search as recommended by experts in the topic areas. Data describing the historical geographic distribution of *B. anthracis* in the environment or human cases of anthrax were summarized along with the emerging understanding of the lifecycle of *B. anthracis*.

2 *Bacillus anthracis* in the Environment and Human Risk

2.1 Overview of *B. anthracis*

B. anthracis is a Gram-positive bacteria responsible for anthrax [5, 6]. The name derives from the Greek word for coal in recognition of the black, dead skin characteristic of the cutaneous form of the disease [7]. This rod-shaped, facultative anaerobe forms chains of boxcar-shaped cells. Two plasmids, pXO1 and pXO2, are required for *B. anthracis* virulence. Three proteins encoded by pXO1 produce the anthrax toxin. Protein necessary for the capsule are produced by pXO2. The antiphagocytic properties of the capsule are important for virulence [8, 9]. The strain of *B. anthracis,* and presence or absence of pXO1 and pXO2 plasmids, provide valuable virulence information. The presence/absence of plasmids largely determine the virulence of *B. anthracis*-pXO1 (coding for a tripartite toxin) and pXO2 (coding for the capsule) for the strains [10]. Seventy-nine *B. anthracis* strains can be distinguished using amplified length polymorphism, corresponded to specific geographic origins [10].

2.2 *B. anthracis* Persistence

The vegetative form of *B. anthracis* does not compete well with other microorganisms in nature [11, 12] and therefore forms spores in response to adverse environmental conditions [5]. The spores enable viable *B. anthracis* to survive for decades under favorable conditions [13]. Generally, spores can resist prolonged desiccation, fluctuations in soil pH, temperature, pressure, and ultraviolet and ionizing radiation [14]. In pond water, *B. anthracis* has been reported to survive 18 years [15]. It survives in moist or dry soil for months to years (3 months to 36 months) [14-16].

Our understanding of the factors that affect persistence of *B. anthracis* in the environment is evolving. As early as 1956, the persistence of *B. anthracis* in the environment was found to depend on a neutral or alkaline pH, sufficient calcium, and adequate moisture [17]. Further studies have supported these findings and added high organic matter as an additional characteristic of soils supporting the persistence of *B. anthracis* spores [13, 14, 18, 19]. *B. anthracis* persistence has been observed in these specific soil and environmental conditions in the United States, Russia, Central Asia, South America, and South Africa [14]. Poorly drained depressions in the land can accumulate organic material and minerals from surrounding “inhospitable” land to create patches in which *B. anthracis* may survive [14, 18]. Generally, it has been hypothesized that *B. anthracis* does not persist in calcium-poor soils, shale or sandstone, or well-drained fields. Anthrax outbreaks are unlikely in areas with acidic soils; *B. anthracis* prefers soils with a pH greater than 6 [14, 18, 20].

A debate has existed as to whether *B. anthracis* persists only as spores, or whether germination of the spores and subsequent vegetative growth might account for persistence. “Incubator areas,” areas of vegetative growth of the *B. anthracis* with subsequent sporulation, were hypothesized by Van Ness [18] to be calcareous/alkaline depressions, rich in organic deposits, with water standing for periods sufficient to kill grass; anthrax also arises in dry streams or calcareous/alkaline hillside seep areas. Incubator areas were hypothesized to form only when rain is sporadic or during dry periods. In an alternative hypothesis, the depressions and seeps referenced by Van Ness [18] as “incubator areas” were referenced by Dragon and Rennie [13] as

"storage areas" for spores. In their hypothesis, rather than reproducing, spores may be transported, on organic matter, to low-lying storage areas, thereby concentrating the spores. Drying of the pools would further concentrate the spores in the soil storage areas [13].

2.2.1 *B. anthracis* Persistence Associated with Microenvironments

Members of the *Bacillus* genus are primarily saprophytic, living in soil [21]. In contrast, *B. anthracis* has been considered an obligate pathogen, unable to reproduce outside of an infected host in nature [22] due to loss of saprophytic capabilities attributed to a single nonsense mutation inactivating a transcription regulator gene [23]. Recent evidence suggests that conditions exist in which reproduction can occur outside of a diseased host. While low levels of soil-borne *B. anthracis* may persist, *B. anthracis* likely depends on mammalian hosts for significant replication and persistence. Recent studies suggest the organism may have a dynamic life cycle in the rhizosphere and soil biome [21, 23]. *B. anthracis* spores, present at low levels, can germinate, multiply, and persist in the rhizosphere when nutrients are available and predation pressures are not too great [21]. Under laboratory conditions, *B. anthracis* spores were shown to germinate and multiply in the rhizosphere of grass (*Festuca arundinaceae*) for up to seven weeks, but not in soil without plants [21]. However, new evidence from outdoor soil experiments with wild type strains in Etosha National Park, Namibia suggest that *B. anthracis* spores significantly promote grass seed germination. While the germinating grass did not appear to enhance multiplication or increase persistence of *B. anthracis*, such a mechanism would attract host ingestion by encouraging foraging, further supporting the importance of mammals in focal pathogen persistence [24].

Hypothetically, biofilms might provide another mechanism by which vegetative *B. anthracis* could persist in soil. Under laboratory conditions, *B. anthracis* was shown to form vegetative biofilms that sporulate under low nutrient conditions [25]. *B. anthracis* reproduces in biofilms present in soil [23, 25].

Dey et al. [26] performed laboratory experiments to determine whether *B. anthracis* would germinate and reproduce in amoebas that would be found in the low-lying areas characteristic of hypothesized "incubator areas". They demonstrated that *B. anthracis* spores inside of amoebas (*Acanthamoeba castellanii*) do germinate and reproduce. Further, they demonstrated that the pXO1 plasmid was necessary for intracellular germination and growth in amoebas, but pXO2 was not. Based on these results they hypothesized a role for amoebas in the persistence of *B. anthracis* in "incubator areas" [26]. *B. anthracis* lacking pXO2 or both pXO1 and pXO2 is observed in the field, with avirulent isolates occurring at spore-contaminated sites within five to eight years [14]. The presence of avirulent *B. anthracis* supports the hypothesis that environmental persistence may arise from incubator areas (assumes microbial reproduction outside of an infected host) [18] rather than spore concentrator areas (assumes no reproduction occurs) [13]. Whether avirulent *B. anthracis* is likely to acquire virulence plasmids in natural settings is not known. Without the pXO2 plasmid *B. anthracis* has a low pathogenicity and is therefore unlikely to be able to infect another animal [22].

Beyond the laboratory, *B. anthracis*-like bacteria were found in earthworm gut in nature [23] and *B. anthracis* multiplied in the intestinal tract of earthworms (*Eisenia fetida*) [23, 27]. Earthworm

abundance and diversity and *B. anthracis* persistence require similar conditions: both are enhanced by slightly alkaline soils with high calcium and high organic matter [27]. It may be speculated that the presence of earthworms is a critical factor for *B. anthracis* persistence at locations where outbreaks have occurred but no spores have been detected in soil samples.

Bacteriophage infection of *B anthracis* may create lysogens (the integration of phage nucleic acid into the bacterial chromosome) to restore functionality necessary to survive and replicate in earthworms, the rhizosphere, or in soil [23]. Such lysogens were observed in nature [23]. Lysogeny with phages (from soil, fern rhizosphere, and earthworm gut) was found to inhibit sporulation, favor biofilm formation, and support persistence in soil and anoxic earthworm gut by activating *B. anthracis* genes [27]. The phages responsible for the lysogens are diagnostic for *B. anthracis* and were shown to be specific for *B. anthracis*-like bacteria (*B. anthracis* Sterne and some related strains of *B. cereus*), but not *B. thuringiensis*, *B. megaterium*, *B. mycoides*, *B. pumilus*, *B. subtilis*, *B. brevis*, *Sporosarcina ureae*, or *Brevibacillus laterosporus* [27].

Whether these, or other, alternative vegetative pathways in the *B. anthracis* lifecycle occur in nature, enabling persistence between outbreaks, remains in debate. The specificity of the bacteriophages for *B. anthracis*, their presence in the natural environment, the observed characteristics of the lysogenic *B. anthracis*, and the detection of avirulent *B. anthracis* in field samples support the hypothesis that vegetative reproduction may occur within the soil environment supporting *B. anthracis* between outbreaks. However, without continuing sporadic outbreaks the disease over time will disappear, suggesting that soil support of *B. anthracis* survival is limited.

2.2.2 Presence of Endemic *B. anthracis* in the United States

Ecological niche models (ENM) can be used to broadly define the geographic range of *Bacillus anthracis* and target surveillance efforts [28, 29]. ENM have been used to predict the potential geographic distribution of *B. anthracis* in the continental United States using confirmed outbreak data from livestock and wildlife between 1955 and 2005 [30]. Additional models described potential shifts in the distribution under climate change, suggesting possible reduced suitable habitat for pathogen survival in southern Texas and expanding habitat in the northern states [31]. Further ENM experiments were performed to refine predictions by modeling the dominant lineage (Western North America – WNA or A1.a [32]) in the contiguous United States [33]. Broadly, such models define the geographic potential for pathogen persistence [34]. Coupling spatial statistics of outbreaks with ENM outputs allows for the identification of high risk transmission areas (where the statistically significant clusters occur) and areas where passive surveillance should increase (where niche models predict persistence in under-investigated areas) [34]. Much of the recent spatial modeling of anthrax, including ENM experiments, has relied on mortality data to understand the disease, which likely underestimates the extent of the disease [35]. More recently, it has been suggested that serological surveillance could delineate additional anthrax enzootic zones [36] by including species with high survival rates.

Recent soil samples were collected along a transect from Manitoba to New Mexico and Texas and analyzed using polymerase chain reaction (PCR) technology to look for persistent *B. anthracis* [37]. PCR analysis was used to look for *B. anthracis* within the range of persistence that has been identified by the ENM [30]. A BA-SF primer (*rpoB* sequence) [38] was used to

distinguish *B. anthracis* from other *Bacillus* species, including *B. cereus*. *B. anthracis* was present in samples collected at five of 104 sample sites tested (one site each in Manitoba, Minnesota, South Dakota, Colorado, and Nebraska) [37]. The transect generally paralleled the eastern edge of the geographic range for *B. anthracis* as determined by Blackburn et al. [30] and showed *B. anthracis* was occasionally present. Whether the detected *B. anthracis* was virulent (and a risk to human and livestock health) was not assessed.

Louisiana and Mississippi are areas with soil and other characteristics such that ecological niche models do not predict *B. anthracis* would be likely to persist [30], yet there has been a history of natural outbreaks of anthrax in the Mississippi River delta [17]. *B. anthracis* was found in soil samples collected in New Orleans after hurricane Katrina in 2005. The samples were analyzed by PCR using the BA-SF primer specific for *B. anthracis* [37]. The PCR-positive samples were cultured and confirmed to be *B. anthracis*. Virulence markers (pXO1 and pXO2 plasmids) were evaluated using PCR. *B. anthracis* were found in 26% of the samples tested. All five *B. anthracis*-positive post-flood samples from New Orleans were positive for pXO1, but only one sample had both the pXO1 and pXO2 plasmids needed for virulence [37]. Two years later (2007) no samples from New Orleans were positive for *B. anthracis* [37]. While the general environmental conditions in the Mississippi delta would be expected to inhibit *B. anthracis* persistence, sporadic outbreaks and detection of *B. anthracis* occurred there. One potential explanation was provided by Van Ness [18], who reported that in a study of the 1959 Pearl River Mississippi outbreak, areas high in calcium were found to be required for anthrax to occur. Thus hospitable microenvironments for *B. anthracis* may exist within an otherwise unlikely area for persistence, resulting in recurrent anthrax in the Mississippi delta. But, the non-reappearance of disease in this area suggests that these later outbreaks may have been due to contaminated feed, not persistent soil contamination.

2.3 Overview of the Classic *B. anthracis* Lifecycle

The classic *B. anthracis* lifecycle is summarized in Figure 1. A necessary condition for anthrax outbreaks is exposure of a susceptible animal to *B. anthracis* with both pXO1 and pXO2 plasmids (required for virulence). In locations with recurrent outbreaks, the presence of *B. anthracis* is generally attributed to persistence in the environment and the presence at novel locations may arise from the transport of spores to the location. Given the potential for persistence of *B. anthracis* spores at a location, outbreaks tend to occur in certain seasons and under specific environmental conditions. When the specified conditions exist, an initial case or cases might occur. With a resulting death, release of blood containing *B. anthracis* and subsequent sporulation might occur at the carcass site. Other animals could then be exposed to *B. anthracis* spores at or near the carcass, or through mechanisms that transport *B. anthracis*, resulting in exposure of other animals and multiplication of cases. Over time, conditions change, resulting in the termination of the outbreak.

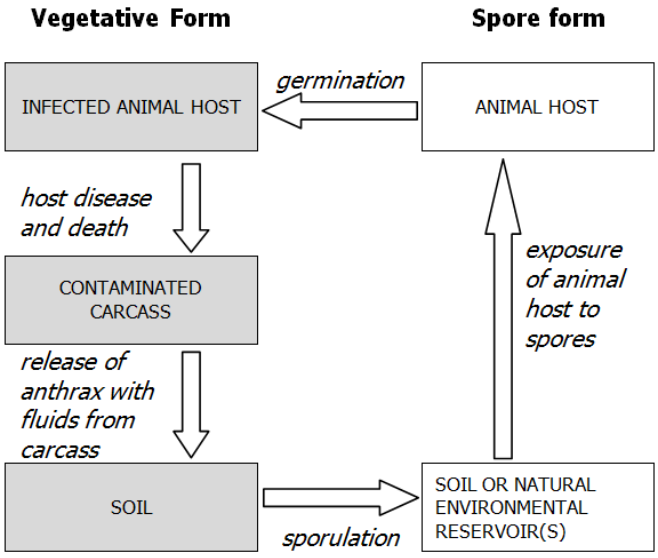

Figure 1. Summary of *Bacillus anthracis* lifecycle adapted from Schuch et al. [23].

2.3.1 Initial Host Acquisition

Anthrax can infect a wide variety of livestock, wildlife, and, rarely, birds (see [39] and [36] for extensive lists of taxa). Susceptible animals are mostly grazing mammals, but include both grazing and non-grazing mammals. Susceptible animals include cattle, horses, sheep, pigs, dogs, bison, elk, white-tailed deer, goats, mink, and numerous other mammals. Ruminants appear to be most susceptible [40]. Among livestock, cattle generally have higher case and mortality rates than other herbivores. Swine and carnivores are relatively resistant to lethality resulting from anthrax infections [40-43]. However, a wide range of carnivores do contract anthrax [39, 44]. Serologic data show almost 90% of carnivores (e.g., lions and hyenas) in some areas have survived anthrax infection, whereas no zebras survived anthrax in the same areas [41] based on serology data. It is notable though that zebras had high seroprevalence rates elsewhere in Africa [45]. Note that in addition to surviving anthrax, high antibody titers might result from sub-clinical anthrax or toxin-induced stimulation of antibody production [36]. In the case of zebras in Namibia, titers fluctuated in animals captured during the anthrax season and recaptured in the non-season [45]. In birds, there are only a handful of reports of anthrax mortality resulting from natural or oral exposures including a few reports of ostriches on ostrich farms [39], of a few captive birds in zoological gardens [39], and of a single vulture among many feeding on carcasses in natural outbreak [46].

There is a high level of uncertainty as to the factors triggering the initial case or cases of an epizootic, whether changes in environment or behavior increase host exposure, changes in stressors increasing host susceptibility, or changes in environmental concentrations of *B. anthracis* [47]. It should not be overlooked that anthrax is typically a summer disease, a time of heat stress that will negatively impact the innate resistance of host species [48], thereby allowing low exposure levels to become infective. An outbreak may arise from one such stressed animal that has been grazing contaminated soil or that has been harboring a latent infection. Generally the index case (or early cases) occurs at or near a former carcass site [49]. Several theories exist

as to the exact route of exposure. The spores may be ingested with grazing and there may be seasonal and behavioral changes that result in greater ingestion of soil containing spores [47] or ingestion of grass at carcass sites locally [24]. Contributing to the initial case in an outbreak, ingesting grit and thorns could cause lesions in the digestive tract providing unimpeded entry of *B. anthracis* into the blood or lymph system allowing escalation to bacteremia [40]. Beyer and Turnbull [40], citing Fox et al. [50], states the lesion theory is not consistent with empirical data. Beyer and Turnbull [40] stated inhalation of spores while grazing over dry contaminated soils could lead to inhalation anthrax, though there is a lack of empirical evidence. Ground disturbance of an old carcass site could lead to an outbreak [19] from inhalation or ingestion. More research is needed to test these infection hypotheses.

2.3.2 Carcass, Sporulation, Spores, and Vegetative Survival at Carcass Sites

B. anthracis is present at high levels, a million to a billion CFU per milliliter (mL) in the blood of animals when they die of anthrax [51]. High carbon dioxide concentrations found in decomposing carcasses reduce sporulation. *B. anthracis* in carcasses appears to die within four or fewer days under conditions supporting anaerobic digestion [Minett [52] cited in [14]] and in competition with other microorganisms [13]. Opening of the carcass by scavenging birds or other mammals, allows body fluids to be drained from the carcass and causes dispersal of vegetative cells into the surrounding environment [14, 19, 52, 53]. Introduction of the vegetative cells to nutrient-poor environments induces sporulation [13]. Initiation and speed of sporulation depend on the temperature and relative humidity, at least in the spore microenvironment [14, 40, 46]. A temperature of 39 °C with high relative humidity (100%) is ideal for sporulation to occur. In Namibia, sporulation in soil was reported to occur at temperatures above 25 °C [51]. At a temperature of 18 °C sporulation is inhibited; the vegetative form of *B. anthracis* dies [14]. In locations and/or conditions not conducive to sporulation, spilled blood with high *B. anthracis* CFU/mL may result in few or no spores in soil; only a small proportion of the *B. anthracis* successfully sporulate [51]. Vegetative *B. anthracis* survival outside of a host has been considered to be poor (<24 h) [54] although recent evidence (discussed in Section 2.2.1) suggests that vegetative persistence in microenvironments might be possible.

Carcass sites are the most likely locations to find *B. anthracis* spores. Spores may persist in soil at some locations for years [51, 55]. Spore titers vary greatly in the area around a carcass site and between anthrax carcass sites [51, 53]. In some environmental conditions, *B. anthracis* spore levels at carcass sites tend to remain high for years (10^4 – 10^6 colony forming units [CFU]/gram [g] soil) [56]. At other carcass sites, *B. anthracis* is observed to quickly disappear from heavily contaminated soil, becoming undetectable in soil or bone marrow from buried carcasses within a year, whether analyzed by culture or by polymerase chain reaction [56]. In other locations spore persistence at carcass sites appears to be transient [51]. More recently, investigations in Texas recovered viable *B. anthracis* with both plasmids (pXO1 and pXO2) from soils around a carcass 12 months after an outbreak and that same genotype was recovered from a fresh deer carcass [55]. In Montana, viable spores were recovered from a bull bison skull 27 months after the animal died during an epizootic (Blackburn, unpublished data). In a study of enzootic areas in Namibia, Africa [51], few soil or water samples not associated with a carcass site were positive for *B. anthracis* and the number of spores, where detected, was low: 3.3% of water samples (1 spore/mL) and 3.0% of soils samples (8 – 80 spores/g). Sampling 106 carcass sites, 65% of the samples had 1 to 10,000 spores/g; 10% had >10,000 spores/g, and 4% had >1 million spores/g.

In another field study in Kenya [57], *B. anthracis* spores were found in 43% of ash samples from burned carcasses, but not in soil samples within three meters of the carcass or samples from ephemeral pools and dry river bed channels. In contrast to the Kenya study, *B. anthracis* spore concentrations were highest within two meters of old or recent carcasses and specifically in locations where the soil was saturated by body fluids from the carcass [53]. The cause of this difference is unknown, but may arise from differences in ecosystems or environmental conditions. Multiplication is not observed at carcass sites [51].

2.4 Conditions Characteristic of Outbreak Initiation

Broadly, recurrent outbreaks are thought to be associated with areas with frequent host-pathogen-soil-host transmission. Anthrax outbreaks in animals in the United States are generally limited to specific geography (discussed above), geology/soils, and seasons [19]. Van Ness [18] hypothesized livestock grazing on soils with pH above 6.0 and ambient temperature above 15.5 °C are in favorable conditions for pathogen exposure.

Under conditions conducive to an outbreak, multiple independent outbreaks may occur [58-60]. For example, in 2004 in Italy there were two outbreaks, 23 kilometers (km) apart, each caused by a different *B. anthracis* strain, suggesting the outbreaks were independent [59]. In the northern hemisphere, anthrax is primarily a summertime disease, with cases occurring after May [61]. Generally, environmental conditions leading to acute outbreaks involve a wet period (typically) followed by an extended drought or hot, dry conditions (see [14] for an in-depth review). In Texas, Blackburn and Goodin [61] quantified spring green-up conditions, showing statistically significant differences in years with epizootics. Using satellite measures of vegetation greenness, the study confirmed that epizootic years greened up earlier and more intensely than years with just sporadic cases. Vegetation indices suggested that short-term green-up events precede large epizootics after prolonged (several week or month) dry periods, supporting that rain events in otherwise dry times may lead to explosive outbreaks. A study from southern Canada suggested that large rain events preceded cattle outbreaks [62]. Similar conclusions were reported from Australia following an acute regional epizootic in Victoria in 1997 [63].

2.4.1 Spore Transport and Case Multiplication

Under conditions supporting sporulation, the index case(s) may provide heavy spore loads resulting in infection of other animals leading to cluster(s) of cases [34, 58]. Spores from the index case infect other animals when they ingest blood containing *B. anthracis* or ingest vegetation contaminated with spores [49]. *B. anthracis* spores may be transported from a carcass site by animals or insects, expanding the area in which anthrax is observed. Spore transport vehicles and mechanisms include movement of infected animals, insects, and predators; physical transport of parts of the carcass; transfer of spores to feathers and feet; and spore passage through the digestive tract and deposition into the environment at remote locations in vomit and feces. Spores may also be transported from the carcass site in runoff and by wind [13, 40, 51]. Insects may play a key role in the spread of anthrax and transmission of spores to animals or to vegetation subsequently eaten by animals. Delay in disposal of carcasses (two to four days) may provide time for insects and especially blow flies to feed on the animals and then serve as vectors of transmission [55]. Peaks in fly populations (biting or non-biting) are associated with

seasonality of anthrax and suggest a role for flies in case multiplication [64, 65]. Laboratory experiments using houseflies (*Musca domestica*) fed even briefly on anthrax infected blood subsequently deposited *B. anthracis* in fly spots (feces and vomitus). The spores germinated and reproduced in the fly gut, raising the titer from less than 10,000 per spot at two hours to about 25,000 – 40,000 per spot at 10 hours. The observed *B. anthracis* populations in fly spots later crashed between 10 hours and 12 hours and declined to about zero per spot 24 hours after feeding [66]. In Africa, fly spots from blowflies feeding on carcasses deposited on browse near the carcass resulted in high levels of *B. anthracis* on leaves. Browsing of leaves may cause new cases, but is likely limited to within several days of an animal death [55]. Additionally, the species of flies, and their physiology, may play an important role in pathogen survival [67] and much work is needed to more fully understand the role of insects [55, 64].

Heavy rains preceding droughts in large anthrax epidemics (noted previously) may provide conditions resulting in the production of large numbers of flies. Large populations of hemophagic ("biting") flies (e.g., *Stomoxys calcitrans)* are hypothesized to cause the broad spread (about 5-10 km) of an anthrax epizootic [39], although to date, published evidence is lacking to confirm this. Available evidence suggests that biting flies may locally amplify risk within an outbreak [64]. A human case of cutaneous anthrax occurred after a gadfly bite contemporaneous with an outbreak that had resulted in the death of sheep. The infected man had been in a pasture that was used by the herd of sheep in which the outbreak occurred [68]. However, he had had no contact with sick animals or carcasses. DNA analysis showed no differences between the strain that killed the sheep and the strain that infected the man. This case supports the hypothesis that there is risk of human anthrax associated with bites by contaminated flies. Historical work in Russia suggested that biting fly risk was associated with shrub land environments and less so with open grasslands [69] but it should be noted that female tabanids lay their eggs in running water, which can be scarce in steppes. The enzootic zone defined by Blackburn et al. [64] meets this shrubland definition. Ticks and mosquitoes may also ingest the bacteria [14]. While tick transmission would be limited to host-jumping species, transmission via mosquitoes would be dependent on the density and numbers of mosquitos [14]. Animals newly infected by ticks and mosquitos, however might travel considerable distance. Both explanations for the transmission and geographic spread of anthrax need further investigation.

Exposure of anthrax carcasses to carnivores may be a key factor in the transmission and continuation of outbreaks in Africa, Argentina, and Canada [51, 54, 70]. Carnivore and avian scavengers are less susceptible to anthrax than herbivores. Both may deposit spores in the vicinity of the opened carcass [53] and transport spores long distances, releasing them in feces [13, 41]. In Namibia, >50% of fecal samples from scavengers near carcasses were positive for anthrax spores. The spore density in the fecal material was highly variable, reaching a high of 20,000 CFU/g of fecal material [51]. Avian scavengers, like vultures [46, 71], and predators, like hawks [70], may disseminate *B. anthracis* spores via fecal material and via contaminated feet and feathers. While a transmission role for *B. anthracis* by African vultures was proposed, a study of vultures in southern Africa did not result in the detection of *B. anthracis* spores and suggests a limited (or no) role in the spread of anthrax [46]. Field investigations from west Texas were unable to recover *B. anthracis* from feces, but did recover spores from feathers and water troughs frequented by vultures feeding on carcasses (Blackburn, unpublished data) supporting early hypotheses on water contamination in Africa [72]. It should be remembered that vegetative

cells will not survive passage through the scavenger gastrointestinal tract. It is when they have consumed spores, e.g., from an already opened carcass a few days old, that they can deposit spores in their feces.

Spores in soil resist movement in spite of high wind and rain events. High levels may remain at carcass sites for years [Canada [53]; Etosha National Park, Namibia [56]]. Wind movement of spores from carcass sites may occur, with concentrations of 7.1×10^{-3} CFU/liter air measured at 18 meters from the center of the site [56]. These low levels would result in few microbes being inhaled, well below the normal infective dose number, and windborne spores from carcass sites are therefore believed to have little impact on the spread of anthrax [56]. Increased entrainment of dust (enhanced by increased animal contact with soil during rutting aggression) has been suggested as a potential cause of increased cases of anthrax through aerosolization of spores [13]. It should be noted that large soil particles will not penetrate far into the lung because of particle size and particles capable of causing anthrax may be of low density in the atmosphere, as was observed in Namibia [5, 56]. Rutting causality is questionable because anthrax outbreaks in wood bison in Canada [13] and deer in Texas [49] begin before rutting season (in northern hemisphere summer).

While the importance of flooding in anthrax epizootics is recognized, the transport role in geographic dispersion is uncertain. In some cases, flooding and natural drainage are assumed to disperse spores over large geographic areas [58, 63]. Alternatively, multiple independent outbreaks over large geographic areas may be due to a large area of optimal conditions in which *B. anthracis* persisted before the flood event [59]. Floods and high runoff could wash soil and spores to depressions ("concentrator sites") [13] and onto seasonally flooded water meadows. Rain may also splash spores onto grass [47]. Water may transport spores to locations of initial cases, but the subsequent dispersion pattern around carcass sites is most consistent with dispersion by scavengers via movement of scavenged body parts and spores in fecal material [53] or by insects [64]. Summarizing the strongest evidence for spore dispersion mechanisms found in published reports points toward (1) herbivores ingesting spores near carcasses and traveling some distance before dying (creating a new carcass site); (2) dispersion by insects, through their biting, in their feces and vomitus; and (3) predator transport in their feces and by physical dispersion of parts of the carcass.

Humans can transmit spores and enable case multiplication of livestock cases by using contaminated bone meal in animal feeds and by administering animal vaccinations containing virulent organisms [73, 74]. However, in the United States, these two causes of anthrax in animals are no longer observed because of requirements for treating imported bone meal and improved vaccines. Strains of *B. anthracis* observed in outbreaks in the United States include enzootic strains and imported strains. Industrial importation of animal products resulted in the introduction of characteristic 'industrial' genotypes into the United States, which resulted in cases of anthrax. These imported strains resulted in a small number of human cases. Some of the imported strains become ecologically established [32, 75].

2.4.2 Temporal Characteristics and Termination of Natural Outbreaks.

During an epizootic, new cases may occur for weeks or months following the initial outbreak [19, 49, 58, 63, 76]. In natural outbreaks impacting multiple farms in the United States, the maximum number of newly affected premises is normally identified in weeks 3 to 5 after the initial case, and all affected premises are identified within 9 weeks. The typical number of livestock deaths per premise is five, with cattle mortality about 6% and horse morality about 17% [19]. In recent U.S. wildlife outbreaks in Texas, deer mortality rates have varied from ~1-7% [64], with some ranches reporting a near 100% loss [55]. The disease is present in all years, but in the sporadic years mortality is ten or fewer deer [64].

Mortality in wildlife may result in higher death rates when wildlife densities are high [14], though this has not been quantified or modeled. In a major outbreak in wood bison, ~10% of a herd of 1,800 died in a single outbreak. By the following year, 93% of surviving adults had developed high antibody titers with sub-clinical anthrax, survival of the infection resulting in immunity, or absorbing toxin components that stimulate antibody formation (see [36]). High percentages of carnivores in an enzootic area may exhibit elevated titers to *B. anthracis* indicating sub-clinical anthrax or survival [41, 77]; other animals, e.g., zebras, appear to have much lower survival rates in the Serengeti ecosystem [41].

In natural settings, outbreaks can continue until conditions change, such as beginning of seasonal rains [14] or cold weather [13]. For example, it has been posited in South Africa, Tanzania, and West Texas, that the end of outbreaks is coincident with seasonal rain [14]. Such weather may remove spores deposited by insects from vegetation by washing *B. anthracis* from the leaves [55]. In the latter case, field evidence suggests this may be in fewer than 21 days [55]. Outbreaks in wood bison in northern Canada end with cold weather [13]. Cold weather termination of an outbreak may be due to unfavorable conditions for sporulation, change in animal behavior, movement of the animal to an area free of spores, loss of fly populations, or other causes. Outbreaks may be ended or mitigated by active management actions such as quarantine, insect control, soaking carcasses in 10% formaldehyde to discourage scavengers and disinfect surface of the carcass, removing and/or incinerating carcasses, use of antibiotics to stop incubating infections, and vaccination of animals in the area outbreaks [59, 60, 76, 78-80]. If vaccination is adequately administered, lower mortality rates have been reported during outbreaks [19, 79]. Delayed germination of spores can result in anthrax cases after an antibiotic regimen has been completed [81].

The rapid removal of carcasses reduces the likelihood of a widespread epidemic by reducing the transport and transmission potential of insects [66], reduces soil contamination and reduces scavenger dispersal of spores [76]. Burning the carcass or using quicklime, or agricultural lime, to cover the carcass [57, 82] may not be effective or effectiveness may not have been determined [82]. Because the persistence of *B. anthracis* spores is enhanced in some instances by alkaline conditions with high levels of calcium, lime may support persistence of viable spores. More recently, a critical review has suggested that lime be discontinued as a decontaminant and considered a spore promoter [83].

2.5 History of Naturally Occurring Anthrax Outbreaks in Animals in the United States

Anthrax, or anthrax-like, outbreaks have been reported in the historical record for thousands of years [84] and have been reported in livestock and wildlife nearly worldwide [85]. Anthrax has a long history in the United States dating back at least 200 years [32], although it might have arrived on the continent prior to European exploration [86]. While the initial introduction to the U.S. is and will remain unclear until more genomic studies are performed, the presence of multiple divergent genetic lineages, as defined by single nucleotide polymorphisms (SNPs), confirms multiple introductions over time [32, 87]. Anthrax epizootics continue to occur in the United States in livestock and wildlife [14], with climatic drivers associated with severity (less green and drier summers found during epizootic years) [61]. Generally, animal outbreaks were associated with historic livestock pastures or farms. In particular, many of these outbreaks occurred at known historical outbreak sites [19]. There may be years or decades between outbreaks at a given location [79]. However, it is likely that livestock and wildlife outbreak reports underestimate the number of cases due to biases in search efforts [35], difficulties in finding carcasses [61], and lack of diagnosis and underreporting [85, 88]. Recent serological reports from Africa [41, 45] and Ukraine [89] further indicate frequent, regular infection. In the latter example of Bagamian et al. [89], serology confirmed infection in wild boar in the absence of livestock or other wildlife reporting. In Namibia, wildlife outbreaks occur annually in Etosha National Park [47]. Across these studies, there is evidence for frequent transmission events. Clearly, viable *B. anthracis* populations persist in areas of prior outbreaks and, under favorable conditions, initiate sporadic or seasonal outbreaks in susceptible livestock or wildlife hosts [13, 30].

Prior to 1950, anthrax in animal populations was reported regularly in several areas of the United States, including parts of California; Texas to Missouri west of the Mississippi River; along the borders between North and South Dakota, Minnesota, Iowa, and Nebraska; along the New York/Pennsylvania and New York/New Jersey borders; and the northeastern border of New York [73]. Historical enzootic sites along the New York border were near transportation terminals where livestock densities were high. Historically, cattle were driven from central Ohio to Dunkirk in southwest New York. There they were loaded on trains to Buffalo [90], and Canada, and shipped via the Black River Canal. Both the New York and Erie Railroad [91] and the Black River Canal [92] connected with the Erie Canal, which carried livestock to New York City. Areas near Dunkirk, New York, the Black River Canal at Carthage, and New York City were historically considered enzootic [73].

From 1945 through 1950, anthrax outbreaks were widespread, with reports in 32 states, of which 16 had no prior reported cases. The outbreaks were primarily attributed to animal feed containing bone meal contaminated with *B. anthracis* [73]; in some cases infection was attributed to live (attenuated) vaccinations [93]. The epizootiology of outbreaks due to bone meal and vaccines was different from endemic outbreaks in several ways [73]: 1) cases occurred in new states where anthrax was not considered enzootic; 2) outbreaks associated with bone meal or vaccinations occurred in every season including winter months; 3) outbreaks occurred across many states; and 4) cases were predominantly or solely in animals eating the contaminated feed or receiving vaccine. Anthrax following vaccinations with killed or weakened bacteria exhibited an incubation period of 3 to 120 days and swelling occurred around the injection site [73].

The geography of anthrax in the United States contracted through the 1960s continued to decrease in area as efficacious animal vaccine and restrictions on international bone meal were introduced [94]. The current enzootic areas are south and west Texas [14, 64] and the Dakotas region [95, 96]. Whether viable and virulent *B. anthracis* spores survive at, and could be released by disturbance of, old carcass sites in the northeastern states is unknown. Outbreaks in wildlife [97] and livestock [98] in Mississippi suggest there might be localized areas of enzootic anthrax in the southwestern Mississippi delta.

From 2000 to 2005, outbreaks were confirmed in North Dakota, South Dakota, Minnesota, Oklahoma, Texas, Nevada, Louisiana, and California [82, 94, 99]. Since 2006, livestock and wildlife outbreaks have been reported from Texas [55, 64] and Montana [14, 42], and livestock outbreaks have occurred in the Dakotas, Minnesota [95], and Colorado [100]. Outbreaks from the past decade have been concentrated in areas defined as having soil conditions for pathogen survival [17, 20]. With the exception of the 2008 epizootic in plains bison (*Bison bison),* Rocky Mountain elk (*Cervus elaphus nelsoni),* and white-tailed deer [42], outbreaks in Montana have only been reported from eastern Montana in the last century [42]. Evidence of confirmed bacteremia in bison from western Montana in 2010 confirms the area may be a re-emerging zone [42]. Large and periodic outbreaks in wildlife species in Texas [55] and Montana [42] suggest that wildlife surveillance is critical to fully defining the extent of ongoing anthrax risk in the United States. Livestock outbreaks have occurred at legacy sites, for example, at a burial site for infected hides at a leather manufacturer; at locations 2.5 km downstream of a contaminated facility; and in plaster with horsehair as a binder [101].

2.6 Historical Incidence of Unintentional Human Anthrax

Humans are susceptible to four anthrax infection types from four routes of exposure: cutaneous anthrax, the most common form of the disease, by direct contact with spores in open lesions or insect bites; gastrointestinal anthrax, by ingesting contaminated meat or possibly drinking contaminated water; inhalation anthrax, by breathing in spores [22]; and, recently identified, injection anthrax by injection of contaminated heroin [102, 103]. The range of incubation periods in humans depends on the route of exposure. For cutaneous anthrax, the incubation range is 1 to 12 days; for gastrointestinal anthrax, the range is 1 to 7 days; and for inhalational anthrax the incubation period is 4 to 45 days, consistent with prolonged spore dormancy within the lung [104].

Typically 20,000 to 100,000 human anthrax cases occur annually [105], with 95% of cases being cutaneous [106]. Most human cases occur in developing countries and are associated with infected animal slaughter or contaminated meat [107]. Despite recent increases in human anthrax incidence in developing countries such as Georgia [2] and Bangladesh [108], unintentional cases in the United States are rare [96]. Environmental locations in the United States where human exposures to *B. anthracis* may occur are not ubiquitous, but are discrete locations where spores were transported or persist.

For human cases of anthrax to occur: 1) *B. anthracis*, most likely spores, must be produced at, or transported to, the site of the outbreak; 2) virulent *B. anthracis* must persist, as spores or through vegetative growth, at the site until a host is infected; 3) conditions must exist supporting

exposure and infection of animals or of the direct exposure of humans; 4) if humans are not directly infected from the persistent *B. anthracis* at the site (unusual), *B. anthracis* must be transferred to humans from the infected animals, animal products, or fomites (from transfer), or via aerosolization, e.g., through soil disturbance. Several sources of unintentional exposure are summarized in Table 1 and discussed in the sections below.

Table 1. Representative Human Anthrax Cases from Unintentional Exposures in the United States.

Location, Year	Source of Exposure	Number of Cases and Route of Exposure	Reference(s)
PA, MA, NJ, NY, DE 1919 - 1925	Leather industry (Tannery)	Not reported	Smyth et al. [109]
PA and other states, 1919-1925	Wool	Not reported	Smyth et al. [109]
PA and other states, 1919-1925	Hair and brush	Not reported	Smyth et al. [109]
United States	Animal contact	Not reported	Smyth et al. [109]
NY and other states, 1919 - 1925	Shaving brush	Not reported	Smyth et al. [109]
Philadelphia, PA 1929 - 1942	Goat skins	Not reported	Smyth et al. [110]
Philadelphia, PA 1929 - 1942	Goat hair	Not reported	Smyth et al. [110]
Philadelphia, PA 1939, 1942	Horse hair	Not reported	Smyth et al. [110]
Philadelphia, PA 1929 - 1942	Wool	Not reported	Smyth et al. [110]
Philadelphia, PA 1929 - 1942	Wool and hair	Not reported	Smyth et al. [110]
Philadelphia, PA 1941	Fur pelts	Not reported	Smyth et al. [110]
DE, MA, NJ, NY, PA, and other states 1939-1943	Hides and skins	Not reported	Smyth et al. [110]
NJ, NY, PA, and other states, 1939-1943	Wool and hair	Not reported	Smyth et al. [110]
U.S., 1939-1943	Shaving brush, fur coat/collars, others	Not reported	Smyth et al. [110]
CA, LA, SD, TX, and other states, 1939-1943	Agricultural	Not reported	Smyth et al. [110]
Northeastern States (PA, NY, NJ, MA, CT, NH), 1945-1951	Mostly industrial exposure	372 cases	Steele and Helvig [73]
FL, 1951	Exposure to skinning dead cow	5 cases	Steele and Helvig [73]
Manchester, NH, 1957	Goat hair	5 cases of inhalation anthrax 4 cases of cutaneous. anthrax	Cohen and Whalen [111]
Manchester, NH, 1957	Across street from goat hair processing mill	1 case inhalation anthrax	Cohen and Whalen [111]
U.S., 1957	Workers at hide or hair mills	4 cases of inhalation anthrax	Cohen and Whalen [111]
U.S., 1948-1957	Near tannery	3 cases of inhalation anthrax	Cohen and Whalen [111]
Four mills in Northeastern U.S., 1955 - 1959	Goat hair processing	21 cases cutaneous 5 cases inhalation	Brachman et al. [112]
U.S., 1961	Secretary in goat hair processing mill	1 case of inhalation anthrax	Bales et al. [113]

Location, Year	Source of Exposure	Number of Cases and Route of Exposure	Reference(s)
U.S., 1966	Truck driver who unloaded goat hair	1 case cutaneous anthrax	Bales et al. [113]
U.S., 1957 – 1971	Veterinarians who necropsied infected animals	6 cases of cutaneous anthrax	Bales et al. [113]
OH, 1964	Pipe insulator goat hair exposure	1 case	Bales et al. [113]
CA, 1976	Weaver with goat hair exposure	1 case	Bales et al. [113]
ND, 2000	Livestock; infected animal carcass	1 case cutaneous anthrax	Centers for Disease Control and Prevention (CDC) [114]
TX, 2002	Laboratory worker handling *Bacillus anthracis*	1 case cutaneous anthrax	Page et al.[115]
NY, 2006 CT, 2007	Made drum heads from African animal hides	1 case inhalation anthrax	CDC [116] ProMED Mail [117]
NH, 2009	Exposed to drumming on “African” drum skin	1 case gastrointestinal anthrax	Adalja [118]

2.6.1 Unintentional Occupational Exposure in the United States Associated with Contaminated Animal Products

Virtually all human cases of anthrax in the United States, other than those due to intentional releases, are cutaneous and are typically associated with the handling and preparing of contaminated animals, processing carcasses, or handling contaminated animal products (i.e., meat, skins/hides, hair, bones, other components of infected animals, products made from contaminated animal parts such as goat hair insulation, imported yarn, knitted sweater, goat hair from horse saddle pads, goatskin handicrafts, bone meal, and drum heads made from contaminated goat hides) [40, 113, 119-121]. Anthrax cases in agricultural settings in the United States typically involve ranchers, veterinarians, or others exposed to diseased animals from direct animal contact during handling, slaughter, butchering, necropsy, ingestion of contaminated meat, or disposal [113]. Trade in infected animals and animal products can transport *B. anthracis* over long distances [40]. Transport of crops in contaminated animal hair sacks, with contaminated hides, or in contaminated ship hulls or containers has resulted in cross-contamination [122, 123] cited in Turnbull et al. [5]). Likewise, bone meal associated with outbreaks has been shown to be contaminated with *B. anthracis* [73, 74]. With the introduction of anthrax vaccines for livestock and encouragement of their use, by 1945 human cases of anthrax attributed to contact with infected animals had declined [124].

In the U.S. there have been 32 documented inhalational anthrax cases between 1900 and 2005. Except for the cases associated with letters from a terrorist containing *B. anthracis* spores (2001) and four cases with no known connection to contamination (1923 – 1947), all human cases of inhalation anthrax were associated with animal hide processing, proximity to animal hide processing, or proximity to animal hair processing [73, 111, 113]. Anthrax occasionally, but rarely, occurred in persons who did not directly handle the contaminated animal parts, such as a

secretary in the main office of a goat-hair processing mill, delivery personnel, and other persons living or working near the contaminated animal processing facility [113]. During a North Carolina outbreak, one textile worker's home was positive for *B. anthracis* out of four textile workers' homes sampled [113]. Routine occupational exposure (daily inhalation of 600 particles containing *B. anthracis* spores) of unvaccinated workers rarely resulted in diagnosed cases of inhalation anthrax. Where a cluster of cases of occupational anthrax was observed, such as in a Manchester, New Hampshire processing plant in 1957 (five cases of inhalation anthrax and four cases of cutaneous anthrax), an unusually high level of *B. anthracis* spores was assumed to be present on the goat hair in use and introduced into the air [111].

Eating meat contaminated with *B. anthracis* was reported to present a low risk factor in the U.S. [113], but has been an important source in the former Soviet Union [107, 125]. While a claim of "low risk" is supported by only one confirmed case of human gastrointestinal anthrax in the United States [118], the risk of gastrointestinal anthrax may be higher than cutaneous anthrax in rural areas of the world where anthrax is enzootic and where one is culturally more likely to eat undercooked meat from ill animals [80, 126]. A Thai outbreak of anthrax associated with an outbreak in cattle and water buffalo resulted in both gastrointestinal anthrax and cutaneous anthrax [126]. Contaminated water has been suspected as a source of infection, but there is no evidence to support the conjecture [113].

2.6.2 Unintentional Occupational Exposure Associated with Laboratory Exposure and Legacy Bioweapon Sites

In addition to accidental exposure to contaminated animal produces, humans have received accidental exposure to spores at historic bioweapon test sites or after release from a laboratory [e.g., Sverdlovsk outbreak from facility release [104]]. Most, if not all, publicly available information on human exposure after a release of *B. anthracis* spores from biological warfare facilities comes from an accidental release at Sverdlovsk, Union of Soviet Socialist Republics (now Ekaterinburg, Russia) [127]. The release resulted from an accidental venting of spores from the bioweapons facility into the outside air where it was dispersed by the wind. Downwind of the facility, at least 68 people, age 24 or older, died from anthrax [104]. The characteristics of the outbreak cases were mapped and the following was observed:

- Human cases (at least 90%) were people who resided or worked within a narrow zone 4 km long extending from the assumed site of the release and consistent with the prevailing wind direction on the date of release (determined to be April 2, 1979)
- Livestock anthrax cases were on the same vector to a distance of 67 km [128]
- Human cases began 2 to 3 days after the release; the last fatal case occurred after an incubation period of 45 days
- Animal cases preceded human cases [104]
- No anthrax cases were known to occur in the region after 1979 [104].

Legacy sites represent a potential source for the introduction of *B. anthracis* spores into the environment. Spores buried at legacy biological weapon production sites may persist. The U.S. biological warfare program included sites in Maryland (Ft. Detrick/Edgewood Arsenal), Mississippi (Horn Island), Indiana (Vigo Ordnance Plant), and Utah (Dugway Proving Ground), and, for animal diseases, New York (Plum Island). Although *B. anthracis* was used at some of

the sites, including field testing, (e.g., Dugway Proving Ground), at other sites *B. anthracis* was not present (e.g., Vigo Ordnance Plant was never operational for *B. anthracis* production) [129]. The risk of human exposure to *B. anthracis* in the environment at a legacy site would depend on many factors including (but not limited to) access to historic weapons sites, the strain *B. anthracis* established in the environment, and whether conditions favor persistence of the spores.

Deliberate releases of *B. anthracis* spores, such as documented on Gruinard Island, Scotland, present a different release and dispersal scenario compared to Sverdlovsk. Small bombs containing *B. anthracis* spores were repeatedly detonated from a gantry over-ground for a prolonged period, resulting in a spore pattern in the residual soil that was highly directional and assumed to reflect the prevailing wind at the time of release. After 40 years, spores were detected more than 50 meters from the point of detonations. Spore concentration in the soil did not show a consistent pattern of decrease with distance; pockets of spores of relatively high concentration were observed beyond areas of lower concentration [130]. The soil at Gruinard Island is a sandstone base overlaid with acidic peat bog topsoil [131], conditions in which *B. anthracis* spores would not be expected to persist [14, 19]. The reason that the spores remained viable has not been reported in the literature and may not be known. Of note, no spores were found in the area in which sheep had died of anthrax after deliberately being exposed to spores dispersed from the bomb [132]. Thus, the soil and environmental conditions did not appear to support persistence of the "natural" spores released from the dead sheep. Persistence of spores intended and prepared as weapons might be different from natural spores, but the original spore-release concentration was very dense.

Occupational anthrax infections in diagnostic and research laboratories in the United States have historically been cutaneous in nature [133]. There continues to be a risk of accidental laboratory-related exposure, primarily for laboratory workers, such as by receiving putative non-viable *B. anthracis* samples containing viable *B. anthracis* or by discarding animal bedding from infected laboratory animals as solid waste rather than as infectious waste [134]. Such exposure occurred without any laboratory workers becoming ill, although most of the exposed laboratory workers were treated with antibiotics once the live *B. anthracis* was detected [134]. Centers for Disease Control and Prevention (CDC) confirmed that *B. anthracis* samples believed to be non-viable were shipped to laboratories that subsequently determined that the samples contained viable *B. anthracis*. A recent transfer of *B. anthracis* within the CDC was inadvertently transported between agency laboratories without confirmation that the spores were completely inactivated [135].

2.6.3 Other Sources of Unintentional Human Cases in the United States

Insect bites (hemophagic flies, mosquitoes, or others) were implicated in the transmission of anthrax in Texas, South Africa, Zimbabwe, and India [14, 65]. In laboratory studies, the potential for such transmissions was demonstrated [65]. In a recent human case in Italy, a hematophagous fly bite was identified as the most likely source of infection [68]. In that report, a sheepherder several kilometers from an anthrax-confirmed sheep death presented with a cutaneous lesion at a fly bite on his arm. Molecular sub-typing linked the herder's strain with that isolated from the dead sheep. No other animal cases were found and the insect bite was the most plausible explanation for infection. That report, coupled with the spatial relationship between biting flies

and deer cases reported in Texas [64], support the need for more empirical work on the role of biting insects in transmission.

While not observed in the United States, intravenous drug users in Scotland, Norway, and Germany contracted anthrax; most cases [15] were in Scotland [102, 103]. Speculation attributes these cases to have resulted from the transport of heroin in contaminated animal skins [102, 103] or to heroin being cut with contaminated bone meal [118].

2.7 Vaccination Efficacy

Vaccination of mammals with live attenuated Sterne strain has been successful in livestock since its introduction in the 1930s [136, 137]. However, cases of anthrax in vaccinated animals may occur [138] for a variety of reasons and has recently been reported in a bison in Montana [42]. Residual virulence due to unpredictable attenuation occasionally results in outbreaks [73, 139]. Vaccinations may be effective in animals for only about six months to a year. A multiple dose regimen is required to ensure effectiveness following an outbreak. Administering vaccine and antibiotics concurrently may prevent development of immunity [79].

While regular vaccination of animals may be effective at preventing outbreaks when used consistently, but because of the high persistence of *B. anthracis*, outbreaks can recur if the vaccinations are terminated [59, 140], even 30 years after a prior outbreak [140]. Commonly an outbreak absence after 10 years without vaccination is considered sufficient to indicate a pragmatic cessation of risk.

Because of occasional severe reactions at the injection site, as well as animal deaths from live vaccine, the Anthrax Vaccine Absorbed (AVA) is used for humans in the United States. The AVA vaccine is a cell-free extract of a non-encapsulated strain prepared from an aluminum potassium sulfate precipitation that contains Protective Antigen [136, 138]. Protective antigen is a component of the genesis of both *B. anthracis* toxins: lethal toxin and edema toxin [141]. Antibodies to protective antigen provide protection against anthrax. The vaccination consists of a series of three subcutaneous injections over four weeks, injections at 6, 12, and 18 months, and subsequent annual booster injections [138].

Vaccine efficacy data for human anthrax are limited to animal model studies and one field study [142]. On the basis of those studies, AVA vaccine is considered to be efficacious in humans and primate models [112, 138, 139, 142, 143]. Results using the rhesus monkey model indicate a two-dose regimen is sufficient to provide protection for almost two years [143]. Depending on the strain of *B. anthracis*, up to 100% of vaccinated rabbits (which have a similar response to rhesus macaques) survived after an aerosol challenge of approximately 100,000 spores, except for one death from a Namibian isolate [142]. Bacteremia was observed in 0% - 80% of the vaccinated rabbits, depending on the isolate. The two most vaccine-resistant isolates (ASIL K7978/Namibia and ASIL K9729/Turkey) were used to provide an aerosol challenge to vaccinated rhesus macaques. Survival was 90% with 25% experiencing transient bacteremia [142].

Human efficacy data is limited to a field study involving comparison of mill workers with occupational exposure to anthrax receiving an AVA-like vaccine to those receiving a placebo (or

neither vaccine nor placebo) [112]. Brachman et al. [112] concluded the vaccine was 92.5% effective at preventing anthrax. Brachman et al. [112] also noted there were no infections among individuals with prior anthrax infections. Some mill workers with only two doses of the anthrax vaccine and an occupational exposure did contract anthrax; three doses are therefore assumed to be necessary [139].

3 Model assessing *Bacillus anthracis* Natural Outbreaks, Unintentional Releases or Intentional Releases

Reviewing the literature on anthrax outbreaks provided insight into characteristics of intentional releases that may distinguish them from natural or unintentional occurrences. Recent studies have provided insight for distinguishing natural from non-natural outbreaks of animal anthrax using specific epizootiological and ecological characteristics [19] and distinguishing natural from non-natural outbreaks of animal diseases using a broad range of characteristics [144-146]. A few of the common criteria across the studies for distinguishing natural from non-natural outbreaks included geographic distribution of the anthrax cases [19, 144-146], season or time that the outbreak occurred [19, 144, 145], appearance of an unknown strain [144-146], presence of an epidemic in a specific population (animal or human) [19, 144, 145], peculiarities in the clinical manifestation of the disease [19, 144, 145], and identification of the agent as a biological warfare agent [144].

An outbreak of anthrax or the detection of *Bacillus anthracis* in an unexpected environment could trigger local, state, and federal responses, particularly when there is the potential for human exposure. Response to the event and subsequent risk management might depend on whether the occurrence of *B. anthracis* is natural, e.g., associated with wildlife or livestock; unintentional, e.g., exposure to a naturally contaminated animal hide or a laboratory-related exposure; or an intentional release of *B. anthracis*. "Intentional release," as used herein, could arise from a variety of scenarios in which an individual or group use a disease agent (i.e., *B. anthracis*) to deliberately cause harm in which the release is no longer considered accidental or unintended, and creates a crime scene [147]. An intentional release could result from exposures arising from criminal production, packaging, handling, or transport of *B. anthracis.* An intentional release could be masked to appear as a natural or accidental event. A claim of responsibility for an intentional release is not proof of anything other than opportunism. Factual linkage must be demonstrated, not assumed or presumed, to establish responsibility for the release. However more weight might be given to such a claim if it were made before the outbreak is recognized and its scale appreciated.

The correct classification of a *B. anthracis* exposure incident as natural, unintentional, or intentional in origin is needed to elicit the appropriate governmental response. This paper's primary concern is the recognition of an intentional event involving *B. anthracis* in the United States compared to naturally occurring outbreaks or unintentional releases. The following categories have been identified to provide examples of potential causes of natural, unintentional, and intentional occurrences that could result in anthrax cases or outbreaks:

[1] Example causes of naturally occurring animal outbreaks:

- unvaccinated animals, or animals without timely boosters, grazing in a pasture where previous outbreaks had occurred or infected carcasses were buried, especially in relation to ground disturbance (ditch clearing, pipe laying, repeated leveling, excavated tannery waste);
- sporadic outbreaks in enzootic areas of west and south Texas, and central and eastern Dakotas, Montana, Saskatchewan, Manitoba;

- hay made in such a pasture contaminated with grave soil, resulting in distant cases in herds where the purchased hay is consumed;
- displacement of contaminated grave soil by floodwater and deposition on downstream water meadows;
- riverbed dredging downstream of an old tannery and deposition on meadows;
- animals fed mineral/salt supplements contaminated with spores from bone meals;
- pigs and dogs with access to carcasses;
- biting-fly infected animal(s) near unreported case;
- animals browsing blow fly-contaminated scrub/bush leaves in proximity to a carcass.
- heavy spring rains and dry summer resulting in excess tabanid biting-fly hatching with access to moribund or dead animals which could transport infection to neighboring or distant ranches [14];
- multi-herd access to a large contaminated batch of commercial feed, minerals or salt;
- feeding captive carnivores meat and bones from a purchased infected carcass;
- sporadic cases in wildlife (i.e., long history in and around to the Wood Bison National Park, Northwest Territories, Canada, and in the exotic wildlife and deer ranches of west Texas and additional reemergence in elk and bison in western Montana).

[2] Examples of unintentional occurrences:

- skinning and butchering, handling of sun-dried hides, or careless disposal an infected carcass;
- contaminated drum heads;
- processing or exposure to contaminated horse hair in old plaster, wool or goat hair ('Bradford Disease');
- buying, receiving, or eating contaminated meat;
- addict injecting contaminated heroin;
- bitten by a tabanid (biting) fly with contaminated mouthparts;
- laboratory exposure;
- inadequate hand washing after handing contaminated objects or wearing contaminated clothes;
- immunocompromised individual in a contaminated environment;
- inexplicable singular cases with no known cause, e.g., tourist hospitalized in Minnesota with anthrax infection with unusual strain from unidentified exposure; serological studies will presumably reveal more such incidents.

[3] Examples of intentional occurrences:

- white powder (hoax) letters to an abortion clinic, mixed with ricin and sent to prison officials, judges, and prosecutors in a recent instance or mailing of contaminated letters [148];
- targeting a much loved or valuable animal, e.g., racehorse, or the devastation of a herd, with a high financial cost;
- using *B. anthracis* with the intent to cause fear in a targeted community, irrespective of the number of victims, whether structured or chaotic.

3.1 Screening for the Likely Cause of a *Bacillus anthracis* Occurrence

This section describes an approach for rapidly screening whether detection of the bacterium or outbreak of the disease is likely an intentional release. While much of what is described here may also apply to Canada and Western Europe, it is too restricted for use in regions where the disease is poorly controlled.

The presence of *B. anthracis* in the environment can be identified in a variety of ways: culture or molecular assay (i.e. PCR) of environmental samples; diagnosis of anthrax through the animal health systems [19, 144]; and BioWatch[1] Actionable Result (detection via PCR) [149]. Confirmation of the presence of *B. anthracis* may lag two to three days or more from the time of an exposure to *B. anthracis* spores (by humans, animals, or air sampler) and their isolation/identification. Some of these methods to identify *B. anthracis* are based on culture while others use more subjective evaluations including observation or direct testing of samples with molecular assays. Direct testing of environmental samples is risky because of the ever-present possibility of multiple organisms contributing to positive responses in the molecular assays. Both immunological assays for *B. anthracis* spores and PCR assays for pXO1 and pXO2 genes have resulted in the misidentification of *Bacillus* species other than *anthracis* as *B. anthracis* [150]. Consequently, use of culture for *B. anthracis* detection remains important for confirmation of positive molecular tests from direct sample testing [150]. Diagnosis of anthrax through the health care and public health systems or syndromic surveillance detecting an increase in anthrax-like symptoms [149] might also indicate that an exposure might have occurred. The recovery of *B. anthracis* spores from a place is not proof of clinical exposure, only of a potential for exposure. Genotyping of the isolates from the environmental location and the clinical samples, and then demonstrating they are identical, confirms the exposure.

A screening approach, including four categories for analysis (shown in Figure 2), is proposed to rapidly and systematically distinguish whether, in the United States, a surveillance detection of *B. anthracis* or clinical diagnosis of anthrax is due to a natural, unintentional, or intentional occurrence. Statements shown in italics are summary statements of information that, collectively, might discriminate intentional from natural or unintentional occurrences (screening statements). These are supplemented by keys leading to indicators suggesting an event may be intentional. The screening approach considers whether:

- In a natural occurrence, observed *B. anthracis* strain(s) is/are consistent with those previously recovered at the site or area, or is/are consistent with a likely source of spores [10, 86]. Normal isolates in a natural occurrence will show no evidence of sophisticated spore modification, e.g., microencapsulation for environmental protection and for rendering spores invisible to biosensors; of multiple antibiotic resistance; or of having been genetically modified to remain fully virulent in spite of prior vaccination.

[1] Department of Homeland Security BioWatch System is a program for detection of biological warfare agents in the air via air filters in major U.S. cities.

- An intentional occurrence is indicated when immunized livestock and/or people are contracting anthrax at a higher than expected incidence [21, 73, 79, 112, 139, 142, 143, 151]. However, immunity is naturally short and in livestock it can be minimal if they were vaccinated while being treated with antibiotics.
- A natural or unintentional occurrence is indicated when the location of the occurrence is at the site of a previous outbreak or along a cattle trail in a region with calciferous alkaline soils, or near a biological laboratory, near a tannery or other animal processing facility, or where a potentially contaminated animal product is or was in use [18, 40, 113]
- Natural outbreaks in animals often coincide with the time of year for grazing or browsing livestock or wildlife (late spring, summer, or early fall) [14, 19, 76]; winter outbreaks are not unknown but are common only when livestock are fed commercial feeds containing contaminated bone meal.
- The environmental factors are appropriate for an outbreak. For example, during a natural outbreak, the outbreak is often preceded by heavy rains followed by drought [13, 58, 59, 63, 140]; during a hot-dry period outbreaks will follow when there is a brief shower that stimulates growth of vegetation for grazing. Sporadic outbreaks will occur during a drought but characteristically seldom involve secondary cases.
- The livestock most commonly affected are cattle, both from enteric and cutaneous disease during a natural occurence. Anthrax is also common in sheep and goats. Pigs are semi-resistant but will be affected with contaminated fibrous feeds or from scavenging. Horses, though they can be infected from grazing, textbook-traditional cutaneous lesions would result from being infected by biting flies radiating out from a previous case [152]. Hyenas, wolves, and coyotes are to differing degrees resistant when scavenging carcasses. African wild dogs (*Lycaon pictus*) appear more sensitive to anthrax [153]. Serological surveys confirm that domestic dogs, which are moderately resistant, can act as sentinels when index cases are missed, though dogs do succumb to infection [19, 30, 40, 41, 44, 51, 54, 76, 79, 154].
- Epidemiology and pattern of cases are consistent with a natural cause: the number of human or animal cases, pattern of cases over geographic region and time (e.g., radiating from an index case when biting flies are involved, or a number of farms more or less at the same time when a contaminated feed product is involved and the distribution reflects sales), and type of human infections (e.g., mostly cutaneous) [13, 14, 19, 58, 60, 76, 79, 104, 155].
- Unintentional occurrences such as laboratory-based infections are not uncommon though this depends on the organism: for example, brucellar infections are a constant threat. Between 1941 and 1975, laboratories in the United States suffered 40 anthrax infections with three deaths in 1941, 1951, and 1958, in spite of the use of laminar flow cabinets since 1950 [156, 157]. As anthrax is non-contagious, accidental laboratory infections will be limited to a direct or indirect exposure, not infrequently from centrifugation, and will be from a documented laboratory strain and will usually result in the cutaneous form of the disease. Cases more often arise in those individuals working with the organism, then from accidents, infected animals/carcasses, and contaminated discarded glassware. Clerical and maintenance personnel are known to have been exposed [158]. A cutaneous case occurred in 2002 in a laboratory worker as a result of unknowingly handling contaminated vials of *B. anthracis* when not wearing gloves [159, 160]. Exposure is usually limited but an exception is the Sterne vaccine strain, which has a reputation for

environmental contamination. Unless the laboratory is handling specially processed dry or microencapsulated spores, a lung infection would not be expected.

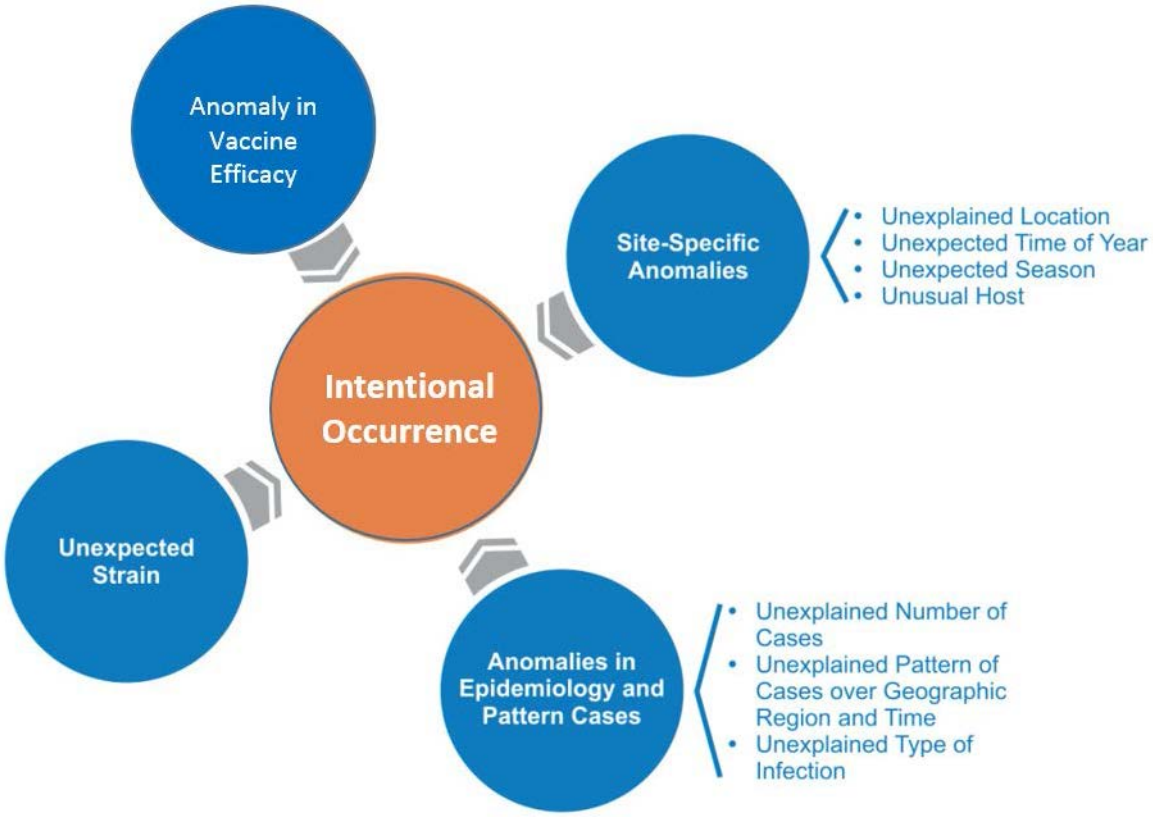

Figure 2. Screening categories for rapidly evaluating likelihood that a *B. anthracis* occurrence in the United States is intentional.

3.2 Screening Category I: Unexpected genetic strain?

Bacillus anthracis is a highly clonal bacterium, typified by a high degree of genetic homogeneity [32, 161]. Genetic diversity has most often been defined using single nucleotide polymorphisms (SNPs; [162]), multilocus variable number tandem repeat analysis (MLVA) using eight [161], 15 [32], 25 [163], or 31 [164]) markers. More rapidly evolving changes are often measured with single nucleotide repeats (SNR; [95]). Broadly, MLVA types fit within major lineages defined by canonical SNPs (those that identify terminal branches within a phylogenetic analysis) and SNRs are interpreted within MLVA-types [165] progressive hierarchical resolving assays using nucleic acids scheme). Geographically, canonical SNPs identify broad lineages, usually at the national or regional scale. MLVA diversity appears to be related to more regional spatial scales [32, 87], with SNRs most useful locally and within MLVA-types. SNRs are subject to homoplasy and less useful when like SNR types are defined between MLVA-types. With this in mind, it is reasonable to expect the genetic diversity during an outbreak in an enzootic region to be consistent with that observed in previous outbreaks at that location or neighboring locations within the region. Multiple MLVA and SNR types have been observed during a natural outbreak

when environmental conditions are favorable suggesting multiple historical introductions [59]. However, the genetic diversity observed will be dependent on the geographic scale of the outbreak, with larger numbers of farms or wildlife populations equating to a potential for higher diversity. Within-farm diversity is often quite limited and more likely detected with SNRs once MLVA types are established. However, unpublished observations by Hugh-Jones indicate that when the strains recovered are from an area historically exposed to contaminated feed or salt, or from a recent distribution of a contaminated feed, there might be interfarm variance, differences between animals within a herd, and even sometimes multiple strains recovered from a singular animal.

Phylogenetically, *B. anthracis* is divided into five lineages (A-E following Lista et al. [163] or A-D and Aβ following Maho et al. [166]). Many available isolates in the global strain collections represent three major lineages, designated A branch (A.Br.), B branch (B.Br.), or C branch (C.Br.) based on canonical SNPs and their respective sub-lineages or sub-groups based on MLVA types. Genetic analyses of *B. anthracis* samples from a variety of North American sources associated with livestock and wildlife outbreaks, confirmed the presence of a single strain (i.e., A.Br. Western North America [WNA]) in 91% (352 WNA of 387 strains analyzed) of SNP samples tested [86], although sample selection in that paper under-represented diversity of Ames and Sterne-like strains from west and South Texas [32, 55, 87]. As shown in Table 2, the 167 samples analyzed by Kenefic et al. [86] showed that most were from western states where anthrax is enzootic (Colorado [CO], Minnesota [MN], Montana [MT], North Dakota [ND], New Mexico [NM], Nevada [NV], and South Dakota [SD]) and were exclusively A.Br.WNA (using canonical SNPs from Van Ert et al. [32]).

Table 2. Strains of *B. anthracis* associated with livestock and wildlife outbreaks in the United States as adapted from Kenefic et al. [86]

State	Observed Strains	No. of Samples	State	Observed Strains	No. of Samples
CA	B.Br.001/002	2	**OK**	A.Br.Aust94	1
CO	A.Br.WNA	1	**SD**	A.Br.WNA	53
FL	A.Br.Ames	1	**TX**	A.Br.001/002	3
IA	A.Br.WNA	1	**TX**	A.Br.Vollum	1
LA	C.Br.A1055	1	**TX**	A.Br.WNA	4
MD	A.Br.Vollum	1	**UT**	A.Br.WNA	2
MD	A.Br.WNA	1	**WT**	A.Br.WNA	1
MN	A.Br.WNA	23	**WY**	C.Br.A1055	1
MS	A.Br.Vollum	1	**WY**	A.Br.WNA	2
MT	A.Br.WNA	1	**USA***	A.Br.001/002	1
NC	A.Br.WNA	1	**USA***	A.Br.Aust94	1
ND	A.Br.WNA	26	**USA***	A.Br.003/004	1
NM	A.Br.WNA	1	**USA***	A.Br.Vollum	2
NV	A.Br.WNA	5	**USA***	A.Br.WNA	28

Source: Kenefic, L.J. et al., (2009). Pre-Columbian origins for North American anthrax. *PLoS One*, 4 (3)**:** e4813.
*No state specified. WNA, Western North America. No specific years for outbreaks given.

What is clear from the North American phylogenetic analyses is that the A.Br.WNA group is well established from Canada into Texas, matching its wide distribution throughout the world [32, 154, 163]. Other genetically-divergent strains of *B. anthracis* found in the United States are assumed to have originated from handling, processing, and using imported animal hides, hair, wool, bone meal, and other animal parts contaminated with *B. anthracis* spores indicative of their regions of origin [32]. Where justified by suspicion of an intentional release, additional genetic testing of a strain (A.Br.WNA for example) may be useful to compare the suspect strain to the local strain(s) at a higher level of genetic detail [167]. Over time the reassessment of archived reference strains have shown instructive minor differences. For example, SNR diversity has been used to differentiate *B. anthracis* strains within large multi-property epizootics in Italy with related minor strains of the singular major epidemic strain taking advantage of the epidemic to infect a few spatially related herds [59].

B. anthracis Ames (A.Br.Ames) has been broadly used in biodefense research [32] as a challenge strain. The organism used in the 2001 Amerithrax attacks was a laboratory strain of Ames [168]. The natural distribution of the classic Ames strain is limited in the U.S. and localized to an area west of Uvalde, Texas; it was originally recovered from a single cow carcass in 1981 in south Texas [32, 86, 169]. Ames-like strains have also been reported from Kazakhstan and Kyrgyzstan [154] and across Inner Mongolia in China [87], and would have reached the U.S. in imported contaminated goat hair or hides sometime in the latter 20th century. Presence of an Ames-like strain in any location except western Texas (defined as the Enzootic Zone by Blackburn et al. [64]) and southern Texas, within Jim Hogg County, could indicate human-cultured spores and (in the absence of an accidental laboratory exposure or release, or import of contaminated animal products from southwest Texas) could signal a possible intentional release. Additional genetic testing of an A.Br.WNA strain may be useful to compare the suspect strain to the local strain(s) at a higher level of genetic detail [167]. Following Lista et al. [163], a high number of markers, such as MLVA-25, should be considered.

The stage in the analysis considers whether there is a likely source for the exotic or rare strain that has not been intentionally released. Genetic data are required on the identified background strains, strains in use in nearby laboratories, or strains endemic to the origin of potentially contaminated animal products at that location. *The presence of an unexplained genetic isolate of B. anthracis is a sufficient anomaly to be subject to intentional release suspicion.*

3.3 Screening Category II: Anomaly in Vaccine Efficacy?

Vaccination of mammals with live Sterne strain (lacks the pXO2 plasmid) has been successful in livestock since its introduction in the 1930s [136, 137]. The Sterne vaccine is currently the most widely used anthrax vaccine and is generally safe and effective for most animals [137, 139]. Reduced doses are needed when vaccinating llamas and goats because of their susceptibility to the low pathogenicity of Sterne. Animals acquire immunity from a single dose in 7-10 days and protection lasts about 9-12 months [136]. When used to protect animals during an outbreak, deaths from incubating infections are observed to decline substantially by the tenth day after vaccination [60]. Anthrax cases among effectively vaccinated livestock or individuals are of concern if only because it reflects exposure, but it can also indicate inadequate vaccine protection. For as yet unknown reasons this is a constant problem with certain Sterne livestock vaccines [170].There are limited studies on the efficacy of the human vaccines [139]. Also

livestock deaths are sometimes seen in purchased unvaccinated stock added to a vaccinated herd grazing a pasture with a history of previous anthrax cases.

Cases of anthrax in vaccinated animals may occur for a variety of reasons. Residual virulence due to unpredictable attenuation in the Pasteur vaccine resulted in a 3% mortality in vaccinated animals, which is why it was abandoned [73, 139]. Anthrax cases appearing soon after vaccination of animals could be due to a lethal incubating infection prior to the onset of protection. If due to the vaccine, the strain can be isolated and confirmed as the vaccine strain (e.g., Sterne). Vaccinations are effective in animals for six months to a year. During an epidemic, a multiple dose regime may be required to ensure effectiveness especially with high value stock. Administering vaccine and antibiotics concurrently may prevent the development of immunity [79]. However some have argued that as spore germination is not simultaneous, sufficient spores will germinate late after the antibiotic blood titer has fallen to an ineffective level and thus an adequate immunity will follow. Although it is better to wait seven to ten days after prophylactic antibiotic treatment before vaccinating, working a herd twice carries both an added stress cost to the livestock and financial cost to the owner.

A cell-free antigen vaccination method is used for humans in the United States [139, 170]. AVA efficacy was tested against several strains of *B. anthracis* in animal models. For certain isolates, AVA was highly protective in both rhesus monkeys and rabbits, but only variably effective in guinea pigs [142]. The only placebo-controlled study of the cell-free human anthrax vaccine evaluated effectiveness among workers in four textile mills who experienced chronic occupational exposure to *B. anthracis* spores on contaminated goat hair. The study reported 92.5% effectiveness with a lower 95% confidence limit of 65% [112]. The duration of immunity from the two-inoculation human vaccine is believed to be 1-2 years [136, 171], with a three-inoculation series protecting up to four years [139]. A five-dose series of a cell-free vaccine (BioThrax™ Anthrax Vaccine Adsorbed, Emergent BioSolutions, Rockville, MD) over 18 months is currently the human vaccination approach approved by the U. S. Food and Drug Administration [172]. According to the BioThrax™ product insert, incomplete vaccination results in a decline in protective antigen antibodies thereby lowering protection.

Effectively vaccinated animals are those that received the vaccine within the past six months and were not receiving antibiotics at the time of the vaccination or the weeks thereafter. Effectively vaccinated humans are those who are within the expected period of immunity for the series of vaccine they have received. Anthrax in effectively vaccinated animals or humans during the expected period of protection (minimally six months) should be considered suspect. Given the high efficacy of vaccines, anthrax occurring among a significant number of effectively vaccinated animals may indicate the presence of a particularly virulent strain of *B. anthracis* in the environment, or a deliberate release of spores of unknown origin for which existing vaccines may not be effective. *Intentional releases should be suspected in outbreaks among effectively vaccinated animals by an unexpected strain, e.g., genetically modified B. anthracis [173]. Anthrax cases in vaccinated humans, especially those without known health compromises, should be considered an indication of possible intentional contamination*. However as present human vaccines require a discipline of multiple injections stretched over time, incomplete series are always a possibility.

3.4 Screening Category III: Site Anomalies Observed?

Normal anthrax outbreaks are not ubiquitous, but occur at discrete locations where *B. anthracis* was transported or persists due to an alkaline soil with a high calcium content. Locations where anthrax outbreaks may occur include those near:

- Historic outbreak sites or carcass burial sites, and downstream (as in water) from such sites – downwind risk is limited to <20 meters with high wind speeds or mechanical soil disturbance, and minimal risk because of large particle sizes [56];
- Historic cattle trails or wildlife (herbivore) grazing habitats within the historical range of anthrax;
- Historic or current industrial animal processing sites (e.g., slaughter houses, tanneries, wool processing, hair processing, fur processing, feed manufacture); nearby locations where spores are carried by wind or washing waters, or where waste has been buried;
- Biodefense laboratories, culture collections, testing ranges, or historic biological laboratories; off-site handling of contaminated laboratory clothing can result in reaerosolization, which can pose a hazard [174];
- Laboratories with United States Department of Agriculture (USDA)/CDC approval for research on virulent *B. anthracis*

Recently there have been two singular human pneumonic anthrax cases, neither from an identified exposure. Fortunately, both survived. Their exposures were probably accidental and not purposeful. One case in the United States is an amateur rock collector while on holiday and traveling [96]. The other is a vehicle maintenance mechanic in the United Kingdom armed forces and previously vaccinated [175]. Over time and random serum testing others may be identified with no known exposure.

In addition, human anthrax may occur during the transport of contaminated animal products, e.g., containers, vehicles, and storage areas; or the use of such animal products, e.g., drum heads, bone meals, and horse hair plaster binding. Human cases of anthrax arising from exposure to contaminated animal products or accidental laboratory release can occur at any time of the year and in any weather. The key factor is exposure to *B. anthracis* spores from such contaminated sources by contact, ingestion, or inhalation. Exposures have been associated with disturbance of legacy carcass sites [5], playing drums made from contaminated skins [118], and disturbing pipe insulation made with contaminated goat hair [113].

In contrast, human cases arising from exposure to sick livestock or wildlife are most likely to occur in the United States during drought conditions following a wet spring [14]. Such conditions can also trigger high biting fly activity, which may expand the size of an epizootic [55] and lead to subsequent human cases, particularly those in close contact with animals, like herders [68]. However, regional drought/rain conditions may not trigger region-wide outbreaks. Recently, Blackburn and Goodin [61] illustrated that epizootics are associated with localized changes in vegetation greenness (a proxy for moisture) that appeared to promote anthrax in one

area of a region and not another. In the United States, such natural outbreaks, as well as singular sporadic cases, most often occur during the late spring through early fall, and end with the onset of cold weather [14, 61]. Human cases of anthrax arising from contact with wildlife or livestock during the winter months or cold weather in enzootic regions should be considered a possible intentional occurrence when other explanations for the initial wildlife or livestock exposure have been ruled out, e.g., livestock consumption of contaminated feed. Wintertime livestock anthrax is not usually expected in the United States, though cases among grazing animals are known to have occurred.

Anthrax can and does infect a wide variety of livestock, wildlife, and rheas and ostriches [19, 22, 40, 58, 140]. In the United States, the typical livestock hosts for anthrax are grazing animals, primarily cattle [19], followed by plains bison [14]. Other susceptible animals include wood bison, white-tailed deer, and antelope, kudu, and lions in exotic animal ranches (common in Texas and elsewhere), and horses, sheep, pigs, dogs, goats, and mink [19, 39, 40, 44, 60, 70, 79, 140]. Cattle generally have more cases and higher mortality rates than other domestic herbivores; mortality rates in white-tailed deer can reach 100% and though only 10% of wood bison will die, another 70% may serologically reveal exposure [36]. However, the presence of anthrax illness in large numbers of animals thought to be resistant to anthrax illness (i.e., require extremely high doses to contract illness) may suggest an intentional release-related exposure. For example, dogs and pigs are moderately resistant to anthrax [176]. Thus an outbreak among dogs in the United States might indicate that affected dogs were exposed to high levels of aerosolized *B. anthracis* spores (as was noted in Sverdlovsk in 1979 [104]), or had scavenged on undocumented carcasses [41], or had been fed contaminated meat (as recently reported in Ukraine [44]). Efforts should be made to determine the sources of dog infections. Thus, in the United States, canine exposure in a farmyard in an enzootic area is not unexpected but, in an urban area, canine exposure is notable.

Laboratory based infections with *B. anthracis* do occur even when care is taken. Unless a specially processed strain is involved that would have increased its aerosol potential, e.g., microencapsulation, it is usually just a minor cutaneous infection. But centrifuge accidents, e.g., broken vials, can and do result in aerosol exposure as well as laboratory contamination. Workers must be aware that outer gloves used within a laminar flow biosafety cabinet should be removed prior to removing hands from the cabinet and outer gloves replaced before touching any other surface outside of the cabinet. However, a review of select agent theft, loss and release reports in the U.S. between 2004 and 2010 revealed 727 such reports of which 12% were losses (primarily due to sample mislabeling or misplacement within the lab) and 88% were release reports – or one to nine reports per 1,000 worker years – of which only 11 resulted in laboratory acquired infections, none of which were with *B. anthracis*; ten were other bacterial infections and one a fungal agent. Thus, laboratory infections with select agents appear to be rare and, therefore, of consequence when observed, but unrecognized releases are by definition unrecorded [177]. That should not stop investigations to look for releases, for example, through routine blind swabs of surfaces likely to get contaminated.

3.5 Screening Category IV: Anomalies in Epidemiology?

Human cases of anthrax in the United States are rare [114]. Most anthrax cases in the United States (other than those arising from the 2001 letters) are cutaneous, traditionally from skinning an infected carcass. However, a few pneumonic cases have been associated with contaminated-

hide drum heads. When wool and hide mills were common, up to 60 years ago, processing imported contaminated wool, hair, and hides, pneumonic anthrax posed a constant threat but they have been shut down. Two cases with no known exposure have occurred in the United States and United Kingdom (see Section 3.4) and the source of their infections remains unknown. With prompt treatment, cutaneous anthrax is rarely fatal; without treatment, the fatality rate for cutaneous anthrax ranges between 10% -20% and is virtually certain if systemic symptoms are present [114]. Only one case of gastrointestinal anthrax has been confirmed recently in the United States and it was associated with contaminated skins used on drums rather than eating contaminated meat [118, 178]. This was in a community hall that had witnessed some 30 years of public drumming events. In 2000 six clinical cases were suspected following the consumption of some contaminated meat but not confirmed because of prior antibiotic treatment [179]. While family and community-based outbreaks of cutaneous and gastroenteric anthrax occur in Central Asia and Africa due to the slaughter and butchering of sick animals and the scavenging of meat from dead animals, such events are not seen in the United States and Canada – the Minnesota incident was an exception. Recently injection anthrax has been seen multiple times in the United Kingdom and Europe from the use of contaminated heroin, and has resulted in high death rates [180]. While many rural physicians are aware of the risk, especially in North and South Dakota, Texas, and the Canadian prairie provinces, urban doctors are less likely to recognize the disease until it is severe or at autopsy.

The immunity to anthrax, whether from a natural exposure or from vaccination, is short-lived [36]. Therefore any unvaccinated person demonstrating antibodies has been exposed sometime in the previous six months; the same applies to a previously vaccinated person with a higher than expected titer. Not all animals die from anthrax and thus animals exposed during an outbreak or epidemic, with or without clinical disease, may demonstrate antibody titers [36, 45, 89]. This can also be in the absence of known coincident outbreaks. For example, as part of the annual brucellosis surveillance, repeated random bison bleedings at the MacKenzie Bison Sanctuary over many years demonstrated sporadic protective antigen antibody positive animals even though the routine aerial surveillance for anthrax carcasses had found no cases at the time (Betty Golsteyn-Thomas, CFIA - ADRI Lethbridge, personal communication, April 11, 2014). *This represents a silent or paradoxical epidemiology but note should be taken when anthrax occurs in the absence of any logical normal explanation, e.g., an urban office worker distant from any hypothetical natural exposure.*

Most cases of human anthrax occur within seven days of exposure [151]. The incubation period range for cutaneous anthrax is from one to 12 days; for gastrointestinal anthrax one to seven days; and for inhalational anthrax one to 45 days, consistent with prolonged spore dormancy within the lung [40, 104, 151, 181-183]. While inhalation anthrax incubation is generally reported as about one to five days, the incubation period is dose-dependent, with a lower dose corresponding to a longer incubation period [184]. Long latency periods, e.g., 30 days, have been occasionally observed in humans [104] and over months in animals [14, 185]. *However, a continued high level of human cases beyond day seven of an initial outbreak may indicate possible on-going intentional releases and exposures. Given the rarity of anthrax cases in the United States today, if multiple human cases cannot be attributed to a common source of exposure such as a contaminated animal, carcass site, laboratory, or a contaminated animal product, an intentional contamination should be suspected. Cases of human inhalation anthrax*

are extremely rare and typically occur as individual cases. Multiple cases of inhalation anthrax are likely to be intentional or associated with an unintentional laboratory release.

As a result of the 2001 contaminated letters incident, the United States Postal Service® (USPS) set up a Biohazard Detection System (BDS) to provide mail security without disrupting the flow of mail from individuals and small organizations. The BDS assumes that all bulk mail from commercial mailers is safe. The BDS is attached to a specific piece of automated postal processing equipment, Advanced Facer Canceller System (AFCS) machines, which are used to scan mail for their proper postage. As the mail is processed by the AFCS, air samples are continually collected by the BDS and analyzed for the presence of *B. anthracis* spores. A positive test signal stops the mail processing, triggers alarms, and informs emergency responders. But absent a positive signal the air sampling does not affect the mail flow. The unique characteristic of the AFCS machines is that they exclusively process collection mail put into street collection boxes, from homes, small businesses, and individuals. Other equipment, such as Delivery Bar Code Sorters are used for bulk mailings from large mailers. BDS was not the only security system to appear after the anthrax letters. The USPS introduced a suite of services known as 'Intelligent Mail' to confront bioterrorism. It is an assemblage of interlinked technologies, including bar codes, scanning equipment, and software that generate, store, and manipulate real-time data from the postal network. The Intelligent Mail barcodes contain unique identifier information indicating mailer and recipient, routing details, and service type. The real-time processing and distribution data generated can be used to isolate cross-contaminated mail and reroute other mailings away from cross-contamination sites. This wealth of Intelligent Mail data would aid investigators in retrospectively tracing the movement of contaminated mail through the system [186]. *Assuming it is as efficient and reliable as claimed, the postal system not only identifies individual pieces of contaminated mail, but provides a holistic view of the event, whether it is unintentional or intentional, as it evolves.*

3.6 Screening Approach for Location-Specific Anomalies and Epidemiology

There are a number of significant singular events which alone indicate a possible intentional event in the United States and are worthy of further investigation:

[1] multiple unrelated cases of anthrax in humans, especially inhalational anthrax;
[2] an outbreak in livestock east of the Mississippi;
[3] a human outbreak that does not involve drums or an obvious occupational exposure;
[4] an outbreak of inhalational anthrax of no obvious cause;
[5] simultaneous or near-simultaneous outbreaks in multiple locations (multi-foci) without an obvious common cause;
[6] cases in individual animals or persons who had been fully vaccinated within 6 months with a recognized valid vaccine;
[7] isolation of multidrug-resistant strains of *B. anthracis*;
[8] an incident that involves envelope(s)/packages containing loose dry *B. anthracis* spores;
[9] the recovery of loose spores in an atypical location (e.g., as in purposefully contaminated animal feed, in HVAC filters, on urban surfaces) or in a vial, outside of a laboratory, especially with evidence of sophisticated spore modification, e.g., microencapsulation;
[10] *the recovery of an unexplained genotype or of genetically manipulated spores.*

Figures 3-7 present keys incorporating both location-specific characteristics and epidemiology for evaluating the likelihood that a *B. anthracis* occurrence is intentional. The keys are not strictly dichotomous; rather, they constitute a systematic path for gathering indications that an occurrence is (or is not) intentional. Therefore, in some cases a statement to "suspect intentional activity" is followed by a loop back into the key to answer additional questions that may lead to additional indicators of an intentional occurrence. As described following the keys, cumulative indicators of suspicion lead to an overall conclusion as to whether suspicion of an intentional release is warranted. Only in specific cases, e.g., presence of spores displaying sophisticated spore modification, will a single indicator lead to a strong conclusion that an intentional release is likely.

The quick screen is biased toward an assumption of an intentional release. There are exceptions to the logic that is used. For example, long latency of *B. anthracis* could cause an outbreak in an unexpected season. Further, individual cases of anthrax sometimes occur with no suspicion of an intentional act, but without a source of exposure ever being found.

Questions in the key in Figure 3 point to the location-appropriate key (in Figures 4 through 7). If the location of an occurrence falls outside of the logical locations where *B. anthracis* might be expected, the occurrence should be interpreted as a potential intentional release.
Using the keys above in the sequence shown, the following interpretation is proposed. A single response of "assumed to be an intentional exposure" suggests a high likelihood of an intentional release incident. Two or more questions in the keys yielding "suspect intentional exposure" outcomes likewise suggest a high likelihood of an intentional incident. A single "suspect intentional exposure" coupled with other answers suggesting a deliberate act as a possible explanation requires further investigation beyond the screening approach presented here to distinguish whether the occurrence is likely an intentional release. The screening approach provides a rapid indication of whether the occurrence is likely intentional. It is not intended for making decisions regarding whether the act was criminal in nature or to be used for public health decisions.

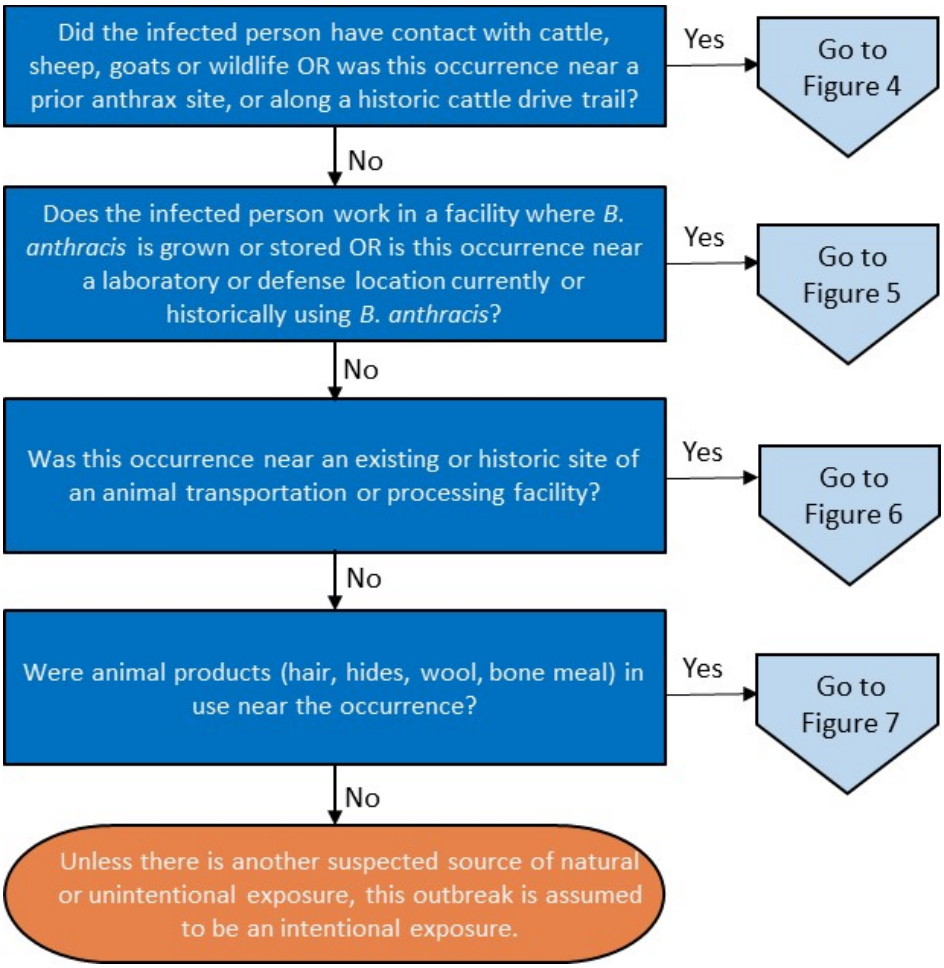

Figure 3. Key to location-specific screening for intentional occurrences.

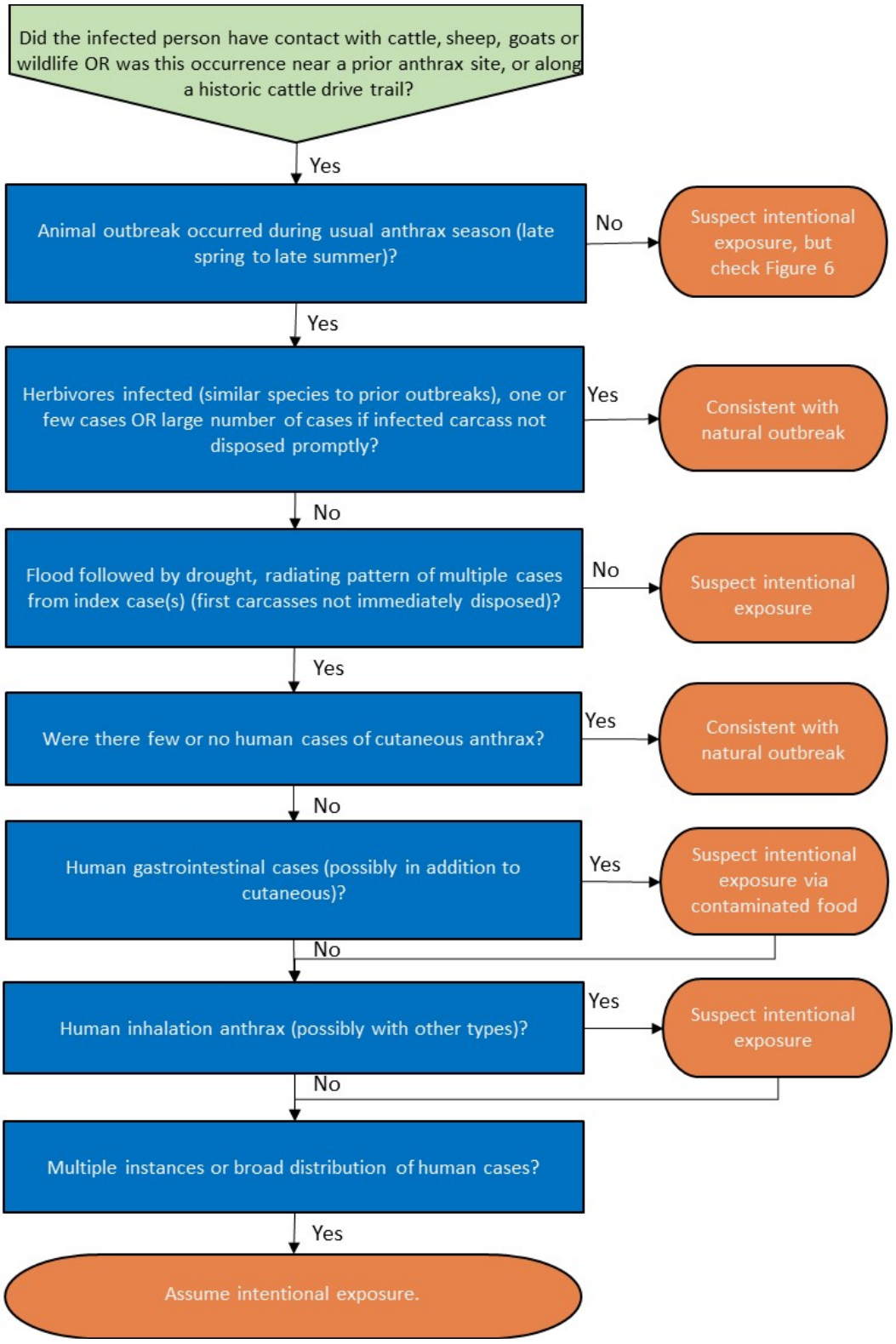

Figure 4. Screening for intentional occurrences in an agricultural location OR occurrences in natural locations (wildlife).

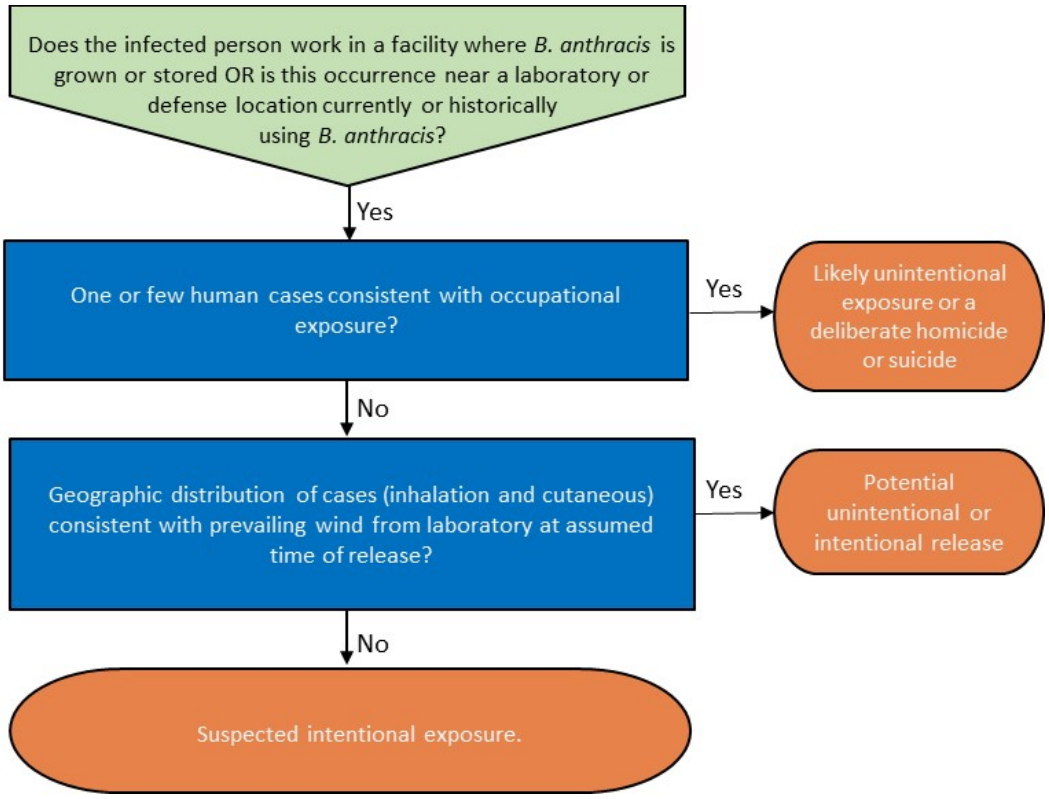

Figure 5. Screening for intentional occurrences in a laboratory location.

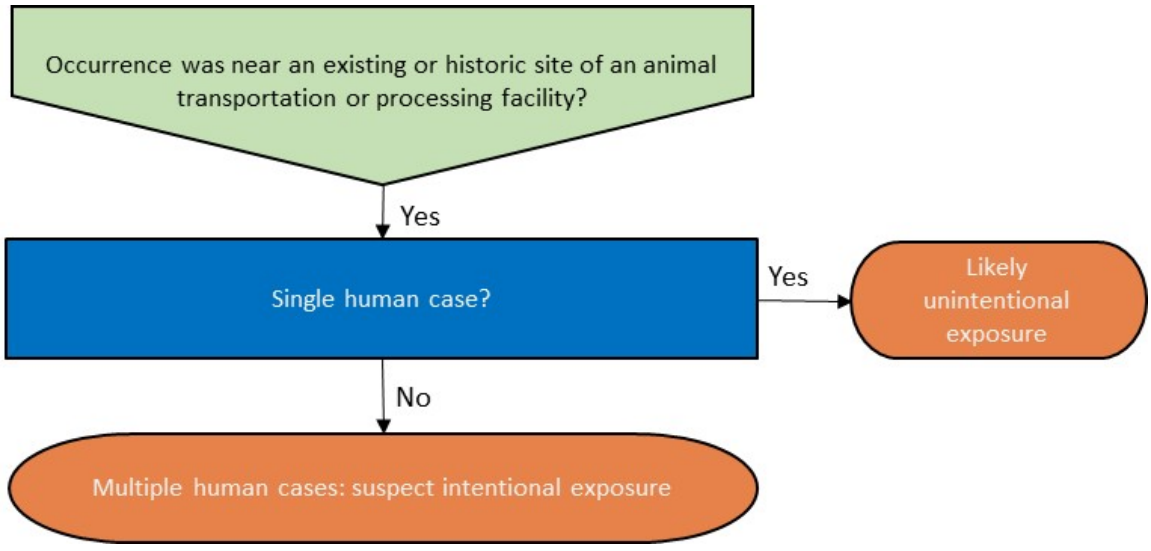

Figure 6. Screening for intentional occurrences in processing plant or animal transportation locations.

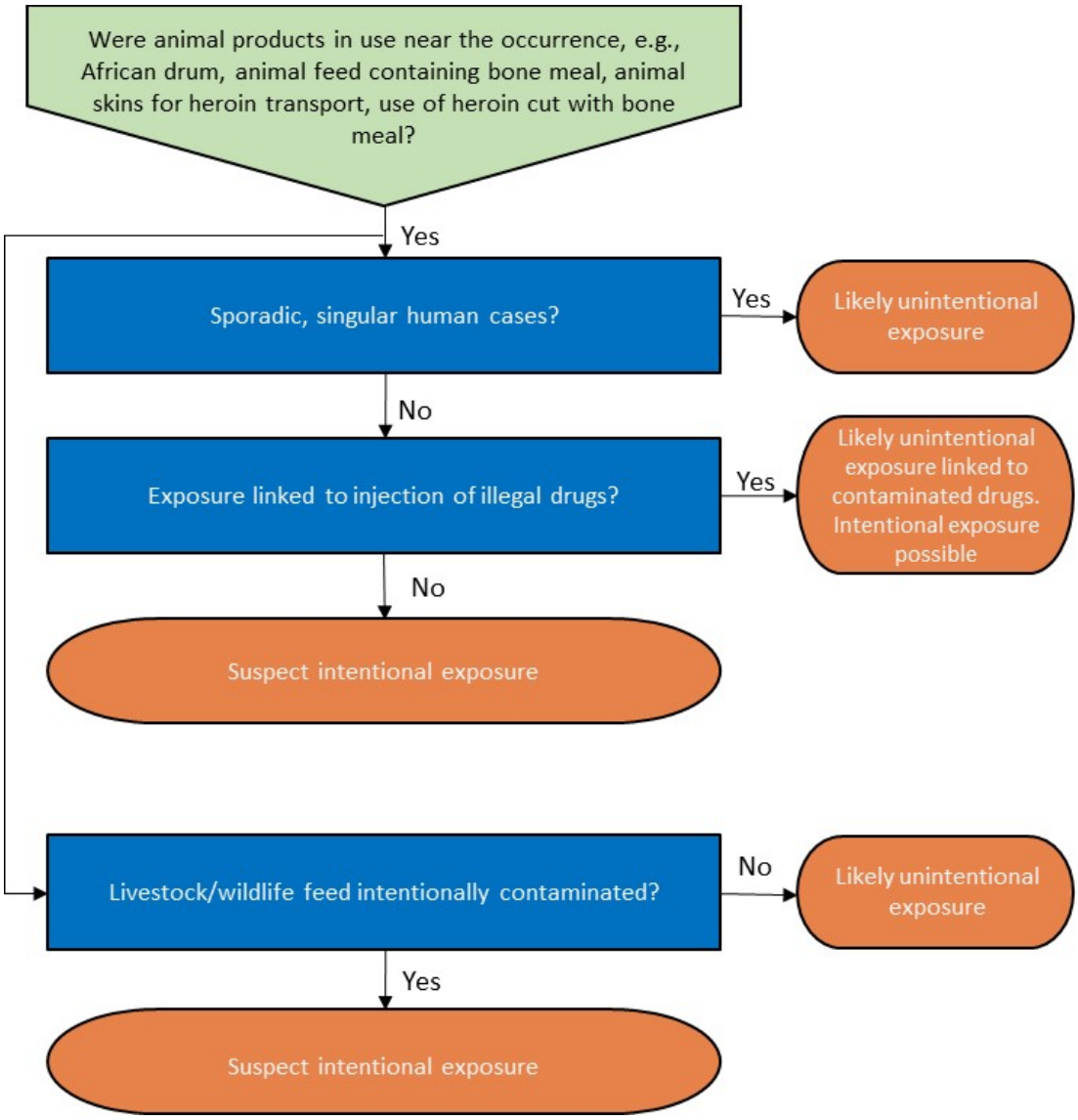

Figure 7. Screening for intentional occurrences in locations where animal products are in use.

3.7 Conclusions

Compared to a natural outbreak or epidemic, an intentional anthrax spore release will occur in the wrong place, at the wrong time, in the wrong group, or with the wrong strain; if from a massive aerosol, there may be multiple cases. Ignoring the repetitive and imitative talcum-powder attacks on abortion clinics, such an attack with anthrax spores is, at the moment, more a matter of theory than reality but ricin has appeared in letters [187]. The footprint for an intentional attack is unpredictable. While the great majority of anthrax related events might be naturally occurring, understanding of the nature of the occurrence might be confused by our still evolving knowledge of the disease, the agent, and its epidemiology. The screening approach presented here is designed to help rapidly and systematically determine the likelihood that a

detection or exposure is intentional. A limited number of parameters need to be considered in this screening process and, given ready access to necessary data, an initial evaluation can be completed quickly, except for identifying the strain of the *B. anthracis*, which will require about 48-96 hours using current technologies.

The culture collections associated with anthrax sites in the United States are limited. It is possible, even likely, over the coming years new and unexpected strains of *B. anthracis* will be discovered in natural environments that support spore survival. This will not reflect an intentional release event, just the discovery of new pre-existing strains in the environment. Following an intentional release, recovery of the organism might be possible from multiple surfaces in the exposed zone; in ranch land it might be recoverable from trees, flowers, grass, soil, and fruits from trees; in urban locations, from windowsills, air condition filters, exposed granite surfaces, benches, automobiles, and so on. Typically, *B. anthracis* is difficult to isolate from environmental surfaces so having multisource samples all yielding *B. anthracis* in the absence of carcasses points to an intentional release.

4 Implications for Clean-Up of Occurrences of *B. anthracis* and Identified Gaps

Roles and actions in the response to an occurrence of biological threat agent depends both on the nature of an occurrence and the risks associated with an occurrence. It is important to know whether an occurrence of biological threat agent is natural, unintentional, or an intentional human act. The risk to humans (or animals) of a biological threat agent detected in the environment needs to be understood to inform response and decontamination decisions. Risk is a function of dose and exposure. If the detected biological threat agent was deliberately released there might be associated factors that increase the risk, e.g., high concentration of spores for exposure, and/or sophisticated spore modifications to enhance aerosolization and retention in the lungs.

Among biological threat agents, *B. anthracis* has unique characteristics that make it attractive for deliberate use: under simple storage conditions the spores remain viable and virulent for decades; the disease is not contagious (not transmitted person to person) thereby limiting spread beyond the target; the spores can be prepared in a form that is readily aerosolized; and inhalation anthrax in humans has a high mortality rate. For these reasons, significant effort has been focused to both understand human exposure to *B. anthracis*, as well as to understand the risks associated with exposures. Identifying factors that trigger timely and appropriate responses could be challenging.

A survey of scientific literature was conducted to determine the current state of the science regarding the presence of *B. anthracis* and corresponding natural or unintentional outbreaks of anthrax. Appropriate response, as well as both the actual and perceived human health risk, is different when a release of *B. anthracis* is deliberate rather than natural or unintentional. During the literature review of natural and unintentional occurrences of anthrax, a variety of characteristics became apparent that would enable a screening of information regarding anthrax outbreaks to rapidly assess whether the occurrence was intentional. The screening would inform the evaluation as to whether an intentional release had occurred. The screening approach is not intended to be a tool for performing the criminal investigation or for the extensive public health assessment, although the information and logic may inform both endeavors.

Knowledge related to exposure from occurrences of *B. anthracis* were identified. It is important to understand under what circumstances human exposure to *B. anthracis* occurs and how *B. anthracis* may attenuate or persist after a deliberate release into the environment. Questions include:

- Why do dormancy periods occur between anthrax outbreaks at a given location and what synergies occur to break dormancy?
- What causes and what prevents natural attenuation of virulent *B. anthracis* in the environment?
- What are the natural reproductive cycles? What is the role (if any) of lysogeny?
- How can detection of virulent *B. anthracis* in the environment be improved?
- How susceptible are human populations to anthrax? What factors influence susceptibility? How can susceptibility data be incorporated into human dose-response relationships?
- How does the background level of virulent or avirulent *B. anthracis* in the environment (and other factors) determine the likelihood of sporadic outbreaks?
- What is the role of seasonal environmental conditions in the severity of epizootics and subsequent risk to humans?
- What is the role of mechanical vectors, and associated spatial and ecological factors, in transmission risk to both animals and humans during outbreaks?
- What is the likelihood and particle size distribution of reaerosolized *B. anthracis* spores in the natural environment?

To rapidly and accurately evaluate whether an occurrence in an intentional release, necessary data must be obtained quickly, including:

- data from CDC/USDA and the Department of Defense on strains of *B. anthracis* in use or historically used at laboratories in the region;
- list of historic outbreak sites and of carcass burial and burn locations in the region;
- environmental background levels of *B. anthracis* typically found at the burial and burn locations;
- determination of the *B. anthracis* strains present.

To reduce uncertainty associated with the proposed rapid evaluation, key knowledge and technology gaps need to be filled. For example, accurate knowledge of the locations of anthrax outbreaks in the last two decades is available. However, locations of outbreaks during the 1950s and 1960s and the locations where the carcasses were buried that might be contributing to outbreaks today are known with less certainty. Information on the strains of *B. anthracis* in use in laboratories may not be readily accessible. Although most reliable laboratories use MLVA24 typing system, SNP assays and SNRs, there is no standard, and some use just the MLVA8 typing system. Fortunately the MLVA8 is included in the MLVA25 thereby identifying the same clade and subclade but missing the finer detail. The result is that a backup genomic identification sometimes has to be organized to more fully characterize the *B. anthracis* strains present at an occurrence. Ensuring that positive diagnostic cultures are not destroyed but promptly shared with the CDC strain archive would retain valuable information.

When non-industrial cases of human anthrax occur in the United States, they are usually cutaneous [5]. The locations of such outbreaks typically correspond to endemic areas from North Dakota to Texas. Outside of these areas anthrax is rarely seen in the United States in this century. Occasionally anthrax is associated with contaminated animal products, typically imported, such as animal-hide drumheads. Even more rare are cases of human inhalation anthrax in the United States and, in some cases, no source of exposure is ever identified. While all human anthrax cases are a cause for concern, natural and unintentional occurrences are generally addressed through routine USDA and CDC sampling procedures. When the outbreak results in significant environmental contamination, EPA might assist in cleanup, requiring appropriate and timely response to an intentional incidents. Use of the proposed systematic assessment of the likelihood that an event was intentional, might help indicate what type of response might be needed.

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