

## Risk of Occupationally Acquired Illnesses from Biological Threat Agents in Unvaccinated Laboratory Workers

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### ABSTRACT

Many vaccines for bioterrorism agents are investigational and therefore not available (outside of research protocol use) to all at-risk laboratory workers who have begun working with these agents as a result of increased interest in biodefense research. Illness surveillance data archived from the U.S. offensive biological warfare program (from 1943 to 1969) were reviewed to assess the impact of safety measures on disease prevention (including biosafety cabinets [BSCs]) before and after vaccine availability. Most laboratory-acquired infections from agents with higher infective doses (e.g., anthrax, glanders, and plague) were prevented with personal protective measures and safety training alone. Safety measures (including BSCs) without vaccination failed to sufficiently prevent illness from agents with lower infective doses in this high-risk research setting. Infections continued with tularemia (average 15/year), Venezuelan equine encephalitis (1.9/year), and Q fever (3.4/year) but decreased dramatically once vaccinations became available (average of 1, 0.6, and 0 infections per year, respectively). While laboratory-acquired infections are not expected to occur frequently in the current lower-risk biodefense research setting because of further improvements in biosafety equipment and changes in biosafety policies, the data help to define the inherent risks of working with the specific agents of bioterrorism. The data support the idea that research with these agents should be restricted to laboratories with experience in handling highly hazardous agents and where appropriate safety training and precautions can be implemented.

**K**NOWLEDGE OF THE MEANS to protect laboratory workers has evolved over time, with several safety measures having been implemented to decrease the risk of infection and intoxication, including biological safety cabinets (BSCs) and vaccines (mostly investigational

vaccines).<sup>1-30</sup> A recent review of 234 persons evaluated for potential laboratory exposures to potential agents of bioterrorism at USAMRIID from 1989 to 2002 demonstrated only five confirmed laboratory-acquired infections during this period, with the majority of individuals

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(78%) in this review having been vaccinated (including many investigational vaccinations) before the exposure.<sup>31,32</sup> As supplies of many of these investigational vaccines are now limited and they are no longer produced, the impact of safety measures alone in preventing occupational illnesses is important to examine as research on potential agents of bioterrorism becomes more widespread.<sup>33-43</sup> Recent events such as the death from Ebola virus of a Russian researcher, a cutaneous anthrax infection in a researcher in Texas, and potential exposure to *Bacillus anthracis* at a research laboratory in California demonstrate the pertinence and importance of this information.<sup>44,45</sup>

From 1943 to 1969, the United States conducted a biological weapons research and development program headquartered at Fort Detrick in Maryland. During this program, individual laboratory workers were periodically diagnosed with accidental infections or intoxications from potential warfare agents. The research in this program often involved production of high concentrations of organisms and experiments involving aerosolization of agents, which placed laboratory workers at a high risk for exposure and disease. A total of 423 occupationally acquired infections were diagnosed until the program ended in 1969.<sup>1</sup> During this period, numerous safety measures, engineering controls, and medical measures were introduced at various time points to help prevent laboratory-acquired infections.

Illness surveillance data, medical records, and medical literature archived from Fort Detrick during this time period were reviewed to attempt to define the impact of personal protective measures, engineering controls (including BSCs), and vaccines on the decrease of occupational illnesses caused by specific infective agents. While the current biodefense research will not produce quantities of organisms as large as those produced in the past biological warfare program, the research may be of higher risk than has been experienced in recent years, as it will necessitate frequent animal aerosol experiments to test the efficacy of biodefense vaccines and other medical countermeasures in order to satisfy requirements of the Food and Drug Administration's (FDA) animal rule for product licensure. The animal rule allows use of animal efficacy data when the risk for research subjects would be too great to ethically perform human efficacy trials. Laboratory-acquired infections in the current lower-risk biodefense research setting are not expected to occur frequently because of improvements in biosafety equipment and changes in biosafety policies. However, the occupational illness data from the offensive biological research program describes the inherent risks of working with specific agents of bioterrorism and stresses the importance of a biosafety program for ensuring implementation of protective measures for at-risk individuals.

## METHODS

Illness surveillance data and medical records on file in USAMRIID's medical archives were reviewed to determine the number and time of occurrence of occupational illnesses caused by *Bacillus anthracis*, *Yersinia pestis*, *Burkholderia mallei*, *Francisella tularensis*, *Brucella* species, *Coxiella burnetii*, *Chlamydia psittaci*, VEE (Venezuelan equine encephalitis) virus, botulinum toxin, and staphylococcal enterotoxin B (SEB) during the U.S. offensive biological warfare research and development program from 1943 to 1969. Infections were diagnosed when people presented with clinical disease (i.e., diagnosis confirmed by cultures or serological testing) or, retrospectively, by an increase in surveillance titers and/or skin test seroconversions, which were performed routinely for most diseases approximately every 3 to 6 months.

Therefore, infections listed in this review include both symptomatic and asymptomatic infections. The annual number of people infected by each agent was determined and correlated with interventions such as BSCs, vaccination, and other personal protective measures. Medical records of individuals diagnosed with an occupational illness after being administered a potentially protective vaccine were reviewed to verify the vaccination status of the individual and to evaluate the circumstances of the vaccine "breakthrough."

### Vaccines

Earlier versions of Q fever, brucellosis, VEE, psittacosis, and anthrax (whole-cell vaccine) vaccines were used in the initial years of the program, but they offered limited or no protective benefits [unpublished data, USAMRIID]. While a phenolized *F. tularensis* vaccine (Foshay vaccine) administered from 1945 to 1959 ameliorated symptoms, the vaccine did not prevent disease after exposure.<sup>36,40,46</sup> The anthrax vaccine (1952) and bivalent botulinum (serotype A/B) toxoid (1944) were the only two vaccines considered to offer potential protection available in the early years of the program. Newer vaccines considered to be protective were not available until 1959 or later (e.g., live tularemia, VEE TC-83, pentavalent [A-E] botulinum toxoid [PBT], and inactivated Q fever vaccines).<sup>33-39</sup> The plague vaccine, available to at-risk laboratory personnel beginning in the early 1950s, did not offer protection against pneumonic plague.<sup>47</sup>

### Laboratories

From 1943 to 1950, only Building 524 was used for the higher-risk experiments involving microbial aerosols and aerosol challenge of animals.<sup>1</sup> Aerosol challenges in laboratories in Building 524 were performed using a

Reynier's germ-free chamber with gloves attached at arm level.<sup>1</sup> Animals were kept in ventilated closed cages with exhaust air passed to oil-fired incinerators. Laboratory conditions in Building 524 (and most other laboratories) also included secondary barriers, such as restricted access, air locks, exit autoclaves, refuse incineration, negative-pressure conditions, clothing changes, and exit showers.<sup>1</sup> However, research was performed on benchtops because BSCs were not available before 1950. Infections also occurred in individuals working in the pilot production plant, where pilot lot production, purification, and agent preparation were performed.

Biosafety cabinets became commercially available in 1950, and were installed from 1950 through 1954. Cabinets were initially installed in laboratories where *Brucella* research was conducted, as treatment for brucellosis during this time was limited and associated with relapses.<sup>1</sup> The last laboratories to convert to using BSCs were those working with *F. tularensis*, as individuals working with this organism generally presented with ameliorated disease due to partial protection of the Foshay vaccine, and because the disease also responded readily to antibiotics. Based on the risk of the research procedures, some laboratories used both Class I BSCs (an open-fronted biosafety cabinet where air flowing into the cabinet was filtered prior to exiting the cabinet via an exhaust duct, but the cabinet did not provide protection from external contamination of materials) and Class III gastight BSCs. Other laboratories used only the Class III gastight BSCs. Laminar flow (unidirectional airflow within a confined space that entraps any created particles into the airstream and subsequently into the media of the air filter) was not introduced into the laboratories until the late 1960s.

Laboratories were defined as P4 and P3 levels beginning in the 1950s.<sup>1</sup> P4 conditions were required for extremely infectious and hazardous agents and required a negative-pressure environment with airlocks, protective laboratory clothing, exit showers and clothing changes, and systems in place to inactivate agents in waste and exhaust air. Infectious material was handled only within BSCs, with exhaust air incinerated or passed through high-efficiency particulate air (HEPA) filters.

P3 laboratory conditions were required for potentially hazardous organisms. P3 conditions generally required the handling of infectious agents within BSCs if procedures were likely to produce aerosols, a ventilation system that resulted in an inflow of air, exhaust air to be discharged outside to prevent laboratory reentry, gloves when handling infectious agents, and protection of vacuum lines with filters.

#### *Other protective measures*

Personal protective measures and improvements in safety practices were continuously implemented during

the program as mechanisms of exposures were determined for laboratory incidents or accidents resulting in occupational illnesses. Postexposure antibiotic prophylaxis, when available, was given after some but not all high-risk exposures.

## RESULTS

A total of 423 infections were diagnosed during the offensive biological warfare program, with tularemia, brucellosis, Q fever, VEE, and anthrax the most commonly occurring infections. Between 1944 and 1945, 23 cases of cutaneous anthrax occurred, with the majority of lesions involving the forearms or hands (12 cases) or the lower facial area or neck (six cases).<sup>48</sup> The number of cases of anthrax markedly decreased in 1946 and 1947, coinciding with the pause in research after World War II (laboratory-acquired infections from all agents decreased during this 2-year break) (Figure 1). In 1946, a policy change required using long-sleeve operating gowns with the gloves taped to the sleeves, replacing the short-sleeve operating gowns previously used, to prevent cutaneous anthrax cases. After anthrax research resumed in 1948, anthrax cases occurred infrequently, with only two cases reported in the 5-year pre-vaccine period from 1948 to 1952. The decrease in infections was attributed to changes in personal protective measures. However, a fatal case of inhalational anthrax occurred in an unvaccinated researcher in 1951. Only three cases of anthrax were diagnosed during the 18 years after anthrax vaccine was implemented in 1952.

Only one case of plague was diagnosed during the offensive biological warfare program.<sup>49</sup> In 1959, a vaccinated laboratory worker (who had received the fourth vaccine dose 1 month earlier) was diagnosed with pneumonic plague, most likely acquired after a high-risk exposure that occurred during the centrifugation of viable *Y. pestis* 5 days earlier.

Six of the seven cases of glanders occurred in the years 1943 to 1945, with a total of only 13 persons engaged in laboratory work with *B. mallei* during this time.<sup>50</sup> Although protective clothing was worn and extreme caution was exercised in working with this agent, all six cases had significant risk of aerosolized exposure to the agent before their diagnosis of glanders (i.e., dropping a flask with cultures, opening lids to containers of aerated cultures in liquid medium immediately after turning off the air current). With changes in personal protective measures and safety education, only one further case (resulting from a percutaneous exposure in 1953) was diagnosed. All seven cases of glanders occurred in researchers working directly with the organism.

Tularemia infections accounted for the greatest number of laboratory-acquired infections during the offensive

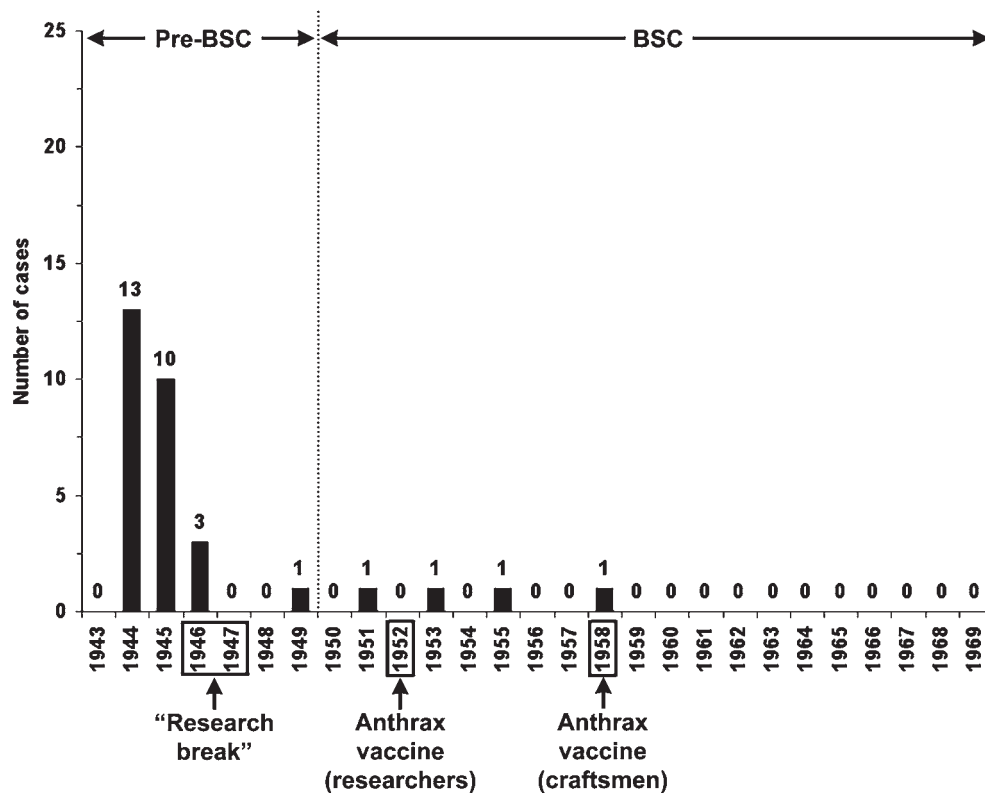


FIGURE 1. ANTHRAX CASES DIAGNOSED AT FORT DETRICK FROM 1943 TO 1969.

biological warfare program (Figure 2). Cases of tularemia occurred in individuals vaccinated with the Foshay vaccine, given from 1945 to 1959, and continued to occur even after installation of BSCs, with an average of 15 infections per year from 1955 to 1959. Once administration of the live tularemia vaccine began in 1959, there was an immediate and dramatic decrease in tularemia infections to an average of one case per year for the remaining 10 years of the program.

An average of 13 cases per year of brucellosis were diagnosed from 1948 to 1951, with aerosol exposure as the suspected route of exposure in nearly all cases. There was a decrease in the number of cases of brucellosis in the 1950s, corresponding with a decline in *Brucella* research beginning in 1952 (Figure 3). Therefore, the decrease in brucellosis cases cannot be attributed solely to the use of BSCs, which were installed beginning in 1950.

Q fever infections decreased from an average of 3.4 cases per year while BSCs were in use (Figure 4) to no infections occurring after initiation of the Q fever vaccine in 1965. All but 1 of the 55 cases were most likely acquired by the respiratory route. VEE infections also occurred during the use of BSCs, with an immediate decrease from an average of 1.9 cases per year (excluding cases of vaccine-acquired disease) to 0.6 cases per year after the introduction of the live, attenuated VEE TC-83

vaccine in 1963 (Figure 5). All but 3 of the 25 laboratory-acquired infections were believed to have been acquired by a respiratory route.

Twelve infections from psittacosis were diagnosed from 1945 to 1954, occurring mostly in laboratory researchers working directly with *Chlamydia psittaci*.<sup>51</sup> Six infections were diagnosed between 1957 and 1961, all occurring in individuals who did not work in laboratories with *C. psittaci*; it is therefore unclear whether the cases were laboratory-acquired. Two of these infections were acquired from nonoccupational sources, three occurred in craftsmen who had not entered a suite containing the organism, and one occurred in a researcher who did not work with *C. psittaci*.

Bivalent botulinum toxoid was given to at-risk people beginning in 1944 and replaced with the PBT in 1959.<sup>52</sup> No cases of botulism occurred during the entire program from 1943 to 1969, even with documentation of 50 potential exposures to the toxin. While prophylactic bivalent (A and B) botulinum equine antitoxin was administered to the first three botulinum toxin exposures occurring between 1945 and 1947 (two exposures from toxin ingestion during pipetting and one aerosol exposure), this practice was discontinued with increasing confidence in botulinum toxoid efficacy and because of concerns of serum sickness from equine antitoxin.

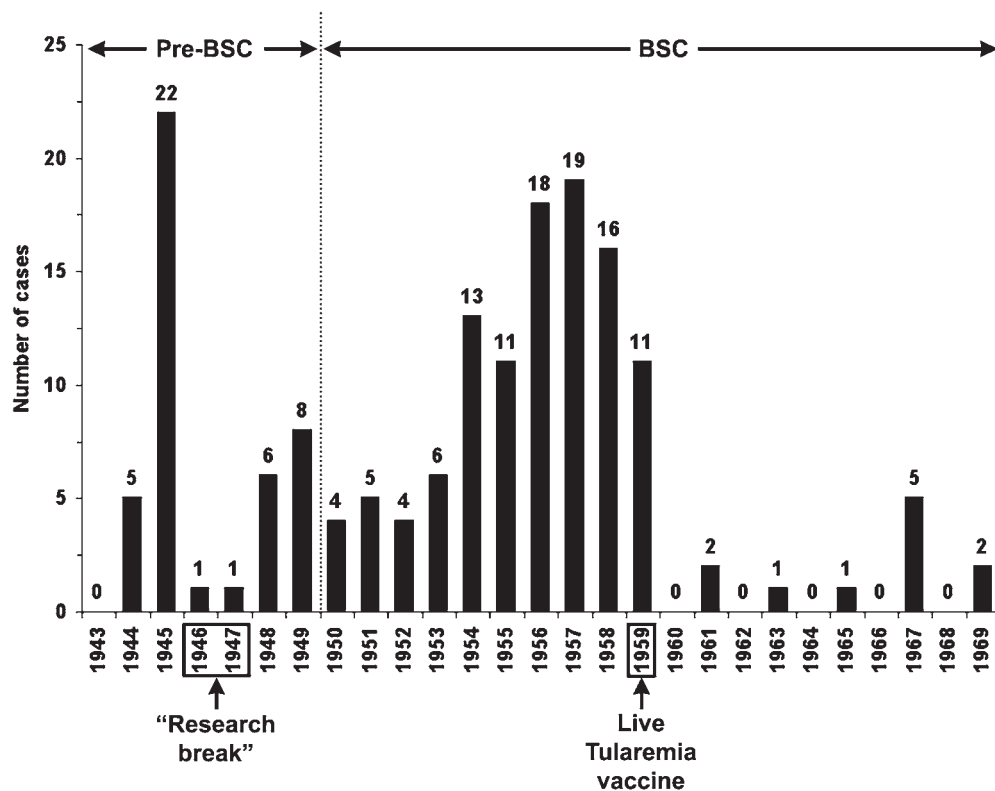


FIGURE 2. TULAREMIA CASES DIAGNOSED AT FORT DETRICK FROM 1943 TO 1969.

Three historical events resulted in aerosol exposures to SEB and subsequent intoxication.<sup>28</sup> In early 1963, two individuals became intoxicated when they were exposed to aerosols of SEB as a result of a ruptured hose that contained a crude filtrate of SEB under moderate pressure. In June 1963, five of seven persons became ill within 24 hours of exposure to a highly purified SEB aerosol, with four persons requiring hospitalization. The source was probable aerosolization of residual SEB from the fur of monkeys, as the monkeys' heads were not wiped after receiving an SEB aerosol exposure.<sup>53</sup> The third event occurred in August 1964, when 15 persons were exposed to SEB due to a leak in the tubes carrying aerosolized SEB for an animal aerosolization experiment. Ten individuals became symptomatic, with nine persons requiring hospitalization. Additionally, a contractor report commented on mild toxic reactions in six persons performing SEB purification studies on open laboratory benches (i.e., conjunctivitis, nondescript chemical irritation of one eye, general skin reaction, severe facial skin reaction, dermatitis, and cold symptoms), and another report noted symptoms mainly of conjunctivitis and acute pharyngitis in 23 persons wearing surgical masks or face shields while working with SEB on open benches, but not in people working with SEB within a BSC.<sup>1</sup>

Organisms known to be environmentally stable occasionally caused illness in people who did not work in laboratories during the offensive biological warfare program.<sup>54</sup> One case each of cutaneous anthrax (1946), Q fever (1952), and psittacosis (1951) was diagnosed in spouses of laboratory workers. A spouse presented with a facial lesion from cutaneous anthrax; her only risk factor was her husband, who worked in the laboratory with *B. anthracis*. Q fever in the spouse might have been acquired from exposure to the organism on her husband's scalp or hair. Although he always showered and changed clothes before exiting the laboratory, he did not always wash his hair (and his head was uncovered while in the laboratory). Finally, a case of psittacosis occurred in a spouse whose husband had contracted psittacosis in the laboratory. As human-to-human transmission of *C. psittaci* is rare but may occur, this was not absolutely confirmed as an infection resulting from environmental contamination of her husband or his clothes.

#### *Breakthroughs of disease after initiation of vaccination*

*Anthrax.* There were two cases of vaccine breakthrough after initiation of anthrax vaccination in 1952.

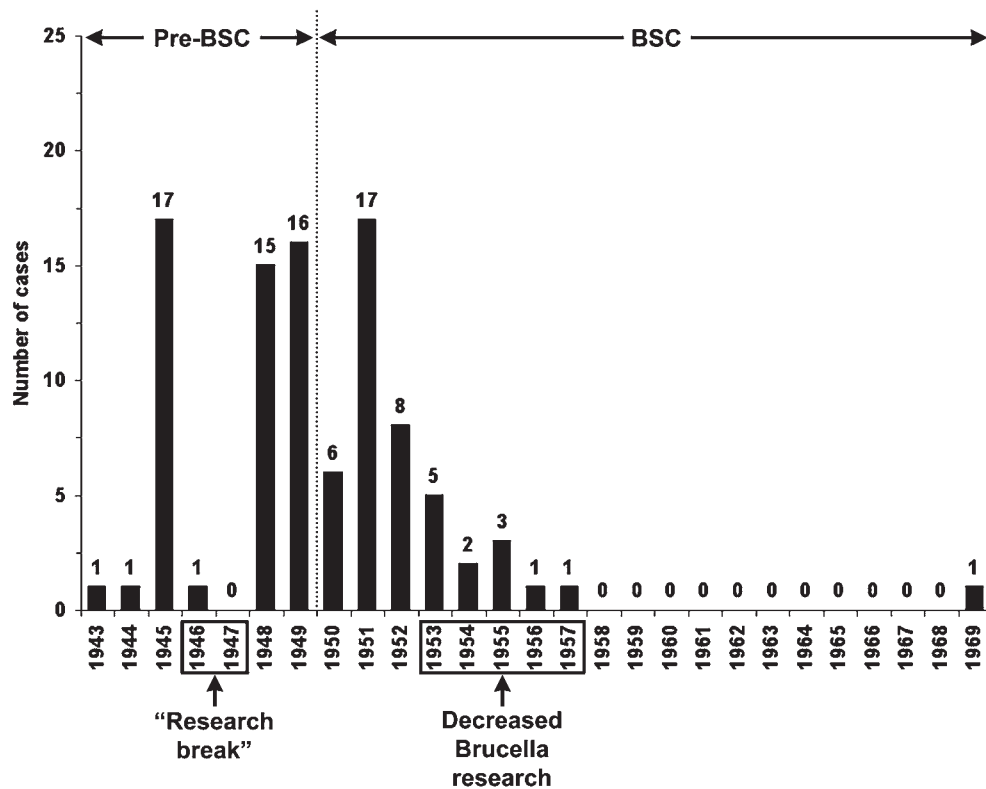


FIGURE 3. BRUCELLOSIS CASES DIAGNOSED AT FORT DETRICK FROM 1943 TO 1969.

One case occurred in an individual who had received three doses of the primary series of anthrax vaccine, and just a few days before the due date of his 6-month dose. The other case occurred in a person who had received only two doses of the primary series. Cutaneous anthrax occurred in this person just a few days after he had received his third dose at 6 months.

A case of inhalational anthrax occurred in 1958 in an unvaccinated craftsman whose exact source of exposure was undetermined after an extensive epidemiological evaluation. This event led to a change of policy to extend anthrax vaccination of all craftsmen beginning in 1958. No subsequent cases of anthrax were diagnosed.

*Tularemia.* Nine of the 11 cases of tularemia diagnosed after initiation of the live vaccine in 1959 occurred in vaccinated individuals. Five cases of vaccine breakthrough involved the ulceroglandular form of tularemia, and they occurred at 5 months and 1.5, 6, 8, and 10 years after vaccination and with tularemia agglutination titers ranging from 1:40 to 1:320 before the illness. Two persons with a past history of pneumonic tularemia, and persistently elevated antibody titers of 1:160 and 1:640 before exposure, accounted for the cases of tularemia occurring in unvaccinated people (one case of ulceroglandular and one case of recurrent pulmonary tularemia). The individual with recurrent pulmonary tularemia was

engaged in pelleting, drying, milling, and packaging large quantities of organism before his infection, but he denied any known break in physical protection.

Four individuals were diagnosed retrospectively with asymptomatic or mild presumed typhoidal tularemia by rises in serological titers and skin test seroconversion. Two individuals denied symptoms during the period of suspected infection, and the other two individuals recalled nonspecific illnesses in the interim period (occurring 2 and 6 years postvaccination) that may have represented mild typhoidal tularemia. One individual had been recently diagnosed with gripe with secondary bronchitis, and the other presented with a sore throat accompanied by chest and back pain. Both individuals were given tetracycline, and their symptoms resolved.

*VEE.* Four breakthrough VEE infections in people vaccinated with the live VEE TC-83 vaccine were retrospectively diagnosed by rises in hemagglutinin-inhibition (HAI) titers. Only one of these cases most likely represented a confirmed symptomatic infection. Seven days after exposure to a leaking container with live VEE virus, the individual developed a fever, sore throat, and the characteristic headache and backache consistent with VEE infection. HAI titers rose from a baseline titer of 1:20 to 1:2560 on day 11 after the onset of symptoms. Symptoms resolved within 6 days, and the individual did

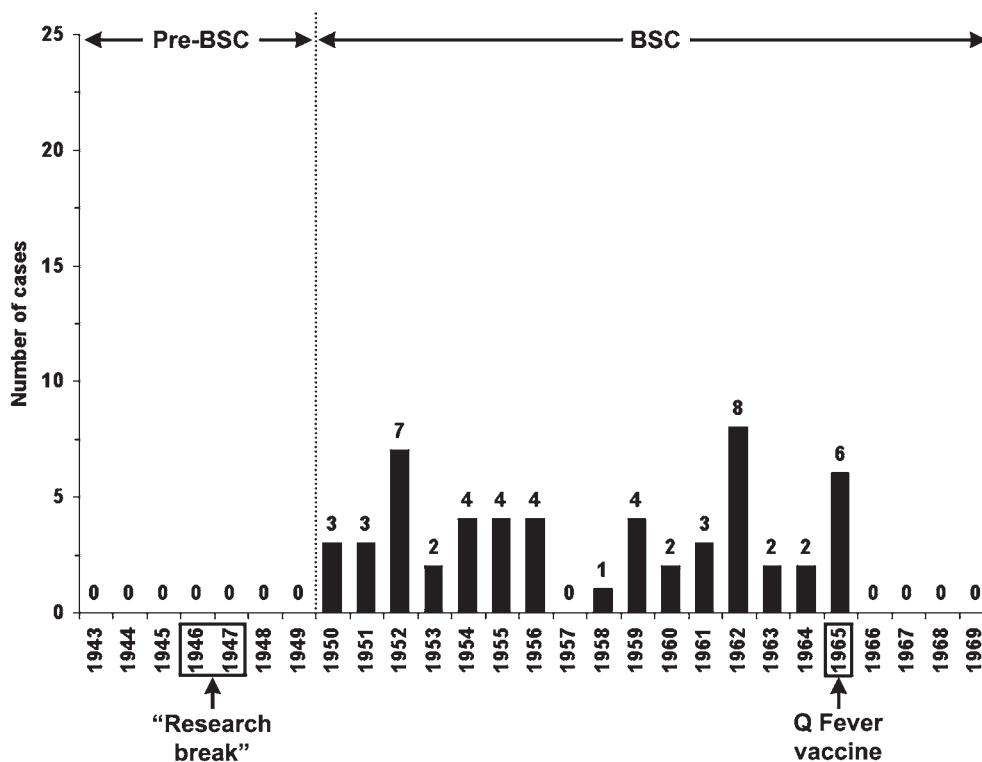


FIGURE 4. Q FEVER CASES DIAGNOSED AT FORT DETRICK FROM 1943 TO 1969 (*C. BURNETII* RESEARCH INITIATED IN 1950).

not require hospitalization. The strain was not confirmed as either an epizootic or an enzootic strain of VEE (the vaccine may not be fully protective against enzootic strains).<sup>38,55</sup>

The three remaining individuals with rises in VEE titers worked with VEE virus but could not recall any laboratory exposure or breach in laboratory technique. Although all three individuals had experienced mild, self-limited, febrile symptoms before the rise in HAI titers, the symptoms were not characteristic of VEE infection, which commonly presents with a severe headache, backache, marked periorbital or retrobulbar ophthalmic pain, and photophobia. The rise in titers in these individuals was felt to represent asymptomatic exposure to VEE, but the possibility that the illnesses represented ameliorated clinical symptoms of VEE resulting from partial protection from the vaccine could not be excluded.

## DISCUSSION

Only in recent years has the civilian public health community become concerned about the potential threat of biological weapons use. With increased research into

countermeasures against bioterrorism, institutions working with these agents will need to confront the risks of occupational exposure to these agents and initiate programs to protect at-risk laboratory workers.

Before the availability of BSCs in 1950, it appears that personal protective measures alone had a significant impact on reducing occupational illnesses from agents with higher infective doses (e.g., *B. anthracis*, *Y. pestis*, and *B. mallei*). However, illnesses continued to occur from agents with lower infective doses (e.g., *F. tularensis*, *C. burnetii*, and VEE) even with the availability of BSCs.<sup>56</sup> The availability of botulinum toxoids early in the program may have been responsible for the lack of occurrence of cases of botulism, and BSCs for the decrease in mild intoxications from SEB.<sup>1</sup> The data support that vaccination may have been a necessary component for the near-elimination of laboratory-acquired infections from agents with lower infective doses in the high-risk research setting of the offensive biological warfare program.

Anthrax cases declined significantly before the availability of BSCs and an effective anthrax vaccine, with personal protective measures and modifications of work practices alone accounting for this decline. Although it is difficult to retrospectively document all historical

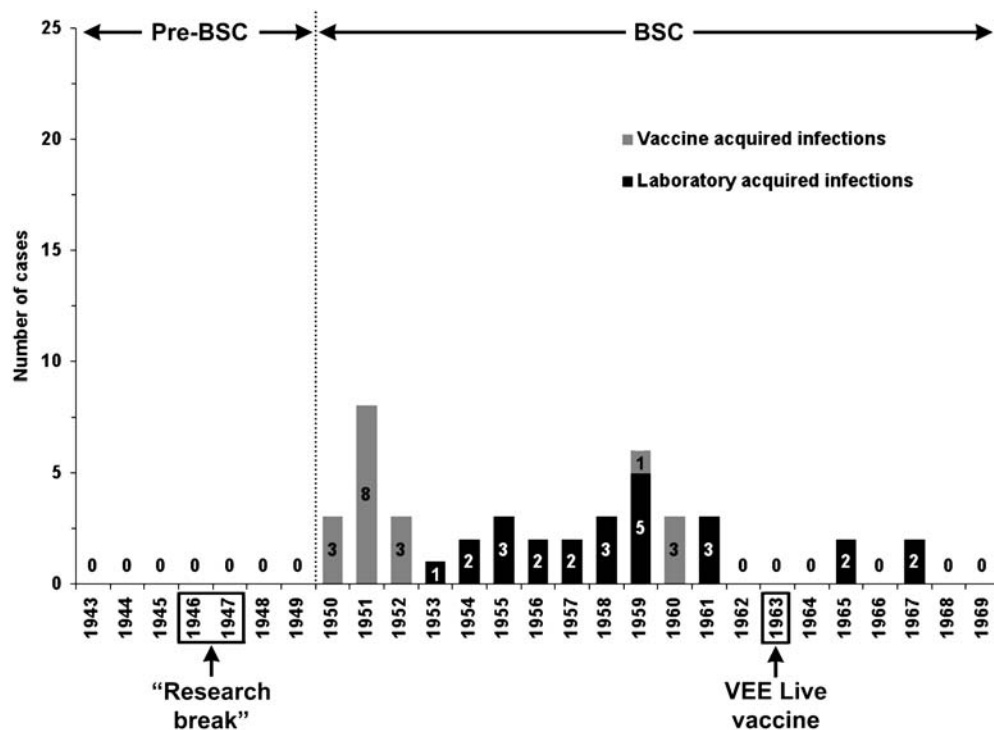


FIGURE 5. VEE CASES DIAGNOSED AT FORT DETRICK FROM 1943 TO 1969. LABORATORY-ACQUIRED AND VACCINE-ACQUIRED FROM EARLIER VEE VACCINE CANDIDATES BEFORE THE TC-83 VACCINE (VEE VIRUS RESEARCH BEGAN IN 1950).

changes that were significant in reducing anthrax infections, the adoption of long-sleeve operating gowns alone would have prevented cutaneous anthrax from occurring on the hands and forearms, which accounted for more than half of the 23 cases occurring from 1943 to 1944. The decline in anthrax infections with personal protective measures alone, before the availability of an anthrax vaccine or BSCs, may reflect the higher infective dose for *B. anthracis*. However, anthrax vaccination to at-risk individuals was important, as demonstrated by two fatal cases of inhalational anthrax in unvaccinated individuals.

Laboratory-acquired infection from *Y. pestis* was uncommon. Only one case occurred during the program, and only three cases of laboratory-acquired plague were reported in the medical literature in the 37-year period from 1925 to 1962.<sup>49</sup> The low frequency has been attributed to the higher infective dose of the organism and the instability of the organism in the environment. The organism is not generally viable in low humidity, sunlight, or warm weather, with outbreaks of pneumonic plague restricted mainly to colder climates and generally in winter months.<sup>49,57</sup>

The decrease in glanders in 1945, occurring after safety education on modifications in work practices and before the availability of BSCs, may be related to a potentially higher infective dose with this agent. Only one

subsequent glanders case occurred during the program, resulting from a percutaneous exposure in 1953.

Tularemia infections continued despite the use of BSCs. The increase in the number of cases of tularemia diagnosed from 1955 to 1959 (even with availability of BSCs) was possibly related to more frequent work with lyophilized cultures (dried preparations of the organisms that may more readily aerosolize).<sup>4</sup> A notable decrease in infections was seen after vaccination began with the live, attenuated tularemia vaccine in 1959. The decrease in laboratory-acquired infections after use of this vaccine at Fort Detrick has been previously reported, with typhoidal tularemia in civilian personnel decreasing from 5.7 cases to 0.27 cases per 1,000 at-risk employee-years.<sup>36</sup> However, the rate of ulceroglandular tularemia remained unchanged postvaccination (from 0.76 to 0.54 cases per 1,000 at-risk employee-years), consistent with the observation that natural disease does not confer immunity to subsequent infections with ulceroglandular forms of the disease.<sup>36,43</sup> Because protection from tularemia is mainly cell-mediated, the occurrence of disease with elevated agglutination titers was not unexpected.<sup>58</sup>

Before 1959, most tularemia cases were probably acquired by inhalation, as laboratory-acquired disease was principally of the typhoidal form.<sup>46,59</sup> Laboratory-acquired disease observed at Fort Detrick appeared to be

milder than that typically seen with naturally occurring disease, with only 19% of persons classified as severely ill.<sup>46</sup> This may be attributed to a number of factors: (a) earlier diagnosis due to a high index of suspicion for disease; (b) obtaining routine serological titers three to four times yearly, allowing for diagnosis of milder and also asymptomatic disease; and (c) the use of the phenolized (Foshay) vaccine, which may have modified symptoms.<sup>36,46</sup> Ulceroglandular tularemia in people vaccinated with the live tularemia vaccine was also milder than that observed in Foshay vaccine recipients (individuals exhibited fewer constitutional symptoms and were unlikely to require hospitalization).<sup>36</sup>

Q fever was the third most frequently diagnosed disease, with 55 cases occurring from 1950 to 1965, before the availability of the Q fever vaccine.<sup>60</sup> The environmental survivability and dissemination capacity of *C. burnetii* is well demonstrated by the number of individuals infected who did not work directly with the organism. Only 42% of people diagnosed with Q fever actually worked with *C. burnetii*; 32% of cases occurred in laboratory workers in adjacent laboratories not involved in *C. burnetii* research, and 26% of cases occurred in nonlaboratory personnel such as clerical workers, craftsmen, and janitors.<sup>60</sup> All but one case of Q fever were believed to have been acquired by the respiratory route. Control of Q fever with BSCs alone was not effective, again perhaps due to the low infective dose and to the organism's ability to survive in the environment for prolonged periods of time.<sup>61</sup>

BSCs alone also did not prevent infections from VEE, another organism with a low infective dose.<sup>56</sup> However, vaccination with the live VEE vaccine was associated with an immediate decrease in the number of infections. Breakthrough infections when specific serologies or cultures were performed generally have been from enzootic strains, to which the vaccine has demonstrated relatively poor immunogenicity in both horses and humans.<sup>38</sup> The live VEE TC83 vaccine, derived from the homologous TC-83 strain of the virus, is more closely related to enzootic serotypes (Trinidad IA, IB, and IC) and is antigenically distinct from enzootic serotypes (i.e., IE and IIIA).<sup>38</sup>

Brucellosis was the second most frequently diagnosed infection during this period.<sup>62</sup> As research with *Brucella* decreased beginning in 1952, the decline in laboratory-acquired cases of brucellosis may be attributed primarily to a decrease in the number of people working with the agent and not necessarily to use of BSCs. As brucellosis has a low infective dose of 10 organisms, one may have expected continued occurrence of some infections from *Brucella* despite the use of BSCs.<sup>56</sup>

The data did not allow conclusions regarding the effect of BSCs on prevention of psittacosis infections. With the

exception of a wife of a laboratory worker diagnosed with psittacosis, the 12 infections diagnosed from 1943 to 1954 occurred in people working directly with the organism.

Review of the literature concerning biosafety at Fort Detrick during the offensive biological warfare program gives further insight into the effect of safety interventions on disease risk in laboratory workers. During the initial 3 years of the program, from 1943 to 1946, there were approximately 4,000 at-risk employees at Fort Detrick, with 159 reporting accidental laboratory exposures (resulting in illness only in one person), 60 laboratory-acquired infections, and no deaths.<sup>63</sup> A review by Wedum revealed that during these early years of the program from 1943 to 1950 (before the availability of BSCs), only one infection per year occurred in laboratories within Building 524 where higher-risk aerosolization experiments were conducted (using Reynier's germ-free chambers and ventilated closed cages for animals), compared to 2.5 to 4 infections per year in laboratories not performing aerosolization experiments during this same time period.<sup>1</sup> However, even before the many personal protective measures resulting from knowledge acquired during the first 3 years of the program were initiated, there were only 35 infections per million person-hours (based on 1,789 annual hours per person) in laboratory workers during this period (1943 to 1945).<sup>30,64,65</sup> Individuals working in the process research laboratories (pilot plants) had a higher occurrence of infections, with 143 infections per million person-hours during this period.<sup>30,64</sup>

From 1954 to 1958, after the installation of the BSCs but before use of the live tularemia vaccine, the infection rate for all laboratory-acquired infections in laboratory workers was reduced to 9.10 infections per million person-hours (yearly range from 8.1 to 15.1).<sup>30,64,65</sup> After the initiation of the use of the live tularemia vaccine in June 1959, the rate of infections was further reduced to 2.01 cases per million person-hours (1960 to 1962).<sup>64</sup> The rate of infections in pilot plant workers also decreased markedly with adoption of BSCs, from the previously reported 143 cases per million person-hours (1943 to 1945) to 6.40 cases per million person-hours (1953 to 1955).<sup>30,64</sup>

Using Class I BSCs in combination with Class III gastight cabinets without vaccinating laboratory personnel (1954–1958) in one laboratory was not totally effective in preventing infections and was associated with approximately two to four infections occurring per year in personnel working in that laboratory.<sup>1</sup> However, using only Class III gastight cabinets in another laboratory (similar organisms and similar risk research) by unvaccinated laboratory workers during this same time period was associated with only one infection per year.<sup>1</sup> Nearly all infections occurring in the above two laboratories during this time were from organisms with low infective

doses, such as *F. tularensis*, *Brucella*, *C. burnetii*, and VEE virus. With vaccinations available beginning in 1959 (e.g., live tularemia, Q fever, and VEE vaccines), the number of infections was further decreased, with only one or two infections occurring in the subsequent 10 years (1959–1969) in each of these laboratories. Similarly, Wedum noted that installation of six Class I BSCs in a P3 containment laboratory working primarily with *F. tularensis* resulted only in a mild decrease from 3.2 cases (1944–1953) to 2.7 cases per year (1954–1956), whereas vaccination with the live tularemia vaccine in a laboratory with 12 Class I BSCs (1957–1959) resulted in a decrease from two cases per year to no cases in the 10 years postvaccination (1960–1969).<sup>1</sup>

In 1963, a risk analysis of laboratory workers revealed that 80% of infections occurred in trained scientific personnel and laboratory assistants.<sup>6</sup> Attack rates were 1.0 per 1,000 at-risk persons in scientific personnel and laboratory assistants as well as in maintenance workers, but rates were lower for administrative and clerical workers, animal caretakers, and dishwashers at 0.4 per 1,000 persons.<sup>6</sup>

During the program, investigations were conducted to identify the mechanism and risk of exposure from specific laboratory procedures or laboratory accidents that resulted in infections. These yielded interventions and safety practices to reduce the risk of exposure and cases of disease in the future. For example, atmospheric sampling studies performed at Fort Detrick demonstrated that significant aerosolization of organisms occurred from performing routine laboratory procedures such as removing lids or stoppers (e.g., from Waring Blenders or a shaken culture), flaming inoculation needles, pipetting fluids, lyophilization, animal inoculation, egg inoculation and harvesting, and disturbing contaminated animal cage litter.<sup>1,3,4,9,11,14–17,22–24</sup> Consequently, a number of other interventions may have resulted in decreases in exposures and infections, including the discontinuation of mouth pipetting, stricter requirements for wearing long-sleeve operating gowns, use of nonbreakable plastic Petri dishes, and use of Luer-Lok syringes (to prevent exposures resulting from the separation of the syringe and needle).<sup>4,11,23</sup>

Occupational illnesses occurring in biodefense research today are expected to be less frequent than observed in the offensive biological warfare program for several reasons. Biodefense research programs will not be working with comparably high concentrations of organisms or performing aerosolization experiments as frequently as in the biowarfare program. Also, the laboratory practices and safety measures that have evolved over the years have become more stringent, bringing improvements in engineering controls (e.g., directional airflow

and better air-handling systems), advances in biosafety equipment (e.g., needleless systems), new vaccines, and applications of lessons learned from historical laboratory exposures. Increased worker awareness and modifications of laboratory practices may also contribute to the decrease in exposures and infections. However, an increase in the number of potentially higher-risk animal aerosol-challenge efficacy experiments may be expected in the ensuing years, as required for vaccine and other countermeasure approval using the FDA animal rule. This may increase the risk for occupationally acquired illnesses in laboratory workers performing biodefense research.

The infective dose of the organism is an important factor in assessing the risk of disease in laboratory workers in today's environment, as demonstrated during the offensive biological warfare program. While most infections from agents with higher infective doses such as *B. anthracis*, *Y. pestis*, and *B. mallei* were prevented even in a high-risk research setting primarily with personal protective measures and safety education alone, the two deaths from inhalational anthrax in unvaccinated individuals highlight the importance of vaccinating people working with or around *B. anthracis*. Improved control of infections from agents with lower infective doses (e.g., *F. tularensis*, *C. burnetii*, and VEE virus) in unvaccinated workers was observed with restriction of work to gastight Class III BSCs. However, unvaccinated laboratory workers employing Biosafety Level 3 or 4 practices may still be at risk from these agents from percutaneous exposures or from unexpected aerosolization accidents (e.g., spills outside the BSC occurring when infectious agents are carried to and from the Class III cabinets).

Vaccination, if safe and effective, offers an additional measure of protection in at-risk laboratory workers. Because the actual exposure is unknown in 80% of laboratory-acquired infections,<sup>30,66</sup> postexposure antibiotic prophylaxis, if available and effective, could potentially be offered to only a small proportion of exposed unvaccinated people. While the use of gastight BSCs and powered air-purifying respirators (PAPRs) will protect against respiratory exposure in unvaccinated individuals, the unpredictability of animals and the need for using sharps still leaves a risk of percutaneous exposures (e.g., animal bites or scratches, or needlesticks). Additionally, wearing PAPRs may restrict vision and manual dexterity, potentially resulting in a higher number of accidents involving needlesticks, which have been responsible for up to 25% of accidents in some laboratories. Although post-exposure prophylaxis can be given to prevent bacterial or rickettsial infections, with viral agents there may be no postexposure prophylaxis available. Safer needle systems are available. In the clinical setting, the safety

mechanism (needle retraction or sheath) is employed after only one injection or blood draw. However, in a research laboratory, where syringes may be used for multiple injections, the safety advantage gained from the safer needles systems may be decreased. Improved gloves that are less likely to be penetrated by a needle or blade but produce only minor impairments in dexterity (i.e., Kevlar gloves) may be helpful in decreasing percutaneous exposures.

Many of the vaccines against agents of bioterrorism are investigational and can only be administered under Investigational New Drug (IND) protocols, thus limiting both the number of facilities that can administer the vaccines and the number of workers who can receive the vaccines. USAMRIID (Fort Detrick, Md.) is currently the only site in the United States administering investigational vaccines for tularemia, Q fever, and VEE. Existing vaccines in use are no longer manufactured and are limited in supply, and newer investigational vaccines (e.g., tularemia, VEE) that may soon enter Phase I clinical trials are unlikely to be licensed in the near future.

Success in limiting occupational illnesses in a high-containment laboratory relies on a number of layered safety measures, including engineering controls, safety training and awareness, personal protective equipment, and medical measures (vaccination and postexposure prophylaxis) to reduce the risk of disease. However, given the hazards inherent in working with the pathogenic organisms of concern, which in many cases are the most virulent organisms in existence, it may not be possible to completely eliminate the risk of exposure or disease in laboratory workers.

## CONCLUSION

With the improvements in environmental controls, personal protective measures, and biosafety equipment available today, laboratory-acquired infections are expected to occur infrequently in the current biodefense research setting. Employment of Biosafety Level 3 and 4 practices is necessary for prevention of exposures to agents of bioterrorism in biodefense research. However, vaccination may provide an additional measure of protection, particularly against percutaneous exposures and unexpected aerosol exposures resulting from leaks, malfunctions of equipment, or human error. Vaccination, if safe and effective, may be particularly important to minimize illness when working with (a) agents with lower infective doses (such as *F. tularensis*, VEE, and *C. burnetii*); (b) agents such as *B. anthracis*, where environmentally stable spores could result in unrecognized exposure and inhalational disease with a high mortality if diagnosis and

treatment are delayed; and (c) highly virulent viral agents where postexposure prophylaxis is not available.

## REFERENCES

1. Wedum AG. The Detrick experience as a guide to the probable efficacy of P4 microbiological containment facilities for studies on microbial recombinant DNA molecules. *Applied Biosafety: J Am Biological Safety Assn* 1996;1:7–25.
2. Wedum AG, Barkley WE, Hellman A. Handling of infectious agents. *J Am Vet Med Assoc* 1972;161:1557–1567.
3. Wedum AG. II. Airborne infection in the laboratory. *Am J Public Health Nations Health* 1964;54:1669–1673.
4. Barbeito MS, Alg RL, Wedum AG. Infectious bacterial aerosol from dropped Petri dish cultures. *Am J Med Technol* 1961;27:318–322.
5. Wedum AG. Control of laboratory airborne infections. *Bacteriol Rev* 1961;25:210–216.
6. Wedum AG. Laboratory safety research with infectious aerosols. *Public Health Rep* 1964;79:619–633.
7. Kruse RH, Wedum AG. Cross infection with eighteen pathogens among caged laboratory animals. *Lab Anim Care* 1970;20:541–560.
8. Wedum AG. Prevention of laboratory-acquired infections. *Am J Med Technol* 1956;22:311–315.
9. Wedum AG. Defensive aspects of biological warfare. *JAMA* 1956;162:34–37.
10. Hanel E, Miller OT, Phillips GB, Wedum AG. Laboratory design for study of infectious disease. *Am J Public Health* 1956;46:1102–1113.
11. Reitman M, Wedum AG. Microbiological safety. *Public Health Rep* 1956;71:659–665.
12. Wedum AG. Bacteriological safety. *Am J Public Health* 1953;43:1428–1437.
13. Wedum AG. Nonautomatic pipetting devices for the microbiologic laboratory. *J Lab Clin Med* 1950;35:648–651.
14. Reitman M, Moss ML, Harstad JB, Alg RL, Gross NH. Potential infectious hazards of laboratory techniques. II. The handling of lyophilized cultures. *J Bacteriol* 1954;68:545–548.
15. Reitman M, Alg RL, Miller WS, Gross NH. Potential infectious hazards of laboratory techniques. III. Viral techniques. *J Bacteriol* 1954;68:549–555.
16. Reitman M, Frank, MA Sr, Alg R, Miller WS. Modifications of the high-speed safety blender. *Appl Microbiol* 1954;2:173.
17. Barbeito MS, Taylor LA. Containment of microbial aerosols in a microbiological safety cabinet. *Appl Microbiol* 1968;16:1225–1229.
18. Barbeito MS, Mathews CT, Taylor LA. Microbiological laboratory hazard of bearded men. *Appl Microbiol* 1967;14:899–906.
19. Barbeito MS, Taylor LA, Seiders RW. Microbiological evaluation of a large-volume air incinerator. *Appl Microbiol* 1968;16:490–495.
20. Barbeito MS, Gremillion GG. Microbiological safety eval-

- uation of an industrial refuse incinerator. *Appl Microbiol* 1968;16:291–295.
21. Sinski JT. Use of the microscope in a gastight safety cabinet system. *Appl Microbiol* 1968;16:659–660.
  22. Phillips GB, Broadwater GC, Reitman M, Alg RL. Cross infections among *Brucella* infected guinea pigs. *J Infect Dis* 1956;99:56–59.
  23. Phillips GB, Bailey SP. Hazards of mouth pipetting. *Am J Med Technol* 1966;32:127–129.
  24. Phillips GB, Jemski JV. Biological safety in the animal laboratory. *Lab Anim Care* 1963;12:13–20.
  25. Jemski JV, Phillips GB. Microbiological safety equipment. *Lab Anim Care* 1963;13:2–12.
  26. Phillips GB. Safety education for students of microbiology. *Biochem Biophys Res Commun* 1962;9:291–296.
  27. Decker HM, Geile FA, Harstad JB, Gross NH. Spun glass air filters for bacteriological cabinets, animal cages, and shaking machine containers. *J Bacteriol* 1952;63:377–383.
  28. Rusnak JM, Kortepeter MG, Ulrich R, Poli M, Boudreau E. Laboratory exposures to staphylococcal enterotoxin B. *Emerg Infect Dis* 2004;10:144–149.
  29. Sewell DL. Laboratory-acquired infections and biosafety. *Clin Microbiol Rev* 1995;8:389–405.
  30. Wedum AG. Responsibility and practice in laboratory safety. In: *Proceedings of the Second Symposium on Gnotobiotic Technology, May 8–9, 1959*. Notre Dame, Ind.: University of Notre Dame Press; 1959:105–119.
  31. Rusnak JM, Kortepeter M, Hawley R, Boudreau E, Aldis J, Pittman P. Management guidelines for laboratory exposures to agents of bioterrorism. *J Occup Environ Med* 2004;46:791–800.
  32. Rusnak JM, Kortepeter MG, Aldis J, Boudreau E. Experience in the medical management of potential laboratory exposures to agents of bioterrorism based on risk assessment at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). *J Occup Environ Med* 2004;46:801–811.
  33. Brachman PS, Gold H, Plotkin SA, Fekety FR, Werrin M, Ingraham NR. Field evaluation of a human anthrax vaccine. *Am J Public Health* 1962;52:632–645.
  34. Brachman PS, Plotkin SA, Bumford FH, Atchison MM. An epidemic of inhalation anthrax: the first in the twentieth century. *Am J Hyg* 1960;72:6–23.
  35. Nigg C, Hottle A, Coriell LL, Rosenwald AS, Beveridge GW. Studies on botulinum toxoid, types A and B. *J Immunol* 1947;55:245–253.
  36. Burke DS. Immunization against tularemia: analysis of the effectiveness of live *Francisella tularensis* vaccine in the prevention of laboratory-acquired tularemia. *J Infect Dis* 1977;135:55–60.
  37. Pittman PR, Makuch RS, Magniafico JA, Cannon TL, Gibbs PH, Peters CJ. Long-term duration of detectable neutralizing antibodies after administration of live-attenuated VEE vaccine and following booster vaccination with inactivated VEE vaccine. *Vaccine* 1996;36:337–343.
  38. Burke DS, Ramsburg HH, Edelman R. Persistence in humans of antibody to subtypes of Venezuelan equine encephalomyelitis (VEE) virus after immunization with attenuated (TC-83) VEE virus vaccine. *J Infect Dis* 1977;136:354–359.
  39. Benenson AS. Q fever vaccine: Efficacy and present status. In: Smadel JE, ed. *Symposium on Q fever by the Committee on Rickettsial Diseases*. Washington, DC: Armed Forces Epidemiology Board; 1959:47–60.
  40. Foshay L, Hesselbrock WH, Wittenburg MJ, Rodenberg AH. Vaccine prophylaxis against tularemia in man. *Am J Public Health* 1942;32:1131–1145.
  41. Saslaw S, Eigelsbach HT, Prior JA, Wilson HE, Carhart S. Tularemia vaccine study, II: respiratory challenge. *Arch Intern Med* 1961;107:134–146.
  42. Saslaw S, Eigelsbach HT, Prior JA, Wilson HE, Carhart S. Tularemia vaccine study, I: intracutaneous challenge. *Arch Intern Med* 1961;107:121–133.
  43. Eigelsbach HT, Downs CM. Prophylactic effectiveness of live and killed tularemia vaccines, I: production of vaccine and evaluation in the white mouse and guinea pig. *J Immunol* 1961;87:415–425.
  44. Page EH, Martinez KF, Seitz TA, Bernard BP, Tepper AL. Update: cutaneous anthrax in a laboratory worker—Texas. *MMWR* 2002;51:482
  45. Shane S. Bioterror fight may spawn new risks. *Baltimore Sun* June 27, 2004:1A,10A–11A.
  46. Overholt EL, Tigertt WD, Kadull PJ, et al. An analysis of forty-two cases of laboratory-acquired tularemia. Treatment with broad spectrum antibiotics. *Am J Med* 1961;30:785–806.
  47. Williams JE, Cavanaugh DC. Measuring the efficacy of vaccination in affording protection against plague. *Bull World Health Organ* 1979;57:309–313.
  48. Ellingson HV, Kadull PF, Bookwalter HL, Howe C. Cutaneous anthrax. Report of twenty-five cases. *JAMA* 1946;131:1105–1108.
  49. Burmeister RW, Tigertt WD, Overholt EL. Laboratory-acquired pneumonic plague. Report of a case and review of previous cases. *Ann Intern Med* 1962;56:789–800.
  50. Howe C, Miller WR. Human glanders: report of six cases. *Ann Intern Med* 1947;26:92–115.
  51. Rosebury T, Ellingson HV, Meiklejohn G, Schabel F. A laboratory infection with psittacosis virus treated with penicillin and sulfadiazine, and experimental data bearing on the mode of infection. *J Infect Dis* 1947;80:64–77.
  52. Nigg C, Hottle A, Coriell LL, et al. Studies on botulinum toxoid, types A and B. *J Immunol* 1947;55:245–54.
  53. Henderson DW. An apparatus for the study of airborne infections. *J Hyg (Lond)* 1952;50:53–67.
  54. Carr EA, Rew RR. Recovery of *Bacillus anthracis* from the nose and throat of apparently healthy workers. *J Infect Dis* 1957;100:169–171.
  55. Dietz WH Jr, Peralta PH, Johnson KM. Ten clinical cases of human infection with Venezuelan equine encephalomyelitis virus, subtype I-D. *Am J Trop Med Hyg* 1979;28:329–334.
  56. Franz DR, Jahrling PB, Friedlander AM, et al. Clinical recognition and management of patients exposed to biological warfare agents. *JAMA* 1997;278:399–411.
  57. Teih TH, Landauer E, Miyagawa F, Kobayashi G, Okayasu G. Primary pneumonic plague in Mukden, 1946 and report of 39 cases with 3 recoveries. *J Infect Dis* 82:52:1948.
  58. Tarnvik A. Nature of protective immunity to *Francisella tularensis*. *Rev Infect Dis* 1989;11:440–451.

59. Van Metre TE, Kadull PJ. Laboratory-acquired tularemia in vaccinated individuals: a report of 62 cases. *Ann Intern Med* 1959;50:621–632.
60. Johnson JE, Kadull J. Laboratory-acquired Q fever. A report of fifty cases. *Am J Med* 1966;41:391–403.
61. McCaul TF, Williams JC. Development cycle of *Coxiella burnetii*: a model for host-parasite interaction. *Microbiol Rev* 1983;47:127–149.
62. Howe C, Miller ES, Kelly EH, Bookwalter HL, Ellingson HV. Acute brucellosis among laboratory workers. *New Engl J Med* 1947;236:741–747.
63. War Department Press Release on Biowarfare, January 1946. Reproduced in: U.S. Army Activity in the U.S. Biological Warfare Programs 1977;1:1–4.
64. Wedum AG. Disease hazards in the medical research laboratory. *Am Assoc Ind Nurses J* 1964;12:21–23.
65. Phillips GB. Microbiological hazards in the laboratory. Part one—control. *J Chem Educ* 1965;42:A43–A48.
66. Sulkin SE, Pike RM. Survey of laboratory-acquired infections. *Am J Public Health Nations Health* 1951;41:769–781.

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