

ity in which the work is to be performed. No matter how much you may want to do it, you cannot use someone else's risk assessment.

Does anyone out there have a biosafety manual I can use/copy/plagiarize?

Simply copy the BMBL word-for-word and your work is done. Wrong! The BMBL publication is by definition "guidelines" and minimal guidelines at that. Each facility and each laboratory within the facility is a unique entity, and the biosafety manual that serves the facility, or laboratory must be equally unique. This concept fits with the concept of risk assessment discussed above. It is important for the biosafety manual to reflect the needs of the risk assessment. The BMBL is the skeleton upon which you and the PI place the meat of the specific safety requirements for the laboratory. Those requirements are based on the potential for release of an organism, or its product to the environment, and the hazard to workers associated with performing the specific protocols in the specific laboratory.

Do you want to build a good biosafety manual? If so, start with the BMBL; then take each section and determine how you are going to specifically meet those require-

ments within your laboratory while performing your particular protocol. For example, the BMBL states that access to the laboratory must be controlled while experiments are being performed. Your manual should indicate that access to the laboratory is controlled by placing appropriate signage at the door to the laboratory prior to initiating work with the agents, and must be enforced by laboratory personnel working in the laboratory. Note that the BMBL states what should be done; the safety manual states how you will do it in your laboratory.

The days of starting an experiment that was just dreamed up without significant planning on the part of the PI and the lab staff are long over. If we are going to have to develop a protocol for conducting an experiment, then we should take the time to complete the risk assessment and to develop appropriate safety protocols to match. Safe operation is not only good for lab workers; it improves the science and the experiment.

Remember, the biosafety manual is not a static document that is developed, put on the shelf, and never looked at again. It is a dynamic document that must change as the experiments progress and protocols change. It is work, but it is work well worth doing.

Biosafety Tips

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Biosafety Tips brings you practical approaches to biosafety or "news you can use." If you are looking for a useful and sensible solution to a biocontainment problem, or perhaps a reference to help convince a skeptical researcher of the need for caution, this is the place to look. In this column, I share biosafety insights for managing a variety of workplace situations. I welcome feedback and suggestions for future topics. Please e-mail any comments or suggestions to karen_byers@dfci.harvard.edu or to Co-Editor Barbara Johnson at barbara_johnson@verizon.net.

Brucella Outbreak in Clinical Microbiology Laboratories

An overview of laboratory-associated infections reported in the past 75 years is published in the Chapter entitled "Epidemiology of Laboratory-Associated Infections" (Harding & Byers, 2006). The source literature on these infections provides detailed case reports that are useful for training purposes. *Biosafety Tips* in Volume 12,

Number 1, summarized reports of laboratory-acquired infections with *Neisseria meningitidis* in clinical microbiologists. In every case, a single microbiologist was infected through droplet, or aerosol transmission from routine identification procedures conducted on the open bench. This column describes a report in the *Journal of Clinical Microbiology* of an airborne Brucella outbreak in 31% of the clinical microbiology staff of a community hospital (Staszkiwicz et al., 1991) and two publications (Sue et al., 1989; Ruben et al., 1991) that describe secondary cases of Brucella infection.

Background

Brucella is a zoonotic pathogen and, outside of the laboratory, presents an occupational risk for farmers, veterinarians, and abattoir workers. Approximately 200 cases of human brucellosis are reported annually in the U.S. In Michigan, where this outbreak occurred, eight cases were reported between 1983 through 1987 (Staszkiwicz et al., 1991).

Index Case

One clinical microbiologist had an illness in July described as “self-limited and hepatitis-like.” Ten weeks later the same microbiologist was hospitalized and diagnosed with brucellosis based on blood cultures positive for presumptive *Brucella* species and an anti-*Brucella* antibody titer of 1:640.

Outbreak Description

When the diagnosis was reported to the hospital, all staff in the microbiology laboratory, as well as adjacent clinical laboratories, were given serological tests and a questionnaire for clinical symptoms and non-laboratory risk factors for *Brucella* infection. The serologic tests indicated that eight of the 26 microbiologists had antibodies to *Brucella*. The survey revealed that one microbiologist had been ill in May, in addition to two in June, and one in July. Three staff members later became ill in August, and one fell ill in September. All had received an initial diagnosis of nonspecific viral illness. Clinical features included myalgia and back pain; in addition, 75% had abnormal liver functions and weight loss. Five of the seven staff who reported an illness on the survey had a positive blood culture (63%), even though only one of the staff members (the index case) was acutely ill when the blood cultures were drawn. None of the 49 staff in adjacent laboratories were infected; all eight infected staff members were clinical microbiologists.

Source Investigation

Staff members had no risk factors other than working in the laboratory, and no one recalled working with *Brucella* in the previous two years. The story of this actual investigation is as thorough as any in the scripts of the CSI television series. The only possible source was a single culture in the freezer with a label dated April 1, 1988. The isolate was from a patient diagnosed with *Brucella* in 1985; the culture had been thawed and plated for viability in the last few days of March, about six weeks before the first case of brucellosis occurred. The Centers for Disease Control and Prevention (CDC) confirmed that the original patient isolate, and five employee isolates, were *B. melitensis*, biotype 3. All infected staff were treated with antibiotics; one had a relapse and was retreated with a different combination of antibiotics. All staff members recovered completely.

How Did the Exposure Occur?

No spills or incidents were reported, but all 8 employees worked in the lab on March 30 and 31, as opposed to five of 18 seronegative employees. The article includes a floor plan with Xs marking the predominant laboratory workspace of the *Brucella* cases, as well as the location where the *Brucella* manipulations occurred—on the open bench. The laboratory did have a biosafety cabinet in the Parasitology/Mycobacteriology laboratory; how-

ever, it was not used for the *Brucella* manipulations. This resulted in the airborne transmission of *Brucella*.

Outbreak Identification

This case study illustrates the difficulty in identifying laboratory-acquired infections that do not have a distinctive diagnostic feature. The infected staff had a wide range of non-specific symptoms (from serious illness requiring hospitalization to a subclinical infection) and incubation times after exposure ranging from six weeks to over five months. Fortunately, the source investigation included a review of freezer contents, since no log records were kept for stock manipulations. This community hospital did not have much experience handling Biosafety Level 3 (BSL-3) pathogens. To prevent re-occurrence, the microbiology laboratory adopted the policy that all work on presumptive, or confirmed Biosafety Level 3 organisms such as *Brucella* would be conducted in the biosafety cabinet at all times. In addition, plates of these BSL-3 organisms are sealed when not in use.

Secondary Laboratory-Acquired Infection

The Staszkiwicz article has a chilling addendum: “In March 1989, a laboratory worker at the Centers for Disease Control became infected while working with our isolates. A break in technique was not identified. Inspection of the laboratory revealed that the biologic safety cabinet was in working order.” Biosafety professionals attending the 32nd ABSA conference in 1989 heard a presentation entitled “Investigation of a Laboratory-acquired *Brucella melitensis* Infection: Confirmed Case Due to Biovar 3, A Strain Responsible for a Previous Outbreak at Another Institution” (Suen et al., 1989). The talk described the first laboratory-acquired infection in the 26-year career of a microbiologist; it was also the first infection in 27 years at this CDC reference laboratory. It was reported that the strain manipulated was an isolate from an outbreak of eight cases in a clinical laboratory outbreak of *Brucella*. Although the geographic location of the outbreak is not named it seems likely that this is the same *Brucella* outbreak. In describing this secondary case, the authors stated: “No specific accident or incident could be identified as leading to the infection, but several of the biosafety recommendations for this agent (referring to BMBL) were not being practiced” (Suen et al., 1989).

Secondary Transmission to Spouse

Another secondary case is described in a third publication (Ruben et al., 1991). One of the clinical microbiologists in the laboratory outbreak was hospitalized on July 18 and released on July 24 with oral antibiotics. Two days later, he was re-admitted and discharged after additional IV medication. The blood cultures drawn during both hospitalizations were negative; urine and sputum cultures were normal. After the laboratory outbreak was confirmed, and his serum titer for *Brucella* antibody was determined to be 1:640, the lab worker was reexamined

and treated with an additional three months of antibiotic therapy. All symptoms resolved; he returned to work in September 1988, and his antibody titer dropped to 80, indicating a resolved previous infection. However, in February 1989, his wife was admitted to the hospital with fevers, chills, headache, and myalgias. *Brucella* was isolated from her blood culture and the CDC reference laboratory confirmed that her blood culture isolate was identical to the outbreak strain. Person-to-person transmission does not commonly occur in *Brucella*; however, it was suspected in this case. The wife had not visited the microbiology laboratory in years and had no other risk factors for *Brucella* infection. Sexual relations with the infected husband had occurred infrequently during his acute illness June through September, but resumed in October 1988, before his antibiotic treatment for brucellosis was completed. No culture evidence for sexual transmission was obtained from either spouse, but the author advises that it would be prudent to abstain from unprotected intercourse during treatment for *Brucella*.

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Molecular Biosafety

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The molecular biology and biotechnology fields are growing by leaps and bounds. Molecular Biosafety aims to shed light on how these cutting-edge techniques impact safety. Please e-mail your insights and questions to Margy Lambert at mlambert@fpm.wisc.edu or Co-Editor Barbara Johnson at barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Safety Advance: Transposon Gene Delivery Systems

What do the terms “piggyBac,” “*Sleeping Beauty*,” and “*Frog Prince*,” have to do with biological safety? These transposons as well as others, such as *Tol2*, *Mos1*, and *Himar1*, are being used in the development of nonviral DNA/gene delivery systems. The relevance to safety is that nonviral delivery methods have the primary advantage over viral vectors of being noninfectious.

Transposons, or transposable elements, are DNA elements that can move or “transpose” from one location in a DNA molecule to another location, either on the same or a different DNA molecule. The phrase “jumping genes,” coined from Nobel Prize winning scientist Barbara McClintock’s research with maize, may be a more

recognizable expression. Transposases are the enzymes that catalyze the DNA movement. The development of gene delivery systems, commonly referred to as vectors, is often driven by the goal of creating safer and more effective human gene therapy options.

Viruses have the capacity to enter host cells and direct host machinery to produce the components necessary for infectious viral particles (Knipe et al., 2007). Some viruses, such as retrovirus, lentivirus, and adeno-associated virus, can integrate into the host genome with the potential result of chronic or latent infections. Integration can also result in insertional mutagenesis if the viral DNA integrates near a gene such as an oncogene.

Use of replication-deficient viral vectors greatly minimizes the chance that infectious progeny viruses will be generated. The possibility of infectivity is not eliminated; however, because replication-competent viruses can arise through a recombination mechanism. Viral vectors’ major advantage over nonviral methods is the ability to achieve stable expression. In particular, retroviral and lentiviral vectors can integrate into the host genome to achieve long-term expression of the gene of interest. Another potential advantage of viral vectors is the ability to target specific tissues. Tissue tropism is exhibited because viral entry is limited by the type of receptors displayed on