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 will share some biosafety insights for managing a variety of situations that arise in the workplace. I welcome feedback or suggestions for future topics. Please send them by e-mail to karen_byers@dfci.harvard.edu or to the Editor, Ira F. Salkin, at irasalkin@aol.com.

I have found that using reports of actual laboratory incidents as part of training programs can help pique audience interest and motivate lab staff to incorporate good microbiological practices into their daily routine. In this issue’s Biosafety Tips, I’ve provided citations and descriptions of some recent laboratory-acquired infections which may be useful in your safety training sessions.

Case 1


A laboratory worker sustained a needlestick while conducting a viral purification procedure using cells infected with vaccinia virus (strain WR). Although the individual had been vaccinated against smallpox in childhood, a pustule appeared on her thumb 3 days after the injury. By days 5 and 6 post-needlestick, new pustules appeared on the worker’s fourth and fifth fingers and she developed axillary lymphadenopathy. On day 8 after the incident, a large erythematous patch developed on her forearm and tissues around the finger lesions became necrotic. On day 9, the lesions worsened and antibiotics (amoxicillin/clavunate) were prescribed due to the suspicion of secondary bacterial infection. The necrotic tissues on the hand lesions were surgically excised. Gram stains on the pustular fluid from the lesions were negative, but diluted pustular fluid inoculated into BSC-40 (monkey kidney) cell cultures produced poxlike cytopathic effects. Virus recovered from the lesions was analyzed with Western blot and polymerase chain reaction (PCR)—restriction length polymorphism and was found to be identical to the strain used in the laboratory. The patient made a full recovery 4 weeks after the initial injury.

The authors state that their “study supports the need for vaccination for laboratory workers that routinely handle orthopoxvirus.”

Further guidance and thoughts for training workers:

- The images in Figure 1 in this online paper may convince researchers working with virulent strains of vaccinia to get immunized.
- The following document lists various vaccinia strains commonly used in laboratories and provides specific vaccination recommendations: Vaccinia (smallpox) vaccine recommendations of the advisory committee on immunization practices (ACIP), 2001, MMWR, Vol. 50, No RR10;1 (6/22/2001) available is online at URL: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5010a1.htm.
Case II


Here is another cautionary tale in which the authors report a vaccinia virus infection in a laboratory staff member following laboratory exposure to a genetically modified vaccinia virus strain. The worker had been vaccinated during childhood and had no personal history of atopy or chronic skin disease. While he was unaware of any accidental injury or exposure incident, he did have a "mild skin barrier disturbance from working with unprotected hands in cold temperature over a prolonged period." This staff member processed vaccinia cell cultures with high concentrations of recombinant virus (10⁹ plaque forming units [PFU]/ml). He noticed a vesicular lesion on the second finger of his right hand which slowly progressed to become a 15 mm infiltrated, inflammatory nodule with central hemorrhagic necrosis. A second inflamed lesion developed 2 days later on the third finger of the left hand. After unsuccessful surgical therapy, a skin biopsy and serum samples were taken. The lesions were treated with topical disinfectants and healed completed in 2 weeks.

The skin biopsy sample was split to allow its analysis by electron microscopy, as well as to inoculate cell cultures. Electron microscopy observation of the tissue sample revealed a poxvirus. The tissue culture yielded a poxvirus, which was subsequently studied by PCR, digest electrophoresis, and sequence analysis. The isolate was identified as the Western Reserve strain of vaccinia virus, genetically modified to contain a functionally inactivated cytohesin-1 gene of human origin—the same recombinant virus used in the laboratory. The gene in this recombinant virus has been shown to impair leukocyte adhesion.

This report could illustrate that:

- Genetic modification does not necessarily make the virus less virulent; careful consideration must be given to the potential effects of each insert.
- Vaccinations received at infancy and again 28 years prior to this infection were not fully protective.

Case III


A 25-year old laboratory technician who had not been previously vaccinated cut her finger on a coverslip. Since a lesion did not develop at that site until 12 days postinjury, the authors assumed that the cut itself was not an exposure incident. They reasoned that the cut site must have been contaminated with the virus at a later time. Unfortunately, the staff member squeezed the "pimple" which developed at the cut site and pus squirted onto her chin. Two days later, a lesion developed on the chin, and she was diagnosed with generalized vaccinia. Thirty-six days later, she felt "almost back to full strength, despite the blackened eschar on her finger." Electron microscopic studies confirmed the presence of pox virus in the lesions.

Conclusions:

- The authors concluded that staff "should be educated about the potential hazards of the work environment, including autoinoculation and the potential spread to contacts."
- Staff must seriously consider the potential risks of the organisms with which they work.
- The authors also advised compliance with the CDC recommendation of smallpox vaccination within the previous 10 years for staff handling the Western Reserve strain of vaccinia.

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Case IV


A 40-year-old microbiologist worked in a bacteriological laboratory processing wound swabs from
which he routinely isolated strains of methicillin-resistant Staphylococcus aureus (MRSA) and EMRSA-15 (endemic MSRA) daily. At one point, he had an upper respiratory infection which caused him to blow his nose in a handkerchief while handling plates and doing the slide coagulase test on the bench. Sometime later, he went out bushwhacking in Australia and cut his leg on a plant. This cut became infected and MRSA (EMRSA-15) was isolated from it. The person had no risk factor for MRSA (no chronic illness, injecting drug use, direct contact with patients) other than working with the organisms. Several weeks after remission of the leg infection, EMRSA-15 was isolated from a nasal swab recovered from the individual. This isolate was indistinguishable from the one isolated from his leg wound. The authors plan to study colonization of laboratory workers with MRSA.

Staphylococcus aureus is normally transmitted by direct skin contact or fomites; however, the authors assume that the transmission and subsequent nasal colonization occurred when the scientist, without prior hand washing, blew his nose while working with MRSA strains.

Topics for discussion:
- The authors advise thorough hand washing to prevent transmission; there is no discussion of whether gloves were routinely worn during these procedures.
- Nasal colonization is always a concern when working with MRSA strains, especially when there are breaks in hygiene.
- This example is particularly useful if workers neglect to wear gloves to handle potentially contaminated plates or fail to wash their hands after glove removal.

Case V


In the UK, strains of Brucella isolated from marine mammals are studied in a BSL3 containment laboratory. Blood tests of one researcher who developed continuing headaches, lassitude, and severe sinusitis indicated positive titers against Brucella that rose during the course of the infection. The strain of Brucella isolated from the researcher’s blood had been previously isolated only from sea mammals.

Although antibiotics relieved the clinical symptoms in a week, a full 6-week course of treatment with doxycycline and rifampicin was completed. The staff member remains symptom-free but seropositive. No other staff members seroconverted. All work was done in a Class III cabinet and, according to the author, “despite a detailed investigation by an independent authority” no specific problem was identified with its operation. The author cites an unpublished study indicating that 27% of stranded sea mammals were seropositive for Brucella, and reminds us of the zoonotic potential of sea mammals.

You might use this example to illustrate:
- Probable aerosol exposure
- Laboratory-acquired infections can occur in the absence of a specific exposure incident
- Zoonotic infections from marine mammals

Case VI


Vibrio parahaemolyticus is an important cause of bacterial food-poisoning outbreaks in Taiwan and had been previously isolated from abalones. In this study, a V. parahaemolyticus isolate (designated 880713) recovered from a human stool sample during a probable foodborne infection was injected into abalones to determine its virulence in this animal model. The laboratory also isolated V. parahaemolyticus (isolated 880915) from the hemolymph of a diseased small abalone and injected it into another set of abalones. The animals were kept in laboratory aquaria and observed daily. Moribund animals were removed by a lab member and samples were obtained for isolation and identification of bacteria. After the experiment had been set up, an earthquake occurred in Taiwan and the laboratory did not have water or electricity for a week. As a result, the authors state that “the usual higher standards of hygiene could not be maintained.” However, work on
This report could be used to emphasize the following points:

- That hand washing is important, even though the use of gloves was not discussed in this publication.
- Disaster planning should address safety procedures for the staff to follow.
- A new animal model may be a new source of zoonosis.

this project apparently continued and the lab member who removed moribund abalones from the aquaria suffered two episodes of acute gastritis during the week of the utility outage. Both strains of *V. parahaemolyticus* 880915 and 880713 were isolated from the lab worker's stool samples. This is the first recorded instance of infection of abalones with a human isolate of *V. parahaemolyticus* and the first laboratory-acquired infection from laboratory-infected abalones.