Laboratory-Confirmed Transmission of Vaccinia Virus Infection through Sexual Contact with a Military Vaccinee

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A laboratory-confirmed, inadvertent transmission of vaccinia virus from an unusual source highlights the importance of epidemiologic tracing, proper biosafety practices in the clinical diagnostic laboratories, and educating clinicians and laboratorians to potential bioterrorism-initiated outbreaks as well as look-alike disease discrimination.

CASE REPORT

A female in her early 20s presented at a local medical center with a vaginal lesion. The patient reported that she first noticed the lesion approximately 10 days after sexual contact. The painless bump had become fluid filled, although there was no apparent lymphadenopathy. Clinical examination revealed a circular indentation approximately 0.5 cm in diameter on the posterior side of the vaginal opening. Urine culture and sensitivity tests were performed, and a swab of the lesion was sent to a viral testing laboratory, where the vaginal swab tested negative for herpesvirus but produced viral cytopathic effect (CPE) in cell culture. The sample was then sent to the Wadsworth Center Virus Reference and Surveillance Laboratory for identification.

The viral sample produced a CPE in primary rhesus monkey kidney, A549, and MRC-5 cell lines. Viral culture supernatants tested negative for herpes simplex virus 1 (HSV-1) and HSV-2 by PCR and enzyme-linked immunosorbent assay, for enterovirus by PCR and immunofluorescence assay (IFA), for adenovirus by IFA, and for varicella-zoster virus by PCR. The virus was also hemadsorption negative, thereby ruling out orthomyxo- and paramyxoviruses. Based on the CPE pattern, no further work was done in the clinical virology laboratory, and the sample was sent to the Biodefense Laboratory for testing for the presence of orthopoxvirus.

Nucleic acid extraction of the viral cell culture was performed in a biosafety level 3 laboratory. Isolated nucleic acid was interrogated by a non-variola virus orthopoxvirus assay and an endogenous control *Escherichia coli* 16S real-time PCR (rtPCR) assay provided by the Centers for Disease Control and Prevention Laboratory Response Network. The 16S assay, which is used to detect PCR inhibitors that may be in the sample, targets residual *E. coli* DNA present in the recombinant *Taq* polymerase used in the

rtPCR. The rtPCR was performed using an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, Calif.) with the following PCR parameters with 9600 emulation option off: *Taq* activation (1 cycle) for 8 min at 95°C and thermal cycling (45 cycles) consisting of denaturation for 15 s at 95°C followed by annealing/extension for 30 s at 63°C. Data were analyzed with the ABI Prism 7000 SDS software, and the resultant cycle threshold (C_t) values are reported.

Non-variola virus orthopoxvirus DNA from the vaginal lesion was detected (Fig. 1A). Strong amplification was seen, and there was no inhibition present in the sample (Fig. 1B). These results were confirmed by a vaccinia virus-specific rtPCR assay (data not shown) previously developed at the Wadsworth Center. The presence of orthopoxvirus virions was additionally confirmed by electron microscopic visualization of characteristic “brick-shaped” virions from the rhesus monkey cell culture (Fig. 1C). Samples were mounted on 400-mesh copper Formvar grids, stained with 2% phosphotungstic acid, and visualized in a LEO 910 (Carl Zeiss Inc., Thornwood, N.Y.) transmission electron microscope operating at 80 keV (Wadsworth Center Microscopy Core Facility).

These results were reported to the hospital submitting the specimen and to the local health department. A subsequent public health investigation and patient exposure interview were conducted. In the course of the interview, the patient reported that she had had sexual contact with an individual in the U.S. military 2 weeks prior and that, approximately 1 h before sexual contact, the soldier had shown the patient his recent smallpox vaccination site and had subsequently rebandaged the area. The patient reported no other sexual partners in the recent past and indicated that she lives alone. Ten days after she was initially seen at the medical center, the lesion had completely healed. The investigation also included interviews with the laboratory personnel who had come in contact with

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FIG. 1. (A) Detection of orthopoxvirus by rtPCR. rtPCR analysis of a viral isolate recovered from a vaginal lesion was performed. A target that detects orthopoxvirus (Vac1) and an inhibition assay (16S) were used to amplify viral DNA. Each assay was performed in duplicate. NTC, no-template control; Rn, normalized reporter signal minus baseline signal. (B) Analytical results from the cultured vaginal lesion isolate. The test column indicates the method used for identification. The Laboratory Response Network rtPCR assay can detect orthopoxviruses, excluding smallpox virus. In the PCR result columns, the numbers shown are the average C_t values from duplicate assays. Higher concentrations of target DNA yield lower C_t values. (C) Electron microscopy showing typical brick-shaped virions, ~250 nm long, embedded in the sodium phosphotungstate stain.

the specimens. There were no reported laboratory exposures or any reported secondary infections from this case.

In this case report, an inadvertent transmission from a military vaccinee to a civilian was not initially diagnosed, due to

the unusual clinical presentation of the infection and the lack of information concerning smallpox vaccine contact. In early 2003, the United States initiated a smallpox vaccination campaign for healthcare workers and first responders. Military personnel have been continuously vaccinated since the late 1940s, with only a brief hiatus from 1990 to 1997 following the

fall of the former Soviet Union. The smallpox vaccine is a live vaccinia virus, which can be transmitted from person to person through physical contact of pustular lesion fluid. Lack of hand washing is presumed to be the mode of transmission of vaccinia virus in this case report. There have been several other reports indicating that inadvertent inoculations have occurred in both current and previous vaccination campaigns (4, 5, 9). Interestingly, reports of sexual or familial transmission of vaccinia virus resulting in vaginal lesions have occurred previously, and there have been 10 reported inadvertent inoculations associated with the recent Israeli and U.S. vaccination programs that are presumed to have been due to sexual contact (1, 3, 5).

In areas with large numbers of military personnel who must participate in the smallpox vaccination program, healthcare providers should include vaccination information on patient history questionnaires and should include vaccinia virus in their differential-diagnosis algorithm. While this case self-resolved and was a minor vaccination adverse event, the importance of incorporating both vaccination status and close-contact history into clinical diagnoses is obvious, especially considering the potential for other, more severe or deadly adverse events that are possible following vaccinia infection.

This case report highlights the importance of vigilance within the public health system to ensure rapid and correct diagnosis and handling of potentially highly infectious agents. Appropriate biosafety practices and procedures were followed in this case, and these limited the real potential for a laboratory-acquired infection. There have been several nosocomially acquired and laboratory-acquired orthopoxvirus infections reported, in which infections occurred with recombinant vaccinia virus strains or other orthopoxviruses, such as cowpox virus (6–8). The Advisory Committee on Immunization Practices report (2) states “Vaccinia vaccine is recommended for laboratory workers who directly handle a) cultures or b) animals contaminated or infected with vaccinia, recombinant vaccinia viruses, or other orthopoxviruses that infect humans (e.g., monkeypox, cowpox).” It is important to remain vigilant about using appropriate universal precautions and good microbiological practices to ensure the safety of laboratorians as well as

their daily contacts. Vaccinia virus is a fast-growing virus with rapid CPE and hence can potentially seriously affect the functioning of the clinical virology laboratory. Cell lines infected with vaccinia virus should be segregated from other cell lines used for clinical laboratory work.

To our knowledge, this is the first report of a laboratory-confirmed inadvertent inoculation associated with the current smallpox vaccination program. While the lesion was self-limiting and resolved without treatment, this report underscores the necessity for health practitioners, laboratories, and public health communities to include vaccinia virus and other orthopoxviruses in their diagnostic algorithms for rash illness and unusual lesions. With the increased awareness of agents that may be used in a bioterrorist attack, there will undoubtedly be more research devoted to the study of these agents. Since it is very likely that healthcare providers will see more unusual lesions from vaccinia virus infections, they should be advised to include work history in a differential diagnosis to determine whether this virus may be a cause of illness.

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