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Laboratory Confirmation of Generalized Vaccinia following Smallpox Vaccination

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The reinitiation of smallpox vaccination has renewed interest in implementing modern diagnostic methods to assess orthopoxvirus infection and adverse events following vaccination. We report here the laboratory confirmation of vaccinia virus in pustular lesions of a healthy adult vaccinee by use of a two-tier algorithm incorporating TaqMan PCR and electron microscopy.

CASE REPORT

In the spring of 2003, health care workers and first responders across the United States volunteered to be vaccinated against smallpox as part of a national security initiative to deter bioterrorism. Extensive prescreening of volunteers was performed to minimize the potential for vaccine-associated adverse events. As part of this initiative, a previously (>25 years earlier) vaccinated 37-year-old healthy female received 18 skin pricks (a standard series of 15 skin pricks for a revaccinee, followed by 3 additional skin pricks when blood was not apparent following the initial series) of Dryvax with a bifurcated needle. Two days postinoculation, the individual developed headache, chills, chest pain, recurrent vomiting, and small lesions (<0.5 cm) on her face, trunk, and legs (Fig. 1A). A stiff neck and a low-grade fever (temperature of <100°F taken orally) appeared and persisted for several days. Approximately 30 lesions were apparent 5 days postinoculation, when a Public Health Response Team from the New York State Department of Health (NYSDOH) was deployed for specimen collection (Fig. 1A). The rapid onset of symptoms was unusual relative to the typical manifestation reported at 5 to 12 days postinoculation in >66% of generalized vaccinia (GV) cases, although a small percentage (<3%) of GV cases have been reported within 2 days after inoculation (5). The rapid onset may have been due to the dose inoculated (i.e., 18 pricks) versus the usual 3 pricks for typical first-time vaccinees.

The decision to collect samples as part of the Adverse Event algorithm was made upon consultation with the NYSDOH Biodefense Laboratory, the county health department, and the NYSDOH regional epidemiology office. Representatives from each of these units were present during the hour-long sample collection procedure, at which point the samples were immediately escorted to the NYSDOH Biodefense Laboratory for testing. Transport and laboratory analysis were completed within 6 h from the time of sample collection.

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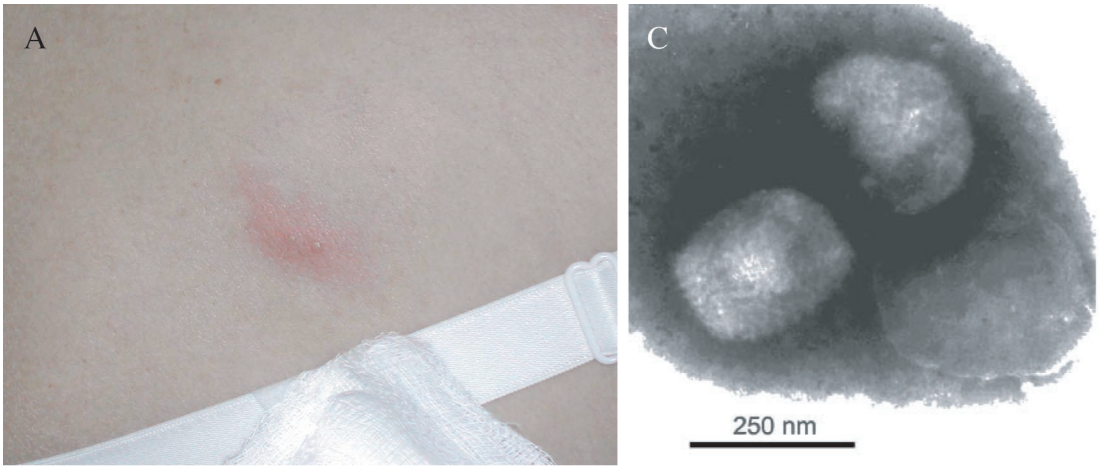
Isolated nucleic acid from two lesions was analyzed using real-time PCR (rtPCR) assays provided by the Centers for Disease Control and Prevention Laboratory Response Network (CDC LRN): a vaccinia virus-specific assay, a varicella-zoster virus (VZV)-specific assay, and an endogenous control *Escherichia coli* 16S ribosomal DNA assay to identify PCR inhibition. The 16S assay detects residual *E. coli* DNA present in recombinant *Taq* polymerase. The VZV assay was performed because this virus is the most likely cause of pustular rashes which could be misdiagnosed as smallpox or vaccinia. The vaccinia virus (strain WR) and VZV positive controls were obtained from the Wadsworth Center Viral Culture Collection and were included, in duplicate, for each assay performed. Controls were spiked at a concentration that produced a final cycle threshold (C_t) value of 30 to 33 to serve as a reliable indicator that the rtPCR assay was functional even at low concentrations of virus. The rtPCR was performed using an ABI Prism 7000 Sequence Detection system (Applied Biosystems, Foster City, Calif.) with the following PCR parameters: *Taq* activation for 8 min at 95°C, denaturation for 15 s at 95°C (45 cycles), and annealing and extension for 30 s at 63°C (45 cycles), with the 9600 emulation option OFF. Data were analyzed using the ABI Prism 7000 SDS software, and the resultant C_t values are reported (Fig. 1B).

VZV genomic sequences were undetectable in lesion fluid, and sample matrices exhibited no inhibition following extraction. Vaccinia virus-specific DNA detected in the lesions supported the clinical diagnosis of GV. The presence of disseminated orthopoxvirus was secondarily confirmed by electron microscopy of material eluted from swabs taken from the base of each lesion. Samples were mounted on 400-mesh copper Formvar grids, stained with 2% phosphotungstic acid, and visualized on a LEO 910 (Carl Zeiss Inc., Thornwood, N.Y.) transmission electron microscope operating at 80 keV. The size and morphology of the particles visualized were definitively characteristic of an orthopoxvirus (Fig. 1C). The final laboratory result was reported in less than 6 h from the receipt of specimens, confirming the usefulness of this algorithm for rapid diagnosis of adverse events following vaccination. Therapy with vaccinia immune globulin or cidofovir was not re-



B

	VACCINIA	VZV	16S Internal Positive Control
Neat extract	25.7	Undetected	31.7
1:10 Dilution	28.8	Undetected	31.8
Target Positive Control	30.8	33.5	32.1
Negative Extraction Control	Undetected	Undetected	31.8
No Template Control (-)	Undetected	Undetected	31.9

FIG. 1. Identification of vaccinia virus in generalized lesions. (A) Digital photo of a lesion on the volunteer's back prior to sampling. (B) rtPCR identification of vaccinia virus DNA in pustular fluid of suspected GV lesions. Numbers are average C_t values from duplicate assays performed on each lesion. If no amplification was seen, results are reported as "Undetected." Higher concentrations of target DNA yield lower numerical C_t values. (C) Electron microscopy shows typical "brick-shaped" virions, ~250 nm long, embedded in the sodium phosphotungstate stain.

quired, because the lesions resolved within 8 days and the patient showed no additional side effects.

Since its development by Edward Jenner in 1796, the live vaccinia virus vaccine has saved countless lives that would otherwise have been lost to smallpox. The cross protection afforded by the vaccine led to the eradication of smallpox virus from the environment as declared by the World Health Organization on 9 December 1979. This declaration justified the termination of routine vaccination of civilians around the world. Declared stocks of smallpox virus are maintained at two World Health Organization reference laboratories in the United States and the former Soviet Union, but unfortunately, virus cultures may also be possessed by other organizations (3). The perceived smallpox threat was minimal until September 2001, when anthrax was used as a terrorist weapon to kill five people in the United States. The possibility of biological terrorism precipitated a reevaluation of the smallpox threat and prompted the United States and other countries to reinitiate vaccination programs. The live virus vaccine used in the current U.S. vaccination program is the same Wyeth Dryvax preparation that was used in the United States for the eradication effort.

The mortality rates of ~0.002% (5, 6) associated with smallpox vaccination were minimal compared to the mortality rate (>30% [3]) associated with variola virus infection when smallpox was endemic. The severe adverse events (SAE) associated with the live virus vaccine were believed to be due to underlying defects in the immune system (progressive vaccinia, vaccinia necrosum, or GV) or dermatologic disorders (eczema vaccinatum or erythema multiforme) (5, 6). Contraindications, including immunosuppression, skin disease, and pregnancy, were not considered during the eradication effort. GV is defined as dissemination of virus to sites distal to the vaccination site. It is differentiated from eczema vaccinatum by the absence of diagnosed eczema or other skin conditions and is considered life-threatening only in cases involving underlying immunodeficiency (2). Although true generalized spread as evidenced by peripheral pustules containing virus is believed to be uncommon (4), recent clinical trials showed that 14.3% of vaccinees exhibited rashes, the majority of which were pustular or vesicular rashes on the chest and back (1).

This is the first reported case of a vaccinia SAE confirmed by using CDC LRN rtPCR methods. The absence of contraindications and the rapid clearance of generalized lesions suggest that vaccinia virus dissemination from an inoculation site is not necessarily dependent on a compromised immune status. The

mild clinical presentation of this rash (small, pustular lesions on the chest and back) was similar to that described by Frey et al. (1), who did not diagnose it as GV. This similarity suggests that generalized spread of vaccinia virus from the site of inoculation may be more common than previously thought, and it supports the use of the CDC LRN methods to differentiate GV from postvaccination rashes. This event also provides real-world validation of the CDC LRN system and its implementation in New York State. The evolution of the Laboratory Response Network over the past 2 years has provided a strong foundation for public health infrastructure and will enable public health laboratories to respond rapidly and effectively to emerging infectious diseases and bioterrorism events.

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