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Persistence of Vaccinia at the Site of Smallpox Vaccination

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Persistence of vaccinia at vaccination sites may help determine the risk associated with secondary transmission. Culture, PCR, and antigen detection were performed on serial vaccination site swab specimens. On day 21 after vaccination, 37% of volunteers were culture positive, most of whom had received vaccine for the first time. Vaccinia is detectable at least through day 21 after vaccination.

In the United States, routine smallpox vaccination ended in 1972 but was reinstituted in January 2003 in response to concerns of potential bioterrorism attacks. Secondary vaccinia transmission has, fortunately, been a rare event [1–3], although a recent case of eczema vaccinatum occurring in a child whose parent had been vaccinated 21 days previously and whose scab had healed serves as a reminder that inadvertent transmission can be catastrophic [4]. A live virus, vaccinia can be transmitted from a vaccine recipient through direct contact or through indirect contact via fomites [2, 5, 6]. In a seminal study from 1975, Koplan et al. [7] reported that cultures of specimens from vaccination sites demonstrated virus to be present for a mean duration of 7.8 days (range, 0–18 days). A more recent study demonstrated that vaccinia-naive patients shed virus from vaccination sites 2–6 days longer and had higher peak mean viral titers, compared with non–vaccinia-naive vaccinated persons, and vaccinia was recovered from a small number of patients up to 23 days after vaccination [8]. The current study sought to readdress the question of persistence of vaccinia at sites of vaccination.

Materials and methods. This was a prospective trial conducted at the Walter Reed Army Medical Center (Washington, DC) in January 2003. The protocol was approved by the institutional review board, and all subjects provided written informed consent. The guidelines for human experimentation of the US Department of Health and Human Services and those of Walter Reed Army Medical Center were observed in the conduct of the study.

Healthy adults receiving the smallpox vaccine were eligible for participation in the study. Subjects were inoculated in the standard manner by multiple puncture vaccination (scarification) using an individually wrapped, sterile, bifurcated needle. The vaccine used in this study was the NYCBOH strain (Dryvax, Wyeth Laboratories; lot no. 4020071; 1×10^8 plaque-forming units per mL); the vaccine was reconstituted on the day of administration in accordance with the package insert instructions. Patients presenting for their primary vaccination (i.e., patients receiving their first smallpox vaccination; hereafter referred to as primary vaccinees) received 3 perpendicular insertions within a 5-mm area, and patients who had previously been vaccinated (hereafter referred to as revaccinees) received 15 insertions.

Volunteers had swabs of the planned vaccination site performed before vaccination and on days 3, 7, 9, 14, and 21 (± 1 day) after vaccination. Vaccination sites were covered with a semipermeable bandage (Coverlet, BSN-Jobst) in accordance with hospital policy, and cultures were performed at the time of dressing change.

Swabs were performed using a sterile rayon tipped applicator (Pur-Wraps, Puritan Medical Products), and swab specimens were placed in viral transport media (Bartels ViraTrans, Trinity Biotech) and frozen at -70°C until analysis. Electrochemiluminescence antigen detection assay was conducted using the Origen immunoassay system (Igen), as described elsewhere [9]. Antibodies used in the antigen detection assay consisted of a mixture of monoclonal antibodies prepared against vaccinia virus envelope proteins. Samples for nucleic acid detection were prepared using the IsoCode Stix DNA isolation device (Schleicher and Schuell) according to the manufacturer's instructions. Vaccinia virus nucleic acid detection was performed using rapid PCR (LightCycler [Roche] and SmartCycler [Cepheid]), as described elsewhere [10]. Viral culture was performed on Vero

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Table 1. Results of viral culture, electrochemiluminescense (ECL), and PCR for detection of vaccinia at the site of vaccination with NYCBOH strain, by number of days after vaccination.

No. of days after vaccination	No. of specimens with a positive result/no. of specimens tested (%)								
	Primary vaccinees			Revaccinees			Total		
	Culture	PCR	ECL	Culture	PCR	ECL	Culture	PCR	ECL
3	10/13 (77)	13/13 (100)	10/13 (77)	13/14 (93)	14/14 (100)	11/14 (79)	23/27 (85)	27/27 (100)	21/27 (78)
7	13/14 (93)	14/14 (100)	14/14 (100)	11/13 (85)	13/13 (100)	13/13 (100)	24/27 (89)	27/27 (100)	27/27 (100)
9	11/12 (92)	12/12 (100)	12/12 (100)	10/13 (77)	13/13 (100)	13/13 (100)	21/25 (84)	25/25 (100)	25/25 (100)
14	10/13 (77)	14/14 (100)	13/14 (93)	3/14 (21)	13/14 (39)	12/14 (86)	13/27 (48)	27/28 (96)	25/28 (89)
21	8/13 (62)	10/13 (77)	8/13 (62)	2/14 (14)	6/14 (43)	8/14 (57)	10/27 (37)	16/27 (59)	16/27 (59)

NOTE. Because of missing data (volunteer no-show or a lost sample), all 28 volunteer samples were not assessed for all time points.

cells with 100 μL of inoculum according to standard procedures [11]. Cultured cells exhibiting cytopathic effect within 14 days after inoculation were submitted for nucleic acid and/or electrochemiluminescence testing for agent identification. Comparison of rates of positive culture results between primary vaccinees and revaccinees at different time points was performed using Fisher's exact test (2-tailed).

Results. Thirty volunteers enrolled, and 28 completed the study and were included in the analysis; 1 volunteer withdrew prior to vaccination, and 1 was withdrawn from the study at day 10 because no reaction to the vaccine was observed (as evidenced by the lack of pustule formation at the vaccination site). Fourteen volunteers were primary vaccinees, and 14 were revaccinees. All baseline swab specimens tested negative for vaccinia. Table 1 shows the results of viral culture, electrochemiluminescence, and PCR for the detection of vaccinia at the site of vaccination, by time point and by vaccination status (primary vaccinee vs. revaccinee). Because of missing data (volunteer no-show or a lost sample), all 28 volunteer samples were not assessed at all time points. Primary vaccinees were significantly more likely than were revaccinees to have a positive culture result at days 14 ($P = .007$) and 21 ($P = .018$). Of interest, the volunteer who was withdrawn because of lack of reaction to vaccine had detectable virus by PCR through day 9 but never had a positive culture result.

Discussion. Similar to findings published >30 years ago, our study reports the persistence of vaccinia at sites of vaccination for at least 3 weeks after vaccination. Our rate of virus culture at $\geqslant$16 days (37%) is higher than the rate previously reported (5%) [7]; vaccinia occurred at a higher rate among primary vaccinees than among revaccinees at days 14 and 21. The longer shedding time of vaccinia from the vaccination site in primary vaccinees has also been observed in other studies [7, 8]. PCR and electrochemiluminescence detected vaccinia virus at a higher rate, compared with culture, at the later time points of the study, and this presumably relates to the recovery of noninfectious vaccinial fragments. Because of the recent report of transmission of vaccinia from a vaccinee to his child with eczema and the resultant eczema vaccinatum, our results highlight that a "safe" time for absence of transmission after vaccination is not well-defined, and providers should adhere to the current recommendations to avoid administration of vaccinia vaccination to patients with at-risk contacts [12].

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Potential conflicts of interest. All authors: no conflicts.

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