given region can be small. This would make it difficult to recruit enough controls within the small areas affected by most outbreaks, particularly within specific age strata. A control bank may therefore be more practical for use in large communitywide outbreaks, outbreaks that occur over large regions (i.e., an entire state), or in densely populated areas. Additionally, the lengthy start-up time required for questionnaire programming with a CATI system also supports the view that the CATI method may be maximally applicable in large-scale investigations.

LeAnne M. Fox,*
D. Charles Hunt,†‡
and Michael J. Beach*

*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; †Kansas Department of Health and Environment, Topeka, Kansas, USA, and ‡University of Kansas Medical Center, Kansas City, Kansas, USA

References

1. Kirk M, Tribe I, Givney R, Raupach J, Stafford R. Computer-assisted telephone interview techniques. Emerg Infect Dis. 2006;12:697.
2. Fox LM, Ocfemia MCB, Hunt DC, Blackburn BG, Neises D, Kent WK, et al. Emergency survey methods in acute cryptosporidiosis outbreak. Emerg Infect Dis. 2005;11:729–31.

Address for correspondence: LeAnne Fox, Division of Infectious Diseases, Children's Hospital, 300 Longwood Avenue, Boston, MA 02115, USA; fax: 617-730-0254; email: Leanne.Fox@childrens.harvard.edu

EID Online
www.cdc.gov/eid

Lack of Transmission of Vaccinia Virus

To the Editor: Recently, the US government completed a targeted vaccination strategy limited to healthcare workers, first responders, and the military because of concern that variola virus, the etiologic agent of smallpox, might be used as a biowarfare agent (1). A concern in such programs is the potential for unintended spread of the vaccine virus (vaccinia) from the primary vaccinee to contacts who may be at the greatest risk of having adverse reactions resulting from secondary transmission (2,3).

Contact spread of the live attenuated vaccinia virus is considered the predominant method of secondary transmission. The conventional methods of preventing a secondary transmission event in the household of a smallpox vaccine recipient include the use of bandages and long sleeves to limit direct contact with the lesion and immediate hand-washing when contact occurs (4).

Several recent reports have measured the presence of vaccinia virus on the dressings or hands of vaccinated persons; however, the recovery of vaccinia virus in the environment has not been evaluated after vaccination in a controlled setting (5–7). We present the first reported attempt to recover live vaccinia virus from the homes of recently vaccinated persons. This study was approved by the St. Louis University Institutional Review Board. We hypothesized that live vaccinia virus shed from the skin reaction could not be recovered in the natural environment, and as a result, constitutes a limited risk for contact transmission.

Three hundred eighty-seven environmental swab samples were collected on 3 different study days from 43 persons (mean age 24 years) with major cutaneous reactions. Persons who participated in this study were selected from a randomized, double-blind, single-center study that compared the safety, tolerability, and immunogenicity of 3 smallpox vaccines (8,9). Following vaccination and after each study visit, the vaccination site was covered with an OpSite Post-Op dressing (Smith and Nephew, Massilon, OH, USA). On postvaccination days 7, 10, and 15, a sterile Calgiswab type 2 applicator (Harwood Products Co., Guilford, ME, USA), moistened in sterile water, was rotated over the linen from the study participant's bed (approximate location of sleeping area), the middle of his or her bath towel, and the inside area of a shirt sleeve adjacent to the vaccination bandage (before laundering). These sampling areas were chosen on the basis of the likelihood of exposure to the semipermeable bandage and the potential for another person to come in contact with the vaccinia virus in these areas. An additional 129 samples from the palm of the study participant's hand used to take the environmental samples were taken to serve as a control mechanism.

After sampling, the tip of the swab was stored in a 15-mL conical tube containing 3 mL multimicrobe transport media (Remel, Lenexa, KS, USA). The 15-mL conical tubes were returned to the clinic in a cooler on cold packs the same day. Recovery of vaccinia virus was determined by infectivity assay. Samples were tested for infectious vaccinia virus by inoculation of fluid cultures of Vero cells grown in 12-well plates. A sample was defined as positive if cytopathic effects were observed (10).

Concurrent with the environmental sampling, the lesion and the outside of the bandage covering the inoculation site for each study participant were swabbed with a Calgiswab Type 2 sterile applicator, and the samples were analyzed by infectivity assay. These samples served as a positive control, indicating that the method

used to sample the environment was appropriate and sensitive.

All 516 environmental samples from designated sampling areas in the homes of recently vaccinated vaccinia-naive persons were negative for live virus as determined by plaque infectivity assay (Table). Only 1 (0.78%) of 129 dressing samples tested on day 7 had measurable titers of vaccinia.

Contact with live vaccinia virus from the lesion at the site of vaccination is the underlying cause of secondary transmission. Common mechanisms for transmission include contact with contaminated bandages and intimate sexual contact. Recent studies have compared a variety of bandages used to cover the vaccination site to determine which class of bandage provides the greatest protection against disseminated virus. Talbot et al. observed that <1% (N = 918) of dressing samples were positive for vaccinia (an initial semipermeable OpSite Post-Op dressing and an outer semipermeable Tegaderm bandage) (5). In a single-blind randomized trial design, Waibel et al. compared the presence of vaccinia virus on the external surface of 3 different types of bandages and noted that the semipermeable membrane with gauze had the smallest proportion of recoverable virus compared with the groups that used a Band-Aid or double gauze with adherent tape (6). Despite the difference in types of bandages from these

studies, the results were remarkably consistent with regard to the limited dissemination of vaccinia virus outside the dressing. In concordance with our results, the semipermeable bandage provided significant protection from exposure to the virus on the outside of the bandage.

This study presented many challenges regarding the sampling and collection of specimens. Collection times, sampling technique, and environmental conditions may have contributed to the absence of viral recovery. In addition, we chose to measure live vaccinia virus as opposed to noninfectious viral genomes (by polymerase chain reaction) because we were concerned with transmission and infectivity. If we had chosen to measure noninfectious viral genomes, a positive outcome may have been likely. However, from a public health standpoint, such information would have been less valuable. Further studies will need to examine the viability of vaccinia virus in the environment to evaluate the possibility of contact transmission.

Acknowledgments

**James H. Stark,* Sharon E. Frey,†
Paul S. Blum,*
and Thomas P. Monath***

*Acambis Inc., Cambridge, Massachusetts, USA; and †St. Louis University School of Medicine, St. Louis, Missouri, USA

References

1. Centers for Disease Control and Prevention. Vaccinia (smallpox) vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2001. MMWR Morb Mortal Wkly Rep. 2001;50:1–26.
2. Sepkowitz KA. How contagious is vaccinia? N Engl J Med. 2003;348:439–46.
3. Neff KM, Lane JM, Fulginiti VA, Henderson DA. Contact vaccinia–transmission of vaccinia from smallpox vaccination. JAMA. 2002;288:1901–5.
4. Department of Defense Military Vaccines. Smallpox vaccine trifold brochure. [cited 2003 May 4]. Available from http://www.smallpox.army.mil/media/pdf/Familybrochure.pdf
5. Talbot TR, Ziel E, Doersam JK, LaFleur B, Tollefson S, Edwards KM. Risk of vaccinia transfer to the hands of vaccinated persons after smallpox immunization. Clin Infect Dis. 2004;38:536–41.
6. Waibel KH, Ager EP, Topolski RL, Walsh DS. Randomized trial comparing vaccinia on the external surfaces of 3 conventional bandages applied to smallpox vaccination sites in primary vaccinees. Clin Infect Dis. 2004;39:1004–7.
7. Hepburn MJ, Dooley DP, Murray CK, Hospenthal DR, Hill BL, Nauschuetz WN, et al. Frequency of vaccinia virus isolation on semipermeable versus nonocclusive dressings covering smallpox vaccination sites in hospital personnel. Am J Infect Control. 2004;32:126–30.
8. Weltzin R, Liu J, Pugachev KV, Myers GA, Coughlin B, Blum PS, et al. Clonal vaccinia virus grown in cell culture as a new smallpox vaccine. Nat Med. 2003;9:1125–30.

Table. Sampling outcomes of 43 study participants who received 1 of 3 smallpox vaccines

Variable	All participants (N = 43)	Vaccine group		
		ACAM1000 (n = 14)	ACAM2000 (n = 15)	Dryvax (n = 14)
Mean ± SD titer from lesion*†‡				
Day 7	3.84 ± 0.98	3.77 ± 1.15	3.85 ± 1.06	3.91 ± 0.74
Day 10	3.75 ± 0.89	3.62 ± 0.54	3.58 ± 1.21	4.07 ± 0.74
Day 15	4.19 ± 1.40	4.08 ± 1.24	3.67 ± 1.57	4.86 ± 1.18
Mean ± SD titer from dressing§				
Day 7	0.63 ± 0.85	0.9 ± 1.50	0	0
Day 10	0	0	0	0
Day 15	0	0	0	0

*Viral shedding results reported as <X were converted to X/2 before calculating.
†Titers are reported as $\log_{10}$ PFU/mL.
‡There were no significant differences among the groups (p>0.05).
§Limit of detection was 1.0 $\log_{10}$ PFU/mL.

9. Monath TP, Caldwell JR, Mundt W, Fusco J, Johnson CS, Buller M, et al. ACAM2000 clonal Vero cell culture vaccinia virus (New York City Board of Health strain): a second-generation smallpox vaccine for biological defense. Int J Infect Dis. 2004;8S2: S31–44.

10. Graham BS, Belshe RB, Clements ML, Dolin R, Corey L, Wright PF, et al. Vaccination of vaccinia-naive adults with human immunodeficiency virus type 1 gp160 recombinant vaccinia virus in a blinded, controlled, randomized clinical trial. The AIDS Vaccine Clinical Trial Network. J Infect Dis. 1992;166:244–52.

Address for correspondence: Thomas P. Monath, Acambis Inc., 38 Sidney St, Cambridge, MA 02139, USA; fax: 617-494-0924; email: tom.monath@acambis.com

Discrimination between Highly Pathogenic and Low Pathogenic H5 Avian Influenza A Viruses

To the Editor: To thoroughly investigate avian influenza outbreaks, identifying highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI) is essential. Currently, determination of inserted basic amino acids within the hemagglutinin cleavage site of HPAI relies on nucleotide sequencing (*1–3*). Direct sequencing is relatively time-consuming and laborious and thus is not suitable for local and regional diagnostic laboratories that receive large numbers of samples that may contain HPAI or LPAI subtype H5N1. Therefore, a rapid diagnostic assay was developed to discriminate between HPAI and LPAI subtype H5 viruses by 1-step real-time reverse transcription–polymerase chain reaction (RT-PCR) with melting curve analysis.

H5 primers flanking the cleavage site were designed from conserved regions among HPAI and LPAI strains by using nucleotide sequences obtained from GenBank and our previous studies (*4–6*). The primers consisted of a forward primer H5F3+ (nucleotides 1001–1021: 5′-AACA-GATTAGTCCTTGCGACTG-3′) and a reverse primer H5R2+ (nucleotides 1124–1103: 5′-CATCTACCATTCC-CTGCCATCC-3′), which yielded products of ≈124 bp and ≈112 bp, corresponding to HPAI and LPAI, respectively. Consequently, the sizes of the amplicons and percentage guanine-cytosine content were different, allowing discrimination between HPAI and LPAI by melting curve analysis (*7*).

One-step, real-time RT-PCR with melting curve analysis was performed in an ABI 7500 system (Applied Biosystems, Foster City, CA, USA). In each reaction, 3.0 μL of RNA sample was combined with a reaction mixture containing 10 μL 2× SYBR Green PCR Master Mix (Applied Biosystems), 0.5 μL 40× MultiScribe (Applied Biosystems) and RNase inhibitor, each primer (at final concentration of 0.5 μmol/L), 1.5 mmol/L $MgCl_2$, and RNase-free water in a final volume of 20 μL. The thermal profile began with incubation at 48°C for 45 min (reverse transcription), then incubation at 95°C for 10 min (predenaturation), followed by 40 cycles of amplification alternating between 94°C for 15 s (denaturation) and 68°C for 40 s (annealing/extension). The SYBR Green I fluorescent signal was obtained once per cycle at the end of the extension step. After amplification, melting curve analysis was performed by heating the sample to 95°C for 15 s, then cooling it to 70°C for 1 min, followed by a linear temperature increase to 95°C at a rate of 0.5°C/s, while continuously monitoring the fluorescent signal. Data were analyzed by the 7500 System SDS Software version 1.2 (Applied Biosystems).

To develop the assay, samples previously identified as influenza A/chicken/Nakorn-Patom/Thailand/CU-K2/04 (H5N1) served as a control for HPAI, and A/duck/Hong Kong Special Administrative Region, People's Republic of China/308/78 (H5N3) served as a control for LPAI. The H5 genes (nucleotides 914–1728) of each strain were inserted into pGEM-T Easy Vector and then transcribed in vitro by using RiboMAX Large Scale RNA Production System-T7 (Promega, Madison, WI, USA). Serial 10-fold dilutions of the standard H5 RNA were subjected to a sensitivity test (*8*). The fluorescent signal can be detected at RNA dilutions as low as 10^2 copies/μL. To assess the specificity, viral RNA extracted from other subtypes of influenza A viruses (H1–H4 and H6–H15) was tested. The assay was specific for the H5 subtype, since no amplification was detected from other subtypes.

Three preliminary melting curve analyses showed that this assay was effective in discriminating between the melting peaks of HPAI and LPAI (Figure). The variations of melting temperature (T_m) between runs were experimentally determined. The mean (standard deviation) of T_m values for HPAI and LPAI were 77.43°C (0.21°C) and 79.57°C (0.23°C), respectively. This assay provided high reliability and reproducibility, since the coefficients of variation were <0.30.

Seventy-eight specimens of influenza A virus were used to validate the assay. The 75 HPAI samples were isolated during the 2004 outbreak in Thailand; 3 LPAI samples, including A/avian/NY/01 (H5N2), A/Chicken/Mexico/31381–3/94 (H5N2), and A/shoveler/Egypt/03 (H5N2), were provided by the Centers for Disease Control and Prevention. The viruses were isolated in embryonated chicken