ORIGINAL ARTICLE

The Detection of Monkeypox in Humans in the Western Hemisphere

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N Engl J Med 2004;350:342-50.

ABSTRACT

BACKGROUND

During May and June 2003, an outbreak of febrile illness with vesiculopustular eruptions occurred among persons in the midwestern United States who had had contact with ill pet prairie dogs obtained through a common distributor. Zoonotic transmission of a bacterial or viral pathogen was suspected.

METHODS

We reviewed medical records, conducted interviews and examinations, and collected blood and tissue samples for analysis from 11 patients and one prairie dog. Histopathological and electron-microscopical examinations, microbiologic cultures, and molecular assays were performed to identify the etiologic agent.

RESULTS

The initial Wisconsin cases evaluated in this outbreak occurred in five males and six females ranging in age from 3 to 43 years. All patients reported having direct contact with ill prairie dogs before experiencing a febrile illness with skin eruptions. We found immunohistochemical or ultrastructural evidence of poxvirus infection in skin-lesion tissue from four patients. Monkeypox virus was recovered in cell cultures of seven samples from patients and from the prairie dog. The virus was identified by detection of monkeypox-specific DNA sequences in tissues or isolates from six patients and the prairie dog. Epidemiologic investigation suggested that the prairie dogs had been exposed to at least one species of rodent recently imported into the United States from West Africa.

CONCLUSIONS

Our investigation documents the isolation and identification of monkeypox virus from humans in the Western Hemisphere. Infection of humans was associated with direct contact with ill prairie dogs that were being kept or sold as pets.

Monkeypox is an uncommon viral zoonosis caused by a member of the genus orthopoxvirus.[1] Monkeypox was initially recognized in 1958 as a viral eruption of captive primates. The first cases in humans were reported in 1970 in Zaire (now the Democratic Republic of Congo).[1] Since then, monkeypox has occurred sporadically in humans throughout that region[2-9] but has not been reported outside Africa.

During May and June 2003, an outbreak of febrile illness with skin eruptions occurred among residents of the midwestern United States.[10] All patients reported having contact with sick pet prairie dogs (cynomys species) obtained through a common distributor. Zoonotic bacterial or viral pathogen transmission was suspected, and studies were conducted to identify the etiologic agent. This report summarizes the initial epidemiologic, clinical, and laboratory investigation of the outbreak in Wisconsin.

METHODS

DESCRIPTION OF THE OUTBREAK

On May 24, 2003, the Wisconsin Division of Public Health (DPH) was notified of a three-year-old girl (Patient 1) hospitalized in central Wisconsin with cellulitis and fever after a bite from Prairie Dog 1 on May 13. The prairie dog had been purchased on May 11 at a swap meet where animals are bought or traded. It became ill on May 13 and was noted to have ocular discharge, lymphadenopathy, and papular skin lesions. The animal died on May 20, and an enlarged submandibular lymph node was submitted to Marshfield Laboratories for bacterial culture. On May 24, a gram-negative bacillus was isolated, raising the suspicion of tularemia or plague. It was ultimately identified as an acinetobacter species and considered to be a contaminant. No similar illnesses were reported, and the case appeared to be an isolated event.

On June 2, the DPH was notified by the Milwaukee Health Department of an illness in a meat inspector (Distributor 2, who was also Patient 4) who resided in southeastern Wisconsin and also worked as a distributor of exotic animals. He had been bitten and scratched by a prairie dog on May 18, a nodular skin lesion developed at the scratch site on May 23, and fever, chills, sweats, and lymphadenopathy began on May 26. He was examined and released from a local emergency department, but his illness worsened. On May 31 he was hospitalized, and tularemia and plague were considered in the differential diagnosis. By June 3 it was determined that he had sold two prairie dogs to the index patient's family at the swap meet. An epidemiologic link between the two cases in different regions of Wisconsin was established, and public health case-finding and animal "trace-back" and "trace-forward" activities were initiated (Fig. 1).

On June 2, the DPH was notified that Marshfield Laboratories had electron-microscopical evidence of a poxvirus in a skin lesion from Patient 2 (the mother of Patient 1), who became ill on May 26. On June 4, orthopoxvirus was visualized by negative-stain electron microscopy of cell-culture supernatants from Patient 2 and Prairie Dog 1, and the DPH was informed. The DPH arranged confirmatory testing of tissue and skin-biopsy specimens from Patient 4 (Distributor 2) and Patients 7 and 8 by the Centers for Disease Control and Prevention (CDC) Poxvirus Section on June 5.

On June 6, polymerase-chain-reaction (PCR) testing at the CDC of specimens from Patients 4, 7, and 8 revealed monkeypox-virus DNA signatures. On June 7, the complete sequence of the hemagglutinin gene derived from the virus from Distributor 2 proved identical to hemagglutinin gene sequences derived from one of two reference monkeypox-virus clades. On June 9, PCR analyses of tissue- and virus-culture supernatants from Patient 2 and Prairie Dog 1 were positive for monkeypox-virus DNA signatures.

PRAIRIE DOGS IN WISCONSIN

From April 15 through May 17, Distributor 2 had purchased 39 prairie dogs from a distributor in northeastern Illinois (Distributor 1). On May 3, Distributor 2 agreed to transport an ill Gambian giant rat (cricetomys species), which had recently been imported from Ghana, from the premises of Distributor 1 to an exotic-animal veterinarian in Wisconsin. The distributor, 15 prairie dogs, and 94 other animals purchased from other suppliers that day reportedly had no direct contact with the rat, which was in its own cage, during the three-hour trip. Subsequently, the rat died, but necropsy did not provide a specific diagnosis. The carcass was incinerated and was not available for further testing.

On May 5, Distributor 2 delivered 2 prairie dogs to Pet Store 1 and 10 prairie dogs to Pet Store 2. Pet Store 2 had received 15 healthy prairie dogs from Distributor 2 in mid-April. The prairie dogs received on May 5 had thinner coats and did not appear as

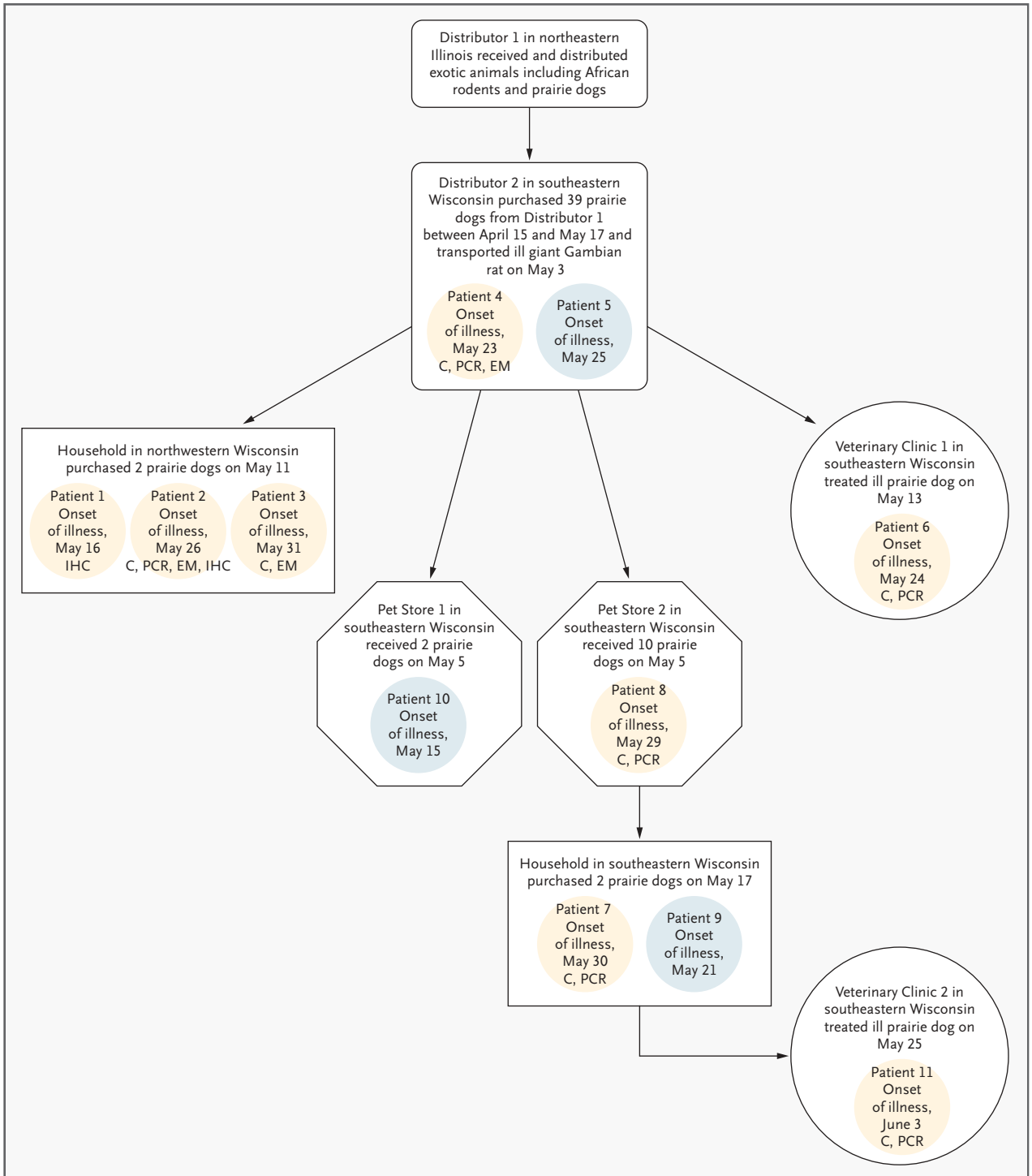

Figure 1. Laboratory-Confirmed Cases (Yellow Symbols) and Probable and Suspected Cases (Blue Symbols) of Monkeypox Reported among Wisconsin Residents as of June 7, 2003.

Case status was determined by means of clinical and laboratory data obtained on or after June 7, 2003. Laboratory-confirmed cases of monkeypox were confirmed by means of viral culture (C), polymerase-chain-reaction assay (PCR), electron microscopy (EM), and immunohistochemical analysis (IHC). Rounded rectangles denote distributors, rectangles households, octagons pet stores, and circles veterinary clinics.

robust as the animals in the earlier shipment, and these animals were housed with the unsold animals from the first batch.

On May 8, Distributor 2 was notified by an owner of Pet Store 1 that the prairie dogs were ill, with watery eyes, congestion, and skin lesions. Distributor 2 retrieved both prairie dogs on May 10; one died, and the other recovered. By May 11, many prairie dogs at Pet Store 2 had watery eyes and nasal discharge, and several had papular skin lesions. The animals were treated by a veterinarian with an oral quinolone and doxycycline. Distributor 2 purchased his final lot of five prairie dogs from Distributor 1 on May 17; all five became ill within a few days after purchase and were not sold. Four of the animals died.

EARLY CONTROL MEASURES

By June 6, control measures invoked by state and local public health and veterinary officials included isolation at home or in the hospital of patients with suspected cases and public health or hospital infection-control surveillance of asymptomatic persons who had been in contact with ill patients or prairie dogs. Recommendations for infection control and personnel protection, similar to those for smallpox, were distributed to health care providers and veterinarians. The premises of affected pet owners and affected business were inspected, infected and exposed mammals were placed under quarantine, and advice about environmental sanitation and personal protection was provided. Seven prairie dogs sold by Distributor 2 that were still at Pet Store 2 were killed on June 5. Four asymptomatic prairie dogs were quarantined at the business location of Distributor 2. By June 6, 38 of the 39 prairie dogs purchased by Distributor 2 from Distributor 1 during April and May had been located. Other mammals that had been in contact with ill prairie dogs were quarantined where they were housed, and humans with suspected infections were isolated where they lived. On June 6, the state epidemiologist for communicable diseases signed an emergency order to prohibit the importation, sale, distribution, or display of prairie dogs or any mammals that had been in contact with prairie dogs that had arrived in Wisconsin after April 1, 2003.

RESULTS

IDENTIFICATION OF CASES

By June 7, 11 patients with confirmed or suspected monkeypox had been identified in central and southeastern Wisconsin. At that time, a suspected case of monkeypox in humans was defined as vesiculopustular skin lesions and fever in persons exposed to animals obtained from Distributor 1 or 2. All 11 patients were linked by direct contact with prairie dogs sold by Distributor 2. The index family (which included Patients 1, 2, and 3) had purchased two prairie dogs from Distributor 2. The eight patients identified in southeastern Wisconsin (Patients 4 through 11) included Distributor 2 and his wife, two employees of different pet stores (1 and 2) that had received prairie dogs from Distributor 2, two veterinarians from different clinics who had treated ill prairie dogs sold by Distributor 2, a person who had purchased two prairie dogs from Pet Store 2, and her houseguest (Fig. 1). The earliest date of onset of illness was May 15.

CLINICAL FEATURES

The 11 initial cases in Wisconsin occurred in 5 males and 6 females ranging in age from 3 to 43 years. Six of the 11 patients had previously received a single dose of smallpox vaccine during childhood. In all cases transmission of monkeypox virus appeared to be by direct contact with an infected prairie dog. However, Patients 2 and 3 provided direct care to their infected child, and the possibility of person-to-person transmission cannot be excluded. Patients 1 and 4 were scratched or bitten by an ill prairie dog. In three patients, the infection appeared to be transmitted directly to open wounds: a cat scratch on the hand of Patient 2, a cut on the hand of Patient 6, and brush scratches on the legs of Patient 7. The incubation period for the infection was difficult to determine owing to the lengthy intervals of exposure to infected prairie dogs and could have ranged from 4 to 24 days (median, 15; mean, 14.5).

Initial signs or symptoms were typically skin lesions or fever (temperature above 38°C) with drenching sweats and severe chills. Frequent signs and symptoms were skin lesions (100 percent), headache (100 percent), fever (82 percent), sweats (82 percent), chills (82 percent), persistent cough (73 percent), lymphadenopathy (55 percent), and sore throat (55 percent). Less frequent signs and symptoms were pharyngitis (27 percent), tonsillar hypertrophy (18 percent), tonsillar erosions (18 percent), malaise (18 percent), mild chest tightness (18 percent), diarrhea (18 percent), myalgias (9 percent), back pain (9 percent), nasal congestion (9 percent), blepharitis (9 percent), and nausea (9 percent). Pulmonary, cardiac, abdominal, neuro-

logic, and musculoskeletal examinations were normal in all patients examined.

In five patients, primary skin lesions appeared as nodular swellings around the margins of bites or scratches (Fig. 2A, 2B, and 2C). In most patients, including the six patients without identifiable primary lesions, 1 to 50 satellite and disseminated skin lesions developed over a period of several days. Lesions evolved from papules to vesiculopustules, some with prominent erythematous flares, and resolved leaving serous-to-hemorrhagic crusts that eventually detached. Different stages in the evolution of the lesions were seen simultaneously in all patients with multiple lesions. Lesion sites included the face, scalp, hands, arms, legs, trunk, perineum, conjunctivae, and buccal mucosa (Fig. 2D, 2E, 2F, 2G, and 2H). Larger lesions left central scars (Fig. 2I). Four of the 11 patients were hospitalized for their illnesses. The clinical course was self-limited in all cases. The median time to crusting of all skin lesions was 12 days (range, 3 to 25). Nine patients received antibiotics (six received ciprofloxacin, and eight were given doxycycline). Patient 1 received intravenous acyclovir, and Patients 2 and 4 received valacyclovir. No patients received vaccinia immune globulin.

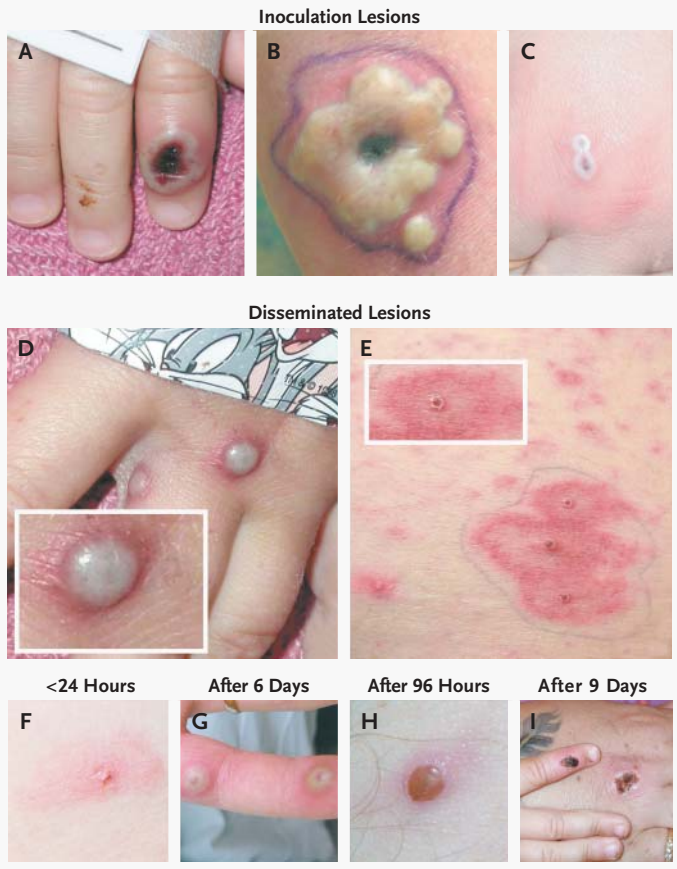

Figure 2. Primary Inoculation Reactions (Panels A, B, and C), Examples of the Smallpox-like (Panel D) and Umbilicated Varicella-like (Panel E) Disseminated Monkeypox Lesions, and the Morphologic Appearance of Disseminated Lesions over Time (Panels F, G, H, and I).

Panel A shows a primary inoculation reaction at the site of a prairie-dog bite on Patient 1, Panel B a prairie-dog scratch on Patient 4, and Panel C a preexisting cat scratch on Patient 2. Panel F shows a disseminated lesion less than 24 hours after its appearance, Panel G lesions after six days, Panel H a lesion after 96 hours, and Panel I a lesion after more than nine days.

LABORATORY INVESTIGATIONS

A battery of laboratory tests was used to identify the etiologic agent of infection. The specific tests used to confirm the diagnosis of monkeypox for individual patients varied depending on the availability of specimens and the stage of illness. Results are summarized in Figure 1.

Sections of formalin-fixed, paraffin-embedded skin-biopsy specimens obtained at the pustular stage from two patients and stained with hematoxylin and eosin showed marked ballooning degeneration of keratinocytes with epidermal necrosis and spongiotic edema (Fig. 3A). In portions of the epidermis, keratinocytes exhibited multinucleation, nuclear clearing with margination of chromatin, and occasional eosinophilic cytoplasmic inclusions (Fig. 3B). A moderate neutrophilic and lymphocytic inflammatory infiltrate was present within the epidermis and superficial dermis.

Orthopoxviral antigen was detected in skin-biopsy specimens by means of immunohistochemical staining with rabbit antivaccinia polyclonal antibody (Virostat).[11] Antigen was most prominent in degenerating keratinocytes and follicular epithelium but was absent in normal-appearing skin at the edges of the specimen (Fig. 3C). Immunohistochemical stains for herpes simplex viruses 1 and 2 and cytomegalovirus were negative.

Tissue samples from seven patients and a submandibular lymph node from Prairie Dog 1 were inoculated onto primary and continuous cell lines, including rhesus-monkey kidney, rabbit kidney, MRC-5, RD, HEp-2, B-SC-40, and VERO cells. Except for the HEp-2 cells, cytopathic changes occurred in all cell lines within one to four days and were characterized by plaques of elongated and rounded cells with prominent cytoplasmic bridging and formation of syncytium.

Transmission electron microscopy was performed on glutaraldehyde-fixed skin-biopsy specimens from two patients.[11,12] Virions in various stages of assembly were observed within the cytoplasm of keratinocytes (Fig. 3D). Cross sections of mature virions contained dumbbell-shaped cores characteristic of poxviruses (Fig. 3E). Negative-stain electron microscopy with phosphotungstic acid[13] was performed on cultures from Patient 2 and Prairie Dog 1 and revealed numerous brick-shaped virions with regularly spaced, threadlike ridges on the exposed surfaces (Fig. 3F). Taken together, the cell-culture and ultrastructural features suggested that the virus was a member of the genus orthopoxvirus.

For molecular diagnosis, DNA was extracted from tissues, specimens obtained with swabs, "touch" preparations (obtained by touching the patients' skin), and cell-culture supernatants.[14] Clinical samples were positive for orthopoxvirus on a PCR assay that amplifies a conserved segment of the DNA polymerase gene (*E9L*) present in all Old World orthopoxviruses except variola. To characterize the virus further, PCR of the orthopoxvirus hemagglutinin and A-type inclusion genes was performed, followed by restriction-fragment–length polymorphism analysis.[15-18] Restriction profiles obtained from samples from the patients and Prairie Dog 1 were identical to reference strains of monkeypox virus and differed from other known orthopoxviruses (Fig. 4A). In addition, a PCR assay for the gene for the monkeypox extracellular-envelope virus protein was positive. In contrast, a real-time PCR assay designed specifically to identify the vaccinia virus cytokine-response modifier B gene sequences was negative.

The combined PCR test results involving independent gene sequences provided strong evidence that the DNA signatures in the clinical samples were caused by a "monkeypox-like" Old World orthopoxvirus. Excluded orthopoxviruses included ectromelia and gerbilpox viruses (both of which have been isolated from rodents but are not considered human pathogens), vaccinia virus (which has the potential to infect some rodents), cowpox virus (which has rodent reservoirs and can cause limited infections in humans), camelpox virus (which is not a cause of human infections), variola (smallpox) virus, and the recognized New World orthopoxviruses.

The remote possibility that an undiscovered Old World orthopoxvirus might have the same DNA fingerprints as monkeypox virus led us to sequence the entire orthopoxvirus hemagglutinin gene from the

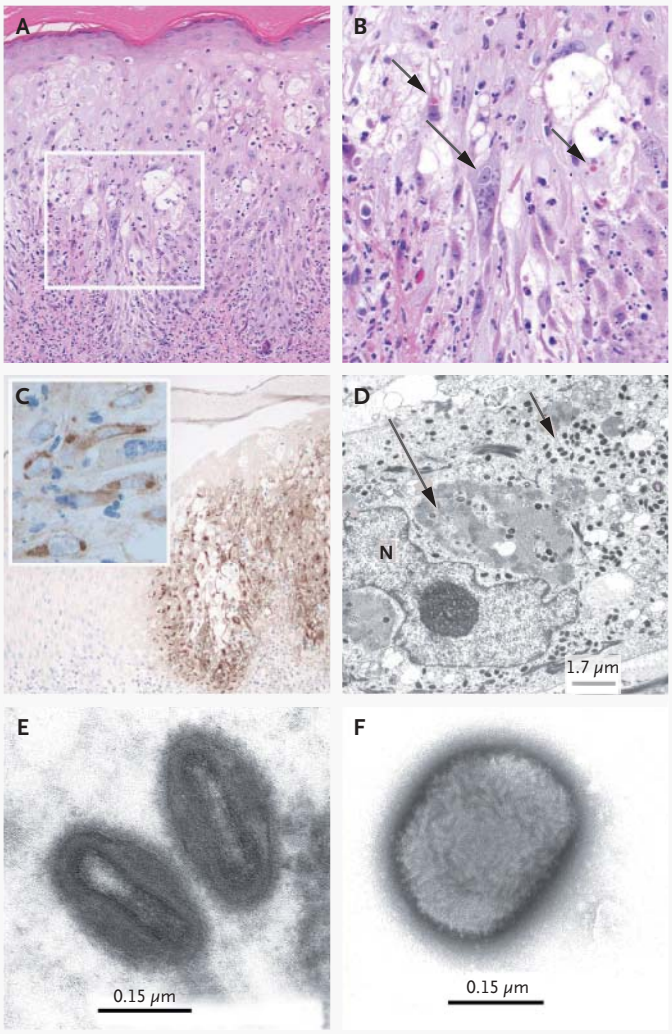

Figure 3. Histologic, Immunohistochemical, and Ultrastructural Evaluation of the Skin-Biopsy Specimen from Patient 2.

Panel A shows scattered degenerating and necrotic keratinocytes within the epidermis and a moderate inflammatory-cell infiltrate within the epidermis and superficial dermis (hematoxylin and eosin, ×50). Panel B shows the boxed area in Panel A at a higher magnification (×200); a multinucleated cell (long arrow) and eosinophilic viral inclusion bodies (short arrows) are evident. Panel C shows immunohistochemical staining of orthopoxvirus antigen within the epidermis (horseradish peroxidase with hematoxylin counterstain, ×40). The inset shows immunoreactivity within individual keratinocytes (×250). Panel D shows virions within the cytoplasm of a keratinocyte and includes immature forms that are being assembled (long arrow) and clusters of mature virions (short arrow). N denotes nucleus. Panel E shows virions with dumbbell-shaped cores characteristic of poxviruses. Panel F shows a negatively stained virion from cell culture (phosphotungstic acid). The brick-shaped particle has regularly spaced, threadlike ridges on the exposed surface.

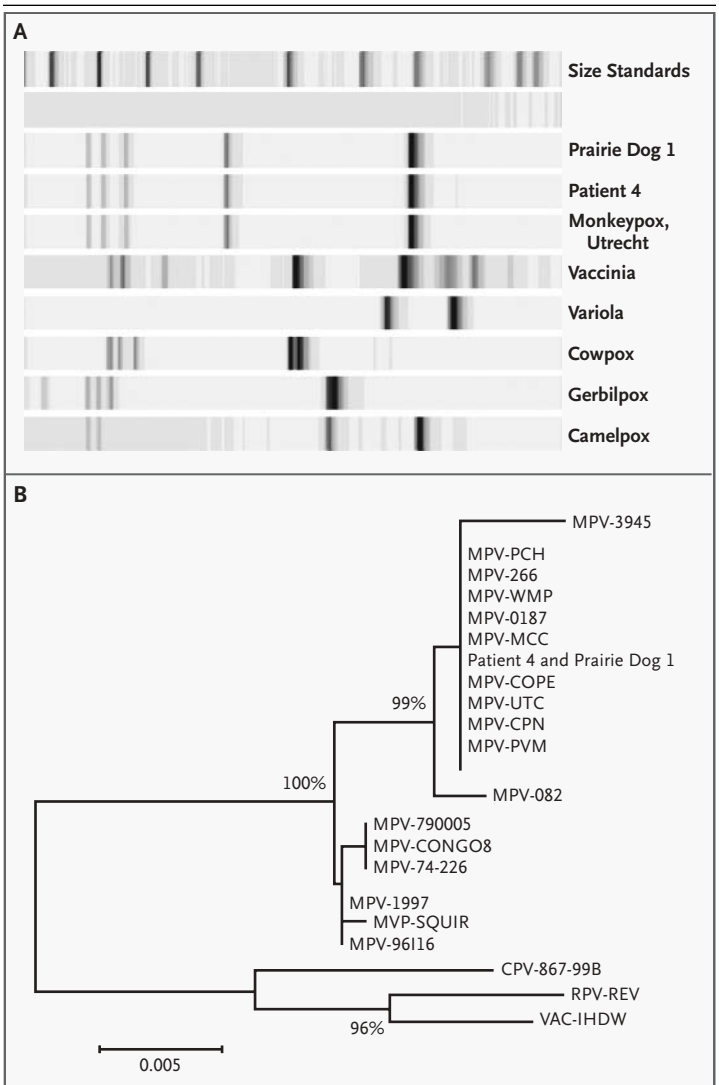

Figure 4. Polymerase-Chain-Reaction Amplification of the Orthopoxvirus Hemagglutinin Gene Followed by Restriction-Fragment–Length Polymorphism Analysis of Samples from Patient 4, Prairie Dog 1, and Reference Isolates of Other Orthopoxviruses (Panel A) and Phylogenetic Tree of Orthopox Isolates (Panel B).

*Taq*I was used for the restriction-fragment–length polymorphism analysis. Panel B shows that the gene sequences of samples from Patient 4 and Prairie Dog 1 shown in Panel A are closely related according to neighbor-joining methods. Significant bootstrap values for major nodes of the resulting hemagglutinin gene dendrogram are also shown. MPV denotes monkeypox virus, CPV cowpox virus, RPV rabbitpox virus, and VAC vaccinia virus. Reference monkeypox strains in the upper node (e.g., MPV-UTC) originated primarily in West Africa, whereas the strains in the middle node (e.g., MPV-CONGO8) are typically of Congolese (central African) origin. Percentages in Panel B indicate the relative similarity of the strains.

samples. The amplified template of both strands was sequenced to eightfold repetitive coverage. The generic primer sequence, first used to amplify the hemagglutinin gene product, was corrected for equivalent monkeypox-specific sequences. The observed hemagglutinin gene sequence obtained from the clinical tissues was compared with GenBank sequences. Closely related gene sequences were compared by means of neighbor-joining methods, and significant bootstrap values were obtained for major nodes of the resulting hemagglutinin gene dendrogram (Fig. 4B). The entire hemagglutinin gene sequence of the novel North American isolates was identical to examples of hemagglutinin genes obtained from monkeypox virus isolated from humans in West Africa and from nonhuman primates in primate colonies.

DISCUSSION

The occurrence of monkeypox among humans and rodents in the Western Hemisphere shows the effect that emerging zoonotic infectious diseases can have on public health. Some consider monkeypox to be the most important orthopoxvirus infection now that smallpox has been eradicated.[3] Although the occurrence of a single case is important, the 72 confirmed or suspected cases of monkeypox reported as of July 30, 2003, represent a large outbreak, as compared with outbreaks of 23 to 88 cases reported in areas of endemic disease.[8,9] Nationally, during the current outbreak, the peak in the onset of illness occurred between May 29 and June 9 (mode, June 3),[19] and there have been no further cases of illness in humans since June 22.[20]

The aggregate clinical signs and symptoms of these early cases in Wisconsin were similar to those described in outbreaks of monkeypox in Africa.[9] Most patients had a prodrome of fever, headache, and sweats before skin lesions and prominent lymphadenopathy developed. In some, a localized lesion was followed by systemic disease. Unique clinical manifestations included focal hemorrhagic necrosis, particularly at the sites of bites or scratches, and erythematous flares. These areas are probably more visible in light skin and differ from areas of focal hemorrhage described in cowpox. No patients died, even though 6 of the 11 patients were born after 1972, after routine vaccination against smallpox was discontinued among civilians. This

figure contrasts with case fatality rates of 4 to 22 percent reported during African outbreaks.[8,9]

The current cases are distinct from poxvirus infections currently or previously acquired in North America. Variola major, variola minor (alastrim), and molluscum contagiosum are transmitted strictly between humans. Volepox, skunkpox, and raccoonpox viruses are not known to cause disease in humans. Infection with orf and bovine stomatitis virus was excluded by the presence of systemic symptoms in most patients and by the fact that the ultrastructural characteristics of the viral particles were not compatible with those of a parapoxvirus.

Federal and state trace-back investigations associated this outbreak with the importation from Ghana, West Africa, of six African rodent species in a large shipment that arrived in the United States on April 9, 2003.[10] The natural history of monkeypox in Africa is largely unresolved. However, on the basis of serologic surveys of antibodies against orthopoxvirus in animals, naturally infected species in Africa may include various rodents (squirrels, rats, mice, and porcupines) and primates.[1,21,22] Experimental infections and observations of animals in zoologic collections have demonstrated that the range of potential hosts for monkeypox virus extends well beyond African species.[1]

We used multiple genomic targets for detection, including generic and species-specific tests, to help ensure the accuracy of virus identification. Monkeypox viruses fall into two genetically distinct virus clades loosely distinguished as either West African or Congolese.[23] Consistent with the origin in a rodent from or near Ghana, gene sequences from the strain responsible for the U.S. outbreak were most closely linked to the West African clade.

There was limited or no spread of monkeypox virus through human contact during this outbreak.[20] Person-to-person transmission documented in African outbreaks has rarely extended beyond three or four transmission cycles. Worst-case–scenario models suggest the disease is not self-sustaining in human populations, even in the total absence of immunity provided by smallpox vaccination.[24]

In Africa, outbreaks of monkeypox in humans are primarily associated with the hunting, skinning, preparing, and eating of infected rodents and monkeys.[9,25] In the United States, the greatest risk was associated with handling exotic and native mammalian wildlife as pets. On June 11, 2003, the CDC and the Food and Drug Administration jointly banned the import of all rodents from Africa, as well as the sale, distribution, transport, and release into the environment of prairie dogs and six African rodent species (tree squirrels, rope squirrels, dormice, Gambian giant pouched rats, brush-tailed porcupines, and striped mice). The CDC recommended that rodents linked to the implicated shipment of African rodents or to the exposed prairie dogs be humanely euthanized and carefully disposed of to prevent further spread of monkeypox to humans or native American mammalian species.[26,27] State and federal bans have curtailed further sale and transport of prairie dogs.[28,29]

The potential remains for subclinical monkeypox-virus infections and possible occult, long-term viral shedding in various African or North American rodent species. Whether monkeypox virus has spread to North American rodent populations is an unanswered question with substantial implications for both human and animal health. It is not known whether mammalian species endemic to North America can maintain a zoonotic sylvan cycle of monkeypox virus. These are the subjects of continuing extensive investigation.

We are indebted to Paul Mitchell, Ph.D., Brad Sullivan, M.D., Ph.D., Kathryn Kolquist, M.D., Molly Hinshaw, M.D., Donald Stoiber, M.S., Lisa Baeten, Laura Bliven, Tom Brunette, and Graig Eldred, Ph.D., of the Marshfield Clinic, Marshfield, Wisc., for clinical care and laboratory testing of the patients and preparation of the photographs and the manuscript; to Carol Kirk, Erik Reisdorf, B.S., and Peter Shult, Ph.D., of the Wisconsin State Laboratory of Hygiene, Madison, for coordinating the transport of samples to the CDC; to Steven Denson, M.D., and G. Richard Olds, M.D. (Department of Medicine), Charles E. Edmiston, Jr., Ph.D. (Department of Surgery), Juliet Gunkel, M.D. (Department of Dermatology), and Paula Traktman, Ph.D. (Departments of Microbiology and Molecular Genetics), of the Medical College of Wisconsin, Milwaukee, for the clinical care and laboratory testing of patients from the Milwaukee area; and to the following persons and institutions for their assistance in the epidemiologic investigations: Susann Ahrabi-Fard, M.S., Herb H. Bostrom, B.A., Donita R. Croft, M.D., Tom Haupt, M.S., and Carl Williams, D.V.M., M.P.H., of the Wisconsin Department of Health and Family Services, Madison; Robert Ehlenfeldt, D.V.M., Ellen Hooker, D.V.M., Shelby Molina, D.V.M., and H. Gregg Smith, D.V.M., of the Wisconsin Department of Agriculture, Trade and Consumer Protection, Madison; Paul Biedrzycki, MP.H., M.B.A., Douglas Gieryn, B.S., Sandy Coffaro, B.S.N., and Bevan Baker, C.H.E., of the Milwaukee Health Department, Milwaukee; Kacey Ernst, M.P.H., of the Milwaukee–Waukesha Consortium for Emergency Public Health Preparedness, Milwaukee; Debbie Schier, R.N., of the Oak Creek Health Department, Oak Creek, Wisc.; Cynthia Tomasello, R.N., of the Shorewood/Whitefish Bay Health Department, Shorewood, Wisc.; Jacqueline Ove, B.S.N., R.N., of the South Milwaukee Health Department, South Milwaukee, Wisc.; Darren Rausch, M.S., Nancy Healy-Haney, Ph.D., R.N., M.P.H., and Rosie Schroeder, R.N., of the Waukesha County Health Department, Waukesha, Wisc.; Nancy Kreuser, Ph.D., of the Wauwatosa Health Department, Wauwatosa, Wisc.; Julie Willems Van Dijk and Tom Wittkopf of the Marathon County Health Department, Wausau, Wisc.; Patty Krug, B.S.N., of the Taylor County Health Department, Medford, Wisc.; Carol Quest, B.S.N., R.N., of the Watertown Department of Public Health, Watertown, Wisc.; Patti Wilson, R.N.,

and Bonnie Grahn, R.N., of the Froedtert Hospital, Milwaukee; Paul H. Hunter, M.D., of the Covenant Medical Group, South Milwaukee, Wisc.; Niraj Sawhney, M.D., of the St. Francis Hospital, Milwaukee; and Tim DeVau, D.V.M., and Jane Mussey, D.V.M., of the Department of Agriculture, Animal and Plant Health Inspection Service, Merrill and Richland Center, Wisc.

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