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Human monkeypox is a zoonotic smallpox-like disease caused by an orthopoxvirus of interhuman transmissibility too low to sustain spread in susceptible populations. In February 1997, 88 cases of febrile pustular rash were identified for the previous 12 months in 12 villages of the Katako-Kombe Health Zone, Democratic Republic of Congo (attack rate = 22 per 1,000; case-fatality rate = 3.7%). Seven were active cases confirmed by virus isolation. Orthopoxvirus-neutralizing antibodies were detected in 54% of 72 patients who provided serum and 25% of 59 wild-caught animals, mainly squirrels. Hemagglutination-inhibition assays and Western blotting detected antibodies in 68% and 73% of patients, respectively. Vaccinia vaccination, which protects against monkeypox, ceased by 1983 after global smallpox eradication, leading to an increase in the proportion of susceptible people.

Methods

Epidemiologic and Clinical Studies

Before civil unrest in the area curtailed the study, a dwelling-to-dwelling search was conducted for cases that occurred from February 1996 and February 1997 in 12 villages (total population 4,057) in the Katako-Kombe Health Zone, Democratic Republic of Congo. The secondary attack rate was 9.3%, and 28% of case-patients reported an exposure to another case-patient during the incubation period. Transmission chains beyond secondary were rare (8,9). A mathematical model to assess the potential for monkeypox to spread in susceptible populations after cessation of vaccinia vaccination indicated that person-to-person transmission would not sustain monkeypox in humans without repeated reintroduction of the virus from the wild (7).

In 1996, 71 suspected human monkeypox cases were reported from the Katako-Kombe Health Zone, Kasai Oriental, DRC. These initial reports suggested predominant person-to-person transmission and prolonged chains of transmission. Two cases were confirmed by monkeypox virus isolation from lesion material (10). In February 1997, an investigation was initiated (11). Our report describes epidemiologic observations and laboratory results supporting the conclusion that repeated animal reintroduction of monkeypox virus is needed to sustain the disease in the local human population.
Epi-Info software (19).

Confidence intervals (CI) for proportions were calculated with exact methods and compared with Fisher's exact tests as appropriate by using Epi-Info software (19).


definite identification.

Animals were processed in a field laboratory (12), identified to genus and species, and bled for serum. Representative voucher specimens of each animal were preserved in 10% formalin for definitive identification.

Laboratory Studies

Human and animal sera were clarified by low-speed centrifugation, immediately stored in liquid nitrogen, and shipped to the Centers for Disease Control and Prevention (CDC) in Atlanta. At CDC, an aliquot of each serum was heated at 56°C for 30 minutes, the following tests were performed: 1) vaccinia virus hemagglutination-inhibition (HAI) assay (13); 2) monkeypox 50% plaque-reduction neutralization assay (13); and 3) Western blot assays for immunoglobulin G (IgG) against monkeypox antigens essentially by using Towbin's and colleagues' methods adapted to mini-transblot and multiscreen apparatuses (Bio-Rad, Hercules, CA) (14).

Western blotting used selected human sera and antigen preparations that consisted of a soluble antigen (20X culture medium concentrate) from monkeypox virus-infected Vero cells (15). Positive controls consisted of sera collected in the 1980s from convalescent-phase monkeypox cases from the prospective study in the DRC and from vaccinia-vaccinated persons; negative controls consisted of sera collected in the 1980s from DRC inhabitants with no history of vaccinia vaccination or monkeypox. Human sera were also tested for antibodies against varicella virus by using kits to detect human IgM or IgG by enzyme-linked immunosorbent assays (ELISA; Wampole Laboratories, Cranberry, NJ). Samples of crusty scab or pustule lesion were cultured for monkeypox virus using the monkey cell lines Vero, LLCMK2, or OMK (13,16), and assayed by polymerase chain reaction (PCR) amplification for monkeypox virus-specific DNA (16,17) and for varicella virus gene 1 (P. Pellett, pers. comm.). In addition, the gene encoding the hemagglutinin (HA) protein of selected monkeypox isolates was sequenced by fluorescence-based methods (Applied Biosystems, Inc., Foster City, CA). Available duplicate coded sera were tested anonymously in Kinshasa, DRC, for antibodies against HIV (Vironostika Human Form II ELISA, Organon Teknika, Denmark).

The relatedness of isolates was examined by comparing DNA restriction endonuclease patterns with patterns of previously mapped monkeypox virus isolates (18) and by comparing the hemagglutinin gene sequences with cognate sequences of other monkeypox isolates.

Statistical Methods

Attack rates were calculated by using a census conducted during the dwelling-to-dwelling case search; information on the age and sex of each person living in Akungula was also obtained. Secondary attack rates within households were calculated by dividing the number of cases that occurred 7 to 21 days following one or more index cases in a household (first-generation secondary cases) by the total number of household members, excluding index cases. Confidence intervals (CI) for proportions were calculated with exact methods and compared with Fisher's exact tests as appropriate by using Epi-Info software (19).

Results

Epidemiologic and Clinical Studies

Eighty-eight clinical cases (7 active and 81 retrospectively identified) were discovered in 9 of the 12 villages (attack rate 22 per 1,000). Fifty (56.8%) of 88 case-patients were male; the median age at onset was 10 years (quartiles 5-19; range 1 month to 62 years).

The number of clinical cases reported per month increased from February to August 1996, followed by a decline until January 1997 and a resurgence of cases in February 1997 (Figure 1). Attack rates in the villages ranged from zero to 105 per 1,000 population in Akungula, where 42 cases were identified in 14 of the 62 village households.

In Akungula, where the age and sex distributions of the population were available, the attack rate was identical for males and females, but was higher (30 of 206 = 146/1,000) in children <15 years of age than in persons 15 to 24 years old (4 of 85 = 47/1,000) and ≥25 years of age (8 of 109 = 73/1,000).

Of 78 retrospectively identified case-patients for whom information was available, 40 (51.3%) reported swelling consistent with lymphadenopathy. All 75 examined patients had scars from a rash (median 50; range 4 to 830; standard deviation 179). Thirteen (15.5%) of 84 patients for whom information was available had a vaccination scar on the upper left arm compatible with prior vaccinia vaccination; all patients were ≥20 years of age. Alopecia was seen in three cases with acute rash illness. Three deaths occurred among 81 of the 88 cases for which follow-up information was available (3.7% case-fatality rate), all in children <3 years of age who died within 3 weeks of rash onset. No information was available to attribute the deaths to monkeypox, superinfection, or other cause.

Seven case-patients (six in a single household) had active disease at the time of the investigation. Each had lymphadenopathy and more than 100 crusty skin lesions. Five had a rash on the soles and palms. Sixty-two (73%) of 85 case-patients for whom information was available reported exposure to another patient 7 to 21 days before onset of their
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Identification of vaccinia-induced antibodies (22). However, sensitivity (96%) than the neutralization test for the (73%) tested positive by the HAI test, which had a higher proportion of sera from retrospectively identified cases with monkeypox virus infection during this study interval. A antibodies and may have been negative (3) in some patients sensitive for the detection of vaccinia vaccine-induced veropoxvirus infections during smallpox eradica-
tion and PCR (17) for monkeypox virus. Because monkeypox is the only known indigenous orthopoxvirus of Africa that infects humans systemically, seropositive cases showing genus-specific antibodies can be reasonably interpreted as cases that had a monkeypox virus infection (20). Of 72 retrospectively identified cases tested, 54% had antibodies by plaque-reduction assay, a preferred test for verifying orthopoxvirus infections during smallpox eradication (20,21). However, this neutralization test was only 83% sensitive for the detection of vaccinia vaccine-induced antibodies and may have been negative (3) in some patients with monkeypox virus infection during this study interval. A higher proportion of sera from retrospectively identified cases (73%) tested positive by the HAI test, which had a higher sensitivity (96%) than the neutralization test for the identification of vaccinia-induced antibodies (22). However, the HAI test may be less specific, and antibody titers detected by it decrease rapidly after infection and would be unlikely to be residual from prior vaccination in the small percentage of patients who had been vaccinated (3,21,23). Finally, 72% of sera from retrospectively identified cases tested positive in a new, nonvalidated Western blot orthopoxvirus antibody test for which sensitivity and specificity have not been determined. Chickenpox, which can be mistaken for monkeypox (24), was reported in the area during the study period. Some patients may have had dual infection by monkeypox and varicella viruses. Within one household, five patients from whom monkeypox virus was isolated showed serologic evidence of recent varicella virus infection, and two of those had detectable varicella virus DNA in skin lesion samples. Thus, a substantial percentage of cases may have been chickenpox, thereby explaining why the case-fatality rate from this study (3.7%) was lower than the 9.6% previously reported (3,24). However, the presence of antibodies against orthopoxviruses by three different assays in 54% of patients suggests that varicella virus was not responsible for most cases during the study period.

Table 2. Species of animals caught in the wild and monkeypox virus plaque reduction neutralization antibody assay results, Katako-Kombe Health Zone, February 23-27, 1997

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Common name</th>
<th>No. Tested</th>
<th>Pos.</th>
<th>Proportion pos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cercopithecus ascani</td>
<td>Spot-nosed monkey</td>
<td>2</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Cercopithecus neglectus</td>
<td>Debrazza monkey</td>
<td>1</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Perodicticus potto</td>
<td>Bosman’s potto</td>
<td>1</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Galagoidea demidovi</td>
<td>Demidoff galago</td>
<td>2</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Genetta rubiginosa</td>
<td>Genet</td>
<td>1</td>
<td>1</td>
<td>100.0%</td>
</tr>
<tr>
<td>Sus scrofa</td>
<td>Domestic pig</td>
<td>1</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Praomyx jacksoni</td>
<td>Forest rat</td>
<td>3</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Cricetomys emini</td>
<td>Gambian rat</td>
<td>3</td>
<td>1</td>
<td>33.3%</td>
</tr>
<tr>
<td>Cavia sp.</td>
<td>Guinea pig</td>
<td>3</td>
<td>1</td>
<td>33.3%</td>
</tr>
<tr>
<td>Petrodromus tetradactylus</td>
<td>Elephant shrew</td>
<td>3</td>
<td>1</td>
<td>33.3%</td>
</tr>
<tr>
<td>Dendrohyrax arboreus</td>
<td>Tree hyrax</td>
<td>3</td>
<td>1</td>
<td>33.3%</td>
</tr>
<tr>
<td>Fundisciusurus anerythrus</td>
<td>Thomas’s tree squirrel</td>
<td>4</td>
<td>2</td>
<td>50.0%</td>
</tr>
<tr>
<td>Funisciurus conicus</td>
<td>Kuhl’s tree squirrel</td>
<td>18</td>
<td>7</td>
<td>38.9%</td>
</tr>
<tr>
<td>Helioseius rufofibrachium</td>
<td>Sun squirrel</td>
<td>2</td>
<td>1</td>
<td>50.0%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>59</td>
<td>15</td>
<td>25.4%</td>
</tr>
</tbody>
</table>

Conclusion

The epidemiologic and laboratory evidence presented here suggests that this monkeypox outbreak is the largest reported. Epidemiologic features of monkeypox that differed from those described during an intensified prospective study in the DRC in the 1980s included an increase in the proportion of patients reporting an exposure to another case during the incubation period (28% in the 1980s, 73% this study) and the clustering of successive cases within households, with as many as eight consecutive cases in one instance (3,10).

Prospective surveillance in the 1980s in the DRC provided opportunities to observe most patients with active-stage monkeypox; thus, skin lesion samples were available for virus isolation to confirm clinical diagnosis. In contrast, the present study identified most patients retrospectively and relied on serologic testing for confirmation of the diagnosis; only seven clinically active cases could be confirmed by virus isolation and PCR (17) for monkeypox virus. Because monkeypox is the only known indigenous orthopoxvirus of Africa that infects humans systemically, seropositive cases showing genus-specific antibodies can be reasonably interpreted as cases that had a monkeypox virus infection (20). Of 72 retrospectively identified cases tested, 54% had antibodies by plaque-reduction assay, a preferred test for verifying orthopoxvirus infections during smallpox eradication (20,21). However, this neutralization test was only 83% sensitive for the detection of vaccinia vaccine-induced antibodies and may have been negative (3) in some patients with monkeypox virus infection during this study interval. A higher proportion of sera from retrospectively identified cases (73%) tested positive by the HAI test, which had a higher sensitivity (96%) than the neutralization test for the identification of vaccinia-induced antibodies (22). However,
the sensitivity and the specificity had been measured using a standard technique and case-control sera. Because information was not available regarding the vaccination status of other members of the patients' households, secondary attack rates within households could not be calculated according to the vaccination status. We did not assess the environmental changes that could have facilitated this outbreak, including increases in household sizes, rates of monkeypox virus infection in the animal population, and a change in rate and type of exposure to wild animals among residents. We were unable to attribute infections in patients who reported an exposure to another patient during the incubation period to person-to-person transmission because some of these patients may also have been exposed to infected animals. Finally, patients with a history of exposure to a patient during the incubation period were the only controls available to generate hypotheses regarding the type of animal exposure possibly associated with infection in patients not reporting an exposure to other patients during the incubation period. This aspect may have caused bias because cases clustered within households where exposures to animals may have been identical and because cases may have been misclassified as to their purported source of infection.

There is no evidence to date that person-to-person transmission alone can sustain monkeypox in the local population. However, our study suggests that in a population with low herd immunity, person-to-person transmission and repeated introduction of virus from the animal reservoir may lead to more and larger clusters of human monkeypox cases in African rain forest areas (25). Further studies are needed to measure the sensitivity and the specificity of the serologic tests currently available so they can be used in the future to better confirm retrospectively identified cases, identify the type of animal or patient exposures associated with acquisition of illness, and evaluate the respective roles of person-to-person and animal-to-human transmission. However, access to the Kasai region has been limited since our study, and little information has become available to better document the current dynamics of monkeypox occurrences or improve World Health Organization recommendations to prevent human monkeypox more substantially.

Acknowledgments
The authors thank T.P. Muamba, G. Mwema, D. Messinger, K. Kilundu, V.B. Mukinda, L.V. Okito, and V.L. Mangindula for assistance in data collection; L. Otshudi for assistance in setup of the investigation; J.D. Muymba-Tamfum for HIV testing; A. Moudi; J.A. Stewart and J. Patton for varicella virus testing; A. Moren, W.C. Reeves, C.J. Peters, and D.A. Henderson for critically reading the manuscript; and J. O'Connor for editorial assistance.

The investigation was partly funded by the U.S. Agency for International Development.

Dr. Yvan Hutin was an Epidemic Intelligence Service officer with the Centers for Disease Control and Prevention at the time of this investigation. He is now at the World Health Organization.

References