Monkeys or Varicella? Lessons from a Rash Outbreak Investigation in the Republic of the Congo


Coordinating Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; District Health Office, Impfondo, Republic of Congo; National Laboratory, Brazzaville, Republic of Congo

Abstract. Monkeypox virus and varicella-zoster virus (VZV) cause visually similar rash illnesses. Monkeypox is more virulent, with fatality rates up to 10%. In June 2007, reports were received of a rash illness outbreak in isolated villages in Likouala district, Republic of the Congo. Blood specimens were obtained from 142 individuals reporting rash illness between January and September 2007 from four villages in Likouala. Thirty-seven cases were identified based on low VZV IgG avidity; cases occurred in all four villages. No probable monkeypox cases with orthopoxvirus-positive IgM responses were observed; however, three possible monkeypox cases, in individuals < 26 years of age, with rash illness occurring > 56 days before sampling and positive orthopoxvirus-specific IgG responses, were identified. Remoteness and delays in reporting limited collection of acute diagnostic specimens. Improvements in rash illness surveillance and infection control, through training of health workers and timely acquisition of diagnostic specimens, are being undertaken.

INTRODUCTION

The elimination of smallpox from Africa led to the recognition of another disseminated orthopoxvirus infection of humans, known as monkeypox. First isolated from captive monkeys in Europe in 1958,1 monkeypox virus was identified to cause human disease in Africa in the early 1970s.2–5 Clinically, human monkeypox has an appearance virtually identical to ordinary smallpox,6 although lymphadenopathy is a prominent sign in monkeypox and not routinely seen in smallpox. Contrasting variola, monkeypox virus is maintained zoonotically, with rodents believed to be the reservoir host, and is geographically limited to heavily forested regions of central and west Africa.7 Outbreaks of monkeypox caused by human-to-human transmission have been well documented, with up to six sequential human-to-human transmissions noted.8 With cessation of routine smallpox vaccination around 1980, population-based immunity to orthopoxviruses is waning, which increases the potential for larger or more prolonged monkeypox outbreaks.

As with smallpox, the possibility for confusion in the diagnosis of monkeypox and varicella-zoster (VZV) exists in areas of the world where both viruses are present.9,10 The occurrence of a febrile prodrome, presence of lymphadenopathy (especially cervical), and manifestation of rash as deep-seated, well-circumscribed lesions at the same stage of development with centrifugal distribution are features of illness associated with monkeypox rather than chickenpox.6 Case fatality is ~10% in human monkeypox, whereas fatalities are rare during primary VZV infection.5,11 Epidemiologic patterns differ as well, because VZV is a highly infectious virus and is spread exclusively through human-to-human transmission, whereas monkeypox can arise sporadically through zoonotic exposure, and secondary human-to-human attack rates tend to be relatively low.11–13 However, monkeypox occurs in remote geographic locations, where diagnosis can be confounded by the lack of trained medical personnel, biased recall of signs and symptoms, and possible effects of other underlying conditions (e.g., a fever associated with malaria can be mistaken as a febrile prodrome). Recent reports detailing studies of monkeypox in central Africa suggest that VZV infection is commonly mistaken as monkeypox, with upward of 50% of suspected monkeypox cases actually being attributable to VZV infection.14,15

Given the global pervasiveness of VZV, strikingly little is known about its epidemiology in Africa. Whereas VZV is a childhood disease in the developed world, studies suggest a different epidemiology in tropical regions of the world, with lower seroprevalence levels in children.16,17 Although VZV infection across sub-Saharan Africa has not been systematically characterized, a study assessing VZV serohistory among refugees ≥ 7 years of age found a seroprevalence of 70% among those from sub-Saharan Africa.18 In contrast, in the United States, seroprevalence between 1988 and 1994, before varicella vaccine introduction in 1995, was 86% among children 6–11 years of age and 99% in adults ≥ 40 years of age.19

In July 2007, we received reports of a widespread outbreak of pustular-rash illness occurring in the Likouala district of the Republic of the Congo (RoC), which was suggested to be monkeypox. Additionally, some cases were reported to have palm and sole lesions,20 a characteristic typical of monkeypox.6 A previous serosurvey in Likouala showed a high seroprevalence of orthopoxvirus-reactive antibodies in the population,21 and furthermore, an outbreak of monkeypox occurred in Likouala in 2003.3 In this report, we describe the events surrounding the study of the outbreak, investigation results, and the lessons learned with regard to the occurrence of, and surveillance for, pustular rash illnesses in rural, central Africa.

MATERIALS AND METHODS

Timing of the investigation. The occurrence of a pustular rash outbreak occurring in Likouala was first reported in June 2007. Attempts of obtain photographs, through local health officials, of patients’ rash were unsuccessful because of limited availability of resources locally. A preliminary study to the villages of Bokpokoto and Congo Malembe was performed in late August 2007. A follow-up study in these villages, as well as Impfondo and Illiangakaka, was performed in late September and early October 2007.
Sample collection. To facilitate collection and storage of clinical diagnostic specimens in the absence of a cold-chain, human blood samples were collected by finger-stick and wicked onto Nobuto blood filter strips (Advantec MFS, Inc., Dublin, CA), which are small paper strips that allow for safe, stable transport of specimens at room temperature for serologic analysis. Specimens were subsequently eluted in the laboratory (CDC, Atlanta, GA) at fixed volume to obtain a 1:40 dilution concentration of sera. Details of the sample collection methodology and use in orthopoxvirus serology are described elsewhere. Because cases occurred over a number of months and sample collection was limited to two short field studies, timing of sample collection relative to disease occurrence was relatively variable. A more detailed description of timing of samples is included below.

Serology. The presence of orthopoxvirus-reactive antibodies was determined by IgM and IgG ELISA, as described previously. To determine background reactivity of orthopoxvirus reactive antibodies, cut-off values (discriminating reactive from non-reactive specimens) were derived from five individuals from Likouala < 10 years of age who had no clinical history of pustular rash illness suggestive of monkeypox.

IgG avidity was used to assess history of infection with VZV, based on the affinity maturation process, in which B cells undergo somatic hypermutation and clonal selection, after infection, to produce high affinity antibodies. This approach has been successfully used to differentiate acute from historic infection for other viral infections, such as cytomegalovirus (CMV). Briefly, VZV IgG avidity was measured using a VZV glycoprotein ELISA (gpELISA) performed according to the protocol of Wasmuth and Miller. Two identical plates were prepared with duplicate wells containing test antigen (purified glycoproteins obtained under a material transfer agreement with Merck and Co., West Point, PA) and normal tissue control antigen. Nobuto filter strip elutants were diluted 1:20 and added to the wells of each plate. One plate received no further additions, and the other was treated with 35 mmol/L diethyl amine (DEA; Sigma Chemical, St. Louis, MO) for 1 minute. Both plates were washed with phosphate-buffered saline (PBS)/Tween20, and anti-human IgG:alkaline phosphatase conjugate was added to wells. Both plates were developed with colorimetric substrate in accordance with the referenced protocol. Optical density (OD) values were adjusted by subtracting the mean normal tissue OD from the mean test OD. Avidity index (AI) was calculated as the ratio of the DEA-treated adjusted OD/untreated adjusted OD × 100. Because of the initial elution of sera from Nobuto filter strips, VZV avidity assays were performed under conditions possibly diluted beyond the range of optimal detection sensitivity. Thus, the absence of an anti-VZV antibody response should not necessarily be interpreted as a negative response.

To examine the utility of this assay in assessing likely temporal occurrence of VZV infection (e.g., recent versus prior VZV infection), we examined VZV antibody avidity among individuals with positive VZV IgG titers at time of sample collection relative to the occurrence of reported rash illness. Individuals with positive IgG titers were grouped as those sampled < 1 month and those sampled 2–8 months after rash onset (cases with sample collection dates ranging from 2 to 8 months after rash onset were aggregated because avidity values were similar across entire time frame). Because the temporal relationship between disease onset and VZV avidity has not been well characterized previously, we examined VZV avidity values relative to reported rash onset, additionally, among a group of eight control individuals who reported a similar rash illness in 2002–2003.

Case definitions. A probable VZV case is defined as an individual reporting rash illness since January 2007 with evidence of recent VZV infection, based on a VZV IgG avidity value of < 50. A probable case of monkeypox is defined as an individual reporting rash illness since January 2007, with a positive anti-orthopoxvirus IgM titer. A possible case of monkeypox is defined as an individual < 26 years of age (born after cessation of smallpox vaccination) reporting rash illness since January 2007, with a positive orthopoxvirus-reactive IgG titer and negative anti-orthopoxvirus IgM titer, whose blood sample was collected > 56 days from the onset of rash illness (individuals without demonstrable anti-orthopoxvirus IgM titers between days 3 and 56 after rash onset are ruled out for monkeypox), and the absence of a VZV IgG antibody response, or a VZV IgG avidity value of > 50.

RESULTS

Overview of the area. The Likouala district is a sparsely populated, heavily forested district, located in northern RoC. Our study focused on four settlements within this district (shown in Figure 1) all located along the Ubangi river, bordering the Democratic Republic of Congo (DRC). Of these, the town of Impfondo, the administrative capital, is the largest, and was readily available access by commercial aircraft. A detailed account of the region has been described elsewhere. The villages of Congo Malembé and Bokpokoto are located 40 and 20 km, respectively, down river from Impfondo. These villages

FIGURE 1. Overview map of Likouala district, villages studied, and timing and number of cases. VZV, Varicella-zoster virus; MPX, monkeypox.
are located on forest clearings along the river, and access is exclusively by boat. A large proportion of the population of these villages is accounted for by refugees, originally from neighboring DRC. Healthcare is limited to rural outposts supported by the United Nations High Commission for Refugees, with care overseen by the non-governmental organization Medicins d’Afrique. The final village, Illiangakaca, is located 35 km north of Impfondo and is accessed by a short boat ride from Dongou, a town accessible by road from Impfondo. Across the entire area, the border between RoC and DRC is relatively porous, with persons frequently traveling across the Ubangi river by canoe or larger vessel.

**Numbers, timing, and epidemiologic characteristics of the outbreak.** We collected clinical, exposure, and demographic data from individuals reporting rash illness onset since January 2007. We also collected blood specimens (by finger prick) for serologic analysis. In total, we studied 142 cases of rash illness; individuals ranged from 1 to 77 years of age (median = 9.5 years); 47% of cases were male. These included 59 individuals from Congo Malembe, 60 from Bokpokoto, 16 from Impfondo, and 7 from Illiangakaca. An additional 18 individuals from Congo Malembe and 2 individuals from Impfondo were interviewed and agreed to provide blood samples, but each reported remote rather than recent rash illness (illness occurred ~4–5 years before).

Geographically, the earliest cases reported during 2007 occurred in Congo Malembe (Figure 1). Rash illness onset among cases investigated in Congo Malembe occurred between January and July 2007. Illness onset dates in Illiangakaca ranged from March to July and in Bokpokoto from July to August. Two individuals in Impfondo reported rash onset in April 2007, and the remaining cases from Impfondo had onset dates ranging from August to September 2007.

Although detailed clinical information was not available, descriptions of severe illness were limited, and no deaths associated with pustular rash illness were reported in any of the communities. In one cluster of cases, evidence of palm and sole lesions was observed.20

**Orthopoxvirus serology results.** Of the 142 individuals studied, orthopoxvirus serologic results were obtained from 140 individuals; 2 individuals from Impfondo had insufficient samples. Sixty individuals from Bokpokoto and 12 individuals from Impfondo had samples collected within 3–56 days after onset of rash, allowing interpretation of IgM results relevant to the diagnosis of monkeypox.2 None of these individuals had orthopoxvirus-reactive IgM titers, indicating the absence of recent monkeypox virus infection.

Across all villages, positive anti-orthopoxvirus IgG responses were markedly higher for those likely to have received childhood smallpox vaccination. Among those ≥ 26 years of age, 61% (10 of 17) of individuals showed anti-orthopoxvirus–specific IgG titers, likely reflecting the durable IgG response seen after childhood smallpox vaccination. In contrast, 16% (7 of 44) of individuals between the ages of 11 and 26 and 4% (3 of 73) of individuals ≤ 10 years of age had positive anti-orthopoxvirus IgG titers. Among those < 26 years of age, four individuals from Bokpokoto and one individual from Impfondo with positive anti-orthopoxvirus IgG titers had samples drawn within 56 days after onset of rash illness and did not have an anti-orthopoxvirus–specific IgM titer, indicating that positive IgG response was caused by previous orthopoxvirus exposure and that the acute rash studied was not caused by monkeypox. Of those who had samples taken > 56 days after rash onset, three individuals < 26 years of age had positive anti-orthopoxvirus–specific IgG titers, indicating possible cases of monkeypox. All possible cases of monkeypox occurred in Congo Malembe.

**VZV serology.** VZV IgG avidity testing was obtained from 55, 19, 14, and 3 individuals from Congo Malembe, Bokpokoto, Impfondo, and Illiangakaca, respectively. Of these, 23 individuals from Congo Malembe, 6 from Bokpokoto, 3 from Impfondo, and 3 from Illiangakaca had a VZV-specific IgG response.

Median avidity values for individuals with illness onsets of < 1 month, 2–8 months, and ~4–5 years before were 11.5, 36.5, and 64.1, respectively (Figure 2). Avidity values differed significantly between the < 1 and 2–8 month groups (P < 0.01; Wilcoxon rank sum), and similarly, differed significantly between the 2–8 month and ~4–5 year groups (P < 0.01; Wilcoxon rank sum). Based on observed avidity values, the cut-off for probable VZV infection was assigned as an avidity value < 50 for individuals reporting rash illness during 2007. This is supported by the observation that a group of eight control, distant infection individuals (with rash illness ~4–5 years before) had avidity values > 50. However, some individuals reporting rash illness in 2007 had an avidity value > 50. If these individuals represent probable VZV cases, the avidity cut-off of < 50 would yield a sensitivity of 79% in this study.

A total of 37 cases met the probable VZV case definition. This included 18, 11, 6, and 2 individuals from Congo Malembe, Bokpokoto, Impfondo, and Illiangakaca, respectively. Although probable VZV case counts were highest among those 6–10 years of age, a sizable proportion of cases were among older individuals (56% of documented cases were > 10 years of age; Figure 3).

**Overall outbreak summary.** In summary, a total of 37 cases studied were classified as probable VZV, 0 cases were classified as probable monkeypox, and 3 cases were classified as possible monkeypox (Figure 1). Probable cases of VZV occurred in all villages studied. Chains of transmission of probable VZV cases were identified in the communities of Bokpokoto and Impfondo. In contrast, possible cases of monkeypox were limited to Congo Malembe.

**DISCUSSION**

In this report, we described the investigation of an outbreak of a rash illness in a rural district of the RoC. The

WE REPORT THE OCCURRENCE OF NUMEROUS PROBABLE CASES OF VZV IN LIKOULA. THE TEMPORAL CLUSTERING OF RASH ILLNESS CASES BY VILLAGE DURING 2007 SUGGESTS THAT THE OCCURRENCE OF VZV REPRESENTED AN OUTBREAK, AS OPPOSED TO ENDEMIC TRANSMISSION OF VZV. ALTHOUGH WE CANNOT DIRECTLY ASSESS POPULATION-BASED SEROPOSITIVITY THROUGH THE DATA FROM THIS INVESTIGATION, THE OCCURRENCE OF A SIZABLE OUTBREAK IN MULTIPLE VILLAGES AND AGE DISTRIBUTION OF CASES OF PROBABLE VZV SUGGEST THAT A CONSIDERABLE PORTION OF THE POPULATION IS NAIVE TO VZV. OF NOTE, 56% OF CASES DOCUMENTED WERE > 10 YEARS OF AGE. IN CONTRAST, BEFORE VARICELLA VACCINATION IN THE UNITED STATES IN 1995, INCIDENCE OF VZV WAS HIGHEST IN CHILDREN < 10 YEARS OF AGE, WITH 90% OF THE CASES OCCURRING BEFORE AGE 15 YEARS.

IN THIS STUDY, VZV INFECTION WAS ASSESSED BY VZV IgG avidity. TO OUR KNOWLEDGE, THIS IS THE FIRST REPORT TO MAKE USE OF VZV IgG avidity for the characterization of cases of VZV in an outbreak investigation. Median avidity values increased with time from rash onset to sample collection, indicating the utility of this assay to assess the temporal occurrence of VZV infection. Although data from this study suggest an IgG avidity cut-off value of 50 as specific for acute VZV infection, the sensitivity of the assay may have been limited. Further studies are warranted to characterize the sensitivity of this assay to identify acute VZV infection. Supporting the utility of our approach, IgG avidity assays for CMV are commonly used to differentiate acute from historic CMV infection.

ALTHOUGH HUMAN MONKEYPOX WAS NOT DEFINITIVELY IDENTIFIED BECAUSE OF THE DELAY IN SAMPLE COLLECTION RESPECTIVE TO RASH ONSET, WE NOTED POSSIBLE CASES OF MONKEYPOX IN THE VILLAGE OF CONGO MALEMBE. THIS SCENARIO IS CLEARLY PLAUSIBLE, GIVEN THE PREVIOUS DOCUMENTED CASES OF MONKEYPOX AND HIGH SEROPREVALENCE OF ORTHOPOXVIRUS REACTIVE ANTIBODIES IN LIKOULA. IN ADDITION, CONGO MALEMBE IS AN ISOLATED VILLAGE, LOCATED ON THE URBANGI RIVER AND SURROUNDED BY DENSE RAINFOREST, AND RESIDENTS REPORTED FREQUENT CONTACT WITH WILDLIFE. FURTHERMORE, THE

PREVIOUS DOCUMENTED CASES OF MONKEYPOX AND HIGH SEROPREVALENCE OF ORTHOPOXVIRUS REACTIVE ANTIBODIES IN LIKOULA. IN ADDITION, CONGO MALEMBE IS AN ISOLATED VILLAGE, LOCATED ON THE URBANGI RIVER AND SURROUNDED BY DENSE RAINFOREST, AND RESIDENTS REPORTED FREQUENT CONTACT WITH WILDLIFE. FURTHERMORE, THE

presence of a large number of cases of human monkeypox in Equateur province, DRC, which is located directly across the Ubanghi river from Likouala, has recently been documented (unpublished observations). Given the porous nature of the countries’ borders in this area and frequent cross-river travel, as well as the sizable refugee population in Congo Malembe, importation of human monkeypox by infected humans or by sale of bushmeat to this area is possible.

This study underscored a number of limitations associated with managing public health surveillance and outbreak investigation in underdeveloped areas of rural Central Africa, and as a result, are currently being addressed in Likouala district by local, national, and international partners. First is the delay in outbreak reporting and response. In some instances, the time between rash onset and this investigation was a number of months. Although reporting is somewhat limited by the absence of a telephone-based communication infrastructure, a partnership has been created between national partners, international partners, and medical organizations that provide healthcare at rural outposts to improve communication. Furthermore, health workers operating in the area have since been provided with rash illness investigation kits that include rash illness case investigation forms, sample collection supplies, and detailed guidelines for rash illness recognition and infection control.

Second, the absence of appropriate sample collection supplies, lack of cold-chain, and the fact the most cases had recovered by the time of study necessitated Nobuto collection strip-based serologic investigation of the outbreak. The small volume of blood collected limited the sensitivity of the VZV IgG avidity assay. Although this assay was useful in identifying recent probable cases of VZV, the limited sensitivity did not allow for ruling out rashes caused by other etiologies. Additionally, the time frame between illness onset and sample collection, namely in the villages of Congo Malembe and Illiangakaca, did not allow us to examine acute orthopoxvirus infection, because the illness occurred outside of the window in which a negative anti-orthopoxvirus IgM response is meaningful. It was therefore not possible to determine whether the presence of an anti-orthopoxvirus IgG response was actually caused by the illness in question or from an earlier orthopoxvirus exposure. To improve diagnostic capabilities, the rash illness investigation kits (described above) contain supplies for collection of direct lesion material samples. This approach is advantageous, because samples that are collected and stored at room temperature can yield meaningful diagnostic results, both for monkeypox virus and VZV.

Finally, there was a difficulty among health personnel in the clinical differentiation between monkeypox virus and VZV. Compounding this problem, we observed the appearance of VZV in Likouala with unusual manifestations, namely the occurrence of VZV lesions on the palms of the hands and soles of the feet. As described, attempts are being made to improve sample collection and assist in laboratory diagnoses of rash illnesses in Likouala. In addition, through the use of pictures and clinical descriptions, we have provided training to local health personnel on the recognition and differentiation of monkeypox and VZV infection.

Monkeypox virus is highly pathogenic in humans, and, with declining population immunity to orthopoxviruses and increasing prevalence of immunocompromising conditions such as HIV, is an important emerging infection that necessitates rapid public health response. In this report, we described
the study of a rash illness outbreak in Likouala district, RoC, which underscored a number of challenges associated with surveillance in isolated parts of the developing world, such as timeliness of reporting and response and clinical and laboratory-based diagnostic capacity. Through laboratory testing, we noted VZV cases with spatial and temporal clustering in multiple rural villages. To facilitate timely investigation of future outbreaks, we attempted to enhance rash illness surveillance in Likouala. Surveillance programs modeled in a similar manner may assist in improved response to other infections in these parts of the world.

Received September 30, 2008. Accepted for publication December 14, 2008.

Financial support: This work was funded entirely by the US Centers for Disease Control and Prevention.

Disclaimer: The findings and conclusions in this report have not been formally disseminated by the CDC and should not be construed to represent any agency or policy.

Authors’ addresses: Adam MacNeil, Mary G. Reynolds, Darin S. Carroll, Kevin Karem, Zach Braden, Ryan Lash, and Inger K. Damon, Poxvirus and Rabies Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road NE, Mailstop G-43, Atlanta, GA 30333. Amba Moundeli and Jean-Vivien Mombouli, Department of Microbiology, Marien Ngouabi University, Mailstop G-18, Atlanta, GA 30333.

Reprint requests: Adam MacNeil, The Centers for Disease Control and Prevention, 1600 Clifton Road NE, MS G-43, Atlanta, GA 30333.

REFERENCES


