A One Health Perspective on Disease Dynamics: Human Monkeypox Transmission in

Sankuru District, Democratic Republic of Congo

by

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Duke University

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Anne Rimoin

Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the Duke Global Health Institute in the Graduate School of Duke University

2015
ABSTRACT

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Abstract

Background: Reports from the first monkeypox (MPX) active surveillance program in the Democratic Republic of Congo (DRC) in the 1980s determined that the disease was not of epidemic potential, with $R_0 < 1$. However, during an active surveillance period from 2005-2007, researchers found a 20-fold increase during the last 30 years. The purpose of this study was to analyze the contact data from 2005-07 and compare characteristics to those of the 1980s, and to assess the change in $R_0$ of MPX. Methods: Contact tracing information and samples from active lesions were collected. Samples were screened by PCR and positive cases were ranked by generation and grouped into chains of transmission according to date of rash onset, contact tracing, and location. $R_0$ was determined using calculations provided in the 1980s study and chain size distribution was compared. Results: Of 1407 suspected cases of MPX investigated in 2005-07, 287 provided contact information with an average of 6.22 (range, 1-20) contacts each. Among the 703 positive cases, 408 distinct chains of transmission were identified. Average chain size was 1.75 cases (range, 1-12), with the longest reaching six generations. The crude secondary attack rate (AR) was 0.092, with an effective $R_0$ of 0.576. Discussion: Contact characteristics and types of contacts differed from those of the 1980s program. This analysis found a higher crude secondary attack rate and effective $R_0$. This could be the result of a higher proportion of unvaccinated contacts, or that the virus is better able to transmit between humans with a more limited amount of contact.
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1. Introduction

1.1 Background

Human Monkeypox (MPX) is an emerging zoonotic disease endemic to forested regions of central and western Africa. The MPX virus is a member of the Poxviridae family, in the genus Orthopoxvirus.\(^1\) Since the eradication of smallpox, MPX is considered the most important orthopoxvirus with respect to human infection.\(^2\)\(^-\)\(^4\)

MPX was recognized as a distinct human disease in 1970 during smallpox eradication efforts in the Democratic Republic of Congo (DRC) when its continued presence was confirmed in rural, forested areas.\(^5\)\(^,\)\(^6\) After the global cessation of smallpox vaccination in 1980, there were concerns that MPX could emerge from central Africa to replace smallpox in the absence of vaccination programs, and that increased surveillance was necessary to assess this risk.\(^3\)\(^,\)\(^7\) As a result, the World Health Organization (WHO) initiated the first active MPX surveillance program in the DRC from 1981-1986 to better understand the public health significance of MPX as the population with cross-protective immunity from smallpox vaccination decreased.\(^3\) Epidemiological data and stochastic models of human-to-human transmission produced from this study concluded that the reproductive number (R\(_0\)) of human MPX was less than 1, with most cases believed to have been infected through contact with forest animals. Viral evolution would be necessary for sustained human-to-human transmission and endemicity.\(^4\)\(^,\)\(^8\)\(^-\)\(^11\)
However, more recent studies have raised concerns about both the initial and long-term validity of these conclusions.\textsuperscript{7}

Since 1986, there have been limited studies published on the burden of MPX infection in DRC due to civil war and the extreme remoteness of its native ranges. Its epidemiology, reservoir species, and potential for inter-human transmission remain poorly understood. However, there has been an increased research interest in MPX since 2003 when MPX-infected Gambian rats imported to the US from West Africa infected prairie dogs at exotic pet stores which subsequently infected 47 human pet owners, highlighting its ability to spread rapidly in naïve populations.\textsuperscript{12,13}

During active surveillance carried out between 2005 and 2007, researchers found that annual incidence of MPX within a forested health zone, Kole, increased 20-fold compared to the 1980’s WHO surveillance in the same zone (0.72 to 14.42 per 10,000).\textsuperscript{7} The increase is potentially influenced by many factors, such as the decreasing population carrying cross-protective immunity from smallpox vaccination, mass human migration into endemic forested regions due to prolonged conflict, a subsequent increased reliance on bushmeat as a main source of protein and thus increased exposure to potential animal reservoirs.\textsuperscript{7,14,15} Disease surveillance systems have improved since the war ended in 2002 and have contributed to the increased reporting of suspected cases, however, a recent study has shown that there was a 4-fold increase in MPX cases between 2001-2013, independent of improved surveillance and reporting systems.\textsuperscript{14}
1.2 Clinical Characteristics and Epidemiological features of MPX

1.2.1 Clinical Characteristics

Monkeypox is the most similar orthopox virus to smallpox, albeit with a lower case-fatality rate between 1.5% and 17%, and a lower rate of human-to-human transmission (R₀ < 1 vs R₀ = 3.5-6). After exposure to the virus, the disease progresses with a 7-17 day incubation period followed by the onset of fever, malaise, and swollen lymph nodes. The typical rash usually appears after 1-3 days of this initial phase, spreading inward from the patient’s extremities (palms of the hands, soles of the feet, or the face) to the trunk of the body, and progresses from macules to papules, vesicles, pustules and umbilications, to scabs and desquamation over a period of 2-4 weeks (Figure 1). Other typical symptoms include chills, headache, backache, sore throat, cough, shortness of breath, and can also include secondary skin infections (19%), pneumonitis (12%), ocular complications (4-5%), and encephalitis (<1%).

Lymphadenopathy is a distinguishing feature of MPX, as it is uncommon in both smallpox and chickenpox.

Figure 1. Female child in the Sankuru district with acute MPX showing characteristic lesions on palms of hands
To definitively distinguish and diagnose MPX from other rash illnesses, primarily chickenpox, a laboratory confirmation is necessary. However, diagnosis can prove difficult, as specimens must be collected within a short period of time during active disease when virus can be isolated. Cases identified late risk false-negative viral detection results; however, cases in the recovery stage can be tested for IgM antibodies in the weeks following illness, and IgG antibodies for years after by ELISA (Figure 2).\(^{21}\)

\[
\text{Figure 2. Kinetics of MPX infection, viral detection and antiviral response}^{22}
\]

### 1.2.2 Epidemiological Features

Major risk factors for acquisition of MPX include contact with known animal reservoirs, proximity to primary forest, and smallpox vaccine status. MPX can infect a range of species based on antibody studies, but live virus has only been isolated from a single rope squirrel found in the Equateur Province of DRC.\(^{23,24}\)
Studies since 1981 report that the vaccinia (smallpox) vaccine confers 80-85% protection for MPX, leaving vaccinated individuals 5.2-fold less likely to acquire the disease than those unvaccinated.\textsuperscript{4,7} Though vaccine-derived immunity is thought to be long-lasting, only 24.5% of the current population located in surveillance regions have received the vaccine, compared to 84.7% in 1981, thus drastically reducing herd immunity and increasing the proportion of susceptible individuals.\textsuperscript{7,19,25}

Epidemiological curves depicting outbreaks of human MPX are typically bimodal. Frequency of primary cases peaks after introduction of a point source of infection and is separated from the peak of secondary cases based on the length of “rash-to-rash interval,” or the average incubation period, which is believed to be 12 days (range, 7-21) (Figure 2).\textsuperscript{9,26} Individuals are believed to be infectious from the time of rash onset, until desquamation, usually 2-4 weeks. Transmission can occur through direct contact, and less efficiently, through respiratory droplets.\textsuperscript{27} It is also assumed that MPX virus is similar to the smallpox virus in that it is large and stable enough to survive on fomites for long periods of time.\textsuperscript{28}

1.2.3 History of Human to Human Transmission

In the WHO sponsored active surveillance program from 1981-1986, 338 cases were investigated, with 245 (72%) considered primary or co-primary, and 93 (28%) secondary, assumed human-derived cases. The secondary attack rate among 431 unvaccinated household contacts was 9.3% (Table 1).\textsuperscript{19} The attack rate among contacts of
secondary cases was only 1.3%, meaning that most chains of transmission died out after 1-2 generations, with the exception of one chain which lasted for four generations.\(^8\)

The crude absolute secondary attack rate was found to be 0.030, with the basic reproduction rate (\(R_0\)) averaging 0.815.\(^4\) However, it should be noted that this \(R_0\) was calculated using the unvaccinated attack rates, 0.093 and 0.048 respectively.\(^4\) Therefore, it may be assumed that this would be the \(R_0\) in a completely susceptible population.\(^29\) To estimate the effective reproductive number (\(R_{\text{eff}}\)), which accounts for the difference in attack rate between vaccinated and unvaccinated contacts and is more representative to the actual transmission potential at the time of the study, one should use the overall attack rates for household and non-household contacts, 0.037 and 0.019 respectively (Table 1). This method determined \(R_{\text{eff}}\) to be 0.323, which represents the average number of secondary cases caused by each case, accounting for differences in vaccination status.

**Table 1. Secondary attack rates of household and non-household contacts by vaccination status. Contacts were reported by 147 cases in the 1980s MPX surveillance period\(^4\)**

<table>
<thead>
<tr>
<th>Vaccination Status</th>
<th>Household</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Non-household</th>
<th></th>
<th></th>
<th></th>
<th>All Contacts</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contacts</td>
<td>Cases</td>
<td>Attack rate</td>
<td>Contacts</td>
<td>Cases</td>
<td>Attack rate</td>
<td>Contacts</td>
<td>Cases</td>
<td>Attack rate</td>
<td>Contacts</td>
<td>Cases</td>
<td>Attack rate</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>431</td>
<td>40</td>
<td>0.093</td>
<td>292</td>
<td>14</td>
<td>0.048</td>
<td>723</td>
<td>54</td>
<td>0.075</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td>989</td>
<td>13</td>
<td>0.013</td>
<td>566</td>
<td>2</td>
<td>0.004</td>
<td>1555</td>
<td>15</td>
<td>0.010</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1420</td>
<td>53</td>
<td>0.037</td>
<td>858</td>
<td>16</td>
<td>0.019</td>
<td>2278</td>
<td>69</td>
<td>0.030</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Passive reporting of MPX cases between 1986 and 1995 resulted in only 13 case reports containing no contact information,\(^30\) however, in 1996-1997, the WHO and CDC investigated a large reported outbreak with sustained human-to-human transmission in
the Katako-Kombe and Lodja health zones of the Sankuru (Figure).\textsuperscript{26,31} Of the 419 cases investigated in 78 distinct villages, only 94 (22\%) were considered primary, with a case mortality rate of 1.3\% and an average attack rate between 5\% - 12\%.\textsuperscript{31} It should be noted however, that the non-specific case definition used in the investigations resulted in the inclusion of varicella (chickenpox) cases, which characteristically are less severe and more transmissible between humans.\textsuperscript{32}

More recently, a chain of transmission containing seven generations of secondary cases was observed in the Republic of Congo in 2003,\textsuperscript{33} suggesting that human-to-human transmission is increasing. It is difficult to ascertain whether this increase is due to a true increase in virus transmissibility ($R_0$) as a result of viral adaptation, or other factors that could increase incidence without changing $R_0$, such as increased frequency of introduction from animal reservoirs, or simply the result of an increased proportion of the population being susceptible to infection as a result of smallpox vaccination cessation.\textsuperscript{29} In the case of the latter option, longer chains of transmission would be observed among susceptible household contacts, but would die out after this close population is depleted. This possibility would obscure the detection of a true increase in $R_0$.\textsuperscript{29}

An active surveillance program from 2005-2007 was completed in the same region as the 1980’s WHO program. Of 760 MPX cases confirmed by quantitative RT-PCR, MPX virus was isolated and sequenced from 60 clinical specimens deemed
representative of all ranged of disease severity, transmission and geography.\textsuperscript{34} Four clades were detected, and in one of the lineages, significant genomic reduction was detected in a region associated with adaptation for human-to-human transmission.\textsuperscript{35} Although the study authors could not confirm that this genomic reduction resulted in increased disease severity and human transmission, there was a correlation, suggesting that rapid viral adaptation may be occurring and should be investigated further.\textsuperscript{34}

\textbf{1.2.4 Models for Human to Human Transmission}

Jezek et al (1988) developed a stochastic model based on the 1980s WHO surveillance data, which attempted to predict the effect of dwindling immunity on inter-human transmissibility of MPX.\textsuperscript{9} This model used parameters derived from data gathered from the observed population, including proportions of vaccination coverage, primary vs. secondary cases, average number of contacts per case (10.7), and secondary attack rates. The stochastic Monte Carlo model simulation was run 100 times for three levels of vaccine coverage: 70\%, 50\%, and 0\%.\textsuperscript{9}

With 70\% vaccination coverage, 147 cases produce an average of 62.7 secondary cases (range, 37-88) in 2-6 generations, with 28\% of primary cases infecting at least one secondary case. With 0\% coverage, 147 primary cases infected an average of 257.2 secondary cases (range, 182-355), with 4-11 generations. In this model, 54\% - 67\% of primary cases would infect secondary cases.\textsuperscript{4}
Limitations of this model include its purely stochastic nature with homogenous contact and transmission dynamics, the lack of depletion of susceptible population due to both clinical and subclinical infections, its small population size, and the inability to distinguish multiple primary cases in the same cluster. Although the authors concluded that there was a limited potential for MPX to be sustained in human populations, they did not take into account the large-scale anthropogenic changes which occurred during the last two decades, such as prolonged conflict, mass human migration into forested areas, and increased reliance on bushmeat. This means that their critical assumption that the annual rate of introduction from animal reservoirs would remain low at 0.35 per 100,000 is most likely flawed. Additionally, the model does not take into account the possibility of viral evolution and adaptation, which would increase its ability to transmit between human hosts.

A more recent study argues that stochastic techniques cannot adequately model infectious diseases characterized by “stuttering chains,” which have a non-zero \( R_0 \) \((0 < R_0 < 1)\), including diseases close to elimination, and many types of zoonotic infections. Rather, models for these diseases should include measures of dispersion, or heterogeneity in transmission dynamics, which is crucial for reliably detecting a statistically significant change in transmissibility. A model for secondary transmission using a two-parameter negative binomial distribution with a mean \( R_0 \) and a dispersion parameter \( k \) determined that it is possible to robustly simulate outbreaks using infection
chain size and accurate primary case data. Results found $R_0$ to be 0.30 (95% CI: 0.21-0.42) and $k$ to be 0.33 (95% CI: 0.17-0.75), which supports previous conclusions that MPX will have limited endemic ability without viral adaptation, even given the highly heterogeneous transmission dynamics of MPX.\textsuperscript{36}

Another component of this recent study concluded that transmission heterogeneity can be compared between two distinct but related populations.\textsuperscript{29} This modeling exercise confirmed the heterogeneous nature of human MPX transmission, but found animal-to-human transmission to be fairly homogenous, which is an important determination considering that anthropogenic changes in animal contact patterns could increase the incidence of human MPX without changing the $R_0$. Additionally, the study authors suggest that data from 760 confirmed cases during the 2005-2007 surveillance program should be able to detect a 0.3 to 0.55 change in $R_{eff}$ with 95% power.\textsuperscript{29}

### 1.3 Project Objectives

This study was conducted using data collected from 2005 – 2007 during an active surveillance program in the Sankuru district of Kasai Oriental province, central DRC. The original purpose of the project was to strengthen the national MPX surveillance system and laboratory testing, to further define the MPX case definition and disease epidemiology, and to determine the disease burden in a highly endemic region.

Analyses using these data have been previously conducted to determine associations between confirmed disease and known and suspected risk factors.
Therefore, this study builds on those analyses by incorporating contact tracing information and geographical information to conduct a spatiotemporal analysis of disease transmission. We classified cases as primary, co-primary, or secondary, and we compared the characteristics and frequencies of human transmission from primary to secondary cases. With this information, we determined the difference in $R_0$ between the 1980s study and the current one.

### 1.4 Significance

Information obtained in this study will help to conserve the limited funds available for surveillance activities by identifying priority locations and/or typical patterns of disease spread. This will allow health zone, district, and national officials to determine locations of highest importance in terms of prevention, surveillance and sampling efforts. It will also give more information on primary vs. secondary transmission, which will allow for comparison to the 1980’s data and help determine if virus transmissibility is changing.
2 Methods

The UCLA-DRC Research Group, in coordination with DRC’s Ministry of Health (MOH), facilitated an active surveillance program to identify cases of human MPX in the Sankuru district of Kasai Oriental province, in central DRC from 2005-2007. The original purpose of the project was to strengthen the national MPX surveillance system and laboratory testing, to further define the MPX case definition and disease epidemiology, and to determine the disease burden in a highly endemic region.

The study team provided technical training, equipment, materials and supervision to the local health offices participating in this prospective, population-based study. The training of an MPX surveillance team, including district and health zone doctors and nurses, who subsequently trained health center nurses and village volunteers, consisted of skills in clinical case identification, outbreak investigation, and reporting of all suspected cases. Material support was provided in the form of cool boxes for each health zone, standard questionnaire forms, specimen collection materials, motorcycles, and bicycles.7

2.1 Setting

Active surveillance took place within 9 health zones of the Sankuru district of Kasai Oriental province in the DRC (Figure 3). The Sankuru has an area of 105,378 km² and is comprised of dense canopy rainforest, mosaic/gallery forest and savannah.24
most populous cities, Lodja, Kole and Lomela are connected to supply roads and waterways, however, most villages contain fewer than 1000 people, are difficult to access, and are established in small forest clearings or traditional agricultural land on the forest edge. \textsuperscript{7} Since 2001, this area has consistently reported the largest incidence of MPX cases and is thus considered an endemic region for MPX.\textsuperscript{7}

\textbf{2.2 Participants}

In 2007, an estimated population of 851,574 people lived in the 9 participating health zones, with 14.28 inhabitants per square kilometer (Table 1).\textsuperscript{37} The inhabitants typically rely on subsistence farming and hunting to provide nutrients, with virtually all
protein obtained from hunted wildlife, the most common sources being duikers, monkeys, and rodents.\textsuperscript{2,25,38,39}

As MPX is one of the nationally reportable diseases in the DRC, all suspected cases were supposed to be primarily investigated as part of the national MPX disease surveillance program. Cases investigated as part of this program were recruited to participate in the study from 23 November 2005 – 31 November 2007. An individual was eligible if he or she met the Ministry of Health’s case definition (fever \( \geq 38 \) °C, and a vesiculopustular rash), and he or she lived within the targeted 9 health zones of the Sankuru district (Table 1). Cases investigated during the study were included in this analysis if their biological sample tested positive for MPX, as determined by PCR assays.

### Table 2. Population estimates of the target health zones of the Sankuru District in 2005-2007

<table>
<thead>
<tr>
<th>Health Zone</th>
<th>Population</th>
<th># Health Centers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Djalo Ndjeka</td>
<td>78900</td>
<td>10</td>
</tr>
<tr>
<td>Katako Kombe</td>
<td>126040</td>
<td>17</td>
</tr>
<tr>
<td>Kole</td>
<td>112795</td>
<td>11</td>
</tr>
<tr>
<td>Lodja</td>
<td>143895</td>
<td>19</td>
</tr>
<tr>
<td>Lomela</td>
<td>99155</td>
<td>18</td>
</tr>
<tr>
<td>Omendjadi</td>
<td>73789</td>
<td>10</td>
</tr>
<tr>
<td>Ototo</td>
<td>103100</td>
<td>17</td>
</tr>
<tr>
<td>Tshudi Loto</td>
<td>53900</td>
<td>11</td>
</tr>
<tr>
<td>Vangakete</td>
<td>60000</td>
<td>14</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>851574</strong></td>
<td><strong>127</strong></td>
</tr>
</tbody>
</table>
2.3 Procedures

For each eligible case identified through active surveillance, an informed consent was administered. The zone MPX surveillance officer conducted a clinical examination on all cases and collected biological samples in the form of crusted scabs and vesicle fluid to send for laboratory confirmation.

Participants were also administered an oral questionnaire in their local dialect, which collected demographic, clinical and epidemiological data, as well as information about recent exposure to wild and domestic animals three weeks prior to illness. Information about human contacts before and after the illness was collected from individuals available for follow-up. Data were collected from all participants prior to obtaining laboratory results, however, only MPX cases confirmed by PCR were included as cases in this analysis.

2.3.1 Ethics

Ethical approval for this study was obtained from participating institutions (University of California and the Kinshasa School of Public Health), and informed consent was obtained from all participants. All researchers completed required trainings and were approved as key personnel by the institutions.

Informed consent was administered orally in local dialect due to a high prevalence of illiteracy, and participation was strictly voluntary. Parental and individual consent was obtained for all participants <18 years of age. All suspected cases and
caretakers were provided with information about the disease, how to care for patients, and supportive treatment in the form of paracetamol, antibacterial soap and antibiotics, as per DRC Ministry of Health guidelines. Because of logistic and budgetary constraints, we were unable to consistently conduct follow-up visits, thus precise information on mortality and secondary transmission could not be ascertained.

2.4 Measures

2.4.1 Clinical Examination and Questionnaire

The health zone MPX surveillance officer conducted a clinical examination on all suspected cases and administered an oral questionnaire in their local dialect. The questionnaire was designed to collect socio-demographic and clinical information in addition to data on frequency and types of exposures to various risk factors, in order to further the understanding of MPX epidemiology and risk factors. Demographic data included age, sex, occupation, geography and population of the location, and clinical data included dates of fever and rash onset, and type and severity of symptoms. Additionally, GPS coordinates were collected for approximately 58% of cases investigated.

2.4.2 Contact Tracing Questionnaire

At the time of case investigation, patients were asked to provide details about contact with individuals displaying a similar illness in the three weeks prior to disease onset (before contacts), as well as contact with all sick or healthy individuals in the weeks
after disease onset (*after contacts*). Contacts were identified by name, village, and head of household. The name and code data were used to link cases when follow-up was not completed. If the contact was also a case that was actively investigated, their case ID was provided. All contacts were assigned a new, second unique identification number after data collection to protect their identity, and to link individuals who had been assigned multiple ID codes. Data collected for symptomatic before contacts included the date of last contact, sex, the presence of fever, the type of contact (household, or non-household), and the relationship to the case. Data collected for after contacts included contact type (household, parcel, school, neighborhood, or other), relationship, dates contact started and ended and types of activities (play, work, sleep, eat with, and care provision). See Appendix A for the Contact Tracing Questionnaire.

### 2.5 Analysis

#### 2.5.1 Laboratory Analysis

All biological samples were tested by the US Army Research Institute for Infectious Diseases, where they were screened for OPX viruses by PCR. Positive samples underwent a second PCR assay for specific DNA sequences to discriminate MPX from other OPX. Laboratory methods are described in full by Rimoin et al (2010).7
2.5.2 Data Management

Data from the Clinical and Demographic Questionnaire were entered between 2005-2008 using Epi Info and Microsoft Access, and subsequently exported as an Excel dataset. Data from the Contact Tracing Questionnaire were entered into an electronic Filemaker Pro database in 2014 with all contacts linked to the appropriate case by Case ID. Case information was confirmed to be identical to the information provided in the clinical and demographic dataset entered previously. Information from contacts who were also investigated as cases was confirmed to match their case investigation records, and any missing Case IDs were entered in the contact record. Both contacts and cases were de-identified before statistical analysis. All data cleaning and statistical analyses were completed using R version 3.1.3.40

2.5.3 Definitions

Similar to algorithms described previously,49,41 cases were classified as primary if s/he was the first person investigated in an independent outbreak chain, or has no history of contact with a symptomatic human within three weeks before rash onset. Primary cases were assumed to be caused by an animal source. Onset is defined as the first day of rash presentation, and also marks the first day of infectivity and exposure to close contacts. A chain of transmission was defined as the primary case, and all subsequent cases stemming from this initial infection. A cluster of cases may contain chains arising from multiple primary cases.29 A case in the same chain or cluster with an
onset less than seven days after exposure to the primary case were classified as co-
primary. Any cases with an onset 7-34 days after reported contact with a symptomatic
primary case was classified as a secondary case of the first generation, and assumed to be
caused by a human source. The generation of secondary cases were determined by their
location within the temporally classified chains of transmission.29

Secondary attack rates are defined as the proportion of contacts exposed to an
active case that developed the disease within the known incubation period. Attack rates
can be compared to the effective reproductive number (R_{eff}), which is defined as the
average number of secondary cases caused by each individual case, with regard to
population susceptibility.10,27 This is as opposed to the basic reproductive number (R_0),
which is the average number of secondary cases caused by each case in a completely
susceptible population.29 Contacts are defined as individuals coming into direct contact
with a case, and household contacts are those residing in the same household. Contacts
were stratified into before and after contacts, as defined above.

2.5.4 Contact Tracing Analysis

Clinical, demographic and contact tracing data were used to determine
characteristics of contact with active MPX cases, and to estimate outbreak cluster sizes.
We calculated the average number of contacts per case, proportions of contacts
according to relationship to the case, proportion of household contacts, and secondary
attack rates among household and non-household contacts. Proportions of household
contacts and secondary attack rates were compared to the 1980s data using a chi-square test for population probabilities.

We classified cases as primary, co-primary, or secondary based on their timing, proximity, and reporting of contact with other confirmed cases, according to the definition provided above. Case classifications were compared to whether each case thought they acquired MPX from a human, animal or other source, as well as whether there was another case of rash illness in their village in the month prior to disease onset.

Outbreak cluster or chain size was tabulated according to the number of cases in each generation of transmission and the number of generations in each chain. Chain size distribution was compared to that of the 1980s. Chains were then stratified by whether they contained co-primary cases, or just a single primary case, and these chain size distributions were compared. Significant differences were determined using Pearson’s Chi-squared test with Yate’s continuity correction.

2.5.5 Disease Transmission Analysis

The crude absolute secondary attack rate \( AR_{crude} \) was found by dividing \( n_{inf} \), the number of contacts subsequently infected by MPX, by \( n_{total} \), the number of contacts reported (Equation 1).

\[
AR_{crude} = \frac{n_{inf}}{n_{total}}
\]  

(1)
To determine the difference in $R_{eff}$ between the 2005-07 surveillance period and the WHO 1980’s surveillance period, we employed the equation used by Fine et al (1988) (Equation 2). Components included $\bar{n}$, the average number of contacts per case, $x_n$, the proportion of household or non-household contacts, and, $AR_h$, their respective crude secondary attack rates. The sum of components for household and non-household contacts was calculated to determine $R_{eff}$.

$$R_{eff} = \sum \bar{n}x_n (AR_h)$$

(Equation 2)
3 Results

3.1 Characteristics of MPX Cases and Contacts

During the 2005-2007 active surveillance program, 1272 suspected cases were investigated, and 703 cases (55.3%) were confirmed positive for MPX. Of these cases, 134 individuals (19.1%) reported having contact with one or more symptomatic human in the three weeks before illness onset, for a total of 212 before contact reports. 287 individuals (40.9%) reported having close contacts in the three weeks after illness onset, for a total of 1786 after contact reports. Contacts were partitioned into household or non-household contacts, with household contacts comprising 67% of before contacts and 84% of after contacts (Table 3). There were significantly more household after contacts compared to the 1980s, when only 53% were household ($X^2 = 300.7, P < 0.001$). The average number of before contacts per case was 1.58 (range, 1-6), and the average number of after contacts was 6.22 (range, 1-20) contacts per case.

<table>
<thead>
<tr>
<th>Type of Contact</th>
<th>2005 - 2007 Program</th>
<th>1981 - 1986 Program</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Before Contacts</td>
<td>Total After Contacts</td>
</tr>
<tr>
<td>Household</td>
<td>143 0.67 1509 0.84</td>
<td>1420 0.62</td>
</tr>
<tr>
<td>Other</td>
<td>69   0.32 277 0.16</td>
<td>858 0.38</td>
</tr>
<tr>
<td>Total</td>
<td>212  0.32 1786 0.16</td>
<td>2278</td>
</tr>
</tbody>
</table>
The majority of before and after contacts were reported to be siblings (55% and 50% respectively), with parents comprising 22% of after contacts and only 9% of before contacts (Figure 4). Individuals external to family were only 4% of the reported after contacts, however, 13% of the symptomatic before contacts were external, suggesting instances of household-to-household transmission. Differences in relationship frequencies in before and after contacts may also represent differences in caretaking and susceptibility patterns; for example, no grandparents were indicated as before contacts while 4% of after contacts were grandparents (Figure 4).

![Contact Relation to Case](image)

*Figure 4. Proportions of before and after contacts by relation to case*
3.2 Characteristics of Disease Clusters

Among the 703 confirmed cases (plus an additional 10 cases in the month before or after the surveillance period who were part of an identified chain), 408 distinct chains of transmission were identified. Of all chains, 261 (64%) consisted only of a single primary case with no secondary spread, while 53 (13%) chains contained one or more co-primary cases, but no secondary generations (Table 4). A further 27 (6.7%) chains were overlapping, meaning they contained one or more co-primary cases as well as at least one generation of secondary cases, making it difficult to distinguish which primary case infected which secondary. There were many chains that may have included additional suspected cases who did not have samples collected because they were either investigated too late, or there was an inadequate supply of collection materials. For example, in the village of Bolengo in the Kole health zone, 17 cases were investigated over a period of four months. The health worker visited the village three times, 1-1.5 months apart. Due to the long intervals between visits, samples were only collected from 6 out of 17 cases, meaning that the remaining 11 cases could not be definitively linked to the chain of transmission.
Table 5. Distribution of primary and secondary cases by generation. The cluster type refers to the number of cases in each generation (i.e. 10210000 corresponds to 1 primary, 0 co-primary, 2 1st generation secondary, and 1 2nd generation secondary case). Chains with no co-primaries are in the top half of the table, while chains with co-primaries are in the bottom half. The total number of cases in each generation is summed in the bottom row.

<table>
<thead>
<tr>
<th>Cluster Type</th>
<th>Primary</th>
<th>Co-primary</th>
<th>1st Sec</th>
<th>2nd Sec</th>
<th>3rd Sec</th>
<th>4th Sec</th>
<th>5th Sec</th>
<th>6th Sec</th>
</tr>
</thead>
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<td>261</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>10100000</td>
<td>39</td>
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<td>0</td>
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<td>0</td>
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<td>10200000</td>
<td>5</td>
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<tr>
<td><strong>Total No Co-primaries</strong></td>
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<td><strong>91</strong></td>
<td><strong>26</strong></td>
<td><strong>10</strong></td>
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</tr>
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<td>12210000</td>
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<td>11121000</td>
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<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total with Co primaries</strong></td>
<td><strong>80</strong></td>
<td><strong>113</strong></td>
<td><strong>40</strong></td>
<td><strong>15</strong></td>
<td><strong>3</strong></td>
<td><strong>1</strong></td>
<td><strong>0</strong></td>
<td><strong>0</strong></td>
</tr>
<tr>
<td><strong>Total All Chains</strong></td>
<td><strong>408</strong></td>
<td><strong>113</strong></td>
<td><strong>131</strong></td>
<td><strong>41</strong></td>
<td><strong>13</strong></td>
<td><strong>3</strong></td>
<td><strong>2</strong></td>
<td><strong>2</strong></td>
</tr>
</tbody>
</table>
Chains were stratified by whether they contained co-primary cases or not to determine whether there was a significant difference in chain size distribution between chains with co-primaries and chains with just a single primary case. Overall, primary and co-primary cases made up 73% of total cases, and 77% of all chains only contained primary or co-primary cases, not spreading to secondary generations. Approximately 19.6% of chains contained one or more co-primary cases, of which 66.2% did not spread to subsequent generations. This is significantly different than the chains that contained only a single primary case, 79.6% of which did not spread to secondary generations (Figure 5). Overall, the average chain size was 1.75 cases (range, 1-12) and the longest chain reached six generations. Chains with one primary case contained an average of 1.41 cases (range, 1-11), as opposed to those with co-primaries with 3.15 cases (range, 2-12).
Figure 5. Percentage of chains containing cases in each generation, stratified by whether they contain co-primary cases or not. Chains containing only primary and co-primary cases were classified as not spreading.

3.3 Monkeypox Transmission Dynamics

Of the 287 confirmed MPX cases reporting after contacts, 87 individuals (30.3%) started a chain of transmission, infecting 98 individual secondary cases, who represented 165 reported contacts due to their contact with multiple cases. Each contact was listed by an average of 1.68 cases (range, 1-8) (Table 4). The crude absolute secondary attack rate was found to be between 0.069 and 0.092 (Table 5).
Table 4. Numbers of confirmed cases reporting contacts in the three weeks after onset of illness, and proportion of after contacts that were infected. Individual contacts were listed by multiple cases. Each time a contact was listed, it was considered a report

<table>
<thead>
<tr>
<th>Contacts of all MPX+ Cases</th>
<th>n</th>
<th>Infected Contacts of MPX+ Cases</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive cases with contact tracing</td>
<td>287</td>
<td>Cases infecting after contacts</td>
<td>87</td>
</tr>
<tr>
<td>Total contact reports</td>
<td>1736</td>
<td>Total contact reports infected</td>
<td>165</td>
</tr>
<tr>
<td>n individuals listed as contacts</td>
<td>1425</td>
<td>n individuals became cases</td>
<td>98</td>
</tr>
<tr>
<td>Contact of n cases on average</td>
<td>1.25</td>
<td>Contact of n cases on average</td>
<td>1.73</td>
</tr>
</tbody>
</table>

Upon stratification into household and non-household contacts, 1214 individuals were listed in 1509 reports of household contacts, and 211 individuals were listed in 277 reports of non-household contacts. We found $AR_{crude}$ for both individual contacts and total reported contacts to determine the best method of comparison to the 1980s reports. Individual $AR_{crude}$ was 0.072 for household contacts and 0.047 for non-household, while total reported $AR_{crude}$ was 0.093 and 0.090 for household and non-household contacts, respectively (Table 5). Using Equation 2, we found the individual $R_{eff}$ to be 0.425, and the total $R_{eff}$ to be 0.576. These values indicate the average number of secondary cases caused by each case, accounting for differences in attack rates between household and non-household contacts.
Table 5. Crude secondary attack rates stratified by household and non-household contacts. Attack rates were found using individuals as well as total contacts reported and are compared to the attack rate found in the 1980s. Crude absolute secondary attack rate was found by dividing total cases by total contacts for each category.

<table>
<thead>
<tr>
<th></th>
<th>Household</th>
<th>Cases</th>
<th>Attack Rate</th>
<th>Non-household</th>
<th>Cases</th>
<th>Attack Rate</th>
<th>Crude Absolute Secondary Attack Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual Contacts(^a)</td>
<td>1214</td>
<td>88</td>
<td>0.072</td>
<td>211</td>
<td>10</td>
<td>0.047</td>
<td>0.069</td>
</tr>
<tr>
<td>Contact Reports(^b)</td>
<td>1509</td>
<td>140</td>
<td>0.093</td>
<td>277</td>
<td>25</td>
<td>0.090</td>
<td>0.092</td>
</tr>
<tr>
<td>Contacts (1980s)(^b)</td>
<td>1420</td>
<td>53</td>
<td>0.037</td>
<td>858</td>
<td>16</td>
<td>0.019</td>
<td>0.030</td>
</tr>
</tbody>
</table>

\(^a\) Does not differentiate between vaccinated and unvaccinated contacts
\(^b\) Vaccinated and unvaccinated contacts, where 70% were vaccinated

### 3.4 Monkeypox Transmission Dynamics

By using contact tracing data, we can more easily visualize the spread of MPX through an affected population. By looking at contact durations compared to dates of illness onset within villages, cases were classified as primary or secondary. However, it is known that case identification, investigation, and follow up was imperfect, meaning that there could be errors in case classification due to missed cases, errors in data collection or entry, or non-investigated asymptomatic carriers. Looking at the network graph of the Lodja health zone in Figure 5, we can see that many cases classified as primary cases reported contact with another primary case during the active surveillance period. Furthermore, there were also many 1\(^{st}\) and 2\(^{nd}\) generation secondary cases with no reported contact with primary cases, as well as a few interesting instances where primary cases are connected indirectly to other primary cases through an asymptomatic
contact. All of these situations could indicate error in case classifications due to the limitations listed above.

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**Lodja Health Zone Network of MPX Transmission 2005–2007**

![Diagram of Lodja Health Zone Network of MPX Transmission 2005–2007]

**Figure 6.** Contact tracing network of the Lodja health zone during 2005-2007. Figure does not include cases that did not submit contact tracing information.
4 Discussion

Since the first active MPX surveillance study in the 1980s, annual incidence in parts of the Sankuru district has increased more than 20-fold. Although researchers in the 1980s determined that there was a low potential for epidemic threat by MPX, this conclusion is now questionable due to anthropometric changes in human contact with animals and other humans, declining vaccination coverage, and possible viral adaptation to humans. This study aimed to determine whether the 1980s $R_{eff}$ and the current $R_{eff}$ are significantly different.

The surveillance study found 703 PCR-confirmed MPX cases, almost twice as many as the 5-year study in the 1980s. The proportion of primary and co-primary cases versus secondary cases was nearly identical to that of the 1980s, at 73% and 27% respectively. However, we found evidence to support that inadequate contact tracing and follow up of cases could have led to an overestimation of primary cases. Many chains are likely to contain additional suspected cases who were investigated within the appropriate incubation period, but did not have samples collected or tested. This would occur as a result of late follow-up visits or inadequate supplies needed to collect samples. As Blumberg et al (2013) have reported, while changes in $R_{eff}$ can be estimated from chain size and $k$ alone, it relies on the assumption that the correct proportion of primary cases per chain is known. Contact tracing helps to connect primary cases to
subsequent generations of cases that may have been missed, thus increasing the robustness of model.

Chain size ranged from 1 to 12 cases, with an average size of 1.75 cases. The longest chain reached six generations and consisted of 13 cases, including one vaccinated woman over the age of 60. This is interesting, as the longest chain in the 1980s only reached 4 generations, and the longest chain every recorded only reached 7 generations. Compounded with the observation that many chains may contain additional cases due to imperfect observation, it is likely that some chains may include generations past the sixth. More analysis could yield these chains by implementing different techniques to classify cases.

Using data from the 1980s surveillance period, Fine et al (1988) reported that the $R_0$ of MPX was 0.815. However, this was found using only the attack rates of unvaccinated household and non-household contacts. When the overall secondary attack rates were substituted into the equation, $R_{eff}$ was found to be 0.323. We determined that the $R_{eff}$ during the 2005-2007 surveillance period was either 0.425 when calculated with the individual contact attack rates, and 0.576 when calculated with the attack rate of total contacts reported. We calculated both of these values in order to determine the high and low estimates of $R_{eff}$, as the individual attack rate assumes that each individual was infected by just one case while the total contact attack rate treats contacts reported by multiple cases as if each report represented a unique individual.
The true $R_{eff}$ is likely to be between these two values; however, it is impossible to determine which case infected contacts listed by multiple cases. The 1980s $R_{eff}$ was calculated using total reported contacts rather than individuals; thus, for the purpose of comparison, we used our upper limit, 0.576.

Although the $R_{eff}$ is still less than 1, this study shows that the human-to-human transmissibility of MPX virus has increased since the WHO surveillance period in 1981-1986. It is likely that an increased rate of zoonotic introduction, along with decreasing vaccination coverage is contributing to the increased incidence seen over the past 30 years. This is of great interest to public health, as it is recognized that poxviruses are readily able to infect and adapt to naïve species. It is suspected that smallpox originated in an animal species, and slowly transitioned to humans as its only host species. Along the way, the virus is thought to have shed parts of its genome unnecessary for human transmission, as it is the shortest of the orthopoxviruses. This is applicable to the epidemiology of MPX today since it has been found that certain clades sequenced from the 2005-07 study have both lost parts of their genome and are more infectious or severe. It is possible that MPX could follow a similar route to its ancestor smallpox; however, it would most likely take centuries to become an exclusively human virus. For this reason, the current MPX virus would be more difficult to eradicate than smallpox due to its wide range of potential host species. The best course of action would therefore be to install control mechanisms to prevent or delay further adaption to humans.

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4.1 Implications for policy and practice

Information gathered from surveillance programs is crucial for governments to be able to respond efficiently and adequately to disease outbreaks. Surveillance in the DRC is particularly lacking, and it has been noted that significantly fewer cases of reportable diseases are found under passive surveillance compared to active. Thus, data from this very thorough, albeit with certain flaws, active surveillance study can help to inform national and international health bodies on the status of MPX epidemiology as well as the necessary precautions and actions to control the spread of the virus and adaptation to humans.

It is known that the smallpox vaccine confers 80-85% long-lasting immunity against MPX; however, its use has been discontinued since the eradication of smallpox. There have been discussions as to whether it should be reintroduced in Central Africa. While results from this study suggest that this would be an appropriate next step in the control of human MPX, there are significant risks and side effects with the vaccine, especially in immune-compromised individuals who represent a growing proportion of the population in Central Africa. Additionally, DRC has one of the world’s most ill-equipped vaccination programs, and already struggles to vaccinate large proportions of its population, especially in remote locations where re-introduction would be most needed.
As MPX has a significantly lower annual incidence than other vaccine-preventable diseases, such as measles, an is for the most part non-fatal, DRC and Central Africa cannot realistically take on the burden of adding another vaccine to their programs. Therefore, we propose that extensive community education programs be implemented in the most affected regions. These programs would sensitize populations to the signs and symptoms of MPX and proper case isolation procedures to limit household spread. They would also explain the risk of contact with animals, and methods of hunting, butchering, and raising animals that limit the risk of infection by MPX and other zoonotic illnesses. We cannot immediately change the diet of populations or community traditions, but we can work with communities to develop safer methods and habits.

4.2 Implications for further research

The implications of this study suggest the need for new, well-informed investigations to determine how to most accurately distinguish primary vs. secondary cases. While contact tracing is a useful source of data, one cannot definitively determine the exact source of infection without investigating the genetic viral phylogeny of all hosts infected within an observed outbreak population. This includes the animal reservoirs in addition to human cases from each generation. To accomplish this, it will be necessary to gain more understanding of the many potential reservoir hosts and the disease dynamics between all potential hosts through serosurveys of wildlife, and
behavioral studies on types and frequencies of animal contact within at-risk human populations. These studies would further the understanding of MPX prevalence, viral genetics, and host immunophysiology, and would also help to elucidate the mechanisms of zoonotic spillover and the implications of animal-to-human transmission.

Concerning the data from 2005-07, the modeling studies completed by Blumberg and Lloyd-Smith (2013) should be replicated and compared to the results of the 1980s. This will return a more accurate estimate of $R_{eff}$ than the one reported here, as their model accounts for heterogeneity in transmission. Additionally, a phylogenetic analysis of the 60 isolates already sequenced and available on GenBank may provide a good deal of information about the steps involved in viral adaptation, as well as chains of transmission to clarify primary vs. secondary cases.

4.3 Study strengths and limitations

This study has many limitations stemming from the study location, staff, study design, data collection, and data entry. The DRC has very weak health and disease reporting systems as a result of massive government corruption and inadequate payment of staff, years of widespread conflict, and an extreme dearth of transportation routes. These factors mean that healthcare staff is overworked, with little incentive to adhere to guidelines and transmit information regarding disease outbreaks to district and national health officials. Although this study supplemented staff with an additional salary, it was still difficult for them to investigate all reported cases in a timely manner,
and similarly, to monitor affected villages for subsequent cases, potentially resulting in many missed primary and/or secondary cases. As cases were confirmed by the detection of MPX virus in crusts or vesicles by PCR, samples could not be collected from cases whose lesions had cleared before investigation. This may have resulted in an underestimation of primary cases and missed cases within chains. Additionally, the study team was not able to analyze serum from MPX survivors or asymptomatic cases, so only individuals with active lesions could be laboratory confirmed and included in the study. There was variation in the level of knowledge among health workers who were able to investigate in a timely manner, such that more knowledgeable workers may have been better able to distinguish chicken pox from MPX, while others may have included all cases of rash illness in the study. This potentially led to a selection bias in participants in some areas.

Contact information was not collected for all cases. Of the contact information collected, the potential for bias in prediction or recall was great, as the information may only have been collected at the initial case investigation, or during the follow-up visit. This could have resulted in incomplete information about the number of contacts and duration of time spent with each. Additionally, as not all cases were visited for follow-up, infected contacts were not always indicated or provided Case ID’s on the primary case’s contact tracing form. Finally, this study cannot prove causation or provide definitive case classifications. There is no way to prove that an infection was caused by a
human vs. animal source, and thus no way to definitively determine primary vs. secondary cases.
5 Conclusion

Human monkeypox is increasing in incidence in Central Africa. As a member of the orthopoxvirus genus, it is capable of adaptation to human hosts. MPX virus seems to be in the midst of this adaptation process, as documented human transmission chains are growing longer while the virus is still being maintained in multiple animal species. The geographic range of MPX is expanding, which is allowing the virus to take advantage of naïve populations of both animal and humans. As it would be very difficult to protect people living in or on the edge of forests from contact with animals, and there are significant risks associated with the re-introduction of the smallpox vaccine in terms of health and overburdening the region’s current vaccination program, the best control and containment strategy would be to implement large-scale community education programs to reduce the risk of zoonotic spillover in at-risk populations. By improving the knowledge and safety of these people, we would hope to limit the number of human cases occurring and reduce subsequent human-to-human transmissions, in order to prevent further viral adaptation and to protect humans both in and outside Central Africa.
Français

**FICHE D'INFORMATION DES CONTACTS DU CAS**

**FICHE 2**

**NO D'IDENTIFICATION DU CAS**

Global Network for Women's and Children's Health Research

NO D'IDENTIFICATION DU CAS

Projet de Surveillance Epidémiologique du Monkeypox
Ministère de la Santé
ESP / INRB / UNC-DRC

No d'identification du cas (Ex: RDCKOR/LO/05/001) ▶ Cette fiche doit être remplie par l'infirmière superviseur de la zone de santé ou l'infirmier du centre de santé de santé pour tous les cas éligibles qui acceptent de participer à l'étude Monkeypox afin d'assurer le suivi des contacts. Elle renferme des informations confidentielles (nom) et devra être gardée en sécurité dans un endroit fermé. Les informations de cette fiche ne seront pas entrées dans la base de données de l'étude.

1. Dans les trois semaines précédant la maladie, le malade avait-il été en contact avec une ou des personnes qui présentaient les mêmes éruptions ou la même maladie?  □ Oui  □ Non (SI NON, ALLEZ À Q3)

2. Si OUI, remplissez les rubriques du tableau ci-dessous pour toutes les personnes ayant eu des éruptions et avec qui le cas a été en contact.

<table>
<thead>
<tr>
<th>N°</th>
<th>Nom et Prénom</th>
<th>Numéro d'identification si ce cas était déjà investigué</th>
<th>Sexe</th>
<th>Présence de maladie?</th>
<th>? = Oui</th>
<th>2 = Non</th>
<th>Cette personne vit elle dans le même ménage que le patient?</th>
<th>1 = Oui</th>
<th>2 = Non</th>
<th>Relation avec le patient (voir codes en bas du tableau)</th>
<th>Estimer la date du dernier contact avec cette personne (jjmm/aa)</th>
<th>Information sur le contact</th>
<th>Nom du chef de ménage et nom du village</th>
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**CODES SUR LA RELATION CONTACT/PATIENT:**

1 = Épouse/Portemine  4 = Grand-parents  7 = Beau-père/Belle-mère  10 = Autre employé
2 = Enfant  5 = Frère/Sœur  8 = Beau-fils/Belle-fille  11 = Autre parent
3 = Parent  9 = Née/Neveu  9 = Employé domestique  12 = Autre personne extérieure à la famille

<table>
<thead>
<tr>
<th>No</th>
<th>Nom et Prénom des contacts</th>
<th>Type de contact (1)</th>
<th>Relation avec le patient (2)</th>
<th>Date du début du contact (jj/mm/aa)</th>
<th>Date de la fin du contact (jj/mm/aa)</th>
<th>Nombre de jours du contact</th>
<th>Type d'activités pratiquées (3)</th>
<th>Cas ou non-Cas ?</th>
<th>Numéro d'identification si ce cas était déjà investigué</th>
<th>Information sur le contact: Nom du chef de ménage et nom du village</th>
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(1) TYPE DE CONTACT:
1 = Ménage
2 = Parcelle
3 = Ecole
4 = Volainage
5 = Inconnu

(2) RELATION CONTACT-CAS:
1 = Epouse/Partenaire
2 = Enfant
3 = Parent
4 = Grand-père/Beau-père
5 = Frère/Sœur
6 = Neveu/Nièce
7 = Beau-père/Beau-frère
8 = Beau-frère/Beau-sœur
9 = Employé domestique
10 = Autre employé
11 = Autre parent
12 = Autre personne extérieure à la famille

(3) TYPES D'ACTIVITÉS : Cocher toutes les possibilités en utilisant « x »

4. Nom de la personne qui a rempli le questionnaire: ________________
5. Date de remplissage du questionnaire: [__][__][__] [jj/mm/aa]
References


Hoff, N. A. Utilization assessment of infectious disease surveillance data to enhance methods for better understanding disease occurrence, trends and gaps in disease reporting in a resource limited setting: Monkeypox in the Democratic Republic of Congo, University of California, Los Angeles, (2014).


Ministère de la Sante de la République Démocratique du Congo. (ed Insititut National de la Statistique) (Ministère de la Sante de la République Démocratique du Congo Kinshasa, 2009).


network: Classes for Relational Data v. R package version 1.11.3 (Irvine, CA, 2014).


