Dr ANNE LAUDISOIT CIFOR/KISANGANI
CIFOR, Université de Kisangani, DRC
Antwerp University, BE
Liverpool University, UK
Mob : +243(0) 820 170 710

SCIENTIFIC REPORT

RESEARCH
BUSHMEAT AND MONKEYPOX

YAHUMA HEALTH ZONE – AKETI HEALTH ZONE - BOMBONGOLO HEALTH AREA
15 JANUARY 2016 - 29 JANUARY 2016

Participants
Dr Anne LAUDISOIT
CIFOR, Kisangani University, Kisangani, Democratic Republic of the Congo
EVOECO, University of Antwerp, Antwerp, Belgium
EEID, School of Biological Sciences, University of Liverpool, Liverpool, United Kingdom
Dr Innocent AKONDA, Ministry of Health, Democratic Republic of the Congo
Michel KOMBA, UNIKIS/CSB, Kisangani University, Democratic Republic of the Congo

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Cover picture : *Dr Innocent leaving the plot of a family who lost two young schoolgirls.*

ACRONYMS

BHC	Bombongolo Health Centre
CF	Congolese franc
COWV	Cowpox virus
CTB	Belgian Technical Cooperation
DRC	Democratic Republic of the Congo
HGR	General hospital of Reference (*Hopital Général de Référence*)
HMPX	Human Monkeypox
INRB	National Institute for Biomedical Research (in Kinshasa)
IT	Titular nurse (*infirmier titulaire*)
IUCN	International Union for Nature Conservation
MPX(V)	Monkeypox (virus)
MCZ	Chief Medical Doctor (*Médecin chef de zone*)
PEE	Program study and expertise (*Programme Etude et Expertise*)
RECO	Community network (Réseau du relais communautaire)
VACV	Vaccinia virus
VARV	Variola virus
VZV	Varicella-Zoster Virus
WHO	World Health Organisation

ABSTRACT

The research mission investigated an eruptive disease cluster in Aketi health zone and more specifically we tried to identify the index case of the outbreak in the Bombongolo health area, in Bogbengo locality. In Aketi town before conducting the outbreak investigation in Bogbengo, human monkeypox was suspected in the Itimbiri neighbourhood and x skin biopsies were taken on active suspected cases. In Bogbengon seven skin biopsies (vesicles, crusts) were collected from active cases. We also gathered the epidemiological data for suspect HMKX cases notified for the entire Aketi health zone from 2014 to 2016 as older data were not available. Biopsies were sent to the National Institute of Biomedical Research (INRB) to verify the nature of the outbreak (infection with either VZV or MKXV). On a total of 12 skin biopsies analysed, 2/5 were positive for VZV and 3/5 for MPX in Aketi town and 3/7 were positive for VZV and 4/7 for MPX in Bogbengo, illustrating once more i) the occurrence of a mixed outbreak, ii) the confusion between the eruptive skin diseases and iii) difficult diagnostic *in situ* and with undertrained health staff. By sampling local wildlife, we aimed also to understand the nature of this outbreak (Orthopoxvirus infection or something else), its origin (imported or locally acquired infection, and which hosts were involved) and potential risk factors. All samples were shipped to Belgium to the EVOECO laboratory (University of Antwerp) to be screened for OXP and MPX DNA presence and positive PCRs fragments have been sequenced and compared to known sequences of the virus.

A. CONTEXT

In 2013, the chief medical doctor (MM) of the Yahuma health zone in DRC reported 282 suspect cases of HMKX with a case fatality rate of 6%. Most of the cases originated from the Hembe health area that reported 29.4% of the total number of cases within the Yahuma health zone with a case fatality rate of 11.4% (Mussaw, internal report). In April 2014, a suspect HMKX geographic cluster was again reported from the Hembe health area to the Provincial Ministry of health (Mussaw, internal report).A first outbreak investigation had taken place in June 2014, and a multidisciplinary expedition planned since the area was extremely interesting both in terms of people (presence of Aka pygmies) and biodiversity. In this region, bushmeat is hunted mostly in the nearby Makila forest linked to the Lopori region in the neighbouring Equateur province. The nearby riverside village of Yafoli hosts a bushmeat market every Saturday; most bushmeat arrive fresh, due to the proximity to the hunting sites.

On 15th January 2016, a team of 22 people left on 3 dugouts Kisangani to reach Yafoli beach 17th January. Motorbikes were then rented in order to send the whole team to Likoso where the camp was going to be established. On the road we stopped by the Yafoli health centre. The nurse recognized me and said that he had two cases of the disease, Monkeypox right now in the neighbourhood. The team saw them, took pictures, and the nurse took biopsies the next day (later confirmed as HMPX by INRB). In Likoso, the welcome ceremony was perfect but in the evening a group of older men suggested they stay awake by the camps as young people and thieves are currently causing troubles in the area. The following day, 18th January, when we started to get ready with traps, camera traps, sticks, cages and buckets people suddenly started to shout at the chief of the village. A conflict started between 5 clans, there is no police, there is no respect of any authority and we got threatened with machetes, spears and bows. After 6 hours discussion, we finally manage to

convince them to let us work. We started choosing guides and helpers of each clan, including the young virulent people. When after 8h negotiations and explanations the first team left they didn't even go 100m in the forest that they had 5 men surrounding them with machetes, and bows. Prof Dudu Akaibe was threatened. « Blood will flow and head will roll if you step in our forest». Women were threatened and finally at 2pm we had to leave, pack again everything, find 10 motorbikes to drive us to Yafoli beach where we slept on the flooded Congo river bank. We managed to take the interviews and samples of the patients on the way back ; both are confirmed Monkeypox cases ; they had eaten a squirrel as last bushmeat. The 19th January, the team left upstream for Monbongo and we – 2 UNIKIS/CSB assistant, Kris Pannecoucke, a photographer and myself – rented a rowing dugout to go down to Bumba with the 2 motorbikes put on the boat (40km, 7h).

We then went from Bumba to Aketi (132km, 7h on the bikes) where a suspected Monkeypox outbreak was reported by the chief medical doctor (Dr Innocent Akonda) of the Aketi health zone and performed an outbreak investigation and bushmeat sampling.

The outbreak was notified to and posted on promedmail www.promedmail.org/post/4014697.

B. CALENDAR

DAY	PLACE
21/01/2016	Arrival in Aketi (by moto) Meeting with Dr Innocent AKONDA Planning of the activities and outbreak investigation
22/01/2016	Aketi HGR : visit and meeting with nurses and doctors of all the health areas Official donation of material from ROTARY grant and CTB grant Meeting and interview with MKX patients
23/01/2016	AS Ahaupa : focus groups and discussions about past epidemics Data given by Gilbert Bodipi (HN CS Ahaupa) Meeting with Akuma – Angengi population Bushmeat sampling
24/01/2016	Aketi river exploration Aketi bushmeat market : bushmeat sampling Aketi CS Itimbiri – case investigation of MKX patients in Aketi township
25/01/2016	Departure from Aketi to AS Bombongolo : current outbreak epicentre site Meeting with chiefs, authorities, RECO (*relais communautaire*) and nurses Summary of the patients list and admission dates Planning of the activities
26/01/2016	Outbreak and case investigation in Bogbengo : outbreak epicentre Identification of index case and contacts Random prevalence survey in the traget locality Bushmeat sampling
27/01/2016	Outbreak and case investigation in Bogbengo : outbreak epicentre Identification of index case and contacts Random prevalence survey in the target locality Bushmeat sampling Back to Aketi
28/01/2016	Debriefing and reporting
29/01/2016	Aketi – Kisangani (by moto)

Logisitics notes

In Aketi, the team slept at the sisters Covent, where the NGO ASBL Aketi in collaboration with Pf. Guy-Crispin Gembu (UNIKIS) recently installed solar panels and internet connection. In Bombongolo (78.7km from Aketi, 3h by moto), the team camped on the chief plot. There is a river nearby to swim and shower, no phone network but a solar panel to charge batteries. The final budget is annexed (Appendix 1).

C. CASES AND OUTBREAK INVESTIGATION

The samples and data are currently being analysed and will be submitted for publication in due time in a peer reviewed journal (Emerging infectious diseases or Ecolhealth).

INTRODUCTION

Poxviruses are double-stranded DNA viruses with large genomes (up to 300 kb) belonging to the family Poxviridae. Smallpox, is a Poxvirus, of the Orthopoxivurs genus, caused by the Variola virus (VARV) and was one of the most devastating diseases known in human history. It claimed the life of 1/10 children in the 18th century Europe and was still responsible for 50 million infections in the Western world in 1950. Smallpox was declared eradicated in 1980.Besides VARV, three other major members of the Orthopox genus have been found to infect humans where they cause smallpox-like rashes: Vaccinia virus (VACV), Monkeypox virus (MPXV) and cowpox virus (COWV). Monkeypox virus (MPXV) is endemic to western and central Africa causing largely unreported outbreaks – particularly the Democratic Republic of Congo (DRC) - of a smallpox-like illness, with development of a typical pustular rash involving the palms and soles. In DRC, HMPX is a reportable disease to the Integrated Disease Surveillance and Response (IDSR) unit (Hoff, 2015). Between 1980 and 2015, HMPX has been notified to the national bureau of the Ministry of health in 264/516 health zones of the country (Hoff, 2015). Two main genetic clades of MPX could be discerned: a Western clade (WC) from countries west of Nigeria and a Congo Basin (CB) clade from central Africa. MPX-CB has been shown to be more virulent in humans, allowing up to 6 consecutive inter-human transmission steps, whereas MPX-WB is likely milder with no inter-human transmission having been observed. (Likos et al, 2005, Nakazawa et al, 2015).

A major difference with the Variola virus - an anthroponose – is that the other three viruses have natural vertebrate reservoirs. Activities such as traditional trapping, hunting and bushmeat consumption, could be a major the risk of acquiring the infection (e.g. Wolfe et al.2005). The exact reservoir - or the reservoirs – of the virus in nature is still under discussion but as elaborated below MPX has previously been detected in rope squirrels (*Funisciurus* spp.), dormice (*Graphiurus sp.),* giant rats (*Cricetomys sp*.), elephant shrews (*Petrodromus sp*.) and to a lesser extent African primates (Kodakevich et al, 1987; Fuller et al, 2011; Reynolds et al, 2012).

The major problem in studying HMKX outbreaks in DRC (and elsewhere) are that they often occur in remote areas. This makes the differential diagnosis, the identification of the index case to track the origin of the outbreak and thus the source of infection, the description of the transmission chain (secondary cases or co-primary cases) difficult. In the absence of smallpox, the main clinical diagnostic problem is the differentiation of HMKX from chickenpox, Varicella-Zoster Virus (VZV) infection (Jezek et al, 1988). In DRC, there are however atypical VZV infections involving palm and sole manifestations in Monkeypox endemic areas, which make it more difficult yet to differentiate from Monkeypox diagnosis (MacNeil et al, 2009).

The research mission investigated an eruptive disease cluster in Aketi health zone and more specifically we tried to identify the index case of the outbreak in the Bombongolo health area, in Bogbengo locality. In Aketi town before conducting the outbreak investigation in Bogbengo, human Monkeypox was suspected in the Itimbiri neighbourhood and x skin biopsies were taken on active suspected cases. In Bogbengo seven skin biopsies (vesicles, crusts) were collected from active cases. We also gathered the epidemiological data for suspect HMKX cases notified for the entire Aketi health zone from 2014 to 2016 as older data were not available.

MATERIAL AND METHODS

Study area : Aketi Health Zone and Bombongolo

The outbreak area is located within the Aketi Health Zone (16.230 km² ; Fig.1), in the Bas Uele Province, in the Democratic Republic of the Congo. The Bombongolo Health Area (BHA) which reported most cases and at the origin of the outbreak consists of 8 localities with the Mobati tribe representing 95% of the total population of the health zone (Table 1).

Division	Site	Population (2016, health division data; hab.)
Health Zone	Aketi Health Zone	149 215
Health Area	Bombongolo Heath Area	7921
Locality	Bogbengo	1445
	Bombongonzele	1304
	Bombongolo	739
	Bodadua	376
	Bodima	718
	Bolela	2178
	Bongobe	528
	Bozamba	633

Table 1. Population data of the Aketi Health Zone and Bombongolo Health Area.

Figure 1. Local map of the Aketi Health Zone (SOURCE : Aketi HGR).

The historical HMPX data of the Aketi HZ could not be found or are fragmentary. But from 2012, the Aketi HZ MCZ managed to identify 111 cases of rash illness classified as epidemic MKX (suspect; 3 deaths) in 2013, 9 cases (0 deaths) in 2014 35 cases (1 death), in 2015, 135 cases (7 deaths). The Aketi HZ reported nearly 87% of the total HMKX suspected cases of the Bas Uele province as per 4th March 2016. The Aketi HZ recorded 107 cases including 7 deaths, 3 deaths having occurred in the same family. The 107 cases in 2016 are not all cases of HMKX; it was confirmed by our pilot mission results (see RESULTS) that a mixed episode of HMPX and chicken pox (VZV) was occurring.

Since 2006, the Aketi HZ has been witnessing a dramatic geographic expansion of "eruptive disease clusters" classified as suspected HMPX along two main axis The fist axis runs from Aketi to the left bank of the river Itimbiri to Makoko and Akuma localities where hunters come to a weekly (Saturday) bushmeat market from the neighbouring ex-Equateur province and 2) right bank of the river, from Aketi to Bombongolo and Aboso localities which connect the Bas Uele province to Central African Republic with a dense rainforest. These details show the intensity and the risk associated with possible exchanges both commercially and in terms of pathogenic strains.

Data collection

Focus group, interviews and prevalence study

We proceeded to informal focus group interviews, gathering medical staff, hunters and villagers in Aketi HGR, Ahaupa health center and Bombongolo (chief plot), addressing questions about the symptoms, the potential seasonality and perceptions of the sources of contamination. A prospective prevalence study was performed in Bogbengo locality, Bombongolo health area, Aketi Health zone , Bas Uele province, DRCongo. between 26th and 27st January 2016 in 34 households using a newly designed question form (Appendix 2).

Starting from the house of the presumed index case, every three house was visited. They presence of one of the parents or head of the household was chosen as a selection criterion. If the person was not present the household was geolocalized (GPS Garmin 60cx) and visited later during the day. We gathered data on family structure, age and sex, and for the active cases the potential source of infection. We used the "suspected case" definition of the National Ministry of Health guidelines for Monkeypox surveillance; namely "a patient presenting with sudden onset of high fever, followed - after a few days - with a vesiculopustular rash predominantly on the face, palms of hands, and soles of feet, or the presence of at least 5 smallpox-like scars". We calculated a suspect HMPX score corresponding to the addition of a series of 7 symptoms. Three relatively specific symptoms (0=absent and 1=present) and 4 unspecific symptoms (0=absent and 0.5=present). Unspecific symptoms were high fever (as we get no reliable answers as for the situation before the onset of the disease), lymphadenitis, general vesiculopustular rash (as this can be mistaken for VZV or other eruptive disease), and ocular lesions. The specific symptoms were a rash on palms of hands, soles of feet, and the presence of at least 5 smallpox-like scars. The highest score is then 6 and lowest set to 1 with at least one eruption sign or 2 unspecific symptoms. One problem is the rare observation of a lymphadenitis as people do not notice it unless very severe; this resulted in missing data.

Animal sampling and human samples

Freshly killed animals were sampled as hunters were returning from the forest to sell their bushmeat, and at the Aketi bushmeat market. Tissues samples (vital organs biopsies, muscle and/or tongue) in 85% ethanol and shipped to the University of Antwerp, Belgium. Geographical coordinates of the place where bushmeat was sampled were recorded and the origin and mean of trapping the animal asked to the seller or hunter.

In Aketi market, when authorized by the seller, the weight (gr/kg) of the piece of meat or whole carcass was also recorded and the retail price noted. A limited number (N=12) of human biopsies (crusts or vesicles) collected from suspected cases were sent to INRB in Kinshasa for diagnostic confirmation and were later shipped to the University of Antwerp for sequencing. Patients were given antibiotics at the Itimbiri health centre to treat and prevent super infections of the lesions.

DNA extraction and wildlife species identification

DNA was extracted from animal samples (vital organs, throat swabs or tongue depending on the circumstances – capture or fresh bushmeat) and human biopsies using a NucleoSpin tissue kit (Machery Nagel, Düren, Germany) according to the manufacturer's instruction.

For each bushmeat sample, DNA was extracted to confirm the host species identity using mitochondrial DNA cytochrome *b* sequencing using L7 and H6 primers (Montgelard et al., 2002). The sequences obtained were compared with known sequences in GenBank and the *AfricanRodentia* database (http://projects.biodiversity.be/africanrodentia/). All new sequences will be depositd in Genbank.

Orthopoxvirus detection and sequencing

An in house *Orthopoxvirus*-specific (OXPV) real-time PCR assay and a VZV was performed for diagnostic confirmation on the human samples at the INRB in Kinshasa as recommended in the national guidelines for disease surveillance.

A pan-OPXV nested PCR targeting the FP gene (Chantrey et al., 1999) were performed at the University of Antwerp (Belgium). The fragments were purified and sequenced in one direction using the FP3 primers. Sequences were compared to Genbank reference sequences and aligned in MEGA6. All new sequences will be deposited in Genbank.

Analysis are ongoing but the first results are given in the appropriate sections.

PRELIMINARY RESULTS

Molecular diagnostic and general sequencing preliminary results

Before presenting the outbreak in detail and the families involved, we present the molecular screening results of skin biopsies from Aketi town and the Bombongolo outbreak area that were performed at INRB. In total, 5 samples from Aketi town and 7 samples from Bombongolo outbreak area were analysed. The results are summarized in the table 2 below.

HOUSEHOLD CODE	AS	Locality	Sex	Age	MPX score	INRB	STATUS
PAKE001	ITIMIBIRI	AKETI	F	17	2	VZV	active
EBA	BOMBONGOLO	BOGBENGO	M	2.8	2.5	VZV	active
DIK	BOMBONGOLO	BOGBENGO	M	12	4	VZV	C
MAN	BOMBONGOLO	BOGBENGO	M	21	4	VZV	C
PAKE002	ITIMIBIRI	AKETI	M	6	4	VZV	C
PAKE002	ITIMIBIRI	AKETI	F	3.5	4	MKX	active
PAKE002	ITIMIBIRI	AKETI	M	13.5	4	MKX	active
PAKE002	ITIMIBIRI	AKETI	M	14	4	MKX	active
MAG	BOMBONGOLO	BODUMA	M	12.5	4.5	MKX	active
MAZ	BOMBONGOLO	BOGBENGO	F	9	4.5	MKX	active
ZED	BOMBONGOLO	BOGBENGO	F	22	4.5	MKX	C
ZED	BOMBONGOLO	BOGBENGO	M	5	4.5	MKX	active

Table2. General results of the molecular screening perfomed at INRB to confirm either a VZV or MPX infection. C:crusts. Dm: missing data.

It reveals that once again , there was a mixed outbreak going on. Five patients were diagnosed with chickenpox (VZV) and 7 confirmed with human Monkeypox ; with one family in Aketi presenting with both diseases in the same household. Interestingly, despite the small number of records for which both biopsies and the score of HMPX definition were obtained, no HMPX case got a score below 4. In Bombongolo, all MPX cases got a score strictly higher than 4. The differential diagnosis is problematic in the field. Indeed, despite some specific symptoms, there are reports of VZV infection causing lesions on the soles of feet or palms of hands in the DRC as illustrated by one patient (PAKE001, F, 17y.o. Fig. 2) while the neighbour had a super infection of his MPX lesions (PAKE002, M, 13.5y.o.).

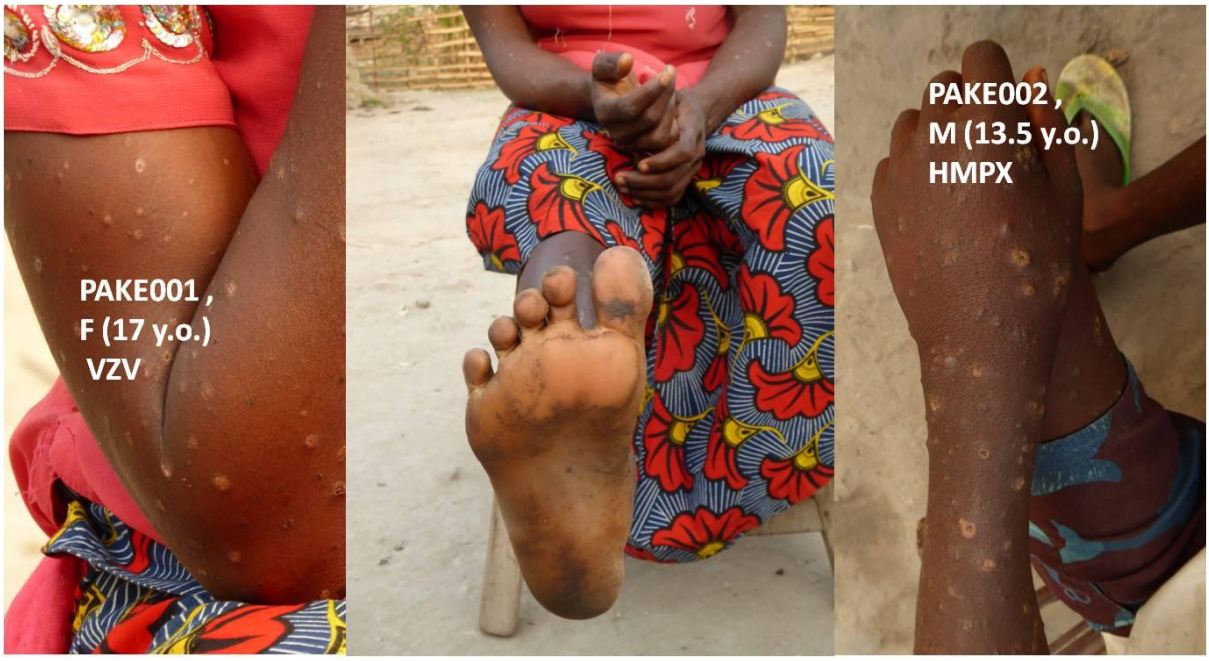

Figure 2. Chicken pox lesions in patient PAKE001 with flat lesions on the sole of feet and super infected MPX lesions in patient PAKE002.

Preliminary genetic analysis of MPX virus DNA sequenced (167bp -

ACGCGAGCATTGTTAAGCCTATGGAGACGACAATGAGGAAACTCTCAAACAACG

GCTAACTAATTTGGAAAAAAAGATTACTAATATAACAACAAAGTTTGAACAAAT

AGAAAAGTGTTGTAAACACAACGATGAAGTTCTATTTAGGTTGGAAAATCACGC

TGAA) in patients show 98% homology with a strain (Genbank accession Number : gi|819325161|KP849471.1) detected in human patients in Yambuku in 1985 (Nakazawa et al, 2015). Yambuku is located at 130km from Aketi and is also the place where Ebola virus emerged in 1976. A total of 35 bushmeat samples were screened (PCR) and sequenced; 19 (54.2%) of 10 different species (6 primate, 4 carnivore, 4 rodent and 5 artiodactyle species) were positive for MPX DNA.

All human sequences were 100% identical to each other and to the wildlife sequences (see below) and belong to the CB clade of the MPX virus.

Mission day-by-day

22nd January : Meeting

The team organized meeting with the Chief Medical Doctor (MCZ) and the 22 titular nurses (IT) of the Aketi Health Zone at the Aketi General hospital of Reference (HGR). The objective of the meeting was to gather information on the history of Monkeypox emergence in the area and decide in which health area to direct the outbreak investigation. Equipment and material (microscope and slides[1], computer and printer) were also officially handed to Aketi HZ. Several patients were also visited and interviewed

22nd January : Bushmeat sampling in Aketi market

In the afternoon, the team headed to Aketi city market to collect bushmeat samples. Seven samples, were collected and three (42.8%) were positive for MPX virus in 3 different species (chimpanzee, duiker and red-tailed monkey ; Table 3).

Species lab. ID	IUCN	Origin	TECH. HUNTING	Processing	US$/kg	MKX+
Monkey sp1	not ID	Angu	gun (C12)	SMOKED	2.0	not tested
Cercopithecus mona denti	LC	Ibembo	gun (C12)	SMOKED	5.0	0
Pan troglodytes schweinfurthii	EN	Ibembo	gun (C12)	SMOKED	4.0	1
Cephalophus silvicultor	LC	dm	Trad.trap	SMOKED	4.3	1
Cercopithecus ascanius schmidti	LC	Bongolu	gun (C12)	FRESH	5.0	1
Pan troglodytes schweinfurthii	EN	Angu	gun (C12)	SMOKED	6.9	0
Cercopithecus mitis doggetti	LC	Akuma	gun (C12)	FRESH	1.9	0

Table 3. Bushmeat samples taken in Aketi market, 22nd January 2016. Legend: gun(C12):rifle ; Trad. trap: traditional trap ; IUCN: red list status: LC:least concern, EN:endangered. The price per kilogram is calculated with an exchange rate US$ and CF = 920.

All sequences were identical and related to the same strain (Genbank accession Number : gi|819325161|KP849471.1) as above. Two chimpanzees samples were present in our sample and were obviously from 2 different individuals as one was positive for MPX and the other wasn't. Rifles are the most common weapon.

[1] Donated by the ROTARY club Seneffe matching grant, Belgium and the EPIRIVE/PEE-CTB grants respectively.

23rd January 2016 : The under-notification of the human cases of Monkeypox

Ahaupa Health Area where the first case of human monkeypox was notified in 2006.

The team headed to the left bank of the Itimbiri rivers to collect data and ideally bushmeat samples. The table 1 shows the data given and centralized by the head nurse of Ahaupa health centre, Mr Gilbert Bodipi. In 2015, in Aboso an outbreak of MKX was investigated by the head doctor of the Aketi health zone (AHZ) as cases were notified to him (Table 4).

Due to distances between remote forest villages, the impossibility to reach them with cars, and the poverty of the populations, most of the people do not reach the official circuit and are hence unnoticed. In total, 30 cases have been notified in 2 years in that health area, with 9 localities involved. Interestingly, reports of ocular lesions – usually reported in 5% - seem to be common with the local MPX strain (Fig. 3, A and B).

YEAR	MONTH	LOCALITY	M 0-11 months	F 0-11 months	M 12 - 59 months	F 12 - 59 months	M 5 - 15 years	F 5 - 15 years	M 15 years +	F 15 years +	TOTAL CASES
2014	JANUARY	ANDEA	0	0	0	0	0	0	1	0	1
2014	FEBRUARY	AHAUPA, GBALA	0	0	0	0	0	0	1	1	2
2014	MARCH	na	0	0	0	0	0	0	0	0	0
2014	APRIL	UNKNOWN	0	0	0	0	0	0	0	1	1
2014	MAY	na	0	0	0	0	0	0	0	0	0
2014	JUNE	na	0	0	0	0	0	0	0	0	0
2014	JULY	AHAUPA, MAKAN	0	0	1	1	0	0	0	0	2
2014	AUGUST	na	0	0	0	0	0	0	0	0	0
2014	SEPTEMBER	GBALA	1	0	0	0	0	0	0	0	1
2014	OCTOBER	na	0	0	0	0	0	0	0	0	0
2014	NOVEMBER	na	0	0	0	0	0	0	0	0	0
2014	DECEMBER	AHAUPA	0	0	0	0	0	0	0	1	1
2015	JANUARY	GBALA	0	0	0	0	0	0	1	1	2
2015	FEBRUARY	UNKNOWN	0	0	0	0	0	0	1	2	3
2015	MARCH	UNKNOWN	0	0	0	0	0	0	1	1	2
2015	APRIL	MAKAN, BOSAYO 2	0	0	0	0	2	1	0	0	3
2015	MAY	na	0	0	0	0	0	0	0	0	0
2015	JUNE	BOKPOTA, GBALA, AKUMA	0	0	2	1	0	0	1	0	4
2015	JULY	AHAUPA, GBALA, AMOKO	0	0	1	0	1	1	0	1	4
2015	AUGUST	na	0	0	0	0	0	0	0	0	0
2015	SEPTEMBER	UNKNOWN	0	0	0	0	1	1	0	0	2
2015	OCTOBER	ABOSO*, BONBANZO	0	0	1	0	1	0	0	0	2
2015	NOVEMBER	na	0	0	0	0	0	0	0	0	0
2015	DECEMBER	na	0	0	0	0	0	0	0	0	0
	2 YEARS	**9 LOCALITIES**	**1**	**0**	**5**	**2**	**5**	**3**	**6**	**8**	**30**

Table 4. HMKX cases notified to the health centre of Ahaupa in 2014 and 2015.

The IT informed us that every Saturday a bushmeat market is held in Akuma locality, 12km inside the forest linking it to the Equateur province and Lopori area. We headed to the site where hunters returning from the market were coming out of the forest to go back to Aketi (30km) and collected fresh bushmeat samples (Fig. 4). Bushmeat samples collected along this axis are diverse and mostly fresh which also shows the species abundance and diversity in this unprotected area of DRC in the vicinity of Aketi.

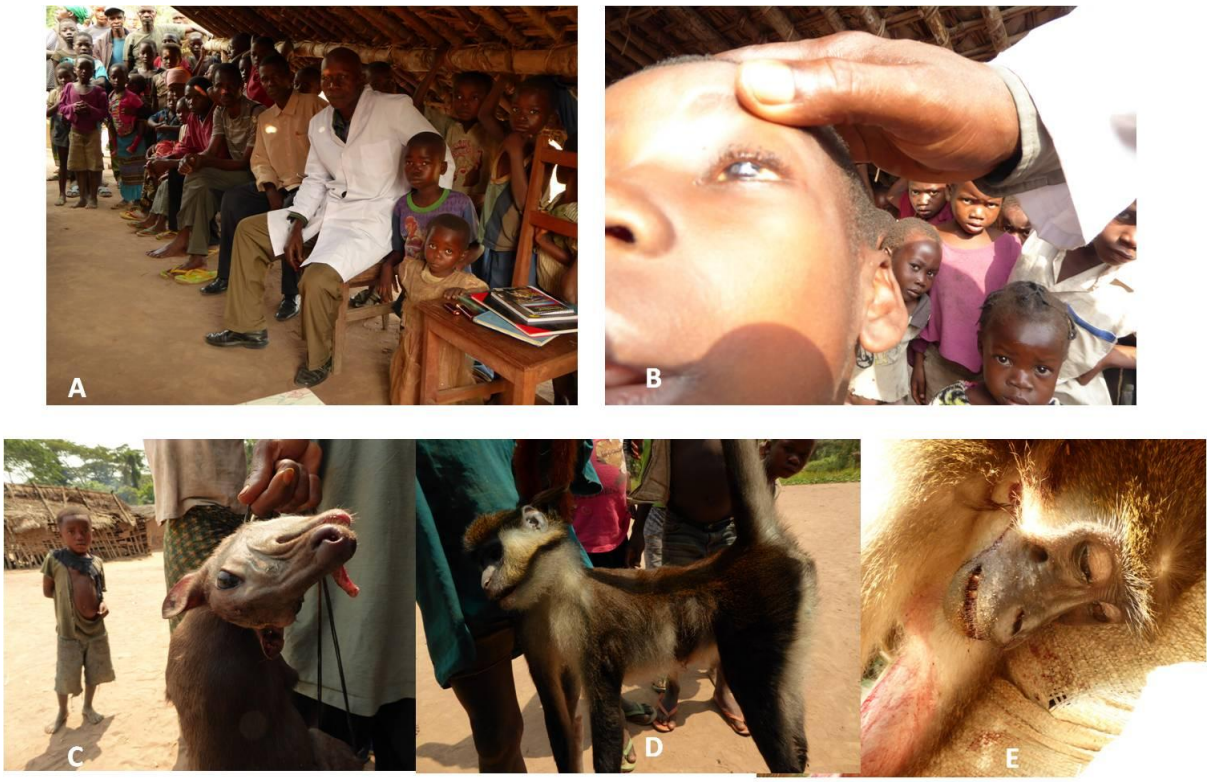

Figure 4. A : Focus group at Akuma health center ; B. Occular lesion caused by MPX in Akuma ; C,D,E some fresh bushmeat sampled, namely C. Duiker (*Cephalophus monticola*) ; D. Monkey (*Cercopithecus ascanius schmidti*) and E: *Cercocebus* sp.

In total, we were granted to sample 6 bushmeat samples, all as either fresh or smoked whole carcass; 4 (66.7%) of them belonging to 3 species (primates and duiker) were positive for MPX virus (DNA evidence) (Table 5).

Species lab. ID	IUCN	Origin	TECH. HUNTING	Processing	US$/kg	MKX+
Cercocebus sp.	dm	Logu Forest - Akuma	gun (C12)	FRESH	na	0
Cercopithecus ascanius schimti	LC	Logu Forest - Akuma	gun (C12)	FRESH	1.8	1
Cephalophus monticola	LC	Logu Forest - Akuma	PT	FRESH	1.1	1
Cercopithecus ascanius schimti	LC	Logu Forest - Akuma	gun (C12)	FRESH	2.2	1
Cercopithecus sp.	dm	Logu Forest - Akuma	gun (C12)	SMOKED	2.2	1
Cercopithecus sp.	dm	Logu Forest - Akuma	gun (C12)	SMOKED	2.5	0

Table 5. Characteristics of the bushmeat sampled along the axis Aketi-Akuma. Legend: gun(C12):rifle ; Trad. trap: traditional trap ; IUCN: red list status: LC:least concern, EN:endangered.

All sequences were identical and related to the same strain (Genbank accession Number : gi|819325161|KP849471.1) as above.

All bushmeat was reported to have been hunted in the Logu Forest. It is likely that the smoked carcass were either hunted further in the forest or earlier during the week.

24th January 2016 : Aketi bushmeat market visit

In the afternoon, the team headed to Aketi city market to collect more bushmeat samples (Fig. 5). Eleven samples, mostly from the Akuma area (Logu forest), left bank of the itimbiri river were collected among which 3 chimpanzees specimen reported to be from 3 sellers (not a same carcass) (Table 6).

Species lab. ID	IUCN	Origin	TECH. HUNTING	Processing	US$/kg	MKX+
Pan troglodytes schweinfurthii	EN	unknown	gun (C12)	SMOKED	5.7	0
Cephalophus dorsalis	LC	unknown	gun (C12)	FLAMED	4.7	1
Potamocherus porcus	LC	unknown	gun (C12)	SMOKED	5.4	1
Cercopithecus nictitans nictitans	LC	unknown	gun (C12)	FLAMED	2.5	0
Pan troglodytes schweinfurthii	EN	Akuma	gun (C12)	SMOKED	2.8	0
Pan troglodytes schweinfurthii	EN	Akuma	gun (C12)	SMOKED	0.2	0
Potamocherus porcus	LC	Akuma	gun (C12)	SMOKED	3.1	0
Cephalophus dorsalis	LC	Bodumbe	gun (C12)	SMOKED	3.8	0
Cephalophus silvicultor	LC	Akuma	unknown	SMOKED	5.5	1
Cephalophus dorsalis	dm	Akuma	unknown	SMOKED	3.5	nt
Atherurus africanus	LC	Akuma	PT	SMOKED	4.5	1

Table 6. Characteristics of the bushmeat sampled along the axis Aketi-Akuma. Legend: gun(C12):rifle ; Trad. trap: traditional trap ; IUCN: red list status: LC:least concern, EN:endangered.

Four samples (36.3%) were positive for MPX virus, two duiker species, a bushpig and an African porcupine (Fig. 5). Sequences were as above.

Figure 5. Aketi bushmeat market with chimpanzee, bushpig, colobes and porcupine on sale.

Case investigation in Itimibri neighbourhood, Aketi town

While working in the market, we noticed a woman covered with active vesicular lesions shopping with her kid on the back. We asked her if she knew what she was suffering from. She replied she didn't know but that she was not the only case in her neighbourhood , the Itimbiri quarter of town. We visited her in the evening for an interview and enquire about those "other cases" and take skin biopsies. We found 3 households with infected people, and recorded a total of 11 suspect HMPX cases (out of 33 people living within the house of the cases) with lesions; 5 were sampled (Table 7). The three household heads reported that the young girl of PAKE002 (in bold in table 7) was the first case.

HOUSEHOLD CODE	Sex	Age	DATE SYMPTOMS STARTED	VACCINIA SCAR	BIOPSY	INRB
ITI-001	F	39	17/01/2016	1	0	
ITI-001	M	3	19/01/2016	0	0	
PAKE002	F	35.5	21/01/2016	0	1	
PAKE002	M	14	19/01/2016	0	1	
PAKE002	M	6	18/01/2016	0	1	VZV
PAKE002	**F**	**3.5**	**2/01/2016**	**0**	**1**	**MKX**
PAKE002	F	1	22/01/2016	0	1	
PAKE002	M	13.5	Dm	0	0	MKX
PAKE002	M	14	Dm	0	0	MKX
PAKE002	M	13	19/01/2016	0	1	
PAKE001	F	17	10/01/2016	0	1	VZV

Table 7. Patient involved in the Aketi suspect HMPX cluster and results of INRB skin biopsies analysis. Dm: missing data ; could not remember.

The mother confirmed. Her daughter spent Christmas and New Year in a temporary settlement near a crop field in Bodumbe locality with her uncle. They ate bushpig *Potamocherus larvatus* and duiker (*Cephalophus* sp.) ; this is apparently the last meat meal they had before she fell sick. The mother came back with her sick daughter from Bodumbe (see §Follow up of the outbreak) to Aketi. People in the house ITI-001 and PAKE001 had contact with her and other family members (PAKE002) as well. However, biopsies were not taken on all cases and as the molecular diagnostic results show, 2/5 patients had chicken pox. Two PAKE002 family members got infected between the 2nd and 24th January; this is consistent with the known HMPX incubation period of 2 to 21 days.

25t-27th January 2016 : Outbreak investigation in Bombongolo

Monkeypox history in the health area of Bombongolo

In 2006, an eruptive disease outbreak struck the Bombongolo health area, this was the first recorded HMPX outbreak in the Aketi HZ. The archives of the Bombongolo health centre for 2014 and 2015 were handed over by the IT and classified ; some data for 2012 and 2013 were also found. Table 8 presents the cases by locality, age, sex and date of admission when available.

YEAR	EPIWEEK	MONTH	LOCALITY	CASES 0-11 months	DEATH 0-11 months	CASES 12 - 59 months	DEATHS 12 - 59 months	CASES 5 years +	DEATHS 5 years +	TOTAL CASES	TOTAL DEATHS	MORTALITY	NOTES
2012	33		BOGBENGO	0	0	0	0	6	0	6	0	0%	
2012	34		BOGBENGO	0	0	2	0	0	0	2	0	0%	
2012	35		BOGBENGO	0	0	1	0	3	0	4	0	0%	
2012	36	september	BODUMA	0	0	0	0	1	0	1	0	0%	1
2012	36	september	BOGBENGO	0	0	4	0	0	0	4	0	0%	
2013	26		BOGBENGO	0	0	1	0	2	0	3	0	0%	
2013	30		BOGBENGO	0	0	3	0	1	0	4	0	0%	
2013	30		BOBOLONGO	0	0	0	0	1	0	1	0	0%	F
2013	31		BONGOBE	1	0	0	0	0	0	1	0	0%	F
2013	31		BOGBENGO	0	0	5	0	1	0	6	0	0%	
2013	32		BOGBENGO	0	0	3	0	2	0	5	0	0%	
2014	16		BOGBENGO	0	0	1	1	0	0	1	1	100%	
2014	21		BOGBENGO	0	0	0	0	1	0	1	0	0%	2
2015	49	december	BOGBENGO	0	0	6	0	1	0	7	0	0%	
2015	51	december	BOGBENGO	0	0	0	0	2	0	2	0	0%	
2015	52	december	BOGBENGO	0	0	3	0	2	2	5	2	40%	3
2015	53	december	BOGBENGO	0	0	2	0	0	0	2	0	0%	
2016	1	january	BOGBENGO	0	0	1	0	2	0	3	0	0%	
2016	2	january	BOGBENGO	0	0	1	0	3	1	4	1	25%	4
2016	3	january	BOGBENGO	0	0	3	0	5	0	8	0	0%	

1 *a 10 years old F (she is 14 now in 2016) - became totally blind - we found her (see details in report)*

2 *Adult*

3 *two young schoolgirls, neighbours (around 13 years old)*

4 *young 7 years old girl , sister of one of the girl that died in week 52 of 2015*

Table 8: Data found in the archives of Bombongolo health centre, 25th January 2016

We held a focus group with the elders, the RECO, the chiefs and nurses of the locality Bodadua. They claim that the 1st time HMKX appeared was in 2006 in Bodadua locality , with several cases (how many ?). Cases were scatterded and seem to have been occuring since 2006 but not under the form of epidemics ; those data are lost/destroyed since there is no real possibility to preserve documents in the health center as it is right now (Fig. 6).

The majority of the people are of the Mobati tribe, they call such eruptive disease « magbélé ». The second outbreak, the current outbreak, is said to have started in 2015 , during the 49th epiweek in Bogbengo locality[2] The data are available but are clearly showing a huge underreporting as people do not reach the official health circuit but rather see pastors, church notables or priests, non accredited doctors or private nurses (see results of the outbreak) who provide either local , traditional treatment or antibiotics (amoxycilline, ampicilline or chloramphenicol sold by a pastor pharmacian of the village).

Figure 6. Health center of Bombongolo.

[2] Each epiweek = week of the year starting with first week as 1, months of 31 days have 5 weeks others have 4

Outbreak area : Bombongolo health area, Bogbengo locality as the epicentre

Bogbengo is a locality of around 1285 persons and 183 households often grouped by groups of 2 or 3 houses with family bounds on a same plot. A prospective prevalence study was performed in Bogbengo locality, Bombongolo health area, Aketi Health zone, Bas Uélé province, DRCongo, between 26th and 27st January 2016 in 34 household surveyed collected 221 individual data (6.5±3.8 person/household) on current and past history of an eruptive disease that was told to be a MPX infection by local health staff. During this survey, 14 cases of the current epidemics were censed making a prevalence of 6.3% , and 8 from past outbreaks (1 in 2011, and 7 in 2006) of a « MPX-like » eruptive disease - as no laboratory confirmation was received (no access to MSF data) – making a past prevalence of 3.6%, Finally, addressing the level of herd immunity to Poxvirus infection, we noted whether or not people had the Vaccinia scar (interrupted in 1980) and reveal than only a quarter (25.4%) may still benefit from this protection. After a quick analysis of the Bombongolo health centre data, the focus group and interviews with two members of the RECO (Bombongolo and Bogbengo), we finally identified 17 families where cases had been either notified or noticed between the end of November (no precise date could be established) and the 27th January.

History of the outbreak: people perception of the outbreak

We present in details a selection of the families, the suspect index case family (ATU) and 2 families that at have lost children (ZED and ABO) and two more families whose interviews brought some more light on the outbreak. From there, we will try to reconstruct the sequence and chain of transmission. Models aiming at understanding the transmission chain, the basic reproduction number (R_0=number of sick people generated by a single case), using date of symptoms onset, and incubation period data as proxies are currently being tested. The dates of the admission in the health centre are considered reliable, more reliable than the recalling of the people. Indeed, only the mother of one girl who died knew the day of the death of her kid not the date; the father ZED who lost 2 girls didn't know when they died.

The full names of the people are not given in this report only the 3 first letters of their family names.

INDEX CASE ? Interview with the ATU family (N infected / N people = 3/5 ; 0 death)

THE ATU FAMILY – Table 9 – BOGBENGO locality

The son of the ATU family, MOD. (6 y.o. – in bold in Table 9) was apparently the index case and was admitted to Bombongolo Health Centre for a week. He recovered. According to both neighbours, the ZED and the ABO Families, the first kid who got sick is MOD but he is not the first case appearing in the 2015 epidemiological records of the Bombongolo health centre.

Sex	Age	MPX Epidemics 2015	Adm. CSB	DATE SYMPTOMS STARTED	MPX score	INRB	STATUS
M	**6**	**1**	**7/12/2015**	**1/12/2015**	**4.5**	**NT**	**R**
M	2.5	1	NO CS	21/12/2015	3	NT	R
F	36	1	NO CS	28/12/2015	3	NT	R
M	4	0	na	na	0	na	na
M	48	0	na	na	0	na	na

Table 9. Description of the ATU family. Adm. CSB: admission date at Bombongolo health centre.*Legend: NO CS: did not report to the health centre ; INRB : diagnostic result of INRB (if collected), NT: not collected, and. D: dead and R: recovered.na: not applicable.*

The mother confirms that her son MOD (6 y.o.) was the first one to present with a general skin eruption in Bogbengo. MOD was admitted in the Bombongolo Health Centre (BHC) on 7th December and stayed in until 14th December 2015. He recovered. With a score of 4.5, and one week in the BHC, one can suspect HMPX but no biopsies were taken when he got sick. The ATU father used to hunt in a radius of 30-50km from their plot in Bogbengo. In the one month period preceding the infection of his kid he went hunting duikers (*Cephalophus* sp.) and giant rats (*Cricetomys* sp.) ; both father and son recall that the son (suspected index case) played with the heads of both animals while cutting them in pieces. The father and the son usually prepare the big chunks and the mother cooks the meat. They all have had contacts with the animals hunted and with raw palm nuts collected in the surrounding forest.

The ATU family moved to Bonzeli locality after their son recovered, and after the outbreak started but they don't remember the date , it was before the 1st January 2016.

In the new house located in Bonzeli, the son of ATU's sister got infected around 2nd January 2016. They had visited the family before then while MOD when she was already sick. Despite MOD's recovery, they did not attend the official BHC when she and her youngest son got sick.

Interview with chief ZED (N infected / N people =12/12 ; 2 deaths)

THE ZED FAMILY – Table 10 – BOGBENGO locality

The ZED family is the family most stricken by the outbreak as all the members of the ZED plot in Bogbengo got sick and 2 of chief ZED's girls died. At first we suspected the outbreak started with them and it can still be the case as the dates of the onset of the symptoms – knowing the 2 – 21 days incubation time -are not properly remembered by family members and were recorded by the Community health network (RECO), the whole ZED family moved to Boduma where the oldest brother'family (MAG) of chief ZED lives. Transmission to MAG family occurred (see MAG family below).

Sex	Age	MPX Epidemics 2015	Adm. CSB	DATE SYMPTOMS STARTED	MPX score	INRB	STATUS	Date DEATH
F	2	1	30/12/2015	21/12/2015	4.5	NT	R	na
M	33	1	21/12/2015	21/12/2015	4.5	NT	R	na
F	1	1	6/01/2016	23/12/2015	5	NT	active	na
F	22	1	23/12/2015	23/12/2015	4.5	MKX	R	na
F	8	1	28/12/2015	28/12/2015	4.5	NT	D	18/01/2016
M	3	1	3/12/2015	na	4.5	NT	R	na
M	5	1	21/12/2015	UNK	4.5	MKX	active	na
F	3	1	21/12/2015	UNK	4.5	NT	R	na
F	25	1	21/12/2015	UNK	4.5	NT	R	na
F	60	1	3/12/2015	UNK	5	NT	R	na
F	19	1	22/01/2016	UNK	4.5	NT	R	na
F	15	1	14/12/2015	UNK	5	NT	D	24/12/2015

Table 10. Description of the ZED family. Adm. CSB: admission date at Bombongolo health centre. *Legend: NO CS: did not report to the health centre ; INRB : diagnostic result of INRB (if collected), NT: not collected, and. D: dead and R: recovered.na: not applicable.*

According to chief ZED, the outbreak started within the ATU family between the end of November and the beginning of December 2015. The chief ZED and the mother of ABO (see below ABO family) also claimed this fact, namely that the first «*person with an eruption on the whole body* » was the son of their next door neighbour, MOD, of the ATU family. The plot of the ATU family is located next – 10m - from the plot of the ZED family. They are next-door neighbours, and the kids played together. The ATU have moved to another locality once their boy MOD (suspected index case) had recovered[3].

However, the first patients appearing in BHC epidemiological records are the 2 sick girls of chief ZED as he consulted earlier the nurse with his sick children (3rd December 2015 – while MOD was admitted 7th December). Chief ZED claims he went to the BHC because he saw how sick the next door neighbour MOD was. ATU family delayed the visit but MOD recovered while chief ZED attended the BHC earlier but both of his girls died. Chief ZED thinks they must have moved there around 17th December 2015. A week later ELA (F, 24 y.o.) one of ZED wives, got sick , then the grandmother NKL (F, 60 y.o.). The chief himself got sick on 21st December 2015 and the whole family followed (difficult for him to remember the dates) . NKA died on Christmas day 24th December 2015. A second girl of the family, the daughter of chief ZED, NSA (F, 8 y.o.) died around 18th January 2016.

The two confirmed MPX cases in the family, ocular lesions, severe super infection (Fig. 7), and a MPX definition score for all the members between 4.5 and 5, this family cluster (without the brother family yet) can be associated to a virulent MPX virus strain. Thorough genetic analyses are required to investigate whether the virulence or a weakened immunity resulted in such a severe outcome.

[3] We found them in Bonzeli village and collected their testimony.

Figure 7. Lesions of several family members of chief ZED.

Chief ZED's main occupation is hunting , he hunts everyday in the surrounding forests. The majority of the bushmeat hunted is made of duikers and monkeys. The whole family consumes bushmeat, mashed bananas, cassava leaves with homemade palm nut oil on a daily basis. The palm nuts are collected in the forest as the trees (*Elaeis guineensis*) grow anarchically everywhere spread by squirrels and other animals according to him. They have no palm tree plantation as such as those are available in the surrounding forested areas.

The MAG family (N infected / N people = 4/11 ; 0(1*) death)

THE MAG FAMILY – Table 11 – BODUMA locality

The MAG family was the second family with severe cases. MaAG is the older brother of chief ZED. When the first girl of ZED fell sick, the whole family of ZED moved to his brother's plot in Boduma and resulted in the infection of several members , two of which were in a life threatening condition when we left.

Sex	Age	MKX Epidemics 2015	Adm. CSB	DATE SYMPTOMS STARTED	VACCINIA SCAR	BIOPSY	MPX score	INRB	STATUS
F	7	1	1/01/2016	1/01/2016	0	0	4.5	NT	R
M	**12.5**	**1**	**2/01/2016**	**2/01/2016**	**0**	**2**	**4.5**	**MPX**	**active**
F	60	1	12/01/2016	5/01/2016	1	0	4	NT	na
F	1.7	1	27/01/2016	23/01/2016	0	0	4	NT	active
F	5	1	dm	dm	0	0	1.5	NT	R
F	9	0	na	na	0	na	0	na	na
M	9	0	na	na	0	na	0	na	na
F	12	0	na	na	0	na	0	na	na
F	40	0	na	na	0	na	0	na	na
F	44	0	na	na	1	na	0	na	na
M	51	0	na	na	1	na	0	na	na

Table 11. Description of the ZED family. Adm. CSB: admission date at Bombongolo health centre. *Legend: NO CS: did not report to the health centre; INRB : diagnostic result of INRB (if collected), NT: not collected, and. D: dead and R: recovered.na: not applicable.*
*A child of MAG family died in February.

The confirmed case M12.5 y.o. had a score of 4.5, as well as his sister (F, 7 y.o.) and all typical lesions. Since they developed the disease after contacts with other sick members of the ZED family, we can suppose that a second family cluster was developing when we visited the family. The follow up was not performed at individual level as funding was not available. However, the MCZ confirmed that a young boy of MAG family died around 15th February.

Interview with the ABO family (N infected / N people = 6/8 ; 1 death)

THE ABO FAMILY – Table 12 - BOGBENGO locality

The ABO family lost one 9 year old daughter. They were friends with the two daughters of the ZED family who died. They played together in the neighbourhood and at school.

Sex	Age	MPX Epidemics 2015	Adm. CSB	DATE SYMPTOMS STARTED	VACCINIA SCAR	MPX score	INRB	STATUS	Date DEATH
F	9	1	18/12/2015	18/12/2015	0	4.5	NT	D	26/12/2015
F	35	1	21/01/2016	27/12/2015	0	4	NT	R	na
M	1.5	1	3/01/2016	3/01/2016	0	4	NT	R	na
M	0.333	1	na	7/01/2016	0	4	NT	R	na
M	7	1	NO CS	10/01/2016	0	4	NT	R	na
M	4	1	NO CS	22/01/2016	0	4.5	NT	R	na
F	17	0	na	na	0	0	na	na	na
M	48	0	na	na	1	0	na	na	na

Table 12. Description of the ABO family. Adm. CSB: admission date at Bombongolo health centre. *Legend: NO CS: did not report to the health centre ; INRB : diagnostic result of INRB (if collected), NT: not collected, and. D: dead and R: recovered.na: not applicable.*

The ABO mother says that the first kid who started with a visible generalized skin eruption was the son of the ATU family confirming the other testimonies. She remembers that when her daughter fell sick they went to Aketi HGR as her condition was really worsening. She didn't eat or wanted to drink. They left for Aketi (75 km) to get better treatment , she doesn't remember when but said they stayed 3 days in the hospital before she died. She knows that the first daughter of ZED died a Saturday (24th December 2015) and her daughter (F, 9y.o., in bold in table x) died the following Thursday (26th December 2015) in Aketi. Six cases were reported in this household, three went to the official health center but 3 did not go as going to the hospital did not save the child who died.

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Bushmeat sampling in the outbreak area

During our stay in Bombongolo, 12 freshly killed mammals (Table 13) were sampled. Eight (66.7%) were positive for MPX virus and related to the same strain as above. Besides one primate, all three squirrels (*Funisciurus*) and 2 species of small carnivores namely the palm civet (*Nandinia binot*ata) and a genet were positive for MPX.

Genus lab ID	**IUCN**	**ORIGIN**	**TECH. HUNTING**	**Processing**	**MKX+**
Funisciurus anerythrus	LC	Bogbengo	PT	FRESH	1
Funisciurus anerythrus	LC	Bogbengo	PT	FRESH	1
Nandinia binotata (noseq)	LC	Bodadua	natural death	FRESH	1
Nandinia binotata (noseq)	LC	Bodadua	natural death	FRESH	1
Cercopithecus nictitans nictitans	LC	Bolela	gun (C12)	FRESH	1
Funisciurus anerythrus	LC	Bolela	PT	FRESH	1
Nandinia binotata	LC	Bodadua	gun (C12)	FRESH	1
Cercopithecus mona denti	LC	Bolela	gun (C12)	FRESH	0
Cephalophus dorsalis	LC	Bolela	dogs	FRESH	0
Genetta sp.	LC	Bogbengo	gun (C12)	FRESH	1
Cercopithecus sp.	dm	Bolela	gun (C12)	FRESH	0
Cercopithecus ascanius schimti	LC	Bogbengo	gun (C12)	FRESH	0

Table 13. Characteristics of the bushmeat sampled in BOmbongolo health area. Legend: C12:calibre 12 and Trad. Trap: traditional trap.

Follow up of the epidemics

At the end of March 2016, a total number of 160 suspect HMPX cases (of which 58 were detected during the January investigation) and 11 deaths (mortality rate=6.9%) had been recorded in Aketi Health Zone (Table 14). As we have seen above, there is likely an underestimation of the real number of "eruptive disease with fever" or "rash illness with fever" cases. Indeed, people do not trust or attend systematically the official health structures, and often report to unregistered "nurses" or pray for health. Moreover, as sampling kits are not available confirming the diagnostic is not achieved, and as medical staff is not properly trained, there is a proportion of those cases that are likely chicken pox.

WEEK	0-11 Months		12 -59 Months		5 year and above		Total	
	Case	Death	Case	Death	Case	Death	Case	Death
S1	0	0	1	0	2	0	3	0
S2	0	0	1	1	7	1	8	2
S3	0	0	4	0	9	0	13	0
S4	0	0	3	0	11	0	14	0
S5	1	0	17	0	19	2	37	2
S6	1	0	5	1	9	0	15	1
S7	0	0	9	0	11	0	20	0
S8	0	0	7	0	5	0	12	0
S9	1	0	8	0	10	0	19	4
S10	0	0	4	0	4	0	8	0
S11	0	0	6	2	5	0	11	2
TOTAL	**3**	**0**	**65**	**4**	**87**	**3**	**160**	**11**

Table 14. Total number of suspect HMPX reported to official health structures in the Aketi Health Zone between January and March 2016.

Cases were reported from 14/18 health areas making the Aketi Health Zone ; Bombongolo Heatlh area alone reported 86 cases and 9 deaths.

In God we trust

A local church called *Bongola motema* church is known in the Democratic Republic of the Congo to refrain people from seeking medical treatment as prayers will miraculously help recovery and chase the devils and evil spirits from their bodies and souls. The pastors do convince their followers that medical treatment is not from god and should thus pray enough and give enough to the intermediary (himself) to get cured. Villagers prefer giving the church 5 goats or monkeys rather than paying the 1000 Congolese francs (1.1 US $) to be admitted in the local health centre and receive free treatment. This is the case in the Bombongolo Health Area where the pastor refrain the people to go to the official medical structures and centres. The result is a delayed treatment for some but for most they follow their guide.

Some transmission may occur via contacts in the church and some kids do recover naturally. The daughter of the pastor got sick and is being treated with lemon juice. She had super infection of the hand vesicles and finally recovered (MCZ, pers. comm.).

Another problem is linked to the fact that when people go to official health treatment they assume they will be treated and healthy. However, since there is only symptomatic treatment for MPX the situation is not simple. For example, in the ZED family while they had consulted the IT before the index case, the index case recovered and two members of ZED family died. The ABO family sought treatment for the first child and when she died stopped consulting the doctors. Indeed, six cases were reported in this household, three went to the official health centre but the remaining did not as going to the hospital did not save the hospitalized child. This situation is not encouraging for people to seek modern treatment and only good information and education can help save lives. People ignore the inter-human transmission mode or the zoonotic sources so that sick children keep going to school and are not separated or isolated neither from others nor at school, church or in the family and people keep eating bushmeat daily. There is a real need for community education and training in all forested areas of the DRC.

DISCUSSION AND CONCLUSION

The Bombongolo and Aketi outbreaks were both mixed epidemics - the epidemic threshold is defined as one confirmed case- of MPX and VZV. While in Aketi, some VZV cases got a score equal to 4, the VZV score was lower in Bombongolo, where also all MPX cases got a score strictly higher than 4.5.The virulence and symptomatic expression of the strains in both areas that are only 80km apart but separated by geographical or physical barriers such as deforested areas and the Itimbiri river. Such pattern calls for further molecular typing and ideally whole genome sequencing with strains of known virulence. For biosecurity reasons shipping non inactivated samples but due to the quick emergence and spatial spread some measures should be taken to transfer strains in accredited laboratories with WHO sample flow track.

In order to refine the case definition in the field, the state of the lesion (vesicular, pustular, macular, crust), aspect (swollen, flat) and density on the face/palms/hands should be assessed and added to the question form for next outbreak investigations. Other authors facing the problem of incomplete case reporting, notification, and difficulty of contact tracing in DRC have emphasised the need to improve surveillance and response (for a recent review see Hoff, 2015), training and education (Roess et al., 2011). Our observations strongly support these authors' statements. Both in Aketi and Bombongolo the first analysis of the history of the outbreaks show at least one introduction from a zoonotic source followed by sustained interhuman transmission. MOD, the son of ATU may have been the index case who transmitted the virus to the neighbouring ZED and ABO children.

Our molecular analysis of the bushmeat sampled shows that infected wildlife circulate in the Aketi Health Zone and that the prevalence of wildlife infected is relatively high. The fact that both presumed index cases in Aketi town and Bombongolo HA had eaten *Potamocherus porcus* or C*ephalophus* meat is consistent with the PCR and sequencing results; both species are infected in the area and the prevalence of infection in both species is unexpectedly high. There is also a clear need to identify the possible zoonotic sources of such outbreaks as forest populations eat bushmeat on a nearly daily basis and especially with the number and diversity of potential host species (10 species with MPX DNA evidence). Endangered and vulnerable species such as chimpanzees are victims of hunters, can transmit MPX to humans (Mutombo et al, 1983) but may also suffer from the disease to various degrees of mortality and morbidity (Parker & Buller, 2013). A piece of 135gr of chimpanzee meat was worth 500CF (0.54$), which makes as little as 4$/kg for such an endangered and protected species. Guns take most of the animals and that the impact of the hunting on protected species would be greatly reduced if laws regarding gun ownership were implemented.

The above elements call for mixed teams during zoonotic diseases outbreaks investigations. Medical doctors and biologists should be properly trained to investigate together such outbreaks in order to prevent and control future emergence.

Since Orthopoxvirus are highly immunogenic, one can postulate that recovered individuals are naturally vaccinated against future infections by members of the same genus. However, while lifelong immunity allowed for smallpox eradication, more research is required in relation to MPX infection and the variability of the immunity according to the MPX clade involved.

REFERENCES

Chantrey J, Meyer H, Baxby D, Begon M, Bown KJ, Hazel SM, Jones T, Montgomery WI, Bennett M (1999) Cowpox: reservoir hosts and geographic range. *Epidemiology and Infection* 122:455-460

Fuller T, Thomassen HA, Mulembakani PM, Johnston SC, Lloyd-Smith JO, Kisalu NK, Lutete TK, Blumberg S, Fair JN, Wolfe ND, Shongo RL, Formenty P,Meyer H, Wright LL, Muyembe JJ, Buermann W, Saatchi SS, Okitolonda E, Hensley L, Smith TB, Rimoin AW (2011) Using remote sensing to map the risk of human Monkeypox virus in the Congo Basin. *Ecohealth*. 8:14-25

Hoff NA (2015) 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. *PhD dissertation UCLA* 120p [http://escholarship.org/uc/item/51v3n3hx]

Jezek Z, Szczeniowski M, Paluku KM, Mutombo M, Grab B (1988) Human Monkeypox: confusion with chickenpox. *Acta Tropica* 45:297-307

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A (2012)

Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics 28:*1647-1649

Likos AN, Sammons SA, Olson VA, Frace AM, Li Y, Olsen-Rasmussen M, Davidson W, Galloway R, Khristova ML, Reynolds MG, Zhao H, Carroll DS, Curns A, Formenty P, Esposito P, Regnery RL, Damon IK **(**2005**)** A tale of two clades: Monkeypox viruses. *Journal of General Virology* 86:2661-2672

Macneil A, Reynolds MG, Braden Z, Carroll DS, Bostik V, Karem K, Smith SK, Davidson W, Li Y, Moundeli A, Mombouli JV, Jumaan AO, Schmid DS, Regnery RL, Damon IK (2009) Transmission of Atypical Varicella-Zoster Virus Infections Involving Palm and Sole Manifestations in an Area with Monkeypox Endemicity. *Clinical Infectious Diseases* 48 (1): e6-e8

Montgelard C, Bentz S, Tirard C, Verneau O, Catzeflis FM (2002) Molecular systematics of Sciurognathi (rodentia): the mitochondrial cytochrome b and 12S rRNA genes support the Anomaluroidea (Pedetidae and Anomaluridae). Molecular Phylogenetics and Evolution 22:220–233

Mutombo M, Arita I, Jezek Z (1983) Human monkeypox transmitted by a chimpanzee in a tropical rain-forest area of Zaire. Lancet 2;1(8327):735-737

Nakazawa Y, Mauldin MR, Emerson GL, Reynolds MG, Lash RR, Gao J, Zhao H, Li Y, Muyembe JJ, Kingebeni PM, Wemakoy O, Malekani J, Karem KL, Damon IK, Carroll DS (2015) A phylogeographic investigation of African Monkeypox. Viruses 22:2168-2184

Parker S, Buller R (2013) A review of experimental and natural infections of animals with monkeypox virus between 1958 and 2012 Future Virol. 2013 Feb 1; 8(2): 129–157.

Reynolds MG, Carroll DS, Karem KL (2012) Factors affecting the likelihood of Monkeypox's emergence and spread in the post-smallpox era. Current Opinion in Virology 2:335-343

Roess AA, Monroe BP, Kinzoni EA, Gallagher S, Ibata SR, Badinga N, Molouania TM, Mabola FS, Mombouli JV, Carroll DS, MacNeil A, Benzekri NA, Moses C, Damon IK, Reynolds MG (2011) Assessing the Effectiveness of a Community Intervention for Monkeypox Prevention in the Congo Basin. *PLoS Neglected Tropical Diseases* 5: e1356

APPENDIX 1. BUDGET

LAUDISOIT - MISSION MONKEYPOX - DEPENSE MISSION MONKEYPOX								
DATE	**CODE**	**ITEM**	**PLACE**	**COST/UNIT**	**N UNIT**	**TOTAL $**	**TOTAL FC**	
28/02/2016	AK1	Guide/Helpers - Temporary 21/01/2016-28/01/2016	Bombongolo	10	8	80		
28/02/2016	AK2	Guide/Helpers - Temporary 21/01/2016-28/01/2016	Bombongolo	10	8	80		
28/02/2016	AK3	Guide/Helpers - Temporary 21/01/2016-28/01/2016	Bombongolo	10	8	80		
1/02/2016	AK4	Expedition biopsies DH/ INRB	Kisangani	30	1	30		
14/01/2016	AK5	24L Essence	Kisangani	1300	24		31200	
14/01/2016	AK6	Matériel motos/entretiens/rechange	Kisangani	173000	1		173000	
29/01/2016	AK7	24L Essence	Buta	1600	24		38400	
22/01/2016	AK8	Essence 36l + huile moto 3l	Aketi	93000	1		93000	
24/01/2016	AK9	24L Essence + bougies	Aketi	68000	1		68000	
28/01/2016	AK10	26L Essence	Aketi	72000	1		72000	
19/01/2016	na	UNITE VODACOM	Bumba	50	1	50		
3/02/2016	na	Modem + 100$ UNITE AIRTEL	Kisangani	160	1	160		
						480	475600	**TOTAL**
							511.397849	**991.397849**

APPENDIX 2. Prospective prevalence survey form.

PAGE 1 MONKEYPOX INVESTIGATION : DIVISION PROVINCIALE : _____ZS :_____AS_____
DATE ENQUETE : _____ / _____/ _____ INVESTIGATEUR _____

AKE1.Waypoint :

AKE2.NOM DE FAMILLE : _____

AKE3.VILLAGE/LOCALITE :_____ **ETHNIE DU PERE :** _____ **ETHNIE DE LA MERE** _____

AKE4.STRUCTURE DE LA FAMILLE et PREVALENCE

N	SEX	AGE	MPX pour le moment ?	Date début symptômes éruptifs ?	A déjà eu le MPX ?	Si oui, MOIS et ANNEE ?	Cicatrice VARV	Fièvre AVANT éruption	ERUPTION CUTANEE GENERALE	LESION SUR PALME	LESIONS SUR PAUME	ADENITE	LESION OCCULAIRE	REMARK
1			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
2			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
3			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
4			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
5			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
6			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
7			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
8			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
9			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
10			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
11			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
12			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
13			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
14			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
15			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
16			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
17			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
18			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
19			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	
20			☐O ☐N		☐O ☐N		☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐O ☐N	☐UNI☐BI ☐N	

PAGE 2 MONKEYPOX INVESTIGATION : DIVISION PROVINCIALE : _____
ZS :_____AS_____

DATE ENQUETE : _____ / _____/ _____ INVESTIGATEUR _____

QUI EST LE REPONDANT ? (1 à 20)_____

QUELLE EST LA POSITION DU REPONDANT DANS LA FAMILLE ? ☐père de famille, chef de ménage ☐mère de famille, chef de ménage ☐fils du ménage ☐fille du ménage

☐grand père ☐grand mère ☐arrière grand père ☐ arrière grand mère ☐oncle maternel ☐tante maternelle ☐oncle paternel ☐tante paternelle

☐Autre (décrire): _____:

Pour chaque patient, soumettre la page 3.

REMARQUES : LESION OCCULAIRE = unipolaire ou bipolaire (un ou deux yeux) – Stade des lésions : M=macule , P=papule, V=vésicule, Pu=pustule et C=croute

COMMENTAIRE LIBRE :

PAGE 3 MONKEYPOX INVESTIGATION : DIVISION PROVINCIALE : _____
ZS :_____AS_____

DATE ENQUETE : _____ / _____/ _____ INVESTIGATEUR _____

N	BIOPSIE	Nombre de biopsies	T°C	Dernière viande de brousse consommée ?	Contact avec salive ou bouche de la dernière viande consommée ?	Consommation de vin de palme ?	Consommation noix de palme crue ?	NOM ECOLE si applicable	NOM de l'EGLISE	NOTE
1	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
2	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
3	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
4	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
5	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
6	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
7	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
8	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
9	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
10	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
11	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
12	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
13	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
14	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
15	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
16	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
17	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
18	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
19	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			
20	☐O ☐N				☐O ☐N	☐O ☐N	☐O ☐N			

QUI EST LE REPONDANT ? (1 à 20)_____