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RESEARCH ARTICLE

Descriptive Epidemiology of Fatal Respiratory Outbreaks and Detection of a Human-Related Metapneumovirus in Wild Chimpanzees (*Pan troglodytes*) at Mahale Mountains National Park, Western Tanzania

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Over the past several years, acute and fatal respiratory illnesses have occurred in the habituated group of wild chimpanzees at the Mahale Mountains National Park, Tanzania. Common respiratory viruses, such as measles and influenza, have been considered possible causative agents; however, neither of these viruses had been detected. During the fatal respiratory illnesses in 2003, 2005 and 2006, regular observations on affected individuals were recorded. Cause-specific morbidity rates were 98.3, 52.4 and 33.8%, respectively. Mortality rates were 6.9, 3.2 and 4.6%; all deaths were observed in infants 2 months–2 years 9 months of age. Nine other chimpanzees have not been seen since the 2006 outbreak and are presumed dead; hence, morbidity and mortality rates for 2006 may be as high as 47.7 and 18.5%, respectively. During the 2005 and 2006 outbreaks, 12 fecal samples were collected from affected and nonaffected chimpanzees and analyzed for causative agents. Analysis of fecal samples from 2005 suggests the presence of paramyxovirus, and in 2006 a human-related metapneumovirus was detected and identified in an affected chimpanzee whose infant died during the outbreak. Our findings provide preliminary evidence that the causative agent associated with these illnesses is viral and contagious, possibly of human origin; and that, possibly more than one agent may be circulating in the population. We recommend that baseline health data be acquired and food wadge and fecal samples be obtained and bio-banked as early as possible when attempting to habituate new groups of chimpanzees or other great apes. For already habituated populations, disease prevention strategies, ongoing health monitoring programs and reports of diagnostic findings should be an integral part of managing these populations. In addition, descriptive epidemiology should be a major component of disease outbreak investigations. Am. J. Primatol. 70:755–765, 2008.

Key words: Mahale; chimpanzees; *Pan troglodytes*; habituated great apes; metapneumovirus; rotavirus; anthropozoonoses; zoonoses; respiratory disease; infectious disease

INTRODUCTION

Concerns among biomedical scientists, public health professionals and great ape conservationists over cross-species transmissions between primates are mounting as humans are increasingly coming into closer proximity with wild primates for a variety of reasons, including habitat loss, bush meat hunting, research activities and ecotourism [Chapman et al., 2007; Goldberg et al., 2007; Mittermeier & Cheney, 1987; Salzer et al., 2007; Wolfe et al., 1998, 2005, 2007]. The Simian immunodeficiency virus

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(SIV_{cpz}), a likely zoonosis of chimpanzee origin manifesting as the human HIV/AIDS pandemic has led to devastating consequences with great public health significance [Gao et al., 1999; Hahn et al., 2000; Keele et al., 2006]. The highly virulent Ebola virus, a deadly pathogen causing a severe hemorrhagic disease and death, and also reported to be zoonotic, is a common threat to both human and nonhuman primates [Formenty et al., 1999, 2003; Huijbregts et al., 2003; Leroy et al., 2004; Rouquet et al., 2005].

Similarly, great apes are susceptible to human pathogens, including paramyxoviruses [Ferber, 2000; Woodford et al., 2002]. For example, the prototype respiratory syncytial virus (''chimpanzee coryza virus'') was isolated from a young captive chimpanzee in 1956 and antigenically similar human isolates were described subsequently, and signs of upper respiratory tract infections in chimpanzees have been associated with respiratory syncytial virus (RSV) [Beem et al., 1960; Belshe et al., 1977; Clarke et al., 1994; Morris et al., 1956]. Captive-bred chimpanzees have been found to be seropositive for one or both strains (CAN75 and CAN83) of human metapneumovirus (hMPV) [Skiadopoulos et al., 2004]. RSV and hMPV are common human respiratory pathogens found globally and are capable of causing upper and lower respiratory diseases. Using molecular methods, RSV, hMPV and other bacterial respiratory pathogens have now been detected in lung tissue taken from chimpanzees (*Pan troglodytes verus*) that died in association with respiratory outbreaks in Taï Forest National Park, Côte d'Ivoire [Chi et al., 2007; Köndgen et al., 2008]. Our studies indicate that the presence of human-related paramyxoviruses in habituated wild chimpanzees in the Taï Forest is not a regionally based occurrence or the result of isolated events. We show that a human-related paramyxovirus is also present and associated with acute and fatal respiratory illnesses in the habituated population of chimpanzees at Mahale Mountains National Park (MMNP).

The largest remaining population of eastern chimpanzees (*P. troglodytes schweinfurthii*) resides in the Mahale Mountains (latitude 6°S, longitude 30°E) in western Tanzania. Beginning in October 1965, habituation efforts were initiated by researchers from Japan to attract chimpanzees from two unit-groups, referred to as the K and the M-Groups, allowing human observers to approach them in their natural habitat [Nishida, 1990]. Chimpanzee viewing has attracted tourists from around the world since Mahale became a Park in 1985 and tourism has steadily increased [Nishida & Mwinuka, 2005]. People have been observed viewing Mahale chimpanzees as close 1–2 m and chimpanzees do approach, and in some cases, even make direct contact with observers [T. Kaur & J. Singh, personal observations]. The M-Group, once comprised of as many as 101 individuals in 1984, is now estimated to contain only 63 individuals, including 15 chimpanzees who immigrated into the M-Group since 1981 [Nishida et al., 2003]. Since the 1990s, signs of respiratory illnesses have been observed in the M-Group chimpanzees [Hanamura et al., 2008; Hosaka, 1995; Kaur & Huffman, 2004]. Although no interference with normal behavior is allowed and only noninvasive sample collection is permitted for research purposes, a long-term health program has recently been established to monitor their health and well-being.

Here, we provide the descriptive epidemiology on respiratory outbreaks that were observed in 2003, 2005 and 2006 in the M-Group at MMNP, and present our findings that the probable causative agent responsible for the fatal 2006 illness is a human-related paramyxovirus.

MATERIAL AND METHODS

In our studies, M-Group chimpanzee observations are recorded on a daily basis throughout the year. Unique facial features allow us to recognize individuals and assign unique identifiers. Age groups are designated as follows: infants (0–3 years), juveniles (4–8 years), adolescents (9–14 years) and adults (15 years and older). Observations on daily chimpanzee sightings, population structure, grouping and ranging patterns, long absences, immigrations, births and deaths are recorded. Observations of noticeable signs of illness are also recorded. Hands-on contact with live chimpanzees is prohibited and samples for research purposes can only be acquired without any animal contact, such as collection of chimpanzee fecal samples from the forest floor, urine falling from chimpanzees in the canopy and post-mortem examination.

Animal Observations and Sample Collections

During September and October 2003, July, August and September 2005 and June and July 2006, chimpanzees in the M-Group were observed having clinical signs of respiratory illness, such as coughing, sneezing, nasal discharge, respiratory distress and lethargy. Signs observed in the chimpanzees seen, chimpanzees found dead, and those with long absences from the group were recorded.

During the 2005 and 2006 illnesses, fecal samples and post-mortem tissues were collected as follows for laboratory analysis. On July 29, 2005, five affected chimpanzees, two adult males, one adult female carrying her dying infant, her juvenile son and another adult female were observed coughing severely and appeared to be weak. Their fecal samples were collected from the forest floor immediately after the four adults and one juvenile chimpanzee were observed defecating and immediately placed in 10% buffered formalin. Post-mortem tissues were collected from the infant approximately 72 hr after its death, when the mother released the

carcass. From July 13 through July 15, 2006 five fecal samples were collected from chimpanzees traveling together. One chimpanzee was a healthy adolescent female (10 years old) with no overt signs of respiratory disease, and the other was her mother, a 45-year-old adult female carrying her infant. Both the older adult and her infant were coughing severely and appeared to be weak. On July 15th, the sick adult female was again observed coughing and weak and carrying her now dead infant. On the following day, the independent adolescent female, still in good health, was again observed traveling with the older adult chimpanzee whose health was apparently improving. Three samples were collected from the healthy adolescent female, and the other two fecal samples were collected from the older adult female with noticeable signs of respiratory disease. The samples were taken from the forest floor immediately after the chimpanzees were observed defecating and stored frozen at −20°C after several hours. In addition, post-mortem tissues were recovered from one infant and an unidentified adult female and preserved in 10% formalin and ethanol.

Seven fecal samples from affected chimpanzees and post-mortem tissues from the infant from the 2005 outbreak were shipped at ambient temperature to the Virginia–Maryland Regional College of Veterinary Medicine (VMRCVM) and to the Centers for Disease Control and Prevention (CDC). From the 2006 outbreak, the five frozen fecal samples were transferred into RNA later storage buffer and post-mortem tissues in formalin from an infant and an unidentified adult female were shipped at ambient temperature to VMRCVM and CDC. All fecal sample and post-mortem tissue collections and transfers were performed by personnel using gloved hands and face masks, in addition to other protective gear. No symptoms or clinical signs of respiratory disease or diarrhea were present in these individuals.

Fecal samples from 2005 and 2006 were evaluated using electron microscopy, and fecal samples from 2006 were also analyzed using seminested PCR enterovirus assay and pathogen discovery PCR assays for viral respiratory pathogens. The fecal samples collected in 2006 were also tested for rotavirus using a commercial enzyme-linked immunoassay (EIA) (Meridian, Cincinnati, OH). Tissues collected during post-mortem examination were sectioned and slides were prepared for histopathological examination.

Viral Analysis

A sensitive, seminested PCR amplification of the VP1 Sequences for direct identification of all enterovirus serotypes was performed as previously described [Nix et al., 2006]. Broadly reactive PCR assays were designed to detect all members of a given family, or genera (*Adenoviridae, Herpesviridae, Coronavirdae, Paramyxoviridae,* and *Orthomyxoviridae* viral families) by primer targeting at the conserved regions within the given groups. Primers designed were applied from the hexon gene for adenoviruses, the polymerase 1b open reading frame for coronaviruses, the polymerase L gene for the paramyxoviruses and the polymerase PB1 gene segment for influenza virus and the sequence of 5′ untranslated region of enteroviruses (S. Tong, unpublished data). The total nucleic acids in stool suspensions were extracted using NucliSens extractor in accordance with the manufacturer's instructions (BioMerieux, Durham, NC). Samples were first tested individually by each of the four pan viral family PCR assays using the Invitrogen SuperScript III Platinum One-Step RT-PCR kit and the Invitrogen Taq polymerase kit (Invitrogen, Carlsbad, CA). The PCR mixtures contained 50 pmol each of forward and reverse primers, 1 × buffer with final concentration of 2.0 mM $MgSO_4$ and 200 μM each of dNTP, 20 units of RNase inhibitor, a 5 μl aliquot of RNA/DNA extracts and 1 unit of SuperScript III RT/Platinum Taq Mix (Invitrogen, Carlsbad, CA). Water was then added to achieve a final volume of 50 μl. The RT-PCR reaction mixture was sequentially incubated at 60°C for 1 min for denaturing, 44–50°C for 30 min (for RT), 94°C for 2 min (for hot start), then 40 cycles at 94°C for 15 sec; 48–50°C for 30 sec; 72°C for 30 sec and a final extension at 72°C for 7 min. The final PCR products were visualized by UV light after electrophoresis on a 2% agarose gel containing 0.5 μg/ml ethidium bromide in 0.5 × Tris-borate buffer (pH 8.0). The positive PCR products were purified using a QIAquick PCR purification kit (Qiagen, Inc., Valencia, CA). Both strands of the amplicons were sequenced with a BigDye Terminators v1.1 ready reaction cycle sequencing kit on an ABI Prism 3100 automated sequencer (Applied Biosystems, Foster City, CA) using the corresponding PCR primers. The remaining reaction conditions were according to the manufacturer's instructions.

Phylogenetic Analysis

Phylogenetic analysis was only completed on the *Paramyxoviridae* viral family as negative nucleic acid results from the *Adenoviridae, Herpesviridae, Coronavirdae* and *Orthomyxoviridae* viral families precluded further analysis. The primers used for amplification of the partial nucleocapsid gene (N) are hMPV N F2 (TCT TCA AGG GAT TCA CCT AAG TG) and hMPV N R991 (GAC CTG AAG CAT TGC CAA GAA C). The primers used for amplification of the partial glycoprotein gene (G) are hMPV G F14 (GAA CAT TCG AGC GAT AGA CAT G) and hMPV G R635 (GTT CGC TGC TTT GGG TTG TGG). PCR and sequencing were performed using the conditions described as above. Phylogenetic trees were constructed by using Maximum-Likelihood in

the program BEAST [Drummond & Rambaut, 2007]. The sequence alignment of 839 nucleotides of the N protein gene fragment and the sequence alignment of 579 nucleotides of the G protein gene fragment of the chimpanzee MPV were compared with 17 sequences of other human and avian MPVs from the NCBI database

Electron-Microscopy

Negative-stained specimens were prepared for electron-microscopy as described previously [Wang et al., 2007]. Briefly, specimen drops were applied to formvar-carbon coated grids, rinsed with water, re-blotted and applied to a drop of a stain containing metal known to block electrons, thereby facilitating the viewing of particles of interest within a transmission electron microscope (TEM). Two percent methylamine tungstate, pH 6.8 marketed as NanoW^R (Nanoprobes, Yaphank, NY) was used as the negative stain. Post-staining with NanoW^R, the grids were viewed within an FEI Technai BioTwin^R TEM that was operating at 120 KV (FEI Company, Hillsboro, OR). Images were captured using an AMT^R CCD digital camera (Advance Microscopy Techniques, Corp., Danvers, MA).

RESULTS

Observational data show that morbidity occurred in all age groups in 2003, 2005 and 2006 (Table I). The predominant initial clinical signs in all the cases were coughing and nasal discharge; weakness and lethargy were also commonly reported. Early in the 2006 outbreak (prior to fecal sample collection), one adult female with a severe cough and nasal discharge had offspring with a cough and nasal discharge. Her infant was also observed with diarrhea and swelling of the eyelids and subsequently died; her adolescent daughter's face and eyelids were swollen [Nomad Camp Trackers, observations]. Another adult female had diarrhea in addition to a bad cough. Morbidity and mortality rates are provided by gender and age groups in Table I. In total, nine infants died, ranging in age from 2 months–2 years 9 months of age. Nine other chimpanzees have not been seen since the 2006 outbreak and may also be dead in association with the outbreak (four infants, one juvenile and four adults). Epidemic curves showing the magnitude and time trend of each outbreak are provided in Figure 1.

TEM examination of the stool specimens revealed structures resembling infectious agents, including paramyxovirus and rotavirus, and paramyxovirus in 2005 and 2006, respectively (Table II and Fig. 2). Pan viral family PCR assays confirmed the presence of a pneumovirus in fecal specimens collected from the sick adult female in 2006. The partial sequence from RNA polymerase (L) amplicon (329 bp) matched the cognate gene sequence of hMPV isolate NL 1 99 with 98% identity. Partial nucleotide sequences were also determined for nucleoprotein N (839 bp) and attachment protein G (579 bp) genes and were found to share 98 and 97% identities with the corresponding genes from hMPV NL 1 99 strain. As shown in the phylogenetic trees in Figure 3, the partial N and G gene sequences of recent hMPV strains showed that the chimpanzee isolate clustered into group A with the highest sequence identity to hMPV JPS03 194 strain (99% in N and 99% in G). Five nucleic acid mutations were observed in the 839 bp N gene amplicon of the chimpanzee MPV relative to the hMPV JPS03 194 strain (all transitions and synonymous changes). In the 579 bp G gene amplicon, five mutations were present (all transitions); four were nonsynonymous and were predicted to result in amino acid changes at positions of T87I, P105S, L136P and K162E in the G protein.

All five samples taken in 2006 tested positive for rotavirus, and negative for enteroviruses. Histopathology results were inconclusive, as all tissues showed marked autolysis and tissue architecture could not be distinguished.

DISCUSSION

Epidemic curves suggest that the 2003 outbreak was from a single point-source exposure, whereas the 2005 and 2006 outbreaks were propagated, probably by chimpanzee-to-chimpanzee contact after the initial introduction of the virus into the population (Fig. 1). All three outbreaks occurred during the dry season (June through September) which coincides with the peak tourist season at MMNP. In the 2003 outbreak, 98.3% of the M-Group chimpanzees were observed with signs of respiratory tract infection and morbidity decreased in subsequent outbreak years. Although there were earlier reports by Hosaka of respiratory outbreaks, the trend as shown in Figure 1 for 2003, 2005 and 2006 suggests that, if the same causative agent was responsible for illness in more than one outbreak year it was probably introduced from humans in 2003. Transmission from humans thereafter would not have been necessary for the disease to manifest again if it could be propagated over the years from chimpanzee-to-chimpanzee.

Persistent hMPV infection in humans without the presence of respiratory symptoms has also been documented [Debiaggi et al., 2006]. If this also occurs in chimpanzees, this could lead to far more serious consequences. For example, if females are carriers and emigrate to neighboring groups they may introduce the disease to other nonhabituated chimpanzees groups. In regard to the chimpanzees that were not observed with signs but have not been seen since the 2006 outbreak, if they were infected with the pathogen and did not succumb to the illness, it is possible, although unlikely that they have

TABLE I. Cause-Specific Morbidity and Mortality Rates for 2003 (September 10–22), 2005 (July 15–September 7), 2006 (June 2–July 22) and 2006 Including Presumed (May 25–July 22)

	2003[a] $N = 58$		2005[b] $N = 63$		2006[c] $N = 65$		2006 including presumed[d] $N = 65$	
	Morbidity	Mortality	Morbidity	Mortality	Morbidity	Mortality	Morbidity	Mortality
Adult males	7/7 (100%)	0/7 (0%)	7/8 (87.5%)	0/8 (0%)	4/10 (40%)	0/10 (0%)	5/10 (50%)	1/10[e] (10%)
Adult females	19/19 (100%)	0/19 (0%)	12/20 (60%)	0/20 (0%)	8/21 (38.1%)	0/21 (0%)	11/21 (91.7%)	3/21[f] (14.3%)
Adolescent males	5/5 (100%)	0/5 (0%)	3/5 (60%)	0/5 (0%)	1/3 (33.3%)	0/3 (0%)	1/3 (33.3%)	0/3 (0%)
Adolescent females	4/4 (100%)	0/4 (0%)	3/4 (75%)	0/4 (0%)	1/4 (25%)	0/4 (0%)	1/4 (25%)	0/4 (0%)
Juvenile males	4/4 (100%)	0/4 (0%)	2/3 (66.7%)	0/3 (0%)	0/4 (0%)	0/4 (0%)	1/4 (25%)	1/4[g] (25%)
Juvenile females	7/7 (100%)	0/7 (0%)	1/10 (10%)	0/10 (0%)	4/11 (36.4%)	0/11 (0%)	4/11 (36.4)	0/11 (0%)
Infants	11/12 (91.7%)	4/12[h] (33.3%)	5/13 (38.5%)	2/13[i] (15.4%)	4/12 (33.3%)	3/12[j] (25.0%)	8/12 (66.7%)	7/12[k] (58.3%)

[a]Presumed index case is a juvenile male.
[b]Presumed index case is an adult female whose infant died during the outbreak.
[c]Presumed index case is an adult male.
[d]Morbidity and mortality rates include nine other chimpanzees (four infants, one juvenile and four adults) have not been seen since the outbreak. They were not observed with clinical signs, but five had a history of contact with affected chimpanzees [Hanamura et al., 2008]. A different adult male may have been the index case.
[e]Estimated to be 29 years of age.
[f]Estimated to be 26, 34 and 35 years of age.
[g]Eight years of age.
[h]Ages of 2 months–2 years 9 months; all observed having signs of respiratory illness.
[i]Ages 5 months and 7 months; both observed with clinical signs.
[j]Ages 11 months–2 years 4 months; all observed with clinical signs.
[k]Ages of <1 month–2 years 4 months.

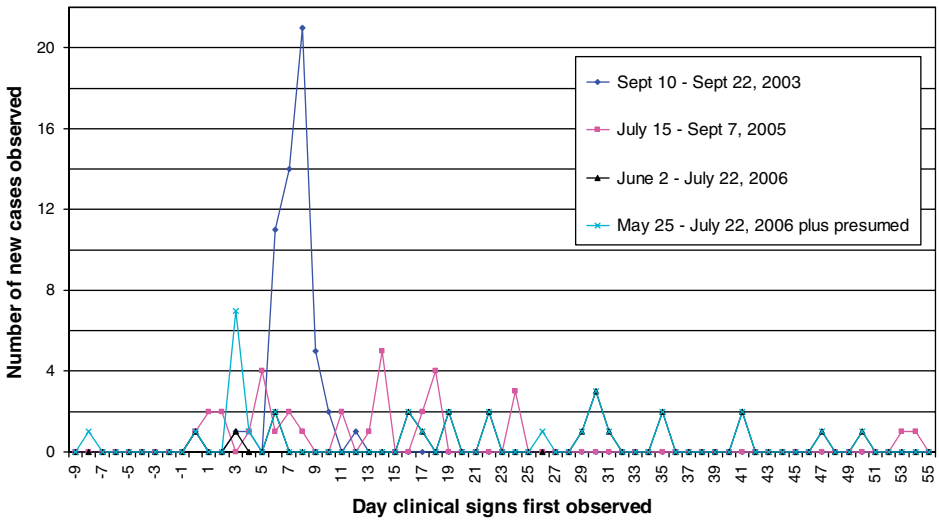

Fig. 1. Epidemic curves showing the magnitude and time trends of the respiratory outbreaks observed for 2003, 2005 (whether or not the two late cases were associated with the outbreak is unknown. However, signs were still persisting in a few chimpanzees including the mother of the infant observed with signs on September 6; hence, these two late cases are considered a part of this outbreak, although exposed or first observed later than most cases) and 2006. Also, shown is 2006 plus presumed cases, where nine additional chimpanzees, although not observed with clinical signs, have not been seen since the time of the outbreak as follows: one on May 25 (day -8), six on June 5 (day 3), one on June 6 (day 4) and one on June 28 (day 26); they are presumed dead possibly in association with the respiratory illness [Hanamura et al., 2008]. These additional chimpanzees appear as new cases on the date they were last seen. On-line version is shown in color.

emigrated to a different Group and may be exposing other (nonhabituated) chimpanzee groups in and around MMNP. At this stage regardless of how careful humans are now to prevent transmission of the causative agent(s), the damage is likely already done. Nevertheless, all possible precautions should be taken and policies enforced to prevent introduction of this and other pathogens.

TABLE II. TEM and Pan Viral PCR Assay Results on 2005 and 2006 Fecal Samples

	Coughing	TEM viral results	Pan viral PCR assays	Rotavirus EIA	Age/gender
2005					
MM05-105	Present	Likely paramyxovirus	ND[a]	ND[a]	16 yrs/male
MM05-106	Present	Likely rotavirus[b]	ND[a]	ND[a]	42 yrs/male
MM05-107	Present	Likely paramyxovirus[b]	ND[a]	ND[a]	7 yrs/female
MM05-108	Present	Likely rotavirus or other reovirus	ND[a]	ND[a]	23 yrs/female
MM05-109	Absent	Negative	ND[a]	ND[a]	23 yrs/female[c]
2006					
MM06-100	Present	Negative	Pneumoviruses[d]	Positive	45 yrs/female[cef]
MM06-101	Present	Likely paramyxovirus	Pneumoviruses[d]	Positive	45 yrs/female[cef]
MM06-102	Absent	Negative	Negative	Positive	10 yrs/Female[eg]
MM06-103	Absent	Negative	Negative	Positive	10 yrs/female[eg]
MM06-104	Absent	Negative	Negative	Positive	10 yrs/female[eg]

[a]ND = no data.
[b]Poor structure likely owing to adverse environment from which the specimens were collected, stored and transported.
[c]Individuals carrying sick infants that died during the sampling period.
[d]Pneumoviriae within the Paramyxoviridae family.
[e]Individuals observed traveling together during the sampling period.
[f]MM06-100 and MM06-101 samples from the same individual.
[g]MM06-102, MM06-103 and MM06-104 samples from the same individual.

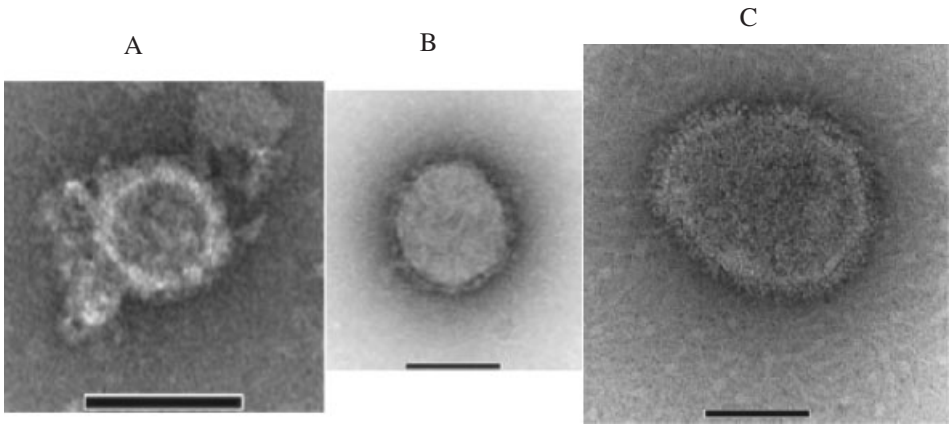

Fig. 2. Negative stain electron micrograph of virus-like structures recovered from fecal samples during the respiratory outbreaks in (**A**) 2005, image obtained from specimen MM05-108 and resembles rotavirus or some other reovirus (~70 nm in diameter), (**B**) 2005, image obtained from specimen MM05-105 resembling paramyxovirus (~130 nm in diameter) and (**C**) 2006, image obtained from specimen MM06-101 suggestive of paramyxovirus (~200 nm in diameter). The apparent morphologic damage to virus-like structures is likely owing to the adverse environment from which the specimens were collected, stored and transported. Methylamine tungstate stain, pH 6.9. Bar in images represents 100 nm.

TEM of fecal samples from affected chimpanzees revealed the presence of viral particles resembling paramyxovirus and rotavirus in 2005 and paramyxovirus in 2006. PCR and sequence analysis of the fecal samples from the same affected chimpanzee in 2006 confirmed the presence of a paramyxovirus, one closely related to the Japanese strain of an hMPV. The sequences were distinct from those of hMPV isolates present in the laboratory but squarely within the sequences of previously reported isolates from humans suggesting that the virus detected is probably of human origin. These results provide evidence that this human-related chimpanzee MPV (hrcMPV) is the likely cause of the fatal outbreak in 2006.

van den Hoogen et al. first identified hMPV in the Netherlands in 2001. Infection with hMPV is associated with acute respiratory tract illness especially in children usually younger than 3 years old, with signs similar to those seen with human RSV infection, ranging from upper respiratory tract disease to severe bronchiolitis and pneumonia [van den Hoogen et al., 2001]. hMPV has since been reported to cause acute respiratory tract infections worldwide in all age groups, with the most severe cases being in infants, the elderly and the immunocompromised [Boivin et al., 2002; Crowe, 2004; Falsey et al., 2003; Hamelin & Boivin, 2005; Hamelin et al., 2004; Honda et al., 2006; van den Hoogen, 2007; van den Hoogen et al., 2001]. Studies show that

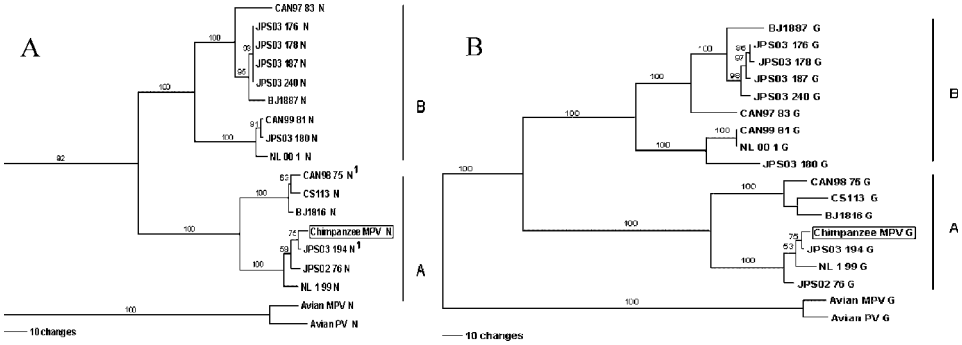

Fig. 3. Comparison of hrcMPV to 17 sequences of other human and avian metapneumoviruses by Estimated Maximum-Likelihood tree (number of bootstraps, 100) based on (**A**) sequence alignment of 839 nucleotides of the N protein gene fragment (NCBI database; NCBI database accession numbers EF199772, NC_007652, AY530091, AY530090, AY297749, AF371337, AY530095, AY530093, AY530092, AY530089, AY530094, AY297748, DQ843658, DQ843659, EF081361, AY525843, AY145286) and (**B**) sequence alignment of 579 nucleotides of the G protein gene fragment (NCBI database; NCBI database accession numbers EF199772, NC_007652, AY530091, AY530090, AY297749, AF371337, AY530095, AY530093, AY530092, AY530089, AY530094, AY297748, DQ843658, DQ843659, AY574224, EF081368, AY296034). Subclades A and B are indicated.

by age 5 years virtually all the children (>90%) in the Netherlands had been exposed and that hMPV has been circulating in humans for at least 50 years; seroprevalence of hMPV-specific antibody in adults has been reported to be nearly 100% [Leung et al., 2005; van den Hoogen et al., 2001]. Serological studies on samples taken in 2002 from children and adults (age range 1 month–35 years) in Japan showed a seroprevalence of 72.5% and that all children had been exposed to hMPV by 10 years of age [Ebihara et al., 2003]. Clinical signs of respiratory illness were observed in all age groups of the M-Group chimpanzees consistent with those reported in humans infected with hMPV. In addition, like humans, chimpanzees under the age of 3 years were more severely affected than other age groups.

Using real-time RT-PCR, RNA from the pneumovirus RSV—the most closely related human pathogen to hMPV—has been detected in feces, nasal secretions, saliva and sweat samples, whereas, hMPV RNA has been detected in nasal secretions, saliva and sweat samples, but not in feces [von Linstow et al., 2006]. Here, we report that the human-related MPV particles can be detected by TEM, as well as PCR in feces from infected chimpanzees. Our findings suggest that the chimpanzees are swallowing virus-laden respiratory secretions that then pass through the gastro-intestinal tract with minimal change in structural morphology of the virions (Table II).

The incubation period in Japanese children estimated by the onset of symptoms was 4–6 days post-infection, and in experimental infection of cynomologous monkeys with hMPV viral shedding began on day 2, peaked at day 4 and lasted until day 9 post-infection [Ebihara et al., 2004a; Kuiken et al., 2004]. Reinfection with hMPV in humans has been considered to occur frequently throughout life based on the risk of reinfection reported to occur with its close neighbor RSV, where host immunity provides incomplete protection for a limited time [Henderson et al., 1979]. In one report, an infant in Japan was infected with two different strains of hMPV in a period of 1 month [Ebihara et al., 2004b]. At Mahale, acute respiratory signs were observed in the same female chimpanzees and their offspring in more than one outbreak year suggesting that perhaps reinfection with hrcMPV occurs, or that perhaps multiple pathogens have been introduced and are circulating in the population. Co-infections with other viruses may be occurring triggering new cases of respiratory disease in the same chimpanzees or perhaps long-term immunity to the same causative agent is not being established. For example, the presumed index case in 2005 was an adult female who was affected in 2003 and again in 2006; she did not have an infant in 2003 but in both 2005 and 2006 her infants died and presumably died, respectively, in association with the illnesses. Her juvenile son was also affected in 2003 and 2005 but was not observed with signs in 2006. Another adult female and her juvenile son were affected in 2003 and 2005. Her infant died in the 2003 outbreak; in 2005, another infant was affected and recovered; and in 2006, both that infant, her juvenile son and the mother were lost to follow-up, all possibly dead in association with the outbreak [Hanamura et al., 2008]. Her adult daughter was affected in 2003, 2005 and 2006 along with her juvenile granddaughter in 2003 and 2006, and her infant granddaughter died in 2006. This trend of repeated acute respiratory illnesses was also observed in adult males. For example, the presumed index case in 2006, an adult male was affected both in 2003 and 2005; if we include the chimpanzees presumed dead, the index case in 2006 was still an adult male affected both in 2003 and 2005. Another adult female and her infant were affected in 2003 and her infant died; she was not observed with signs

herself in 2006 but her infant has not been seen since that outbreak and is presumed dead [Hanamura et al., 2008].

In 2005, structures resembling rotavirus were detected by the TEM, and in 2006, two affected chimpanzees, although not sampled, were observed having diarrhea. All five fecal specimens collected from 2006 (from chimpanzees with and without clinical signs of respiratory illness) tested positive for rotavirus by an EIA kit that has been widely and extensively used in surveillance studies and clinical trials of human vaccines throughout the world. As it detects antigen (not RNA) using a monoclonal antibody specific to a simian rotavirus, it is highly unlikely this is a laboratory contamination as our positive and negative controls gave expected results. Molecular characterization of the strains by polyacrylamide gel electrophoresis, RT-PCR and nested PCR was attempted and all gave negative results; therefore, whether the rotavirus is of simian or human origin is not known. These negative molecular results agree with those of a recent study in which rotavirus was detected only by EIA but not by molecular techniques [Wang et al., 2007]. That study demonstrated that rotavirus was unstable and appeared atypical in stools of nonhuman primates with diarrhea, an observation contrary to general belief by many people. As rotavirus is ubiquitous and neither of the two chimpanzees reported to have diarrhea were tested for rotavirus, whether a co-infection with rotavirus may have played a role in the fatal respiratory outbreaks is not known. In the studies conducted by von Linstow et al., RSV was found in five fecal samples taken from five different children; four of the children had diarrhea [von Linstow et al., 2006]. We speculate that the presence of rotavirus in the chimpanzees may alter transient time through the digestive tract or gastrointestinal function and thereby facilitate the passing of intact virus particles. Whether or not the virions of hrcMPV excreted in the feces are infectious and constitute a potential mode of transmission is not yet known, however, live avian MPV is known to be shed in the feces of nonvaccinated hens [Hess et al., 2004].

hMPV is a newly recognized virus reported to exist worldwide and only a limited amount of sequence data is available on the worldwide distribution of different strains. As shown in Figure 3, the hrcMPV strain detected in Mahale chimpanzees is closest to the Japanese strain JPS03 194 (99% in N and 99% in G genes), whereas, the hMPV P genes isolated from the three Taï Forest chimpanzees are closest to the CAN98 75 strain. There is ~94% identity between CAN98 75 N gene and JPS03 194 N gene. Data on the closely related virus, RSV, would argue against making any claims about the source of hrcMPV. There is much more data on RSV and it demonstrates that similar or identical strains (based on similar amount of sequence data) can be isolated from different countries and continents during the same year, as well as over many years. This probably reflects the extent of global travel as well as the stability of some strains over time, which is interesting given the lack of fidelity of RNA virus replication. In order to draw any conclusions regarding the possible source(s) of hMPV in habituated wild chimpanzee populations, virus-laden samples would need to be sequenced from park staff, researchers, tourists, tourist guides and local villagers. In addition, sequence data from many parts of the world would be required in order to establish local and global distributions of the different strains of hMPV.

Finding human-related paramyxoviruses, like RSV and hrcMPV, in habituated chimpanzee populations is not unforeseen. For the past quarter of a century, humans have been gradually coming into closer proximity with great apes populations. There are many examples of possible transmissions of parasites, protozoa, bacteria and even viruses to habituated great ape populations. In Beni, Zaire and at Gombe Stream National Park, Tanzania, poliovirus transmission from humans to chimpanzees was suspected and several chimpanzees died and others developed limb paresis or paralysis [Goodall, 1986; Kortlandt, 1996]. Transmission to gorillas of *Sarcoptes scabiei* and *Giardia duodenalis* in Bwindi Impenetrable National Park, Uganda and measles virus in Parc National des Volcans, Rwanda have been reported [Ferber, 2000; Graczyk et al., 2001, 2002; Salzer et al., 2007; Sholley, 1989]. At Kibale National Park in Uganda, *Escherichia coli* isolates in the feces of habituated chimpanzees were found to be genetically more similar to isolates from the feces of humans employed in research and tourism than those obtained from humans in a local village who had no regular interactions with them [Goldberg et al., 2007]. This suggests that, globalization and ease and expediency of international air travel, may be increasing the risk of introducing infectious agents into these once remote and inaccessible areas, thereby increasing the risk to habituated great apes in their natural habitats. There is mounting concern now given that great ape populations are diminishing and it is becoming more difficult for these species to recover. Furthermore, in the case of RSV and hMPV, as safe and effective vaccines are not even available, the risk of introduction to habituated great ape populations far exceeds that of other human pathogens where immunization is possible.

With increasing reports on cross-species transmissions between great apes and humans, infectious disease surveillance of great ape populations and other wildlife populations along equatorial Africa are of great significance to both public health and great ape survival. Practices need to be put into place and enforced to prevent new introductions of pathogens from humans. The following recommendations are

being made based on the limited amount of information available at this time, and knowing full well that these recommendations are subject to change as studies progress.

Disease Prevention and Surveillance

1. To prevent airborne transmission, face masks are being worn by researchers and tourists [Hanamura et al., 2006]. However, they are ''single use surgical masks'' and are being worn repeatedly. A new mask needs to be used each day that individuals travel into the forest for chimpanzee viewing; multiple uses need to be discontinued. Those coming into daily contact over longer periods of time (i.e. researchers) should wear N95 particulate filter respirators instead. N95 respirators should be designated for individual use and not be shared among different individuals. They need to be stored in a clean, dry and uncontaminated area in a manner that prevents them from being misshapen or damaged, and discarded if they become damaged, soiled or wet or if breathing becomes difficult.
2. Under no circumstances should anyone who has signs of respiratory illness visit the forest for chimpanzee viewing. Mucous discharge from the nose or oral cavity of humans should never be discharged into the forest; use of tissue paper or handkerchiefs should be encouraged.
3. Proper disposal of human waste generated in the Park should be mandatory to help reduce the risk of pathogen transmission to chimpanzees, especially as chimpanzees are known to come into close proximity to the tourist camps and research camps.
4. Occupational health programs should be established and maintained over the long term and include Park staff, researchers (faculty, staff and students), trackers and guides. The program should include everyone who regularly or frequently observes the chimpanzees and include vaccinations, TB testing and continuing education and training.
5. An educational program should be instituted so local villagers who may come to work in the Park are better informed about the Park being an important National resource. It should highlight why the animals in the Park and Park conditions need to be well maintained. It is crucial that the know-how to prevent and tackle infectious diseases be woven into the local community members so they are rooted in place for the long term. A tourist educational program would also be a good addition to the chimpanzee-viewing program.
6. Routine monitoring for infectious diseases and timely reporting of findings should be ongoing for habituated wild populations. Standardized formats for data collection should be used to facilitate analysis. All chimpanzees observed having respiratory signs should be reported immediately to a designated party responsible for follow-up. Interventions, such as vaccinations (if available), should be considered to mitigate the effects of pathogens of human origin that have been or may be introduced into a habituated population.
7. Habituation of new groups, when necessary, should be conducted taking all possible precautions. Surveillance should be conducted on non-habituated groups whenever possible, as exposures may have already occurred from humans or have spread from habituated groups. Baseline health data should be acquired and food wadge and fecal samples be obtained and banked as early as possible when attempting to habituate new groups of chimpanzees to document pre-habituation status and determine changes.
8. Old policies, as well as any new policies, should be updated, combined into a single document and re-distributed yearly, just before the onset of peak tourist season, to all contingencies viewing chimpanzees (researchers, tourists and Park staff). Policies need to be enforced.

Outbreak Response and Management

An official outbreak response and management plan should be established to tackle any and all disease outbreaks in habituated populations. The plan should be agreed on and put in place immediately so that it is available to guide the response and investigation of the next disease outbreak(s). Standard operating procedures need to be incorporated for standardized sample collection, processing and analysis. Descriptive epidemiology should be a major component of disease outbreak investigations. Outbreak response teams should include in-country medical, veterinary medical and public health professionals.

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