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Original Contribution

Codetection of Respiratory Syncytial Virus in Habituated Wild Western Lowland Gorillas and Humans During a Respiratory Disease Outbreak

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Abstract: Pneumoviruses have been identified as causative agents in several respiratory disease outbreaks in habituated wild great apes. Based on phylogenetic evidence, transmission from humans is likely. However, the pathogens have never been detected in the local human population prior to or at the same time as an outbreak. Here, we report the first simultaneous detection of a human respiratory syncytial virus (HRSV) infection in western lowland gorillas (*Gorilla gorilla gorilla*) and in the local human population at a field program in the Central African Republic. A total of 15 gorilla and 15 human fecal samples and 80 human throat swabs were tested for HRSV, human metapneumovirus, and other respiratory viruses. We were able to obtain identical sequences for HRSV A from four gorillas and four humans. In contrast, we did not detect HRSV or any other classic human respiratory virus in gorilla fecal samples in two other outbreaks in the same field program. Enterovirus sequences were detected but the implication of these viruses in the etiology of these outbreaks remains speculative. Our findings of HRSV in wild but human-habituated gorillas underline, once again, the risk of interspecies transmission from humans to endangered great apes.

Keywords: respiratory disease, respiratory syncytial virus, enterovirus, western lowland gorillas, great apes, noninvasive detection

INTRODUCTION

Great ape habituation projects have been established in Africa since the 1950s (Gruen et al. 2013). They have contributed to our understanding of great ape behavior and societies. Together with great ape tourist sites, they have also contributed significantly to the conservation of great apes and their habitats by bringing revenue to local communities (Macfie and Williamson 2010) and by protecting animals from poaching (Pusey et al. 2007; Köndgen et al. 2008; Campbell et al. 2011).

Anecdotal reports of respiratory disease (RD) outbreaks in habituated great apes reach back to the 1960s

(Lonsdorf et al. 2006). However, no systematic investigations were performed until much later, when the likely etiology of eight outbreaks in western chimpanzees (*Pan troglodytes verus*), eastern chimpanzees (*Pan troglodytes schweinfurthii*), and mountain gorillas (*Gorilla beringei beringei*) was clarified. These investigations identified two common human viruses, the human respiratory syncytial virus and the human metapneumovirus (HRSV and HMPV; family *Paramyxoviridae*, subfamily *Pneumovirinae*), as plausible causative agents (Kaur et al. 2008; Köndgen et al. 2008; Palacios et al. 2011). HRSV and HMPV circulate globally within human populations and are known to cause only mild respiratory symptoms in healthy adults. In infants however, they can lead to severe lower respiratory tract infections with significant mortality (Weber et al. 1998). In six outbreaks in wild-habituated great apes, these infections seem to have led to opportunistic coinfections with *Streptococcus pneumoniae* or *Pasteurella multocida* (Chi et al. 2007; Köndgen et al. 2008, 2011; Palacios et al. 2011). The impact of these outbreaks was often severe, with morbidity and fatality rates as high as 100 and 19%, respectively (Köndgen et al. 2008). Human paramyxoviruses have also been detected during great ape respiratory disease outbreaks in captive settings. In these outbreaks, morbidity and fatality rates reached up to 100 and 10%, respectively, despite treatment (Szentiks et al. 2009; Unwin et al. 2013; Slater et al. 2014).

Many recent studies identify enzootic viruses, such as enteroviruses (EV) and adenoviruses (AdV), in wild and captive great apes, some of which are closely related to human-infecting respiratory pathogens (Wevers et al. 2010, 2011; Harvala et al. 2011, 2014; Hoppe et al. 2015). In addition, serological evidence and experimental studies in captive settings show that great apes are susceptible to a number of other human RD-causing pathogens, such as parainfluenza viruses, rhinoviruses, or measles virus (Kalter et al. 1997; Bennett et al. 1998; Kooriyama et al. 2013; Buitendijk et al. 2014). This raises the possibility that not only human paramyxoviruses are responsible for RD outbreaks in wild great ape populations.

To identify whether other viruses are responsible for RD outbreaks, it is important to investigate the etiology and origin of even more great ape RD outbreaks. Ideally, strong onsite capacities will allow for immediate sample collection and diagnostics of both great apes and neighboring human populations, thereby maximizing the chances of identifying causative agents and possibly therapeutic and preventive options. Here, we report about three RD

outbreaks that struck two habituated western lowland gorilla (*Gorilla gorilla gorilla*) groups at a research and tourism program in Dzanga Sangha Protected Areas in Central African Republic between March 2012 and November 2014. The second outbreak was investigated with a field laboratory onsite, and analyses were performed while the outbreak was active (real-time investigation). This outbreak occurred simultaneously in humans and one habituated gorilla group in August 2012. Respiratory symptoms in the local human population, including coughing, nasal discharge, sneezing, elevated breathing rate, and wheezing sounds during expiration, occurred before the first symptoms in the gorillas were noted.

MATERIAL AND METHODS

Field Sites

Situated in the northwestern Congo Basin, Dzanga Sangha Protected Areas (DSPA) in Central African Republic (CAR) includes the Dzanga-Ndoki National Park, which, together with Lobéké National Park in Cameroon and Nouabalé-Ndoki National Park in the Republic of Congo, form the Sangha Tri-National World Heritage Site (inscribed by UNESCO in 2012). The DSPA is managed by a partnership between the national government of CAR and Worldwide Fund for Nature (WWF). As part of WWF's long-term habituation project, the Primate Habituation Program, where two groups of gorillas have been fully habituated (Makumba and Mayele group) at adjacent sites (Bai Hokou and Mongambe, respectively, approximately 10 km apart); another two groups were still under habituation at the time of the study. Within the National Parks, human activities such as hunting, gathering, fishing, and agriculture are prohibited and access is strictly regulated. Humans do not permanently live in the park but temporarily stay in one of the several research camps. Tourists are not allowed to stay overnight. The people working for the project, and thus entering gorilla habitat, live either in or close to the village of Bayanga, which lies within DSPA, outside of Dzanga-Ndoki National Park (Fig. 1).

Samples

Outbreak 1 (February 2012)

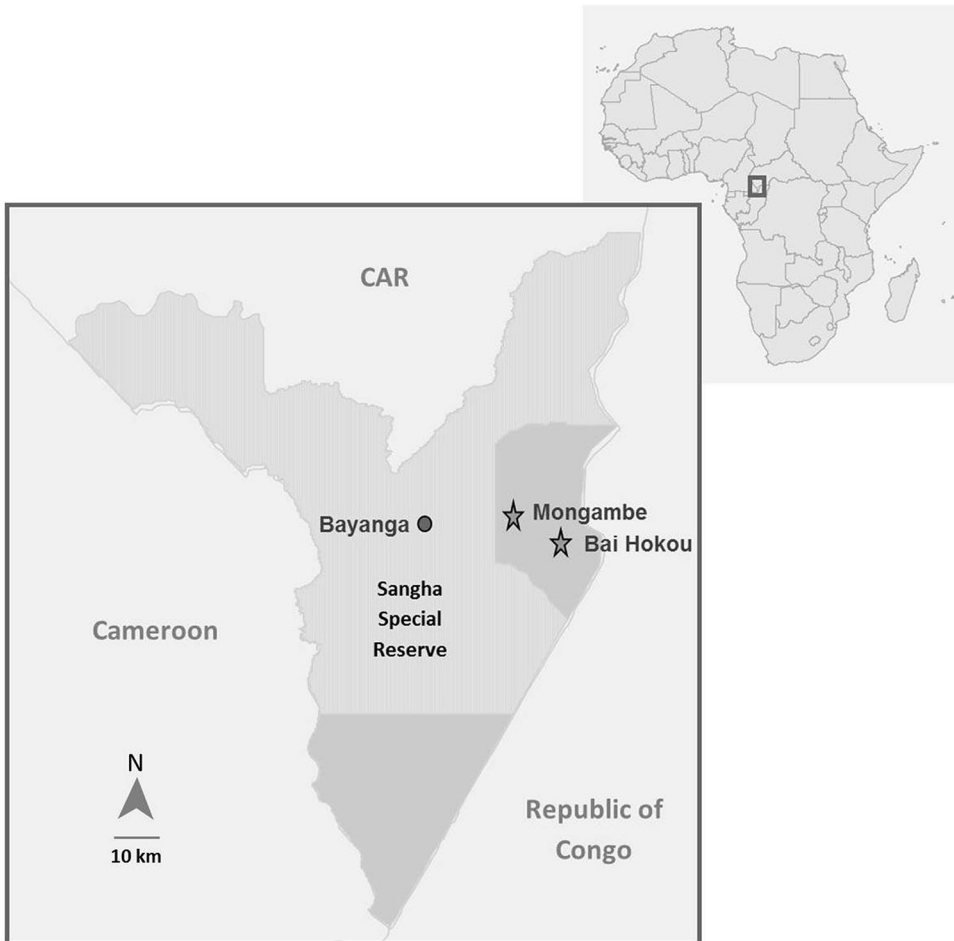Seven (individually identified) gorilla fecal samples, from the seven symptomatic members of the Makumba group

Figure 1. Map of field site.

(group size, N = 9) were collected, put into tubes containing Ambion® RNAlater® Solution (Invitrogen) and kept at ambient temperature. The severe symptoms observed in this outbreak (very productive cough to the point of vomiting and by day 24, 8 out of 9 group members were affected), which continued until April 2012, warranted the temporary halt of tourist visits and a veterinary intervention with antibiotics (described in Vlckova et al, submitted). Additionally, fifteen human fecal samples were collected from the corresponding research camp.

Outbreak 2 (August 2012)

Fifteen gorilla fecal samples from eight (individually identified) symptomatic individuals of the Makumba group were collected. Symptoms included cough, lethargy, and running noses. By day 14, 7 out of 8 group members were affected. Each member of the group was sampled at least once. Individuals with the most severe symptoms (highest

frequency of coughing) were sampled repeatedly. Simultaneously, a total of 80 throat swabs were taken from humans who enter the gorilla habitat (or were in direct contact with those who do), divided into six demographic groups: tracker (guides) (N = 25), symptomatic tracker family members (N = 9), researcher (N = 2), symptomatic researcher family member (N = 1), project/camp assistants (N = 5), and eco-guards (park rangers) (N = 38). The samples were put into tubes containing Ambion® RNAlater® Solution and maintained at ambient temperature for 2–3 days. Afterwards they were frozen at −14°C.

Outbreak 3 (August 2014)

Ten fecal samples from two of the most severely symptomatic gorillas (highest frequency of coughing) of the Mayele group (group size, N = 15), were taken initially with fifteen more samples from ten (individually identified) individuals collected at a later stage of the outbreak

Table 1. Primers Used in This Study

Pathogen	Assay type	Primers	Conditions described in
Adenovirus (AdV)	TaqMan PCR	**AD-024S** GAC GCY TCG GAG TAC CTG AG **AD-024R** RGC CAG IGT RWA ICG MRC YTT GTA **AD-024 probe** 6FAM-CTG GTG CAG TTY GCC CGC-TAMRA	Chmielewicz et al. 2005
Corona virus (CoV)	Conventional PCR	**CoV All F** AAR TTT TAY GGY GGB TGG VAT RAY ATG TT **CoV All R** TGY TGD GAR CAR AAY TCR TGW GGT CC	Nitsche, unpublished data*
Enterovirus (EV)	TaqMan PCR	**E-TM 1 S** GCC CCT GAA TGC GGC TAA T **E-TM 2 R** RAT TGT CAC CAT AAG CAG YCA **EV probe** 6FAM AAC CGA CTA CTT TGG GTG TCC GTG TTT C-TAMRA	Pusch et al. 2005
Human metapneumovirus (HMPV)	Two-step real-time RT-PCR for screening	**hMPV F S** gCTCCgTAATYTACATggTgCA **hMPV F S1** gAAgCTCYgTgATTTACATggTYCA **hMPV F AS** gACCCTgCARTCTgACAATACCA **hMPV F AS1** AgTKgATCCTgCATTTTTACAATACCA **hMPV F TMGB** F-CCYTgCTggATAgTAAAA-MGB **hMPV F TMGB1** F-CCTTgTTggATAATCAA-MGB	Reiche et al. 2014
	Conventional confirmation PCR	**MPVP 01.6 S** ATG TCA TTC CCT GAA GGA **MPVM 02.4 R** GTC TAC TAG GTA GGA CTC CAT	Mackay et al. 2004
Human respiratory syncytial virus (HRSV)	TaqMan PCR for screening	**RSV 1084** GAT GGC TCT TAG CAA AGT CAA GTT **RSV 170** CAT CTT CWG TGA TTA ATA RCA TCR CAC ATA **RSV MGB probe** FAM-ACA GGA GAT ART ATT DAY ACT C-NFQ MGB	Reiche and Schweiger 2009
HRSV A	Semi-nested confirmation PCR	**GPB** AAG ATG ATT ACC ATT TTG AAG T **F1** CAA CTC CAT TGT TAT TTG CC **nRSBG** GTG GCA ACA ATC AAC TCT GC	Sato et al. 2005
HRSV B		**GPA** TTG AAG TGT TCA ACT TCG TTC C **F1** CAA CTC CAT TGT TAT TTG CC **nRSAG** TAT GCA GCA ACA ATC AAC C	
Influenza virus A (Flu A)	Real-time PCR	**M+25** AgA TgA gTC TTC TAA CCg Agg TCg **M-124BB** ccW gCA AAR ACA TCY TCA AgT YTC Tg **M-124sw** CTg CAA AgA CAC TTT CCA gTC TCT g **M+64 MGB** FAM-TCA ggC CCC CTC AA-MGB	Schulze et al. 2010
Influenza virus B (Flu B)	Real-time PCR	**BMP-13** gAg ACA CAA TTg CCT ACC TgC **BMP-102**AN TTC CCA CCR AAC CAR CAR TgT AAT **BMP-72** MGB FAM-CTg CTT TgC CTT CTC-MGB	Schulze et al. 2010
Pan pneumovirus	Semi-nested PCR	**PNE-F1** GTG TAG GTA GIA TGT TYG CNA TGC ARC C **PNE-F2** ACT GAT CTI AGY AAR TTY AAY CAR GC **PNE-R** GTC CCA CAA ITT TTG RCA CCA NCC YTC	Tong et al. 2008
Pan paramyxovirus	Semi-nested PCR	**PAR-F1** GAA GGI TAT TGT CAI AAR NTN TGG AC **PAR-F2** GTT GCT TCA ATG GTT CAR GGN GAY AA **PAR-R** GCT GAA GTT ACI GGI TCI CCD ATR TTN C	Tong et al. 2008
Pan picornavirus	Semi-nested PCR	**OL-26 s** GCA CTT CTG TTT CCC C **OL-27 as** CGG ACA CCC AAA GTA G **JWA-1b as** CAT TCA GGG GCC GGA GGA	Jang et al. 2005

Table 1.   continued

Pathogen	Assay type	Primers	Conditions described in
S. pneumoniae	TaqMan PCR for screening	**SP_autolysin_F** ACG CAA TCT AGC AGA TGA AGC **SP_autolysin_R** TGT TTG GTT GGT TAT TCG TGC **SP_lytA_TM** FAM-TTT GCC GAA AAC GCT TGA TAC AGG G–TAMRA	MacAvin et al. 2001

PCR performed in a total volume of 25 μl with 0.1 μl Platinum Taq polymerase (Applied Biosystems, Darmstadt, Germany), 0.75 μM of each primer in a 300 nM concentration, a 200 μM concentration of each deoxynucleoside triphosphate (dNTP), and 2 mM MgCl₂; cycling conditions: 95°C for 5 min and 45 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 1 min, with a final 7 min of extension at 72°C and an expected amplicon size of 670 bp.nnn

(10 weeks later). A cough persisted in the group over several months, with varying intensities and frequencies in different individuals. Sample collection was attempted from all group members, if possible, with an emphasis on those with a higher frequency or intensity of coughing. Samples were put into tubes containing Ambion® RNA*later*® Solution and kept at ambient temperature. No human samples were obtained during this outbreak. All samples were transported to Germany, where they were stored at −80°C.

Field Lab Analysis/Real-Time Investigation (Performed During Outbreak 2)

Eight gorilla fecal samples and 20 human throat swabs were tested in a field laboratory. RNA was extracted from fecal samples and cDNA synthesized as described by Köndgen et al. (2010). RNA from throat swabs was extracted using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Samples were screened for HRSV and HMPV using generic PCR protocols as described by Reiche and Schweiger (2009) and Reiche et al. (2014), excluding the probes, targeting the N or F protein gene, respectively, with expected amplicon sizes of 142 and 161 bp. Amplification was conducted for 5 min at 95°C, followed by 45 cycles of 95°C for 15 s, and 60°C for 30 s. PCR products were analyzed using electrophoresis in a 2% agarose gel. Positive and negative controls were included in each run. Positive samples were tested with an additional confirmation PCR; for HRSV, a hemi-nested PCR was performed targeting the hypervariable region of the G protein gene as described previously by Sato et al. (2005). Amplification was carried out for 5 min at 94°C, followed by 40 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min, with a final 7 min of extension at

72°C. PCR products were analyzed by electrophoresis in a 1.5% agarose gel, with an expected amplicon size of 458 bp. Human and gorilla samples were processed separately, spaced at two weeks between with gorilla samples tested first.

Confirmation and Additional Testing at the Robert Koch-Institute

In addition to all remaining samples (seven gorilla, 60 human), the eight gorilla and 20 human samples screened in the field were retested upon their arrival in Germany. RNA was extracted as described above; gorilla and human samples were handled separately, on separate days. The samples that had been tested in the field were extracted again. Samples were tested in duplicates, for human respiratory pathogens, following the protocols summarized in Table 1, with the exception of human throat swabs, which were not tested for *S. pneumoniae*. PCR products from positive samples were purified using ExoSAP (USB Europe GmbH, Staufen, Germany) and sequenced using the ABI BigDye Termination Kit (Applied Biosystems, Weiterstadt, Germany). When multiple bands were present, the expected band was cut from the gel, purified, and sequenced. If no clean sequence could be obtained, the procedure was repeated and the purified PCR products were cloned with the Topo TA Cloning Kit (Invitrogen). Colonies were analyzed using colony PCR, and positive samples were purified and sequenced. When confirmation by other systems was not possible, the qPCR products were also sequenced.

All viral sequences generated were analyzed using Geneious R7.1.4 (Biomatters Limited) and blasted against the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Samples were only considered positive for a given pathogen if a sequence of sufficient quality could be obtained.

Phylogenetic Analysis

We did not perform phylogenetic analyses of the HRSV sequences, as the identical sequences found in gorilla and local human samples already substantiated a recent transmission event. In contrast, the enterovirus (EV) sequences did not find any close to perfect match in public databases. We therefore performed phylogenetic analyses on a dataset that also comprised 109 representative sequences of human and nonhuman primate EV sequences. The sequences were aligned using Muscle (Edgar 2004), and conserved blocks of the alignment were selected using Gblocks (Talavera and Castresana 2007). Both steps were performed in SeaView v4 (Gouy et al. 2010). The best model of nucleotide evolution was identified using jModelTest v2.1.4 (Guindon and Gascuel 2003; Darriba et al. 2012), applying the Bayesian information criterion. Phylogenetic analyses per se were performed in maximum likelihood (ML) and Bayesian frameworks. ML analyses were performed using PhyML v3 (Guindon et al. 2010); branch robustness was determined by nonparametric bootstrapping (250 pseudoreplicates). Bayesian analyses were run in BEAST v1.8.2 (Drummond et al. 2012) under a lognormal-relaxed clock and describing tree shape with a coalescent Bayesian SkyGrid model. Two chains were run for 50 million generations; convergence of the runs and appropriate sampling of the posterior were assessed using Tracer v1.6 (Rambaut et al. 2014). Post burn-in trees from the two chains were combined using LogCombiner v1.8.2 before being summarized onto the maximum clade credibility tree identified with TreeAnnotator v1.8.2 (both software programs are distributed with BEAST). Branch robustness was assessed through posterior probabilities.

Results

For an overview of results see Table 2, details in Table 3.

Outbreak 1

All gorilla fecal samples were negative for HRSV and HMPV; one tested positive for EV. The closest hit in the NCBI database, with 90% identity, was Simian agent 5 (strain B165, complete genome; Accession: AF326751.2). All gorilla samples were negative for other pathogens tested.

All simultaneously collected human fecal samples also tested negative for HRSV and HMPV; one also tested positive for EV. The closest hit in the NCBI database was swine vesicular virus (isolate ITL 2/92 5' UTR; Accession: AY875991.1) with 89% identity and human enterovirus 71 (isolate 17001, gene for polyprotein, partial cds; Accession: AB575924.1) with 90% identity.

The gorilla EV differed in 83/234 positions from the human EV.

Outbreak 2

Field Analysis

Of the fifteen gorilla fecal samples from eight individuals, four samples (from four different individuals) tested positive for HRSV in the field laboratory with the generic screening PCR. The electrophorese gels of the confirmation PCRs showed multiple bands, which were difficult to interpret. Three of the 20 human throat swabs (one

Table 2. Overview of Findings

	Outbreak 1 (Start Feb 2012)	Outbreak 2 (Start Aug 2012)	Outbreak 3 (Start Aug 2014)
Gorilla group	Makumba	Makumba	Mayele
No of individuals affected	8	6	5
Duration	Feb–Apr 2012	Aug–Sep 2012	Aug 2014–Feb 2015
Pathogens found in gorilla fecal samples	EV (1/7)	HRSV (4/15), AdV (1/15)	EV (12/25)
Pathogens found in human samples (fecal samples and throat swaps)	EV (1/16), AdV (5/16)	HRSV (4/80), HMPV (1/80), RV (1/80)	N/A

Table 3. Sequences Obtained in Three Independent Outbreaks

	Sample type	No. of samples	Pan Pneu L gene ~240 bp	HRSV N gene ~140 bp	HRSV G gene ~450 bp	HMPV P gene ~350 bp	EV 5'UTR ~320 bp	RV 5'UTR ~390 bp
Outbreak 1								
Gorillas	Feces	7	–	–	–	–	1	–
Humans	Feces	15	–	–	–	–	*1*	
Outbreak 2								
Gorillas	Feces	15	**3**	**4**	1 (poor quality)	–	–	–
Researcher	Throat swab	2	–	–	–	–	–	–
Researcher family member	Throat swab	1	**1**	**1**	1	–	–	–
Camp assistant	Throat swab	5	**2**	**2**	2	–	–	1
Tracker	Throat swab	25	–	–	–	–	–	–
Tracker family member	Throat swab	9	**1**	**1**	1	–	–	–
Eco-guards	Throat swab	38	1	–	–	1	–	–
Outbreak 3								
Gorillas (beginning)	Feces	10	–	–	–	–	6	–
Gorillas (10 weeks later)	Feces	15	–	–	–	–	6	–

Sequences identical for humans and gorillas in outbreak 2 highlighted in bold and for outbreak 1 and 3 in italics.

researcher, one researcher family member, and one tracker family member) tested for HRSV with the generic screening PCR were also positive on the gel.

Analyses at the Robert Koch-Institute

All collected samples were (re)tested in conventional laboratory settings. All field laboratory results from the four gorilla samples could be confirmed by real-time PCR. The sequences obtained from this assay were 100% identical with known HRSV A strains (RSVA/Homo sapiens/USA/TH_10454/2013, complete genome; Accession: KU950698.1). Unfortunately, from the confirmation assays, no sequences of satisfying quality could be obtained for phylogenetic analyses. From three of the four positive samples, sequences could be generated from the pan-pneumovirus assay (targeting the L protein gene) and were 99% identical with known HRSV A strains (human respiratory syncytial virus isolate Kilifi_10028_12_RSVA_2003, partial genome; KP317955.1). All other pathogen tests (as in Table S1) were negative.

Of the 20 retested human samples, two HRSV positive and all negative samples from the field could be confirmed. Only one sample that tested positive for HRSV in the field could not be confirmed. An additional two human throat swabs (from two camp assistants), from the 60, which had not been tested in the field, came up positive for HRSV.

Sequences of relevant length could be obtained, using further PCRs targeting the G and the L genes; sequences of the G gene were 99% identical with known HRSV A strains (RSV/PUNE/NIV1063010/10/A attachment glycoprotein G gene, complete cds; and fusion protein F gene, partial cds; Accession: KF246622.1). All HRSV sequences from the different human individuals were identical, and the L and N protein gene sequences obtained from human and gorilla samples were also identical each. One eco-guard tested positive for HMPV and one camp assistant for rhinovirus. All other human throat swabs were negative for all remaining pathogens tested (note they were not tested for *S. pneumoniae*, because characterization of streptococci from a throat swab, only based on molecular methods, is very challenging and needs careful interpretation).

Outbreak 3

Ten fecal samples from two gorillas tested negative for HRSV and HMPV. EV was detected in both individuals. The sequences were identical with each other and the human sequence obtained from outbreak 1 but clearly distinct from the gorilla sequence from outbreak 1 (Fig. 2). Fifteen samples from 10 individuals of the same group, including the two initially tested individuals, were tested two months after the onset of the outbreak when symptoms were con-
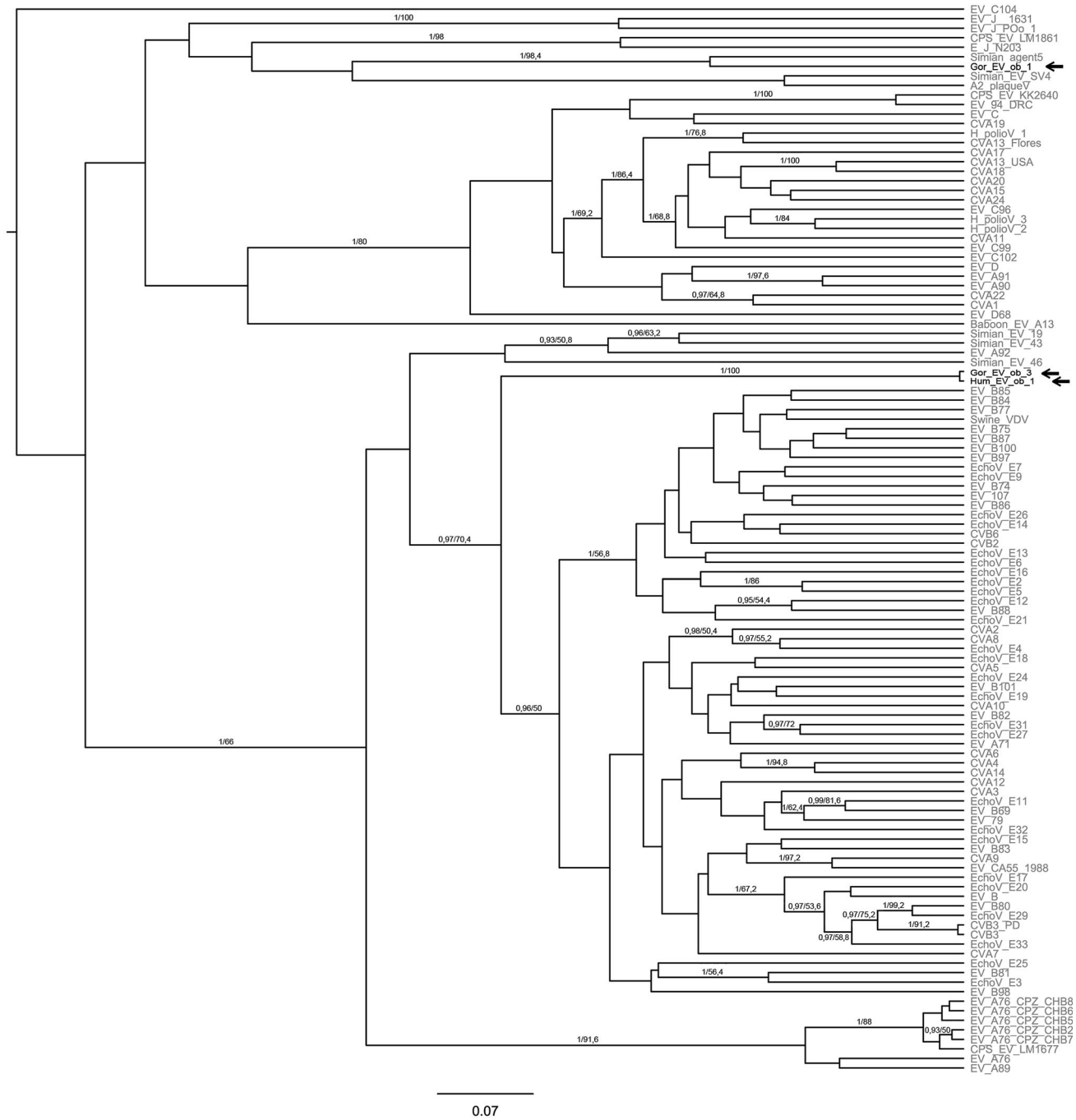
Figure 2. Phylogenetic positions of enteroviruses found in a human and a gorilla and two years later in another gorilla group during respiratory disease outbreaks. EVs were named as follows: 'Gor_EV_ob_1' Gorilla Enterovirus outbreak 1, 'Hum_EV_ob_1' human enterovirus outbreak 1, 'Gor_EV_ob_3' Gorilla Enterovirus outbreak 3. This tree is a maximum clade credibility tree generated from the output of Bayesian Markov chain Monte Carlo (BMCMC) analyses. We also ran maximum likelihood (ML) analyses; the ML tree was topologically very similar. Branch robustness was assessed through posterior probabilities (BMCMC) and nonparametric bootstrapping (ML); posterior probabilities/bootstrap values are plotted above branches.

tinuing or increasing in some individuals. The same EV was found in three gorillas that had the most severe symptoms (i.e., highest frequency and intensity of coughing). All sequences were compared to the nonredundant nucleotide sequence database of the National Center for Biotechnology Information (NCBI), using BLAST. The closest hits were swine vesicular virus and human enterovirus 71 (isolates as above in outbreak 1). In line with BLAST results, the new

gorilla EV sequence did not appear in close sistership with any EV sequence published to date (Fig. 2). Furthermore, it did not cluster with any sequences identified from wild chimpanzees, or other nonhuman primates that could be included in these analyses. All samples were negative for the remaining pathogens tested.

DISCUSSION

Until recently, diagnostics of respiratory pathogens were often only made after the outbreaks had taken place. Yet, timely onsite diagnostics allow for better disease management, ranging from targeted interventions (e.g., treatments by blow pipe using selected antibiotics as in our Outbreak 1) to management decisions (quarantine or similar for humans). Importantly, this approach also greatly enhances the likelihood of tracing back the origin of a given outbreak. The first simultaneous detection of HRSV in humans and wild-habituated great apes, as described for our outbreak 2, represents compelling evidence of a cross-species transmission from humans. That said, a spillback of the virus into the human population also remains possible.

No fatalities occurred in any of the outbreaks. All investigations were performed noninvasively. Successful detection of viruses in great ape feces can vary, however, depending on the viral load excreted, which will change over the time of the outbreak (Köndgen et al. 2010). Unfortunately, RNA degrades quickly in the environment and successful detection might also be influenced by variables such as food intake. This could explain why additional samples from individuals that tested positive for HRSV during outbreak 2 tested negative.

Real-time investigations can be challenging. For this study, during outbreak 2, throat swabs from humans were taken when the gorillas had already started showing symptoms and not at the exact time when the transmission would have occurred. Thus, the infectious pressure that can be determined from the samples, taken after the onset of the outbreak, does not represent the infectious pressure at the time of presumed transmission. However, the virus was circulating in the human population in the same time period as in the gorilla group, and likely before. This, along with the finding of other respiratory viruses circulating in the human population, also demonstrates the importance of an adequate onsite field laboratory to continuously monitor the health of habituation project staff, the sur-

rounding human populations, and potentially any visitors who are brought into close contact with the apes.

Another reason that makes a human-to-gorilla-transmission plausible is that the sporadic contact between gorilla groups makes it highly unlikely that HRSV circulated within the ape population. In the described cases, group composition did not change shortly before the outbreaks started and no encounter with other groups was observed. However, once a pathogen enters a group, there are some distinct ape species differences with a marked effect on the potential outcome. In a habituated western lowland gorilla group, habituation success and group stability largely depend on the silverback. In contrast to chimpanzee communities, if the silverback dies during the course of an outbreak the remaining group members will disperse, which can lead to pathogen-mediated dispersal (Nunn et al. 2008). This would not only bear considerable economic losses for the habituation project but comes with the risk of infected group members joining other groups and thereby spreading the infection.

The habituation project described here, has employed a range of measures to prevent disease transmission from humans, including controlled human health status, no sick people allowed with the gorillas (although this does not mitigate people being nonsymptomatic carriers), minimum viewing distance, small observer groups, no eating, spitting, or leaving anything behind in the forest. Yet, facemasks had not been included in the prevention program, relying instead on behavioral mechanism such as, if provoked, turning away and coughing or sneezing into clothing, not hands. The use of facemasks, as a preventive measure, is widely recommended (Gilardi et al. 2015), based on assumptions made from human hygiene and respiratory disease prevention. For influenza viruses, which show similar modes of aerosol transmission as pneumoviruses, wearing of surgical masks can reduce the overall viral copy numbers of exhaled aerosols by 3.4-fold; however, it cannot completely avoid the shedding of infectious virus beyond the mask (Milton et al. 2013). Commonly used surgical facemasks and N95 respirators have been shown to be equally effective in filtering influenza virus (Johnson et al. 2009). Unfortunately, compliance seems to be a major limitation resulting in lower efficacy of face mask use (Radonovich et al. 2009). In a hot and humid environment under physical strain, wearing a N95 facemask is uncomfortable and it is unlikely that recommendations will be followed at all times. Due to the incomplete protection through face masks, the additional use of a field lab, which

detects virus shedding, and a quarantine system could be an effective asset to existing prevention programs, as it does not rely as much on human compliance—although this would present logistical difficulties for tourism programs. An in-situ field lab could also be used for real-time and onsite diagnostics in other wildlife disease outbreaks, which would allow for timely management decisions. Furthermore it is useful in distinguishing humans infected with (and shedding) a respiratory pathogen from those with, for instance, a noninfectious smokeŕs cough. Importantly, 96% (23/24) of the results from the field were confirmed at the Robert Koch-Institute in Germany, which supports the efficacy of a field laboratory.

In our outbreak 2, eight of the nine human tracker family members tested were infants <5 years of age. 50% of the HRSV positive samples stem from these infants, who made up only 10% of the human sample pool collected during the outbreak. However, in addition to small sample size, this analysis is biased, because only infants with respiratory symptoms were sampled. In contrast, only 30 of the 72 adults showed mild respiratory symptoms, of which most can likely be attributed to smokeŕs cough. Even though only opportunistic sampling of symptomatic family members was performed, infants were the only individuals with severe respiratory symptoms (including wet cough, sneezing, nasal discharge, wheezing). HRSV is believed to be the most important viral pathogen causing acute lower respiratory disease (ALRI) in young children, with ALRI being the leading cause of global child mortality, with 99% of RSV-caused deaths occurring in developing countries (Nair et al. 2010). Thus, the contact of project staff and researchers with young children may represent a risk factor for the transmission of pneumoviruses to wild-habituated great apes. A One Health approach, improving childreńs health around great ape habitat, could help to reduce infectious pressure on wild great apes.

In recent years, several enzootic viruses have been identified in wild great apes, some of which might cause clinical disease, such as EV and AdV. EV are among the most common human viruses, with high rates of subclinical infections (Palacios and Oberste 2005). Yet, they can be the cause of several, sometimes severe diseases and syndromes, such as acute hemorrhagic conjunctivitis, aseptic meningitis, acute flaccid paralysis, myopericarditis, hand-foot-and-mouth disease, and respiratory disease. Harvala et al. (2014) and Sadeuh-Mba et al. (2014) showed that EV are cocirculating in human and wild great ape populations; some are shared virus types and some genetically divergent EV variants. Unfortunately, studies investigating EV

occurrence in wild great apes do not reveal information on the health status of the investigated animals; therefore, it is difficult to determine whether the pathogens had caused any illness. In captive settings, some human EV strains have been shown to cause RD in different nonhuman primates, including great apes (Kelly et al. 1977; Zhang et al. 2011; Nielsen et al. 2012). In our study, we found EV in both gorillas, and a human. The presence of an EV in the human population or wild great apes, respectively, is per se not surprising. However, the finding of the same virus in a human, and two years later in a gorilla group, is an indicator for yet another transmission, even if the directionality cannot be determined. Unlike HRSV, which is rather unstable in the environment, EV infection can occur, for instance, through contaminated water (Harvala et al. 2014). Thus a cocirculation in ape and human populations does not require the same physical proximity. The viruses found are unknown strains, which could be enzootic great ape strains or human EV strains unknown due to underreporting in the region. The fact that the shared EV was detected in the most symptomatic gorilla individuals could point toward its involvement as a causative agent.

In conclusion, our study adds to the growing body of evidence substantiating the early concerns expressed by Woodford et al. (2002). It further demonstrates that onsite real-time health monitoring offers excellent potential to untangle the causes of RD in habituated great apes. Once more, it pinpoints the involvement of human respiratory pathogens as likely causative agents.

Despite the risk of disease transmission that human presence in great ape habitat poses to the animals, research and tourism sites have also been demonstrated to have protective effects (Pusey et al. 2007; Köndgen et al. 2008; Campbell et al. 2011). To minimize the negative effects of human presence, disease surveillance and development of effective prevention strategies are crucial for the conservation of these endangered species.

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INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.

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