ORIGINAL ARTICLES

Absence of paramyxovirus RNA in non-human primate sanctuaries and a primatology center in Gabon

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ABSTRACT

The viruses of the *Paramyxoviridae* family are known to infect a wide range of animals, including primates, birds, rodents, carnivores, bats, ungulates, snakes, cetaceans and humans. This study aims to investigate the circulation of paramyxoviruses in five potential host species groups (humans, non-human primates, rodents, shrews, and bats) living in the same environments in three conservation programs dedicated to non-human primates, namely the Lékédi park, the primatology center of the International Center for Medical Research of Franceville and the Gorilla Protection Program, located in Gabon. We tested 35 workers, 343 NHPs (8 species), 141 bats (4 species), 420 rodents (5 species) and 10 shrews, sampled between 2013 and 2014. Faecal and organ samples were analyzed using three heminested reverse transcription-PCR (hnRT-PCR). All the 1884 samples tested were negative for PV detection. Further studies spanning a greater period of time are needed to investigate PV circulation patterns in these conservation programs.

Key Words: Bats, Confined environment, Humans, Rodents, Shrews, Sympatric species

1. INTRODUCTION

The *Paramyxoviridae* family is one of the eight families assigned that form the Mononegavirales order and is composed of seven genera with the *Avulavirus*, *Henipavirus*, *Morbillivirus*, *Respirovirus*, *Rubulavirus*, *Aquaparamyxovirus* and *Ferlavirus*.[1] Paramyxoviruses (PVs) are known to infect a diverse range of hosts including bats,[2,3] rodents,[4,5] primates,[6] snakes, lizards and tortoises.[7] They are transmitted either directly via inhalation of nasopharyngeal secretions, or indirectly through environments contaminated with faeces, urine or saliva.[8] Frequency of at-risk contact and proximity between individuals/species enhance the spread of pathogens

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which are directly transmitted.[9,10] In primate conservation programs such as the Parc de la Lékédi (LEK; Lékédi parc), the Centre de Primatologie (CDP; Primatology Center) of the Centre International de Recherches Médicales de Franceville (CIRMF; International Center for Medical Research of Franceville) and the Projet Protection des Gorilles (PPG; Gorilla Protection Program), located in Gabon, the proximity and repeated contacts between humans and nonhuman primates (NHPs) are very common. Some cases of zoonotic transmission of paramyxoviruses have already been reported, particularly through the close contact of humans with infected animals (horses for the Hendra virus and pigs for the Nipah virus).[11] In Africa, the identification of the Morbillivirus in frugivorous bats *Eidolon helvum* and *Hypsignathus monstrosus* closely related to the human mumps virus in Congo (Drexler et al. 2012) and the identification by a wildlife biologist in Uganda of Sosuga virus in Egyptian rousette bats (*Rousettus aegyptiacus*)[12] suggests bats could be natural reservoir and confirms the zoonotic potential of PV. In the CIRMF's primatology center, frugivorous bats, rodents and shrews live in sympatry and feed on food leftovers in the feeding areas of NHPs. Frugivorous bats such as *Rousettus aegyptiacus* and *Eidolon helvum* have already been shown to host a wide diversity of paramyxoviruses including Henipa, Morbilli and Rubulaviruses, some of these being closely related to the mumps virus.[2] It is thus expected that such environments, in which different wildlife species mingle, attracted by shelter and food, could enhance the circulation of paramyxoviruses within and between different host species. In general, most studies on wildlife are focused on pathogen exchanges between a single wild species and humans[6,13] or on pathogen characterization in a single wild host species,[3] thus neglecting the spectrum of hosts that could play a major role in pathogen persistence. In this paper, we investigated five potential host species of paramyxoviruses (humans, NHPs, rodents, shrews, and bats) living in the same environments in order to evaluate the circulation of paramyxoviruses in conservation programs dedicated to primates.

2. Methods

2.1 Sites and study population

The sampling was conducted in 2013 and 2014 in three sites 100 km away on average and located in the province of Haut-Ogooué, east of Gabon: le Centre de Primatologie (1°37'59" N/13°34'59" E) of the CIRMF, the Parc de la Lékédi (1°28'0"S, 13°0'0"E), and the Projet de Protection des Gorilles (1°35'03.84"S, 14°15'56.73"E) located in the National Park of the Plateaux Batékés. After a quarantine stay in the CDP, some species of NHPs seized by the Ministry of Water and Forests from private owners are transferred to the LEK (chimpanzees and gorillas) or the PPG (gorillas). The CDP hosts more than 350 NHPs belonging to endemic species such as chimpanzees (*Pan troglodytes troglodytes*), gorillas (*Gorilla gorilla gorilla*), mandrills (*Mandrillus sphinx*), torquatus (*Cercocebus torquatus*), solatus (*Cercopithecus solatus*), cephus (*Cercopithecus cephus*), and imported species such as vervets (*Chlorocebus aethiops*) and macaques (*Macaca muletta* and *M. rhesus*). A team of 22 people (veterinary and caregivers) provides animal care, food and monitoring. NHPs live either in a forest enclosure (*Mandrillus sphinx, Cercopithecus solatus and cephus*) or in large aviaries (for the other NHPs species). Several animal species coexist with NHPs, including frugivorous bats, rodents and shrews, which feed during the night on food and banana leftovers in the feeding or storage areas of NHPs (field observations). The LEK hosts two groups of 15 chimpanzees each, and one group of 3 gorillas. A team of 7 people provides appropriate animal care, food and monitoring. Tourists also visit the park. The aim of the PPG is to reintroduce gorillas in their natural habitat. Animals came from European zoos or were seized by the Ministry of Water and Forests from private owners. In 2013 and 2014, the project was composed of two groups of gorillas, one group which was released, and a second group of 4 individuals which was in a habituation program aimed at ecotourism. The sampling was carried out on this last group. Nine people work in this program and participate in the process of releasing of gorillas.

2.2 Sampling

As recommended by the National Ethics Committee for Research (CNER), human samples were collected by the CIRMF's medical team and based on the workers' voluntary participation, and the NHP samples were collected by veterinaries following routine health procedures and during occasional veterinary consultations. Bats were captured using mistnets (12 m×2.4 m) during 30 nights each year at different locations in the CDP following recommendations in the study by Kunz TH and Parsons S.[14] The rodents and shrews (micromammals) were captured using live traps (Tomahawk and Sherman) as described by Duplantier.[15] The trap grid covered the total area of the CDP and 149 traps were placed at 30m intervals within a radius of 1.2 km around aviaries and enclosures. As described in previous studies,[3,5] the euthanasia of bats and micromammals was performed through the use of an inhalant anesthetic (halothane) and followed by an autopsy through which selected internal organs including spleen, lungs, liver, intestine and kidney were collected. The faecal samples from sanctuaries were kept frozen during the transport to the CIRMF laboratory. Otherwise, faecal samples of NHPs were collected in sterile

tubs by workers and all samples were stored at -80°C until analysis. Bats and micromammals species were identified by trained field biologists according to the identification keys of the species[16, 17] and confirmed by molecular analysis as described by.[18]

Table 1. Overview of specimens collected in different sites and tested by hemi-nested PCR. N number of samples analyzed, n is number of individuals studied, CDP (le Centre de Primatologie), LEK (Park de la Lékédi), PPG (Projet protection Gorilles) and NHP (Non-Human Primates)

Samplingsite	Human			NHPs			Micromammals			Bats			Nature of samples
	species	n	N	species	n	N	species	n	N	species	n	N	
CDP	H. sapiens	22	39	P. t. troglodytes	65	87	Lemniscomys striatus	93	93	Epomops franqueti	106	106	**Faecal**
				G. g. gorilla	5	8	Lophuromys nudicaudus	52	52	Hypsignathus monstrosus	14	14	
				M. sphinx	197	411	Mus musculus	136	136	Eidolon helvum	12	12	
				C. torquatus	2	2	Praomys spp	89	89	Rousettus aegyptiacus	9	9	
				C. solatus	13	13	Rattus rattus	40	40				
				C. cephus	5	5	Sylvisorex ollula	7	7				
				C.aethiops	9	9	Sylvisorex johnstoni	3	3				
				Macaca sp	54	66							
LEK	H. sapiens	7	33	P. t. troglodytes	19	64							
				G. g. gorilla	2	8							
PPG	H. sapiens	9	13	G. g. gorilla	4	4							
Total		**38**	**85**		**375**	**677**		**420**	**420**		**141**	**141**	
CDP							Lemniscomys striatus	93	93	Epomops franqueti	106	106	**Organs**
							Lophuromys nudicaudus	52	52	Hypsignathus monstrosus	14	14	
							Mus musculus	136	136	Eidolon helvum	12	12	
							Praomys spp	89	89	Rousettus aegyptiacus	9	9	
							Rattus rattus	40	40				
							Sylvisorex ollula	7	7				
							Sylvisorex johnstoni	3	3				
Total								**420**	**420**		**141**	**141**	

2.3 Molecular analysis

The extraction of viral RNA was carried out on a BioRobot EZ1 automat (Qiagen, Germany). Samples (spleen, lungs, liver, intestine and kidney) were first processed in pools by species, up to four specimens per pool. Then, for fecal specimens, ca. 100 mg of faeces of each individual were pooled and suspended in 500 μl of phosphate buffered saline (Biological Diagnostic Supplies Ltd, UK) as previously described (Drexler et al. 2009). For organ specimens, ca. 100 mg of spleen, lungs, liver, intestine and kidney of each individual were pooled and crushed as previously described.[19] Total RNA was then extracted using the EZ1 Virus Mini Kit 48 (Qiagen, Germany) and EZ1 RNA Tissue Mini Kit (Qiagen, Germany), respectively for feces and organs, according to the guidelines of the manufacturer. The extraction quality and the RNA quantification were checked by spectrophotometry using the nanodrop (Thermo Fisher Scientific, USA). The search for viral RNA of paramyxoviruses was conducted using three heminested reverse transcription-PCR (hnRT-PCR) assays targeting the polymerase gene as previously described.[20] The sensitivity limit was calculated as between 10 and 100 RNA copies for the Rubulavirus-Avulavirus subgroup-specific PCR, the Morbillivirus-Respirovirus-Henipavirus subgroup-specific PCR, and the Pneumovirinae subfamily-specific PCR and between 500 and 1,000 copies for the Paramyxovirinae subfamily-specific PCR.[20] Positive controls were systematically included in each PCR performed.

3. Results and discussion

Based on the workers' voluntary participation, 35 people (19 in CDP, 7 in LEK and 9 in PPG) were included in this study and 343 NHPs were sampled between 2013 and 2014. In addition, 141 bats and 420 micromammals were captured. A total of 1,884 samples were analyzed, including 1,323 fecal samples from 85 healthy human volunteers, 677 from NHPs, 420 from micromammals, 141 from bats, and 561 organs from 420 micromammals and 141 bats (see Table 1). No paramyxovirus RNA was detected in these samples while the positive controls were revealed as well with organs and fecal samples. This study conducted on 1,884 samples is the first which considers diverse groups of mammals living in the same environment. Based on the previous studies reporting the identification of PVs in humans, NHPs,[6,13] bats[3] and rodents,[4] the detection of PV RNA in the different host communities studied here was expected. Indeed, the detection of PVs in feces and organs from bats,[5] the promiscuity between NHPs and humans[21–23] as well as the high density and strong interactions around food distribution points between micromammals and bats should increase the probability of viral transmission between wild species on the one hand, and occasionally between wild species and humans on the other hand[21,24–26] and thus the persistence of PVs at the host community level. Furthermore, bats have the ability to maintain viruses in low concentrations in organs due to immunoregulatory genes.[27,28] In the Congo basin, Drexler et al.[5] reported the identification of the Morbillivirus (5 cases) which is closely related to the human mumps virus in in organs from frugivorous bats *Eidolon helvum* and *Hypsignathus monstrosus*. But the number of bats sampled here was very low (n = 141) unlike the study of Drexler et al. (4,954 bats from 11 countries over several years). In Gabon, no infection was reported in rodents by Drexler et al.[5] PV infections (14 cases) were reported in bats (*Coleura afra*) by Maganga et al.[3] on a large sample size (985 bats from Gabon over two years). In our study no infection was reported in species *Epomops franqueti*, which is the most abundant species in our sample (106; 75%). These results are similar with a previous study.[5] It could be explained by the fact that *Epomops franqueti* is a solitary species living in very small groups from 1 to 3 individuals[29] unlike *Eidolon helvum* and *Hypsignathus monstrosus* which live in large colonies sometimes comprised of several million animals,[30] and in small colonies from 25 to 132 animals,[18] respectively. Large social group sizes, intense social interactions, and high spatial mobility of many bat species are some key attributes that contribute to a greater occurrence of viruses in bats, unlike other groups of mammals.[31] More generally, virus circulation mainly depends on efficient transmission and sufficient host population size to persist over long periods.[25,27,32] Hence, PV infections could occur epidemically or endemically according to host species and region. Cases reported earlier in bats living in Gabon[3,5] could correspond to an epidemic period. Besides the PV's circulation pattern and species ecology, the lack of detection of PVs in the studied hosts could also be due to a low viral load in the samples associated to the use of degenerate primers. Degenerate primers can indeed cause a drop in test sensitivity.[33] No data of PV in NHPs are available in Gabon. Only few studies reported mumps and measles viruses from the sera of patients.[34,35] Thus, the nature of our biological samples (faecal) from humans and NHPs could also explain the lack of detection of PVs in these species. Human samples were from healthy volunteers and therefore it was difficult to detect any paramyxovirus RNA. A better approach would have been serology on sera of these individuals. Although it would not meet the objectives of this study, serology would have given more information on the circulation of PVs.

4. Conclusion

Longitudinal studies of host communities are needed to identify patterns of spread and persistence of PVs, especially by investigating different periods of the year, if we consider that viral shedding in hosts could occur at specific periods or seasons (e.g. reproductive, gestation or birth periods).

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Authors' contribution

François Renaud and Dominique Pontier contributed equally and supervised this work.

Conflicts of Interest Disclosure

The authors declare no conflict of interest.

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