Supplemental Data

Pandemic Human Viruses Cause Decline of Endangered Great Apes

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Supplemental Experimental Procedures

Molecular Analysis

DNA and RNA were extracted from frozen lung tissue with DNAeasy and RNAeasy tissue kits (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. cDNA was synthesized by using the Superscript Kit (Invitrogen, Karlsruhe, Germany) and random hexamer primers (TIB Molbiol, Berlin, Germany). Various PCR approaches were applied in order to identify relevant bacteria [S1] and viruses. Samples were screened for influenza virus A-H1, A-H3, B [S2], adenovirus [S3], measles virus [S4], coronaviruses (A.N., unpublished data), picornavirus [S5], parainfluenza virus [S6] (with modified nested primers for Parainfluenza 3: ParaIII-1060: 5′-CCCTGGTCC AACAGATGGGT-3′ and ParaIII-1150: 5′-ACACCCAGTTGTG TTGCAGATT-3′), RSV [S7], and HMPV [S8]. MassTag PCR [S9, S10], a multiplex assay that uses small molecular tags and mass spectroscopy for detection of multiple PCR products, was also used.

Positive samples were further characterized with PCR assays targeting phylogenetically relevant DNA fragments. For HRSV, first and heminested PCRs targeting the hypervariable region of the G protein were performed. The primers specific for subgroup B used for the first PCR were primers GPB and F1. Those used for the heminested PCR were primers nRSBG and F1, as described previously [S7, S11]. To amplify the P Gen of HMPV, the primers MPVP01.6 and MPVM02.4 were used, as described by Mackay [S8]. Amplification was conducted for 5 min at 95°C, followed by 35 cycles of 95°C for 30 s, 54°C for 30 s, and 72°C for 1 min, with a final 10 min of extension at 72°C.

PCR products of HMPV and HRSV were purified with the Gel Extraction Spin Kit (Genomed, Loehne, Germany) and then cloned with the Topo TA Cloning Kit (Invitrogen, Karlsruhe, Germany). Colonies were analyzed by colony PCR. Sequencing was performed with the ABI Big Dye Termination Kit (Applied Biosystems, Weiterstadt, Germany).

Phylogenetic Analysis

Viral sequences generated from chimpanzees were compared to sequences amplified from human patients that were available in GenBank. Final data sets contained 99 taxa for HRSV and 36 taxa for HMPV (Tables S3 and S4) and were trimmed in length to 381 and 867 bp, respectively, to avoid large end gaps. Adequate substitution models were selected based on Akaike's Information Criterion from the set of models included in Modeltest [S12] as well as several codon-position (CP) models [S13]. Model likelihoods were calculated in PAUP* v4.0b10 [S14] and in baseml which is part of the PAML package [S15]. The selected models were GTR + G for HRSV and $HKYuf_{112}$ + CP_{112} + G_{112} for HMPV [S16]. Maximum likelihood (ML) trees were found by using heuristic searches in Treefinder [S17] based on the previously estimated model parameters. The same program was used to assess the statistical support for individual nodes based on 1000 bootstrap replicates. Trees were rooted by using the two oldest sequences from 1960 and 1962 as an outgroup in the case of HRSV and by midpoint rooting in the case of HMPV.

Evolutionary rates and the corresponding divergence dates associated with the human virus/chimpanzee virus splits were estimated in BEAST v1.4.8 [S18]. Only taxa for which the year of sampling was available were included in the analysis (HRSV, n = 90; HMPV, n = 25). Six HMPV sequences from 2003 and 2004 were randomly assigned in equal parts to each of the 2 years. Two independent runs with 10 million generations under a constant-population-size model were performed, with the first 1 million generations being subsequently removed as burn-in. Convergence between runs and effective sample sizes for parameters of interest were assessed with the program Tracer [S19]. No date was available for a HRSV sequence from Beijing, which was the human-derived virus in the data set most closely related to the chimpanzee viruses found during the 2006 outbreak. In this case the estimated genetic ML divergence between the two groups was combined with the median evolutionary rate estimate to produce a divergence date. The upper and lower bounds of the 95% highest posterior density interval of the rate estimate were used to provide a confidence interval.

Chimpanzee Habituation Projects and the Suppression of Poaching

To sample chimpanzee and poaching distributions, we used standard line transects methods [S20]. Along each transect, we noted all signs of chimpanzee presence, including direct sightings, vocalizations, nests, and nut cracking sites. We also noted all signs of poaching, including wire snares, shotgun cartridges, machete cuts, meat drying racks, and poaching trails.

Transects were hierarchically clustered at two spatial scales. In the smaller scale, 500 m transects were centered on each edge of a square with sides that were 1 km in length. In the larger scale, clusters of four 500 m transects (4-clusters) were centered on each vertex of a square with sides that were 2 km in length. Clusters of 16 transects (16-clusters) were then systematically distributed across the park, with a distance of 11 km between 16 cluster centers. This produced a total of 734 500 m transects or 46 16-clusters.

Two chimpanzee habituation projects have operated in Taï National Park in recent years. Our research project began chimpanzee habituation in 1979 while an ecotourism project (Guiroutou site in Figure 3) in the southwest corner of the park began habituation in 1990 and operated until 2003. We used two approaches to examine whether these habituation projects had a suppressive effect on the poaching of chimpanzees. In the analyses presented in the main text, we sorted transects by distance from the nearest of the two chimpanzee habituation projects and pooled transects into ten equal size bins. We then measured the strength of the relationship between distance from the habituation projects and poaching-sign encounter rate in terms

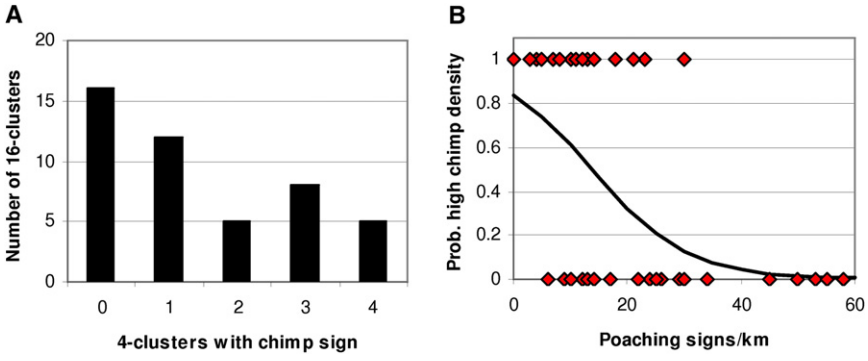

Figure S1. Chimpanzee-Sign Frequency and Chimpanzee-Sign Presence Probability

(A) Frequency of large survey units (y axis; 16 × 500 m) with chimpanzee-sign presence in zero to four of the subunits (x axis; 4 × 500 m).

(B) Probability of chimpanzee-sign presence in at least two of the subunits (y axis) as a function of the number of poaching signs per km (x axis).

Table S1. GLM Predicting Poaching-Sign Encounter Rate

Model	#_par	AIC	Constant	Dist. Village	Dist. Habituation
Null	1	364.3	3.02		
Villages	2	359.8	3.43	−0.108	
Villages + habituation	3	349.8	2.37	−0.039	0.075

Abbreviations: #_par, parameter; dist. village, distance to village; and dist. habituation, distance to habituation sites.

of the Pearson Product Moment Correlation, assuming a power law relationship between poaching intensity and distance to habituation site (i.e., we performed a linear regression on the natural logarithms of the dependent and independent variables). This produced a highly significant positive correlation ($R^2 = 0.94$, $p < 0.001$). We also performed a similar analysis with the poaching-sign encounter rate as a predictor of chimpanzee-sign encounter rate. There was a significant negative correlation between poaching and chimpanzee encounter rates ($R^2 = 0.45$, $p < 0.03$).

In our second analysis we sought to control for the fact that poaching pressure in Africa typically emanates from villages [S21–S23] and, therefore, that the position of both the Taï chimpanzee research project and the Guiroutou ecotourism project near villages just outside the park would normally predispose the habituation sites to particularly intense poaching pressure. To that end, we used both distance from villages and distance from the closest of the two habituation sites as covariates in a generalized linear model (GLM) predicting poaching-sign encounter rate (log link, negative binomial error). Thus, we tested not just whether poaching sign decreased with increasing distance from the habituation sites but also whether poaching intensity around the habituation sites was lower than would be expected given their position near villages. In implementing the analysis, we faced the problem that our 500 m transects were spatially clustered and, therefore, did not meet the assumption of (spatial) independence explicit in the GLM. To ensure independence, we pooled data within 16-clusters, which were systematically placed and, therefore, approximately spatially independent.

We evaluated support for the two covariate effects in terms of Akaike's Information Criterion [S24]. A model including a constant and the "distance from village" covariate improved support by 4.5 AIC units relative to a model assuming constant poaching intensity (Table S1). In a frequentist framework, this would be considered a highly significant effect. Adding distance from the chimpanzee habituation project produced an additional, even larger improvement of 10 AIC units. Thus, the survey data strongly supported the hypothesis that the habituation projects suppressed poaching activity.

We conducted a second GLM analysis to evaluate the importance of poaching as a determinant of chimpanzee distribution. In this model we used poaching-sign encounter rate to predict chimpanzee-sign encounter rate. In this analysis we faced the problem that, as is often the case in ape surveys, there was a large stochastic variance of chimpanzee-sign counts around predicted values. Therefore, instead of using the raw sign counts directly, we evaluated whether each 4-cluster had any chimpanzee sign at all. Within each 16-cluster, we then scored the number of 4-clusters that had at least one chimpanzee sign. This produced a bimodal distribution in which most of the 16-clusters had zero or one 4-cluster with chimpanzee sign, whereas the remainder had two to four 4-clusters with chimpanzee sign (Figure S1A).

We then defined a high chimpanzee density event as any 16-cluster with a score of two or higher and used logistic regression to estimate the relationship between poaching-sign encounter rate and the probability of a high chimpanzee density event. This analysis produced strong support for a strong negative relationship between poaching intensity and chimpanzee density. The model including poaching improved AIC by nine units relative to a model assuming constant chimpanzee density (Table S2), whereas the predicted probability of observing a high chimpanzee density event ranged from 0.82 in areas with no poaching sign to effectively zero in areas of high poaching intensity (Figure S1B). This result suggests that poaching is a major determinant of chimpanzee distribution at Taï, although GLM analyses not presented here suggest that other factors, such as habitat type, also are predictive of chimpanzee distribution (P.K.N., unpublished data).

Table S2. GLM Predicting Chimpanzee-Sign Encounter Rate

Model	#_par	AIC	Constant	Poaching
Null	1	61.4	0.63	
Poaching_signs	2	52.0	1.64	−0.119

Abbreviation: #_par, parameter.

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Table S3. Sequences of the HRSV G Gene Used for Phylogentic Analysis

Origin	Year of Isolation	Strain/Isolate	Accession Number
New Zealand			
	1984	NZB_84_05	DQ171845
	1985	NZB_85_03	DQ171847
	1985	NZB_85_01	DQ171859
	1988	NZB_88_01	DQ171849
	1988	NZB_88_02	DQ171850
	1989	NZB_89_01	DQ171858
	1989	NZB_89_02	DQ171862
	1989	NZB_89_03	DQ171863
	1989	NZB_89_04	DQ171864
	1990	NZB_90_01	DQ171876
	1990	NZB_90_02	DQ171851
	1990	NZB_90_03	DQ171852
	1990	NZB_90_05	DQ171877
	1991	NZB_91_01	DQ171860
	1992	NZB_92_03	DQ171872
	1992	NZB_92_05	DQ171868
	1993	NZB_93_05	DQ171861
	1994	NZB_94_01	DQ171874
	1995	NZB_95_01	DQ171866
	1995	NZB_95_02	DQ171875
	2004	NZB_04_01	DQ171867
	2004	NZB_04_02	DQ171878
	1984	NZB_84_01	DQ171841
	1984	NZB_84_02	DQ171843
	1984	NZB_84_06	DQ171865
Europe			
Belgium	1991	BE/154/91	AY751217
	1991	BE/23/91	AY751216
	1995	BE/14273/95	AY751227
	1996	BE/12015/96	AY751132
	2001	BE/12595/01	AY751107
	2002	BE/1162/02	AY751104
	2002	BE/1613/02	AY751103
	2001	BE/12670/01	AY751086
	2001	BE/12370/01	AY751118
	2001	BE/12670/01	AY751086
UK	12/1995	70870/12/95	AJ290205
	11/1995	70207/11/95	AJ290197
	12/1995	70739/12/95	AJ290204
	12/1995	70319/12/95	AJ290198
	1/1996	70003/01/96	AJ290210
	1960	SW/8/60	M73545
North America			
USA	1994/1995	NY01	AF233931
	1994/1995	MO30	AF233928
	1994/1995	AL19734-4	AF233924
	1990-1995	CH93-18b	AF065252
	1985	WV15291	M73542
	1994/1995	TX69208	AF233933
	1994/1995	MO53	AF233930
	1962	CH/18537/62	M17213
	1980	WV4843	M73540
	1983	WV10010	M73541
	1985	WV/B1/85	AF013254
	1985	WV/15291/85	M73542
	1989	NM/1355/89	M73543
	1990	CH10b	AF065250
	1993	CH93-9b	AF065251
	1993	CH93-53b	AF065253
Canada	1994/1995	CN1839	AF233926
South America			
Argentina	1999	BA/3997/99	DQ227366
	1999	BA/1370/99	DQ227364
	1999	BA/802/99	DQ227363
	1999	BA4128/99B	AY333364
	1999	BA3859/99B	AY333363
	1999	BA3833/99B	AY333362
	1999	BA/3931/99	DQ227365
	1999	BA/1326/99	DQ227398
	2002	BA/1214/02	DQ227379
	2002	BA/770/02	DQ227373
	2002	BA/1461/02	DQ227382
	2004	BA/1526/04	DQ227408
	2004	BA/493/04	DQ227407
	2003	BA/5021/03	DQ227405
	2003	BA/4909/03	DQ227404
	2002	BA/1445/02	DQ227399
	1999	BA/3859/99	AY333363
Uruguay	1999	mon/1/99	AY488794
	1999	mon/7/99	AY488800
	1994	strain 41605	AF251557
	1994	strain 40745	AF251556
	1990	MON/15/90	AY333361
Asia			
China	recent years	Beijing H1123	DQ270230
Japan	2003	NG153/03	AB175821
	2000	Sap/4/00-01	AB117522
India	2003	DEL/609/03/B	DQ248941
Africa			
Kenia	2003	Ken/29/03	AY660681
	2003	Ken/23/03	AY660683
	2002	Ken/109/02	AY524573
Mozambique	1999	Moz 11/99	AF309665
	1999	Moz/205/99	AF309678
	1999	Moz/198/99	AF309676
South Africa	1998	SA98D1656	AF348826
	1999	SA99V800	AF348821
	1997	SA97D934	AF348817
	1999	SA99V429	AF348813
Ivory Coast	1999	Loukoum_99	EU240450
	2006	Candy_06	EU240452
	2006	Ishas Baby_06	EU240453
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Table S4. Sequences of the HMPV P Gene Used for Phylogentic Analysis

Origin	Year of Isolation	Strain/Isolate	Accession Number
Europe			
The Netherlands	2001	00-1	AF371337
	unknown	NL/1/99	AY525843
North America			
Canada	1998	CAN98-78	AY145247
	1998	CAN98-77	AY145246
	2000	CAN00-15	AY145255
	1998	CAN98-73	AY145242
	1998	CAN98-74	AY145243
	1998	CAN98-75	AY145244
	1998	CAN98-76	AY145245
	1998	CAN98-79	AY145248
	2000	CAN00-13	AY145253
	1997	CAN97-82	AY145250
	1999	CAN99-81	AY145249
	2000	CAN00-16	AY145256
	1999	CAN99-81	AY145249
Asia			
China	unknown	CS113	EF081367
	unknown	CS088	EF081364
	unknown	CS058	EF081363
	unknown	CS099	EF081365
	unknown	CS105	EF081366
	unknown	BJ1816	DQ843658
	unknown	BJ1887	DQ843659
Japan	2003-2004	JPS02-76	AY530089
	2003-2004	JPS03-180	AY530092
	2003-2004	JPS03-187	AY530093
	2003-2004	JPS03-176	AY530090
	2003-2004	JPS03-240	AY530095
	2003-2004	JPS03-194	AY530094
Australia			
	unknown	Q02-1071	DQ229372
	2002	Q02-1981	AY256867
	2001	Q01-719	AY321507
	2001	Q01-705	AY321506
	2001	Q01-702	AY256863
Africa			
Ivory Coast	2004	Ophelia_04	EU240456
	2004	Oreste_04	EU240455
	2004	Virunga_04	EU240454