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Frequent foamy virus infection in free-living chimpanzees of the Taï National Park (Côte d'Ivoire)

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Foamy viruses are frequently found in non-human primates and apes in captivity. However, data on simian foamy virus (SFV) infection in apes from the wild are limited. Necropsy specimens were collected from 14 West African chimpanzees (Pan troglodytes verus) from three communities in the Taï National Park, Coˆ te d'Ivoire. PCR analysis revealed SFV-related int- and env-specific sequences in 12/14 chimpanzees. Two young chimpanzees were not infected. Plasma from 'PCR-positive' chimpanzees reacted against Pr71/74gag in Western blot analysis. Phylogenetic analysis demonstrated clustering of all analysed sequences with SFVcpz previously identified from the other P. troglodytes verus, although interestingly the sequences were diverse and no grouping according to a particular animal community was observed. The body compartments of two infected animals were examined and found to contain SFV sequences. Frequent SFV infections in chimpanzees from this area significantly increase the potential risk of zoonotic transmission to rural populations through direct contact, hunting and consumption of bush meat.

Foamy viruses (FVs) belong to the genus Spumavirus and are exogenous retroviruses that frequently infect mammals (Flügel, 1993; Meiering & Linial, 2001). Although members of the family Retroviridae, these viruses are different from the other family members in many aspects, including genome structure, morphogenesis, replication and pathogenesis (Linial, 1999; Rethwilm, 2003; Delelis et al., 2004). FVs can easily be transmitted in vivo, but infection is not associated with clinical manifestation in animals. FVs infect a broad spectrum of cells in vitro, but, in contrast with the situation in vivo, the infection is associated with a strong cytopathic effect, followed by cell death 7–8 days postinfection (Flügel, 1993). Simian foamy viruses (SFVs) were discovered in the mid- 1950s as contaminants of simian cell cultures (Enders & Peebles, 1954; Rustigian et al., 1955). Later, SFVs were detected in non-human primates (NHPs) in captivity (Swack & Hsiung, 1975; Herchenröder et al., 1994; Broussard et al., 1997; Hussain et al., 2003) and in animals from the wild (Barahona et al., 1976; Kaschula et al., 1978; Morozov & Lagaye, 1998; Calattini et al., 2004). The frequency of SFV infection in NHPs might be different depending on species and area. A high prevalence of SFV was reported in wild-caught mandrills from Cameroon and Gabon (Calattini et al., 2004), and free-ranging macaques from Thailand and Singapore (Jones-Engel et al., 2007). The saliva and blood of infected animals are considered the principal routes of SFV transmission (Falcone et al., 1999; Murray et al., 2006; Brooks et al., 2007). SFVs have been described in captive apes (Bieniasz et al., 1995; Schweizer et al., 1995), but SFV infection in apes from the wild is just beginning to be explored (Calattini et al., 2004, 2006). Recently, using a faecal-based assay, a high SFV infection rate was demonstrated in chimpanzees from equatorial Africa (Liu et al., 2008). Zoonotic transmission of SFV to humans is a matter of concern (Gessain & Calattini, 2008) and has been documented on several occasions, including accidents while handling infected animals in primate research facilities (Heneine et al., 1998; Callahan et al., 1999; Boneva et al., 2002, 2007; Brooks et al., 2002; Switzer et al., 2004) and in zoos (Sandstrom et al., 2000) and in the wild through bites and bushmeat hunting (Wolfe et al., 2004; Jones-Engel et al., 2005; Calattini et al., 2007). However, signs of infection-associated disease in infected humans or further human-to-human transmission of SFV was not documented (Boneva et al., 2002, 2007; Bastone et al., 2003; Switzer et al., 2004). Limited data are available regarding the prevalence and incidence of SFV in groups with known kinship and social structure. These analyses are of more than academic interest and may help to reveal some details of SFV interspecies and cross-species transmission. The aim of this study was to estimate the prevalence of SFV in wild chimpanzees from three communities in the north (N; n538), middle (M; n512) and south (S; n563) of the Taï National Park (Côte d'Ivoire, West Africa), habituated to human observation. Each community area is approximately 12–20 km2, with the M area partially overlapping both the N and S areas (Herbinger et al., 2001). Most mating is observed to occur within a community, although there are no natural barriers separating these communities. Necropsy specimens from 14 chimpanzees (Table 1)

including blood samples from six animals were collected (Leendertz et al., 2004b) and deep frozen until use. Available blood samples were examined by Western blotting (dilution 1 : 100) (Table 1) against sucrose density-gradient-purified prototype foamy virus (PFV) from U373 MG cells (Morozov et al., 1997). Antibodies reacting with two related Gag polyproteins, Pr71/74gag, which are principal structural elements of the PFV core, were considered to be positive (Hahn et al., 1994). Whilst the five adult chimpanzees reacted with Pr71/74gag, the juvenile animal was antibody negative (Table 1). Applying all necessary measures to avoid contamination, DNA was isolated from the body compartments of two animals using a DNeasy tissue kit (Qiagen). The quality of the DNA was tested with c-myc-specific PCR primers: 59- GCCAGAGGAGGAACGAGCT-39 (sense, nt 1660–1678); 59-GGGCCTTTTCATTGTTTTCCA-39 (antisense, nt 1740–172) (positions according to GenBank accession no. NM_002467). DNA specimens from the spleen were examined by nested PCR with a set of generic int primers (Schweizer & Neumann-Haefelin, 1995). DNA (500 ng, equivalent to ~7.56104 cells) was analysed by PCR performed in a 50 *m*l reaction mixture with Taq polymerase (InVitek). After initial denaturation at 94 uC for 4 min, the DNA was subjected to 42 cycles of denaturation at 94 uC for 1 min, annealing at 45–60 uC (depending on primers) for 30 s and extension at 70 uC for 1 min. Two microlitres from the first-round PCR was used in the nested PCR. SFV int-like sequences were detected in 12/14 chimpanzees, including all five animals that were antibody positive in the Western blot, whilst the animal that was negative for SFV antibodies was also negative in the PCR analysis. The two chimpanzees that were PCR negative, one of which tested negative in the Western blot, were less than 2 years of age (Table 1). Amplicons were compared with SFV sequences from GenBank by BLAST search. Multiple sequence alignments were performed using CLUSTAL W (Thompson et al., 1994), and a DNA distance matrix was calculated. To determine phylogenetic relationships, neighbour-joining (NJ) phylogenetic trees were constructed (Saitou & Nei, 1987) using the PHYLIP software package. Comparative analysis of the SFV int amplicons showed some diversity of sequences in infected chimpanzees, even between animals living in the same community (Table 2). Whilst the sequences clustered in a NJ phylogenetic tree analysis with several other SFV sequences that had previously been identified in wild-caught Central and Western African chimpanzees (Pan troglodytes verus), they were well separated from SFV sequences recently described in three other subspecies (P. troglodytes troglodytes, P. troglodytes schweinfurthii and P. troglodytes vellerosus) (Fig. 1a). Previously, SFV sequences have been revealed in several body compartments from SFV-infected African green monkeys (Falcone et al., 1999) and macaques (Murray et al., 2006). However, body compartments in SFV-infected great apes have not been analysed for infection. To address this question, we examined DNA specimens isolated from different organs of the chimpanzees Kady (M) and Leo (M). Eight body compartments from Kady (peripheral lymph nodes, spleen, lung, heart, colon, duodenum, jejunum and tonsils) and four body compartments from Leo (peripheral lymph nodes, spleen, lung and jejunum) were examined. In both chimpanzees, SFV sequences were detected in all compartments, indicating ubiquitous virus distribution in the animals' bodies. Amplicons from the spleen and lymph nodes of Kady were cloned and sequenced. Comparative analysis of 12 and seven clones from each compartment, respectively, revealed good clustering of sequences within each compartment and a minor (~1 %) polymorphism between the sequences from the different compartments (data not shown). As analyses of the SFV env sequences in infected NHPs are not frequently carried out, it was of interest to analyse the SFV env polymorphism in infected chimpanzees. A set of generic nested PCR primers was designed to amplify 458 bp long FV env sequences: set 1: 59-TGGGARATTGGATTATAT- 39 (sense, nt 8602–8619) and 59 CTTAGTTGTAGATTTGGCAG- 39 (antisense, nt 9092–9073); set 2: 59-ATGAATTGRKTATACCTAAACA-39 (sense, nt 8621– 8642) and 59-TGGCAGTCGTGGCTCAAA-39 (antisense, nt 9078–9061); positions according to GenBank accession no. U21247. Using nested PCR, SFV env sequences were revealed in 11/ 14 animals; all 11 animals had previously been shown to be PCR positive for int (Table 1). Tita (S) was positive for int but was repeatedly found to be negative for the SFV env sequence. Amplicons from 10 animals were sequenced and, after sequence editing, fragments of 418 bp were analysed. Sequences from Gargantua and Gise`le (both from N and offspring of the same mother), and from Leo (M) and Dorry (N), respectively, were identical (Table 2). The other env sequences were divergent, and more than 3% difference in sequence could be found not only between chimpanzees from different communities, but also within a community. In phylogenetic analysis (Fig. 1b), seven out of nine env sequences formed three different clusters that were well supported by bootstrap analysis. SFV env sequences were determined from five antibody-positive and PCR-positive animals from the N group; however, despite their community affiliation, these sequences were found in all three clusters. Whilst one cluster comprised only the env sequences amplified from Gise`le and Gargantua (N), the two other clusters comprised sequences amplified from animals originating from different communities. Thus, animals from distant communities may harbour SFV with closely related env sequences and vice versa – SFV env from animals living in the same community (e.g. Kady and Leo) may be more divergent.

Studies of the molecular epidemiology of SFV in wild NHPs is in its infancy and many questions remain unanswered. Here, we investigated for the first time the prevalence of SFV in necropsy specimens collected from chimpanzees from three communities with well-established social structures and kinship in the Taï National Park, Coˆ te d'Ivoire. Twelve of 14 chimpanzees were found to be infected with SFV. Given that only the two youngest animals (up to 2 years of age) were not infected, all adult animals were SFV positive, indicating a high prevalence of SFV in free-living chimpanzees in the Taï National Park. SFV sequences found in the Taï chimpanzees were closely related to published SFVcpz sequences detected in other P. troglodytes verus. However, the diversity of viral sequences in infected animals from the same community was unexpected. Notably, SFVcpz sequences in some animals from separate communities were found to be closer to each other than to those in animals living in the same community. For instance, NJ phylogenetic analysis of SFV int sequences from Lefkas (N) and Rafiki (S) formed one cluster. Whilst several other int sequences also clustered, this was the only cluster that was statistically well supported in bootstrap analysis (Fig. 1a). Comparable relationships were obtained by maximum-likelihood and parsimony tree analyses (data not shown). Similar findings were observed with SFV env sequences where one cluster comprised Dorry (N) and Leo (M), and another Gise`le and Gargantua (both from the N community). The largest cluster comprised SFV sequences that had been isolated from Rafiki (S), Lefkas (N), Loukoum (N) and Kady (M). Notably, Lefkas (N) and Rafiki (S) showed a similar clustering in the int tree analysis. Whilst this cluster was also well supported in bootstrap analysis, all other int clusters that differed from the env tree analysis lacked statistical support. The SFV sequence diversity observed in the communities was higher compared with the low SFV diversity described in captive settings (Schweizer et al., 1999). This may be for two reasons. Chimpanzees in captive settings have been caught in the wild as young individuals so are less likely to be infected with SFV. This has probably led to the introduction of a single or a few SFV variants into the captive community. Secondly, these events could have taken place a short time ago in comparison with the situation in the wild where viruses have co-existed with chimpanzees for thousands of years. Thus, in captive settings, artificial bottle-neck effects are probably created, leading to viruses with limited variations. In an attempt to explain the diversity of viral sequences in animals within the same community, we considered several factors: different routes of infection, frequency of reinfection, virus import from distant chimpanzee communities and even trans-species transmission. We speculated that routes of infection might contribute to the virus polymorphism in NHPs. As mentioned above, SFV transmission in NHPs may occur via saliva, blood and, as has been suggested, seminal fluid (Broussard et al., 1997). Although it has not yet been demonstrated, breastfeeding may also contribute to SFV dissemination. The two young animals were SFV negative, and an SFV PCRpositive mother/SFV PCR-negative offspring (Loukoum and Leonardo) pair was revealed. On the other hand, infected Gargantuan and Gise`le (brother and sister) carried the same SFV. Whilst our observations are anecdotal and controversial, we suggest that comparative analyses of body fluids from SFV-infected animals may be useful to provide clarity to observed polymorphisms. Sporadic migration of females into new communities (Boesch & Boesch-Achermann, 2000) and additional rare visits of some animals to nearby communities increase the probability of new sexual contacts and occasions for fights, particularly between animals from separate communities. Thus, the risk of infection and reinfection with 'new' viruses is significantly increased and, as a consequence, may contribute to virus diversity in a given community by homologous recombination. In this regard, the probability of finding more virus variants in old animals may be higher; thus, clonal analyses of SFV sequences from infected animals of different ages could be helpful. Our studies showed that SFVcpz sequences were present in all of the examined body compartments of two chimpanzees, indicating a lack of preferential target organ(s) (or tissues) for SFV in chimpanzees. This result is in full concordance with the observed spread of SFV in African green monkeys (Falcone et al., 1999). Interestingly, our clonal analysis of the amplicons revealed some polymorphisms between SFVcpz sequences in the body compartments of the apes (data not shown). Taken together, we have demonstrated a high prevalence of SFVcpz among free-living chimpanzees from three communities of the Taï National Park, thus supporting recent data on frequent chimpanzee infection in equatorial Africa (Liu et al., 2008). Notably, we revealed a certain variability in SFV sequences in infected animals within a given community, and the variability of SFV within a single community was comparable to that observed between different communities. The reason for this is unclear. High SFV prevalence in chimpanzees from this area and easy interspecies transmission of the viruses pose a significant risk of infection for the rural population. Even when considering SFV as latent, screenings for infection and follow-up studies of the infected native human population are highly desirable.

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Figures

Table 1. Chimpanzees from the Taï National Park (Coˆ te d'Ivoire) analysed for SFV infection and a summary of results

+, Positive result; –, negative result; NS, not sequenced; ND, not done.

Animal	Cause of death*	Area/age (years)/sex (male/female)	PCR *int*	PCR *env*	Western blot
Gisèle	*Bacillus anthracis*	N/~5/f	+	+	ND
Gargantua	*B. anthracis*	N/~10/m	+	+	+
Dorry	*B. anthracis*	N/~10/f	+	+	+
Loukoum	Respiratory infection	N/~27/f	+	+	+
Lefkas	Respiratory infection	N/~8/m	+	+	ND
Leonardo	Respiratory infection	N/~2/m	–	–	–
Leo	*B. anthracis*	M/~29/m	+	+	+
Noah	*B. anthracis*	M/~7/m	+	+	ND
Kady	Respiratory infection	M/~30/f	+	+	+
Rafiki	Leopard	S/~19/m	+	+	ND
Tita	Leopard	S/~20/f	+	–	ND
Virunga	Respiratory infection	S/~25/f	+	+ NS	ND
Ophelia	Respiratory infection	S/~2/f	–	–	ND
Olduvai	*B. anthracis*	S/~8/m	+	+	ND
Total			12/14	11/14	

*Leendertz et al. (2004a); Köndgen et al. (2008).

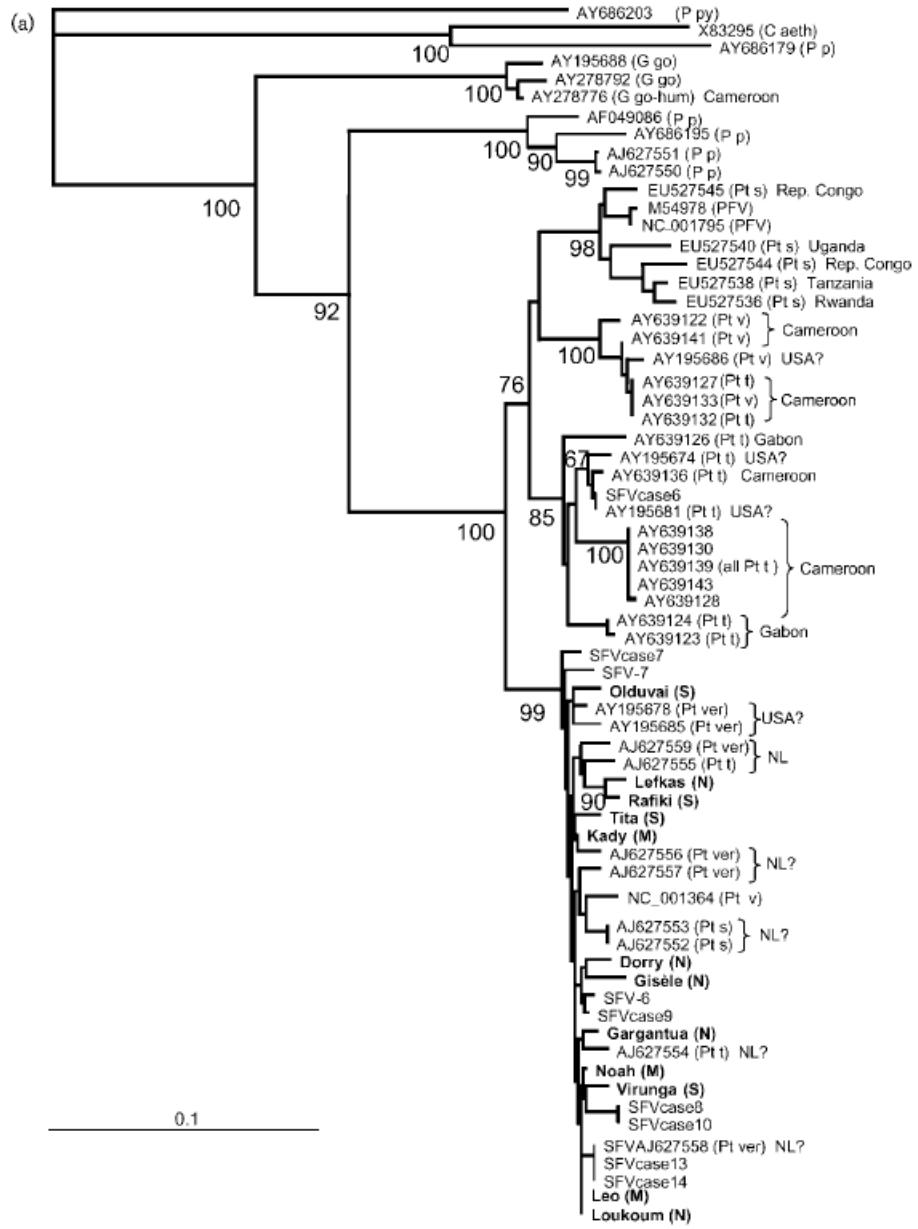
Table 2. Sequence distance analysis of the SFV int and env amplicons from infected P. troglodytes verus from the Tai National Park

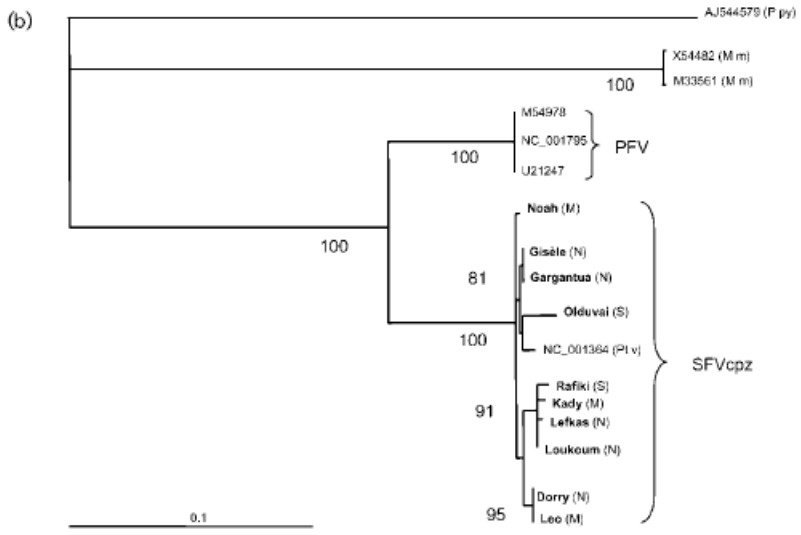
–, Not sequenced

Integrase	Envelope												
	SFVcpz*	Leo (M)	Loukoum (N)	Noah (M)	Gargant-ua (N)	Dorry (N)	Olduvai (S)	Gisèle (N)	Rafiki (S)	Lefkas (N)	Kady (M)	Virunga (S)	Tita (S)
SFVcpz*		98.6	98.3	97.8	99.3	98.6	98.1	99.3	97.8	98.1	97.6	–	–
Leo (M)	98.2		99.4	97.9	99.3	100	97.6	99.3	98.6	98.8	98.3	–	–
Loukoum (N)	98.2	100		97.8	98.8	99.0	97.4	99.8	99.5	99.8	98.8	–	–
Noah (M)	97.9	99.5	99.5		98.3	97.8	96.9	98.3	97.4	97.6	97.8	–	–
Gargantua (N)	97.4	99.2	99.2	98.7		99.3	98.3	100	98.3	98.6	98.1	–	–
Dorry (N)	97.2	98.4	98.4	98.4	97.7		97.6	99.3	98.6	98.8	98.3	–	–
Olduvai (S)	96.4	98.2	98.2	97.7	97.7	96.6		98.3	96.9	97.1	96.7	–	–
Gisèle (N)	96.6	97.9	97.7	97.7	97.2	97.4	96.1		98.3	98.6	98.1	–	–
Rafiki (S)	96.1	97.1	97.2	96.9	96.9	96.1	96.6	96.4		99.3	98.8	–	–
Lefkas (N)	95.9	97.1	97.4	97.2	97.2	96.4	96.9	95.9	98.7		98.6	–	–
Kady (M)	97.2	99.0	99.0	98.4	98.2	97.4	98.2	96.9	97.7	97.4		–	–
Virunga (S)	97.2	99.0	99.0	98.7	98.2	97.9	97.2	96.9	96.4	96.1	97.9		–
Tita (S)	96.6	98.4	98.4	97.9	97.7	96.9	97.7	96.4	96.4	97.2	98.4	97.4	

*SFVcpz (GenBank accession no. NC_001364).

Fig. 1. NJ phylogenetic trees of the SFV int sequences (386 bp) from 12 chimpanzees (a) and SFV env sequences (418 bp) from 10 chimpanzees (b) from the Taï National Park. The names of the Taï chimpanzees are shown in bold and GenBank accession numbers are as follows: SFV int sequences: DQ142644–DQ142647, DQ142659–DQ142666 and EU248953– EU248955; SFV env sequences: DQ142648 and DQ142652–DQ142658. Sequences NC_001795, M54978 and U21247 are variants of SFVcpz that represent a zoonotic transmission to man (referred to as PFV). The SFVggo-like sequence (AY278776) was detected in a Cameroon hunter. C aeth, Cercopithecus aethiops (African green monkey); M m, Macaca mulatta (rhesus macaque), G go, Gorilla gorilla (gorilla), P py, Pongo pygmaeus (orang-utan); P p, Pan paniscus (bonobo); Pt ver, Pan troglodytes verus (western chimpanzee); Pt t, Pan troglodytes troglodytes (central chimpanzee); Pt s, Pan troglodytes schweinfurthii (eastern chimpanzee); Pt v, Pan troglodytes vellerosus (Nigeria and Northern Cameroon chimpanzee). SFVcase 6–10, 13 and 14 are SFVcpz int sequences detected in infected humans (Switzer et al., 2004). GenBank accession numbers are given. The location of some primates is indicated as NL (The Netherlands) and USA. Bars, nucleotide substitutions per site.




(b)
AJ544579 (P py)
X54482 (M m)
M33661 (M m)
100
M54978
NC_001795
U21247
PFV
100
Noah (M)
100
Gisèle (N)
81
Gargantua (N)
Olduvai (S)
100
NC_001364 (Pt v)
SFVcpz
Rafiki (S)
Kady (M)
91
Lefkas (N)
Loukoum (N)
Dorry (N)
95
Leo (M)
0.1