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Nonhuman primate retroviruses from Cambodia: High simian foamy virus prevalence, identification of divergent STLV-1 strains and no evidence of SIV infection

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ABSTRACT

Nonhuman primates (NHPs) carry retroviruses such as simian immunodeficiency viruses (SIV), simian T-cell lymphotropic viruses (STLV) and simian foamy viruses (SFV). Here, we revisited NHPs from Cambodia to assess the prevalence and diversity of these retroviruses using updated viral detection tools. We screened blood from 118 NHPs consisting of six species (*Macaca fascicularis* ($n = 91$), *Macaca leonine* ($n = 8$), *Presbytis cristata* ($n = 3$), *Nycticebus coucang* ($n = 1$), *Hylobates pileatus* ($n = 14$), *and Pongo pygmaeus*) ($n = 1$) by using a Luminex-based multiplex serology assay that allows the detection of all known SIV/HIV and SFV lineages. We also used highly sensitive PCR assays to detect each simian retrovirus group. Positive PCR products were sequenced and phylogenetically analyzed to infer evolutionary histories.

Fifty-three of 118 (44.9%) NHPs tested positive for SFV by serology and 8/52 (15.4%), all from *M. fascicularis*, were PCR-confirmed. The 8 novel SFV sequences formed a highly supported distinct lineage within a clade composed of other macaque SFV. We observed no serological or molecular evidence of SIV infection among the 118 NHP samples tested. Four of 118 (3.3%) NHPs were PCR-positive for STLV, including one *M. fascicularis*, one *P. cristata*, and two *H. pileatus*. Phylogenetic analyses revealed that the four novel STLV belonged to the PTLV-1 lineage, outside the African radiation of PTLV-1, like all Asian PTLV identified so far. Sequence analysis of the whole STLV-1 genome from a *H. pileatus* (C578_*Hp*) revealed a genetic structure characteristic of PTLV. Similarity analysis comparing the STLV-1 (C578_*Hp*) sequence with prototype PTLVs showed that C578_*Hp* is closer to PTLV-1s than to all other types across the entire genome. In conclusion, we showed a high frequency of SFV infection but found no evidence of SIV infection in NHPs from Cambodia. We identified for the first time STLV-1 in a *P. cristata* and in two *H. pileatus*.

1. Introduction

Nonhuman primates (NHPs) are carriers of diverse types of viruses, some of which like simian immunodeficiency viruses (SIVs), simian T-cell lymphotropic viruses (STLVs) and simian foamy viruses (SFVs) can be transmitted to humans as zoonotic infections (Pedersen and Davies, 2009; Sharp and Hahn, 2011; Switzer and Heneine, 2011; Wolfe et al., 2004). Studying the occurrence and circulation of these simian retroviruses in wild primate populations enables us to better understand retrovirus evolution in primates and also provides tools for monitoring possible future retroviral zoonotic events.

Foamy viruses (FVs) are complex retroviruses infecting a wide range of animal species, such as, cats, cows and nonhuman primates (Han and Worobey, 2012; Rethwilm, 2010). Simian foamy virus (SFV), is widely prevalent in wild-caught and captive-born NHP (Meiering and Linial, 2001). Molecular evidence suggests that SFV has co-evolved with NHPs for millions of years (Switzer et al., 2005). Current evidence suggests that all Old and New World monkeys and apes are infected by SFV, at variable proportions (Morozov et al., 2009). Increasing evidence has documented transmission of SFV to persons exposed to the blood and body fluids of NHPs during occupational activities in zoos and primates centers or via hunting or butchering NHPs or keeping them as pets (Betsem

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Table 1
Identification of SFV and STLV in nonhuman primates from Cambodia.

Common names	Scientific names	N	SFV serology	SFV PCR	STLV screening (PCR)
			N Pos/N tested (%)	N Pos/N tested (%)	N Pos/ N tested (%)
Pileated gibbon	*Hylobates pileatus*	14	4/14 (28.6)	0/4 (0)	2/14 (14.3)
Long-tailed macaque	*Macaca fascicularis*	91	42/91 (46.6)	8/42 (19)	1/91 (1.1)
-Infant		22	3/22 (13.6)	2/3 (66.6)	–
-Adult/Juvenile		69	39/69 (56.5)	6/39 (15.4)	–
Pig-tailed macaque	Macaca leonine	8	6/8 (75)	0/5 (0)	0/8 (0)
Orang-outan	*Pongo pygmaeus*	1	0/1 (0)	0	0/1 (0)
Silver langur	*Presbytis cristata*	3	1/3 (33.3)	0/1	1/3 (33.3)
Slow Loris	Nycticebus coucang	1	0/1 (0)	0	0/1 (0)
Total		118	53/118 (44.9)	8/52[a] (15.4)	4/118 (3.4)

[a] There was not enough DNA left for 1 sample SFV antibody-positive to be tested by PCR.

et al., 2011; Calattini et al., 2011, 2007; Huang et al., 2012; Mouinga-Ondeme et al., 2012; Switzer et al., 2012, 2004). High SFV infection prevalence (3.6–24%) were reported for persons who received severe injuries during the hunting of monkeys and apes in Cameroon and Gabon (Calattini et al., 2007; Mouinga-Ondeme et al., 2012). Phylogenetic analysis demonstrated that, in these persons, zoonotic SFV infection originated from diverse ape and monkey species, including chimpanzee, gorilla, macaque, African green monkey, baboon, and mandrill (Murray and Linial, 2006; Switzer and Heneine, 2011). The potential for SFV to cause disease in humans and to be transmitted from person-to-person after cross-species transmission is not fully understood. However, evaluating the prevalence of SFV in wild-caught Asian NHP may give more insight in the dynamics of this retrovirus in this part of the world.

Human T-cell lymphotropic viruses (HTLVs) are the human counterparts of STLV, termed together primate T-cell lymphotropic viruses (PTLVs). To date, four types of HTLV (HTLV types 1–4), have been described in humans with three of them having the simian counterparts identified named STLV 1, 2 and 3, respectively (Courgnaud et al., 2004; Switzer and Heneine, 2011; Wolfe et al., 2005). A highly divergent STLV identified in a *Macaca arctoides* (Van Dooren et al., 2005) was tentatively classified as STLV-5 (Liegeois et al., 2008; Mahieux and Gessain, 2011). The recently discovered HTLV-4 has no simian virus analogue identified yet (Wolfe et al., 2005). HTLV-1 causes adult T-cell leukemia, neurological disorders and has also been associated with inflammatory diseases (Gessain et al., 1985). HTLV-2 is less pathogenic than HTLV-1 (Koralnik et al., 1994) and the clinical consequences of HTLV-3 and HTLV-4 infection are currently unknown (Calattini et al., 2005, 2006a; Switzer et al., 2006, 2009). Among the 79 currently recognized Asian NHP species (http://www.primate-sg.org/asia.spp.htm), STLV-1 has only been identified in several *Macaca* spp. (Ibrahim et al., 1995), *P. p. pygmaeus* (Van Dooren et al., 2007) and *H. syndactylus* (Van Dooren et al., 2007). STLV-2, -3 and -4 have not yet been described in any Asian NHPs. Our knowledge of the prevalence and diversity of STLV in different Asian NHP is thus limited, especially compared to those from Africa where 7% to more than 80% of certain species are infected (Ahuka-Mundeke et al., 2011b; Courgnaud et al., 2004; Liegeois et al., 2012; Leendertz et al., 2010).

Human immunodeficiency virus (HIV) is the result of cross-species transmission of SIV from NHP to humans in Africa (Sharp and Hahn, 2011). The closest simian relatives of HIV-1 are SIVcpz in chimpanzees (*P. t. troglodytes*) and SIVgor in gorillas (*G. g. gorilla*) from West Central Africa (Keele et al., 2006). SIVsmm in sooty mangabeys (*C. atys*) from West Africa are the closest relatives of HIV-2 (Hirsch et al., 1989). At least 45 African NHP species are carriers of an SIV (Li et al., 2012; Locatelli and Peeters, 2012), and infection is generally host specific. This observation is remarkably contrasting with the situation of Asian NHP, for which no evidence of an SIV infection has yet been documented. Earlier seroepidemiological surveys performed in 1986 and 1988 (Lowenstine et al., 1986; Ohta et al., 1988) found no evidence of SIV infection among Asian NHP, including more than 800 samples from *Macaca* sp., Orang-outan and colobus monkeys. However, the antigens used for screening in these two studies consisted of purified HIV-1 or SIVagm proteins, which we now know do not provide adequate cross-reactivity with all currently known SIV lineages and thus a reduced detection sensitivity. Given the improvement of detection tools, revisiting Asian NHP for SIV screening deserves attention. Moreover, recent findings from monkeys on Bioko Island, Equatorial Guinea, indicated that SIV may have been present among African Old World monkeys for more than 32,000 years, which is a longer period of time than that previously calculated with molecular clock methods calibrated using modern sequences (Worobey et al., 2010). Furthermore, the discovery of an ancient endogenous lentivirus in a gray mouse lemur from Madagascar suggested that lentiviruses may already have infected some ancestors of the African and Asian cattarhine lineages (Gifford et al., 2008).

In the present work, we screened 118 different animals from Cambodia, representing six species of monkeys and orang-outans, for SFV, STLV and SIV infection using highly sensitive molecular and serologic tools. We document a high prevalence of SFV infection but found no evidence of SIV infection in these animals. We also identified four NHPs infected with highly divergent STLV-1, including one long-tailed macaque (*Macaca fascicularis*) and for the first time, two pileated gibbons (*Hylobates pileatus*) and one silver langur (*Presbytis cristata*). Our findings highlight the importance of additional studies to investigate the range of simian retrovirus infection in Asian NHPs and also the possibility of zoonotic human retroviral infection in this region.

2. Material and methods

2.1. Animals, blood sampling and dried blood spot (DBS) preparation

Sampled NHPs belonged to six different species and included: 91 long-tailed macaques (*M. fascicularis*), 14 pileated gibbons (*H. pileatus*), 8 Northern pig-tailed macaques (*Macaca nemestrina leonina*), three silver langurs (*P. cristata*), one orangutan (*Pongo pygmaeus*) and one slow Loris (*Nycticebus coucang*), giving a total of 118 NHPs (Table 1). All animals, except the orang-outan which was kept as a pet, were wild caught and were sampled between March 2006 and March 2007 during their quarantine period at the Phnom Penh Zoo, Cambodia. The Cambodian branch of the Wild Aid Programme rescued these NHPs from the illegal trade. After a veterinary examination, NHPs found in good health were released in the wild, while those in poor health remained at the zoo until recovery. Twenty-two long-tailed macaque were infants (18.6%) while the remaining individuals were adults/juveniles at the time of sampling. Dried blood spots (DBS), 3–5 spots per animal, representing about 20 μl of whole blood per spot, were prepared using