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Exposure to SIVmnd-2 in Southern Cameroon: Public Health Implications

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Abstract

Compelling evidence appeared in 2002 of human exposure to a plethora of primate lentiviruses through hunting, handling of bushmeat, and/or animals kept as pets in Cameroon. To determine SIV prevalence in pet animals, an analysis of 28 sera of nonhuman primates found no SIV infection in greater spot-nosed monkeys (0/5) or chimpanzees (0/10), and a prevalence rate of 23.1% (3/13) in mandrills kept as household pets in southern Cameroon. Phylogenetical analysis based on pol-integrase region and mitochondrial cytochrome b gene showed that the newly found SIV from Mandrillus sphinx (SIVmndCM-202, SIVmndCM-211, and SIVmndCM-218) clustered significantly with SIVmnd-2. Questionnaire data were also collected to assess whether owners had experienced bites, scratches, or exposure to blood and/or body fluid. Risk to human health from cross-species transmission of the newly identified SIVmnd-2 to infect humans remains unknown. **(AIDS Rev. 2009;11:135-9)**

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Key words

Zoonosis. Central Africa. Mandrills. SIVmnd-2. Cameroon.

Introduction

Chimpanzees (*Pan troglodytes troglodytes*) are recognized as the reservoir of simian immunodeficiency viruses (SIVcpzPtt) that have been introduced into humans at least three times, resulting in HIV-1 groups M, O, and N (a third HIV-1 lineage)[1,2]. Van Heuverswyn, et al. reported the discovery of HIV-1 group O-like viruses in wild gorillas[3]. The cross-species transmission of SIVcpz is now thought to have occurred through humans being exposed to the blood of chimpanzees infected with SIVcpz during hunting and butchering of nonhuman primates in Central Africa early in the 20th century[3-6]. Caring for captive nonhuman primates has lead to the transmission of a range of infections, including simian foamy virus and herpesvirus B, primate malaria, and tuberculosis[4]. Such behaviors can facilitate transmission of microorganisms from nonhuman primates to humans. Finally, a case of retrovirus transmission from mandrills to humans has already been documented. Simian T-cell lymphotropic virus type 1 (STLV-1) from *M. sphinx* has been described as the simian counterpart of human T-cell lymphotropic virus type 1 (HTLV-1) subtype D[7,8]. Moreover, a close molecular and phylogenetic relationship has been reported between STLV-1 subtype D from mandrills in Gabon and HTLV-1 strains obtained from Pygmies living in Cameroon and the Central African Republic and from a healthy non-Pygmy carrier in Gabon. To assess the potential risk of zoonotic transmission of monkey-possessing SIV to humans, we conducted a serologic and genetic survey among monkeys that were kept as household pets in southern Cameroon.

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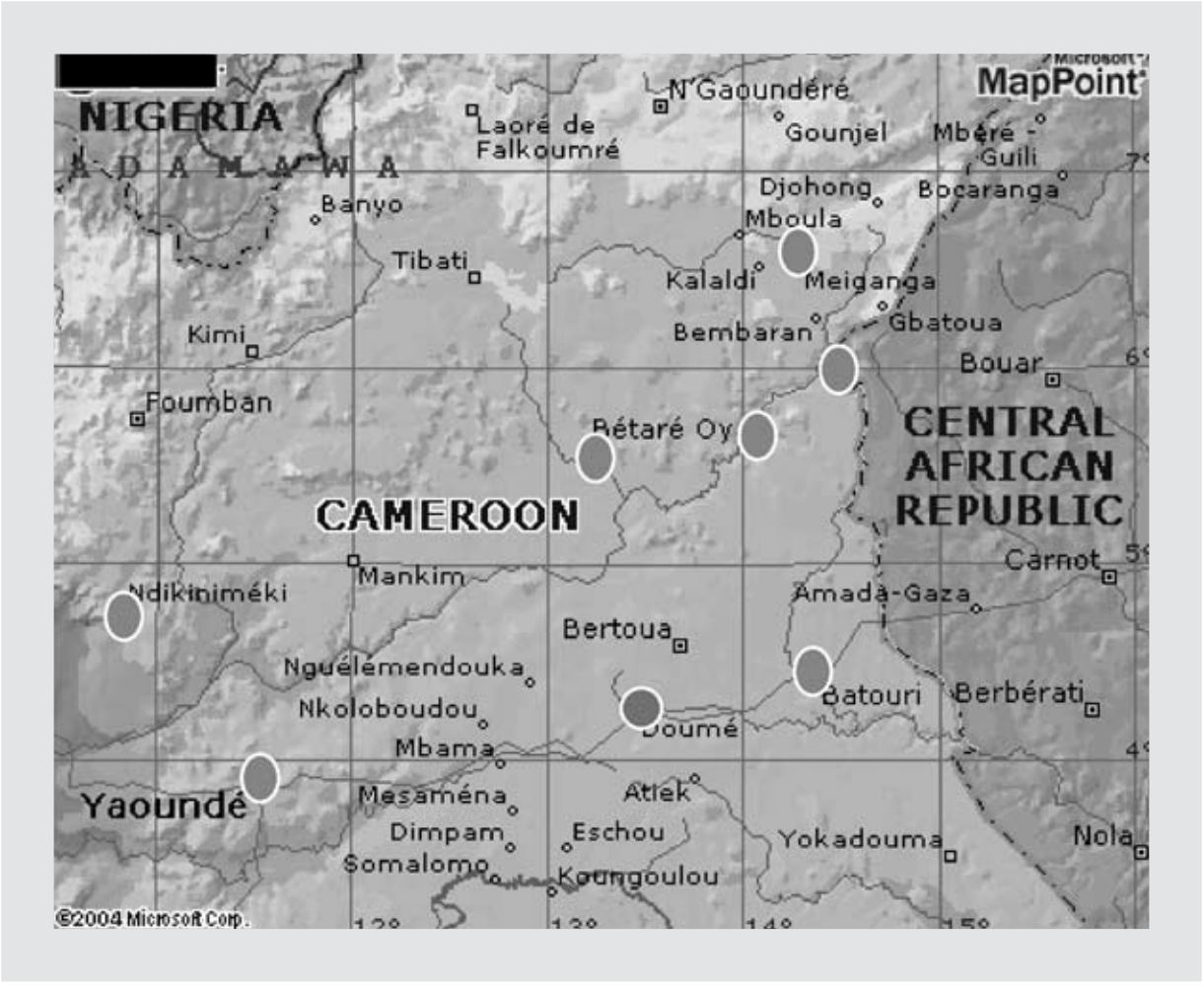

Figure 1. *Locations of pet animals in southern and central Cameroon. Study sites marked with a filled circle indicate areas where SIVmnd-2 were isolated.*

Materials and methods

Blood samples were collected from 28 juvenile/infant nonhuman primates kept as household pets in rural and urban areas of the southern part of Cameroon (Fig. 1). The plasma obtained from nonhuman primates was screened for HIV antibodies by a microparticle enzyme immunoassay kit (AxSYM HIV1/2; Dianabot, Tokyo, Japan) and particle agglutination assay (Serodia HIV-1 and HIV-2; Fujerebio, Tokyo, Japan)[5,6]. All reactive specimens were confirmed by Western Blot (New Lavblot HIV-1 and HIV-2, Sanofi Diagnostic Pasteur, Marnes-la-Coquette, France). DNA was extracted from whole blood (Qiagen, Hilden, Germany) and a part of the *pol* sequences covering the integrase gene (approximately 300 base pair) was amplified by nested polymerase chain reaction (PCR) using the primers, unipol 5 (5'-TGGGTACCAGCACACAAAGGAATAGGAGGAAA-3')/unipol 6 (5'-CCACAGCTGATCTCTGGCCTTCTCTGTAATAGACC-3'), in the first round; and unipol 1 (5'-AGTGGATTCATAGAAAGCAGAAGT-3')/unipol 2 (5'-CCCCTATTCCTTCCCCTTCTTTTAAAA-3'), in the second round. Amplification for the *pol* region was done at 45 °C for 30 seconds and 72 °C for one minute, with a final extension of 72 °C for 10 minutes. To confirm the species of the SIV-infected monkeys identified in this study, we amplified and sequenced the 424 base pair fragment of mitochondrial cytochrome b gene, which can be used to distinguish subspecies of old-world primates[6]. The fragment (corresponding to nucleotides 14725-15148 of human mitochondrial DNA) was amplified in the DNA extracted from peripheral blood mononuclear cells of the SIV-positive monkeys, using Qiagen DNA extraction kit (Qiagen, Hilden, Germany)[6]. Neighbor-joining phylogenetic trees including reference *pol* sequences and appropriate reference sequences for mtDNA were constructed using Clustal W then drawn

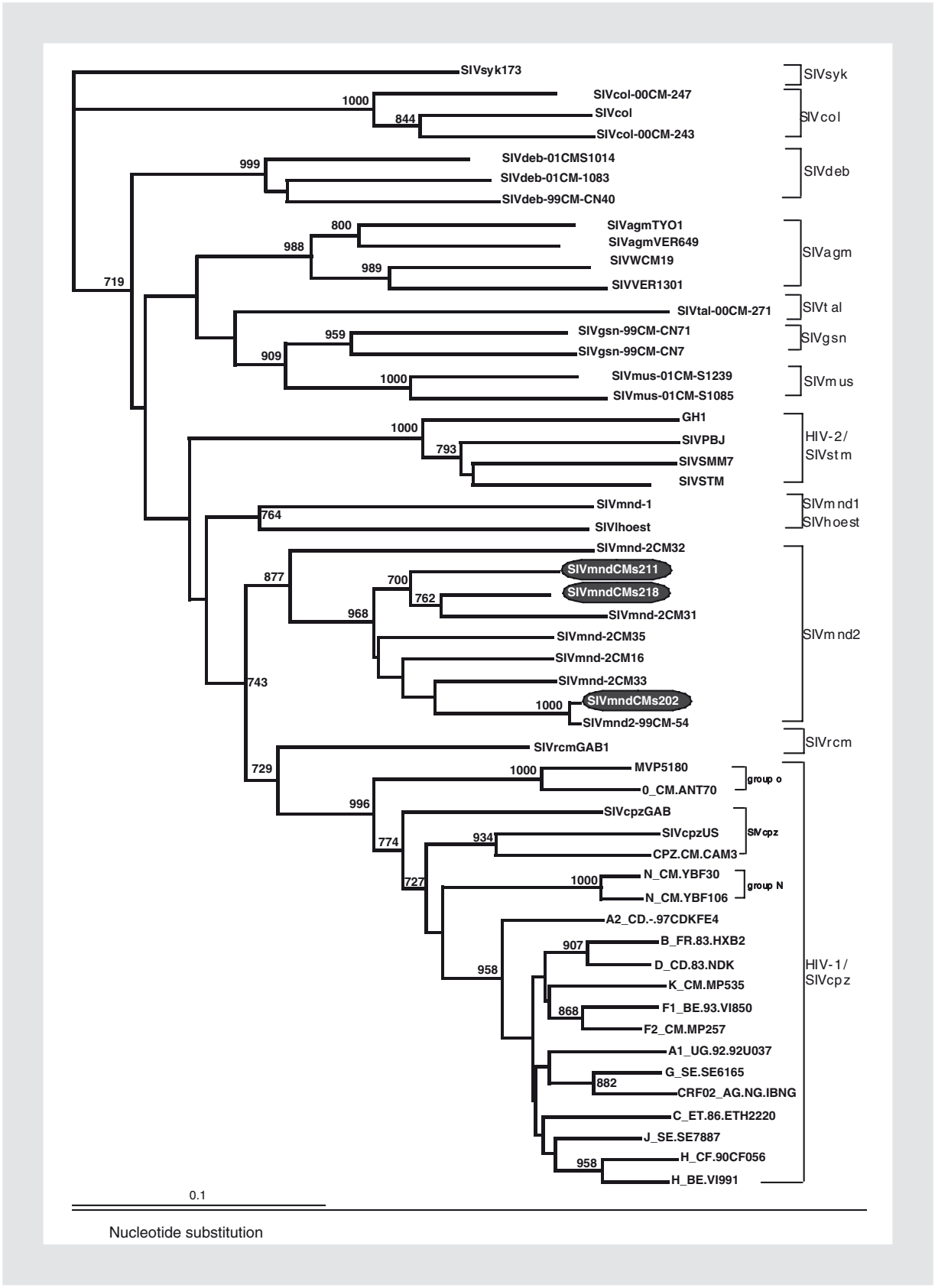

Figure 2. *Phylogenetic tree of pol sequences from three SIVmnd-2 isolates (SIVmndCM-202, SIVmndCM-211 and SIVmndCM-218). The bootstrap value at each node represents the number among 1,000 bootstrap replicates that support the branching order. Bootstrap resampling of 70% or higher is shown. Brackets on the right represent the major Simian Immunodeficiency Virus (SIV) lineages.*

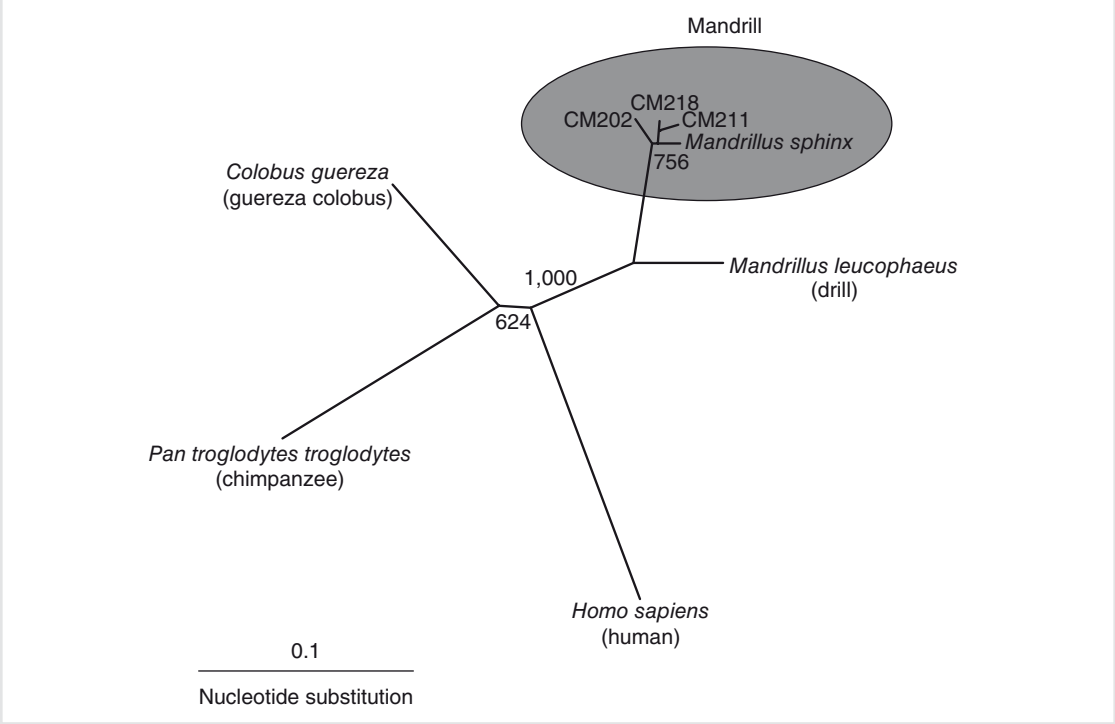

Figure 3. *Un-rooted tree of the 424 base pair (bp) fragment of mitochondrial (mt) cytochrome b gene, which can be used to distinguish subspecies of old-world primates.*

using Treeview PPC version 1.6.6 (Institute of Biochemical and Life Sciences, Scotland, UK), (Fig. 2 and 3). Bootstrap resampling (1,000 data sets) of multiple alignments was performed to test the statistical robustness of the trees. Kimura-2 parameter were calculated with the DNADIST program in the PHYLIP package program[9,10].

Accession numbers

The DNA sequences of SIVmnd-2 and mitochondrial cytochrome b DNA determined as part of this study have been submitted to GenBank (accession numbers to be available soon).

Results and discussion

Most primates kept as household pets we identified were seen in a southern region of Cameroon (Fig. 1). Table 1 shows the results of a serologic survey of pet juvenile and infant nonhuman primates according to species. Since commercially available HIV screening assays contain only a limited number of antigens, we used Western Blot as a confirmatory assay. Analysis of 28 nonhuman primate sera found no SIV infection in *Cercopithecus nictitans* (0/5, 0%), and *Pan troglodytes troglodytes* (0/10, 0%), and a prevalence rate of (3/13, 23.1%) for *Mandrillus sphinx* (Table 1). The PCR amplification of short fragment of *pol* -(integrase) was successful in all sero-reactive samples. Our findings confirm a high prevalence of SIVmnd-2 infection in mandrills as described by Peeters, et al.[11] in 7/20 (35%) of pet animals in southern Cameroon. The mandrill is a large semi-terrestrial primate belonging to the *Papionini* tribe, living in the tropical rain forests of Cameroon and Gabon[9,10]. Behavioral data generated by Wolfe, et al.[4] from a study conducted among 3,971 persons in 17 village sites in southern Cameroon indicated that monkeys were kept more frequently than other types of wild animals. Of people sampled across all sites, 0.6% kept gorillas, 1.5% chimpanzees, 9.9% monkeys, and 1.8% rodents[4]. In our study most of the pets were still juveniles or infants at the time of sampling, and 17.8% were greater spot-nosed monkeys, 35.7% chimpanzees, and 46.4% mandrills. Previous studies also reported a high proportion of greater spot-nosed monkeys (44/215, 20.5%), mustached guenons (29/215, 13.5%), olive baboons (22/215, 10.2%), and mandrills

Table 1. Serological survey and nested PCR amplification of animal kept as pets in southern Cameroon

Species (common name)	SIV lineage	Serology					Nested PCR amplification	
		HIV-1/2 Ag/Ab Combo	AxSYM*	HIV-1 PA†	HIV-2 PA	HIV-1 WB‡	pol-IN§	env-gp41
Mandrillus sphinx (Mandrill)	SIVmnd-2	3/13 (Npos/ Ntested)					3/13	0/13
		(1)-01cmr-202	44.9	256	512	(± gp160, gp110/120, p18)	SIVmndCM-202	(–)
		(2)-01cmr-211	16.7	< 32	512	(± p52, p34, p25, p18)	SIVmndCM-211	(–)
		(3)-01cmr-218	57.6	1,024	2,048	(± gp160, gp110/120, p18)	SIVmndCM-218	(–)
Cercopithecus nictitans	/	0/5	< 1.0	< 16	< 16	/	(–)	(–)
Pan troglodytes (Chimpanzee)	/	0/10	< 1.0	< 16	< 16	/	(–)	(–)

*AxSYM HIV-1/2 , a microparticle assay EIA (Abbott, Tokyo, Japan); signal/cut-off > 1.0 means reactive.
†Particle agglutination (PA), titer against HIV-1 or HIV-2. The antibody titer was measured according to manufacturer instructions (Serodia HIV, Fujirebio, Tokyo, Japan).
‡New Lavblot HIV-1 and HIV-2 (Sanofi Pasteur, Marnes-la-Coquette, France).
§Genotyping based on HIV *pol*-integrase region (288 bp).

(20/215, 9.3%) as domesticated monkeys in southern Cameroon[11,12]. During the 20th century, firearms increased the efficiency and frequency of hunting. Both subsistence and commercial hunting with wire snares and firearms are widespread activities throughout the forests of Central Africa[4]. Most pet monkeys are acquired at a very young age, often when their parents are killed by hunters. Because pets are usually young, the prevalence of chronic diseases in this population may be less than that among adult primates to which hunters and butchers are exposed. Nevertheless, because of the potential for regular contact with pet animals, even a low frequency of infections among pets may be important.

As SIVsm was able to jump to the human population, the possibility that SIVmnd-infected mandrills could also represent a reservoir posing a risk for humans cannot be excluded. Thus, further study will be necessary to clarify if SIVmnd-2 is capable of zoonotic transmission, and if interventions will be necessary to prevent the introduction of an SIV into humans and the appearance of new "HIV" in central Africa.

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