Naturally acquired simian retrovirus infections in central African hunters


Summary

Background Hunting and butchering of wild non-human primates infected with simian immunodeficiency virus (SIV) is thought to have sparked the HIV pandemic. Although SIV and other primate retroviruses infect laboratory workers and zoo workers, zoonotic retrovirus transmission has not been documented in natural settings. We investigated zoonotic infection in individuals living in central Africa.

Methods We obtained behavioural data, plasma samples, and peripheral blood lymphocytes from individuals living in rural villages in Cameroon. We did serological testing, PCR, and sequence analysis to obtain evidence of retrovirus infection.

Findings Zoonotic infections with simian foamy virus (SFV), a retrovirus endemic in most Old World primates, were identified in people living in Central African forests who reported direct contact with blood and body fluids of wild non-human primates. Ten (1%) of 1,099 individuals had antibodies to SFV. Sequence analysis from these individuals revealed three genetically independent human SFV infections, each of which was acquired from a distinct non-human primate lineage: De Brazza's guenon (Cercopithecus neglectus), mandrill (Mandrillus sphinx), and gorilla (Gorilla gorilla), two of which (De Brazza's guenon and mandrill) are naturally infected with SIV.

Interpretation Our findings show that retroviruses are actively crossing into human populations, and demonstrate that people in central Africa are currently infected with SFV. Contact with non-human primates, such as happens during hunting and butchering, can play a part in the emergence of human retroviruses and the reduction of primate bushmeat hunting has the potential to decrease the frequency of disease emergence.

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Methods

Obtained samples from 1,099 people, including 999 human and 100 non-human primates. Selected samples from 29 individuals for analysis. Designed PCR and sequencing assays. Conducted serological testing. Did serological testing, PCR, and sequence analysis to obtain evidence of retrovirus infection.

Findings

Zoonotic infections with simian foamy virus (SFV), a retrovirus endemic in most Old World primates, were identified in people living in Central African forests who reported direct contact with blood and body fluids of wild non-human primates. Ten (1%) of 1,099 individuals had antibodies to SFV. Sequence analysis from these individuals revealed three genetically independent human SFV infections, each of which was acquired from a distinct non-human primate lineage: De Brazza's guenon (Cercopithecus neglectus), mandrill (Mandrillus sphinx), and gorilla (Gorilla gorilla), two of which (De Brazza's guenon and mandrill) are naturally infected with SIV.

Interpretation

Our findings show that retroviruses are actively crossing into human populations, and demonstrate that people in central Africa are currently infected with SFV. Contact with non-human primates, such as happens during hunting and butchering, can play a part in the emergence of human retroviruses and the reduction of primate bushmeat hunting has the potential to decrease the frequency of disease emergence.
workers. SFV is also endemic in most Old World primates. For these reasons, SFV infection can serve as a sensitive marker for the potential for natural infection with other, less transmissible simian retroviruses, such as SIV. Furthermore, SFV is genetically diverse and shows host-specific viral lineages, which facilitate the identification of the non-human primate source species in SFV-infected people. Although global populations have been screened for evidence of natural SFV infection, studies have not focused on individuals reporting contact with non-human primates in the wild, and have so far failed to present evidence of natural infection. Here, we combine evaluation of behaviours, such as the hunting and butchering of non-human primates, that may place individuals at risk for the acquisition of simian retroviruses with examination of blood samples for evidence of SFV infection.

Methods
We did the studies in the context of a community-based HIV-prevention campaign designed to provide information and decrease transmission. Participation was voluntary and participants gave informed consent. The study protocol was approved by the Johns Hopkins Committee for Human Research, the Cameroon National Ethical Review Board, and the HIV Tri-services Secondary Review Board. We made the questionnaires and matching samples anonymous by removing all personal identifiers to provide an unlinked study population.

Procedures
Blood was obtained from participants, transported to a central laboratory, processed into plasma and peripheral blood lymphocyte samples, and stored at -80°C until used. We first screened plasma samples for antibodies to SFV using EIA following standard procedures. We diluted samples 1 in 100 and tested them in duplicate in separate microplates containing antigen from either uninfected canine thymocytes or combined SFV antigen from canine thymocytes infected with SFV from ape (SFVcpz, chimpanzee) or monkey (SFVagm, African green monkey). We averaged the replicate sample optical density values, and calculated optical density ratios of reactivity to SFV over the uninfected antigens. An optical density ratio of greater than 1.32 was set as a cutoff value for seroreactive samples on the basis of assay validation with PCR-confirmed infected and uninfected non-human primates and human beings (data not shown). We further tested EIA-reactive samples by two western blot assays as previously described. Criteria for western blot positivity were reactivity in the SFV blot to Gag p68 and p72, or p70 and p74 proteins (characteristic of monkey-type or ape-type SFV infection, respectively) and absence of similar...
These primers are located within the 5' (TCC AGG ITT GGT AAG 3') and were variable RIU5 region of the LTR and successfully to amplify diverse 3' in the first round of GAG TGT 3') in the second round of amplification with primers PBR2 (5'GGG ATT TTG TAT ATT GAT TAT CC 3'), which were dilutions of DNA lysates prepared from cells infected with an Asian macaque SFV (SFVmac) isolate. SFVmac is specific to Asian macaques and should not be found in African primates, and therefore controls for cross-contamination from positive controls.

We obtained peripheral blood lymphocytes by Ficoll-Hypaque centrifugation and prepared DNA lysates as previously described.

We did not amplify DNA from peripheral blood lymphocytes and confirmed its integrity by 3: Detection of SFV genomic DNA by nested PCR using primer pairs PBR1 and PBR2 (5'GGG ATT TTG TAT ATT GAT TAT CC 3'), which were dilutions of DNA lysates prepared from cells infected with an Asian macaque SFV (SFVmac) isolate. SFVmac is specific to Asian macaques and should not be found in African primates, and therefore controls for cross-contamination from positive controls.

SFV isolates from a mandrill, drill, and olive baboon were propagated on C227 cells and DNA lysates were prepared and amplified as previously described.

Demographic data and risk profiles for ten individuals with confirmed SFV-epizootic results are shown in Figure 1. South African studies, weekly hand and mouth examination, and peripheral blood lymphocytes were processed separately and tested in laboratories in different buildings.

SFV isolates from a mandrill, drill, and olive baboon were propagated on C227 cells and DNA lysates were prepared and amplified as previously described.

We did PCR analysis of DNA from all primates using the first letter of the genus name and the species or subspecies names with their house names or codes within parentheses (panel 1). GenBank accession numbers for the 20 new SFV int sequences, the int sequences used for phylogenetic comparison, and the new SFV LTR sequences from infected humans are shown in panel 2.

Role of the funding source

JGC, FM, and DLB at the US Military HIV Research Program, one of the sponsoring organisations, contributed to the study design, data interpretation, and writing of the report.

Results

We examined 200 individuals from each of nine villages in southern Cameroon, close to natural non-human primate habitats, both forested and non-forested (Figure 1). Individuals were asked to identify and quantify their exposure to a range of non-human primates, which were classified into categories that could be reliably distinguished by the common chimpanzee, gorillas, and spider monkey. 1099 (61%) of 1800 participants reported direct exposure to fresh non-human primate blood and body fluids, mainly through hunting and butchering. We screened these 1099 exposed individuals for SFV antibodies using an EIA capable of detecting divergent monkey and ape SFVs, followed by confirmation with a

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<th>Sex</th>
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NK=SFV gag not known. Study sites are shown in Figure 1. Gu=Gunung, Nh=Ndong, M=Cameroon, N=Central African Republic, G=Gabon. We did not amplify DNA from peripheral blood lymphocytes and confirmed its integrity by 3: Detection of SFV genomic DNA by nested PCR using primer pairs PBR1 and PBR2 (5'GGG ATT TTG TAT ATT GAT TAT CC 3'), which were dilutions of DNA lysates prepared from cells infected with an Asian macaque SFV (SFVmac) isolate. SFVmac is specific to Asian macaques and should not be found in African primates, and therefore controls for cross-contamination from positive controls.

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We prepared DNA from peripheral blood lymphocytes matched with the ten western blot positive plasma samples. Three of these ten (CAM2467, CAM1465, and CAM1083) tested positive for both SFV int and LTR sequences by PCR analysis using generic primers based on available Old World monkey and ape SFV sequences (figure 3). We sequenced the int and LTR amplicons and analysed them phylogenetically using SFVs from non-human primates native to Cameroon and from other Old World monkeys. The int sequences from monkey and ape species all clustered into separate lineages, suggesting co-speciation of host and SFV (figure 4).

We identified identical tree-topologies using maximum likelihood analysis (data not shown), lending further support to the genetic relationships from the neighbour-joining analysis. The sequences from all three Cameroonian hunters were SFV-related and distinct from each other. The SFV int sequences all clustered with a different central African non-human primate SFV lineage with high bootstrap support. CAM1083 clustered tightly with gorilla (SFVgo) in the ape SFV group. CAM1465 and CAM2467 both fell within the monkey SFV clades. CAM1465 clustered tightly with SFV from mandrills (SFVmsp), whereas CAM2467 clustered with the sequences formed by Ceropithecus spp (De Brazza's guenon and Hamlyn's guenon) and had the highest relatedness to SFV from De Brazza's guenon (SFVene) (figure 4).

Similar phylogenetic relations were also apparent in the analysis of the LTR sequences (figure 4). Both CAM1083 and CAM1465 LTR sequences clustered with high bootstrap support with the clades from gorilla and mandrill, respectively, whereas CAM2467 clustered with a ceropithecus sequence from Hamlyn's guenon. LTR sequences from De Brazza's guenons are not available to confirm the closer phylogenetic relatedness seen in int between these sequences and that of CAM2467. This study extends the known range of non-human primates capable of transmitting SFV to man, which before this report included only animals common to zoos and laboratories (baboons, African green monkeys, macaques and chimpanzees*).*9–13)

Each of the three people who tested positive by PCR was from a different rural village in the lowland forest of southern Cameroon, a region of high primate biodiversity. The SFVgo-infected person was a 45-year-old man. He reported that he butchered and consumed monkey, chimpanzee, and gorilla meat, and hunted all these groups, using at various times, guns, bows, and wire snares. The SFVmsp-infected person was a 48-year-old woman who reported that she consumed butchering monkeys, and hunted monkeys with wire fences.
snare. The participant infected with SFVvme was a 25-year-old man who butchered both monkeys and chimpanzees, but who may also have been exposed through contact with a pet monkey. All three species implicated in these zoonotic SFV infections inhabit the geographic range of the study.

We did not detect SFV sequences in seven of the ten individuals who were western-blot positive or in all 12 with atypical western-blot profiles. To distinguish monkey-type from ape-type SFV infection, plasma samples from the seven people who tested positive by western blot and negative by PCR were sequenced by western blot with either ape or monkey SFV antigen. All seven showed stronger reactivity to monkey SFV antigen than to ape antigen, suggesting monkey-type infection (data not shown). The reasons for the negative SFV PCR results in these seven samples are unknown. They might be due to the presence of low proviral loads, divergent viruses, or they may indicate non-specific reactivity with the SFV gag proteins. Previous findings in primates show similar serological and PCR reactivity in monkeys infected with divergent SFV strains. Additional studies with virus isolation and PCR are needed to confirm SFV infection in these people.

**Discussion**

SFVs are known to have the potential to infect laboratory and zoo workers who are occupationally exposed to non-human primates. Our findings show that individuals reporting direct contact with primates are also infected with SFV under natural conditions. They show that people can be naturally infected with SFV originating from many non-human primate hosts (gorillas, mandrills, and De Brazza’s guenon). Of note, mandrills and De Brazza’s guenons, and other monkeys and apes from this geographic region, are infected with unique SIVs,” some of which replicate in human cells in vitro. Therefore the results suggest that the opportunity for cross-species transmission of other retroviruses, such as SIV, also exists in the same exposed population. SFV infections in this study were from several geographically isolated locations, suggesting that—contrary to conventional wisdom—infectious zoos are widespread, arising in various locations where people are naturally exposed to non-human primates. Although we cannot estimate the total number of such infections, widespread contact with such primates throughout rural forested regions of central Africa suggests that many such infections probably exist.

The scarce information that exists suggests that there is no secondary transmission or morbidity and mortality in people with SFV infections. However, since previous studies have been restricted to very few occupationally infected people, and because SFV is not screened for in blood banks, naturally acquired SFV might have spread undetected both within and outside central Africa, or it might be pathogenic. Awareness of this possibility calls for increased follow-up of SFV-infected individuals and further surveillance, since population-level spread raises the potential for viral adaptation and the evolution of pathogenicity.

Our results show simian retroviral zoonosis in people who have direct contact with fresh non-human primate bushmeat, and suggest that such zoonoses are more frequent, widespread, and contemporary than previously appreciated. The increased amount of hunting in central Africa that has resulted from a combination of urban demand for bushmeat and greater access to primate habitats provided by logging roads, has increased the frequency of human exposure to primate retroviruses and other disease-causing agents. In addition to helping conserve endangered species, the reduction of non-human primate hunting activities has the potential to reduce the frequency of cross-species transmission of simian retroviruses and other pathogens.

**Contributors**

N Wolfe, D Burke, W Switzer, T Folks, and W Henle contributed to planning and design of the study. N Wolfe acquired and analyzed primary data and contributed to the writing of the report. W Switzer, V Bhullar, V Shenmugam, A Wright, T Folks, and W Henlé designed and undertook laboratory studies, analyzed laboratory results, and contributed to the writing of the report. J Carr, F McCuschan, and D Bux contributed to the design of the study, interpretation of results, and writing of the report. J Tomimori and E Mpunji-Ngape assisted in the design of the study and interpretation of results. A Premo and T Tamboli designed behavioral questionnaires and contributed to the interpretation of results. All authors approved the final version of the report.

**Conflict of Interest statement**

None declared.

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**References**


