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Short Communication

Lack of Evidence for Infection with Simian Immunodeficiency Virus in Bonobos

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In the discussion on the origin of the human immunodeficiency virus type 1 (HIV-1) it has been hypothesized by E. Hooper in his book *The River* that the oral polio vaccine (OPV) could be held responsible for the introduction of the simian immunodeficiency chimpanzee virus (SIVcpz) into humans, leading to its human counterpart, HIV-1.[1] This theory suggests that oral polio vaccine stocks would have been prepared in chimpanzee kidney tissue contaminated with SIV. During the African vaccination trials in the 1950s these allegedly SIV-contaminated OPVs would have then been administered to people in what is now the Democratric Republic Congo. However, this OPV/HIV hypothesis has recently lost a great deal of its credibility due to the work of Korber *et al.*,[2] Salemi *et al.*,[3] Rambaut *et al.*,[4] Blancou *et al.*,[5] and Berry *et al.*[6] The first three studies show that HIV-1 group M infection in humans originated before the 1930s and thus predates the oral polio vaccination trials, while the last two studies confirm that macaque kidney tissue had been used for the preparation of the early batches of the OPV, and the absence of chimpanzee mitochondrial (mt)DNA and HIV/SIV_{CPZ}-like sequences in these vaccines. However, as R. Weiss[7] mentioned, it could still be argued whether "chimpanzee kidney cells" and bonobo kidney tissue "could have been used locally in Africa to amplify the batches of the OPV," as insinuated by E. Hooper.[1,8,9] As E. Hooper stated in his book, bonobos (*Pan paniscus*) have been reported earlier by us and others,[10,11] to be at the origin of another zoonotic human retrovirus, human T-cell lymphotropic virus type II (HTLV-II). Thus, bonobos and the use of their tissues may pose a potential risk for cross-species transmission of other etiologic agents. SIV infection has been reported in various African monkey species, but no data about the serostatus in bonobos have been published yet. The fact that SIV infection has been described in two subspecies of the bonobo's closest related simian species, the common chimpanzee or *Pan troglodytes*,[12–14] raises the question of whether bonobos are also a natural host for SIV. This is especially interesting since the natural habitat of the affected *P.t. troglodytes* and *P.t. schweinfurthii* subspecies neighbors that of the bonobo, which is situated in the Central Congo basin, between the Congo River, the Lomami and the Kasai/Sankuru Rivers, and the Lake Tumba/Lac Ndombe area (Fig. 1).

To assess the prevalence of SIV in bonobos, we have performed a serologic survey of 26 bonobos, of which 14 were wild caught and 12 were born in captivity. Thirteen animals were housed at the Yerkes primate center in Atlanta, Georgia, 7 in a breeding center of the Antwerp Zoo in Belgium, 2 in the Leipzig Zoo in Germany, and 4, saved from poachers, were housed in an orphanage in Congo Brazzaville. The bonobo plasma/serum samples were screened with either the Genetic Systems HIV-1/-2 peptide EIA (Redmond, WA), the Abbott AxSYM HIV-1/2 microparticle EIA (Abbott Laboratories, Delkenheim, Germany), or an in-house SIV antigen ELISA using SIVmac251 purified viral lysate (Advanced Biotechnologies Incorporated, Columbia, MD). All test results were negative. Additional screening of all 26 bonobo samples with the INNOLIA HIV (Innogenetics, Ghent, Belgium) confirmation test, a line immunoblot assay more sensitive for divergent SIV strains than commercial Western blots,[15] found no reactivity in all 26 bonobos. Previously, these animals were screened for infection with another simian retrovirus, the simian T-cell lymphotropic virus type I/II (STLV) using an HTLV-I/-II particle agglutination test (Serodia-HTLV, Fujirebio, Japan) and/or an

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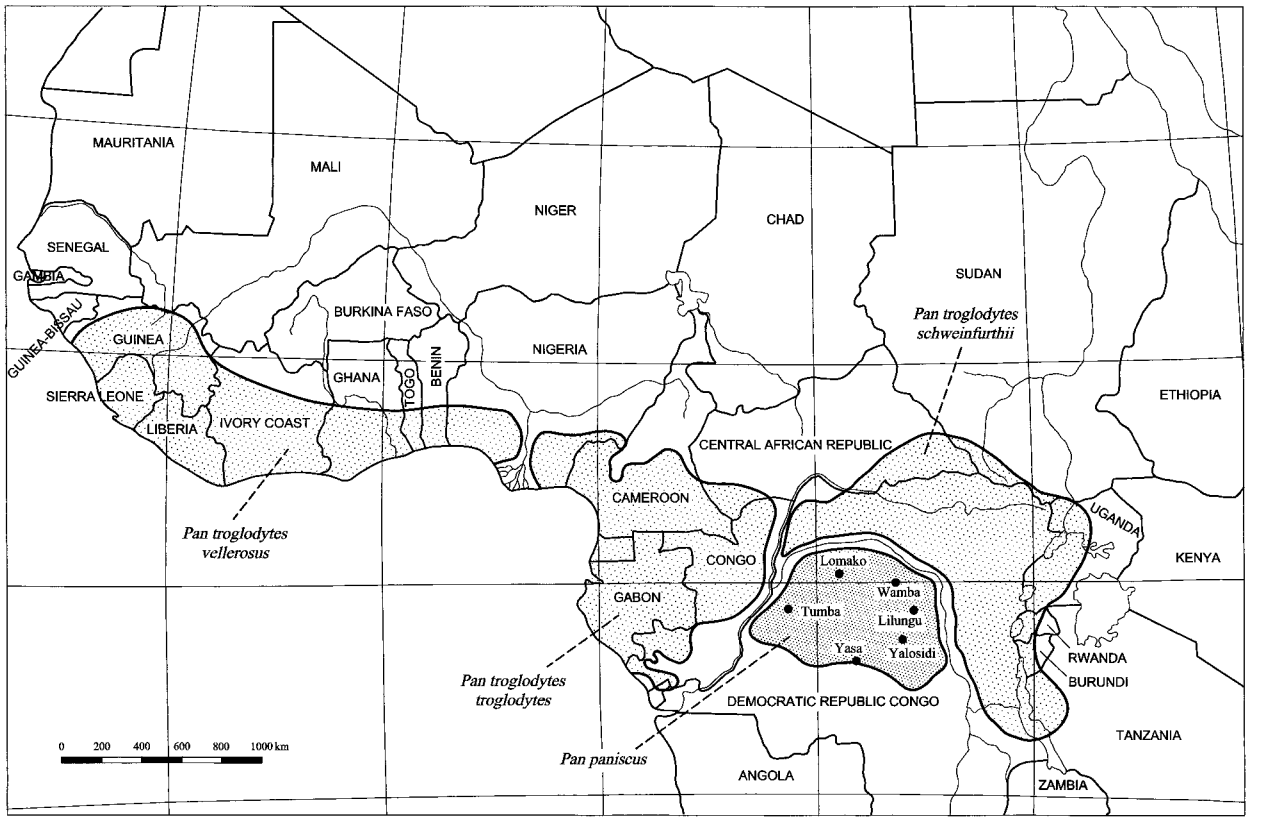

FIG. 1. Map of the probable distribution of members of the genus *Pan* in equatorial Africa around 1900, indicated by dotted regions (adapted from de Waal and Lanting[20]). The three subspecies of *Pan troglodytes* are spread from West to East Africa: *P.t. vellerosus* in West Africa, *P.t. troglodytes* in Central Africa, and *P.t. schweinfurthii* in East Africa. The *Pan paniscus* is restricted to an area south of the Congo River. Six study sites are indicated on the map.

HTLV-I/-II Western blot assay (Genelabs, Diagnostics HTLV Blot 2.3, Singapore, Malaysia). Twelve animals were found to be reactive or indeterminant by these assays, six U.S. bonobos with positive but untypeable Western blot results, 2 Congolese with positive Serodia and indeterminate Western blot results, and 4 from Antwerp, with positive Serodia and positive but untypeable Western blot results. Three out of six U.S. bonobos had documented STLV-II infection by cell culture and one Antwerp bonobo by cell culture and PCR assays.[10,11]

The prevalence of SIV infection among different monkey species and the study of SIV/HIV phylogeny both contribute to the understanding of SIV evolution, cross-species, and zoonotic transmissions. Therefore, it is equally important to report positive and negative findings of SIV infections in different simian species. To put our SIV-negative findings in the proper perspective, we need to consider the following possible origins for a putative SIV infection in *Pan paniscus*. Any SIV infection of the bonobo species has to be either the result of virus–host coevolution or viral cross-species transmissions. The first scenario, that of host-dependent SIV evolution, has been suggested by several groups based on the current knowledge of SIV/HIV phylogeny.[16–19] The finding of distinct monophyletic subspecies clusters such as SIVcpz, SIVagm, and SIVlhoest/sun support this coevolution theory. Since bonobos and SIV-infected chimpanzees speciated only approximately 3 million years ago,[20] the coevolution scenario implies that the bonobo species is a potential natural host for a distinct SIV. A recent study on the origin of HIV-1 group M,[3] however, stated that a simian-to-human transmission of SIV, probably from chimpanzees, must have occurred between 1700 and 1930 in order to give rise to HIV-1 clade M. Based on this time frame, SIV cospeciation seems very unlikely, as the SIVcpz common ancestor, and possibly even the common ancestor of all SIVs, probably diverged less than 3 million years ago. SIV infections therefore most likely all originate from cross-species transmissions. In view of this second scenario, putative SIV infections among bonobos have to be due to cross-species transmissions. Clear cases of cross-species transmissions of SIV have been reported several times for other simian species such as SIVlhoest/sun ⇔ SIVmnd[17,18] and SIVdrl ⇔ SIVrcm.[21] This would imply interspecies transmissions in the wild between SIV-infected species living in overlapping habitats. The only currently known SIV-infected simian species sharing the habitat of the *Pan paniscus* are *Cercopithecus neglectus*, *C. ascanius*, *C. wolfi*, and *Cercocebus agilis*.[15,22,23] Interspecies contacts of the small number of bonobos still living in the wild with other primates have been reported,[24–26] but bonobos are known as a very social and peaceful species,[20] and hunting or aggressive interactions with sympatric primates have not been observed.[24] Therefore, the chance of SIV interspecies transmissions to bonobos seems very low.

Taking into account the time frame of introduction of SIV

into primates, the low chances of SIV transmission to bonobos from sympatric species, and the SIV seronegative status of the 26 bonobos presented here, it seems very unlikely that among the few bonobos at camp Lindi (where chimpanzees and bonobos were kept by the Wistar Institute, which conducted the OPV experiments), one would have carried an SIV strain closely related to HIV-1. It seems even more unlikely that the kidneys of that specific bonobo would have been used to locally amplify the prepared OPV batches.

Although our negative data do not support the hypothesis that bonobos are a natural host for SIV, they must be interpreted in terms of the limitation of the small number of animals tested. However, the detection of STLV-II infection in this population was reassuring and suggests that the ability to detect bonobo-type retroviral infections was not affected by our sampling. Nevertheless, additional screening of larger numbers of bonobos is needed to confirm these results, for example, by testing urine and fecal samples of bonobos in the wild as Santiago *et al.* described for the *Pan troglodytes* species.[27] Since bonobos are an endangered species, it will be very difficult to sample new wild-caught animals. Our results still represent about 20% of the bonobos in captivity (26/125[28]) but only 0.52–0.13% of the bonobo population in the wild.[29] The bonobo population will probably continue to decrease mainly due to increased hunting and slaughter by humans for the bushmeat trade or for personal consumption and because of destruction of the bonobo's habitat.

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