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Lack of Evidence of Simian Immunodeficiency Virus Infection Among Nonhuman Primates in Taï National Park, Côte d'Ivoire: Limitations of Noninvasive Methods and SIV Diagnostic Tools for Studies of Primate Retroviruses

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Abstract

It is now well established that the human immunodeficiency viruses, HIV-1 and HIV-2, are the results of cross-species transmissions of simian immunodeficiency viruses (SIV) naturally infecting nonhuman primates in sub-Saharan Africa. SIVs are found in many African primates, and humans continue to be exposed to these viruses by hunting and handling primate bushmeat. Sooty mangabeys (*Cercocebus atys*) and western red colobus (*Piliocolobus badius badius*) are infected with SIV at a high rate in the Taï Forest, Côte d'Ivoire. We investigated the SIV infection and prevalence in 6 other monkey species living in the Taï Forest using noninvasive methods. We collected 127 fecal samples from 2 colobus species (*Colobus polykomos* and *Procolobus verus*) and 4 guenon species (*C. diana*, *C. campbelli*, *C. petaurista*, and *C. nictitans*). We tested these samples for HIV cross-reactive antibodies and performed reverse transcriptase-polymerase chain reactions (RT-PCR) targeting the *gag*, *pol*, and *env* regions of the SIV genome. We screened 16 human microsatellites for use in individual discrimination and identified 4–6 informative markers

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per species. Serological analysis of 112 samples yielded negative ($n=86$) or uninterpretable ($n=26$) results. PCR analysis on 74 samples confirmed the negative results. These results may reflect either the limited number of individuals sampled or a low prevalence of infection. Further research is needed to improve the sensitivity of noninvasive methods for SIV detection.

Keywords

Microsatellites; Nonhuman primates; Noninvasive sampling; Serologic assays; SIV

Introduction

There are 40 different nonhuman primate species in sub-Saharan Africa that are infected with SIVs (van de Woude and Apetrei 2006; Van Heuverswyn and Peeters 2007). It is now well established that SIVs from chimpanzees (*Pan troglodytes troglodytes*) and gorillas (*Gorilla gorilla gorilla*) in West central Africa and from sooty mangabeys (*Cercocebus atys atys*) in West Africa are the progenitors of human immunodeficiency virus type 1 (HIV-1) and HIV-2, respectively, the etiologic agents for AIDS (Gao *et al.* 1999; Hirsch *et al.* 1989; Keele *et al.* 2006; Peeters *et al.* 1989; Van Heuverswyn *et al.* 2006). These viruses have crossed species barriers on multiple occasions and generated different groups of HIV-1 (M, N, O, and P) and HIV-2 (A–H) (Hahn *et al.* 2000; Plantier *et al.* 2009). SIV infections have been documented in great apes, *Cercopithecinae* (guenons), *Papionini* and *Colobinae*, exclusively on the African continent. Each primate species is infected with a species-specific SIV lineage, and some closely related monkey species are infected with closely related SIVs, suggesting virus–host coevolution (van de Woude and Apetrei, 2006). However, studies have also described cross-species transmission followed by recombination with highly divergent lentiviral strains (Bailes *et al.* 2003; Clewley *et al.* 1998; Salemi *et al.* 2003; Souquiere *et al.* 2001). One species can also be infected with 2 different SIV lineages (Souquiere *et al.* 2001; Takehisa *et al.* 2001) or with SIV variants that result from recombination events between SIVs from cohabiting monkey species (Aghokeng *et al.* 2007).

It has become widely accepted that the handling and consumption of SIV infected primates contributed to the emergence of HIV and that humans are still exposed to SIVs when adopting these practices (Aghokeng *et al.* 2009; Peeters *et al.* 2002). Therefore it is important to investigate wild-living nonhuman primate populations for SIV infection and to determine SIV prevalence. In the last 5 yr, researchers have developed and refined methods to detect specific antibodies and to extract nucleic acids from feces and have investigated the presence of SIV infection in wild-living nonhuman primate populations by analyzing fecal samples collected from the forest floor (Keele *et al.* 2006; Locatelli *et al.* 2008b; Santiago *et al.* 2005; Van Heuverswyn *et al.* 2006, 2007b). Moreover, microsatellite analysis on the fecal samples collected can help to discriminate individuals from the same species: an especially useful tool for cryptic primate species living in the high strata of the forest canopy (Bonhomme *et al.* 2005; Coote and Bruford 1996; Goossens *et al.* 2000).

The Taï National Park in Côte d'Ivoire is inhabited by 9 diurnal primate species: western red colobus (*Piliocolobus badius badius*), black-and-white colobus (*Colobus polykomos polykomos*), olive colobus (*Procolobus verus*), Diana monkey (*Cercopithecus diana diana*), Campbell's monkey (*Cercopithecus campbelli campbelli*), lesser spot-nosed monkey (*Cercopithecus petaurista buettikoferi*), greater spot-nosed monkey (*Cercopithecus nictitans stampflii*), sooty mangabey (*Cercocebus atys atys*), and West African chimpanzee (*Pan troglodytes verus*) (McGraw *et al.* 2007). The human population hunts and eats all these species; the most caught monkey species are red colobus, black-and-white colobus, sooty

mangabeys, and Diana monkeys (Bshary 2001; Caspary *et al.* 2001; Refisch and Koné 2005). Of these species, western red colobus, olive colobus, sooty mangabeys, and chimpanzees have been tested for SIV infection. Despite extensive testing for HIV cross-reactive antibody detection, researchers have not yet detected any naturally occurring lentiviruses in West African chimpanzees (Prince *et al.* 2002; Switzer *et al.* 2005; Van Heuverswyn *et al.* 2007a). However, 80% of western red colobus (Leendertz *et al.* 2010; Locatelli *et al.* 2008b) and 50% of sooty mangabeys are infected with SIV (Santiago *et al.* 2005). Importantly, the sooty mangabey populations from this part of Africa are the ancestors of the HIV-2 strains responsible for the epidemic in West Africa. We suspect the SIV prevalence in these populations to be even higher, as the results are based on virion RNA detection in fecal samples, and this underestimates infection status. For example, a study of fecal RNA detection of SIV in sooty mangabey (SIVsmm) fecal samples revealed 50% decreased sensitivity compared to RNA detection in the corresponding blood samples (Ling *et al.* 2003), and PCR yield from SIV fragments extracted from fecal samples of western red colobus was only 93% and 64% in *env* and *pol* fragments, respectively (Locatelli *et al.* 2008b). Olive colobus are infected with a species-specific SIV lineage, SIVolc, distantly related to SIV in western red colobus (SIVwrc) (Courgnaud *et al.* 2003b). One of 2 individuals tested was positive, but the prevalence of infection in the wild is unknown. There is no information regarding the infection and the prevalence of SIV in the remaining 5 species (Courgnaud *et al.* 2003b; Locatelli *et al.* 2008b; Santiago *et al.* 2002, 2005).

We report the serological and molecular results of 127 fecal samples collected from habituated free-ranging *Colobus* and *Cercopithecus* species in the Taï Forest. We also report on human markers that cross-amplify microsatellite loci in these nonhuman primate species. Our results show that none of the 127 fecal samples collected was SIV positive. These results may reflect the limited number of individuals sampled, the reduced performance and sensitivity of the diagnostic tools identifying not yet characterized SIV in fecal material, or a low prevalence of infection. We also include recommendations to improve the outcome of future studies with similar research goals.

Materials and Methods

Study Site and Sample Collection

Taï National Park measures 4570 km² and is located in the southwestern part of Côte d'Ivoire (6°20'N–5°10'N and 4°20'W–6°50'W). The study site is located near the western border of the park, *ca.* 20 km southeast of the town of Taï (Stoorvogel 1993; Withmore 1990) (Fig. 1).

We sampled 2 social groups (pol1 and pol3) of black-and-white colobus, 3 social groups (ver1, ver2, and ver3) of olive colobus, 3 Diana monkey groups (dia1, dia2, and dia3), 1 group (cam1) of Campbell's monkeys, 1 group (pet1) of lesser spot-nosed monkeys, and 1 group (nic1) of greater spot-nosed monkeys. Researchers have habituated all these groups to their presence since the early 1990s (McGraw *et al.* 2007). We collected data on group size, composition, and range in parallel to fecal sampling from March 2004 through July 2004. During group observation, we collected freshly dropped faecal samples (2–5 g) in 15-ml tubes containing 7 ml of RNAlater (Ambion, Austin, TX). For each fecal sample, we recorded the name of the collector, the species, sex and age class of the individual if known, as well as the date, time, and location in a 3-km² grid system with 100×100 m cells marked with painted dots on trees (Fig. 1). We sampled greater spot-nosed monkeys outside the main study grid. Sampling was biased toward adults and subadults vs. juveniles or infants, because the former were less shy and their sex was more easily identified. We recognized several individuals from the black-and-white and the olive colobus groups and collected

sequential samples from the same individuals. For the *Cercopithecus* species we assessed only the age-sex class. We stored samples at camp for 30–60 d at 4°C and subsequently shipped them to the laboratory in Montpellier, France, where they were kept at –80°C.

Detection of HIV Cross-Reactive Antibodies in Fecal Samples

We recovered IgGs after dialyses of fecal samples by applying methods previously adopted for antibody detection in fecal samples of gorillas, chimpanzees, and western red colobus (Keele *et al.* 2006; Locatelli *et al.* 2008b; Van Heuverswyn *et al.* 2006). Briefly stated, we resolubilized RNAlater-precipitated immunoglobulins by diluting fecal/RNAlater mixtures (1.5 ml) with PBS–Tween 20 (7.5 ml) followed by inactivation of this solution for 1 h at 60°C and centrifugation (3500g for 10 min) to eliminate the presence of salt contained in the RNAlater medium. We then dialyzed it against PBS overnight at 4°C. We tested the reconstituted extracts for HIV cross-reactive antibodies using the INNO-LIA HIV confirmation test (Innogenetics, Ghent, Belgium). This test, which has been successful in identifying the majority of the known SIV lineages, includes HIV-1 and HIV-2 recombinant proteins and synthetic peptides that are coated as discrete lines on a nylon strip. In addition to these HIV antigens, each strip has control lines: 1 sample addition line (3+) containing antihuman immunoglobulin (IgG) and 2 test performance lines (1+ and +/–) containing human IgG. We performed all assays according to the manufacturer's instructions and scored samples as INNO-LIA positive when they reacted with 1 HIV antigen.

Nucleic Acid Extraction from Fecal Samples

We extracted viral RNA from 74 fecal samples using the RNAqueous-Midi kit (Ambion, Austin, TX), as previously described (Keele *et al.* 2006; Locatelli *et al.* 2008b; Santiago *et al.* 2003). We selected samples for RNA extractions based on the amount of material still available after DNA analysis and antibody detection. For fecal DNA extractions, we used the QIAamp Stool DNA mini kit (QIAGEN, Hilden, Germany) and previously described methods (Keele *et al.* 2006; Locatelli *et al.* 2008b). We also cross-checked a subset of samples for DNA quality by amplifying a region of *ca.* 1500 bp in the glucose-6-phosphate dehydrogenase gene (G6PDH) and a *ca.* 390 bp fragment of the mitochondrial DNA spanning the 12SrRNA gene (data not shown).

Amplification of SIV from Fecal RNA

We know from our previous study on *Ptilocolobus badius badius* that the absence of IgG reactivity does not correlate with the absence of virus, because we were able to amplify SIVwrc in 14 individuals (Locatelli *et al.* 2008b). In addition, we could not rule out a lack of sensitivity of the serological tests performed on fecal samples from nonhuman primates species investigated for the first time. Therefore, despite the absence of IgG reactivity, we proceeded to viral RNA detection. We performed RT-PCRs on total fecal RNA using SIV lineage-specific as well as universal primers: primer sequences, annealing temperatures, amplicon sizes, targeted region of the SIV genome are listed in Table I. We tested consensus primers amplifying fragments ranging from *ca.* 200 to *ca.* 800 bp of the SIV *pol* gene (DR1-DR2/DR4-DR5 and polOR-polis4/polis2-uni2) on samples from all species (Clewley *et al.* 1998; Courgnaud *et al.* 2001). We also used more SIV lineage-specific primers amplifying fragments of the *pol* gene of SIVs from the SIVgsn/mus/mon lineage in *Cercopithecus* species (*C. mona*, *C. cephus*, and *C. nictitans*) (Aghokeng *et al.* 2007), which we tested on Diana, Campbell's, and lesser and greater spot-nosed monkeys. We tested specific primers amplifying SIVwrc *pol* and *env* fragments of 650 and 570 bp, respectively (Locatelli *et al.* 2008b), on *Colobus polykomos* and on *Procolobus verus*, based on the hypothesis that SIV would have coevolved within the subfamily of the Colobinae. SIVwrc *pol* and *env* are known to amplify SIVolc (Liégeois *et al.* 2009). In addition, we also tested newly designed primers (olcpol) targeting a smaller region of *pol* of *ca.* 300 bp on olive colobus. We

designed another set of consensus degenerate primers, targeting a fragment of 252 bp in the *gag* region of SIVwrc (Locatelli *et al.* 2008a) from the alignment of the *gag* region of 2 SIVwrc samples from *Ptilocolobus badius badius* (SIVwrc-98CI-04 and SIVwrc-97CI-14), collected in Côte d'Ivoire (Courgnaud *et al.* 2003b). We also tested some black-and-white colobus samples with primers from the SIVcol lineage derived from a blood sample of colobus guereza (*Colobus guereza*) from Cameroon (Courgnaud *et al.* 2001). Based on the alignment of consensus sequences of SIVcolCGU1, SIVwrc-98CI-04, SIVwrc-97CI-14, and SIVcol-97CI-12, we designed additional primers (cgzpol and bwcpol) to amplify fragments of *ca.* 500 and *ca.* 600 bp, respectively. All PCRs performed included tissue samples from red colobus, olive colobus, or colobus guereza, which we had previously identified as SIV positive, as well as negative controls.

We performed PCR amplifications using the Long Expand PCR kit (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's instructions. Each amplification reaction included a manual hot-start at 94°C followed by 35 cycles with a denaturation step at 94°C for 20 s, an annealing temperature set according to the primer melting temperatures, and a variable extension time depending on the size of the expected fragment (1 mn/kb). We purified potential PCR SIV products (Q-Biogene, Illkirch, France) and sequenced them directly using the inner primers on an ABI 3130xl Genetic Analyser (Applied Biosystem, Courtaboeuf, France). We then checked and assembled the sequences via Lasergene (DNASTAR Inc., Madison, WI).

Microsatellite Analyses

To discriminate individuals among the samples collected, we selected human microsatellite loci in which cross-amplification had been conducted successfully in previous studies of various nonhuman primate species. Initially, we conducted separate PCR amplifications at 16 microsatellite loci (Table II) in 10 µl using a Robocycler Gradient 96 (Stratagene, USA). The reaction contained 1 µl of genomic DNA, 1× PCR buffer (Invitrogen, UK), 1–2 mM MgCl₂, 250 µM each dNTP (ABgene, UK), 10 µM each primer (MWG or TAGN, UK), 1 µl of *Taq* DNA polymerase (Invitrogen, UK), 0.4 mg/ml of bovine serum albumin (BSA, Promega, USA) and 10% dimethyl sulfoxide (DMSO, Fisher Scientific, UK). We amplified D7s503, D11s925, D16s420, D17s791 as described in Coote and Bruford (1996). PCR conditions were: 3 min at 95°C; 7 cycles of 45 s at 95°C, 1 min at 50°C; 90 s at 72°C; then 30 cycles with annealing temperature 54°C and finally 72°C for 10 min. For D1s207, D2s141, D3s1768, D4s243, D5s1475, D6s271, D6s311, D6s501, DXs571, DXs8043, DXs6810, DXs6799 initial annealing was 48°C, followed by 40 cycles at 55°C. All sets of amplifications contained negative controls and gorilla and chimpanzee DNA as positive controls. For the initial screening, we tested 2–6 samples of each species per locus, electrophoresed PCR products on an agarose gel, and visualized them using ethidium bromide. Based on the preliminary microsatellite analysis results, we targeted the following microsatellite loci for the discrimination of 127 fecal samples: D3s1768, D4s243, D5s1475, D6s501, D7s503, and D17s791. We labeled the 5'-ends of the forward primers fluorescently as described in Table II and conducted PCRs using the Qiagen Multiplex kit following the manufacturer's Microsatellite Protocol for the reaction mix and thermocycling (annealing temperature: 57°C; number of cycles: 40). We multiplexed 3 primer pairs (2 µM each primer) in a 10-µl reaction volume. We followed a multitubes approach to limit erroneous genotyping (Gagneux *et al.* 1997; Navidi *et al.* 1992; Taberlet *et al.* 1996). We relied on a few high-quality DNA samples as species controls, mostly to verify whether the 2 sources had comparable allele sizes. We extracted and genotyped DNA from hair from 2 researchers involved in this study at each locus to detect possible human contamination at both the sample collection and PCR stage (Allen *et al.* 1998; Vigilant 1999). We separated products via capillary electrophoresis (ABI 3100). We sized alleles relative to an internal

size standard (ROXHD400) using Gene Scan 3.7 (Perkin-Elmer). We calculated alleles frequencies and expected heterozygosity (data not shown) using Genepop v 3.4 online (Raymond and Rousset 1995).

Results

Samples, Animal Identification, and Microsatellite Analysis Results

Table III lists the number of samples collected from the 6 species investigated. Of the 16 loci tested, we detected 2 alleles at 6 loci (D3s1768, D4s243, D5s1475, D6s501, D7s503, D17s791) and used these to genotype a total of 90 samples. We did not genotype the remaining 37 samples of black-and-white colobus, because they were likely to represent duplicates of previously collected samples from visually identified individuals belonging to habituated animals from social group pol3. We genotyped 83 of 90 samples successfully from a total of 65 individuals: 27 *Colobus polykomos*, 23 *C. diana*, 10 *P. verus*, 6 *C. campbelli*, 3 *C. petaurista*, and 2 *C. nictitans* (Table III). Microsatellite analysis results including amplicon sizes for each of the loci are listed in Table SI (supplementary data). We were able to compare genotypes only between individuals of known relationship, i.e., mother–offspring, for black-and-white colobus, and observed no deviation from Mendelian inheritance. There were no instances in which we consistently observed >2 alleles per individual per locus, suggesting that amplification of contaminating mammalian, bacterial, or fungal DNA did not occur (Bradley and Vigilant 2002).

Detection of HIV Cross-reactive Antibodies

We tested 112 of 127 fecal samples belonging to *Colobus* and *Cercopithecus* species for HIV cross-reactive antibodies (Table SI). We did not analyze the remaining 15 samples because the material was degraded or insufficient. We considered samples as degraded whenever we were unable to score alleles via microsatellite analysis. For the black-and-white colobus, 42 samples belonging to 16 individuals revealed a clear presence of IgG, but did not react with any of the HIV antigens. The 15 remaining samples, which we did not genotype, were uninterpretable because the antihuman IgG upper line was absent or gave a considerably weaker signal than that of the lower human IgG line on the strips, and we did not observe any reactivity with any HIV antigens (Table SI). For the olive colobus, only 2 samples belonging to 2 individuals revealed a clear presence of IgG, but did not react with any of the HIV antigens. The 11 remaining samples corresponding to 8 individuals were uninterpretable, as previously described in this paper. For the *Cercopithecus* species, the 42 samples corresponding to 33 individuals revealed a clear presence of IgG, but none of them reacted with any of the HIV antigens and we therefore considered them negative.

Detection of SIV Infection in Tai Primate Communities by Amplification of Viral RNA in Feces

None of the RT-PCRs using species-specific primers, consensus primers for different species of the Colobinae subfamily (SIVwrc, SIVolc, SIVcol) or for some *Cercopithecinae* species (SIVmus, SIVmon, SIVgsn), or universal primers able to detect a large number of SIV lineages in *pol* amplified any fecal viral RNA in the species we investigated.

Discussion

We assessed SIV prevalence using 2 different methods—detection of HIV cross-reactive antibodies and amplification of viral RNA in fecal samples—among 3 monkey species known or suspected to be infected by this virus (olive colobus, Diana monkey and greater spot-nosed monkey) and 3 species living sympatrically with species known to be highly infected (western red colobus and sooty managbeys). We focused mainly on black-and-

white and olive colobus and Diana monkeys. Campbell monkeys, lesser spot-nosed and greater spot-nosed monkeys were more cryptic species and therefore more difficult to follow and sample in a limited amount of time. We found no SIV-positive individuals. This may be due to a reduced performance of the diagnostic tools when identifying a yet uncharacterized SIV, to the limited number of individuals sampled, or to a very low prevalence of infection. Further research is needed to improve the sensitivity of noninvasive methods for SIV detection.

The INNO-LIA HIV I/II confirmation assay that we used has previously identified 9 new SIV lineages in blood and tissue samples from 15 different primate species, including SIVwrc from western red colobus and SIVolc from olive colobus in the Taï Forest and SIVcol from black-and-white colobus in Cameroon (Courgnaud *et al.* 2003b; Peeters *et al.* 2002). It has also successfully identified SIVcpz in west-central chimpanzee and SIVgor in western lowland gorilla fecal samples (Keele *et al.* 2006; Van Heuverswyn *et al.* 2006). This suggests that this test can reliably identify SIV in a variety of nonhuman primate blood samples and in fecal samples of 2 great ape species. However, IgGs were absent or present only in low quantities in our western red colobus fecal samples. We previously amplified vRNA successfully in 14 of 53 individuals (Locatelli *et al.* 2008b), suggesting that the uninterpretable serological results for colobus monkeys do not necessarily reflect the absence of SIV vRNA in fecal samples. We could interpret INNO-LIA HIV I/II profiles reliably in only 2 of 13 olive colobus fecal samples, and PCRs on 10 olive colobus from 3 neighboring groups were all negative. There was a greater percentage of interpretable serological results in black-and-white than in olive colobus (74% vs. 15% displayed detectable IgG values), but RT-PCRs using a combination of specific, consensus, or universal primers did not detect any SIV-positive samples in either species. In contrast to results for *Colobus* species, we detected IgG in all samples of *Cercopithecus* tested via the INNO-LIA assay, but these samples all yielded negative antibody detection results, confirmed by negative RT-PCRs.

Species differences in sensitivity in serological tests may be due to only partial cross-reactivity of antibodies directed against genetically divergent viruses or to the fact that naturally infected animals have lower antibody titers to specific viral antigens than humans. For example, in sooty mangabeys the abundance of virus-specific antibodies decreases from plasma to urine to feces (Ling *et al.* 2003). Thus it is evident that noninvasive molecular epidemiological studies of SIV infection are limited by the nature of the samples analyzed (feces vs. blood) and the quality and quantity of vRNA extracted. Therefore, in addition to feces, whenever possible, researchers should also collect urine samples to increase the chances of antibody detection in nonhuman primate populations. Although the majority of the known SIV lineages have been identified based on cross-reactivity with HIV antigens, using INNO-LIA confirmation tests (Courgnaud *et al.* 2003a; Peeters *et al.* 2002), SIV antibody detection using synthetic peptides would be more specific (Aghokeng *et al.* 2006), although the low sensitivity of SIV lineage specific ELISA and LUMINEX tests in fecal samples compared to INNO-LIA tests limit their use for the moment (Martine Peeters *pers. obs.*).

To be sure that we are not obtaining false-negative results, future studies should also perform several extractions of each sample, or test serial specimens from the same individuals to be able to run a logistic regression model and estimate the prevalence of SIV infection within a 95% confidence interval. This is a difficult task, considering the amount of material available and the challenge of fecal sample collection of small arboreal nonhuman primates in the field. Moreover, future studies should also establish the sensitivity of the PCRs among these newly tested nonhuman primate species: To do so, we would need to compare blood from SIV-positive individuals with the corresponding fecal

samples. This would be best accomplished in a captive setting or under strict veterinary supervision in the field.

Finally, it is difficult to estimate the minimum number of individuals required to test the presence of SIV infection noninvasively in a given monkey species, because SIV prevalence rates vary significantly from species to species. For example, in Cameroon 1% of *Cercopithecus nictitans* (9/859) and *Cercopithecus cephus* (9/864) were infected with SIV, whereas 50% of *Cercocebus torquatus* (6/12) and *Chlorocebus tantalus* (3/6) were SIV positive (Aghokeng *et al.* 2009). Moreover, SIV prevalence rates can also vary in the same species depending on the geographical location, as for SIVcpz from chimpanzees and SIVgor from gorillas in Cameroon, where rates varied 0–32% and 0–4.6%, respectively (Keele *et al.* 2006; Neel *et al.* 2009) and chimpanzee communities in Gombe National Park, Tanzania, where rates varied 9–18% in (Keele *et al.* 2009). We covered 25–100% of the adult population for Diana monkey group dia3, black-and-white colobus group pol3, and olive colobus group ver3, based on censuses of the habituated social groups living in the Taï Forest in 1999 and 2001 (Buzzard and Eckardt 2007; Korstjens 2001) (Table IV). Because the prevalence of infection for a particular species is unknown, better coverage of the adult population within each social group than we were able to obtain in most cases is desirable to ensure that all potentially infected individuals have been tested. If possible, the sampling should also be extended to several neighboring groups for each species investigated, and populations across a wider geographic range.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Location of fecal samples collected for 2 groups of black-and-white colobus: pol1 (*green dots*) and pol3 (*blue dots*); 3 groups of olive colobus: ver1 (*gray triangles*), ver2 (*white triangles*), and ver3 (*black triangles*); 3 groups of Diana monkeys: dia1 (*red rhomboids*), dia2 (*orange rhomboids*), and dia3 (*pink rhomboids*); 1 Campbell's monkey group (*light blue crosses*); and lesser spot-nosed monkeys (*yellow squares*) on a 3-km² grid system with 100×100 m cells. The grid system consisted of lines (trees marked in blue, letters) in a north–south direction and lines (trees marked with yellow paint, numbers) in an east–west direction. (Photo credits: *Colobus polykomos*: Sabrina Locatelli, *Procolobus verus*, *C. diana*, *C. campbelli*: Florian Möllers; *C. petaurista*: Noël Rowe. Right upper corner: Map of Côte d'Ivoire. The study site is depicted with a red dot and it is located near the western border of the park. Map based on p. 63 of the Atlas de la Côte d'Ivoire, 1983, Editions Jeune Afrique-Paris).

Table 1

Species investigated and details of viral primers tested

Species	Primers tested	Sequences	TM (% GC) (°C)	Estimated amplicon size	Region targeted	Reference
<i>C. polykomos</i>	DR1-DR2/DR4-DR5	DR1 (5'-TRCAYACAGGRCWGAYGA-3')	58	800	<i>pol</i>	Clewley <i>et al.</i> 1998
		DR2 (5'-AIADRTCATCCATRTAYTG-3')	42.7			
		DR4 (5'-GGIATWCCICAYCCDGCAGG-3')	60	200		
		DR5 (5'-GGIGAYCCYTTCCAYCCYTGHHG-3')	64			
		polOR (5'-ACBACYGCNCCTTCHCCTTTC-3')	53.1	800	<i>pol</i>	Cournaud <i>et al.</i> 2001
	polOR-polis4/polis2-uni2	polis4 (5'-CCAGCNCACAAAGGNATAGGAGG-3')	55.2			
		polis2 (5'-TGGCARTRGAYTGYACNCAYNTRGAA-3')	54.7	650		
		uni2 (5'-CCCCTATTCTCCTCCCTTCTTTTAAAAA-3')	53.3			
		wrcpolF1 (5'-TAGGGACAGAAAGTATAGTAATHTGG-3')	50.9	1100	<i>pol</i>	Locatelli <i>et al.</i> 2008b
		wrcpolR1 (5'-GCCATWGCYAA TGCTGTTTC-3')	49.7			
	wrcpol	wrcpolF2 (5'-AGAGACAGTAAAGGAAGGAAAGCAGG-3')	54.4	650		
		wrcpolR2 (5'-GTTTCWATTCTTAACCACAGCADA-3')	51.4			
		wrcenvF1 (5'-TGGCAGTGGGACAAAAATATAAAC-3')	50	750	<i>env</i>	Locatelli <i>et al.</i> 2008b
		wrcenvR1 (5'-CTGGCAGTCCCTTCTCCAAAGTTGT-3')	55.3			
		wrcenvF2 (5'-TGATAGGGMTGGCTCTCGGTGATG3'-3')	56.6	550		
	wrcgag	wrcenvR2 (5'-AATCCCCATTYYAACCAAGTTCCA-3')	51.1			
		wrcgagF1 (5'-ATDGAGGATAGAGGNTTTGGAGC-3')	50.2	600	<i>gag</i>	Locatelli <i>et al.</i> 2008a
		wrcgagR1 (5'-GCCCTCTACTCTCTTGACATGC-3')	53.4			
		wrcgagF2 (5'-CCAACAGGGTCAGATATAGCAG-3')	49.7	250		
		wrcgagR2 (5'-ACTTCTGGGGCTCCTTGTCTGCTC-3')	55.9			
<i>C. polykomos</i>	olcpol	olcpolF1 (5'-TAGATACAGGRCAGATGAYACAGTAAT-3')	53.8	700	<i>pol</i>	Unpublished
		olcpolR1 (5'TCCAYCCYTGAGGHARYACATTATA-3')	47.7			
		olcpolF2 (5'-CTAGAAATWATWGGRRGRATAGGRRG-3')	52.6	300		
		olcpolR2 (5'-ATYTTWCCTTCTKCTTCYARTCTRTCACA-3')	50.8			
		cgzpolF1 (5'-CAGTGYTGATATAGGAGATGCC-3')	51.9	700	<i>pol</i>	Unpublished
	cgzpol	cgzpolR1 (5'-ACTGCATAGCCCCCATTTGTCC-3')	48.7			
		cgzpol F2 (5'-CAGCTTTYACAGTGCCCATCAGTG-3')	51.9	500		

Species	Primers tested	Sequences	TM (% GC) (°C)	Estimated amplicon size	Region targeted	Reference
<i>P. verus</i>		cgzpolR2 (5'-TCTTCTGCTTCTGCACTAAGCTG-3')	50.2			
	bwcpol	bwcpolF1 (5'-TAGATACAGGAGCAGATGATACAGT-3')	49.3	1000	<i>pol</i>	Unpublished
		bwcpolR1 (5'-ATTIDCCYCCTATCCCTTTTATGWGC-3')	52.7			
		bwcpolF2 (5'-AGAYTRGAAAGCAGARGGAAAAAT-3')	44.8	600		
		bwcpolR2 (5'-TCCYACCAATTYYTGTAYATCAITTTACTGT-3')	51.1			
<i>C. diana</i>	wrcpol *					
	wrcenv *					
	olepol *					
	DR1-DR2/DR4-DR5 *					
	CNMF1/polOR2					
<i>C. campbelli</i>	CNMF2/CNMR2 *					
	polOR-polis4/polis2umi2 *					
	wrcgag *					
	CNMF1/POLOR2	CNMF1 (5'-TATCCYTCCYTGTGTCATCYCTCTTT-3')	51.35	2750	<i>pol</i>	Aghokeng et al/2007
	CNMF2/CNMR2	polOR2 (5'-ACBACWGCCTCTTCWCCTTTTCCA-3')	70			
<i>C. petaurista</i>		CNMF2 (5'-AATGGAGAAATGYTMATAGATTTCAG-3')	49.4	2050		
		CNMR2 (5'-CCCCYATTCTCCCTTTTITTTA-3')	51.07			
	DR1-DR2/DR4-DR5 *					
	polOR-polis4/ polis2umi2 *					
	CNMF1/polOR2					
<i>C. nictitans</i>	CNMF2/CNMR2 *					
	DR1-DR2/DR4-DR5 *					
	polOR-polis4/ polis2umi2 *					
	CNMF1/polOR2					
	CNMF2/CNMR2 *					
	DR1-DR2/DR4-DR5 *					
	polOR-polis4/ polis2umi2 *					

* Details of primers tested described already for another species. Y = C/T, W = A/T, R = A/G, H = A/C/T, B = C/G/T, S = G/C, K = G/T, D = A/G/T, N = A/C/T/G, Y = C/T, M = A/C, K = G

Table II

Sixteen human microsatellite loci screened using fecal DNA from 6 primate species from Taï Forest, Côte d'Ivoire

Locus	VNTR	PCR primer sequences 5'-3'	Fluorescent label	Marker References	Source of marker information
D1s207 ^a	(CA) _n	F:CACTTCTCCTTGAATCGCTT R:GCAAGTCCTGTTCCTCAAGTCT	—	Z16601	Coote and Bruford (1996)
D2s141 ^a	(CA) _n	F:ACTAATTAATACCCNCACTCCC R:TTTTCCAAACAGATACAGTGAACCTT	—	Z16793	CHLC
D3s1768 ^c	(GATA)	F:GGTTGCTGCCAAAGATTAGA R:CACTGTGATTTTGCTGTGGGA	FAM	G08287	CHLC
D4s243 ^c	(GATA)	F:AAATCCCTTTTCTACCTTTCTATCAC R:GAGAGGAGAGATAAAAAGATGTAATG	FAM	G33465	Smith <i>et al.</i> (2000)
D5s1475 ^c	(GGAT) _n	F:ACTCAAGCTAAGGCCCTCAT R:GCATTTTGGGTCAAAAATTG	HEX	G08488	Goossens <i>et al.</i> (2000)
D6s271 ^b	(CA) _n	F:AAGGTAACAATTGGGAAATGGCTTA R:TTACTTTCATTATCTTAGCATACAGAG	NED	Z16648	Coote and Bruford (1996)
D6s311 ^a	(CA) _n	F:TCATTGGTGTGTGCATTAA R:TTGGAAGGATGAGAAATTAAGG	—	Z17200	Coote and Bruford (1996)
D6s501 ^c	(GATA) _n	F:GCTGGAACTGATAAGGGCT R:GCCACCCCTGGCTAAGTTACT	NED	G08551	Morin <i>et al.</i> (1998)
D7s503 ^c	(CA) _n	F:ACTTGGAGTAATGGGAGCAG R:GTCCCTGAAAAACCTTTAATCAG	HEX	Z16870	Coote and Bruford (1996)
D11s925 ^b	(CA) _n	F:AGAACCAAGTCGTAAAGTCCTG R:TTAGACCATTATGGGGCAA	HEX	Z17002	Coote and Bruford (1996)
D16s420 ^b	(CA) _n	F:ATTTCTGAGGTCTAAAGCA R:TTAGGCCCCAGTCCACACTCA	FAM	Z17069	Coote and Bruford (1996)
D17s791 ^c	(CA) _n	F:GTTTCTCCAGTTATTCCTCC R:GCTCGTCTTTGGGAAGAGTT	NED	Z16689	Coote and Bruford (1996)
DXs571 ^a	(CA) _n	F:AAATATTGGTGCAGGACTGT R:AAATCAGATGCAGTGATGGGT	—	Z17275.1	CHLC

Locus	VNTR	PCR primer sequences 5'-3'	Fluorescent label	Marker References	Source of marker information
DXs8043 ^a	(CA) <i>n</i>	F:AGTTCTCAGAAACATTTGGTTAGGC	—	Z53101	CHLC
		R:AATTATTGGCAAAAGAGTACAGGCAG	—		
DXs6810 ^a	(GATA) <i>n</i>	F:ACAGAAAACCTTTTGGGACC	—	G09983	CHLC
		R:CCCAGCCCTGAATATTATCA	—		
DXs6799 ^a	(GATA) <i>n</i>	F:ATGAAATTCAGAAATTATCCTCATACC	—	G08099	CHLC
		R:GAACCAACCTGCTTTTCTGA	—		

^aPrimers that failed to amplify from fecal samples (7/16)

^bPrimers that amplified these loci, but that did not provide repeatable results (3/16)

^cSuccessful and reproducible amplifications (6/16)

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Table III
Species investigated, number of samples collected, and number of individuals based on successful genotyping

Species	No. of samples collected	No. of samples in microsatellite analysis	No. of samples successfully genotyped	No. of individuals	Serology		
					Positive/tested	Uninterpretable/tested	Negative/tested
<i>Colobus polykomos</i>	70	34	29	27	0/57	15/57	42/57
<i>Procolobus verus</i>	14	13	12	10	0/13	11/13	2/13
<i>Cercopithecus Diana</i>	31	31	30	23	0/31	0/31	31/31
<i>C. campbelli</i>	7	7	7	6	0/7	0/7	7/7
<i>C. petaurista</i>	3	3	3	3	0/2	0/2	2/2
<i>C. nictitans</i>	2	2	2	2	0/2	0/2	2/2
Total	127	90	83	65	0/112	0/112	0/112

Serology analyses are divided into positive, uninterpretable, and negative results

Table IV

Coverage of the adult population of censused social groups (%)

Group	Census 1 (1999) ^a		Census 2 (2001) ^b		Samples collected March–July 2004 ^c			% of the adult population sampled
	Adult male	Adult female	Adult male	Adult female	Adult male	Adult female		
B&W colobus								
Pol1	1–2	4–6	—	—	6	—	75	
Pol3	1	6	2	8	4	—	>100	
Olive colobus								
Ver1	1–2	1–3	2	1	—	—	60	
Ver2	nd	nd	—	2	1	—	na	
Ver3	1	1–2	2	2	—	—	>100	
Diana monkey								
Dia1	1	11	—	3	7	—	83	
Dia2	1	13	1	6	2	—	64	
Dia3	1	11	—	1	2	—	25	
Campbell's monkey								
Cam1	1	6	—	3	3	—	86	
Lesser spot-nosed monkeys								
Pet1	1	8	1	1	1	—	33	
Greater spot-nosed monkeys								
Nic1	1	4	—	1	1	—	40	

^a *Colobus* census (Korstjens 2001)

^b Guenon census (Buzzard and Eckardt 2007)

^c Excluding unscorable microsatellite results; number of individuals according to microsatellite analysis *nd* not determined; *na* not applicable