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# Non-invasive testing reveals a high prevalence of simian T-lymphotropic virus type 1 antibodies in wild adult chimpanzees of the Taï National Park, Côte d'Ivoire

Fabian H. Leendertz,[1,2,3] Christophe Boesch,[2] Heinz Ellerbrok,[1] Wolfram Rietschel,[4] Emmanuel Couacy-Hymann[5] and Georg Pauli[1]

Correspondence
Georg Pauli
PauliG@rki.de

[1]Zentrum für Biologische Sicherheit, Robert Koch-Institut, Nordufer 20, 13353 Berlin, Germany

[2]Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany

[3]Institute for Parasitology and International Animal Health, Freie Universität Berlin, Königsweg 67, 14163 Berlin, Germany

[4]Zoologisch-Botanischer Garten Wilhelma, 70342 Stuttgart, Germany

[5]Lanada/Lcpa, Bingerville, Côte d'Ivoire

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Little information is available on the prevalence of retrovirus infections in populations of non-human primates living in their natural habitats. To gain such information, methods were developed to detect antibodies to simian T-lymphotropic virus type 1 (STLV-1) in urine from wild chimpanzees. Samples from more than 74 chimpanzees living in three communities in the Taï National Park, Côte d'Ivoire, were analysed. The prevalence of STLV-1 antibodies in adults and adolescents was significantly higher (35/49, 71·4 %) than that in infant and juvenile chimpanzees (3/31, 9·7 %).

## INTRODUCTION

Primate T-lymphotropic virus type 1 (PTLV-1) is found in a broad spectrum of different primate species (Koralnik *et al.*, 1994; Slattery *et al.*, 1999; Meertens *et al.*, 2001; Leendertz *et al.*, 2004); the human variant of this retrovirus, human T-lymphotropic virus type 1 (HTLV-1), is associated with adult T-cell leukaemia and myelopathy (Poiesz *et al.*, 1980; Gessain *et al.*, 1985). There is little information, however, on the prevalence of the simian variant of the virus [simian T-lymphotropic virus type 1 (STLV-1)] in populations of wild-living great apes. Previous surveys on the prevalence of STLV-1 infection in chimpanzees have been restricted to wild-caught individuals; however, in most cases, wild-caught great apes are taken from their killed mothers when they are very young; therefore, data from these animals may not represent the overall virus prevalence in the population (Nerrienet *et al.*, 2004). Observation of captive chimpanzees has shown that none of 79 offspring born to STLV-1-positive mothers was infected after weaning (Niphuis *et al.*, 2003). This observation is in contrast to the mother–child transmission of HTLV-1 in humans, where breastfeeding is a high risk factor for transmission (up to 33 % in the southern part of Japan) and transmission rates increase significantly with the length of time that the child is breastfed (Ando *et al.*, 2003). The role of sexual transmission also seems to differ between non-human primates and humans; sexual transmission of STLV-1 in captive chimpanzees and other non-human primate species has only been observed in very few cases, which implies that this route of infection has only a minor role (Georges-Courbot *et al.*, 1996; Niphuis *et al.*, 2003; Parrish *et al.*, 2004). In contrast, in humans, the HTLV-1 infection rate is higher in adult women than in adult men, indicating that male-to-female transmission is a natural route of HTLV-1 infection (Kajiyama *et al.*, 1986; Tajima *et al.*, 1987; Plancoulaine *et al.*, 1998).

Field surveys of well-studied wild primates, such as chimpanzees, are needed to determine the natural prevalence of STLV-1 and to understand the routes of virus transmission. Blood samples from a large number of wild-caught individuals are required for such surveys. Unfortunately, this would be possible only by anaesthetizing or killing the animals, which is contrary to the conservation of wild-living primate species. A previous study that was performed on necropsy samples from 10 chimpanzees (six of them adults) belonging to the study groups that are described in this investigation indicated a high prevalence of STLV-1 infection in the adult individuals that were analysed (5/6). In addition, STLV-1 genome analyses showed a remarkable variance in STLV-1 strains that were isolated from different

chimpanzee groups, within one group or even in strains from a single individual. Two of these new STLV-1 strains had close genetic similarity to strains that were found in two red colobus monkeys (the preferred prey of chimpanzees in this area) from the same region, which suggests interspecies transmission (Leendertz *et al.*, 2004). In order to determine the prevalence of STLV-1, we modified antibody-detection assays to determine STLV-1-specific antibodies in urine samples from all members of three communities that are the subject of long-term, ongoing behavioural research.

## METHODS

**Material and sample collection.** This study included all members of three groups of free-ranging chimpanzees that had been living under human observation for many years. The three groups consisted of 10, 24 and 46 individuals, respectively. The chimpanzees could be distinguished individually and detailed data on relatedness (Vigilant *et al.*, 2001) and behaviour (Boesch & Boesch-Achermann, 2000) have been documented. As part of a project on the elucidation of disease aetiology in these wild-living chimpanzees, urine samples have been collected regularly since 2001. Only samples that were clearly assignable to individuals were collected (by using single-use micropipettes) from leaves that were either lying on the forest floor or growing just above ground level. Urine was transferred to 2 ml cryotubes and stored on ice immediately. These tubes were transferred to liquid nitrogen on the same day upon return to camp and stored continuously at temperatures below −40 °C until analysis. The name of the chimpanzee, date, place, time of urination, time of sampling, time of sample preservation, colour and volume of urine were documented for each sample.

Chimpanzees younger than 5 years were defined as 'infants', those of 6–9 years as 'juveniles', those of 10–15 years as 'adolescents' and individuals older than 15 years as 'adults' (Boesch & Boesch-Achermann, 2000).

**Validation of urine antibody testing.** Multiple urine samples and blood/serum and/or DNA samples that were extracted from spleen or other lymphatic tissues were available from 11 chimpanzees. Blood/serum samples were tested for STLV-1 antibodies by using an HTLV-1/2 ELISA (Murex Biotech) and/or an HTLV-1/2 Western blot (version 2.4; Genelabs Diagnostics Pte.) (Table 1).

Blood that was shed by two chimpanzees following a violent encounter was collected, together with leaves from the ground. The sample from one of these individuals was stored at −70 °C, whereas the sample from the other was dried and stored in a tube that contained silica gel beads. To recover antibodies, leaves were rinsed with 2 ml HTLV-1/2 blotting buffer and the solution that resulted was used for Western blot analysis and ELISA. Matched urine and DNA samples were also available from four chimpanzees living in German zoos. DNA was tested by using a sensitive and specific real-time PCR assay (Leendertz *et al.*, 2003). All HTLV-1/2 ELISA results for blood samples were confirmed subsequently by HTLV-1/2 Western blot analysis.

**Table 1.** Characteristics of chimpanzees and samples that were used to establish the test

| Chimpanzees | | | Sample | | |
|---|---|---|---|---|---|
| Origin | STLV status | *n* | DNA | Blood | Urine |
| Wild* | + | 5 | 4 | 5 | 14 |
| | − | 1 | 1 | 1 | 1 |
| Captive† | − | 5 | 5 | 1 | 6 |

*This group includes three wild chimpanzees that died in 2001 and 2002 from unknown causes and two chimpanzees that bled after in-group fighting.
†Chimpanzees living in German zoos.

**Antibody detection.** Detection of specific antibodies in urine samples from various primates has been described for simian immunodeficiency virus (SIV) (Santiago *et al.*, 2002, 2003; Ling *et al.*, 2003). We used antibody-detection tests that were designed to determine antibodies in human serum or plasma samples. Urine samples were analysed by using a modified version of this protocol for Western blot analysis. After thawing, urine samples were vortexed and then centrifuged at 3000 r.p.m. to remove debris. The following modifications of the test were introduced: 500 µl urine was mixed with 500 µl blotting buffer. Western blot strips were incubated in narrow wells to ensure that they were covered completely by the urine–buffer mixture. Wells were sealed with adhesive tape and incubated overnight on a rocking platform at room temperature; the samples were then aspirated and stored at −20 °C. Strips were washed three times with wash buffer (for 5 min each) and incubated with the enzyme-labelled secondary antibody according to the manufacturer's instructions. Strips were incubated subsequently for 1 h with substrate solution and the reaction was stopped by rinsing with distilled water.

Urine samples that showed reactivity to at least two envelope antigens (rgp46-I and GD21) and at least one core protein (p19 and/or p24) on the Western blots were defined as 'STLV-1 antibody-positive', those that showed reactivity to either envelope or core structural proteins exclusively were described as 'indeterminate' and strips that showed reactivity against the immunoglobulin control only were described as 'negative'. This algorithm follows the recommendations of the HTLV European Research Network for seroepidemiological HTLV studies (Goubau *et al.*, 1996).

Sensitivity and specificity of the test were determined by using an algorithm for the evaluation of diagnostic tests that was developed by Galen & Gambino (1979) and Abel (1993), with a confidence interval of 95 %. The fraction of positive tests per total number of urine samples (rather than number of individuals) was analysed, with confidence limits that were determined given the assumption of binomial sampling. For these calculations, it was assumed that successive samples from one individual were not correlated (Santiago *et al.*, 2003).

**Prevalence estimation.** To investigate the prevalence of STLV-1, we tested 74 urine samples and six blood samples from 80 individuals with unknown STLV-1 antibody status. The blood samples originated from individuals that had died before 2001, the year in which urine sampling was first implemented.

## RESULTS

### Sensitivity of the test

HTLV-1/2 Western blot assays were performed on urine samples from 11 chimpanzees with known STLV-1 infection status and these results confirmed the ability of the test to detect STLV-1-specific antibodies in urine reproducibly (Fig. 1). All urine samples from chimpanzees that had tested negative by using serology and/or PCR ($n=7$) were negative

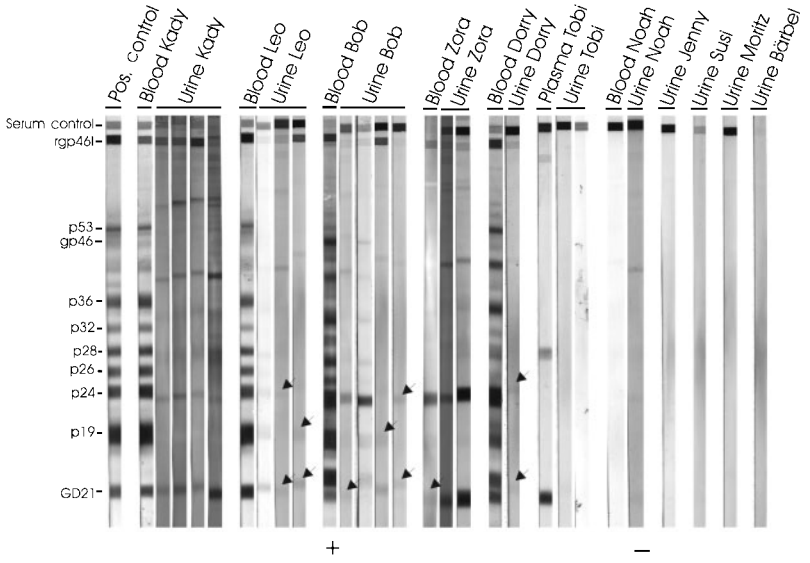


**Fig. 1.** Western blot analysis of 11 chimpanzees with known STLV-1 infection status. Direct comparison of blood and urine samples was made for seven chimpanzees; for 'Jenny', 'Susi', 'Moritz' and 'Bärbel', no blood was available, therefore STLV-1 status was based on DNA samples by using a highly sensitive HTLV/STLV real-time PCR. DNA was extracted from blood from chimpanzee 'Tobi' and was analysed for STLV-1 by using real-time PCR, but STLV-1 was not found. Further analyses for other retroviruses are in progress. Arrows indicate weak bands.

by Western blotting, whereas urine samples from serologically positive chimpanzees ($n=14$) showed various intensities of staining, but distinct reactivities to HTLV-1-specific proteins. It is noteworthy that all reactive urine samples from seropositive individuals stained the envelope proteins rgp46-I and GD21 and at least one of the core proteins (p19 and/or p24). In some Western blot analyses, additional bands were present (p26, p28 or p53) that were related to internal structural proteins. Western blot assays were performed on blood from the 11 chimpanzees and stained the full pattern of HTLV-1-specific proteins (Fig. 1). A high level of sensitivity and specificity was seen with this test, as infection status was determined correctly in all animals when using the urine samples. The sensitivity and specificity limits that were calculated for this modified test were 77–100 and 59–100 %, respectively, when using a confidence interval of 95 % that was based on our results and on the number of samples, i.e. not on the number of individuals, as proposed by others (Santiago *et al.*, 2003). The wide confidence limits for sensitivity and specificity that are seen here are a result of the low sample number available to validate this modified test system.

### Screening of chimpanzees

In total, 80 individuals were assessed for STLV-1 antibody status by using the Western blot assay (Fig. 2). Urine was used as the antibody source for 74 of the chimpanzees. We tested one sample per individual, with the exception of 'Marius', where two independent samples were tested. Blood samples were available from only six chimpanzees. By applying the algorithm that was proposed, an overall STLV-1 antibody prevalence of 47·5 % (38/80) was observed (Table 2). Preliminary analysis showed no significant difference in the infection rate between infants (0–5 years) and juveniles (6–9 years) (exact $\chi^2$ test, $\Xi^2=0{\cdot}3$, $P<1$) and between adolescents (10–15 years) and adults (older than 15 years) (exact $\chi^2$ test, $\Xi^2=1{\cdot}335$, $P=0{\cdot}285$). However, due to the limited number of individuals within each group and age class, individuals were then combined into two categories, infants and juveniles (<10 years old) and adolescents and adults (10 years and older). Division at the age of 10 seemed to be appropriate, as this is the time that female chimpanzees reach sexual maturity, move to a new community, participate in hunting and thus have increased access to meat. Logistic regression analysis (with STLV-1 antibody prevalence as a dependent binary variable and age class, group and sex as independent variables) revealed that age class alone has a significant influence on antibody status (Table 3), with chimpanzees in the older age class being more likely to be infected (Table 4).

When estimating antibody prevalence in 24 adult females of known social position (ranking) in the north and south communities, a trend emerged for high-ranking individuals (R. Stumpf, personal communication). It was found that these females were more likely to be positive for STLV-1 antibodies than lower-ranking individuals [logistic regression: Wald $\Xi^2(1)=2{\cdot}757$, $P=0{\cdot}097$, exp $(\beta)=0{\cdot}552$]. No calculation of social position was possible for positive adult males' test results, due to the low number of individuals.

## DISCUSSION

### Evaluation of the method

All 21 urine samples that were collected from 11 chimpanzees were assayed correctly for STLV-1 infection status, as determined by PCR and/or serology (100 % sensitivity and specificity). Testing of samples that were collected at different time points revealed that antibody content, as determined by intensity of staining of different HTLV protein bands, varied with time. Wide 95 % confidence intervals of 77–100 % for specificity and 59–100 % for sensitivity were calculated, as only a limited number of samples was available for validation of the modified test

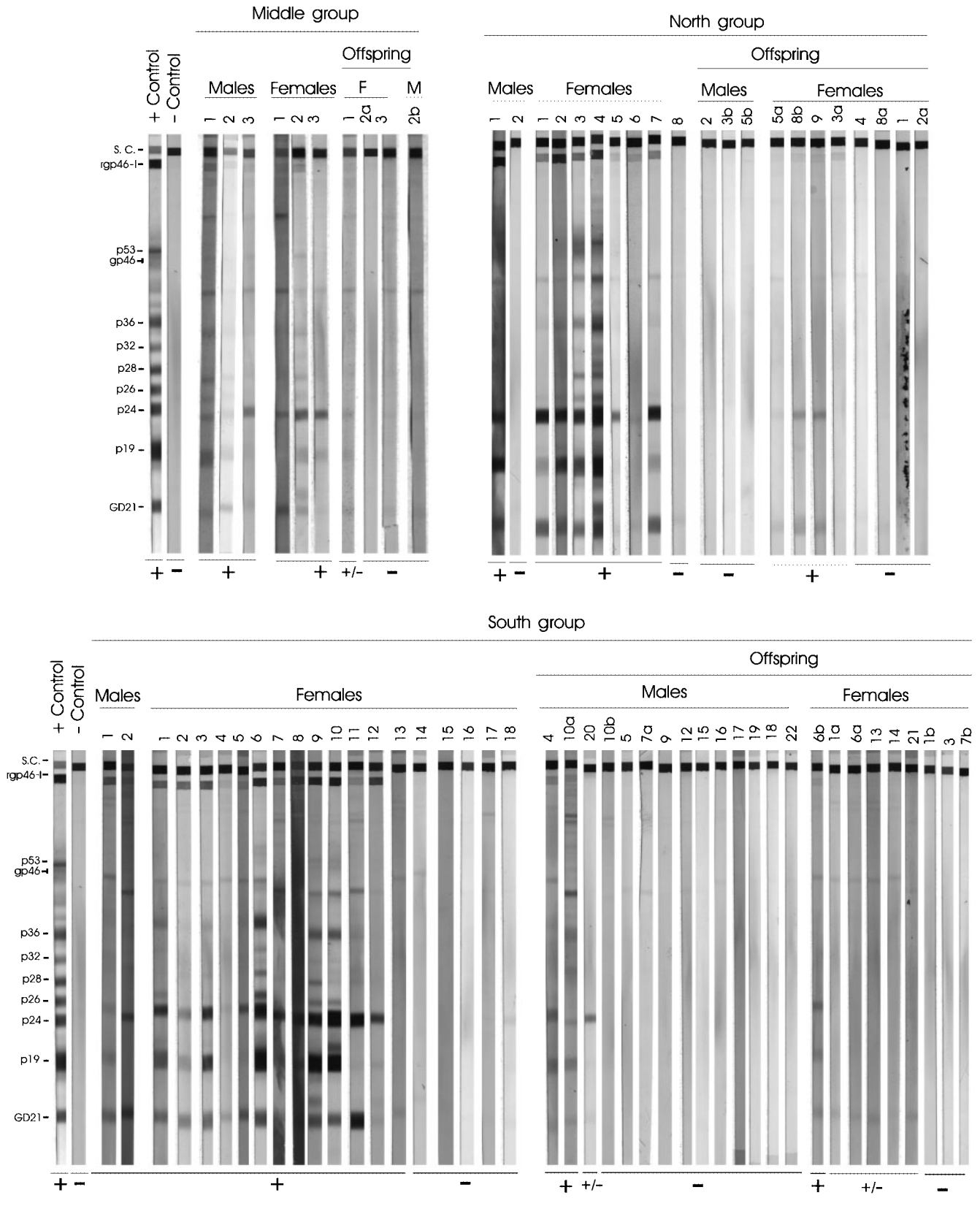


**Fig. 2.** Western blot analysis of urine samples collected from 74 chimpanzees originating from the three study groups. Offspring were numbered according to their mother; offspring without a living mother are represented by numbers following on from the mothers' numbers.

**Table 2.** Prevalence of STLV-1 antibodies

Antibodies were determined by Western blot analysis of 74 urine samples and six blood samples in three groups of wild-living chimpanzees.

| Group | | Age group (years) | | | |
|---|---|---|---|---|---|
| | | 0–5 | 6–9 | 10–15 | >15 |
| North | | | | | |
| No. individuals | 24 | 6 | 5 | 4 | 9 |
| No. positive | 13 | 0 | 2 | 4 | 7 |
| No. indeterminate | 0 | 0 | 0 | 0 | 0 |
| Positive (%) | 54·2 | 0 | 40 | 100 | 77·8 |
| Middle | | | | | |
| No. individuals | 10 | 1 | 1 | 2 | 6 |
| No. positive | 6 | 0 | 0 | 1 | 5 |
| No. indeterminate | 1 | 0 | 0 | 0 | 1 |
| Positive (%) | 60 | 0 | 0 | 50 | 83·3 |
| South | | | | | |
| No. individuals | 46 | 8 | 10 | 6 | 22 |
| No. positive | 19 | 1 | 0 | 2 | 16 |
| No. indeterminate | 6 | 1 | 4 | 1 | 0 |
| Positive (%) | 41·3 | 12·5 | 0·0 | 33·3 | 72·7 |
| All groups | | | | | |
| No. individuals | 80 | 15 | 16 | 12 | 37 |
| No. positive | 38 | 1 | 2 | 7 | 28 |
| No. indeterminate | 7 | 1 | 4 | 1 | 1 |
| Positive (%) | 47·5 | 6·7 | 12·5 | 58·3 | 75·7 |

strategy. Nevertheless, this test can be considered as a useful tool for non-invasive epidemiological screening to estimate the prevalence of STLV-1 infection in chimpanzee populations that would not otherwise be accessible to serological analyses. It can be assumed that antibodies against different STLV-1 strains were detected, as the wild chimpanzees that were used to establish the test were infected with highly divergent STLV-1 strains (Leendertz *et al.*, 2003, 2004). The wide confidence interval does not allow us, at present, to recommend testing urine unreservedly for analysis of the infection status of individual chimpanzees. Although all urine samples that were collected from STLV-positive chimpanzees reacted positively in the Western blot assays, it can be assumed that testing two or more independent urine samples compensates for the differences seen in antibody titres, thus increasing the statistical probability of detecting STLV-1-positive animals. Blood samples from three chimpanzees from the study groups that died of natural causes confirmed the results of the tests on urine (one adult female was positive and two infants, aged 1 and 5 years, were negative).

**Table 3.** Possible variables that may influence STLV-1 infection in chimpanzees of the Taï National Park

| Criterion | Wald $\Xi^2$ | df | *P* | Exp ($\beta$) |
|---|---|---|---|---|
| Group | 2·782 | 2 | 0·249 | |
| Sex | 0·592 | 1 | 0·442 | 2·723 |
| Age class (<10 versus >10 years) | 9·756 | 1 | 0·002 | 51·862 |

**Table 4.** Number of STLV-1 antibody-positive female and male chimpanzees

Age classes: 0–9 years, infant and juvenile; ⩾10 years, adolescent and adult chimpanzees. Indeterminate results are considered to be negative for STLV antibodies.

| Group | Females | | Males | |
|---|---|---|---|---|
| | 0–9 years | ⩾10 years | 0–9 years | ⩾10 years |
| North | | | | |
| Total no. individuals | 6 | 11 | 5 | 2 |
| No. positive | 2 | 10 | 0 | 1 |
| No. indeterminate | 0 | 0 | 0 | 0 |
| Positive (%) | 33·4 | 90·9 | 0 | 50 |
| Middle | | | | |
| Total no. individuals | 1 | 5 | 1 | 3 |
| No. positive | 0 | 3 | 0 | 3 |
| No. indeterminate | 0 | 1 | 0 | 0 |
| Positive (%) | 0 | 60 | 0 | 100 |
| South | | | | |
| Total no. individuals | 8 | 20 | 10 | 8 |
| No. positive | 1 | 13 | 0 | 5 |
| No. indeterminate | 4 | 1 | 1 | 0 |
| Positive (%) | 12·5 | 65 | 0 | 62·5 |
| All groups | | | | |
| Total no. individuals | 15 | 36 | 16 | 13 |
| No. positive | 3 | 26 | 0 | 9 |
| No. indeterminate | 4 | 2 | 1 | 0 |
| Positive (%) | 20 | 72·2 | 0 | 69·2 |

In order to compare the sensitivity of the HTLV-1/2 Western blot version 2.4 (Genelabs Diagnostics) to another Western blot kit that is used frequently for PTLV diagnostics [the INNO-LIA HTLV I/II (Innogenetics)], we tested 13 urine samples by using both methods. Although bands that were observed with the INNO-LIA Western blot were very faint compared to most bands seen by using HTLV-1/2 Western blot version 2.4, most results were consistent between both kits. Five out of six HTLV-1/2 Western blot version 2.4-positive urine samples tested positive in the INNO-LIA assay and the other was indeterminate; four out of four samples tested negative and three samples that had tested indeterminate in the HTLV-1/2 Western blot version 2.4 were negative in the INNO-LIA Western blot. These results suggest that the INNO-LIA Western blot has a slightly lower sensitivity for the screening of antibodies in urine samples than the HTLV-1/2 Western blot version 2.4. We therefore suggest screening for urine antibodies with the HTLV-1/2 Western blot version 2.4.

Analysis of urine samples by using the particle agglutination

test (Serodia HTLV-1; Fujirebio) and ELISA (Murex HTLV-1/2 ELISA) gave no clear results when paired samples of blood and urine were analysed; this may be due to low antibody titres or inhibitors present in urine.

## Prevalence

A high STLV-1 antibody prevalence (38/80, 47·5 %) was detected in wild chimpanzees that live in three defined groups in the Taï National Park by using this method. Sixty-seven of 74 urine samples from chimpanzees with unknown STLV-1 antibody status showed distinct positive or negative Western blot results. According to the criteria that are suggested in this paper, urine samples from 34 individuals were STLV-1 antibody-positive and those from 26 individuals were antibody-negative. On the basis of the test algorithm, urine samples from seven of 74 chimpanzees showed an indeterminate Western blot pattern and were considered to be negative for the epidemiological study. Five of these chimpanzees were juveniles; a follow-up of these individuals will be performed in the future.

It was not possible to discriminate between STLV-1, -2 and -3 from the Western blot results, but no reaction of blood or urine samples from Taï chimpanzees to HTLV-2-specific peptides was observed on Western blot strips. Furthermore, an investigation of 10 animals by using PCR with STLV-2 or -3 primers did not give a positive reaction (Leendertz *et al.*, 2004).

The only evidence for a relatively high prevalence of STLV-1 in chimpanzees is from a study by Niphuis *et al.* (2003), who found that 20 of 37 founder animals (54 %) from a breeding colony of *Pan troglodytes verus* that originated from Sierra Leone were STLV-1-positive. At present, the route of infection of the founder animals is unclear, as the mother-to-child transmission rate in this breeding colony is extremely low. It is possible that infection of the founder animals might be have been caused iatrogenically by treatment with STLV-contaminated material, as these animals were captured in Sierra Leone as infants (E. J. Verschoor & J. L. Heeney, personal communication).

In our study groups, there was previous evidence for a high STLV-1 prevalence from 10 chimpanzees that had died from natural causes. Five of six adult chimpanzees were positive by STLV-1 serology and PCR (Leendertz *et al.*, 2004). The results that were obtained in this study confirm and extend these findings. To our knowledge, screenings of other chimpanzee subspecies (mainly *P. troglodytes troglodytes*) revealed a much lower prevalence (Ishikawa *et al.*, 1987; Georges-Courbot *et al.*, 1996; Nerrienet *et al.*, 2001, 2004). Table 5 gives an overview of published data on STLV-1 prevalence in chimpanzees of different origin. Differences in prevalence may reflect the age at which individuals were caught (Nerrienet *et al.*, 2004), but differences in chimpanzee subspecies susceptibility, range or number of prey, lower prevalence of STLV-1 in simian prey and even biological properties (such as STLV-1 strain infectivity) should also be considered. Further investigations will be performed on the prevalence of STLV-1-directed antibodies in different subspecies of chimpanzees and in other monkey species that originate from different regions of Africa.

## Transmission

Only one of 15 infants (6·7 %) and two of 16 juveniles (12·5 %) were positive for STLV-1 antibodies in Western blot analysis. These findings imply that there is an age-dependent risk for STLV-1 infection, at least in the three groups that were investigated. The risk of infection increases with age: seven of 12 adolescent (58·3 %) and 28 of 37 adult (75·7 %) chimpanzees were STLV-1-positive. For offspring of 17 STLV-1-infected mothers, two of 21 infant and

**Table 5.** Prevalence of STLV-1 reported in different chimpanzee subspecies

Subspecies that have been assumed, based on the geographical origin of the chimpanzees, are indicated by '?'.

| Species | Origin | Living conditions | No. positive/total (%) | Reference |
|---|---|---|---|---|
| *Pan troglodytes verus* | Côte d'Ivoire | Wild-living | 38/80 (47·5) | This study |
| *Pan troglodytes verus* | Sierra Leone | (a) Wild-caught* | 20/37 (54) | Niphuis *et al.* (2003) |
| | | (b) Offspring of (a) | 2/160 (1·3) | |
| *Pan troglodytes* (*verus* and *troglodytes*?) | Gabon, Cameroon, Zaïre, Equatorial Guinea | (a) Wild-caught | 1/40 (2·5) | Georges-Courbot *et al.* (1996) |
| | | (b) Captive-born | 0/21 | Georges-Courbot *et al.* (1996) |
| *Pan troglodytes vellerosus* | Cameroon | Wild-caught | 1/56 (1·8) | Nerrienet *et al.* (2004) |
| *Pan troglodytes* | Different zoos | Captive | 2/37 (5·4) | Hunsmann *et al.* (1983) |
| *Pan troglodytes* (*vellerosus*?) | South Cameroon† | Wild-caught | 1/35 (2·8) | Nerrienet *et al.* (2001) |
| *Pan troglodytes* (*troglodytes*?) | Gabon | Wild-caught | 0/52 | Ishikawa *et al.* (1987) |
| *Pan troglodytes* (*troglodytes*?) | Central African Republic | Wild-caught | 0/3 | Saksena *et al.* (1994) |

*From the paper, it is not clear whether the animals were wild-caught.
†Origin not clear.

juvenile chimpanzees were found to be positive, compared to one adolescent of eight progeny from seven STLV-1-negative mothers. Infant chimpanzees usually suckle for about 4 years or longer (Boesch & Boesch-Achermann, 2000), which represents a long time-exposure for young chimpanzees to the milk of potentially infected mothers. Additional routes of transmission must be considered to be responsible for high STLV-1 antibody prevalence in adult Taï chimpanzees, as in individuals that were $<5$ years old, only one of 15 chimpanzees tested positive.

In young individuals, the possibility of a lower antibody response (and consequently a lower antibody titre in blood and urine) cannot be excluded, which may lead to an underestimation of the frequency of positive infants and juveniles. However, our previous results and additional infant samples that were analysed at a later date showed that tissue/blood samples from six of six chimpanzees that were $<10$ years old had tested negative, whereas, in contrast, five of six adult chimpanzees had tested positive by using serological and PCR methods (Leendertz *et al.*, 2004).

Significant differences in antibody prevalence in adolescent and adult versus infant and juvenile individuals (71·4 vs 9·7 %) may be due to three important factors in chimpanzee life: in-fighting, sexual intercourse and consumption of other primate species.

By studying the distribution of STLV-positive and -negative female individuals around the time of sexual maturity (10 years; at this age they also start to attract adult males), a clear jump in STLV-1 prevalence can be observed. On sexual maturity, male chimpanzees start to be involved in the male hierarchy and female chimpanzees leave their native group to immigrate into new groups. Both changes in behaviour carry a high potential for conflict and create new sexual partner constellations. In captivity, virus transmission from one chimpanzee to another via fighting or sexual contact is assumed to occur (Georges-Courbot *et al.*, 1996; Niphuis *et al.*, 2003; Parrish *et al.*, 2004); these routes also cannot be ruled out as sources of infection for the Taï chimpanzees. Fourteen adult/adolescent chimpanzees were found to be negative for STLV-1 antibodies; among these were high-ranking individuals like 'Marius', the current top-ranking male of the north group (we tested two independent samples from this male). These results suggest a low rate of STLV-1 transmission via aggression and sexual behaviour in the wild chimpanzees of the Taï National Park, despite frequent fighting and copulation within the group (Boesch & Boesch-Achermann, 2000).

In previous studies, we described six new STLV-1 isolates (Leendertz *et al.*, 2003, 2004) in five chimpanzees originating from the same groups that were investigated here. These virus isolates showed surprisingly high heterogeneity, not only in comparison to STLV-1 isolates described previously in other primate species, but also between the different chimpanzee groups, within one group and even between two strains that were isolated from a single individual. We also found STLV-1 in western red colobus monkeys that live in the same area of the Taï National Park. The STLV-1 strains that were detected in these red colobus monkeys were closely related phylogenetically to strains found in two chimpanzees, suggesting that interspecies transmission from prey to hunter via killing and consumption of meat, bones and intestines may be an important route of virus transmission (Leendertz *et al.*, 2004).

Participation in hunting of different monkey species and thus access to primate meat also becomes more frequent with maturity, awarding the maturing chimpanzee a higher social position within the community. Almost the entire prey is ingested, including bones and skull, which may induce wounds in the oral cavity or digestive tract. Adult male and female chimpanzees consume a mean of 186 and 25 g, respectively, of simian prey per day. Adolescent and younger chimpanzees consume considerably less prey than adults and generally obtain only small pieces that have been discarded by their mothers or other adults. The red colobus represents the most frequent prey by far (80 % of simian prey) of the 10 different simian species that are hunted by chimpanzees (Boesch & Boesch-Achermann, 2000).

The potential risk of infection via copulation and aggression may increase with age (i.e. total number of sexual and aggression contacts). In combination with our previously published molecular data (Leendertz *et al.*, 2004) and observations on transmission routes of STLV-1 in captive chimpanzees (Niphuis *et al.*, 2003), the data that are presented here on STLV-1 antibody prevalence in different age groups of chimpanzees suggest an important role of horizontal virus transmission from prey to hunter.

### Conclusions

Further studies on the molecular epidemiology of STLV-1 infections in monkey species that are hunted by chimpanzees, as well as long-term studies on STLV-1 antibody prevalence in chimpanzees, are necessary to gain deeper insights into the epidemiology of STLV-1 in populations of wild-living primates. Analysis of urine samples for the presence of STLV-1 antibodies provides an important tool for non-invasive screening of such populations.

In contrast to other studies, our data indicate that the risk of acquiring an STLV-1 infection in wild *P. troglodytes verus* might be different from that of humans for an HTLV-1 infection. As high-ranking individuals like 'Marius', a vivid hunter that is surrounded by many STLV-1 antibody-positive chimpanzees, are found to be STLV-1-negative, there may be differences in susceptibility to STLV-1 infection at the individual level.

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