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Noninvasive Monitoring of the Health of *Pan troglodytes schweinfurthii* in the Kibale National Park, Uganda

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*We assessed the health status of chimpanzees (*Pan troglodytes schweinfurthii*) of the Kanyawara group in the Kibale National Park in Western Uganda via noninvasive methods. We conducted visual veterinary inspection, parasitological and urine analysis in association with behavioral observations, causing minimal disturbance or stress to individually recognized chimpanzees. We applied multiple parasitological techniques to 252 stool samples to compare their efficacy in detecting parasitic infection and to increase the power of detecting a wide range of parasites at a more sensitive level. We examined 76 urine samples via a quick detection method to evaluate multiple parameters of urine that indicate organ dysfunction. Results of the different analyses are mutually supportive and provided useful information for monitoring bodily condition and diseases. The multifaceted health evaluation*

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system is a beneficial tool for monitoring long-term and short-term changes in health status due to environmental stress, seasonal dietary change, and disease in wild chimpanzee populations. Use of this method to detect changes in health, when employed together with behavioral observations, may also provide important insights into the potential effects of self-medicative behaviors.

KEY WORDS: intestinal parasites; urine analysis; *Pan troglodytes*; health monitoring; Uganda.

INTRODUCTION

Emerging tropical diseases like the Ebola virus have spread across Africa, threatening the stability of wild ape populations (Walsh *et al.*, 2003) and requiring mobilization of scientists of multiple fields to expand our knowledge concerning the risk of transmission to humans. The chimpanzee-related Simian Immunodeficiency (Virus SIVcpz) virus is the origin of HIV-1 in humans (Bailes *et al.*, 2003; Hahn *et al.*, 2000). De Groot *et al.* (2002) hypothesized that in the distant past, chimpanzees experienced a pandemic of SIVcpz or a related retrovirus, which spread through chimpanzee populations across Africa, dramatically reducing their numbers, but leading to its currently benign state. Noninvasive sampling to detect the natural distribution of SIVcpz in wild chimpanzee populations across the continent today, have begun to increase our understanding of the natural ecology of its transmission and may provide a useful model for understanding the emergence and transmission of HIV among humans (Bailes *et al.*, 2003; Hahn *et al.*, 2000; Santiago *et al.*, 2002). These examples serve to reinforce the relationship between conservation and the health of wildlife and human populations (Meffe, 1999; Daszak *et al.*, 2000; Daszak *et al.*, 2001) and the need to better understand the effects of all diseases on the health and behavior of primates in the wild.

An interdisciplinary approach to monitor health status, interfacing medical and behavioral sciences, is necessary to deepen our understanding of disease, intra and interspecific transmission, and its overall effect on the health and behavior of chimpanzees. Capture and/or containment, necessary to collect blood and tissue samples and for clinical investigation, risks injuring chimpanzees, and interferes with long-term behavioral research by disrupting the trust developed between subjects and researchers. Furthermore, stress and manipulation can induce changes in the parameters under study. Some researchers have included longitudinal monitoring of the health of individuals via multiple noninvasive measures such as activity budgets and parasitology to quantify illness (Huffman *et al.*, 1993; Alados and Huffman, 2000). Parasites that can have a significant impact on the health and behavior of chimpanzees (Huffman *et al.*, 1996; Huffman and Caton, 2001) might be detected more easily by periodic health monitoring at both individual and population levels.

The majority of information on the parasitic species infecting chimpanzees is from studies of captive individuals (Van Riper *et al.*, 1966; Jessee *et al.*, 1970; Myers and Kuntz, 1972; Healy and Myers, 1973) or from fecal samples that could not be assigned to identified individuals (Mc Grew *et al.*, 1989; Kawabata and Nishida, 1991; Landsoud-Soukate *et al.*, 1995; Lilly *et al.*, 2002). Exceptionally, File *et al.* (1976), Huffman *et al.* (1997) and Ashford *et al.* (2000) studied at field sites where the primates are habituated to humans, providing the opportunity to collect fecal samples from identified individuals, thereby obtaining more accurate measures of prevalence of infection within the group and seasonal variation of infection in individuals.

Huffman *et al.* (1993), Wrangham (1995), Messner and Wrangham (1996), Huffman *et al.* (1996), Huffman *et al.* (1997), Huffman and Caton (2001) and Dupain *et al.* (2002) used noninvasive parasitological techniques were in primatological field studies to measure and to evaluate the possible affects of behavioral responses to parasite infection, but there is only limited published information concerning results of urinalysis in free-living chimpanzees (Kaur and Huffman, 2004; Kelly *et al.*, 2004). Urinalysis screens for urinary tract infection and renal diseases, which can also reflect the dysfunction of organs and other infections because abnormal metabolites may appear in urine.

Validation of the approach will be an important step toward evaluating the possible curative powers of self-medicative behaviors in apes, linked to the ingestion of medicinal plants infrequently ingested by chimpanzees and associated with the control of symptoms of illness and parasite infections (Wrangham and Nishida, 1983; Huffman and Seifu, 1998; Huffman *et al.*, 1993). Also of value, is the possibility of finding entirely new compounds, like the steroid glucosides in *Vernonia amygdalina* (Jisaka *et al.*, 1992), antimalarial limonoids in *Trichilia rubescens* (Krief *et al.*, 2004b) or useful plant properties such as antibiotic, anti-inflammatory, antipyretic and immunostimulating properties in the diets of apes (Cousins and Huffman, 2002; Huffman *et al.*, 1998; Krief, 2003).

In order to obtain accurate diagnoses of the health status of chimpanzees in wild populations, we combine results of urinalysis with an intestinal parasite survey including evaluation of parasitic infection loads, visual veterinary health inspection and behavioral activity budgets of individuals in the Kanyawara community of Kibale National Park, Uganda. We aimed to evaluate the usefulness of multiple health monitoring methods as a noninvasive tool for monitoring health in chimpanzees.

METHODS AND MATERIALS

Study Site

We conducted field studies at the Makerere University Biological Field Station (MUBFS) in Kibale National Park, Uganda, which occupies 795 km^2 between 0°13′ to 0°41′N and 30°19′ to 30°22′E. The elevation of Kanyawara is around 1500 m. The rainfall averages 1700 mm per year. The vegetation of Kanyawara is a mosaic of mid-altitude moist forest, secondary forest, grassland, swamps and plantations of *Eucalyptus* and pines and includes elements of lowland tropical rain forest, montane rain forest and mixed deciduous rain forest. Kibale has 2 rainy seasons from March to May and from September to November with a drier season in between. We conducted the study from December 2000 to March 2001 (dry season) and in October 2001 (rainy season).

Subjects

We observed chimpanzees (*Pan troglodytes schweinfurthii*) in the Kanyawara community. This group comprised *ca.* 50 individuals, well habituated to observers on the ground at a distance of 5–10 m. Chimpanzees have been monitored daily since 1987 by 1–2 teams of field assistants working in pairs with additional researchers. In June 1999, we counted 10 adult males, 2 adult females without offspring and 14 mothers with 22 dependants (10 females and 10 males, 2 young infants of unidentified sex) in the community.

Behavioral Data

We observed subjects from dawn to dusk via focal animal sampling (450 h in the dry season and 195 h in the wet season) continuously recording activities and food plants eaten in 10 min periods and changing targets every 10 min whenever possible. In addition, observation *ad libitum* (Altmann, 1974) allowed accurate recording of particular sequences related to possible self-medication. We focused on the diet of the identified chimpanzees, and recorded all ingested items in detail.

Veterinary Observations, Sample Collection and Analysis

Veterinary work consisted of daily clinical observations looking for possible pathology of respiratory, digestive, reproductive, urinary and locomotive functions. We conducted urinalysis and intestinal parasite evaluation. When following a focal animal throughout the day, we paid particular attention to any abnormal behavior, especially clear signs of illness such as decreased appetite, long and frequent resting, sneezing, coughing or intestinal disorder, per Huffman *et al.* (1997). Whenever possible, we collected feces and urine from all clearly identified individuals.

Parasitological Analysis

We inspected fecal samples immediately after discharge to check for the presence of macroscopic parasites and consistency, then collected and stored them individually in vials. We used 4 levels to describe stool consistency: (1) diarrheic feces, which are liquid or runny, spreading on the ground immediately after discharge, sometimes with undigested food, (2) soft or pasty feces, deposed in a heap without form, (3) solid and well-formed feces, (4) dry and hard feces from constipation. Were analyzed samples microscopically via the McMaster flotation method with magnesium sulfate (Gordon and Whitlock method: Bailanger, 1973) within one day of collection. We counted eggs and larvae in a McMaster slice. We stored 2 g of each sample in 18 ml of 10% formalin and fixed 0.5 g in merthiolate-iodo-formalin (MIF staining). We analyzed the fecal samples within ≤6 mo of collection. We analyzed formalin samples microscopically by a direct method to ascertain the load and species present. We counted the total number of eggs, larvae, adults and ciliates from an aliquot of 200 μl and multiplied the number by 50 to obtain the parasite content per g of feces. We performed a diphasic ether-formalin concentration on each sample in order to search for rare eggs. We checked MIF samples for the presence of protozoan cysts. We corrected parasite loads as counted by the McMaster method and direct examination according to stool consistency by multiplying the count by a coefficient of 2 if the dung was soft and pasty and 3 if it was runny or liquid (Hercberg *et al.*, 1986): a corrected parasite load. We used the various methods to increase the opportunity of finding rare parasites and to obtain higher sensitivity for the detection of parasitic infestation.

We collected 252 dung samples from identified chimpanzees, including 187 during the dry season and 65 during the rainy season. They came from 38 individuals, mainly adults (127 samples from 18 females and 125 samples from 20 males).

Urinalysis

We collected fresh urine evacuated from chimpanzees in the trees either in a plastic bag hanging from a forked stick, or by pipeting the urine from leaves. We used only urine samples uncontaminated by feces or soil matter and stored them in dry clean containers. We tested them for bilirubin (a degradation product of hemoglobin), urobilinogen (a degradation product of bilirubin), glucose, hemoglobin (an indication of hemolysis), ketones (a by-product of fat metabolism), nitrites, and proteins via a commercial dry reagent dipstick (Multistix 10 SG Bayer©) immediately upon returning to the field station. We also measured urine pH, specific gravity, and quantity of red blood cells and white blood cells (leukocytes) via reagent pads. We noted the identity of the donor, date and hour of collection. Urine obtained when the chimpanzee urinated from a night nest tends to be highly concentrated and is thus the most likely to be diagnostic of abnormality. We considered place and means of collection (leaves pipeting, urine-stick use), amount collected, macroscopic aspects of urine such as color or turbidity, and presence of crystals to be useful information.

We analyzed 76 urine samples from 32 chimpanzees, including 45 samples (21 from males and 24 from females) collected during the dry season and 31 (13 from males and 18 from females) collected during the rainy season.

STATISTICS

We used parametric tests for data following a Gaussian distribution, this assumption being tested via the Kolmogorov and Smirnov method. When values were assumed to be sampled from a Gaussian population, we used a 2-tailed t-test when variances of 2 samples tested were equivalent or the Welch t-test when not equal. If data failed the normality test with $p < 0.05$, we used nonparametric tests via a Mann-Whitney test (M-N test) to compare 2 groups of data and a Kruskal-Wallis test (K-W test) to compare >2 groups of data. We performed Student's t-test to evaluate percent comparisons.

RESULTS

Fecal Examination

The proportion of samples without nematodes or cestodes detected by McMaster flotation or by direct examination was low (6.5%, $n_1 = 181$, in dry season and 4.6%, $n_2 = 54$ in rainy season, p n.s., $p = 0.54$). Among the samples collected in the dry season, parasites were detected more efficiently by direct examination than by flotation ($p < 0.001$, $n_1 = 182$, $n_2 = 187$). (Table I). A reverse trend occurred in the wet season ($p = 0.02$, $n_1 = 65$,

Table I. Prevalence of helminths for samples and individuals, following 2 methods of fecal analysis in 2 seasons (see table II for species)

	Dry season				Rainy season			
	MacMaster		Direct examination		MacMaster		Direct examination	
	%	*n*	%	*n*	%	*n*	%	*n*
Positive samples	69	187	84	182	89	52	63	65
Positive individuals	100	38	95	38	91	23	77	23

$n_2 = 52$), probably because eggs, detected more efficiently by flotation, were more abundant in the rainy season than in the dry season. The number of positive individuals (with ≥1 positive fecal sample) was high during both wet and dry seasons (no significant difference), regardless of the method used (Table I).

Parasitic Load by Direct Examination

Mean parasitic loads measured by direct examination are not significantly different between the dry and the rainy season (301, $n_1 = 182$, $SD_1 = 820$ and 197, $n_2 = 65$, $SD_2 = 296$ respectively, M-W test, $p = 0.058$). Of all samples analyzed, 96% contained <1000 helminths/g of feces, and the 10 samples with >1000 parasites/g were produced by 10 different individuals. We monitored 6 of them for parasitic load from a mean of 12 samples (6 to 22 samples each). In each case, only one sample exhibited a high parasic load among all the samples collected. Parasitic loads were uniform throughout the study.

There is no significant difference in the mean parasitic load (number of parasites (eggs, larvae, adults) per g wet mass of feces) in females versus males (dry season: m (mean) = 369, $n = 86$, SD = 1129 for males, $m = 249$, $n = 83$, SD = 375 for females; wet season: $m = 231$, $n = 26$, SD = 291 for males, $m = 175$, $n = 39$, SD = 301 for females; M-W test, $p = 0.96$ and $p = 0.54$). Less than 8% of the feces were liquid. Corrected parasitic loads for runny, soft and normal feces are not significantly different (normal consistency feces: $m = 226$, $n = 184$, SD = 355, soft feces $m = 491$, $n = 47$, SD = 1485, runny feces: $m = 168$, $n = 16$, SD = 188; K-W test $p = 0.085$). Nevertheless, parasitic load may be only weakly affected by consistency, because soft feces had a significantly higher corrected parasitic load than that of normal stools (M-W test, U = 3481, $p = 0.039$). Neither sex nor runny consistency had a significant relationship with parasitic load.

Parasite Load by McMaster Flotation

The corrected number of helminths determined by McMaster flotation is significantly higher during the wet season (5.7, $n_1 = 188$, $SD_1 = 20$ in the dry season; 12.4, $n_2 = 53$, $SD_2 = 18$ in the wet season, M-W test, U = 2688 $p < 0.001$). In both seasons, male samples contained more parasites than female ones (dry season: for males: $m = 7.0$, $n = 97$, SD = 25.3, for females: $m = 4.3$, $n = 91$, SD = 12.2, M-W test, U = 3472, $p = 0.011$; rain season for males: $m = 14.9$, $n = 25$, SD = 14.7, for females: $m = 10.2$, $n = 28$, SD = 20.9, M-W test, U = 239, $p = 0.048$). The number of parasites as estimated by McMaster flotation is significantly different according to feces consistency. Liquid and soft feces contained more parasites than normal feces (normal consistency feces: $m = 6.8$, $n = 176$, SD = 22.6, soft feces: $m = 8.5$, $n = 47$, SD = 9.5; runny feces: $m = 7.6$, $n = 17$, SD = 7.9, K-W test, KW = 13.5; $p = 0.001$).

Prevalence of the Species Observed

We identified ≥5 species of helminths and noted larvae and eggs from unidentified strongyle species. Like Ashford *et al.* (2000), we rarely found *Trichuris trichiura* and *Bertiella studeri*. Contrarily, *Oesophagostomum stephanostomum* and *Strongyloides fulleborni* were common (Table II).

We detected protozoa by direct examination. *Troglodytella abrassarti* was the more common of 2 species of entodiniomorph ciliates, which could be considered either a parasite or as a symbiont (Collet *et al.*, 1984). We also observed a small entodiniomorph, which presumably is the same as that described in Kibale (Ashford *et al.*, 2000), Gombe (File *et al.*, 1976), Mount Assirik (Mc Grew *et al.*, 1989) and La Lopé (Landsoud-Soukate *et al.*, 1995).

We observed seasonal differences in prevalence of 10 species of helminths and protozoans (Table II). Most species occurred more frequently in the rainy season. Via the flotation method, the prevalence of eggs of *Oesophagostomum stephanostomum, Strongyloides fulleborni* and the larvae of *Probstmayria* sp. was higher during the rainy season ($p < 0.05$). There also was higher prevalence of eggs of an unidentified nematode in the rainy season via direct examination and flotation ($p = 0.0016$ and $p < 0.001$ respectively). *Troglodytella abrassarti*, was more frequent ($p = 0.02$) in the wet season (77%) than in the dry season (61%). As observed for *Iodamoeba bütschlii* by Ashford *et al.* (2000), there was an increased prevalence of *Endolimax nana* in the wet season.

Conversely, via McMaster flotation and direct examination, the unidentified nematode larvae occurred more frequently in the dry season

Table II. Prevalence of intestinal parasitic species in Kibale chimpanzees

	Dry season				Statistical difference according to method used	Rainy season				Statistical difference according to method used	Higher prevalence and statistical difference according to season	
	MacMaster flotation		Direct examination			MacMaster flotation		Direct examination				
	n	%	*n*	%		*n*	%	*n*	%		MMF	DE
	187		182			52		65				
Positive samples												
Nematodes												
Oesophagostomum stephanostomum	91	48.7	59	32.4	**	41	78.8	17	26.2	***	RS***	n.s.
Strongyloides fulleborni	25	8.6	17	9.3	n.s.	12	23.1	5	7.7	*	RS**	n.s.
Unidentified eggs	14	7.5	1	0.5	**	20	38.5	6	9.2	***	RS***	RS***
Trichuris sp.	4	2.1	1	0.5	n.s.	0	0	0	0	n.s.	n.s.	n.s.
Probstmayria sp.	0	0	20	11	**	2	3.8	6	9.2	n.s.	RS*	n.s.
Nematode larvae of unidentified sp.	60	32.1	125	68.7	*	3	5.8	33	50.8	***	DS**	DS*
Cestodes												
Bertiella studeri	7	3.7	7	3.8	n.s.	0	0	0	0	n.s.	n.s.	n.s.
Protozoa												
Troglodytella abrassarti	0	0	111	61	***	0	0	50	77	***	n.s.	RS*
Small entodiniomorphs	0	0	49	27	***	0	0	8	12.3	*	n.s.	DS*
Pseudolimax sp.	0	0	37	20.4	***	0	0	1	1.5	n.s.	n.s.	DS***
Endolimax nana	0	0	4	2.2	n.s.	0	0	9	13.8	**	n.s.	RS***
Entamoeba coli	0	0	2	1.1	n.s.	0	0	3	4.6	n.s.	n.s.	n.s.
Coccidia	0	0	11	6	***	0	0	0	0	n.s.	n.s.	n.s.

Note. n.s. (p not significant) $p > 0.05$; *$0.01 < p < 0.05$; **$0.001 < p < 0.01$; ***$p < 0.001$. MMF: Mac Master Flotation, DE: Direct Examination RS: rainy season; DS: dry season.

($p < 0.05$). The small entodiniomorph was also more common in the dry season (27% vs 12%, $p = 0.02$). Finally, coccidia were present only in the dry season. However only 11 samples yielded coccidia and 9 were from individuals of the same maternal family, which suggests that the seasonal difference in coccidia prevalence may have been due to chance.

Urinalysis

Results from urinalysis are in Table III. High values may indicate a medical problem but alternative explanations are possible. The association of several high values increases the probability that the individual's condition is compromised. Accordingly, depending on the sex of the chimpanzee and the nature of the abnormal values, we may classify an individual as sick or healthy.

Leukocytes and blood were in 45% and 34% of the samples, respectively ($n = 76$), and were often associated together. In females, aside from urinary pathology, menstruation might explain some of the results. Fifty-two samples (68%) had $\geq$1 abnormal value but if samples of cycling females with blood or leukocytes or both without other abnormal values are excluded, 41 samples (53%) could be reasonably suspected of showing some form of pathology. In order to evaluate the effect of the estrous cycle on blood and leukocytes, we analyzed samples from cycling and noncycling females separately. Of the females sampled, 50% were positive for blood versus 15% of the male samples ($p = 0.002$), but samples from noncycling females were also more often positive than samples from males were ($p = 0.02$). For leukocytes, there is no significant difference between males and noncycling females, but leukocytes are significantly more frequent in cycling females than in noncycling females. Extreme values for specific density were in 25 samples; 18 exhibited a specific density of 1.00 and 7 appeared very concentrated (1.03). All but 2 samples had an alkaline pH (>7). To better estimate the state of health of the chimpanzees, we took into account the number of abnormal values in the same urine sample and searched for them in other samples from the same individual (Table III).

The most extreme cases are as follows. First, one sample (#U17) from a 3-yr-old female, OT, on 16 January 2001 exhibited 4 abnormal values: nitrites, leukocytes and proteins were positive and urine glucose was also detected. We classified her as being in poor condition. Similarly, the presence of nitrites associated with blood and leukocytes suggested a urinary tract infection in sample #U28 (7 February 2001) of an old female, AR. A urine collection from AR, on 9 February 2001 was also positive for nitrites, and

Table III. Summary of urine analysis by number of detected abnormal values

Number of abnormal values	Abnormal values	Number of samples	Female samples	Male samples	Identity of female individuals	Identity of male individuals
1	Blood	6	5	1	NL;LP;TG; AS;AS	PB
	Leukocytes	14	5	9	BU;LP;JK;L R; OK	ED;BE;LK;A J;AJ;LK;YB; LK;AJ
	Nitrites	2	1	1	NL	KK
	pH	2	0	2		KK;LB
	Proteins	1	0	1		PB
	Ketones	1	1	0	EK	
		26	*12*	*14*		
2	Blood & leukocytes	14	12	2		TU;TJ
	Blodd & nitrites	3	2	1	AR;OU	ST
	Leukocytes & nitrites	2	1	1	UM	BB
	Leukocytes & proteins	2	1	1	LP	LK
	Nitrites & ketones	1	0	1		SL
	Urobilinogen & blood	1	0	1		LK
		23	*16*	*7*		
3	Blood & leukocytes & nitrites	1	1	0	AR	
	Urobilinogen & blood & nitrites	1	0	1		LK
		2	*1*	*1*		
4	Leukocytes & nitrites & proteins & glucose	1	1	0	OT	
		1	*1*	*0*		

there were traces of blood. Another sample was positive for 3 measures: #U42 (25 February 2001) from LK that was positive for urobilinogen (1) and nitrites with traces of blood. Previous and subsequent samples from LK (#U41: 21 February 2001 and #U45: 25 February 2001) confirmed a urinary tract infection with traces of blood and leukocytes. A previous sample from LK, on 11 December 2000 (#U01) was also positive for urobilinogen. He is the only tested individual to have been positive for this parameter, suggesting a liver problem. Associations of abnormal leukocyte/nitrite and leukocyte/protein values were present in 4 individuals (2 females and 2 males). The presence of ketones was rare (2 samples) and was only once associated with nitrites. Proteins and leukocytes were in the urine sample of LP collected on 3 February 2001.

Diagnosis of Illness as Evaluated from Behavioral Data, Coprological and/or Urinalysis

Usually, chimpanzees looked healthy even if many of them suffered from mutilations and injuries from poachers' snares, e.g., locomotor disabilities (Table IV).

Wound of LB

On 15 February 2001, a fight occurred between 2 adult males: YB and LB. YB bit LB's foot and the 5th toe was severely cut, hanging from the foot by a strip of skin. The metatarsal bone was visible. On 16 February 2001, LB frequently inspected his toe; the wound was wet and oozing. On 19 February 2001, locomotion appeared to be painful and LB did not join the male group. On 20 February 2001, the toe was black and dry. LB moved with difficulty in the trees. On 21 February 2001, the wound on the edge of the foot extended 1cm deep and 4 cm wide. He did not use his foot to move in the trees and spent much time inspecting his wound and removing pus. On 24 February 2001, his toe fell off and the wound looked clean. A urine sample collected on 16 February showed a low pH value but no other sign of ill health. The low pH value is possibly related to the health status of LB.

Influenza-like Illness (KK)

On 15 February, 2001 a 17-yr old male, KK, frequently coughed. On the previous day, he looked weak and lethargic. His breathing rhythm was high; he had a deep cough, dyspnea and wheezing that was more pronounced

Table IV. Health status of possibly sick chimpanzees from the Kanyawara community evaluated by noninvasive methods

	Age	Disease	Veterinary observations	Urinalysis	Coprological analysis	Behavioral observations
LB	~35 yrs	Toe wounded after a fight	16/02/01: wound sweeping; 20/02/01: locomotion painful, toe black and dry; 24/02/01: the toe fell off	16/02/01: low pH		Did not join male group
KK	17 yrs	Influenza-like illness	15/02/01: frequent coughing; 16/02/01: weak, apathetic; increased breathing rhythm, dyspnea, sneezing, thick discharge	15/02/01: low pH	16/02/01: feces rich in *Probstmayria* sp., strongyle eggs and larvae, trichuris eggs	16/02/01: resting for 77% of 7:15 h of observations (average of 33% for the 13 other individuals of the party)
AR	>50 yrs	Influenza-like illness	09/02/01: deep coughing, sneezing, weak and very thin individual	7/02/01: nitrites, blood, leukocytes; 9/02/01: nitrites and blood	7/02/01 and 9/02/01: multispecific parasite infection (*Strongyloides fulleborni* eggs, ancylostoma and *Probstmayria* sp. larvae), soft consistency. F#108: macroscopic and microscopic *Bertiella studeri* infection	7/02/01: frequent resting in day nests; 9/02/01: left her nest 1:20 h after her daughter
LP	~50 yrs	Digestive discomfort		3/02/01: leukocytes, proteins	2/02/01: coccidiosis and multispecific parasite infection	5/02/01: seed-eating from two fresh elephant dungs + geophagy; 10/02/01: consumption of fine fiber material from a hollow trunk
OK	6 yrs	Intestinal disorder	16-20/02/01: alternately dry, soft and runny stools	No sample collected	High parasite numeration by direct examination and Mac Master flotation, mutispecific infection including *Probstmayria* sp.	20/02/01: Bark consumption of *Albizia grandibracteata* never recorded as food before

when he lay down. Sneezing was frequent and discharge was thick with mucous. Fecal sample #141 from KK, on 16 February was very rich in *Probstmayria gombensis* (1750 parasites/g) and we found strongyle eggs and larvae and eggs of *Trichuris* via the flotation method. The activity budget of KK versus 13 other individuals from the same party from 11:35 to 18:50 h shows that KK rested 77% of the time versus 33% for the rest of the group and fed during only 16% of the time (versus 48% for the other individuals). KK was the only individual from the party to feed on immature figs *(Ficus capensis)*. Urinalysis on 15 February revealed one of the 2 lowest pH values of all 76 samples in our study.

Influenza-like Illness (AR)

On 9 February, 2001 AR did not leave her nest before 08:10 h whereas her daughter AS was out at 06:47 h. AR sneezed and coughed in her nest and when out of the nest, she had a deep cough and looked weak and very thin. Nitrites and occult blood were in 2 urine samples (#38 and #U31), fecal samples from the same dates (7/02/01 and 9/02/01, respectively) were all positive for parasites, and one of them was soft. One sample (#F108) contained macroscopic and microscopic *Bertiella studeri.* Eggs of *Strongyloides fulleborni*, *Ancylostoma* sp. and larvae of *Probstmayria* sp. were in the other samples. In this case, increased parasitic burden is likely related to induced weakness by a flu-like disease.

Atypical Behaviors and Clinical Signs of LP

LP had concomitant abnormal urinalysis (proteins and leukocytes), coccidiosis and high parasitic load around the beginning of February 2001. On 5 February at 14:05 h, she rummaged through fresh elephant dung and for 6 min, carefully removing unidentified seeds, crunching and swallowing them. She did the same 10 min later for 1 min. At 15:40 h, she ate soil for 2 min, which is a longer duration than usual at Kanyawara. Five days later, at 9:50 h for 5 min, she inserted her head into an old hollow trunk lying on the ground, inspecting it and removing handfuls of fine fibrous material, which she ate. The 3 behaviors were unusual. During the study period, LP was the only chimpanzee that we observed to eat seeds from dung. Chimpanzees ate soil only on 3 occasions during the survey period. The consumption of rotten wood is also exceptional. In addition to the high parasitic levels and abnormal urinalysis her dung sample on 5 February was soft and contained high amounts of ciliates (*ca.*, 32,000 *Troglodytella abrassarti*/g).

Intestinal Disorder (OK)

From 16 October 2001, OK, a 6 yr-old female, suffered from an intestinal disorder with alternately dry, soft and diarrheic stools and parasitic infection. Feces that day (#225, 8:20 h) were solid stools with 3 species of parasites detected by McMaster flotation, with a corrected parasitic load of 300 (mean corrected parasitic load for October 2001: 175) and 2 strongyle species of larvae detected by direct examination. The next day, feces (sample #227, 8:45 h) were dry and yellow with *Probstmayria* sp., which was not detected before. Feces on 19 October (#236, 10:55 h) were diarrheic with *Probstmayria* sp. and 2 other strongyle species. The corrected McMaster load was high (21).

Unusual eating behavior occurred in association with the infection. OK ate bark soft tissues (probably phloem and cambium) of *Albizia grandibracteata* on 20 October 2001 at 09:42 h. Kanyawara chimpanzees had never before been seen to eat bark of *Albizia grandibracteata*, though consumption of its leaves occurred infrequently. She was the only one of the group to ingest the plant, stopping to eat it for 3 min while her mother with 2 other youngsters waited for her.

Feces collected on 22 October (#254, 7:25 h), about 46 h following the ingestion of the bark had a normal consistency. The parasitic load had dropped to 0 and only 4 eggs of 2 species were detected by McMaster flotation.

DISCUSSION

The combination of behavioral and unusual feeding observations with urinalysis and fecal parasitological measurements, often insufficient by themselves to indicate a disease state precisely, enabled us to assess the health status of Kanyawara chimpanzees.

Fecal Evidence

For almost all the infected individuals, parasite numbers were low and the highest parasite loads (>1000/g) appeared only once in multiple samples from a single individual. Parasite loads have rarely been evaluated in this way in chimpanzee surveys. However, Hercberg *et al.* (1986) studying the parasite infection in human subjects from a rural zone of Benin, considered that 2000 parasites/g is a moderately high load. Only 1% of the chimpanzees had a similar or higher parasite load. Froment and Koppert (pers. comm.) studying 517 human subjects from a forest area in Cameroon reported that, 15% had >2000 parasites/g of feces, whereas 59% of them had <300 parasites/g. In Kanyawara chimpanzees fecal samples examined via McMaster flotation, consistency is related to parasitic burden, with liquid and soft feces being richer in parasites than feces of normal consistency. This was confirmed in the case of soft feces by direct examination. The frequency of liquid feces was lower for Kanyawara chimpanzees than for the Cameroonian human population, 14% of whom had diarrhea (Froment, 2001). Thus, symptoms associated with parasite infection appeared less frequently in the Kanyawara chimpanzees than in a forest human population.

In terms of specific prevalence, our results obtained by direct examination are similar to those of Ashford *et al.* (2000) for many species (*Trichuris* sp., *Strongyloides fulleborni, Probstmayria* sp., *Bertiella studeri*, and *Ascaris* sp.) (Table V). Their samples were collected in the Kanyawara community between 1992 and 1994.

Contrarily, results from other communities are often different. Some parasitic species absent in our study were common in chimpanzee communities: Gombe (*Physaloptera* sp., *Ascaris* sp.) and Mount Assirik (*Physaloptera* sp. and *Enterobius* sp.) (File *et al.*, 1976; Mc Grew *et al.*, 1989; Murray, 1990 cited in Ashford *et al.*, 2000). The probable endobiont *Troglodytella* sp. was less prevalent in Kanyawara than in some other studies (35% in La Lopé: Landsoud-Soukate *et al.*, 1995; 14% in Gombe: Murray, 1990, in Ashford *et al.*, 2000). It was noted absent in Mahale by Kawabata and Nishida (1991), but Huffman (unpublished data) found it

Table V. Intestinal parasites recorded from two surveys of faeces of chimpanzees in the Kibale National Park, Uganda

Study period	1992–1994 Ashford *et al.* (2000)	2000–01 present study	2000–01 present study
Number of samples	123	247	239
Number of chimpanzees	45	38	38
Methods used	Volumetric dilution, iodine staining	Direct examination	Mac Master flotation ($MgSO_4$)
Samples found positive (%) for			
Troglodytella sp.	91	65	0
Small ciliate	81	23	0
Bertiella sp.	1.6	2.8	0
Trichuris trichiura	0.8	0.4	1.7
Strongyloides sp.	4.9		
Strongyloides fulleborni		8.9	15.5
Unidentified strongyles	31	64	26.3
Oesophagostomum sp.		30.7	55.2
Probstmayria sp.	7.3	10.5	0.8
Enterobius sp.	0.8		
Ascaris sp.	0	0	0

in a later survey. Combining our data with results of previous surveys, Kanyawara chimpanzees appear to be relatively healthy, and the prevalence of parasites is low there compared to other sites. The balance between a host and parasites can be affected by various factors, including infections from external injury, disease, and poor nutrition due to low food availability. Plant secondary compounds can also affect the balance. Kanyawara chimpanzees experience regular shortages of fruit lasting up to several months at a time, but they are not predictably related to seasonal variation in rainfall (Wrangham *et al.*, 1996). Nevertheless, intersite variations in parasitic infection can be affected by differences in the seasonality of rainfall, temperature and other ecological parameters that directly affect the reproduction of some parasitic species (Huffman *et al.*, in prep). In such cases, parasitic infection may be an indicator of general health status and immunity. Nevertheless, parasitic egg and larva loads in the feces do not necessarily reflect intestinal parasitic load, as egg output is different for each species. In human parasites, there is great variation in fecundity: an *Ascaris lumbricoides* pair lays 1000 eggs/day whereas an *Ancylostoma duodenale* pair lays only 10 eggs/day. Because these parameters are not available for chimpanzee parasites, our data should be interpreted with care, but it provides information on the severity of infection with regard to previous samples collected on the same individual. Moreover, as underlined by Ashford *et al.* (2000), the techniques used in different studies are often diverse, complicating comparisons further. The several methods that we used and the results confirm differences in selectivity and sensitivity for the detection of eggs and larvae.

Seasonal differences in infection rates are indicated by McMaster flotation, with higher numbers in the wet season for *Oesophagostomum stephanostomum, Strongyloides fulleborni* and *Probstmayria* sp. Direct examination gave similar results for both entodiniomorph and amoeba levels. Conversely, in the study of Ashford *et al.* (2000), prevalence was seasonally stable. In surveys by Huffman *et al.* (1997) and Dupain *et al.* (2002), prevalence of *Oesophagostomum stephanostomum* and of an unidentified species of larvae was higher in the rainy season. Such results combined with screening of the biological properties of plants eaten (Huffman *et al.*, 1993; Ohigashi *et al.*, 1994; Krief *et al.*, in prep) suggest that in addition to climate, diet may affect parasitic load and may balance the health status of individuals.

Urinary Evidence

In urinalysis, interpretations of positive results were not always obvious. Interpretation may be biased by observer effect and an unusual color in the urine of chimpanzees may also be due to food intake. Muller and

Wrangham analyzed >1300 urine samples from Kanyawara chimpanzees between 1994 and 1999 (unpublished data). Leukocytes and blood were less frequent in the samples than in ours. Beyond the bias induced by observer effect in interpreting the color of the reagent pads, the fact that our collection contained a higher proportion of samples from adult females (51% *vs* 41%) might partly explain such differences. Among 385 samples collected between 1995 and 1997, leukocytes were significantly more frequent in samples from females than males ($p < 0.01$), whereas blood was not significantly more frequent in samples from females ($p = 0.07$). According to our results, the estrous cycle appeared to increase the frequency of positive samples for leukocytes. The females seemed to be more affected by urinary tract inflammation (detected by the presence of blood) than males were. The high urinary pH also observed by Kelly *et al.* (2004) at Kibale and by Kaur and Huffman (2004) at Mahale, may reflect the large amounts of plants in their diet (Cotard, 1993).

As an additional problem, urine pH is not controlled as rigidly as blood pH and can be affected by various factors after collection. For example, bacteria contain enzymes that can break down urea to ammonia, which then combines with hydrogen ions, increasing the urine pH (alkaline urine). Several etiologies may be involved in acidic urine: (1) respiratory acidosis which is due to respiratory failure such as obstructive pulmonary disease and (2) metabolic acidosis which might be a consequence of renal failure, mellitus diabetes, diarrhea, starvation, severe vomiting and severe infection (Cotard, 1993). The concentration of urine (specific gravity) depended on the time of day, the amount of food and fluids ingested, and recent activity of the chimpanzee. It may also indicate a kidney problem. Nevertheless, alkaline urine interfered with the measurement of specific gravity. Moreover, the dipstick values for gravity were unreliable vis-à-vis results measured by a refractometer (Kelly *et al.*, 2004). Accordingly, our specific gravity values are not reliable.

According to the urinalysis of our samples, few individuals were sick. Only 3 individuals had >2 abnormal parameters. Two components may indicate a disease other than urinary tract infection. First, ketones (from SL and LK samples) may indicate metabolic abnormalities such as uncontrolled diabetes or abnormal nutritional condition, including anorexia, starvation, high protein and fat or low carbohydrate diets, or might be a consequence of increased metabolism, including fever, acute illness, pregnancy or lactation. Further, urobilinogen detected in 2 samples from the same individual may be a sign of liver problems: bilirubin in the urine indicate biliary strictures, gallstones in the biliary tract, a tumor in the liver, or hepatitis with bile duct obstruction. Bilirubin is further metabolized to urobilin by bacteria in the intestines. A small percentage of them are reabsorbed and eventually appear in the urine where they are referred to as urobilinogen.

Most of the other abnormal samples probably indicated urinary tract infection or kidney problems. Kidney disease may cause an increase in protein level. Nitrites and leukocytes are other indications of urinary tract infection. We should note that ≥ 2 parameters were abnormal in the case of 2 samples from an old female chimpanzee (AR), coinciding with a period during which she suffered from respiratory and parasitic infections. Nitrite and leukocyte levels were also abnormal as were protein and glucose levels in one sample collected from a young female (OT). When the level of glucose in the blood is very high, some of the glucose may appear in the urine.

Finally, our results of urinalysis are consistent with the general good health status of Kanyawara chimpanzees as evaluated by fecal analysis. Nevertheless, some results indicated that chimpanzees are sometimes in poor health without any obvious behavioral sign.

Benefits of Multimethod Investigation

Our detailed examples (Table IV) underscore the relationship among the results of diverse methods to evaluate the physiological conditions of chimpanzees. A low pH of urine samples characterized 2 ill chimpanzees. First, the painful and infected wound of LB associated with a decrease in feeding likely made his urine more acidic. In KK acidic urine may have resulted from his obstructive respiratory disease. Activity budget and parasite analyses are also convergent with the clinical diagnosis of illness. We had in this particular case the opportunity to collect urine and fecal samples in addition to observe symptoms of the respiratory illness, which emphasizes the possibility of detecting health problems by multiplying the methods used in a general health survey.

Ficus capensis consumed by KK is used by people in the Ivory Coast and South Africa to treat strong coughing and in Zimbabwe for rhinitis, and people use the immature fruits to treat infection in the Congo (Bouquet, 1969). Feeding on mature figs of *Ficus capensis* is not unusual for Kanyawara chimpanzees, but KK was the only individual in his party feeding on immature fruits. Chimpanzees usually carefully select the ripest ones.

Under free-ranging conditions, the consumption of secondary compounds or other non-nutritional items such as soil may maintain the health of the individual or counteract the discomfort of parasite-related diseases on both (Huffman, 1997; Lozano, 1998). In the case of AR, a urinary tract infection together with an infection by *Bertiella studeri*, which was rare during our study, were associated with coughing and sneezing. In the examples of LP and OK, we suggest that relief of intestinal discomfort may have occurred after consuming non-nutritional items with possible medicinal value.

In the case of LP, the presence of coccidia in her stools and proteins and leukocytes in her urine may indicate her weakness, and her digestive discomfort could be the cause, or at least in some way related to, the 3 atypical dietary choices she displayed: prolonged soil-eating, seed-eating from elephant dung and consumption of tree fiber. Moreover, in spite of the possible role in cellulose digestion of the ciliate *Troglodytella*, a symbiont (Collet *et al.*, 1984), such a high number might cause spoliation and suggest an imbalance in the digestive microfauna. O'Donoghue et *al.* (1993) observed numerous *Troglodytella abrassarti* in several diarrheic fecal samples from siamangs, which is consistent with this hypothesis. Soil samples regularly selected and ingested by Kibale chimpanzees are mineralogically and chemically similar to pharmaceutical Kaopectate™ (Mahaney *et al.*, 1997) and to material consumed by other primates such as mountain gorillas (Mahaney *et al.*, 1990, 1995a) and rhesus macaques (Mahaney *et al.*, 1995b). Knezevich (1998) proposed that the pharmaceutical properties of clay may counteract the effects of parasitic infections in free-ranging rhesus macaque populations. Foraging in elephant dung to select digested seeds, as practiced by LP, is also unusual for Kanyawara chimpanzees. There are few documented cases of coprophagy in wild animals: Magliocca *et al.* (2003) observed that in a Congolese forest clearing, foraging in elephant dung for seeds accounted for 33% of feeding time for sitatungas and 20% for hogs. Krief *et al.* (2004a) described a strong correlation between the ingestion of seeds from their own feces by reintroduced chimpanzees in the Congo and their consumption of fruits of *Dialium* sp. In sitatungas, hogs and the reintroduced chimpanzees, regular seed consumption probably constitutes a significant nutritional supplement. In the case of LP, seed consumption from elephant feces was brief and occurred only once, suggesting something other than nutritional benefit.

Consumption of medicinal foods could sometimes keep infections at subclinical levels (Huffman, 1997; Huffman *et al.*, 1998). When infection levels increase to a point that individuals are visibly ill, they may eat particular plants that could have therapeutic effects, such behavior being likely learned within the group and passed down as a behavioral tradition (Huffman and Hirata, 2004). With regard to OK, the consumption of bark of *Albizia grandibracteata* occurred in a period of relatively heavy parasitic infection. The bark of *Albizia grandibracteata* is used traditionally by people in Uganda against bloating (Heine and König, 1988) and in the Democratic Republic of Congo against intestinal parasites and amoebae by small ruminants (Balagizi Karhagomba and Ntumba Kayembe, 1998) and humans (Defour, 1994). We noted a significant drop in the parasitic load and normal consistency of the feces 2 days after bark ingestion. Chemical investigation of the bark of *Albizia grandibracteata* directed by bio-assays revealed bioactive saponins with anthelminthic properties tested against *Rhabditis pseudoelongata* (Krief, 2003), which adds a possible case to the growing body of evidence of the use of medicinal properties of plants by animals. The methods evaluated in this study enable us to pinpoint such critical health related periods of time for the potential use of medicinal plants and to select new plants for further phytochemical studies.

Investigations of diverse parameters of health provide a better assessment of bodily condition and aid in the interpretation of behavior. We have demonstrated in the present study the feasibility of using urinalysis for diagnostic purposes without disturbing the study subjects. Urinalysis provides a supplementary measure of physical condition and long-term surveys will enable us to evaluate the effect of diet on different parameters of health. Combining different methods for parasitic detection seems useful to describe eggs and larvae present in stools, especially in the case of mild infections. Detailed urinary, parasitological, behavioral and veterinary investigations provide a strong basis for identifying and following the evolution of diseases in wild populations for conservation purposes. From a wildlife conservation standpoint, monitoring of the health status of individuals by noninvasive methods may also assist to measure the impact of poaching, ecotourism or environmental degradation on chimpanzees in the natural habitat.

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