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Facial dysplasia in wild forest olive baboons (*Papio anubis*) in Sebitoli, Kibale National Park, Uganda: use of camera-traps to detect health defects

Running title: Malformations in wild olive baboons

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tables: 4

figures: 1

Abstract

Background: Primate populations are in decline, mainly affected by agriculture leading to habitat loss, fragmentation but also chemical pollution. Kibale National Park (Uganda), Sebitoli forest, surrounded by tea and crop fields, is the home range of chimpanzees presenting congenital facial dysplasia. This study aimed to identify to what extent the same phenotypical features are observed in baboons (*Papio anubis*) of this area.

Methods: A total of 25,390 clips recorded by 14 camera traps between January 2017 and April 2018 were analyzed.

Results: We identified 30 immature and adult baboons of both sexes with nose and lip deformities. They were more frequently observed in the North-Western part of the area.

Conclusions: A possible effect of pesticides used in crops at the border of their habitat is suspected to alter the embryonic development. This study emphasizes the importance of non-invasive methods to detect health problems in wild primates that can act as sentinels for human health.

Key words: malformation, primate, camera-trap, endocrine disruptor, anthropogenic pressure

Introduction

Most primate species are threatened with extinction or declining populations resulting from escalating and unsustainable human activities[1]. These activities lead to anthropogenic environmental modifications that could affect wild primates by cascading effects. Non-human primates can act as sentinels of environmental toxics caused by anthropogenic activities. However, exposure to pollution is rarely measured and cases of intoxication rarely reported. Accumulation of heavy metals and levels of lead were measured in different locations and species such as howler monkeys (*Alouatta pigra*)[2], in urban free-ranging rhesus monkey (*Macaca mulatta*)[3] and in long-tailed monkeys (*Macaca fascicularis)*[4]. Levels of serum dioxin were measured on douc langurs (*Pygathrix nigripes*)[5,6] in Vietnam to understand the consequences of the use of Agent Orange during the war. However, no consequence on the health was observed associated to the levels of lead and dioxin in these studies.

The leading causes of primate extinction i.e. habitat loss[1] are mainly due to the conversion of natural forests into agricultural systems. Environmental pollution due to conventional agricultural practices and use of pesticides could directly threaten the health of the surrounding wildlife[7]. Yet few surveys have been conducted to show evidence of exposure to pollutants on animal health due to close proximity to agricultural areas (birds studies[8-10] but no primates).

The direct or indirect observation of primates is a way to assess their health by looking at potential wounds or malformations. Most primates' deformities described in literature are the result of falls, fights, or wounding by predators[11]; and some are the collateral consequences of being caught in snares set by poachers[12]. However, in Sebitoli area in the northern part of Kibale National Park, Uganda, it has been suggested that the malformations observed have different causes. Indeed, exposition to chemicals used on crops, arboricides such as Agent Orange and pollution from combustion exhaust gases from vehicles could be the cause of the high number of congenital malformations observed in the primates including 16 chimpanzees and six baboons (*Papio anubis*)[12-15]. While chimpanzees are monitored daily in this area, baboons are opportunistically seen and Krief et al.[15] described facial deformities in six baboons captured in a one minute video by a single camera trap.

The objective of this survey was to expand spatially and temporally the camera trap network to confirm, better describe the lesions in baboons and evaluate the number, age and sex class of affected individuals sharing the habitat with Sebitoli chimpanzees.

Material and methods

The study site was the Sebitoli area located at the extreme North part of the Kibale National Park, Western Uganda (795 km², 0°13'-0°41'N and 30°19'-30°32'E[16]). The chimpanzees, and more generally wildlife have been monitored since 2009 by the Sebitoli Chimpanzee Project. This Park is composed of a mid-altitude forest with high plant and animal diversity[17]. The Sebitoli area is surrounded by human activity, and in particular, agriculture comprising in the western fringes, small farms with food crops, tea and eucalyptus, and to the East, tea estates and eucalyptus plantations. Phytochemical use differs according to the crops and thus to the area[15]. In order to understand if the location and the crop type in contact with the territory of a group of baboons could influence the number of affected individuals, we divided the Sebitoli area into four zones delimited by the tarmac road crossing the chimpanzee home range (20 km²) in the south[18] and two survey transects that divided the region into equivalent areas in west, east, north and south (Figure 1). We assumed that East and West are differently affected by pesticides and that the road play a "natural" limits between groups of North and South. We counted the malformed individuals encountered in the video clips recorded by the cameras localized in each of the four zones to understand if malformed individuals frequent more often one of the four zones.

From January 2017 to April 2018, up to 14 camera traps (CT) equivalent to 4746 CT days were set in the forest (named hereafter "forest-CT") and in the border of cultivated fields of maize (named hereafter "garden-CT") that surround the Park (Figure 1). The locations are recorded with a GPS Garmin 64CS and projected on a map[19]. The recorded clips are collected every two weeks. We used 13 HD video-traps Reconyx XR-6 Ultrafire™ and 2 HD video-traps Bushnell Trophy Cam HD Max™ with a day/night auto sensor and sound recordings. The settings were: high definition video of 1280 × 720 pixels, video length of 30 s for Reconyx™ and 60 s for Bushnell™. Video recording started when motion was sensed at a distance of up to 21 m for Reconyx™ and 18 m for Bushnell™; there is a 5 s delay between consecutive triggers. Camera traps were set at least 1 m above the ground and at least 1 m off the trail or on an animal track where we expected the animals to pass. Three CT were set and active at the same location during the entire duration of this study. Other CT were moved in the forest according to (1) presence of mature crops at the edge of the forest in order to record crop feeding events by chimpanzees, baboons and elephants or (2) presence of bees nests visited by chimpanzees in order to obtain footage of tools used for honey harvesting by chimpanzees (3) different events

such as stealing and breaking of CT by animals or poachers resulting of some CT being not active during some periods.

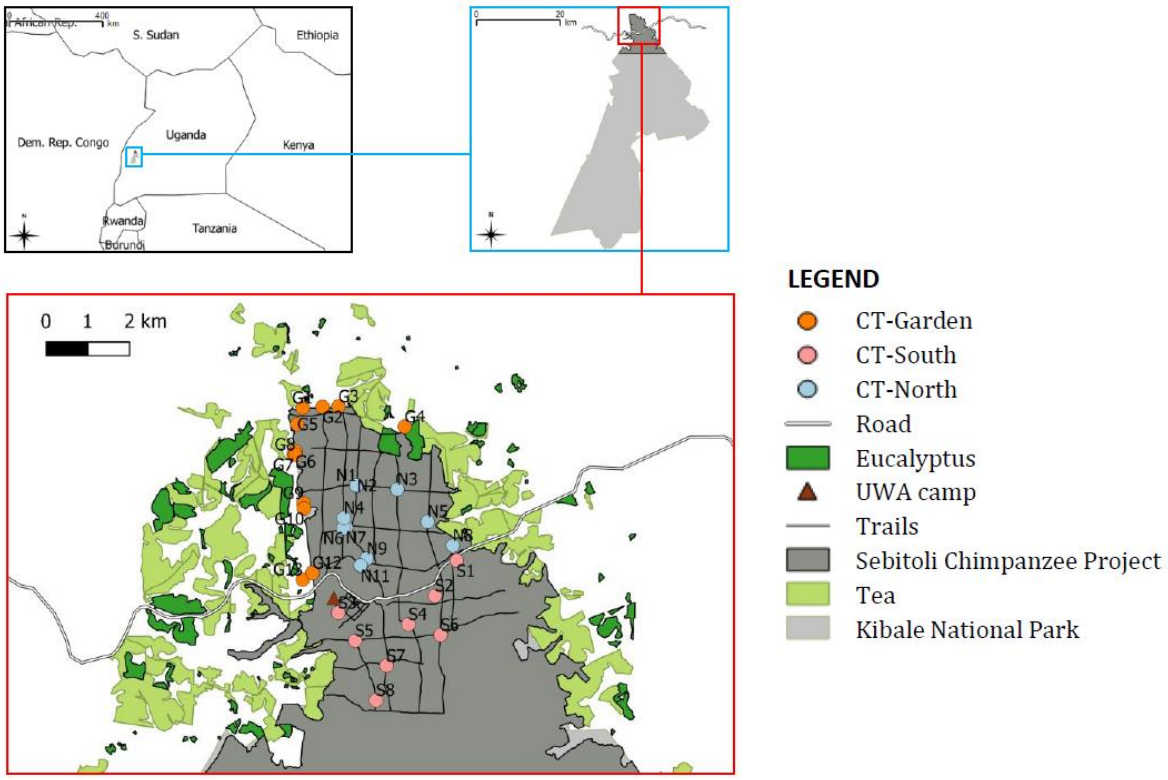


Figure 1. Map of the camera-trap study in the Sebitoli Chimpanzee Project, Kibale National Park in Uganda. We placed 13 camera-traps in the garden (CT-Garden), 19 camera-traps in the forest: 11 in the Northern part (CT-North) and 8 in the Southern part (CT-South) of the road that cross the area. The forest is surrounded by eucalyptus and tea plantations.

For each clip we identified the animal species observed, the number of individuals of each species seen, their activities and the health impairments observed. When successive clips started less than 5 minutes apart with the same species being present, we considered them not to be independent and thus animals were considered to be part of the same group. If it was more than 5 min and less than 10 minutes between the clips and we could identify again clearly an individual previously identified (known or because of some features) we also considered those clips as non-independent. For those non-independent clips, we counted the number of individuals that appear for the first time in the given clip and were not seen in the previous clip. By adding the number of individuals that appeared to be different, we could estimate the size of the group. We calculated the relative abundance of the presence of baboons by dividing the number of individuals seen (could be the same individuals over the month) by 100 CT days.

The CT days are the days for which the camera was fully operational and could register. It excludes events of CT's stealing and memory card full in few days because of vegetation movement in front of the High Sensitivity Image sensor.

We tried as far as possible to determine the sex and the class of age (adapted from Packer[20]; Rose[21]) for the olive baboons: infant (0 to 1 year, carried by mother), juvenile (1 to 4 years, small and not carried by mother) and mature (i.e., sexually mature individual including adult and sub-adult more than 4 years). The class of age of an individual reported corresponds to its first observation during the study period. Sex determination of juveniles and infants from camera trap videos was often difficult; therefore, this age category is generally classed as unknown sex. When individuals looked very similar and cannot be morphologically differentiated, we assumed that they were the same individual seen on different occasions. We defined mother-infant pair when an infant has been seen carried by its mother.

For individuals with physical features related to health impairment, we extracted a screen shot from the video-clip and described them as precisely as possible. We classified the abnormalities related to fur color (depigmentation), malformation or wound (presence of blood, pus or scab). We specified the body part affected: the head, the limbs, the trunk, the tail or the genitals. For facial malformations, parts of the face affected are mentioned as followed: nasal bone, nostrils, lip, ectopic apertures on the nasal bone and others features.

The baboons were observed without resorting to invasive methods and without interaction with the researchers. We adhered to the research protocols defined by the guidelines of the Uganda Wildlife Authority (UWA), which were also approved by the Muséum national d'Histoire naturelle (Memorandum of Understanding MNHN/UWA/Makerere University SJ 445-12).

Results

Out of 34 species identified, olive baboons were observed on 3,238 clips out of 25,390 clips recorded in 4,746 days by 14 cameras over an area covering about 20 km². We saw 4,906 baboons over 2,204 clips in the forest-CT clips (abundance relative per 100 CT-days = 118.8) against 1,645 baboons over 1,034 clips in the garden-CT clips (abundance relative per 100 CT days = 266.2).

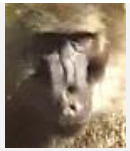
Table 1. Individuals observed with particularities classed by the localization and the type of the particularity.

Localization	Face	Limbs	Trunk	Tail	Genital	Total
Depigmentation	1	-	-	-	-	1
Malformation	30	1	1	-	-	32
Malformation/wound	-	-	-	6	-	6
Wound	4	7	2	1	1	15
Total	35	8	3	7	1	54

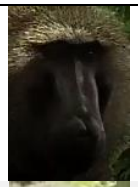
Out of the 6551 occurrences when a baboon can be seen, we identified 54 different individuals affected by physical defects (Table 1). Among them, 30 individuals were displaying facial dysplasia: 25 had malformed nostrils, 12 had ectopic apertures on the nose, 10 had an interruption of the nasal bone and three had others defects. The detailed description of the individuals presenting facials deformities is available in Table 2 as well as a screen capture of the face for each of them. Each individual was seen a mean of 2.8 times ranging from 1 to 14 times in 12 different locations (seven in the forest and five in the garden) over 33 CT locations during the entire study period. None of the individuals with facial dysplasia had wounds or infections. The lesions observed at different occasions did not change: they did not increase or decrease in size or severity. Most of the individuals with facial deformities were mature baboons (n=20) with nine males, eight females and three of unknown sex. We observed nine juveniles and one infant carried by a non-malformed mother. For two adult females affected (Ind 2 & Ind 3), we could properly observe their infants who did not have visible malformations. The females weremostly affected at the nostrils (n=7), the nasal bone (n=3) and by having ectopic apertures (n=2). The males had also mostly malformed nostrils (n=8), but ectopic apertures (n=7) were more frequent than nasal bone deformity (n=3). There is no apparent effect of gender.

Sixteen individuals (8 adult males, 3 adult females, 1 adult unknown sex and 4 immature unknown sexes) were seen in association on 17 times in non-independent clips (Table 3). The troops of baboons were not habituated and clearly identified in this study but those associations of recognizable baboons allowed us to establish a draft of troops. We identified a possible troop that included those sixteen individuals only visible in five locations of CT in the North.

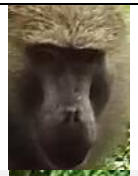
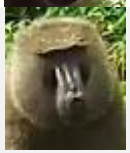
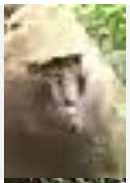
Table 2. Listed individuals with facial malformations with the picture taken from a screen capture of a video clip.

Description of the individual			Description of the individual malformed features						Number of times seen	Location of camera trap concerned
Code	**Class of age**	**Sex**	**Nasal bone**	**Number of nostril**	**Shape of nostril(s)**	**Additionnal Appertures**	**Other**	**Picture**		
Ind 1	Inf	X	Interrupted	1	central & deformed	-	Lips deformed		3	S8
Ind 2	Ad	F	Interrupted	1	central & round	Bottom left	-		12	N4, N5, N6, G3, G9, G11
Ind 3	Ad	F	Interrupted	1	central & triangular	-	-		14	N4, N6, G2, G8, G9, G11, G10
Ind 4	Juv	X	Interrupted	2	asymmetric (left larger)	-	Lips twisted		1	S8
Ind 5	Juv	X	-	1	central & triangular	-	-		3	S1, N4, N8
Ind 6	Ad	M	Interrupted	2	-	Bottom central	-		1	N6

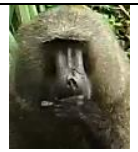
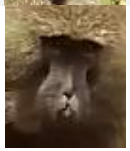
196 **Table 2.** (Continued)

Description of the individual			Description of the individual malformed features						Number of times seen	Location of camera trap concerned
Code	**Class of age**	**Sex**	**Nasal bone**	**Number of nostril**	**Shape of nostril(s)**	**Additionnal Appertures**	**Other**	**Picture**		
Ind 7	Ad	M	-	2	asymmetric (right larger)	Slit in the middle of the nose	-		6	N4, N6, S8
Ind 8	Ad	F	-	1	central & round	-	-		1	S8
Ind 9	Juv	X	-	2	-	-	Asymmetric frown		6	N4, N6, G2, G9
Ind 10	Ad	M	-	2	asymmetric (left larger)	Slit in the middle of the nose	-		5	N4, N6, G2, G9
Ind 11	Juv	X	-	1	central & round	-	-		5	N4, N6, S2
Ind 12	Juv	X	-	1	central & deformed	-	-		2	N4, G2

197 **Table 2.** (Continued)

Description of the individual			Description of the individual malformed features						Number of times seen	Location of camera trap concerned
Code	Class of age	Sex	Nasal bone	Number of nostril	Shape of nostril(s)	Additionnal Appertures	Other	Picture		
Ind 13	Ad	F	-	2	narrow and asymmetric (left higher)	-	-		2	N4
Ind 14	Ad	M	Interrupted	2	-	Bottom	-		1	N6
Ind 15	Juv	X	-	1	no nasal septum	-	-		1	N4
Ind 16	Ad	M	Interrupted	2	-	Bottom central	-		2	N4
Ind 17	Ad	M	-	1	no nasal septum	-	-		2	N4, G2
Ind 18	Ad	M	-	2	asymmetry (right larger)	Slit in the left side of the nose	-		2	N4, N6

198 **Table 2.** (Continued)

Description of the individual			Description of the individual malformed features						Number of times seen	Location of camera trap concerned
Code	**Class of age**	**Sex**	**Nasal bone**	**Number of nostril**	**Shape of nostril(s)**	**Additionnal Appertures**	**Other**	**Picture**		
Ind 19	Ad	M	Pleated	2	narrow and asymmetric (left longer)	-	-		1	N5
Ind 20	Ad	M	-	2	asymmetry (left larger and higher)	-	-		3	N8
Ind 21	Ad	M	-	1	no nasal septum	In the top middle of the two nostrils	-		2	N8, S2
Ind 22	Ad	M	-	2	flat nose	-	-		1	G2
Ind 23	Juv	X	Interrupted	2	-	Slit on top of the nose	-		1	G2
Ind 24	Ad	X	-	2	flat nose	-	-		1	G9

199 **Table 2.** (Continued)

Description of the individual			Description of the individual malformed features						Number of times seen	Location of camera trap concerned
Code	**Class of age**	**Sex**	**Nasal bone**	**Number of nostril**	**Shape of nostril(s)**	**Additionnal Appertures**	**Other**	**Picture**		
Ind 25	Ad	F	-	2	asymmetry (left higher)	Slit in the left side of the nose	-		1	N8
Ind 26	Juv	X	-	1	central & round	Slits under the nose	-		1	G9
Ind 27	Ad	F	-	2	twisted on the right side	-	-		2	G9, N6
Ind 28	Ad	F	Interrupted	1	central & triangular	-	-		1	G9
Ind 29	Ad	X	-	2	flat nose and asymmetric (left larger)	-	-		1	G11
Ind 30	Ad	X	-	1	central & round	Slit on top of the nose	-		1	G11

200 *Note*: Ad : mature individuals, Juv : Juveniles, Inf : Infant, M : Male, F : Female, X : Sex unknown, - : normal feature

Table 3. Association of individuals with facial malformations observed in non-independent clips.

Individuals together	Group size	Where
Ind 2 & Ind 3 & Ind 5 & Ind 10 & Ind 15	29	N4
Ind 2 & Ind 3	13	N6
Ind 2 & Ind 3	18	G9
Ind 2 & Ind 3	20	N6
Ind 2 & Ind 13	4	N4
Ind 2 & Ind 14	22	N6
Ind 2 & Ind 18	20	N6
Ind 3 & Ind 7	25	N4
Ind 3 & Ind 23	43	G2
Ind 3 & Ind 24	8	G9
Ind 5 & Ind 21	58	N8
Ind 7 & Ind 9	32	N4
Ind 9 & Ind 10	21	N6
Ind 9 & Ind 11	25	N6
Ind 11 & Ind 13 & Ind 16 & Ind 17	24	N4
Ind 16 & Ind 18	23	N4

Individuals with facial defects are relatively more abundant in the two zones in North and more precisely the North-Western area of Sebitoli (Table 4).

Table 4. Distribution in Sebitoli of malformed individuals.

Position	Number of individuals seen	Number of ct	Number of days filmed	Abundance
N/W	23	21	1406	1,63584637
N/E	6	4	804	0,74626866
S/W	3	4	1085	0,2764977
S/E	5	4	833	0,6002401

Note: Abundance = Number of individuals seen/ 100 CT-days

Discussion

Facial deformities affected 30 olive baboons of both sexes and all the classes of age in Sebitoli, confirming the opportunistic footage by camera trap in 2014 of six olive baboons presenting facial malformations in a troop of 35 individuals[15]. There were no wounds, pus or blood likely excluding infection by *Treponema pallidum*[22]. For individuals seen several times, there was no evolution of the lesions during the study period, and the naso-oro-facial defects are observed in

very young individuals suggesting congenital origins of those malformations. The baboons appeared otherwise to be healthy, integrated into social groups, and apparently reproducing as two females with dysplasia were carrying non-malformed infants. Malformations also occured in other parts of the body as an infant had severely malformed limbs causing locomotor issue and an adult male had a hunchback but those could be related to malnutrition or trauma. Spontaneous malformations occur in a variety of non -human primates in captivity but at low rate (0.3%-0.9%) (*Papio sp.*[23,24]; *Macaca sp.*[24,25];). Clefts of the palate and/or of the upper lip are very rare in wild non-human primates but have been observed in several species in captivity includingStrepsirrhines (*Varecia variegata*[26]) as well as in Haplorhines, including New World monkeys (*Callithrix jacchus*[27]; *Saimiri sp.*[28,29]), Old World monkeys (*Macaca sp.*[30,31]; *Papio sp.*[32]; *Mandrillus sphynx*[33]), and great apes (*Gorilla sp.*[34]) . In Sebitoli, the large number of individuals of all age and sex classes with facial defects (n=30) observed in the relatively small area studied (20 km^2), the absence of other cases reported in the national park covering 795 km^2 likely indicate an etiology related to a local cause. Olive baboons usually live in multi-male, multi-female groups or "troops", ranging in size from 15 to 150 individuals[35-37]. In adjacent troops of olive baboons, all males emigrated from their natal troop in order to mate after they reach sexual maturity and can return for short period of time[20,38-40] whereas nearly all females remained in their natal troop[41] making the females the basis of troop identity. One-male groups also exist and can be the majority encountered[42]. The home range of forest olive baboons has been rarely studied and was estimated at roughly 390 ha and 520 ha[35] which is small compared to the average home range of 3,890 ha given by DeVore[43] for savannah baboons. The five locations of CT where we had seen the sixteen individuals in association represented an area of 522 ha, so we can estimate that they are part of the same larger troop. Thus, in a single group estimated to count 58 individuals, one out of four individuals is affected.

Only one other case of facial deformity concerning an olive baboon has been documented in the south of the park where a female was missing the most basal part of the upper jaw and nose[45]. In addition, there are no cases of similar lesions described in areas without exposition to agrochemicals. This indicates that pesticide exposure could contribute to the facial dysplasia observed in olive baboons as it was suspected for chimpanzees[12,13,15]. The fact that the two primate species -chimpanzees and olive baboons- in exactly the same range suffer from the same deformities strengthens the chemical pollution hypothesis. In addition, both species regularly feed on crops[46,47]. In this study area, the crop fields are mostly in the North-western part, between 2 and 4 km away from the road. The most abundant proportion of malformed

individuals was also found in the north-western patches suggesting that the chemicals on food cropsassociated with those sprayed on tea, could be indeed responsible. The most affected troop of baboons is living in the Northern location where they are not exposed to road pollution. This is in favor of an etiology related to pesticides rather than combustion gas exposition. According to previous analyses, maize used in this area and consumed by this troop was coated with imidacloprid[15]. In addition, maize stem and maize seed samples from Nyakabingo and Kyawankada, the two locations at the far north of the study area, were tested positive for chlorpyrifos[15]. In these two locations, farmers also reported using 2,4 D amine on maize. Glyphosate is used in every tea estate including the one in Eastern part of the home range[15]. The fact that affected baboons are more often observed in the Western part than Eastern part of the range seems to support an effect of pesticides used in maize or glyphosate associated with other chemicals rather than an effect attributed to glyphosate alone. It is the possible unique combination of pesticides, fungicides, herbicides, and fertilizers used in those food crops that could have contaminated water, soil and plants and in consequence affected the wildlife of the park[13].

In response to crop-raiding, farmers usually chased primates, probably causing the baboons of this area to fear human contact. They tend to rapidly flee when humans are near[48] making them difficult to observe, individually identify, and study. The use of camera traps is very convenient to overcome this difficulty[49]. It is an important tool that allows studying primates without exacerbating potential threats to the species like the transmission of zoonotic diseases[50-52], and the potential exposure to poachers[53]. While also mitigating the negative impacts of habituation, the use of camera-traps can overcome the limitations of direct observations by filming in accessible areas[54,55] and rare or elusive individuals without the potential human presence bias[56,57]; in a relatively short period of time[49] However the counting of individuals in a group, the individual health and behavioral monitoring to estimate the effect of the phenotypical defects would be necessary; there are impossible with this type of indirect survey. Technological advances have led to widespread adoption of camera traps to survey wildlife distribution, abundance and behavior[58,59] and recently health[15]. Indeed, an opportunistic footage by camera trap in 2014 had raised the issue of capturing facial malformations by camera trap with six olive baboons in Sebitoli[15]. Our study on a larger scale confirmed the interest of using camera-traps to survey the health of wildlife and improve our knowledge about the insidious threat of pollution.

Acknowledgement

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