



Agricultural expansion as risk to endangered wildlife: Pesticide exposure in wild chimpanzees and baboons displaying facial dysplasia

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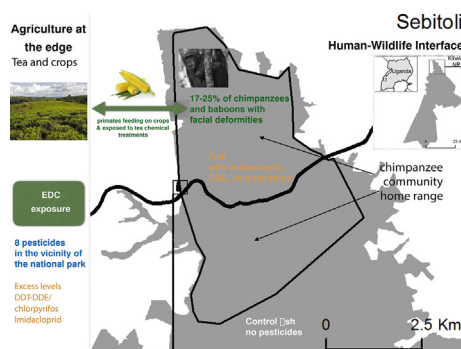
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HIGHLIGHTS

- Agricultural land expansion towards parks exposes wildlife to environmental pollution.
- We studied the human-wildlife interface in Kibale National Park, Uganda.
- Methods used were environmental chemistry, ethnological enquiries, and primatology.
- Numerous chimpanzees and baboons display similar facial deformities and are exposed to agricultural pollutants.
- We propose that the high levels of EDC pollution represent an underestimated threat to endangered chimpanzees.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 23 December 2016

Received in revised form 14 April 2017

Accepted 14 April 2017

Available online xxxx

Editor: D. Barcelo

Keywords:

Endocrine disruptors

ABSTRACT

Prenatal exposure to environmental endocrine disruptors can affect development and induce irreversible abnormalities in both humans and wildlife. The northern part of Kibale National Park, a mid-altitude rainforest in western Uganda, is largely surrounded by industrial tea plantations and wildlife using this area (Sebitoli) must cope with proximity to human populations and their activities. The chimpanzees and baboons in this area raid crops (primarily maize) in neighboring gardens. Sixteen young individuals of the 66 chimpanzees monitored (25%) exhibit abnormalities including reduced nostrils, cleft lip, limb deformities, reproductive problems and hypopigmentation. Each pathology could have a congenital component, potentially exacerbated by environmental factors. In addition, at least six of 35 photographed baboons from a Sebitoli troop (17%) have similar severe nasal deformities. Our inquiries in villages and tea factories near Sebitoli revealed use of eight pesticides

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Pesticides
Non-human primates
Apes
Monkeys
Uganda

(glyphosate, cypermethrin, profenofos, mancozeb, metalaxyl, dimethoate, chlorpyrifos and 2,4-D amine). Chemical analysis of samples collected from 2014 to 2016 showed that mean levels of pesticides in fresh maize stems and seeds, soils, and river sediments in the vicinity of the chimpanzee territory exceed recommended limits. Notably, excess levels were found for total DDT and its metabolite *pp'*-DDE and for chlorpyrifos in fresh maize seeds and in fish from Sebitoli. Imidacloprid was detected in coated maize seeds planted at the edge the forest and in fish samples from the Sebitoli area, while no pesticides were detected in fish from central park areas. Since some of these pesticides are thyroid hormone disruptors, we postulate that excessive pesticide use in the Sebitoli area may contribute to facial dysplasia in chimpanzees and baboons through this endocrine pathway. Chimpanzees are considered as endangered by IUCN and besides their intrinsic value and status as closely related to humans, they have major economic value in Uganda via ecotourism. Identifying and limiting potential threats to their survival such be a conservation priority.

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1. Introduction

The conversion of natural forest to agricultural land exposes wildlife to multiple threats including forest fragmentation, bushmeat hunting, zoonotic diseases, and often environmental pollution due to excessive pesticide usage. In Europe, pesticide-linked poisoning accounted for 6.5% of identified causes of death in wildlife between 1986 and 1998 (data from the SAGIR network, Lamarque et al., 2000). More recent data show reduced fitness of animals living in close proximity to agricultural zones, including areas in Africa (Bro et al., 2015; Ciliberti et al., 2011; Millot et al., 2015). In Africa, the health of non-human primate populations may serve as a valuable indicator of successful ecosystem management, notably for sentinel species (Goldberg et al., 2012). The emblematic chimpanzee (*Pan troglodytes*) is an important species, not only because of the urgent need for its protection, and its phylogenetic position as the closest living relative of humans, but because this species may provide significant information about risks for the health of humans living in the same environment. Furthermore, chimpanzee ecotourism represents an important potential economic resource for a number of countries when correctly conserved and managed.

Kibale National Park is a protected area in Western Uganda that is one of the most biodiverse forests in Africa and home to 12 primate species, including the endangered eastern chimpanzee (*Pan troglodytes schweinfurthii*) (Chapman and Lambert, 2000). Kanyanchu, an area situated in the middle of the national park, is an important international chimpanzee ecotourism site. Thirty kilometers north of Kanyanchu lies the northern most region of the park, Sebitoli. The home range of the Sebitoli chimpanzee community is surrounded by industrial tea plantations, small scale agriculture including maize gardens, and is split by a tarmac road. In a buffer 2.5 km zone around their home range, tea plantations cover 24.1% (21.5 km² out of 89.2 km²) and eucalyptus plantations cover 3.8% of the buffer (3.4 km²; Bortolamiol et al., 2013). Thus, this community of chimpanzees and other sympatric wildlife have to cope with proximity to high-density human populations (~300 inhabitants/km²; Hartter, 2009) and dense traffic on the tarmac road crossing their home range (Cibot et al., 2015). Potential inbreeding due to spatial isolation, unsustainable agriculture practices, and misuse of pesticides are among the issues that may directly threaten health of wildlife in this area and in Africa generally.

During the first years of our monitoring, we discovered that 12 out of the 60 identified wild chimpanzees in Sebitoli suffered from anomalies, including reduced nostrils, cleft lip, and limb deformities. In addition, some individuals exhibited reproductive problems (no cycle and swelling of the genital area, no dependent offspring) and hypopigmentation. This syndrome likely has a congenital basis that could be due to or exacerbated by environmental factors (Krief et al., 2014, 2015). Within the same park, primates, including chimpanzees, have been observed and studied for more than 25 years. In three sites (Ngogo, Kanyawara, Kanyanchu, home ranges of more than 300 chimpanzees), wild chimpanzees are daily monitored, and no anomalies of this type have been reported before we discovered the deformities observed in chimpanzees in Sebitoli.

However, as soon as we noticed that chimpanzees were exhibiting unusual phenotypes in Sebitoli, we did a bibliographical review on wild primate cases of such deformities and interviewed our colleagues about such anomalies in their study sites. Two primate cases were reported. First, D. Watts and J. Mitani from Ngogo research site sent us several photos of a male chimpanzee with an abnormal lip that we submitted for diagnosis to a specialized medical team, who reported the condition as a cleft lip (Krief et al., 2015). To our knowledge and after careful bibliographical review, this is the only reported case of wild chimpanzees having a facial dysplasia outside the Sebitoli area. Secondly, there is a documented case of a female baboon in Kibale National Park missing all but the most basal part of her upper jaw and nose, with the authors proposing that the arhinia was congenital (Struhsaker et al., 2011).

Here, our goal is to determine the cause of the phenotypical impairments of the primates in this area and to understand whether they can be related to the environmental pollution of their habitat. Motivated by the larger proportion of the individuals that suffer from anomalies in Sebitoli in contrast to other regions in Kibale National Park and Africa, we explored the hypothesis that chimpanzees and other primates in Sebitoli may be exposed to chemicals used on neighboring crops that could instigate or exacerbate developmental impairments. This hypothesis is strengthened given that Sebitoli, chimpanzees regularly visit crop fields and feed on maize (stems and seeds) and plantains and they live in close proximity with tea monocultures. Small fields and gardens with varied, rotating crops, including maize, are also found in the surrounding area. Frequently, between two and six maize gardens are close enough to the edge of the forest to be accessible to chimpanzees and baboons.

As chimpanzees are a threatened species, no invasive biological sampling is allowed. Therefore, as a first step, we verified whether or not Sebitoli chimpanzees are exposed to pollutants that could affect development and contribute to their abnormal phenotypes. To this end we used environmental analysis on the one hand and fish from different areas of the park as sentinels on the other hand.

2. Material and methods

2.1. Study site

The Sebitoli area is located in the north of Kibale National Park (795 km²; 0°13' to 0°41'N and 0°19' to 30°32'E; Chapman and Lambert, 2000). This section of Kibale was commercially logged in the 1970s, leading to damage of about 50% of the trees (Struhsaker, 1997). Today, a large proportion (70%) of this area of the park is degraded or regenerating forests and only 14% represents old-growth forest (Bortolamiol et al., 2014).

2.2. Methods

2.2.1. Description and records of non-human primates (NHP) affected by physical anomalies

The chimpanzees and 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).

2.2.1.1. Chimpanzees. Chimpanzees live in multi-male multi-female communities where individuals divide in parties that are frequently changing size and composition over time each (fission-fusion system; Chapman et al., 1995). They usually fear humans and are very difficult to approach in natural conditions. To observe wild chimpanzees for research or tourism (without provisioning which is currently forbidden in Uganda), a process of “habitation” over 10 to 15 years is necessary. After habituation, chimpanzees will accept human presence at close range during all their activities and are relatively easy to follow as they move.

In Sebitoli, teams work daily from 6 am to 8 pm to monitor chimpanzee's behavior and health. Habituation by the Sebitoli Chimpanzee Project (SCP) started in 2009. We documented approximately 80 to 100 chimpanzees in a range of 25 km² (Fig. 1), meaning that the chimpanzee density is high in this area, reaching 3 individuals/km². More than 80% of perimeter of their home range is in contact with anthropogenic development (Bortolamiol et al., 2014). Sixty of the individuals are identifiable based on morphological features and they were assigned with names for monitoring: 15 adult males, 18 adult females (estimated to be 15 years and older), 7 subadults (10–14.9 years old), 15 juveniles (3.0–9.9 years old), 5 infants (0–2.9 years old; age classes according to Pontzer and Wrangham, 2006) and are well habituated (habituation levels according to Bertolani and Boesch, 2008).

When chimpanzees are located, the observers describe the composition of the party and the behavior and the health status of each individual during the contact time. Total contact time with chimpanzees from 01 January 2009 to 31 May 2015 was 6879 h out of 29,515 h spent by the observers in the forest. During this monitoring we accumulated direct observations of chimpanzees over 6 years, for which we recorded and detailed any signs of limb or facial deformities. By February 2015, we had determined that at least 10% of the chimpanzees displayed congenital facial dysplasias (Krief et al., 2014, 2015), and 30% of them exhibited limb deformities due either to poaching or congenital disease (Fig. 2). However, the following year, even more cases were observed (Table 1).

2.2.1.2. Baboons (*Papio anubis*). To determine if other species were affected by the same type of impairment in Sebitoli area, we set up several HD video-traps (Bushnell Trophy Cam HD Max™) with a day/night autosensor and sound recordings. Video recording started when motion was sensed at a distance up to 18 m. The settings were: high definition video of 1280 × 720 pixels, video length of 30 s, trigger interval of 1 s. One of the cameras, which was fixed from July 22, 2014 to August 11, 2014 on a tree facing the forest side near the entrance to a small maize field in Nyamigere area, recorded a group of baboons with facial anomalies (Fig. 3). We observed all individuals facing the camera and visible in each 30 second-clip and described their deformities (Table 1). We could not obtain direct observation or better photos because baboons are not monitored and not habituated to observers in Sebitoli. In general, baboons fear human contact as they are chased by farmers when they raid crops.

2.2.2. Interviews of villagers, tea society managers, and agrochemical retailers

Interviews were conducted with 23 volunteers: 3 agrochemical retailers in Fort Portal (the nearest town to Sebitoli having a major retail sector) 9 tea growers, 8 maize farmers, and 3 villagers cultivating both maize and tea in locations close to the park. Retailers were asked to list the chemical products sold in their shop and to rate the frequency of the request for each product on a 4-point scale (rare, common, high, very high). Farmers and tea growers using land near the border of the park were asked which chemicals they use, for which purpose,

the frequency of their use, and the year of their first use of each product. Most of farmers and villagers growing crops near the park were not born in the region. Local villagers prefer to farm elsewhere as the fields abutting the park are too prone to raids and crop destruction by wild animals. Thus, the land is most often used by migrant farmers from other Ugandan districts, who cultivate for free or for low rents.

For this reason, there is no “historical data” about the use of chemical products in the area immediately adjacent to the park. Because of the low level of education and the poverty many of these people do not have watches, do not use calendars and so answers were often imprecise. Despite these drawbacks, the interviews provide useful information to better understand the local context and especially the lack of systematic and methodic use of these products by farmers.

2.2.3. Sample collection and storage

Fig. 1 presents the locations where the samples were collected in and around the Sebitoli chimpanzee home range.

- Fresh maize (seeds and stems) and soil samples were collected from January to July 2014. Maize stem (ca. 20 cm each) (n = 40) and fresh seed (n = 22) samples (1.3 m tall and at maturity) were collected from maize gardens neighboring the forest in Sebitoli area (Fig. 1). Soil samples (each ca. 200 g) (n = 56) were collected using an auger at 15 cm depth from (1) tea plantation neighboring the forest (n = 20), (2) maize gardens when the maize had just been planted (20 samples), and (3) abandoned fields and from the elephant trenches in the forest. River sediment samples (n = 11) were collected using a grab sampler from sites that either lay at the foot of neighboring tea plantations or in the forested area where Sebitoli chimpanzees range. All the samples were wrapped in aluminum foil and placed in labeled airtight bags, kept in ice-coolers at approximately −4 °C and transported to the Pesticide Residues Analytical laboratory at Makerere University where they were kept in a freezer at −20 °C before extraction and analysis.
- Ten individuals of the fish species *Barbus neumayeri* were collected at Mpanga River, within Sebitoli home range, at 300 m from the border of the Park in May 2016. Ten control individuals of the same species were collected inside the park 10 km from the first collection site in a stream that originates within the park and thus receives no run-off from agricultural or tea fields.
- Dry maize seeds that are planted in the gardens at Sebitoli are coated with an unknown red product. They are labeled as “Hybrid H520 (Kenya-seeds™)” but we could not find any information about the composition or the nature of the coat. In order to analyze the coat, we bought coated maize seeds at Fort Portal Market in July 2016.

2.2.4. Chemical analyses

Table 2 summarizes the methods used by the Ugandan and French laboratories to analyze the samples.

Fresh maize seeds and stems, soil, and sediments were analyzed for chlorpyrifos, profenofos, dimethoate, cypermethrin and DDT and its metabolites and for ethylenethiourea (ETU), a metabolite of mancozeb.

Fish and dry coated maize seeds were analyzed for organochlorines, organophosphates, pyrethroid insecticides, herbicides including the following substances: carbofuran, chlorpyrifos-methyl, chlorpyrifos-ethyl, diazinon, dichlorvos, malathion, methiocarb, mevinphos, parathion-ethyl, pyrimifos, terbufos, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, permethrin, tefluthrin, alachlor, atrazine and desethyl-atrazine, cyanazine, metolachlor, simazine, terbutylazine, trifluraline.

Samples were extracted, cleaned up and analyzed with GC/MS (see detailed methods for chemical analyses in Appendix 1).

An extended and confirmatory screening was performed on fish muscle for 150 substances in an accredited laboratory (Phytocontrol®) (see Appendix 2 for the list of substances).

The significance of differences in the pesticide residue levels of soil, sediments, maize stems and maize seeds, from different sources, tea

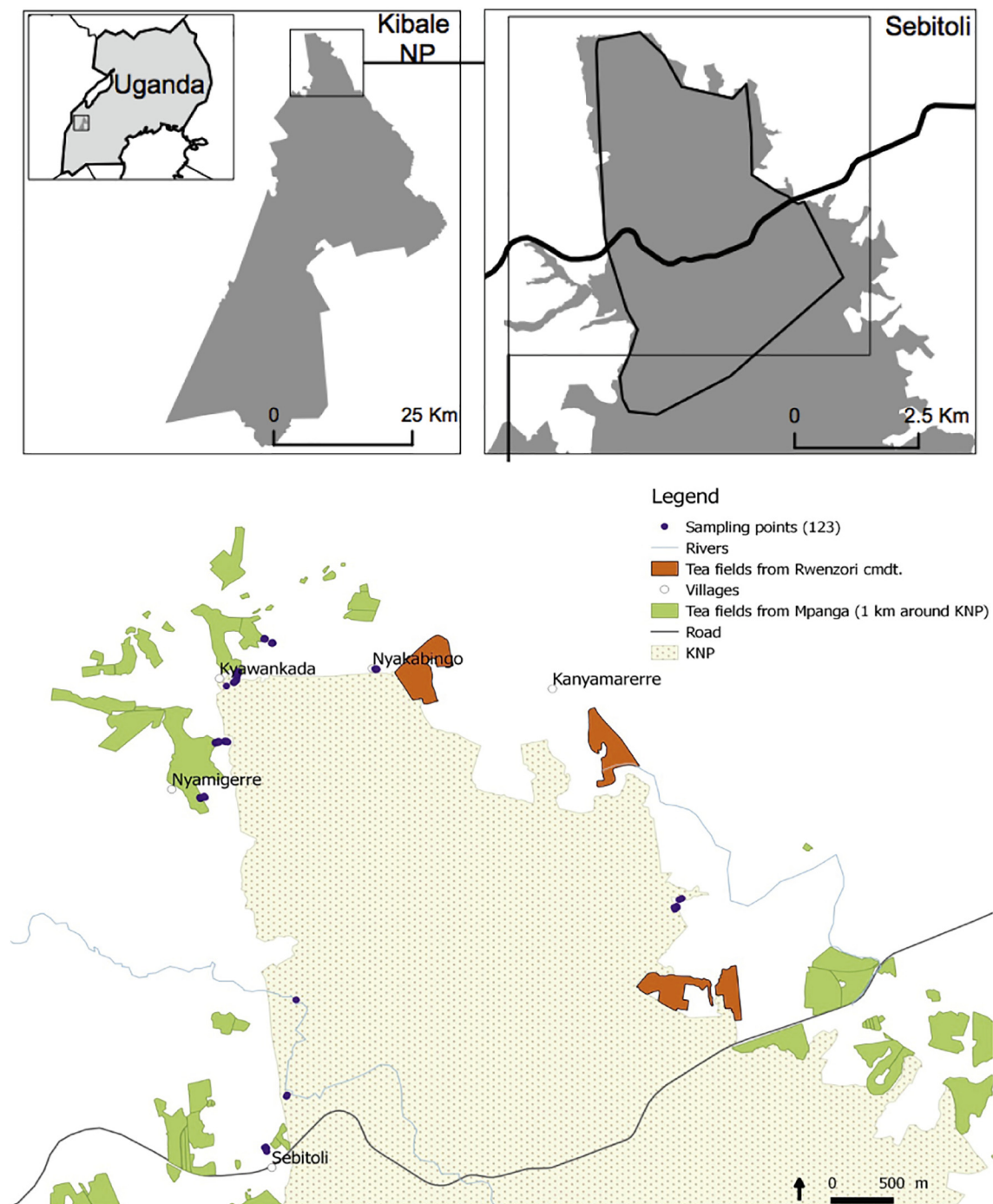


Fig. 1. Map of Sebitoli area, in the northern part of Kibale National Park, Uganda : locations of tea fields, villages and sampling locations for pesticide analyses.

garden, maize garden, bush, trench, and forest was assessed using the Student's *t*-test. Descriptive normal probability plots as described by Kutner et al. (2003) were used to verify the normality of the environmental data. A straight diagonal indicated that the data normally distributed (see Appendix 3 for representative normal probability plot).

3. Results

3.1. Types of facial anomalies recorded in NHP

3.1.1. Chimpanzees

By July 2016, we had identified 16 chimpanzees out of 66 monitored individuals with abnormal facial phenotypes: one adult female has a

cleft lip (KY), others nasal deformities: one adult male (AG), two sub-adult females (NO, SA) and two subadult males (KB, ZL), five juvenile males (KW, LV, MN, KR, PR) one juvenile female (MB), and four infant females (GA, KJ, NT, BR) (Fig. 2 and Table 1). Some of these individuals also have limb deformities (KY), and/or reproductive function problems (two females, GL and PP).

3.1.2. Baboons

Of 79 videos recorded between July 22 and August 11, 2014, the presence of baboons was detected on seven consecutive videos on July 22 and were from the same group (Fig. 3). They entered the maize garden one after the other using a small path from 17:05 to 17:08, then left the maize garden running. Of the 35 different individuals recorded, at

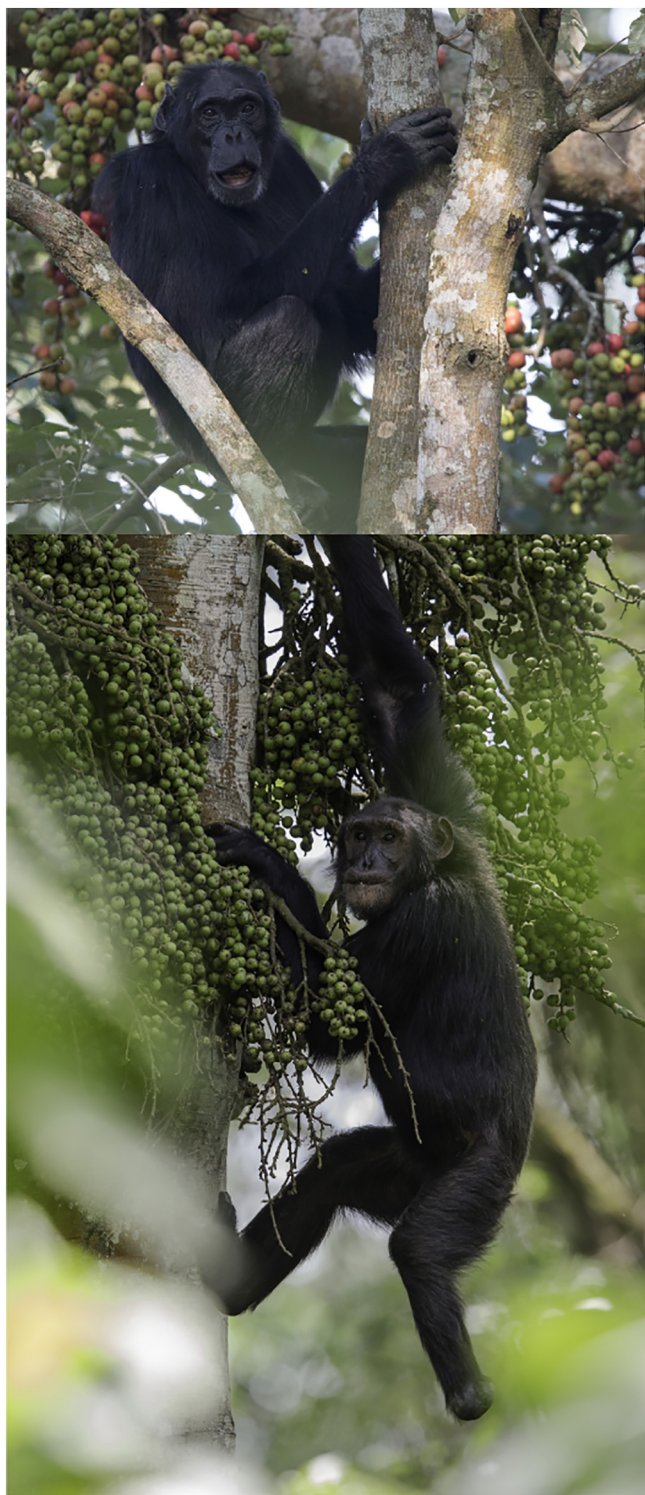


Fig. 2. Two chimpanzees with facial deformities at Sebitoli, Kibale National Park, Uganda (©Jean-Michel Krief). Upper: Kyara, adult female chimpanzee affected by cleft lip. Lower: Aragon adult male chimpanzee affected by nasal and limb deformities.

least six individuals (four adults and two immature individuals – 17%) displayed abnormal facial phenotypes. One was an adult male, two were adult females with infants having normal phenotype, one adult female with abnormal phenotype was accompanied by an immature with severe facial lesions and another immature was carried by a female without any facial deformity. Lesions varied from a simple cleft nostril to complete absence of nostril and unique aperture at the middle of the nasal bone.

3.2. Use of pesticides and herbicides around Sebitoli forest

Farmers interviewed commented that they frequently plant maize seeds from Kenya coated with an unknown red product. They also use different products for treating their crops including: mancozeb, cypermethrin, glyphosate, paraquat and 2,4 D amine. The two first products have been used regularly over the past 12 years and glyphosate has been used for more than 30 years. The two major tea factories (Mpanga Growers tea factory and Rwenzori Commodities Ltd) growing tea in the vicinity of the forest are still using glyphosate as herbicide. Farmers growing tea in the villages of Sebitoli and Kanyamarere use Paraquat (Tables S1 & S2).

The Fort Portal shops reported selling other products, but the local farmers did not report using them (Table S1). Oddly, the reported demand for chlorpyrifos, profenofos, metalaxyl-M, dimethoate, diammonium phosphate was very high or high, but none of the farmers cultivating maize or industrial growing tea mentioned using them. The shop owners also do not know the nature of the red product coating the maize seeds they sell and nothing related to the nature of product use for coating the seeds was mentioned on the label of the bag “Hybrid H520 (Kenya-seeds™)”.

3.3. Pesticides identified in samples collected in the environment of Sebitoli forest

3.3.1. Fresh maize seeds and stems, soil, and sediments

The 35 fresh samples, comprising fresh maize seeds and stems, soil, and sediments were analyzed for profenofos, dimethoate, cypermethrin, chlorpyrifos, DDT and its metabolites (*o,p'*-DDT, *p,p'*-DDT and *p,p'*-DDE). Pesticides, chlorpyrifos and DDT metabolites, were identified, confirmed, quantified, and compared to established quality assessment criteria (Fig. 2; see detailed results in Table S3). The recorded values of chlorpyrifos in maize stems and seeds were higher than MRL (Maximum Residue Limit) authorized (0.372 ± 0.363 and $0.352 \pm 0.026 \text{ mg kg}^{-1} \text{ w.w.}$, respectively). *p,p'*-DDT and *o,p'*-DDT residues were detected in maize stem samples with mean values of 0.007 ± 0.028 and $0.002 \pm 0.009 \text{ mg kg}^{-1} \text{ w.w.}$, respectively. Profenofos was not detected in seeds and stems of maize. Residue levels of ETU were below limits of detection in all the samples tested, while detectable non-quantifiable values of *p,p'*-DDD and *p,p'*-DDE were detected (qualitative analysis), though not quantified. Maize stem samples from Nyakabingo and Kyawankada, the two locations at the far north of the study area sampled tested positive for chlorpyrifos. Maize seed samples were positive for chlorpyrifos in all locations sampled (Nyakabingo, Kyawankada and also in Sebitoli). Both total DDT and chlorpyrifos levels tended to be higher in the stems than in the seeds, but no statistical difference was found between maize stems and seeds - chlorpyrifos, $df = 15$, $t_{\text{stat}} = -0.205$, $t_{\text{crit}} (2\text{-tail}, \alpha = 0.05) = 2.131$; total DDT— $df = 14$, $t_{\text{stat}} = -1$, $t_{\text{crit}} (2\text{-tail}, \alpha = 0.05) = 2.145$. Detectable non-quantifiable values of *p,p'*-DDE were found in all the soil and sediment samples. Residue levels of other the targeted chemicals were below detectable levels in all the soil and sediment samples.

3.3.2. Fish and dry coated maize seeds

Chlorpyrifos ($0.030 \pm 0.005 \text{ mg kg}^{-1} \text{ w.w.}$), *pp'*-DDE ($0.078 \pm 0.010 \text{ mg kg}^{-1} \text{ w.w.}$) and imidacloprid ($0.016 \pm 0.008 \text{ mg kg}^{-1} \text{ w.w.}$) were detected in fish from Sebitoli area, in the home range of chimpanzees while the control fish sampled from the central part of the park had not residue out of the 150 tested (Table S4, Appendix 1). Imidacloprid was also present in the coated maize seeds used by farmers in Sebitoli area at a high concentration ($460 \pm 25 \text{ mg kg}^{-1} \text{ w.w.}$) (Fig. 4).

4. Discussion

Two previous publications reported facial dysplasia affecting the nose in 10% of Sebitoli chimpanzees and presented arguments for a

Table 1

Facial deformities in baboons (recorded clips in July 22, 2014) and chimpanzees (direct observations) in the North of Sebitoli chimpanzee home range.

Species	Individual code or case (number for baboons)	Sex	Age class or estimated age in July 2016	Lesions	Relatives
Chimpanzee	AG	Male	Adult (18)	Concave midface profile, flat nose, nostrils absent, concave shape of the area of nasal spine, 3 deep notches, asymmetry of the nasal fossae, missing left foot	
Chimpanzee	SA	Female	Adult (15)	Concave midface profile, flat nose, absence of nasal wings	
Chimpanzee	KB	Male	Subadult (11)	Absence of nasal wings and round fossae of nose, exophthalmos	KT (mother): no anomalies
Chimpanzee	GA	Female	Infant (3)	Flat nose (died from unknown cause at age of 3 years in 2015)	KT (mother): no anomalies
Chimpanzee	KR	Male	Juvenile (9)	Flat nose	KL (mother): no anomalies
Chimpanzee	LV	Male	Juvenile (5)	Asymmetrical and reduced nostrils, three median notches on the nose, swollen areas both sides of the nose	LS (mother): no anomalies LK (½ brother): no anomalies
Chimpanzee	KW	Male	Juvenile (7)	Flat nose, ventral depigmentation, terminal phalanx of digit 2 and 5 of right hand missing	KE (mother): no anomalies
Chimpanzee	KJ	Female	Infant (3)	Flat nose	KE (mother): no anomalies KW (brother): flat nose
Chimpanzee	KY	Female	Adult (35)	Cleft lip, no sexual swelling, digits 2, 3 and 4 missing, 5th digit atrophied, no anogenital swelling, no copulation, no dependent offspring since at least 9 years	
Chimpanzee	ZL		Subadult (10)	Flat nose (disappeared from unknown cause in 2016)	
Chimpanzee	NO	Female	Subadult (12)	Flat nose	NK (mother): no anomalies
Chimpanzee	NT	Female	Infant (3)	Flat nose	NK (mother): no anomalies NP (½ sister): no anomalies
Chimpanzee	MN	Male	Juvenile (7)	Flat nose	Orphan
Chimpanzee	BT	Female	Infant (3)	Very flat nose	BR (mother): no anomalies
Chimpanzee	KP	Male	Juvenile (8)	Very flat nose	mother unknown
Chimpanzee	MB	Female	Juvenile (8)	Flat nose	MJ (mother): no anomalies
Chimpanzee	PP	Female	Adult (40)	no anogenital swelling, no copulation, no dependent offspring since at least 9 years	
Chimpanzee	GL	Female	Adult	Thumb missing on left hand, fingers 2 and 5 missing in right hand; no anogenital swelling, no copulation, no dependent offspring since at least 9 years	
Baboon	#1 (clip 35)	Male	Adult	Cleft right nostril	
Baboon	#2 (clip 35)	Female	Adult	Both nostrils deformed	
Baboon	#3 (clip 36)	Female	Adult	Interrupted nasal bone with unique aperture at middle nose and another aperture on the right lower side of the nose	#4 (offspring?)
Baboon	#4 (clip 36)	ND	Immature	Interrupted nasal bone, both nostrils deformed with aperture at middle nose	#3 (mother?)
Baboon	#5 (clip 36)	ND	Immature	No nasal septum	Carried by its mother who has no deformities
Baboon	#6 (clip 36)	Female	Adult	Two additional apertures near nostrils	

congenital origin (Krief et al., 2014, 2015). The results reported here not only update the situation after three more years of daily observations but also investigate other potential causes. Results show that the proportion of chimpanzees affected is much higher than previously reported, reaching 25%. We also describe for the first time similar nasal lesions in baboons in the same area. The lesions were observed in 17% of both adult and immature baboons in the group.

In neither species did the affected individuals have any infectious lesions nor any form of ulcer, or papule that might argue for a congenital origin. Hence our decision to search for environmental factors as potential causes. Chemical analysis showed that levels of pesticides in maize stems and seeds, soils, and river sediments in the vicinity of the chimpanzee territory exceed recommended limits and that fish from Sebitoli area contain at least three chemicals (chlorpyrifos, *p,p'*-DDE and imidacloprid) which are absent from control fish from the central part of the park.

Imidacloprid was also found in coated seeds that farmers plant in field bordering the primate habitat. Baboons' lesions appeared much more destructive than in chimpanzees. The etiology of this facial dysplasia in both species needs be better understood and explored and we cannot rule out the fact that different causal agents may result in quite similar symptoms or that one set of factors exacerbates another.

Facial dysplasia resulting from destructive processes with perforation or collapse of nasal septum due to yaws (*Treponema pallidum*) in chimpanzees and baboons follows a phase with lesions (Giacani and Lukehart, 2014; Rinaldi, 2008). Yaws is a suspected cause of facial dysplasia in red colobus in Kibale (Goldberg, unpublished data), but the spirochete bacterium has yet to be identified. We need to monitor this group of baboons to assess whether the facial dysplasia has a congenital origin or due to environmental factors; however, none of the individuals has the types of lesions observed in the red colobus. Confirmation of the origin of yaws is important because the World Health Organization (WHO) (2012) set a goal for yaws eradication by 2020. The disease is transmitted by direct contact with infectious lesions in humans; however, the mode of transmission between primates and humans is not established (Giacani and Lukehart, 2014). Flies can inoculate the pathogen through wounds, thus in areas where baboons and chimpanzees leave food remains in the field, risks of cross species transmission is high. Thus, if it is present in the Sebitoli and other populations of primates, it could impact the success of the WHO yaws eradication campaign, especially in areas where non-human primates feed on human crops and leave food remains that are fed on by flies that also have contact with humans. The genetic and ecological relationships between *Treponema* infections in humans and primates require clarification. However, given that two primate species exposed to chemical

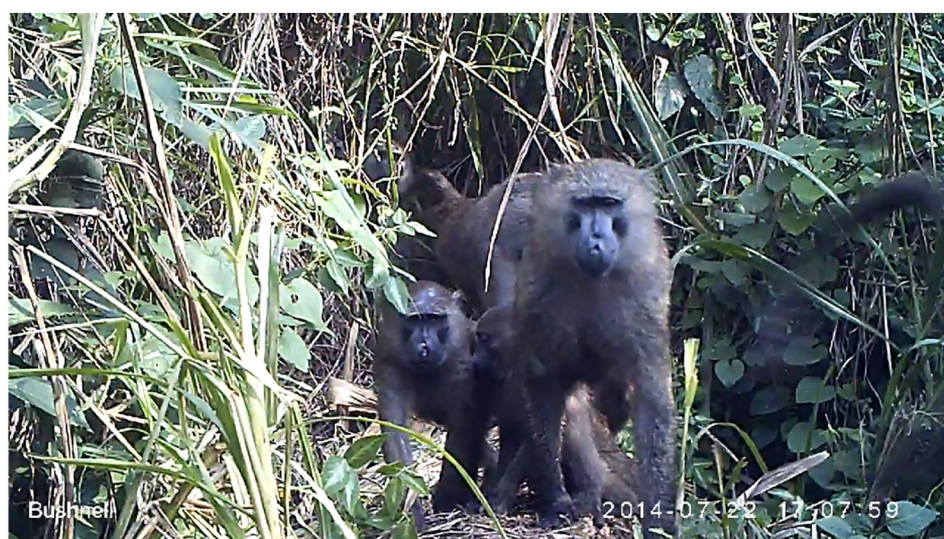


Fig. 3. Adult and immature baboons affected by nasal deformities, Sebitoli, Kibale National Park, Uganda (images extracted from camera trap ©Jean-Michel Krief/Sebitoli Chimpanzee Project).

pollutants in the same area showed deformities without infection and that such lesions were not reported elsewhere where primates are not exposed to agrochemicals, suggests that pesticide exposure may contribute to the facial dysplasia observed in both species.

At least three pesticides (chlorpyrifos, mancozeb, DDT) used in this area affect thyroid hormone (TH) signalling. TH is implicated in cranial facial organogenesis (Bronchain et al., 2017). Abnormal maternal or neonatal levels of TH have been associated with facial dysplasia, including cleft palate and lip in human new-borns (Akin et al., 2014; Kremenová et al., 1981). We detected chlorpyrifos in maize seed samples, as well as chlorpyrifos, *p,p'*-DDT, and *o,p'*-DDT in fresh maize stem samples, with chlorpyrifos being the most frequently detected

residue. Chlorpyrifos and *pp'*-DDE residues were also detected in fish collected within Sebitoli chimpanzee home range while no pesticide were detected in the fish from the control area.

DDT and its metabolites, as well as chlorpyrifos were also detected, the latter being the dominant residue in the area. However, again no farmers reported having used it. The MRL (*Maximum residue limit*) authorized by European Food Safety Authority for chlorpyrifos for human consumption is $0.01 \text{ mg} \cdot \text{kg}^{-1}$ for corn and for farm animals $3 \text{ mg} \cdot \text{kg}^{-1}$ for forage (EFSA, 2012). The acceptable daily dose for humans is $0.001 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. In June 2000, the US Environmental Protection Agency (EPA) announced a ban on the sale of the insecticide chlorpyrifos for residential use. Prenatal exposure to chlorpyrifos, an

Table 2

Chemical methodologies used to analyze the samples collected in Sebitoli area.

Chemical analyzed	Samples	Makerere University Laboratory (Uganda)	Ecotox Lyon Laboratory (France)	Phytocontrol ® (France)
Pesticides	Fresh maize stems and seeds Soil samples from tea plantation, maize gardens, abandoned fields River sediments	GC-MS (Agilent 6890N, CA, USA), & selective mass detector (Agilent 5975, inert XL Quadrupole, CA, USA) Column: HP5-MS		
Organochlorines	Fish samples Coated maize seeds		CG-ECD (Agilent 2850, CA, USA) Column: HP1 GC-MS (Agilent 6890 and 5973 CA, USA) Column: HP5-MS	
Organophosphates, pyrethroids and herbicides	Fish samples Coated maize seeds		HPLC-UV detection (VWR Lachrom Elite) Column: C18, 25 cm	
Nicotinoid insecticides	Nicotinoid insecticides			
Extended screening for herbicides, fungicides, glyphosate + AMPA (150 substances)	Fish samples Coated maize seeds			HPLC-MS/MS-internal confidential methodology-accreditation delivered by the French Committee for Accreditation (Cofrac) in accordance with the international standard ISO/IEC 17025 (Certificate No. 1-1904 rev. 6)

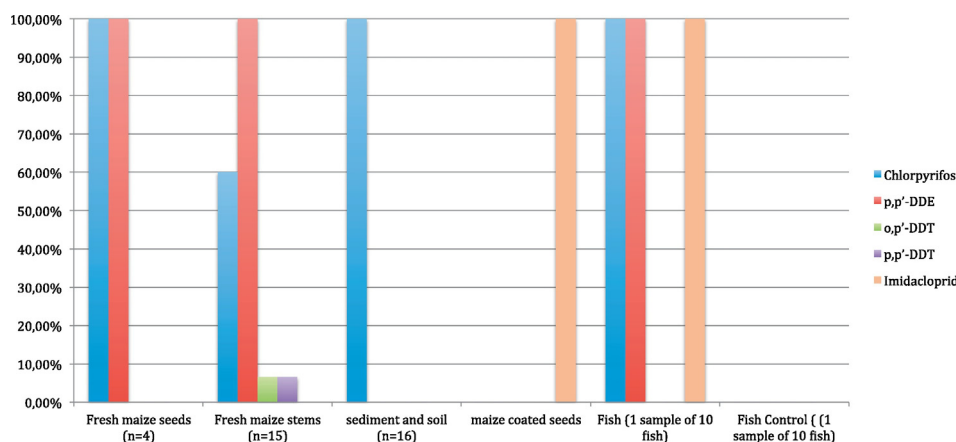


Fig. 4. Chemicals detected in soil, sediment and fresh maize samples collected at the border of the park and their frequency of detection if the samples (%). Detection of chemicals in coated seeds sold in Fort Portal and in fish from Sebitoli area and from a location in the middle of the park.

organophosphate insecticide, has been associated with neurobehavioral deficits in humans and animal models (Eskenazi et al., 1999; Rauh et al., 2006, 2012).

DDT has been associated with a variety of human health effects including reduced fertility, genital birth defects, breast cancer, and diabetes. Damage to developing brains may result from in utero DDT exposure as DDT exposure can be linked to modified TH levels (Eskenazi et al., 2009; Freire et al., 2013), and maternal TH levels are determinants of fetal neuro-development (Korevaar et al., 2016). In 2001, more than 100 countries signed the Stockholm Convention, a United Nations treaty, which sought to eliminate use of 12 persistent toxic compounds, including DDT. Under the pact, use of DDT is allowed only for controlling malaria. A worldwide ban on its agricultural use was formalized. Since then, nine nations including Uganda notified that they are continuing to use DDT. The lower detection frequency of DDT compared to its metabolites in all the matrices suggests the DDT pollution may be from past usage.

DDT and its primary breakdown product, *p-p'* dichlorodiphenyldichloro-ethylene (*p-p'*-DDE), are highly lipophilic, persistent, and bioaccumulate in humans because of their long half-lives (6 to 10 years) (Longnecker, 2005; WHO, 1989; Wolff et al., 2000). An *o,p'*-DDT/*p,p'*-DDT ratio of 0.3 in one of the maize stem samples suggests the DDT pollution is most likely from use of technical DDT and not the dicofol-type DDT (Qiu et al., 2005). A higher concentration value of the *p,p'*-DDT isomer compared to the other isomers, as implied by the *o,p'*-DDT/*p,p'*-DDT ratio of 0.3, suggests recent use of DDT in the environment. Although there was public health application of DDT for indoor spraying against mosquitoes in Uganda in 2008 (Mukasa, 2013), the recent exposure to DDT is majorly attributed to atmospheric deposition (Arinaitwe et al., 2016). Arinaitwe's study showed that there was potential for long range atmospheric transfer of contaminants including DDTs, from emission sources elsewhere, as far as the Indian Ocean, to the Lake Victoria watershed.

It would be necessary to quantify the DDT metabolites and determine the [*p,p'*-DDT]/[DDT metabolite] ratios to rule out continued illegal use of DDT, a restricted chemical in the study area.

Imidacloprid was detected on coated seeds and also in fish from Sebitoli. Contamination of fish is clearly related to water contamination, but also likely contamination of wild plants which are growing close to the source of the contamination as well as aquatic and terrestrial insects on which the fish feed. It is most likely that chimpanzees are contaminated via the food ingested, rather than by direct pesticide spraying. The region is mountainous and rainfall is abundant leading to rapid groundwater runoff. In addition, they feed on leaves, fruits, bark of trees and on stems of terrestrial herbaceous vegetation growing on the riverbank and in swamps.

Imidacloprid is neither considered to be a reproductive toxicant, nor to alter thyroid signalling pathway (EFSA, 2008, 2015). However, some recent reviews suggest that imidacloprid may decrease sperm production, pregnancy rates, or even increase premature birth or embryo death (Gibbons et al., 2015). These effects are also described in the European Risk Assessment Report, but are associated with maternal toxicity. Similarly, there are some short-term toxicity studies indicating a potential for thyroid follicle atrophy in rats (EFSA, 2013) with a NOAEL (No Observed Adverse Effect Level) of $7.8 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. The presence of imidacloprid residues in fish collected in Sebitoli area while absent from fish from control area is consistent with its use as a coating treatment of maize and its long environmental persistence (EFSA, 2013).

The current excessive use of multiple pesticides could lead to cocktail effects in the Sebitoli chimpanzee home range and in the baboon territory. Such contamination from past use or neighboring areas may contribute to the facial dysplasia in the two primate species studied through effects on TH disruption. The reproductive dysfunctions of chimpanzees may also implicate imidacloprid exposure. Wildlife, including primates, living in the central part of Kibale are less exposed to pesticides as confirmed by the absence of contamination of the fish sampled there. Baboons and chimpanzees from these areas do not have lesions, suggesting that a detailed survey of pesticide use and potential results throughout all of Kibale would provide correlative evidence to address potential impacts of these agricultural practices.

Interviews of farmers and tea growers revealed that they are not recording the chemicals nor their treatment protocols. In consequence, both villagers and consumers cannot be aware if they are exposed to these potentially dangerous products.

None of the known mothers of dysplastic chimpanzees were affected, while up to two dependents from a same mother are affected (KJ and KW from KE, NT and NO from NK; KB and KG from KT). AG is affected but according to genetic analysis (Krief, unpublished data), his half siblings (RO and NE) are not. Such findings suggest adverse effects of environmental chemicals during gestation as opposed to genetic findings.

In 2013, the European Food Safety Authority (EFSA) reported that 101 out of 287 pesticides in current usage could affect thyroid signalling. As TH is an endocrine signal, chemicals that affect TH signalling are endocrine disruptors. In 2012, WHO defined an endocrine disrupting chemical (EDC) as "an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function". Embryonic or prenatal exposure to EDCs has been associated with numerous diseases and dysfunctions in offspring and adulthood in wildlife and in human populations. Notably chemical interference with thyroid signalling affects brain development in humans. Numerous TH disrupting chemicals have been documented

as increasing risk of neurodevelopmental disorders and IQ loss (Berbel et al., 2014; Jacobson and Jacobson, 1996).

Iodine is a major component of TH. In plants grown in deficient soils, iodine concentrations may be as low as 10 µg/kg of dry weight, compared with about 1 mg kg⁻¹ in plants from iodine-sufficient soils. Iodine-deficient soils are common in inland regions, mountainous areas and places with frequent flooding like Sebitoli. Even though cretinism is for most societies a disease of the past, historical data are available on severely hypothyroid patients (including cretins) who suffered from facial dysplasia with saddle-nose deformity due to a complete lack of TH and/or iodine during development (Smith, 1894 cited in Morvan-Dubois et al., 2013), i.e., the same phenotypic deformities as seen in Sebitoli chimpanzees. Additionally, iodine deficiencies are still a major public health problem throughout Africa and especially in Uganda. Actually, endemic goiters in sub-Saharan Africa were first described in 1891 among Bantu-speaking populations living in the Semliki River Valley on the western base of the Rwenzori Mountains, within a hundred kilometers of our study area (Ehrenkranz et al., 2011). A 1999 survey of goiter prevalence revealed that the frequency of thyroid enlargement had increased in a number of districts along Uganda's western border (Bimenya et al., 2002). And in 2011, a survey of neonatal Thyroid Stimulating Hormone (an indicator of thyroid status) showed that presumptive iodine deficiency persists in human populations in western Uganda, including the Kibale region (Ehrenkranz et al., 2011). Goiters have not been observed in chimpanzees or baboons in Sebitoli. Folate levels have also been associated with abnormal cranio-facial development, including cleft lip/palate via endocrine regulation and especially thyroid (Colleran et al., 2003; Prescott and Malcolm, 2002; Sittig et al., 2012). A final point is that TH has recently been shown to be involved in neural crest cell migration and cranio-facial defects (Bronchain et al., 2017).

The fact that chimpanzees from the other regions of Kibale are not affected by the deformities suggests that either iodine or folate deficiencies may be aggravating factors, but not the principle factor explaining the observed malformations in chimpanzees and baboons. In many human diseases for which we are currently observing major increases in incidence (autism spectrum disorder, metabolic disease, etc.), both prenatal exposure to pollution and genetic susceptibilities are considered to be key factors.

These findings bolster the possibility that thyroid disruption by pesticides could be implicated in our observations, though we cannot rule out the possibility that others factors could contribute to the dysplasia. Besides iodine and folate just referred to, retinoic pathways disruption may be implicated. Retinoic acid signalling is essential for normal skeleton development could contribute to facial dysplasia (Vieux-Rochas et al., 2007). It is important to stress that the two sets of nuclear receptors, TH receptors (TRs) and retinoic acid receptors (RARs) share the same heterodimeric partner, RXR (9 cis acid retinoic receptor). Thus disruption of one or other signal could be implicated in the cranio-facial dysplasias observed. However, as yet, less is known about perturbation of the retinoic acid signally by pesticides and other endocrine disruptors, whereas numerous pesticides can disrupt thyroid signalling.

5. Conclusions

The excess levels of DDT, DDE, chlorpyrifos, and imidacloprid found in the vicinity of Kibale National Park where two primate species show facial deformities are cause for alarm. Another disquieting finding is that farmers do not report the use of these products and thus are not aware of exposing themselves and their families to these dangerous products. Although the causal nature of the relationship between pesticides and deformities remains to be verified, there is reason for concern. Future research will include pesticide and thyroid hormone analyses in urine samples of chimpanzees. However, sampling urine in wild chimpanzees is not an easy task as the analysis requires quite large volumes and at best will provide evidence for punctual or acute exposure and not provide insight into chronic exposition. Since two species of primates are

affected, inbreeding is unlikely as a main factor contributing to the expression of these traits, but it could be a co-factor exacerbating deleterious effects. We are unaware of any study that has examined the birth defects in connection with pesticide use in the human population in this region, but this should be an important next step in the study of environmental pesticide exposure. Human activity and its impact on wildlife, notably in the form of increasingly intensive agriculture and uncontrolled use of pesticides, require closer monitoring. Finally, this agriculture/forest ecotone represents a form of land usage that is increasingly dominant in tropical forest landscapes.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2017.04.113>.

Acknowledgements

We are grateful to Uganda Wildlife Authority and Uganda National Council for Science and Technology for giving us the authorization to conduct research in Kibale National Park, Uganda. We deeply thank J. M. Krief co-director of Sebitoli Chimpanzee Project, N.F. Nsaben, coordinator of SCP and field assistants for their work in KNP, particularly E. Balinda, D. Kyomuhangi, J. Alinaitwe and N. Kwezi, we are indebted to K. Arinaitwe for support and supervision provided to F. Gumisiriza in the laboratory. Field trips and pesticide analysis were granted by the ATM 2014–2016 of the National Museum of Natural History, Projet pour la Conservation des Grands Singes and the Fondation Prince Albert II. There is no conflict of interest for this publication and the funders had no role in study design, data collection, analysis and interpretation, preparation of the manuscript and decision to submit this publication.

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