REPORT ON THE ILLEGAL IMPORTATION OF MEAT, INCLUDING BUSHMEAT, SEIZED AT ZAVENTEM AIRPORT - 2017/2018

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SUMMARY

The hunting for and consumption of bushmeat is traditional and vital for many communities around the world; it provides food and income as well as holding traditional value. Historically subsistence hunting was sustainable, however increased demand, improved access to forests and more efficient methods of hunting are resulting in unsustainable offtakes of wildlife. The growth in human population and ease with which people can move around the globe are causing an increase in demand, within range countries and internationally. Hunting unsustainably has the potential to cause a species to go extinct, locally or globally. The decrease or complete loss of a population has wider impacts on the ecosystem and so the people who depend on it.

The international bushmeat trade is not fully understood and as such, it is unknown what impact this may be having on wildlife populations. This study aimed to gain a better understanding of the international bushmeat trade by estimating an average monthly weight of bushmeat being imported and determining which species are predominantly involved. Working with customs officers at Brussels airport, flights from Sub-Saharan Africa were targeted and all passengers' luggage searched for both bushmeat and domestic meat (livestock). Visual identification, radiographs and genetic analysis were conducted to determine the species involved and any further information such as the age of the animal and hunting method used. Using the information of bushmeat seized and an estimate of the number of people entering Brussels from West and Central Africa each month, it was estimated that an average of 3.7 tonnes of bushmeat was being brought through Brussels airport each month. A range of species were identified, some of which were CITES listed. Some suggestions are made in order to reduce this importation by raising awareness on penalties and better enforcing those penalties. Besides, reinforcement of routine customs controls and more random schedules for specific actions of reinforced controls should be favoured by adequate budgets, allowing also a good, reiterated information and sensitization of custom's officers. It would be justified that European budgets should be accorded for customs controls to Member States that are main and specific entry gates on the EU and its market. This would also allow the raising and presence of sniffling dogs to detect meat and

other illegal products in passengers' luggage, and the use of mini-technical devices to analyse DNA sequences on the spot.

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INTRODUCTION

Bushmeat (or wild meat) is meat derived from wildlife and includes all wild, terrestrial or semi-terrestrial species (Hoffman and Cawthorn 2012). Primates, pangolins, antelopes, rodents and reptiles, among many other taxa are commonly involved in the bushmeat trade and as such CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) listed and non-CITES listed species are involved. Bushmeat is hunted and consumed worldwide, particularly across Latin America, Asia and Africa (Abernethy et al. 2013; Nasi et al. 2008). The illegal wildlife trade, of which bushmeat makes up just a part, is one of the most lucrative black-markets and is a major threat to wildlife populations (Ngoc and Wyatt 2013).

The international carriage of uncertified meat and fish products is illegal for sanitary reasons under national, European Union, and International Air Transport Association regulations. For example, the European Union prohibits any personal consignment of meat, or meat products, from entering the Union unless specifically authorized and certified as being eligible for import (EC Regulation 745/2004 of 16 April 2004). In addition, international trade in many wild species and their products is prohibited or regulated for conservation reasons under the CITES which is an international and legally binding agreement between governments. Its aim is to ensure that international trade in specimens of wild animals and plants does not threaten their survival. The species covered by CITES are listed in three appendices, according to the degree of protection they need. Trade in specimens of species listed in appendix 1 or 2 can be, respectively, permitted only in exceptional or controlled circumstances, while species listed in the appendix 3 may be imported into or exported (or re-exported) from a State party to the Convention only if the appropriate document has been obtained and presented for clearance at the port of entry or exit.

For some people in rural areas bushmeat provides vital protein and fat and essential micronutrients to a diet that is otherwise high in carbohydrates from crops (Golden et al. 2011; Nasi et al. 2008; Wilkie and Carpenter 1999). Often in these areas there is no substitute for bushmeat, therefore without it people may develop nutrient deficiencies (Golden et al. 2011; Wilkie and Carpenter 1999). Iron deficiency is one possibility of this and a study conducted in Madagascar showed a link between quantity of bushmeat in the diet and haemoglobin levels (Golden et al. 2011). Golden et al (2011), found children with higher quantities of bushmeat in their diet had higher levels of haemoglobin, whilst restricting access to wildlife resources increased the risk of anaemia to the population. The harvest and trade provide not only with a source of nutrition but also provides several different parties such as hunters, traders, restaurant owners and agriculturists with a source of income (Loibooki et al. 2002; Wilkie and Carpenter 1999). The consumption of bushmeat, along with the use of other animal by-products is not only an essential part of the diet but is often deeply rooted in many cultures.

Wildlife has been hunted for millennia, however as the human population increases and advances in infrastructure and weaponry continue, we are reaching unsustainable levels of harvesting, therefore threatening the existence of many wildlife populations (Chaber et al. 2010; Brodie et al. 2009; Gaubert et al. 2015; Milner-Gulland, Bennett, and others 2003; Pailler 2007). The rise in the human population is occurring in both urban and rural areas; whilst for rural communities bushmeat is a necessity, for urban communities it is a delicacy, causing an increase demand so increasing the hunting pressure. Some of this increase is attributed to increased employment opportunities within forest areas, as a result of logging, mining and other industries (Nasi et al. 2008; Willcox and Nambu 2007).

The rise in industry in remote areas, as well as contributing to the rise in human numbers, is resulting in increased construction of roads, railways and other infrastructure (Brashares et al. 2004; Nasi et al. 2008). Not only does this contribute to deforestation and fragmentation but also provides improved access to previously remote areas of the forest (Brashares et al. 2004). This improves hunter chances of coming into contact with wildlife whilst also restricting wildlife movement (Brashares et al. 2004). These improved transport networks connect rural to urban populations, who are then able to travel further, enabling bushmeat harvests to be easily transported (Brashares et al. 2004). While bushmeat is an essential and potentially the only source of protein in rural areas, it is quickly becoming a symbol of social status and wealth in urban areas, with people willing to pay high prices for it. The frequency and ease with which humans travel has resulted in an international bushmeat market, with people wishing to continue parts of their culture as well as being considered a delicacy, where people are willing to pay high prices (Chaber et al. 2010; Falk et al. 2013). While policies and laws are in place in range countries that prevent the unsustainable use of natural resources and the illegal trade of them, the enforcement of these is often insufficient (Nasi et al. 2008).

Therefore, the gain of selling bushmeat is higher than the potential costs (Loibooki et al. 2002; Ogden, Dawnay, and McEwing 2009).

As the rate of harvesting rises, wildlife populations are unable to reproduce fast enough to maintain the population numbers (Nasi et al. 2008). Advances in hunting practices has widened the range of species able to be targeted as well as increasing the likelihood of a successful hunt, therefore decreasing the sustainability (Brodie et al. 2009; Nasi et al. 2008; Pailler 2007). Overhunting has the potential to cause local or global extinction of the targeted species, but it may also affect; non-target species, species interactions, ecosystem structure and function (Brodie et al. 2009). A large proportion of bushmeat hunting comprises frugivorous mammals; being mid trophic level alterations to these populations can impact the entire ecosystem (Abernethy et al. 2013; Brodie et al. 2009). A reduction in prey species results in less food available for predators and increasing inter and intra species competition (Mbotiji and others 2002). Targeting apex predators or large herbivores can affect species lower down the trophic levels; populations may be allowed to boom when released from competition or predation pressures and potentially altering the areas ecology (Abernethy et al. 2013). In tropical forests 70-90% of trees and shrubs depend on animal-mediated seed dispersal, therefore the removal of animals such as frugivorous primates impacts seedling recruitment and plant regeneration, composition, density and diversity of plant species (Beaune et al. 2013; Brodie et al. 2009).

The potential cascading effects on the ecosystem are a growing concern but the threat to individual species has received more focus (Brodie et al. 2009). Hunters will often aim for individuals with large body size, therefore they target adults over juveniles and males over females which can distort the age sex ratios (Kümpel 2006). Due to the removal of adults, annual population recruitment is lowered, and the social structure of the species may be disrupted; male biased harvests will be disruptive to territorial, monogamous species but will have little impact on polygynous groups (Bennett and Robinson 2000; Kümpel 2006). The targeting of large-bodied individuals promotes the reproduction of small-bodied individuals and so may reduce the average body size of the species (Bennett and Robinson 2000). Some evidence also exists that behaviour is altering in order to avoid hunters e.g. duikers are diurnal, solitary animals but are becoming more active at night and found in pairs (Kümpel 2006).

The trade in wildlife and wildlife products has the ability to eliminate populations and often remains unnoticed until it is too late (Wasser et al. 2004). The international demand for bushmeat may be exacerbating unsustainable levels of hunting, this is of particular concern for species that are listed by CITES in order to restrict international trade for conservation reasons (Chaber et al. 2010). The amount of bushmeat entering Europe is largely unknown, although a couple of recent studies conducted in Paris and Switzerland have estimated weekly imports of bushmeat at 5.25 tonnes and 165kg respectively (Chaber et al. 2010; Falk et al. 2013). Taxa seized in previous studies were primates, rodents, crocodiles, pangolins and antelopes, several of which are CITES appendix 1 or 2 listed (Chaber et al. 2010; Falk et al. 2013).

Genetic analysis can determine species, populations and relationships between individuals and has been successfully used to assist with conservation law enforcement (Ogden, Dawnay, and McEwing 2009). Species identification by genetic analysis is the most commonly used wildlife DNA forensics and has been used in cases of illegal poaching and trading of products such as traditional medicines and shark fins (Ogden, Dawnay, and McEwing 2009). Identification of a species using DNA is usually done by amplifying and sequencing a potion of the genome using PCR (polymerase chain reaction) and then comparing it to known sequences in databases such as GenBank (Baker, 2008; Ogden et al, 2009). Another technique for species identification is to create a phylogenetic tree; if the unknown sample lies within a cluster of sequences that all refer to one species then it is assumed to be that species (Baker 2008; Ogden, Dawnay, and McEwing 2009). Initial studies using mitochondrial DNA fragment analysis in African markets showed to be a successful tool to genetically identify the species being sold (Malisa et al. 2006; Ntie et al. 2010). Determining geographic origin is more difficult as DNA analysis looks to identify the specimen's reproductive population of origin; this requires the different populations to be genetically distinct and an extensive database (Ogden, Dawnay, and McEwing 2009; Wasser et al. 2015). In some cases, geographical origin of a specimen can be determined by spatially mapping microsatellites and mtDNA (mitochondrial DNA) sequences and matching unknown samples to the closest location; this has been successful in determining the origin of chimpanzees and poached ivory, as well as identifying between Chinese sika deer subspecies (Ghobrial et al. 2010; Wasser et al. 2015; Wu et al. 2005).

Through the analysis of seizures of illegal meat at Belgian airports, this study aims to estimate:

- risks to the conservation of nature in Africa by: genetically identifying the species involved in this trafficking, determining their CITES status and age of the animals hunted (adults / juveniles) and, when possible, tracing their provenance through phylogeography and biogeography.

- risks to Belgian biodiversity by identifying invasive alien species carried by or with illegally transported meat.

- the nature, scale and driving forces of this trafficking.

METHODS

Collection of Samples from Brussels Airport

Meat was being collected during 3 main types of control: BACON actions, routine customs controls and the Leaking Luggage screening.

BACON (for « Baggage Controls ») actions

The Federal Agency for the Safety of the Food Chain (FASFC) and customs officials determine set dates to perform passenger baggage checks at Brussels-National airports (Zaventem). Inspectors from DG Environment of the Federal Public Service Health, Food Chain Safety and Environment (FPS Health) were closely involved in Bacon actions. The aim is to monitor compliance with the rules on the import of meat, plants, plant and animal products and living animals, including protected species. The actions occurred between the hours of 5 am and 9 am, when most flights from Africa land, during this time all passengers were stopped, and their luggage searched.

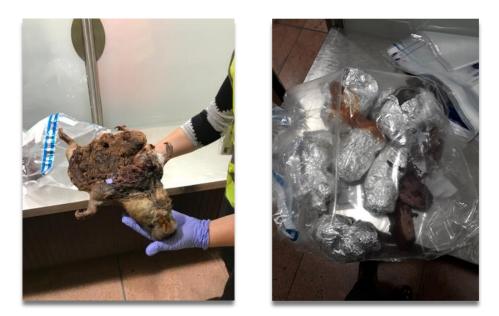


Plate 1. Bushmeat seized during a BACON action - Photo courtesy: FPS Health.

When possible, information recorded at the airport was, for the *i*th port of origin, number of passengers in the flights from this destination (c_i), number of passengers checked (n_i), and the weight of bushmeat carried by the *j*th passenger (k_{ij}). The total estimated weight of fish or meat imported during this period (Jan 2017 to Oct 2018) for a given country is given by:

$K_i = c_i \sum k_{ij} / n_i$

and the total weight imported across all of the routes searched is the sum of the countryspecific weights.

Routine customs controls

Targeted or opportunistic seizures from custom officers including the anti-drugs group were also collected since January 2017.



Plate 2. Flyer from the Belgium customs' airports news.

Leaking Luggage

Passengers who are getting a connecting flight through Brussels airport often have their luggage transferred directly between aircrafts for them. If when the luggage is transferred it appears that there is something leaking from it, the bag is held in order to be searched. These luggage's are stored into a refrigerated container and searched in average once per month by Brussel's airport services.



Plate 3: Leaking luggage screened, and meat seized. Photo courtesy: Dr. Veronique Renault.

All meat found in baggage from Africa during BACON, leaking luggage or routine customs controls was sealed in plastic bags, numbered and put in a sealed plastic drum (specifically labelled for the bushmeat project) for transport to the Faculty of Veterinary Medicine at the University of Liege. The seizures were then visually inspected in the university' necropsy room under strict biosecurity measures. Seizures with bones were radiographed, bushmeat and undetermined meat were sampled for genetic analysis, and raw or well-preserved meat were samples for future pathogens' analysis (figure 1).

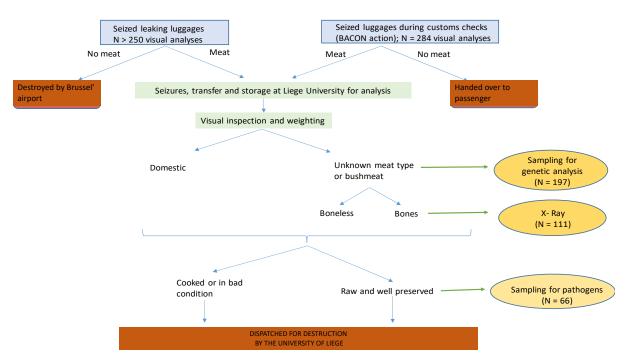


Figure 1. Diagram illustrating the meat selection and sampling process.

Species Determination

The tissue samples were taken for DNA analysis at the Faculty of Veterinary Medicine at the University of Liège and sent, in 80% ethanol, to Institut des Sciences de l'Evolution de Montpellier (ISEM) and later Laboratoire Evolution & Diversité Biologique (EDB) at the Université Paul Sabatier Toulouse III by DHL carrier, both laboratories performed the same genetic analysis following the same protocols.

A total of 197 samples were DNA-extracted using KingFisher Flex (Thermo Fisher +Scientific); this technique uses magnetic beads to extract DNA of 96 samples per capture (Suomalainen, 2009). Following Olayemi et al (2011) and Gaubert et al (2015), we PCR-amplified and sequenced four mitochondrial genes; cytochrome *b* (Cyt b), cytochrome c oxidase I (COI) and ribosomal subunits 12S and 16S, using 'universal' mammalian primers specifically designed for the barcoding of bushmeat (Gaubert et al. 2015). The PCR products were directly sequenced on the ABI 3500XL 24 capillary sequencer of the ISEM molecular biology platform ("Genotyping and Sequencing" - LabEx CEMEB, University of Montpellier, France; <u>http://www.labex-cemeb.org/genseq-genotypage-sequencage</u>). The sequences from the 197 genetic samples (197 x 4 sequence reactions) were cleaned and aligned using the BIOEDIT software (Hall, 1999). The sequences were submitted into DNABUSHMEAT

(Gaubert et al, 2015) and GenBank to identify the species. In brief, sequence identification was based on a tree/similarity approach combined to expert curation of the Genbank database (Gaubert et al., 2015). Sequence identification was considered verified whenever a majority of species attribution among the four genes was reached.

Radiographs

All specimens seized that contained bones were x-rayed at the Medical Imaging Department of the Faculty of Veterinary Medicine at the University of Liège using a direct x-ray machine with a flat sensor (Digivex, Medex, Belgium - 120kV / 300mA). The specimens were kept in sealed plastic bags for biosafety and the equipment was fully disinfected after use. The radiographs were examined by a specialist veterinary radiologist (Valeria Busoni, DMV, PhD, DipECVDI; *Associate Professor in Veterinary Diagnostic Imaging*), to identify anatomical region of the specimen, note whether the animal was an adult or juvenile by looking at whether the growth cartilages were completely or partially opened, identify any bullets present and any other signs of interest such as fractures as a result of trapping.

Illegal meat volume arriving at Brussels airport in passenger's luggage

In order to estimate the average weight of bushmeat being carried in flights from each origin, flight origin, weight of domestic meat and bushmeat (kg) seized was recorded during each of the BACON actions. The total number of flights and passengers from selected countries of origin to Brussels airport was provided by the Belgium Federal Public Service Mobility and Transport (Table 3). This, along with the average weight of bushmeat being carried by each passenger bringing meat illegally from each selected country, allows to estimate the average weight of bushmeat arriving at Brussels airport in passenger's luggage for the study period. The percentage of passengers bringing bushmeat (number of passengers bringing bushmeat versus number of passengers checked) was determined on the following BACON actions 16/5/2017, 30/5/2017, 3/6/2017, 20/6/2017, 4/7/2017 and 1/8/2017 (when members of the scientific team were present to record those data).

Preliminary research on Socio-anthropological aspects of the driving forces and organization of the bushmeat trade and consumption in Belgium.

In 2017, observations were conducted in restaurants, markets and supermarkets of the city of Brussels. During these observations, we initiated casual discussion with several informants. Conducting formal interviews appeared irrelevant at that point, as informants are not very comfortable when it comes to formally discuss the topic of bushmeat.

RESULTS

Illegal meat volume arriving at Brussels airport in passenger's luggage

During the BACON, routine customs controls from 1/1/2017 to 02/10/2018, a total of 839 kg of meat and associated products (such as milk, cheese, insect larvae, etc) from 284 seizures from all countries were seized. A total of 173 (687 kg) seizures equivalent to 82% of the volume seized came from Africa. Meat seized per passenger per flight route were recorded to estimate the total rates of bushmeat import. The mean of the volume of livestock (table 1) and bushmeat seized per passenger carrying bushmeat per country (table 1, figure 2 & 3) were calculated which, combined with the percentage of passengers carrying bushmeat in the controlled flights and with the total number of passengers from selected countries of origin to Brussels airport from January 2017 to Oct 2018 (table 2), allowed us to estimated total rates of import, arriving from African departure points at Brussels Zaventem airport (table 3). Meat seized from non-African countries were not included in this study.

Country of Origin	Ν	Mean	SD	Min	Median	Max
Benin	2	6.00	1.41	5.0	6.00	7.00
Burkina Faso	4	4.13	2.66	1.0	4.25	7.00
Cameroon	11	3.11	2.45	0.2	2.50	7.50
Congo	11	4.61	4.80	0.7	4.00	17.00
Ivory Coast	3	4.67	2.47	3.0	3.50	7.50
Rwanda	11	4.90	9.04	0.6	2.25	32.0
Senegal	4	1.16	0.76	0.3	1.20	2.00
Тодо	8	3.45	2.00	1.5	3.00	7.00
Uganda	1	13.00	NA	13.0	13.00	13.00

Table 1. Mean, Number of seizures (N), Standard Deviation (SD), Minimum (Min), Maximum (Max) of the volume of domestic meat in kg seized per passenger carrying domestic meat per country.

NA: Not Available

Country of Origin	Ν	Mean	SD	Min	Median	Max
Burkina Faso	2	1.35	0.21	1.20	1.35	1.50
Burundi	1	4.00	NA	4.00	4.00	4.00
Cameroon	9	3.66	3.91	1.00	2.00	13.00
Congo	19	1.93	1.68	0.60	1.00	6.60
Ethiopia	1	3.50	NA	3.50	3.50	3.50
Ivory Coast	5	2.83	2.42	1.00	1.50	6.80
Nigeria	1	4.00	NA	4.00	4.00	4.00
Senegal	1	2.00	NA	2.00	2.00	2.00
South Africa	1	0.45	NA	0.45	0.45	0.45
Togo	5	5.64	4.47	1.20	6.50	12.00

Table 2. Mean, Number of seizures (N), Standard Deviation (SD), Minimum (Min), Maximum (Max) of the volume of bushmeat in kg seized per passenger carrying bushmeat per country.

NA: Not Available

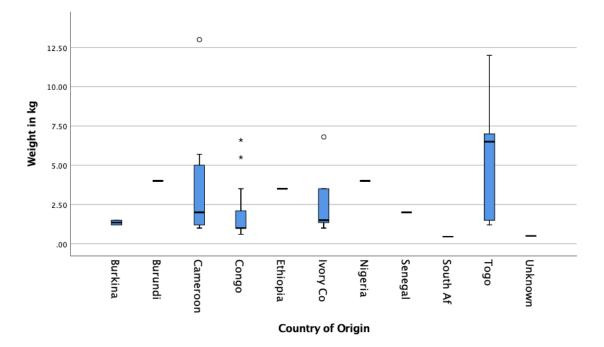


Figure 2. Box plot representation of the volume of bushmeat carried by passengers from targeted African countries.

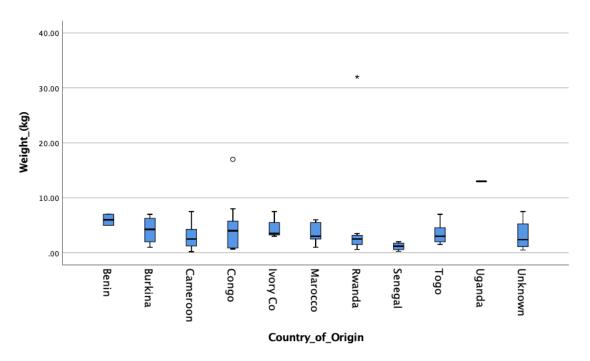


Figure 3. Box plot representation of the volume of domestic meat carried by passengers from targeted African countries.

	Number of flights	
Origin Airport (Country)	(Jan 17 – Oct 18)	Total Passengers on board
Abidjan (Iv. Coast)	350	86021
Accra (Ghana)	5	357
Addis Abeba (Ethiopia)	607	68002
Bamako (Mali)	4	16
Banjul (Gambia)	427	104404
Brazzaville (Republic of the		
Congo)	7	8
Bujumbura (Burundi)	88	18779
Cape Town (South Africa)	2	401
Conakry (Guinea)	275	69896
Cotonou (Benin)	268	62628
Dakar-Blaise Diagne (Senegal)	4	840
Dakar-Int'l (Senegal)	16	3808
Douala (Cameroon)	6	1468
Entebbe (Uganda)	237	51905
Freetown (Sierra Leone)	303	64039
Johannesburg (South Africa)	5	404
Kigali (Rwanda)	425	80317
Kilimanjaro (Tanzania)	1	10

Kinshasa (DRC)	303	61772
Libreville (Gabon)	2	111
Lomé (Togo)	378	94062
Luanda (Angola)	236	47015
Lusaka (Zambia)	1	11
Mahé (Seychelles)	2	6
Monrovia (Liberia)	60	11533
N'Djamena (Tchad)	2	48
Niamey (Niger)	2	40
Nouakchott (Mauritania)	1	30
Ouagadougou (Burkina Faso)	8	1174
Port Harcourt (Nigeria)	1	6
Windhoek (Namibia)	1	2
Yaoundé (Cameroon)	538	135041
Zanzibar (Tanzania)	26	4519
Total	4887	1013754

Table 3. Total number of flights and passengers from selected countries of origin to Brussels airport from January 2017 to Oct 2018 (Belgium Federal Public Service Mobility and Transport).

							Estimated volume in Kg
	Number of passengers carrying	Number of		Estimation of the number of passengers		Std. Deviation of	imported from January 2017 to October 2018
	bushmeat	passengers	of	1 0	carried per		(95%
		checked during the given		bushmeat from Jan 2017	· ·		confidence interval)
Country	0	U			bushmeat	passenger	intervar)
							46,61
Burkina Faso	1	34	2.94%	34,53	1,35	0,21	(+/- 7.32)
Burundi	1	25	4.00%	751,16	4,00		3.004,64
Cameroon	2	41	4.88%	6658,98	3,66	3,91	24.342,55
							8.510,86
DRCongo	3	42	7.14%	4.412,29	1,93	1,68	(+/- 7.417,98)
Ethiopia	1	20	5.00%	3.400,10	3,50	•	11.900,35
							12.171,97
Ivory Coast	1	20	5.00%	4.301,05	2,83	2,42	(+/-10.429,06)

Nigeria		1	25	4.00%	NA	4,00	•	NA
South Africa	NA	NA		NA	NA	0,45	•	NA
Senegal	NA	NA		NA	NA	2,00	•	NA
								20.404,22
Togo		3	78	3.85%	3.617,77	5,64	4,47	(+/- 16.168,24
								80.381,20
								(+/-
								60.036,69)
TOTAL								

Table 4. Summary of the rates of bushmeat, and estimated total rates of import, arriving from African departure points at Brussels Zaventem airport over the study period.

*: The percentage of passengers bringing bushmeat (number of passengers bringing bushmeat versus number of passengers checked) was determined on the following BACON actions 16/5/17, 30/5/17, 3/6/2017, 20/6/2017, 4/7/2017 and 1/8/17.

NA: Not Available

Nota bene: The volume bushmeat imported from Cameroon doesn't follow a normal distribution (non-Gaussian distribution) and thus the 95% confidence interval cannot be calculated.

It is estimated that on a monthly basis 3.653,7 kg of bushmeat land in Brussels airport (80.38,20 kg over 22 months) from those countries. It is important to note that all passengers landing in Brussels don't always exit in Brussels and therefore our calculation is an estimate of the volume of bushmeat entering Europe via Brussels airport.

Species Determination

In total, there were 105 samples taken from leaking luggage, 84 from passengers and 8 from unknown source. Among those, 81 were a priori identified as livestock, 75 as wild game and 41 did not have any identification. Travelers supplied 17 identification hypotheses (ca. 9% of the cases), whereas customs / researchers post-processing the meat supplied 113 (ca. 57%) identification hypotheses. In terms of origin, Cameroon (53), DR Congo (21) and Togo (13) were the most represented countries; 76 samples (ca. 39%) had no traced origin.

The 197 samples extracted on the KingFisher showed heterogeneous DNA qualities. Absorption ratios (260/280), reflecting the quality of the DNA, did not vary significantly between samples from leaking luggage and passengers (Wilcoxon-Mann-Whitney test; Z-Score = -0.01576; *p*-value = 0.98404). However, samples from passengers yielded significantly higher DNA concentrations than leaking luggage (Z-Score = -2.74147; *p*-value = 0.00614; Fig. 4).

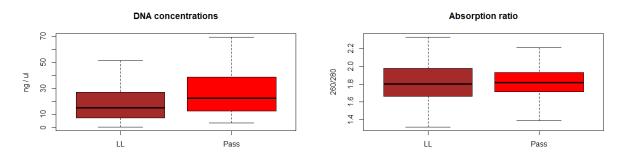


Figure 4. Box plot representation of the DNA concentrations and absorption ratios (260/280) from tissue samples taken from leaking luggage (LL) and passengers (Pass).

The DNA extracts were in their majority successfully sequenced for the four genes (ca. 73%), allowing full congruence analysis. Respectively ca. 18%, 9% and <1.0% of the samples were sequenced for three, two and one gene (see Appendix 1). In 32 cases (ca. 16%), there was at least one incongruence in the species identifications derived from the different genes sequenced for a given sample. Overall, 13 samples (ca. 7%) could not be resolved taxonomically because of a too high level of incongruence among gene identifications. The rest of the samples were confidently identified to the species-level (ca. 90%) or the genus-level (ca. 3%). We could not distinguish among several congeneric species for the following taxa: 4 duikers (*Cephalophus* spp.), 1 monkey (*Chlorocebus* spp.) and 1 cane rat (*Thryonomys* sp.).

Our results showed that there was a certain level of wrong a priori (morphologybased) identifications within the meat categories "Livestock" and "Wild", with 5 out of 81 (ca. 6%) of livestock samples turning out to be wild species after DNA sequencing, and 11 out of 75 (ca. 15%) of the wild samples actually belonging to livestock. Taking into consideration the more specific a priori identifications given by customs / researchers (e.g. Antelope, Beef, Pangolin, etc.), the overall rate of false identification reached ca. 26%. As a consequence, the original declared/supposed balance between Livestock, Wild and unattributed samples, which represented 81, 75 and 41 samples respectively, was reshuffled by the genetic identification, the final spectrum of seized meat actually totalizing 109 Livestock, 75 Wild and 13 unsolved samples (Fig. 5).



Figure 5. Spectrum of meat categories estimated from morphological identification (left) and DNA-typing (right). U = Unknown attribution.

DNA-typing yielded a precise spectrum of the "meat" diversity seized at the Brussels' airport, showing notably that:

(i) Artiodactyla were the dominant taxon (122 samples), followed by Rodentia (26)

(ii) 7 other Orders were minimally represented: Pholidota (12), Primates (7), Lagomorpha

(1), Squamata (4), Testudines (2), Crocodilia (4), Galliformes (5) and Perciformes (1) (Fig.6)

(iii) a majority of samples (109; ca. 55%) represented Livestock (Fig. 5)

(iv) within Livestock, the domestic cattle (*Bos taurus*) was dominant (ca. 44%), followed by the domestic goat (ca. 26%) and the domestic pig (ca. 18%) (Fig. 7)

(v) within the Wild category, Rodentia were dominant (ca. 35%), followed by Artiodactyla (ca. 27%) (Fig. 8)

(vi) within the Wild category, three species represented almost half of the seizures: the African brush-tailed porcupine (*Atherurus africanus*; 12), the greater cane rat (*Thryonomys swinderianus*; 10) and the African common pangolin (*Manis tricuspis*; 12) (Fig. 9)

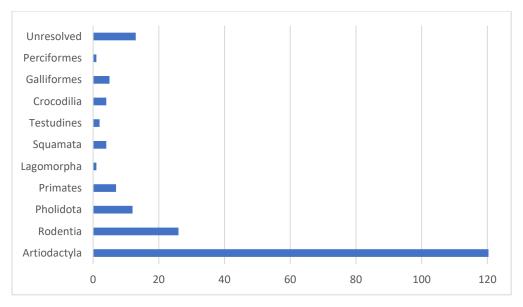


Figure 6. Representation (in number of genetic samples) of the 9 Orders of vertebrates seized at the Brussels' airport.

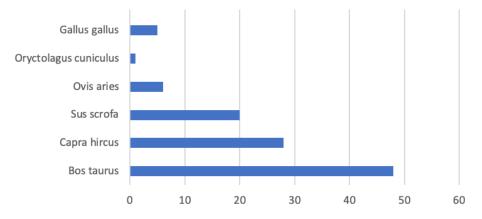


Figure 7. Representation (in number of genetic samples) of the species / taxonomic diversity within the Livestock category.

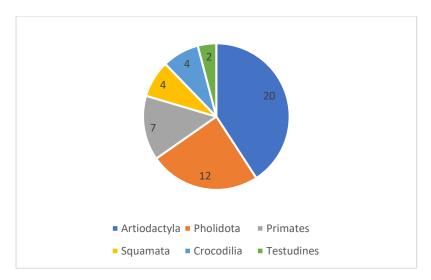


Figure 8. Representation (in number of genetic samples) of the ordinal diversity within the Wild category.

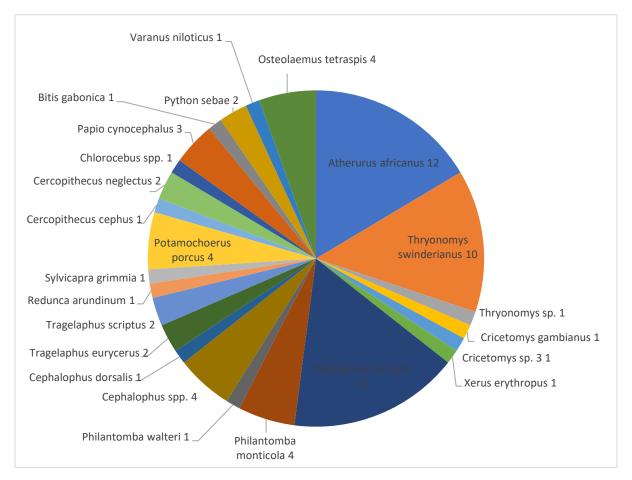


Figure 9. Representation (in number of genetic samples) of the species / taxonomic diversity within the Wild category.

In fine, DNA-typing identified 33 taxa in the meat seized at the Brussels' airport, 29 of which being traceable to the species level. The wild game or bushmeat was represented by 7 Orders of vertebrates and 26 species / taxa, including:

(i) Artiodactyla (9 species / taxa: Cephalophus dorsalis, Cephalophus spp., Philantomba monticola, P. walteri, Sylvicapra grimmia, Tragelaphus eurycerus, T. scriptus, Redunca arundinum, Potamochoerus porcus)

(ii) Rodentia (6 species / taxa: Atherurus, africanus, Thryonomys swinderianus, Thryonomys sp., Cricetomys gambianus, Cricetomys sp. 3, Xerus erythropus)

(iii) Pholidota (1 species: Manis tricuspis)

(iv) Primates (3 species / taxa: Papio cynocephalus, Cercopithecus neglectus, Chlorocebus spp.)

(v) Squamata (3 species: Bitis gabonica, Python sebae, Varanus niloticus)

(vi) Crocodilia (1 species: Osteolaemus tetraspis)

(vii) Testudines (2 species: Pelusios chapini, P. gabonensis)

Within the bushmeat seized at the Brussels' airport, 10 species were CITES-listed:

- Bay duiker Cephalophus dorsalis: Appendix II. One sample from unknown origin.

- Blue duiker *Philantomba monticola* : Appendix II. Four samples, including 3 from Cameroon and 1 from unknown origin.

- African common pangolin *Manis tricuspis*: Appendix I. Twelve samples, including 6 from Cameroon and 6 with unknown origin.

- Yellow baboon Papio cynocephalus: Appendix II. Three samples from Ethiopia.

- Moustached monkey Cercopithecus cephus: Appendix II. One sample from Cameroon.

- De Brazza's Monkey *Cercopithecus neglectus*: Appendix II. Two samples, including 1 from DR Congo and 1 with unknown origin.

- Grivet monkeys Chlorocebus spp.: Appendix II. One sample from DR Congo.

- African rock python *Python sebae*: Appendix II. Two samples, including 1 from DR Congo and 1 from Cameroon.

- Nile monitor Varanus niloticus: Appendix II. One sample from Cameroon.

- African dwarf crocodile *Osteolaemus tetraspis:* Appendix I. Four samples, including 2 from DR Congo and 2 from Cameroon.

Tracing the geographic origin of the seized samples was possible, at the subregional scale, in 12 cases (representing 6 species / taxa):

- Manis tricuspis: West Africa (1 sample) and West Central Africa (11)
- Cercopithecus cephus: West Central Africa (1)
- Cricetomys gambianus: West Africa (savannah) (1)
- Cricetomys sp. 3: West Central Africa (1)
- Philantomba walteri: Dahomey Gap (1)

Except for *P. tricuspis*, the restriction to a sub-region was made possible by the restricted range of the species.

Radiography

111 x-rays were taken from which 94 were relevant. 30% and 56% of the animals radiographed were adults and juveniles respectively, while 14% couldn't been determined. 19% of the samples x-rayed contained bullets. Most of the pangolins (8 out of 12) and rodents (29 out of 38) were juveniles while all primates were adults.

Ballistic

Ballistic expertise was done by Sylvain Dujardin (National Institute of Criminology & Forensic Science, Belgium), Samuel Kalpers (ULg) and Melanie Melnik (ULg) who recovered some pellets in one pangolin (plate 4) and eight pellets in an antelope leg (plate 5).

The projectiles are lead balls similar to the projectiles used in Europe for the hunting of small game (hare, duck, etc). However, it is impossible to give more precision on the weapon used. This type of projectile can be found in almost all calibres used in weapons smooth. It can only be assumed that the weapons used is similar to the picture presented in pictures 1 & 2.

The shots in the antelope leg were unlikely lethal. The majority of shots were stopped by bones without breaking them. The shot was either a weak shot or from a distance, it is nevertheless not possible to conclude as both the type of weapon and ammunition used are unknowns. It can be noted that the common calibres for this type of projectiles range from 410 to 12. The cartridges of these calibres are available in different "lengths / power" from 2 inches (5.08 cm) to 3 inches (7.62 cm).

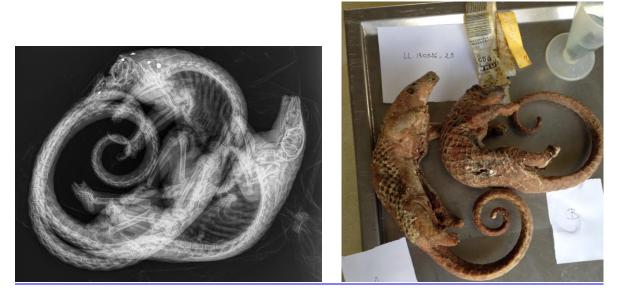


Plate 4. Pangolin' picture and x-ray.

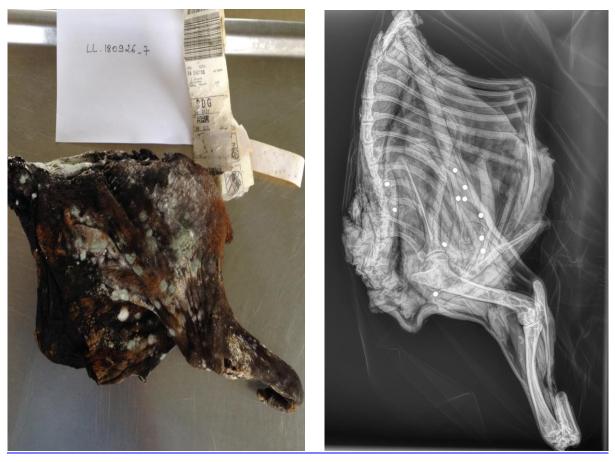


Plate 5. Antelope leg' picture and x-ray.



Picture 1. Pellets recovered from an antelope leg



Picture 2. Pellets recovered from a pangolin



Plate 6. of the type of firearm frequently used by African village hunters (<u>www.vrt.be/vrtnws/nl/2018/0/03/pano-bushmeat</u> - Accessed on the 16/10/2018)

Larvae/ insects collected on bushmeat seizures

Two larvaes and two insects were collected from meat samples and sent to the laboratory of functional and evolutionary entomology (Gembloux Agro-Bio Tech, University of Liege) and observed by Dr. Rudy Caparros Megido and Professeur Frédéric Francis. After observation of the specimens B00818 and 280217 through binocular microscopic. It appears that they are larvae and adults belonging to the order Coleoptera, family Dermestidae, and more specifically individuals of the species: *Dermestes frischii*. The larvae of this species are recognizable by a large amount of hair and are found in different types of stored food (wheat, rice, various cereals, raisins or peanuts, kibble for dogs or cats or dried meats) or on animal tissues containing certain proportion of wool, fur or silk. Larvae are rather necrophagous and normally feed on the carcasses of other animals, mainly insects. This genus is distributed worldwide. The condition of the two insects collected did not allow identification of those specimens.

Collection of samples for future pathogens' detection

A total of 66 samples were achieved and stored at -80°C for a future detection of pathogens by metagenomic analysis.

Preliminary research on Socio-anthropological aspects of the driving forces and organization of the bushmeat trade and consumption in Belgium

One of the first things to consider when investigating the bushmeat trade is the way we introduce ourselves to the informants in the field. Ethically speaking, an anthropologist can't act as an undercover journalist pretending to be someone he isn't. As the actors of the bushmeat trade (importers, vendors, consumers, etc.) are not very inclined to talk freely about their activities, especially if the interlocutor doesn't appear to be a potential customer, entering the field can be a complicated issue. The chosen approach for these preliminary observations in African restaurants (N=2), markets (N=3) and supermarkets (N=5) of the city of Brussels was to introduce the researcher as an anthropologist conducting a study on "food habits in the African diaspora of Belgium", with a special interest in the consumption of imported African commodities. Even if it allowed us to access information about some of the

driving forces of bushmeat consumption, the extent and organization of the bushmeat traffic couldn't be discussed.

In order to get solid, reliable data in the future (especially on the trade itself and its organization), Dr. Melodie Dieudonné recommends a real immersion of the investigator in the field (as a worker on the markets, employee in restaurants, etc.), not undercovered but as a stated anthropologist.

DISCUSSION

Illegal meat volume arriving at Brussels airport in passenger's luggage

The bushmeat trade within Africa has been well documented. However, the international trade of bushmeat has only recently begun to be investigated. This study provides an average estimate of the amount of illegal bushmeat entering Belgium each year on commercial flights from West and Central Africa. Although the estimated annual import of bushmeat is less than half that of domestic meat imports, this study estimates that 3.653,7 kg of bushmeat are entering Europe through Brussels airport every month. The main countries of origin for the importation of bushmeat was found to be Cameroon, Togo, Ivory Coast and the Democratic Republic of Congo. Previous studies conducted in France and Switzerland also found Cameroon to be a large contributor along with Ghana, Central African Republic and the Democratic Republic of Congo (Chaber et al, 2010; Falk et al, 2013).

It is unknown whether the bushmeat seized was intended for personal consumption or commercial purposes. Comparing these results with similar studies; the mean weight of bushmeat per passenger appears to be more to that found in Switzerland but less than was seen in France (Chaber et al, 2010; Falk et al, 2013). Chaber et al (2010), suggested that bushmeat is being imported into Paris for commercial purposes due to the high weight per consignment. From the data collected at Brussels, it could be suggested that the bushmeat is being imported for personal consumption as the average weight seized per passenger carrying bushmeat was 2.0 kg -10 times less than that seized in Paris. The average weight of domestic meat seized per passenger carrying such meat was 3.4 kg. Whilst the comparably low average of bushmeat entering Brussels leans itself towards personal consumption, the high price of bushmeat means that even importing small amounts would be worthwhile for commercial purposes. This study does not present any market information, but it is known that bushmeat can be purchased in a Sunday market behind Brussels train station and, if prices are similar to Paris, one kg of bushmeat may fetch between 20 and 30 euros (Chaber et al, 2010).

Not only is the financial reward for importing bushmeat high, but the penalties if caught are low and rarely enforced (Chaber et al, 2010). In Belgium the penalties for transporting a CITES listed species without the appropriate certificates are: a fine of 26-50,000EUR and potential imprisonment for 6 months to 5 years (Art 5, CITES law, 1981). The likelihood of these

penalties being enforced are low; in order for prosecution, evidence of the species is required (which is not always obvious at first glance with bushmeat, like illustrated by our study and its false "first view" identifications) and meat seized at airports is normally immediately aimed at being incinerated like required by European legislation (Chaber et al, 2010; EC Regulation 745/2004 of 16 April 2004). From the time spent with the customs officers, it is clear that more training is required in all aspects of CITES (this was requested by some of them) in order to provide them with the knowledge to properly enforce violation to the CITES. It was also evident that some officers were more invested than others, this is due to: lack of interest (through lack of information), risk to their health and the fact that bushmeat is unpleasant to handle. It is however important to note that in Belgium, Customs officers are legally capable to issue a notice for infringements to the CITES legislation, but they cannot impose a fine (only a tribunal or the FPS Health if the parquet doesn't prosecute). Fines with regard to infringement concerning the introduction of meat into the EU can only be imposed by FASFC. At this point, there is no strong message to passengers who contravene the law by bringing meat into Belgium. This issue could be tackled by systematically applying administrative sanctions (fines) that normally apply under any Belgian relevant legislation like FASFC legislation or CITES legislation in order not to leave the infringement unpunished (which sends otherwise a wrong message of little importance of the infringement, and encouragement to try it again). In parallel, Parquets need to better inform and prosecute bushmeat importation.

Radiography

Due to their body size (and so amount of meat) adult animals should be targeted for bushmeat over juveniles, however previous studies as well as this one found a large proportion of bushmeat to be juveniles (Chaber, unpublished; Fa et al, 2000; Kumpel, 2006). This could be because: the demand for bushmeat is high enough that it is worthwhile for a hunter to target an individual that may yield less meat than another; the populations have become depleted to the point where juveniles are prominent; or because traditional selective hunting methods are being lost and juveniles are easier to catch (Fa et al, 2000; Kumpel, 2008). As people move in and out of rural areas, traditional hunting techniques passed through families are being lost (Willcox & Nambu, 2007). Traditional traps are set to be species and size specific and people would naturally monitor populations and change their targets depending on abundance, but this is being lost and replaced with non-selective snares and guns (Bennett & Robinson, 2000; Nasi et al, 2008; Willcox & Nambu, 2007). Bullets or debris were found in some of the carcasses

show that guns, among other methods, are used; the shift from trapping to shooting has made hunting more efficient and also allowed for a wider range of species to be hunted (Bennett & Robinson, 2000; Kumpel, 2008). It is important to note that the fact that only 17.7% of the samples x-rayed contained bullets does not mean that the rest (82.3%) of the animals were not killed by bullets (due to the particular technique used to butcher animals). From a ballistic point of view, it seems weapons used are no war machine but rather local/traditional weapons. In addition, risk assessment should be performed to assess the risk of the presence of bullets or debris in the carcasses on human health.

Species Determination

DNA-typing proved critical in determining the taxonomic identity of the meat seized at the Brussels' airport. Many samples did not have a precise ID (e.g. "Sausage", "Livestock", "Meat"; 32 samples) or were literally without ID (Unknown; 41 samples). After DNA-typing, 93% of the samples had their identification reaching the species- or genus-level, which is much higher than a previous study surveying bushmeat across 5 African countries (Gaubert et al. 2015), possibly because most of the species transiting to Brussels were common, well-studied species, registered in DNA databases.

To some extent, DNA-typing also allowed narrowing the origin of the samples without flight attribution to a sub-region in Africa (e.g. one giant pouched rat and several African common pangolins from West Central Africa), although this task was complicated by the fact that (i) DNA registers for African mammal species are still poorly represented and (ii) some unresolved taxonomic debates affecting several bushmeat taxa (notably small antelopes and monkeys) do not allow for a clear taxonomic identification, and in turn for their geographic traceability (Kingdon 2013). DNA-typing would have also been a useful tool for inferring movements of meat between countries of origin (Baker 2008), accurate taxonomy and / or geographic assignment (which was possible in some cases) allowing to check whether a given species or population is actually present in the country of origin. So far, all the species / populations identified were present in the country of origin of the flights, thus not suggesting –but not refuting– trans-national movements of meat before departure to Europe. Unfortunately, the important level of missing data regarding the origin of the flights that affected our dataset did not allow for an exhaustive assessment of this point.

Despite a great level of heterogeneity in terms of DNA quantity and quality between extractions from leaking luggage's and passengers' bags, our 4-gene PCR-based method proved robust to any type of potential DNA degradation (rotten meat, cooked meat, smoked meat), further extending the spectrum of meat type the method is able to cover (Gaubert et al. 2015). It is likely that the short-sized fragments targeted (400-650 bp), the mammalian-specific primers designed, and the organelle used (mitogenome, present in high quantity in the mammalian tissues), played a favourable role here.

The 4-gene approach was critical by providing multiple assessment of species ID ("multi-barcoding"), especially when working on such blind series of data where pictures of the seized meat were generally lacking (or did not allow for a precise identification because of the processing of the carcasses). Indeed, the frequent absence of photographs of the seized meat and the technical separation between the DNA-processing step and the meat seizure-sampling processing chain (i.e. from seizures to sampling) would have misled a single gene approach, still frequently used in DNA barcoding and wildlife trade surveys (Janjua et al. 2017). For instance, 19 COI-based identifications (COI being used as the "universal" barcode for living organisms) proved wrong when compared to the identifications derived from the other genes, possibly because of COI-primers binding preferences for a given taxon when DNA extracts were contaminated by exogenous DNA (a most plausible case as we know that several pieces of meat were conditioned together before sampling). In addition, 30 samples had their genetic identifications in conflict with the a priori morphological identifications (confusion occurred between a rat and a duiker, for instance). In a few cases, the 4 genes identified different species within a single DNA extract (e.g. one "Rodent" was traced to Atherurus africanus using 12S and 16S genes, whereas COI assigned it to Capra hircus, and cyt b did not work). Again, the lack of access to the meat-processing chain was a limitation in diagnosing the relevant issues concerning the genetic identifications, even more since we cannot exclude in every case crosscontamination among the wells of the plates where DNA extracts were stored.

The dominance of domestic cattle (*Bos taurus*) in the Livestock samples is at odds with what is known of the consumption of domestic meat in sub-Saharan Africa, where the consumption of small livestock (notably chicken) seems to prevail (Albrechtsen et al. 2005). Such results could indicate a bias towards beef as the favoured domestic meat to bring from Africa to Europe, and further investigations on the practices related to African beef consumption in Europe –where beef is largely available– could enlighten the motives behind such trend.

On the other hand, the wild meat seized at Brussels' airport showed a taxonomic spectrum quite representative of the bushmeat species commonly found on African markets. Indeed, Rodentia and Artiodactyla were dominant, followed by Pholidota and Primates, which

is a typical trend observable on the African bushmeat markets (Codjia & Assogbadjo 2004, Petrozzi et al. 2016). The presence of a fair level of African common pangolins (16% of the wild meat) is both a reflexion of the traditional presence of the species on the market stalls (Boakye et al. 2016), but could also mirrors a more global, recent trend towards targeting pangolins as a valuable meat item (Mambeya et al. 2018). Having said that, the overall taxonomic spectrum of the wild meat seized in Brussels, which was congruent with the patterns found on African market stalls, could support the idea that the transportation of wild meat from Africa to Europe, using domestic flights, is not done with the purpose of feeding the circuit of a parallel and organized, lucrative market. Rather, people just come with what they like to eat and what they think will please their family and friends. Clearly, wildlife items of high value (rhino and elephant horns, pangolin scales) may take other, organized circuits to reach Europe (Heinrich et al. 2016).

Our study revealed the seizure of 10 CITES-listed species (on Appendices I and II), none of which had been a priori identified on morphological grounds, supporting further the decisive input of DNA-typing in tracing the wildlife trade. The number of CITES-listed samples (31) represented ca. 16% of all the DNA-typed samples, and ca. 41% of the Wild samples. This latter value is similar to the percentage of CITES seizures found in a previous study made at the Roissy airport (39%; Chaber et al. 2010).

Given the large error rate of morphological identification notably related to the processing of the transported meat, and the significant contribution of DNA-typing in reaching species-level ID, it is our belief that the molecular tracing of the bushmeat trade should be regularly implemented in conjunction with seizure actions. Additional, comparative studies including more seizures and controlling through all the points of the meat processing chain (i.e. from seizure to sampling) should allow strengthening the approach that we have implemented here.

While international trade is small relative to in-country trade, the significant volumes reported here, coupled with the presence of species listed in both Appendices 1 and 2, suggest that the issue should be of immediate concern to CITES. In addition, several species not listed on the CITES appendices might not have the resilience to sustain heavy hunting level. They come on the CITES lists when they are actually endangered. Their protection should start before that state.

Anthropological study

Preliminary research on Socio-anthropological aspects of the driving forces and organization of the bushmeat trade and consumption in Belgium:

"Bushmeat definition"

During this short investigation, it was found that the very term "bushmeat" appeared to cover a great diversity of meanings depending on the actors questioned. As scientists interested in this topic, we have a quite common definition of bushmeat, but this definition is definitely not the same depending on where people stand (hunter, carrier, vendor, consumer, law enforcement, scientist, wildlife officer, etc.).

There is a plurality of definitions, and this plurality has to be explored for many reasons. By understanding how people conceive and define bushmeat, we can better understand how they perceive the traffic, its importance, the threat it represents, etc. According to Dr. M. Dieudonné, one of the first things we have to do is to explore, describe and analyze the different and various conceptions of bushmeat in presence. This can also be a way of adapting our behavior to the "types of informants" we interview.

In the field, we met people who conceive bushmeat as simple "wild meat" – versus "domestic meat" – without considering the continent of origin or the CITES status (a Belgian boar or deer thus enters that kind of definition). Others perceive bushmeat as literally "meat from the bush, from the forest", this is the case for many people living in African forest areas, but also many African immigrants. Others will consider bushmeat only as protected wild species, etc.

These differences of definition also influence the reliability of the information shared by the actors. As the importation of African meat in passengers' s luggage is illegal in Belgium, using the word "bushmeat" on the markets can result in the mutism of the informants that will get suspicious and/or frightened. Adapting our vocabulary to the conceptions of the stakeholders is an important step in the collect of reliable data.

It is crucial to understand and document these differences of conception and definition (what it represents, what it means for the different stakeholders) in order to have a clear comprehension of the bushmeat realities.

Reasons for consumption

The few people who accepted to discuss their bushmeat consumption were a good example of the topic's complexity. It would be a mistake to only focus on the "cultural aspects" of this consumption, to think that only people of African origin consume it and that they mainly do it for "cultural" reasons. The reasons for bushmeat consumption of course have some cultural basis, but by paying too much attention to those "cultural habits", the danger is to miss some very important determinants to the bushmeat trade and consumption. Social aspects, micropolitical aspects, taste preferences (that are not necessarily cultural), nostalgia and homesickness, taste for new experiences amongst Europeans and people of African origin, etc. are just a few examples of factors influencing the demand (and inevitably the offer) for bushmeat in Europe. It can appear as a simple "vocabulary issue", but the implications can be important. Paying a close attention to political, economic and social aspects of the bushmeat demand and consumption is therefore mandatory.

In 2018, Brussels Airport initiated a study on "Sensitization of African travelers" with a qualitative exploration, with the aim to influence current travelers' behavior lead by Frank Geers, MaResCon. This research team interviewed 16 African passengers to better understand why people do import those goods and how to sensitize the travellers in order to change current behaviour. Their key results outlined below are in total agreement with our findings:

- No difference in findings when it comes to age, gender, country of origin, education, profession
- There is a very profound connection with the home country
- All food from the home country is so much better. There is an overwhelming belief that the quality, the taste ... is superior to the European products
- Export and import are partly a kind of business model to pay the travel expenses
- The import of meat, fish vegetables ... must be huge (15 out of the 16 participants were active importers)

- There is an awareness of rules and policies, but the specific knowledge is rather poor. A lot of confusion
- The African traveller is reluctant to search for information (the less one knows the better)
- Customs Control is a calculated risk: you win some, you lose some

Table 5. Key learnings - Brussels Airport – Sensitization Africa travellers from Frank Geers.

As already discovered during the study done in France and this current study in Belgium, the meat is generally transported in suitcase (well packed: anti-leak - anti odour) while the legal goods (fruits, dried fish, vegetables, prepared meals) are mainly transported in cool box. More intense control will be needed to increase the chance of being caught and minimise the 'lottery effect. More effort should also be focusing on informing the passengers beforehand of what is prohibited and what is not.

Sanitary risks

Sanitary risks were not investigated in this study but 66 samples were collected for future analyses. However, several studies have presented bushmeat as a potential source for zoonotic viruses (Bachand et al, 2012; Kilonzo et al, 2013; Smith et al, 2012). Non-human primates illegally imported into the United States were found to be carrying retrovirus and herpesvirus DNA, other examples include; monkeypox, ebola and henipavirus (Mann et al, 2015; Smith et al, 2012; Weiss et al, 2012). For many of these the transmission, particularly from human to human, is poorly understood making predictions of spread difficult (Smith et al, 2012). Aside from the threats posed by bushmeat, the illegal importation of domestic meat has the potential to threaten Europes' agriculture and so economy. Diseases such as; foot and mouth disease, African swine fever, classical swine fever and swine vesicular disease, have the potential to enter Europe through illegally imported meat (Pharo & Cobb, 2011; Wooldridge et al, 2006). Outbreaks of these have the potential to threaten health as well as individual's income and countries economy (Wooldridge et al, 2006). Awareness campaign should be promoted on these risks. Recent unexpected event of ASF in Belgium reinforce the usefulness of these campaigns. In addition, within this project, samples for pathogen detection were collected and stored for future analyses.

STUDY LIMITATIONS AND RECOMMENDATIONS

- Targeted flights for the Bacon Actions were not randomized (often same day of the week, never during the WE, due to necessity of organization of the apparently insufficient number of available custom's officers; so some flights may be over-valued while others could be under-estimated. Randomization of flights will be crucial for any further study and for a real picture of the situation. Usual traders may rapidly notice and take benefit of this non-randomization.
- Passengers in transit, cargo and postal services were not investigated. The meat coming via train was also not assessed while several key flights are combining flights and trains journeys. For instance, flights from Nigeria to Brussels often involve a train trip from Paris or Amsterdam (picture 3). Passengers leaving Sub-Saharian Africa may transit through countries (of the Arabian Peninsula, for example) from which the flights were not considered in this study. Any future studies should include other trade routes.

	9:52 AM – 8:05 PM AccesRail, KLM · Operated by Thalys for Acce	10h 13m Flight + Train	2 stops ZWE, AMS	
	7:17 AM – 8:05 PM Air France, KLM · Operated by SNCF	12h 48m Flight + Train	2 stops CDG, AMS	
AF	10:33 AM – 8:30 PM Air France · Operated by SNCF	9h 57m Flight + Train	1 stop 2h 24m CDG	
->	7:40 AM – 5:25 PM British Airways	9h 45m BRU-LOS	1 stop 1h 55m LHR	

Picture 3. Screenshot of the flight option to travel from Nigeria to Brussels.

- The number of BACON actions was not consistent over the entire study period: in 2017, 17 Bacon actions were considered for the study (starting from February till December) which corresponds to 2 actions per month¹; while in 2018, Bacon actions were reduced to one action per month at the Brussels airport at the request of FASFC (9 Bacon actions took place from January till September 2018). This limited number of flights checked is a source of uncertainty which can only be solved by checking more flights. The FASFC and the custom authorities might be inclined to do if illegal meat trade was

becoming a priority. A disease risk analysis linked to this illegal trade might raise the importance given to this issue.

- Information such as nationality of passengers and origin or connecting flights were not always recorded and/or transmitted to the scientific team which is an important limitation to this study. In addition, information recorded were sometimes inaccurate or inexact. A reliable and consistent system to record information need to be designed in collaboration with both the custom authorities and the FASFC to ensure proper record keeping. Regular training for customs officers should be organised to facilitate their work by making the inspection and collection of samples more operational and simpler.
- No one could unfortunately fill up the questionnaire designed to record specific information on passengers and meat imported. The customs didn't have the human capacity to do so and it wasn't part of the FASFC' duties.
- A considerable of non-targeted items such as fish and vegetables were stored in the bushmeat containers which negatively impacted on the scientific team workload.
- Contact persons in both the FASFC and the DG environment changed over the study period which lead to coordination difficulties.
- The genetic identification of species would benefit, notably for the possibly crosscontaminated samples, from a high-throughput sequencing approach (HTS) such as shotgun sequencing, allowing to decipher the different mitogenomes present in single DNA extracts, as expected from the levels of incongruences observed among the four genes for a number of samples.
- More education concerning CITES and Bushmeat is needed by and requested for by the majority of the customs officers.
- Staff capacity is a major issue (this doesn't only apply to Customs but also to other services concerned). Adding to this, most flights from Africa arrive between 5am and 9am while night shift consists of 2 or 3 officers who generally change shifts at 7am, resulting in a reduced surveillance at the time when arrivals from Africa peak.

LIST OF FIGURES AND TABLES

Plate 1. Bushmeat seized during a BACON action - Photo courtesy: FPS Food Health.

Plate 2. Flyer from the Belgium customs' airports news.

Plate 3. Leaking luggage screened, and meat seized. Photo courtesy: Dr. Veronique Renault. Figure 1. Diagram illustrating the meat selection and sampling process.

Table 1. Mean, Standard Deviation (SD), Minimum (Min), Maximum (Max) of the volume of domestic meat in kg seized per passenger carrying domestic meat per country.

Table 2. Mean, Standard Deviation (SD), Minimum (Min), Maximum (Max) of the volume of bushmeat in kg seized per passenger carrying bushmeat per country.

Figure 2. Box plot representation of the volume of bushmeat carried by passengers from targeted African countries.

Figure 3. Box plot representation of the volume of domestic meat carried by passengers from targeted African countries.

Table 3. Total number of flights and passengers from selected countries of origin to Brussels airport from January 2017 to Oct 2018 (Belgium Federal Public Service Mobility and Transport).

Table 4. Summary of the rates of bushmeat, and estimated total rates of import, arriving fromAfrican departure points at Brussels Zaventem airport.

Figure 4. Box plot representation of the DNA concentrations and absorption ratios (260/280) from tissue samples taken from leaking luggage (LL) and passengers (Pass).

Figure 5. Spectrum of meat categories estimated from morphological identification (left) and DNA-typing (right). U = Unknown attribution.

Figure 6. Representation (in number of genetic samples) of the 9 Orders of vertebrates represented in the seizures of the Brussels' airport.

Figure 7. Representation (in number of genetic samples) of the species / taxonomic diversity within the Livestock category.

Figure 8. Representation (in number of genetic samples) of the ordinal diversity within the Wild category.

Figure 9. Representation (in number of genetic samples) of the species / taxonomic diversity within the Wild category.

Plate 4. Pangolin' picture and x-ray.

Plate 5. Antelope leg' picture and x-ray.

Picture 1. Pellets recovered from an antelope leg

Picture 2. Pellets recovered from a pangolin

Table 5. Key learnings - Brussels Airport - Sensitization Africa travellers from Frank Geers.

Picture 3. Screenshot of the flight option to travel from Nigeria to Brussels.

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Dr. Anne-Lise Chaber (alchaber@hotmail.com) is a Doctor of Veterinary Medicine who specializes in wildlife conservation, illegal wildlife, trade and eco-epidemiology. Anne-Lise holds the One Health chair at the University of Adelaide (Australia) and is a researcher associated with the University of Liège. She initiated and coordinated the first project on the illegal importation of bushmeat (VB) from Africa to Europe. This study (http://onlinelibrary.wiley.com/doi/10.1111/j.1755-263X.2010.00121.x/full) revealed the magnitude of bushmeat international traffic from Central and West Africa to Hexagon, and highlighted the threat this traffic poses to biodiversity and public health (http://link.springer.com/article/10.1007%2Fs10393-015-1065-9; http://dx.doi.org/10.1111/tbed.12481). Anne-Lise is also a member of the UK bushmeat working group and research associate at the Zoological Society of London. She is the author of several studies and publications in eco-epidemiology.

Function in the present study: Scientific coordination of the project and related research, communication with the customs authorities and DG Environment, data statistical analysis, drafting of the official report.

Dr. Philippe Gaubert has been a Research Officer at the Research Institute for the Development (IRD) since 2005, where he has developed a collaborative project on the molecular tracing of African bushmeat. This project is unique in its scope (it covers 11 countries in sub-Saharan Africa, from Guinea to the Democratic Republic of Congo) and in the direct involvement of about 15 African academics. It is materialized by seven publications in journals indexed to Current Contents (2011-2016) and the setting up of a bioinformatic tool for the genetic identification of species traded as bushmeat (DNABUSHMEAT). This project is supported by national, international, binational programs and NGOs (e.g. ANR (France), FCT (Portugal), AMRUGE-CI (France – Côte d'Ivoire) and International Foundation for Science), and was recently presented to the ICCB / ECCB "27th International / 4th European Congress for Conservation Biology" (2015, Montpellier).
Function in the present study: Identification of species by molecular typing, tracing of their geographical origin.

Professor Claude Saegerman has a veterinary training, a master's degree in animal epidemiology, a PhD in veterinary science and is a diplomat from the European College of Veterinary Public Health. He specializes in complex data processing and eco-epidemiology (https://www.ncbi.nlm.nih.gov/pubmed/?term=Saegerman+C). Its field of activity concerns several continents (Europe, America, Africa and Asia). He has extensive field experience and experimental infections with zoonotic, exotic and epizootic diseases. He is responsible for courses in epidemiology, quantitative risk analysis, prevention and control of communicable diseases and biosafety in the Faculty of Veterinary Medicine of the University of Liège and is also President of the Faculty of Biosafety Unit of the same faculty. He has also taught zoonotic diseases at the Institute of Tropical Medicine in Antwerp for many years. He is the Belgian representative of EFSA's Emerging Risks Exchange Network (EREN) and a member of numerous scientific committees around the world.

Function in the present study: Epidemiologist in charge of the biosafety component. Administrative coordination of the project

Dr. Valéria Busoni graduated from the European College of Medical Imaging in 1999. She has 17 years of experience as a clinical radiologist practicing in all species (pets, exotic animals, large animals) but also as a radiologist expert in various fields (expertise of X-rays for the selection of horses, analysis of fish radiographs for research in biology of fluvial species, expert for the Higher Council of Health for veterinary radiology ...). Dr. Busoni is a professor of medical imaging at the ULg. She also teaches in French Schools, Universities in Italy, Switzerland and Austria and teaches numerous courses and conferences around the world.

Function in the present study: Realization and interpretation of radiographs of the bushmeat.

Dr. Mélodie Dieudonné is an anthropologist specializing in the analysis of the social, economic and political dimensions of the problem of bushmeat in Central Africa. Mélodie has just completed his doctoral dissertation at the ULg on the theme: The meat of some, the fauna of others. Anthropological analysis of wildlife conservation in three villages of the forest zone in East-Cameroon. Her expertise in the African sector provides a solid basis for understanding the European aspect of bushmeat trafficking, and more particularly its organization in Africa. Belgium.

Function in this study: Anthropologist.

Harriet Green graduated from The Royal Veterinary College with an MSc in Wild Animal Biology in 2018, having previously gained a BSc (Hons) Zoology from the University of Roehampton. Since graduating Harriet has undertaken a role as a research and development intern within Frontier/The Society for Environmental Exploration's London office and a Fundraising Assistant at The Gorilla Organisation. Both roles provided valuable experience and great opportunities to get involved with the management of non-profit organizations. Tasks ranged from funding applications and preparation of science reports to interacting with the public to raise awareness and promote the beneficial work that is carried out by the companies. Harriet aims to continue working in the field of Wildlife Crime & Trade and from January 2019 will be starting a new job in the police force, aiming to join the National Wildlife Crime Unit.

Function in this study: Research assistant – Report writing support

APPENDICES

Appendix 1. DNA-based identification of the 197 analysed samples based on the four mitochondrial genes (Cyt *b*, COI, 16S, 12S).

ID researcher: Morphological-based identification. Meat cat. Morpho: Morphological-based classification of meat. Meat cat. DNA: DNA-based classification of meat. Final ID: DNA-based identification based on the level of congruence among the four genes. CITES: Appendix number where the taxon is listed. IUCN: IUCN Red List category

Appendix 2. Spectrum of the wild meat diversity seized at the Brussels' airport.