Public health risks from illegally imported African bushmeat and smoked fish

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Large-scale importation of bushmeat from West and Central Africa into Europe was reported in 2010. We sampled 18 illegal African bushmeat consignments seized at Charles de Gaulle airport, Paris, France and tested for the presence of bacteria. Additionally, five smuggled smoked fish were analysed for polycyclic aromatic hydrocarbons, which are known carcinogens. All bushmeat samples had viable counts of aerobic bacteria above levels considered safe for human consumption. We also identified zoonotic bacterial pathogens in bushmeat and unsafe levels of carcinogens in fish. The illegal importation of meat is a potential risk for the introduction of pathogens.

KEY WORDS:

Public health, illegal trade, bushmeat, Europe

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(Intro)

Bushmeat hunting has been identified as a source of zoonotic disease transmission, including the emergence of novel human pathogens (Smith et al., 2012; Wolfe et al., 2004).The international bushmeat trade from West and Central Africa into Europe (Falk et al., 2013; Chaber et al. 2010) might pose human or animal health risks due to infectious agents. In addition, bushmeat and fish are often smoked and this method of preservation has been identified as a source of exposure to polycyclic aromatic hydrocarbons (PAH), which are known carcinogens for consumers (Akpambang et al., 2009). During a study carried out at Roissy-Charles-de-Gaulle airport (CDG), France in 2008, we sampled illegally imported bushmeat and fish that had been seized from passengers by customs officers. Here, we report the findings of our analyses of potential public health risks associated with this trade.

(Material and methods)

Twenty-nine Central and West African Air France flights were checked from the 3rd to 20th of June 2008. Customs inspections were run opportunistically based on custom officer availability and passenger arrival at the gate. Passengers' luggage was opened for manual inspection and bushmeat was seized when present. Such seizures almost always comprised whole carcasses which were either smoked (80 % of the bushmeat seized) or fresh (especially crocodiles and pangolins).

One 30 gram sample was excised from each seized bushmeat carcass, from the skin to deep in the muscle. Each sample was placed in sealed triple plastic bags, labelled, frozen at -20°C and stored for up to one month until analysed. Ninety samples were collected in this way, 18 of which were selected at random and submitted to a commercial laboratory (MC Labo, Saint-Sever, France) for bacteriological analyses. Samples were thawed at 4°C for 24-36 hours and were analysed for Aerobic Viable Count (AVC), *Enterobacteriaceae*, *Listeria monocytogenes* and *Salmonella* spp. For AVC and *Enterobacteriaceae* counts, the respective procedures were followed: ISO 4833 (2004) and AFNOR NF V08-054 (1999). The isolation of *Salmonella spp.* was carried out in accordance with ISO 6579 (1993). For the detection of *L. monocytogenes*, the procedure described in ISO 11290-1 (1996) was followed. Bacterial isolates characteristic of *Staphylococcus* spp. or *Streptococcus* spp, were further identified using protocols ISO 6888-1(1999) for *Staphylococcus spp*. identification and colonial

appearance, Gram stain, catalase test, Lancefield grouping and optochin sensitivity for *Streptococcus* spp. identification.

One smoked fish was randomly selected from each of five different flights (two from Central African Republic, and one each from Angola, Guinea and Cameroon), and sent to the French National Reference Laboratory (LABERCA, Nantes, France) for polycyclic aromatic hydrocarbons (PAH) analysis. The samples were analyzed for15 PAH using an accredited method (Veyrand et al., 2007).

(Results)

All samples taken deep in the muscle were bacteriologically sterile, but a range of bacteria was cultured from the surface of each sample submitted. Not all bacteria cultured were identified due to financial restrictions. The bacteriology results are presented in Table 1. Briefly, mesophilic bacteria were abundant in all 18 samples tested, with AVCs ranging from 1.4×10^6 to 8.18×10^8. No *Salmonella* spp. were isolated and *E.coli* was cultured from only two samples. *Listeria* spp. (including the human pathogen, *Listeria monocytogenes*) were cultured from 10 samples. *Streptococcus* spp. and *Staphylococcus* spp. were identified in nine of the samples analysed. Potentially zoonotic pathogenic biotypes of these Gram-positive bacteria, such as *S. aureus*, were detected in four samples (Table 1). PAH values in the five fish samples tested ranged from 133.12 to 406.43µg/kg of fresh weight (Table 2).

(Discussion)

The high abundance of the total mesophilic bacterial flora was probably due to unhygienic handling of the meat prior to seizure and sampling. Most of the bacteria found and identified were environmental and few were known pathogens, but the sample size was small and a wider range of zoonotic pathogens (e.g. *Salmonella* spp.) might be found if a larger sample size was tested. The absence of bacterial growth from samples taken deep in the muscle indicates that bacterial contamination was superficial. Bacterial identification revealed various biotypes of which *Listeria monocytogenes, L. grayi* and *S. aureus* are associated with food-borne illnesses (Scallan et al., 2011).

All of the bushmeat analysed, had AVC above the 5 log cfu/cm2 limit set by the European Regulation (EC) No. 1441/2007, (2007,7,12) and would therefore be considered unfit for human consumption in Europe. Although zoonotic pathogens, such as *L. monocytogenes*,

were isolated from the bushmeat, the risks from these hazards can be adequately controlled through basic kitchen hygiene, which includes taking steps to avoid cross contamination, and thorough cooking. Most traditional African meat dishes involve long periods of stewing which should kill any bacteria present.

The fifteen PAH tested for have been identified as a priority for food hygiene measures because they show clear evidence of mutagenicity and genotoxicity in somatic cells, both *in vivo* and in experimental animals (European Commission, 2005). PAH are produced by natural and anthropogenic processes, principally pyrogenic (incomplete combustion of coal, wood or other organic substances) and petrogenic (incomplete combustion of petroleum products) inputs (Hodgeson, 1990). PAH contamination of fish (and bushmeat) is linked to the smoking process. Our results showed extremely high levels of PAH contamination. Cancer risk estimates for oral uptake of PAH are based on those for benzo[a]pyrene (BaP). In Europe, the maximum allowable levels for BaP in fish and meat products is 5 micrograms/kg and only 1 microgram/kg in children's food (JEFCA, 2005). BaP values in the five fish we tested were three to > 5 times higher than the maximum allowed in the European Community (Table 2). Alonge (1988) implicated food-borne PAH from smoked dried meat in the high incidence of primary liver and stomach cancer reported in Nigeria.

(Conclusion)

Although our sample size was low, we identified unsafe levels of bacteria and zoonotic bacterial pathogens in bushmeat and unsafe levels of carcinogens in fish illegally imported to Europe. The illegal importation of fresh meat should not be overlooked as a potential risk for the introduction of pathogens. A larger sample size and the inclusion of virological analyses could identify additional public or livestock health threats from this illegal trade. Further research is needed to fully understand the scale of, and risks associated with, the international illegal meat trade.

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Table 1. Bacteriology results from illegally imported bushmeat skin samples seized at Charles de Gaulle airport.

Species sampled	AVC [a]	Totals Coliforms [a]	*Listeria* spp. [a]	Other
Pangolin	$4.52\ 10^7$	0[b]	$4.85\ 10^4$ *L. monocytogenes*	*Staphylococcus aureus* *Streptococcus sp.*
Pangolin	$1.01\ 10^8$	0[b]	$7.23\ 10^4$ *L. ivanovii*	not detected
Pangolin	$9.3\ 10^6$	0[b]	$2.23\ 10^3$ unidentified species	*Klebsiella oxytoca* *Enterobacter sp* *Staphylococcus sp*
Porcupine	$8.18\ 10^8$	0[b]	0[b]	not specified
Porcupine	not countable[c]	0[b]	8711 *L. ivanovii*	*Streptococcus sp.*
Porcupine	$3.51\ 10^7$	0[b]	$3.34\ 10^7$ *L. grayi*	not specified
Primate	$1.31\ 10^7$	0[b]	$1.49\ 10^6$ unidentified species	*Staphylococcus sp.*
Primate	$3.89\ 10^6$	0[b]	$1.25\ 10^5$ unidentified species	*Staphylococcus sp.*
Crocodile	$2.62\ 10^6$	0[b]	$2.77\ 10^5$ unidentified species	*Staphylococcus sp*
Crocodile	$1.50\ 10^7$	0[b]	$2.00\ 10^3$ *L. grayi*	*Citrobacter freundii* *Staphylococcus sp.*
Crocodile	$4.5\ 10^6$	0[b]	$1.15\ 10^5$ *L. welshimeri*	*Staphylococcus sp.*
Duiker	not countable[c]	$1.89\ 10^3$	not countable[c] *L. grayi*	*Citrobacter freundii*
Duiker	$3.15\ 10^8$	$1.69\ 10^5$	$6.64\ 10^7$ unidentified species	*Staphylococcus sp.* *Staphylococcus aureus*
Duiker	not countable[c]	0[b]	not countable[c] *L. grayi*	not specified

Unknown	1.18 10^8	0[b]	0[b]	not specified
Great cane rat	not countable[c]	0[b]	8.07 10^4 *L. ivanovii*	not detected
Red hog	1.4 10^6	0[b]	1.32 10^5 *L. monocytogenes*	*Staphylococcus aureus*
Mix fish/ meat	6.7 10^6	0[b]	3.35 10^5 unidentified species	*Staphylococcus aureus* - *Klebsiella ozonae* Bacillus sp. (non *anthracis*)

[a] Colony forming units per gram of bushmeat.

[b] Below the detectable limit of 30 colony forming units per plate.

[c] Above the maximum limit of 300 colonies forming units per plate.

Table 2. Concentrations of the sum of 15 polycyclic aromatic hydrocarbons and of benzo[a]pyrene in illegally-imported smoked fish seized at Charles de Gaulle airport.

Sample	∑PAHs µg/kg of fresh weight	Concentration in benzo(a)pyrene (BaP) µg/kg of fresh weight (± SD)
1	133.12	17.35 ± 2.72
2	209.27	20.40 ± 3.19
3	406.43	45.02 ± 7.07
4	134.66	15.27 ± 2.40
5	190.91	22.43 ± 3.52