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SCIENTIFIC REPORT OF EFSA

An update on the risk of transmission of Ebola virus (EBOV) via the food chain[1]

European Food Safety Authority[2, 3]

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ABSTRACT

Several animal species were found to harbour Zaïre Ebola virus (ZEBOV), mainly non-human primates and fruit bats. The risk for persons in Europe linked to the transmission of ZEBOV via handling and preparation (by consumers or staff handling the food in kitchens immediately prior to consumption), and consumption of bushmeat illegally imported from Africa was assessed. The outcome was the probability for at least a single human case of ZEBOV in Europe due to transmission via bushmeat. This probability results from a combination of several steps: 1) the bushmeat has to be contaminated with ZEBOV; 2) the bushmeat has to be (illegally) introduced into the EU; 3) the imported bushmeat needs to contain viable virus when it reaches the person; 4) the person has to be exposed to the virus; and 5) the person needs to get infected following exposure. Due to lack of data and knowledge, which results in very high uncertainty, it is not possible to estimate this risk. Considering all these elements, and based on: (i) the limited number of outbreaks confirmed to date in Africa in spite of the routine consumption of bushmeat in that continent, (ii) the handling of bushmeat in Europe not involving high risk practices such as hunting and butchering, and (iii) the assumed low overall consumption of bushmeat in Europe, it can be assumed that the potential for introduction and transmission of ZEBOV via bushmeat in Europe is currently low. The public health consequences of such an event would be very serious given the high lethality and potential for secondary transmission. Hardly any information on ZEBOV infectivity is available on the effect of salting, smoking or drying of meat. Therefore, a conclusion cannot be reached regarding the effectiveness of these methods for virus inactivation. Thorough cooking (100 °C) will destroy the virus.

KEY WORDS

Zaïre Ebola virus, ZEBOV, bushmeat, survival, inactivation, transmission

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SUMMARY

Following a request from the European Commission, EFSA was asked to provide scientific and technical assistance on the risk of transmission of Ebola virus (EBOV) via the food chain. More specifically, EFSA was asked to review the risk for persons in Europe linked to transmission of EBOV via handling and preparation (both carried out by consumers immediately before consumption) as well as consumption of bushmeat illegally imported from Africa. EFSA was also asked to assess the survival of EBOV in meat or meat products, the range of species that are able to transmit or carry the virus, and whether any data exist on physical or chemical treatments that would inactivate the EBOV in products of animal origin and especially in meat.

The outcome of the assessment is the probability for at least a single human case of Zaïre Ebola virus (ZEBOV) in Europe due to transmission via handling and preparation (carried out by consumers or staff handling the food in kitchens immediately prior to consumption) and consumption of bushmeat illegally imported from Africa. This probability is the result of a combination of several necessary steps: 1) the bushmeat has to be contaminated with ZEBOV at the point of origin; 2) the bushmeat has to be (illegally) introduced into the EU; 3) the imported bushmeat needs to contain viable virus when it reaches the person; 4) the person has to be exposed to the virus; and 5) the person needs to get infected following exposure.

Due to lack of data and knowledge, which results in very high uncertainty, it is not possible to estimate this risk. However, considering all the elements in these steps, and based on: (i) the limited number (n = 27) of outbreaks that have been reported to date in Africa in spite of the routine consumption of bushmeat in that continent, (ii) the handling of bushmeat in Europe not involving high risk practices such as hunting and butchering, and (iii) the assumed low overall consumption of bushmeat in Europe, it can be assumed that the potential for introduction and transmission of ZEBOV via bushmeat in Europe is currently low. It should be noted that the public health consequences of such an event (a single human case of ZEBOV occurring in Europe) would be very serious given the high lethality and potential for secondary transmission. In addition, it should be noted that the information considered in this report is largely based on historic Ebola outbreaks and not the current outbreak in West Africa.

Some studies on physical or chemical treatments have been performed on cell-cultured virus as starting material, in some cases diluted with serum. These studies indicate that ZEBOV can survive in liquid media for many days. Survival is better at low temperature (4 °C) than at room temperature. In addition, freezing or refrigeration will preserve infectivity of ZEBOV; ZEBOV can survive multiple freezing/thawing and a long-term storage when frozen. Inactivation of this virus occurs when heated at 75 °C for 30 minutes, after ultra violet (UV) and gamma irradiation, and with 1 % formaldehyde or β-propiolactone. ZEBOV is also susceptible to 3 % acetic acid (pH 2.5), 1 % glutaraldehyde, alcohol-based products, and dilutions (1:10-1:100 for ≥ 10 minutes) of 5.25 % household bleach (sodium hypochlorite) and calcium hypochlorite (bleach powder). Hardly any information is available on the effect of salting, smoking or drying of meat on ZEBOV infectivity. Therefore a conclusion cannot be reached regarding the effectiveness of these methods for virus inactivation. Thorough cooking (100 °C) will rapidly and efficiently destroy the virus.

Considering that ZEBOV infection in some animal species, such as non-human primates, is characterised by a haemorrhagic disease, it is reasonable to expect that an extensive viraemia occurs and that the virus is present in blood and in all organs, secretions and excretions at the height of infection or when the animal has died. The virus has also been isolated from the muscle of non-human primates. It can, therefore, be assumed that virus will be present in the meat of such animals immediately after slaughter. However, there is no information on survival of EBOV in meat or animal products, although it is expected that survival is better at low temperature (4 °C) than at room temperature. The probability of the contaminated bushmeat having viable virus on arrival into the EU would be higher in fresh or frozen meat after a short transport time, and lower in well dried or smoked meat exposed to higher temperatures during transport.

In the very limited number of studies to date, EBOV has only been detected in carcasses of gorillas (*Gorilla gorilla*), chimpanzees (*Pan troglodytes*), duikers (*Cephalophus* spp.) and from live individuals of some species of Old World fruit bats (*Epomops franqueti, Hypsignathus monstrosus, Myonycteris torquata*), small rodents (*Mus setulosus*, *Praomys* spp.) and in one species of shrew (*Sylvisorex ollula*). In addition, antibodies against EBOV have been reported in these and other fruit bat species (*Epomophorus gambianus, Eidolon helvum, Micropterus pusillus, Mops (Mops) condylurus* and *Hipposideros gigas, Roussetus aegyptiacus* and *Rousettus (Rousettus) amplexicaudatus*) and dogs (*Canis lupus familiaris*).

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

In April 2014 the Commission requested the ECDC to assess *"What is the risk of transmission of Ebola virus through contact with bushmeat irregularly transported by passengers coming from areas affected by Ebola virus disease. Has such a transmission mode been documented in the past?"*

The ECDC consulted EFSA on the food safety aspects and identified a low risk in bushmeat but with high uncertainties.

Import into the EU of any fresh meat from Western African countries is not authorised. Member States and EFTA countries have been alerted to increase vigilance on personal passengers' luggage.

EBOV is thought to circulate in wild animals in sub-Saharan Africa. It has been found in fruit bats, chimpanzees, gorillas and duikers. Human infections have been linked to direct contact with such animals. WHO recognises that people in some parts of the affected countries rely on bushmeat for their livelihood and do not avoid eating meat from animals found dead in the "bush". Import of non-human primates is not harmonised (national rules apply) but they can only be introduced into approved bodies, centres and institutes in the EU. From the EU TRACES system, it appears that no imports have taken place from the affected countries.

The websites of WHO and ECDC mention that initial cases of Ebola were contracted by handling infected animals or carcasses, secondary cases occur by direct contact with the body fluids of an ill person, either through unsafe case management or unsafe burial practices.

The Commission requested that the EFSA provides the technical assistance by the end of October 2014.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In view of the above I request EFSA to provide a technical assistance, in the framework of Article 31 of Regulation (EC) No 178/2002 in order to:

1. Review the risk linked to transmission of EBOV via bushmeat. This was considered low, although with a high level of uncertainties. Would new scientific information/evidence lead to conclude an increased risk of EBOV via bushmeat as a source of contamination or is the earlier assessment still valid?

2. What is the persistence/transmissibility of EBOV through meat or animal products?

3. WHO recommended for risk reduction, amongst others, "*to reducing the risk of wildlife-to-human transmission from contact with infected fruit bats or monkeys/apes and the consumption of their raw meat. Animals should be handled with gloves and other appropriate protective clothing. Animal products (blood and meat) should be thoroughly cooked before consumption*."

   Is meat from these species able to transmit/carry the EBOV? Are there other species potentially dangerous?

   Are there any data available on physical (especially heating) or chemical treatments that would inactivate the EBOV in products of animal origin and especially in meat?

4. In the event of possible future outbreaks, what would be the drivers for occasional spillover event, including ecological factors?

Clarification provided by the European Commission

The European Commission informed that imports of bushmeat and meat products thereof, into the EU originating from Western or Central African countries are not authorised in accordance with European Union import legislation[4].

Terms of Reference 1 has been clarified as the risk of transmission of Ebola virus to persons in Europe arising from the handling and preparation (both carried out by consumers immediately before consumption), as well as consumption, of bushmeat from Africa. Therefore, occupational risks are to be excluded. In addition, 'persistence' in Terms of Reference 2 relates to the survival of the virus in meat.

[4] Commission Decision of 29 November 2007 laying down the animal and public health conditions and model certificates for imports of certain meat products and treated stomachs, bladders and intestines for human consumption from third countries and repealing Decision 2005/432/EC. OJ L 312, 30.11.2007, p. 49-67.

ASSESSMENT

1. Introduction

Infections with some Ebola viruses (EBOV) may cause a severe disease in humans called Ebola virus disease (EVD). There are five species of the genus Ebolavirus (Filoviridae family): Zaïre Ebola virus (ZEBOV), Sudan Ebola virus, Reston Ebola virus (REBOV), Taï Forest Ebola virus and Bundibugyo Ebola virus. These viruses, except for REBOV, cause acute and highly lethal enterohaemorrhagic fever in humans (Kaplan and Olinger, 2013). To date, there have been 27 reported outbreaks of EVD in Africa (CDC, online). The current outbreak in West Africa (impacting mainly Guinea, Liberia and Sierra Leone), first reported to the World Health Organization (WHO) on 22 March 2014, was caused by ZEBOV. Since December 2013 and as of 31 October 2014, 13 703 cases of EVD, including 4 920 deaths have been reported by the WHO (ECDC, 2014). A concurrent EVD outbreak was declared on 26 August 2014 in the Democratic Republic of Congo. The two outbreaks are not connected, as determined by comparative analysis of the genome sequence.

As described in the ECDC report (ECDC, 2014), the incubation period (the period between infection and first symptoms) in humans is usually four to ten days but can be as short as two days and as long as 21 days. Case fatality rates have varied from 44 % to 90 % in past outbreaks, depending on factors such as the strain of Ebola virus, the level of or access to care, reliability of baseline (denominator) data collected during outbreaks, etc. Ebola viruses are considered to be highly transmissible to and among humans by direct contact with infected blood, and other bodily fluids/secretions (e.g. stool, saliva, sweat, semen, milk), tissues, organs from dead or living infected persons, although quantitative information on rates and levels of shedding by these different potential sources of infection is sparse. Transmission via inanimate objects contaminated with infected bodily fluids (fomites) is possible. The principal mode of transmission in human outbreaks is human-to-human transmission through direct contact with a symptomatic or dead EVD case or with surfaces and materials (e.g. bedding, clothing) contaminated with body fluids from an infected case. The risk for transmission is considered low in the early phase of human disease (prodromal phase), but viral loads in blood and secretions rapidly increase during the course of illness, with the highest levels of virus shedding observed late in the course of illness of severely ill patients. Burial ceremonies and handling of dead bodies play an important role in transmission. Ebola virus genome has been detected in semen up to 91 days after onset of disease, and replicative Ebola virus has been detected in semen 41 days after onset of disease.

Transmission of ZEBOV to humans in Africa is thought to occur by contact with dead or living infected animals, e.g. non-human primates (such as gorillas and chimpanzees), forest antelopes (duikers) and bats (see Section 2), although most detailed studies traced cases back to the skinning and butchering of carcases. Hunting and butchering of chimpanzee and fruit bats has been identified in previous outbreaks as a potential source of infection (Feldmann and Geisbert, 2011; Muyembe-Tamfum et al., 2012).

EFSA's Unit for Biological Hazards and Contaminants (BIOCONTAM Unit) addressed the Terms of References (ToRs) 1 to 3 in this current report. The answer to the ToR 4 will be delivered by the Unit for Animal and Plant Health (ALPHA Unit) at a later stage in a separate report.

Bushmeat was defined as "*meat taken from any animal native to African forests, including species that may be endangered or not usually eaten outside Africa*"[5]. This assessment only concerns the risk of transmission of ZEBOV via bushmeat to persons in Europe, which for the purposes of this report is considered as arising only from the *handling, preparation and/or consumption* of this meat. In the context of this report, when referring to *handling and preparation* this should be interpreted as the handling of bushmeat carried out by consumers or staff handling the food in kitchens that occurs immediately prior to consumption. It should be noted that the information provided in this report is

[5] http://www.collinsdictionary.com/dictionary/english/bushmeat

largely based in historic Ebola outbreaks and not the current one described above. Data are lacking in many aspects of the transmission of ZEBOV, as described in this report.

To answer the TOR 1, elements of the import risk assessment framework described in Chapter 2.1 of the World Organisation for Animal Health (OIE) Terrestrial Animal Health Code (World Organisation for Animal Health (OIE), 2013) were combined with the CODEX food safety risk assessment (CODEX, 2007). These steps are: 1) Entry assessment (until the illegally imported bushmeat bypasses the border controls), 2) Exposure assessment, 3) Hazard characterisation, and 4) Risk characterisation.

2. Animal species that can harbour or be contaminated with EBOV

Pigott et al. (2014) have recently summarised the information available in the scientific literature on EBOV infections reported in animals. The species implicated are gorillas (*Gorilla gorilla*), chimpanzees (*Pan troglodytes*), Old World fruit bats (*Epomops franqueti, Hypsignathus monstrosus, Myonycteris torquata, Epomophorus gambianus, Eidolon helvum*) and duikers (*Cephalophus* spp.). Fruit bats were the only species in which the virus was detected (by polymerase chain reaction-PCR) in live animals, and remain the most likely, but still unconfirmed, natural reservoir host for EBOV (Hayman et al., 2012; Leroy et al., 2005; Pourrut et al., 2007; Wood et al., 2012), and usually do not show clinical signs (Swanepoel et al., 1996).

Olson et al. (2012) also reviewed the results of animal sampling during EBOV outbreaks in humans and reported a EBOV detection rate of 32.7 % (18/55) in 'carcasses', i.e. animals found dead (non-human primates and duiker) and 0.2 % (13/5 309) in live-captured animals (of which all the positive samples were from fruit bats). In addition to the above-mentioned animal species, this article reported antibodies against EBOV in other bat species (*Micropterus pusillus, Mops (Mops) condylurus* and *Hipposideros gigas*[6]*, Roussetus aegyptiacus* and *Rousettus (Rousettus) amplexicaudatus*). A retrospective study describing the presence of antibodies in dogs (*Canis lupus familiaris*) during/following EBOV outbreaks was also included (Allela et al., 2005). The review by Olson and colleagues (2012) also includes negative findings in the following animal orders: Afrosoricida (5 samples tested for antibodies), Hyracoidea (7 samples tested for the presence of both antibodies (a) and virus (v)), Macroscelidae (28 a, 29 v), Pholidota (66 a, 95 v), Proboscidea (2 carcasses v), Soricomorpha (105 a, 123 v), Class Aves (85 a, 421 v), and Class Reptilia (30 a, 155 v).

EBOV nucleic acid has also been detected during ecological studies, i.e. in areas not affected by human outbreaks, in small rodents (*Mus setulosus*, *Praomys* spp.) and in one species of shrew (*Sylvisorex ollula*) (Morvan et al., 1999).

Another study investigated animals found sick or dead during the course of Ebola-related investigations and other biological research projects conducted from November 1994 to November 2003, based on reports from local people residing in human outbreak areas (Lahm et al., 2007). A wider range of species (primates such as *Cercopithecus* spp., *Mandrillus sphinx. Colobus* spp., African brush-tailed porcupine (*Atherurus africanus*), African civet (*Civettictis civetta*) and Red river hog (*Potamochoerus porcus*)) was reported, but no EBOV was isolated from the few samples available.

The countries included in these studies are Côte d'Ivoire, Central African Republic, Democratic Republic of Congo, Gabon, Ghana and the Republic of Congo. In addition, it has to be noted that Old world fruit bats, belonging to the Pteropodidae family, are distributed throughout sub-Saharan Africa, including Madagascar (Mickleburgh et al., 1992).

In this assessment, more weight was given to evidence of the presence of virus in either live animals or carcasses than to the detection of antibodies. The findings in these studies are summarised in Table 1. The least reliable information was considered to be the reports of moribund or dead animals in areas where human outbreaks had occurred, as the presence of EBOV was not confirmed by laboratory analysis of the samples collected from the animals.

[6] In this particular case, *Mops (Mops) condylurus* and *Hipposideros gigas* were reported together.

Table 1: Reported detection of Ebola virus or antibodies in wildlife (adapted from Olson et al., 2012 and Morven et al., 1999)

Order (species which tested positive)	Virus detected[(c)], (samples positive/samples taken)	Antibodies detected[(d)], (samples positive/samples taken)
Chiroptera[(a)] (bats)	**Yes** 13/1 418 (alive)	**Yes** 158/4 883
Non-human primates[(a)] (gorilla, chimpanzee)	**Yes** 0/285 (alive) 17/33 (carcasses)	No 0/275
Artiodactyla[(a)] (duikers)	**Yes** 0/58 (alive) 1/13 (carcasses)	No 0/23
Carnivora[(a)] (dogs)	No 0/49 (alive) 0/6 (carcasses)	**Yes** 21/87
Rodentia[(a)] (scaly-tailed squirrel)	No 0/2 540 (alive)	**Yes** 1/2 431
Rodentia[(b)] (mice)	**Yes** 6/163 (alive)	N/A
Soricomorpha[(b)] (shrew)	**Yes** 1/56 (alive)	N/A

(a): Adapted from Olson et al. (2012).
(b): Adapted from Morven et al. (1999).
(c): By one of these methods: histopathological examination of tissues, virus isolation in Vero cell culture, antigen capture assays, or virus-specific polymerase chain reaction.
(d): Using enzyme-linked immunosorbent assays (ELISA) targeting virus-specific host immunoglobulin G (IgG) antibodies.

Pigs have been infected with ZEBOV in laboratory settings (Kobinger et al., 2011; Weingartl et al., 2012), after which they developed clinical signs. Weingartl and colleagues (2012) were also able to observe aerosol transmission from ZEBOV-infected pigs to non-human primates.

REBOV was originally reported in wild-caught cynomolgus monkeys *(Macaca fascicularis)* imported from the Philippines into the United States (Rollin et al., 1999), and has also been reported in pigs both in the Philippines (Barrette et al., 2009) and China (Pan et al., 2014) as an infection concurrent with porcine reproductive and respiratory syndrome virus (PRRSV), so it was not clear if REBOV was causing any clinical signs. Marsh and colleagues did not observe clinical signs in pigs experimentally infected with REBOV (Marsh et al., 2011). REBOV has not been shown to cause symptomatic infections in humans. The animal and public health implications of this EBOV species are yet to be determined.

Despite the numerous references to Ebola viruses in different animal species in the scientific literature, prevalence or epidemiological studies in wildlife during normal sylvatic cycles (i.e. not related to EBOV outbreaks) are lacking. It is therefore very difficult to conclude on the prevalence of ZEBOV in any of the animal species that are used to produce bushmeat. Due to these gaps in current knowledge, it is not possible to exclude that other species are able to carry the virus.

Concluding remarks: In the very limited number of studies to date, EBOV has only been detected in carcasses of gorillas (*Gorilla gorilla*), chimpanzees (*Pan troglodytes*), duikers (*Cephalophus* spp.) and from live individuals of some species of Old World fruit bats (*Epomops franqueti, Hypsignathus monstrosus, Myonycteris torquata*), small rodents (*Mus setulosus*, *Praomys* spp.) and in one species of shrew (*Sylvisorex ollula*). In addition, antibodies against EBOV have been reported in these and other fruit bat species (*Epomophorus gambianus, Eidolon helvum, Micropterus pusillus, Mops (Mops) condylurus* and *Hipposideros gigas*[6]*, Roussetus aegyptiacus* and *Rousettus (Rousettus) amplexicaudatus*) and dogs (*Canis lupus familiaris*).

3. Sensitivity of EBOV to physical or chemical agents

Studies on the sensitivity of EBOV to physical (Section 3.1) and chemical agents (Section 3.2) are summarised below. All these studies were performed on cell-cultured virus as starting material, in some cases diluted with serum.

3.1. Physical agents to inactivate Ebola virus

EBOV can survive in liquid media (tissue culture media possibly diluted in serum) for many days. Survival is better at low temperature (4 °C) than at room temperature. As for any virus, freezing or refrigeration will preserve infectivity of Ebola virus (ECDC, 2010; WHO, 2013) and the virus can survive multiple freezing/thawing (5 cycles) and long-term storage when frozen (Chepurnov et al., 1995).

When cell-cultured virus was held at 20 °C, a 4 log reduction in virus titre was observed within the first 26 days. After 46 days, titres were just above the detection limit, implying a 5 log-reduction. At 4 °C a decrease of 2-3 logs over 46 days was observed. No differences were observed when cell-cultured virus was diluted in guinea pig serum (Piercy et al., 2010).

EBOV, when dried in the same media on solid surfaces (plastic and glass) and stored at 4 °C, showed a decrease in the viral titre of 4 logs over 14 days. When virus-containing tissue culture media was dried onto glass, the virus survived for over 50 days (Piercy et al., 2010).

In another study (Sagripanti et al., 2010), cell-cultured Ebola virus remained infectious for more than six days (4 log reduction observed) when dried onto glass, polymeric silicone rubber, or painted aluminum alloy, and when stored in the dark under ambient conditions (between 20 and 25 °C and 30 - 40 % relative humidity) .

Mitchell and McCormick (1984) tested the inactivation of cell cultured EBOV diluted in human serum samples (1/10, final concentration of 5 to 7 $\log_{10}$ plaque-forming unit (PFU)/ml). Complete inactivation of the virus in serum samples occurred at 60 °C, but not at 45 °C or 56 °C. The time required to inactivate 5 logs PFU/ml at 60 °C was 22 min. Bray et al. (1999) achieved the inactivation of EBOV by heating it at 75 °C for 30 min.

Inactivation of EBOV can also be achieved by ultra violet (UV) and gamma radiation (Klenk et al., 1999). For example, 27×10^6 rads of Gamma radiation inactivated the Ebola virus in human serum (Mitchell and McCormick, 1984). Complete inactivation of EBOV was achieved by Gamma irradiation with 6×10^6 rads (Bray et al., 1999), 0.15×10^6 rads (4 °C) or 0.22×10^6 rads (-60 °C) (Elliott et al., 1982) and 3×10^4 rad/min for 30 min (Lupton, 1981). Gamma radiation was found more efficient in killing Ebola virus compared to UV light and β-propiolactone inactivation (Elliot et al., 1982). In another study (Sagripanti and Lytle, 2011), Ebola virus has been shown to be moderately sensitive to germicidal UV (UVC, 254 nm radiation). It has to be remembered that, unlike gamma radiation, UV radiation has no or very low penetrating effect to inactivate the virus in tissue suspensions.

3.2. Chemical agents to inactivate Ebola virus

Inactivation of Ebola virus can be achieved by 1 % formaldehyde or with β-propiolactone. Since the virus has an envelope as outer membrane, brief exposure to phenol disinfectants and lipid solvents like deoxycholate and ether (Klenk et al., 1999) results in a rapid inactivating effect.

When freshly drawn human blood was mixed with EBOV at a concentration of 5 to 6 $\log_{10}$ PFU per ml and a 1/100 dilution was made with acetic acid (pH 2.5), total virus inactivation was obtained after 15 minutes, while this was not observed in the phosphate-buffered saline control (pH 7.3) (Mitchell and McCormick, 1984). The pH value at which the virus was inactivated was not measured but it can be concluded that EBOV infectivity is sensitive to acid pH. The virus is also susceptible to 1 % glutaraldehyde, alcohol-based products, and dilutions (1:10-1:100 for ≥ 10 minutes) of 5.25 %

household bleach (sodium hypochlorite), and calcium hypochlorite (bleach powder) (WHO, 2008, 2010, 2014).

The WHO guidance (WHO, 2014) for cleaning environmental surfaces or objects contaminated with blood or other body fluids suggest the use of standard hospital detergents/disinfectants (e.g. a 0.5 % chlorine solution or a solution containing 5 000 parts per million of available free chlorine).

4. Survival of Ebola virus in meat and animal products

Considering that a ZEBOV infection in some animal species, such as in non-human primates, is characterised by a haemorrhagic disease, it is reasonable to expect that an extensive viraemia occurs and that the virus is present in blood and in all organs, secretions and excretions at the height of infection or when the animal has died. The virus has also been isolated from the muscle of non-human primates (Rouquet et al., 2005). It can, therefore, be assumed that the virus will be present in the meat of such animals immediately after slaughter or death following infection. It can also be assumed that the viral load can be high, but there is no available information in the literature in this respect. There is no clear information on the persistence of Ebola virus in meat or animal products. Leroy et al. (2004) stated that animal carcasses left in African forests were not infectious after 3 to 4 days, however details were not provided. Therefore the methodology used to assess infectivity is not available, neither is it known whether these carcasses had been sampled earlier for this virus.

A decline in pH accompanying *rigor mortis*, which might have an inactivating effect on acid-labile viruses in meat after slaughtering, only occurs when animals are healthy at slaughter and may not occur when they are stressed, have fever or when they naturally die from disease. Moreover, exact data on the pH value at which EBOV virus is inactivated are not available, so it cannot be concluded if *rigor mortis* has any effect on the virus in meat after slaughter or death.

Bushmeat is derived from hunted wild animals and dressed in the field without any veterinary inspection. There is a high potential for the meat to be contaminated with a wide range of potential pathogens depending on the species and its origin. However, most bushmeat is often dressed and smoked because smoking substantially extends the shelf life of the meat (Cowlishaw et al., 2004). Bushmeat could also be dried or salted, and because of these treatments the initial load of viable organisms on the bushmeat would be expected to be reduced. To preserve the bushmeat it may also be frozen on arrival in the EU.

Since experimental data on the inactivation of EBOV in meat, organs or animal products are completely lacking, only general statements can be made. The results given earlier on the effect of inactivating agents on survival of EBOV in cell-cultured medium, whether or not mixed with serum (Piercy et al., 2010), are considered higher than what could be achieved in meat. Inactivation by chemical agents would only reduce the surface contamination of meat, and may be less effective when the virus is embedded in tissues or organs compared to the virus in cell-culture medium, because organic materials may bind to the inactivating chemical and lower its efficacy.

Hardly any information is available on the effect of salting, smoking or drying of meat to eliminate EBOV infectivity, and general statements cannot be made since the methods used are not standardised. The effect on inactivation of viruses throughout the meat or meat products upon smoking or drying may thus be highly variable. Thorough cooking (100 °C) will rapidly and efficiently destroy the virus.

Concluding remarks: Considering that ZEBOV infection in some animal species, such as in non-human primates, is characterised by a haemorrhagic disease, it is reasonable to expect that an extensive viraemia occurs and that the virus is present in blood and in all organs, secretions and excretions at the height of infection or when the animal has died. The virus has already been isolated from the muscle of non-human primates. It can, therefore, be assumed that the virus will be present in the meat of such animals immediately after slaughter or death. There is no information on the survival of EBOV in meat or animal products; however, it is expected that survival is better at low temperature (4 °C) than at room temperature. Hardly any information is available on the effect of salting, smoking

or drying of meat on survival of ZEBOV. Thorough cooking (100 °C) will rapidly and efficiently destroy the virus.

5. Risk linked to transmission of ZEBOV via bushmeat

Contact with raw meat from an animal infected with ZEBOV can potentially result in transmission of the virus to humans. As the virus can survive for several days in liquid or dried material in the laboratory, it is assumed that the virus may remain viable for several days in unprocessed meat, and perhaps longer in frozen or refrigerated unprocessed meat.

Skinning and chopping monkey cadavers have been identified as a source of contamination. However, only those people who have been in contact with the dead monkey before it was cooked were affected; nobody was infected by eating the cooked meat (Georges et al., 1999). To date, there have been no reported cases of transmission of EBOV from handling, preparation and consumption of illegally imported bushmeat in the EU or elsewhere. However, the infection with EBOV through oral mucosa is still a possibility (Jaax et al., 1996), so if a food serving was contaminated with viable ZEBOV (i.e. through insufficient cooking), the risk of infection could not be excluded.

Importation of bushmeat may be a potential source of contamination by pathogens such as EBOV (Smith et al., 2012). The risk of ZEBOV transmission to persons in the EU is influenced by the availability of contaminated bushmeat. Bushmeat cannot be legally imported into the EU, but illegal importation does occur. Whether bushmeat illegally imported from Africa to the EU is contaminated with ZEBOV at the point of origin will be heavily influenced by several factors: the epidemiological situation regarding ZEBOV in the geographical area where the animals were hunted and the bushmeat produced, the animal species from which the bushmeat was obtained (i.e. whether the species are able to carry the virus or not), and the processing, if any, applied to the bushmeat prior to export (e.g. cooking, drying, smoking).

Other factors that will influence the risk are the likelihood of virus surviving in the meat until it reaches the persons, as well as handling, preparation and cooking practices of the bushmeat in Europe. There is a lot of uncertainty about these factors as not a lot of data are available given the illegal nature of these imports, as described below.

5.1. Risk pathway for ZEBOV to reach European persons via bushmeat

To assess the risk linked to transmission of ZEBOV via bushmeat, elements of the import risk assessment framework described in Chapter 2.1 of the World Organisation for Animal Health (OIE) Terrestrial Animal Health Code (World Organisation for Animal Health (OIE), 2013) were combined with the CODEX food safety risk assessment (CODEX, 2007). These steps are: 1) Entry assessment (until the illegally imported bushmeat bypasses the border controls), 2) Exposure assessment, 3) Hazard characterisation, and 4) Risk characterisation. Figure 1 represents all the necessary steps for ZEBOV to reach a European person.

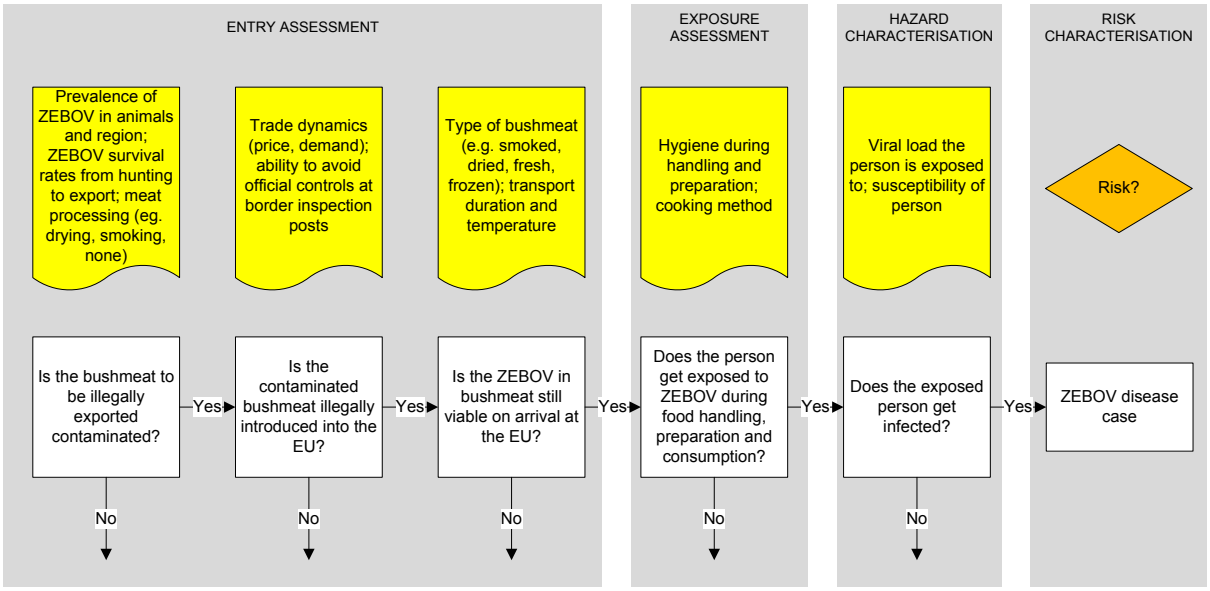

Figure 1: Risk pathway for transmission of ZEBOV to persons in Europe via handling and preparation (both carried out by consumers or staff handling the food in kitchens immediately before consumption), as well as consumption, of bushmeat from Africa. Factors included in the upper yellow boxes are considered to influence the probability of the step immediately below.

5.1.1. Entry assessment

In this report, the entry assessment describes the pathways required to introduce ZEBOV into the EU via bushmeat, estimating the probability of this event. It can be further subdivided as follows:

a) Probability of bushmeat being contaminated with ZEBOV at the point of origin

This probability is a combination of several necessary steps:

1. The animal species from which bushmeat is produced is susceptible to ZEBOV infection, and
2. The animal was hunted in an area where ZEBOV is circulating, and
3. The animal has to be infected with ZEBOV, i.e. carrying the virus, and
4. ZEBOV has to survive during any processing, if any, of the fresh meat (e.g. smoking, salting or cooking) at the point of origin.

Of the many animals (BCTF, 2001) that have been linked to the production of bushmeat, serologic or virological evidence of ZEBOV infection has only been reported in a few species (see Section 2). Few studies are available regarding virus prevalence, which of course will depend on the epidemiological situation in the area under study. Rouquet et al. (2005) found increased mortality of large primates in association with human EBOV outbreaks, suggesting the prevalence in wildlife may vary significantly over time. For many species of animals consumed as bushmeat, there is no evidence of whether or not they can be infected.

The occurrence of ZEBOV in bushmeat is considered unknown, and as mentioned above depends on several factors such as the area where the animals were obtained from, the animal species and whether these animals were hunted or found already dead. The probability of the meat being contaminated will be higher if it originates from species that are susceptible to infection with ZEBOV and come from areas where ZEBOV is present, especially if the meat is not subject to any processing treatment (i.e. exported fresh or frozen). The highest probability would be for bushmeat sourced from animals from

areas experiencing active virus transmission in wildlife, especially from animals found dead. As mentioned in Section 2, Olson et al. (2012) reported a detection rate of 32.7 % (18/55) positive samples for EBOV in 'carcasses', i.e. animals found dead. From these, the majority of positive animals were non-human primates (17 positive out of 33 animals sampled), the other positive sample belonging to a duiker (1/13 sampled). In live-captured animals, 0.2 % (13/5 309) were found to harbour the virus (fruit bats were the only positive animals, with 13/1 418 samples taken).

Another aspect to consider is the amount of virus that can be found in infected animals. High levels of virus are found in the internal organs of non-human primates (Feldmann and Geisbert, 2011), and virus has been isolated from the muscle of non-human primates (Rouquet et al., 2005), but there are no specific data about the level of virus in the muscle of infected animals, whether these are symptomatic or not. Data from experimentally infected non-human primates shows widespread systemic infection and high titres in many organs, which would indicate that the virus would be present in muscle in at least these species. In addition, most bushmeat will likely have been processed at the point of origin to prolong shelf life. Treating methods may vary, including salting, drying and smoking. However, hardly any information is available on the effect of these methods on ZEBOV infectivity, although they are expected to reduce it.

Concluding remarks: Given the scarcity of data, the probability of bushmeat being contaminated with ZEBOV at the point of origin cannot be accurately assessed. Many species providing bushmeat are not known to be sensitive to infection with ZEBOV, and the current evidence of occurrence of ZEBOV is limited to Western and Central Africa. The virus has been detected in approximately 1/3 of carcasses of animals found dead (almost exclusively from non-human primates) in areas where human outbreaks took place. No studies have been conducted outside these areas, but the virus is expected to circulate less frequently when no human or animal outbreaks occur. Bushmeat is frequently processed before exporting, reducing or eliminating viral loads. The probability of infectious ZEBOV being present in bushmeat is considered higher if it originates from species that are susceptible to infection with ZEBOV and comes from areas where ZEBOV is present, especially if the meat is not subject to any processing treatment (i.e. exported fresh or frozen). The highest probability would be for bushmeat sourced from animals from areas experiencing active virus transmission in wildlife, especially from animals found dead.

b) Probability of illegal importation of bushmeat into the EU

Few data are available on the illegal importation of bushmeat into the EU that escapes the border controls. As mentioned above, of public health relevance is the specimen condition and the species composition.

In a recent study (Schoder et al., 2014), spot-checks were carried out on the luggage of 61 355 passengers from 240 flights from non-EU countries arriving at the Vienna International Airport (Austria), with the objective to estimate the prevalence of zoonotic agents in products of animal origin introduced to the EU via uncontrolled imports. Over a period of eight months (August 2012 – March 2013), 262 meat and meat products were detected, and six bushmeat samples. The bushmeat samples originated from Nigeria (n = 3), South Africa (n = 2) and Ethiopia (n = 1). There were no instances of raw meat. Of the six samples, one (head of beef) was cooked, two were smoked and three (biltong, game, one not defined) were dried.

Another study (Rodríguez-Lázaro et al., 2014) assessed the presence of Methicillin-resistant *Staphylococcus aureus* in food confiscated from passengers on flights from 21 non-EU countries at the Bilbao International Airport (Spain). A total of 195 food samples were confiscated, of which 117 were meat samples of diverse animal origin (including antelope, beef, chicken, duck, guinea pig, pork, rodents and turkey). The origin of the samples was diverse and seven samples were derived from West Africa.

In the study by Falk et al. (2013) during the period 2008 to 2011, the quantity of meat illegally imported into Switzerland in air passengers' luggage was estimated, including a separate estimation for bushmeat. The study revealed that meat from a wide array of animal species was imported into Switzerland. The average annual weight of meat seized during the period analysed was 5.5 tonnes, of which 1.4 % (249 kg) was bushmeat. However, in a stochastic model the total annual inflow of illegal bushmeat imports was estimated at 8.6 tonnes (95 % CI 0.8 to 68.8). In this model, the frequencies of illegal import of bushmeat (nIllegalBm) was derived from nIllegalBm = Npass × P1 × P2 × P3 × P4 × P5, where Npass is the total number of passengers entering Switzerland via Zurich and Geneva airports, and P1 to P5 denote probability distributions of the different steps of the scenario tree. These probabilities are: probability that the passenger will not declare anything (P1, PERT distribution with mode 0.9961); probability that the passenger's luggage will not be searched at the Swiss custom borders (P2, PERT distribution with mode 0.9959); probability that the passenger whose luggage is searched is importing an animal product (P3, PERT distribution with mode 0.037); probability that the imported animal product is meat (P4, PERT distribution with mode 0.68), probability that the imported meat is bushmeat (P5, PERT distribution with mode 0.005). West Africa (including Cameroon, Côte d'Ivoire, Benin, Ghana) was the greatest contributor to these bushmeat imports. The bushmeat often came in relatively large quantities: the median weight of the confiscated bushmeat imports was 4.5 kg (interquartile range 2 to 10). The processing methods for bushmeat were known for only two imports: one was fresh bushmeat from Cameroon and the other was dried meat from South Africa. The species from which the meat was derived was recorded for 60 % of seizures and included diverse groups (see between brackets with the figure in square brackets referring to meat derived from West Africa) such as primates (apes) (N = 1 [1]), antelopes (N = 3 [1]), pangolin (N = 1 [1]), birds (N = 1 [1]), porcupines (N = 3 [3]), other rodents (N = 5 [3]), and unspecified game animals (N = 4 [2]).

In France (Chaber et al., 2010), a systematic survey of customs seizures of bushmeat (and livestock meat and fish) carried by passengers arriving from sub-Saharan Africa at Paris Roissy-Charles de Gaulle airport was performed, combining targeted and random luggage inspections. It was found that 7 % out of 134 passengers carried bushmeat, with individual consignments over 20 kg. Eleven bushmeat species were found, including two primate, two ungulate, three rodent, two crocodile, and two pangolin, with rodents and blue duiker making up 75 % of the total number of carcasses found. It was estimated that 273 tonnes of bushmeat is illegally imported per year on these flights. Central African Republic, Cameroon, and Republic of Congo were the main sources of bushmeat, with a small amount from Ivory Coast, and none from any other country. According to the authors, the volume and nature of import and trade suggests the emergence of a luxury market for African bushmeat in Europe. Imports are supplying an organised system of trade and are not solely being brought for personal consumption. This is indicated by the large size of many individual bushmeat consignments, and the presence of traders within Paris who are able to supply bushmeat to order.

In the United States (Smith et al., 2012), a pilot project to establish a surveillance methodology for zoonotic agents in confiscated wildlife products revealed that samples collected at several international airports (2008-2010 period) identified parts originating from non-human primate and rodent species, including baboon, chimpanzee, mangabey, guenon, green monkey, cane rat and rat. Pathogen screening identified retroviruses (simian foamy virus) and/or herpes viruses (cytomegalovirus and lymphocryptovirus) in the non-human primate samples, although samples were not tested for EBOV. Specimens varied in condition, including items that were fresh, raw transported in a cooler, lightly smoked, or well dried. Most items contained moist inner tissue.

The above studies only consider illegal importation via airports. However, bushmeat could also be imported through other routes, chiefly sea freight. A study by Hartnett et al. (2007) estimated the mean flow of illegally imported meat (i.e. not just bushmeat) contaminated with foot and mouth virus from West Africa to be around 10 kg per year for the UK. The total flow of illegal meat per year from West Africa was estimated as 1 213 tonnes (95 % CI 399 to 3 082). This study considered the following mode of import: passenger baggage, post and courier, air and sea freight. There are no studies looking

specifically at the amount of bushmeat illegally imported through routes other than air passenger luggage.

Concluding remarks: Based on existing studies on checks of personal passengers luggage, the frequency of finding bushmeat illegally imported into the EU has been estimated as one out of 5 000 pieces of luggage from flights from non-EU countries arriving in Austria. The total annual inflow in Switzerland has been estimated at 8.6 tonnes (95 % CI 0.8 to 68.8) and at a major airport in France at 273 tonnes per year. These estimates are uncertain as indicated by the broad confidence interval. The species from which the bushmeat was derived included diverse groups: primates (apes), antelopes, pangolin, birds, porcupines, other rodents, crocodile, blue duiker and unspecified game animals. The processing method for bushmeat was not usually recorded.

c) Probability of the contaminated bushmeat having viable virus on arrival into the EU

Assuming the bushmeat is contaminated at the point of origin, the probability of having viable virus on arrival at place of consumption in the EU is dependent on the processing the meat has been subject to (e.g. higher for fresh and frozen bushmeat, lower for dried or smoked bushmeat), and the duration and conditions of transport (e.g. vacuum packing, temperature, etc).

Apart from a few studies on survival of EBOV in media and dry surfaces (see Section 3 above), there are no data on survival of the virus in different meat products, so this probability is currently unknown. Depending on the type of product, the survival would be higher (e.g. in fresh or frozen meat after a short transport time) or lower (in well dried or smoked meat exposed to higher temperatures during transport). From the information in b) above it seems that the range of bushmeat products in terms of processing methods is wide (specimens included items that were fresh, cooked, smoked, or well dried), although some studies conducted in West Africa suggest that most carcasses are sold in local markets in a processed state (e.g. smoked) to ensure meat preservation (Kamins et al., 2011; Minhos et al., 2013).

Concluding remarks: Bushmeat specimens seized at European borders varied in condition (see Section 5.1.1. b) above), including items that were fresh, cooked, smoked, or well dried. It was noted that these items could contain moist inner tissue, which could favour the survival of the virus. There is no information on the survival of EBOV in meat or animal products; however, it is expected that survival is better at low temperature (4 °C) than at room temperature. The probability of the contaminated bushmeat having viable virus on arrival into the EU would be higher in fresh or frozen meat after a short transport time, and lower in well dried or smoked meat exposed to higher temperatures during transport.

5.1.2. Exposure assessment

This step relates to the survival during storage in Europe and the exposure (probability and numbers of infectious virus) of European persons to ZEBOV during handling and preparation (both carried out by consumers or staff handling the food in kitchens immediately prior to consumption), as well as consumption, of bushmeat.

An important aspect of the risk of infection with EBOV is the possibility of humans to get infected during handling and preparation of contaminated bushmeat in Europe. Practices prior to consumption (such as hunting, butchering, and preparation), rather than consumption of the meat *per se*, seemed to be a key risk factor for people that became infected in previous Ebola outbreaks in Africa, although the cooking method was not described (Pourrut et al., 2005). The importance of the handling and preparation step is determined by the high infectivity and the route of infection with ZEBOV (see Section 5.1.3 below). In addition, cross contamination needs to be considered, especially in the case of fresh bushmeat, as persons might get exposed to ZEBOV through consumption of a meal that does not contain bushmeat but has been prepared together with bush meat (e.g. salad).

There is no published information on how these meat products are handled, prepared or consumed and the levels of consumption in Europe. The probability of the virus surviving the cooking or the food preparation step is therefore difficult to estimate. Bushmeat tends to be purchased dressed and smoked. One can therefore expect a minimal handling during preparation. Anecdotal evidence in Europe suggests that the meat, which is usually smoked and may be up to one week old, is washed in salted water. The meat is chopped into small pieces and boiled for a considerable amount of time.

Whether the virus survives in the meat or meat product or not will depend on the method of food preparation, with complete inactivation expected in thoroughly cooked meat and no inactivation for those meat products consumed without further cooking.

The consumption of bushmeat by Europeans can be assumed to be low compared to the routine consumption of bushmeat in Africa. For example, in Africa's Congo Basin, people eat an estimated 4.5 million tonnes of bushmeat per year (Nasi et al., 2011).

Concluding remarks: There is no published information on how bushmeat products are handled, prepared or consumed, or about the levels of consumption in Europe. Based on African studies, handling and preparation of bushmeat are key risk factor for ZEBOV infection. The handling in Europe does not involve hunting or butchering the wild animal.

5.1.3. Hazard characterisation

In this step, the **probability of the person to get infected following preparation, handling and consumption of a meal containing contaminated meat** is described.

Non-human primates have been reported to be infected with viral haemorrhagic fever by 1-10 infectious viruses when exposed to aerosols (Franz et al., 1997). Johnson and colleagues (1995) reported a higher dose (400 PFUs) when infecting EBOV in rhesus monkeys by inhalation. The lethal dose of EBOV inoculated into the peritoneal cavity of mice was one virus. Mice were resistant to large doses of the same virus inoculated subcutaneously, intradermally, or intramuscularly (Bray et al., 1999). In another experiment, three out of four orally inoculated rhesus monkeys were infected when using a dose of 5.2 $\log_{10}$ of ZEBOV suspended in 1 mL of serum (Jaax et al., 1996).

It is therefore reasonable to assume that the probability of human infection after being exposed to even low doses of virus particles could be high.

However, no definitive information is available about the infectivity dose of EBOV to humans. The infectivity dose will depend on the exposure route, e.g. ingestion, skin contact, mucosal surfaces, etc.

The health consequences of a person infected with ZEBOV are serious. Case fatality rates have varied from 44 % to 90 % in past outbreaks, depending on factors such as the strain of Ebola virus, the level of or access to care, reliability of baseline (denominator) data collected during outbreaks, etc. In addition, as mentioned in ECDC (2014), secondary transmission to caregivers in the family and in healthcare facilities is possible.

Concluding remarks: Based on non-human primate studies, agents causing viral hemorrhagic fevers are believed to be highly infectious. The probability of infection will depend on the exposure route, e.g. ingestion, skin contact, mucosal surfaces etc. The public health consequences of a human case of ZEBOV linked to transmission from bushmeat occurring in Europe would be very serious given the high lethality and potential for secondary transmission.

5.1.4. Risk characterisation

The outcome of the risk assessment is the probability for at least a single human case of ZEBOV in Europe due to transmission via bushmeat. The remit of the assessment was restricted to the risk of transmission of Zaïre Ebola virus (ZEBOV) to persons in Europe arising from the handling and

preparation (both carried out by consumers or staff handling the food in kitchens immediately prior to consumption) as well as consumption of bushmeat from Africa.

Due to lack of data and knowledge, which results in very high uncertainty, it is not possible to estimate this risk. However, considering all the elements in the pathway discussed above (in Section 5), and based on: (i) the limited number (n = 27) of outbreaks that have been reported to date in Africa in spite of the routine consumption of bushmeat in that continent, (ii) the handling of bushmeat in Europe not involving high risk practices such as hunting and butchering, and (iii) the assumed low overall consumption of bushmeat in Europe, it can be assumed that the potential for introduction and transmission of ZEBOV via bushmeat in Europe is currently low. It should be noted that the public health consequences of a human case of ZEBOV linked to transmission from bushmeat occurring in Europe would be very serious given the high lethality and potential for secondary transmission. In addition, it should be noted that the information considered in this report is largely based on historic Ebola outbreaks and not on the current outbreak in West Africa, first reported to the WHO on 22 March 2014.

The risk is considered higher if the bushmeat would originate from species that are susceptible to infection with ZEBOV and comes from areas where ZEBOV is present, especially if the meat is not subject to any processing treatment (i.e. exported fresh or frozen). The highest risk would be for bushmeat sourced from animals from areas experiencing active virus transmission in wildlife, especially from animals found dead**.**

Although information is lacking in volumes and patterns of consumption of bushmeat in Europe, it is assumed that this is not equally distributed in the overall population. The risk linked to transmission from bushmeat exists only for specific groups who choose to handle or eat bushmeat, thus it is assumed that these groups would be at a higher risk than the overall population.

CONCLUSIONS AND RECOMMENDATION

CONCLUSIONS

1. **Review the risk linked to transmission of EBOV via bushmeat. This was considered low, although with a high level of uncertainties. Would new scientific information/evidence lead to conclude an increased risk of EBOV via bushmeat as a source of contamination or is the earlier assessment still valid?**

- The import of bushmeat and meat product thereof into the EU originating from Western and Central African countries is not authorised. To date there have not been any reported cases of transmission of EBOV from handling, preparation and consumption of illegally imported bushmeat in the EU or elsewhere.

- The remit of this risk assessment is restricted to the risk of transmission of ZEBOV to persons in Europe that is arising from the handling and preparation (both carried out by consumers or staff handling the food in kitchens immediately prior to consumption) as well as consumption of bushmeat from Africa.

- The outcome of the assessment is the probability for at least a single human case of ZEBOV in Europe due to transmission via bushmeat.

- This probability is the result of a combination of the following necessary steps as described in the risk pathway. These steps include: 1) the bushmeat has to be contaminated with ZEBOV at the point of origin; 2) the bushmeat has to be (illegally) introduced into the EU; 3) the

imported bushmeat needs to contain viable virus when it reaches the person; 4) the person has to be exposed to the virus, and 5) the person needs to get infected following exposure.

- The conclusions on these steps are:
    - Given the scarcity of data, the probability of bushmeat being contaminated with ZEBOV at the point of origin cannot be accurately assessed. Many species providing bushmeat are not known to be sensitive to infection with ZEBOV, and the current evidence of occurrence of ZEBOV is limited to Western and Central Africa. The virus has been detected in approximately 1/3 of animal carcasses (almost exclusively from non-human primates) in areas where human outbreaks took place. No studies have been conducted outside these areas, but the virus is expected to circulate less frequently when no human or animal outbreaks occur. Bushmeat is frequently processed before exporting, reducing or eliminating viral loads.
    - The probability of infectious ZEBOV being present in bushmeat is considered higher if it originates from species that are susceptible to infection with ZEBOV and comes from areas where ZEBOV is present, especially if the meat is not subject to any processing treatment (i.e. exported fresh or frozen). The highest probability would be for bushmeat sourced from animals from areas experiencing active virus transmission in wildlife, especially from animals found dead.
    - Based on existing studies on checks of personal passengers luggage, the frequency of finding bushmeat illegally imported into the EU has been estimated as one out of 5 000 pieces of luggage from flights from non-EU countries arriving in Austria. The total annual inflow in Switzerland has been estimated at 8.6 tonnes (95 % CI 0.8 to 68.8) and at a major airport in France at 273 tonnes per year. These estimates are uncertain as indicated by the broad confidence interval. The species from which the bushmeat was derived included diverse groups: primates (apes), antelopes, pangolin, birds, porcupines, other rodents, crocodile, blue duiker and unspecified game animals. The processing method for bushmeat was not usually recorded.
    - Specimens seized at European borders varied in condition, including items that were fresh, cooked, smoked, or well dried. It was noted that these items could contain moist inner tissue, which could favour the survival of the virus. There is no information on survival of EBOV in meat or animal products, however it is expected that survival is better at low temperature (4 °C) than at room temperature. The probability of the contaminated bushmeat having viable virus on arrival into the EU would be higher in fresh or frozen meat after a short transport time, and lower in well dried or smoked meat exposed to higher temperatures during transport.
    - There is no published information on how bushmeat products are handled, prepared or consumed, or about the levels of consumption in Europe. Based on African studies, handling and preparation of bushmeat are key risk factors for ZEBOV infection. The handling in Europe does not involve hunting or butchering the wild animal.
    - Based on non-human primate studies, agents causing viral hemorrhagic fevers are believed to be highly infectious. The probability of infection will depend on the exposure route, e.g. ingestion, skin contact, mucosal surfaces etc. The public health consequences of a human case of ZEBOV linked to transmission from bushmeat occurring in Europe would be very serious given the high lethality and potential for secondary transmission
- Due to lack of data and knowledge, which results in very high uncertainty, it is not possible to estimate this risk. However, considering all the elements in the pathway discussed above, and

based on: (i) the limited number (n = 27) of outbreaks that have been reported to date in Africa in spite of the routine consumption of bushmeat in that continent, (ii) the handling of bushmeat in Europe not involving high risk practices such as hunting and butchering, and (iii) the assumed low overall consumption of bushmeat in Europe, it can be assumed that the potential for introduction and transmission of ZEBOV via bushmeat in Europe is currently low. It should be noted that the public health consequences of such an event (a human case of ZEBOV linked to transmission from bushmeat occurring in Europe) would be very serious given the high lethality and potential for secondary transmission.

- In addition, it should be noted that the information considered in this report is largely based in historic Ebola outbreaks and not on the current outbreak in West Africa, first reported to the WHO on 22 March 2014.

2. What is the survival of EBOV through meat or animal products?

- Considering that ZEBOV infection in some animal species, such as in non-human primates, is characterised by a haemorrhagic disease, it is reasonable to expect that an extensive viraemia occurs and that the virus is present in blood and in all organs, secretions and excretions at the height of infection or when the animal has died. The virus has also been isolated from the muscle of non-human primates. It can, therefore, be assumed that the virus will be present in the meat of such animals immediately after slaughter or death.

- There is no information on survival of EBOV in meat or animal products; however, it is expected that survival is better at low temperature (4 °C) than at room temperature. The probability of the contaminated bushmeat having viable virus on arrival into the EU would be higher in fresh or frozen meat after a short transport time, and lower in well dried or smoked meat exposed to higher temperatures during transport.

3. WHO recommended for risk reduction, amongst others, "*to reducing the risk of wildlife-to-human transmission from contact with infected fruit bats or monkeys / apes and the consumption of their raw meat. Animals should be handled with gloves and other appropriate protective clothing. Animal products (blood and meat) should be thoroughly cooked before consumption*."

Is meat from these species able to transmit/carry the EBOV? Are there other species potentially dangerous?

Are there any data available on physical (especially heating) or chemical treatments that would inactivate the EBOV in products of animal origin and especially in meat?

- In the very limited number of studies to date, EBOV has only been detected in carcasses of gorillas (*Gorilla gorilla*), chimpanzees (*Pan troglodytes*), duikers (*Cephalophus* spp.) and from live individuals of some species of Old World fruit bats (*Epomops franqueti, Hypsignathus monstrosus, Myonycteris torquata*), small rodents (*Mus setulosus*, *Praomys* spp.) and in one species of shrew (*Sylvisorex ollula*). In addition, antibodies against EBOV have been reported in these and other fruit bat species (*Epomophorus gambianus, Eidolon helvum, Micropterus pusillus, Mops (Mops) condylurus* and *Hipposideros gigas*[6]**Error! Bookmark not defined.**, *Roussetus aegyptiacus* and *Rousettus (Rousettus) amplexicaudatus*) and dogs (*Canis lupus familiaris*).

- All available studies on physical or chemical treatments have been performed on cell-cultured virus as starting material, in some cases diluted with serum. These studies indicate that:

    - ZEBOV can survive in liquid media for many days. Survival is better at low temperature (4 °C) than at room temperature.

  - Freezing or refrigeration will preserve infectivity of ZEBOV; ZEBOV can survive multiple freezing/thawing and a long-term storage when frozen.
  - Inactivation of ZEBOV occurs when heated at 75 °C for 30 minutes.
  - Inactivation of ZEBOV can be achieved by UV and gamma irradiation.
  - Inactivation of ZEBOV can be achieved by 1 % formaldehyde or with β-propiolactone.
  - ZEBOV is susceptible to 3 % acetic acid (pH 2.5), 1 % glutaraldehyde, alcohol-based products, and dilutions (1:10-1:100 for ≥ 10 minutes) of 5.25 % household bleach (sodium hypochlorite), and calcium hypochlorite (bleach powder).

- Hardly any information is available on the effect of salting, smoking or drying of meat on ZEBOV infectivity. Therefore a conclusion cannot be reached regarding the effectiveness of these methods for virus inactivation. Thorough cooking (100 °C) will rapidly and efficiently destroy the virus.

RECOMMENDATIONS

- The prevention of all illegal imports of bushmeat into the EU should be strictly enforced, as this would be the most effective measure to prevent the transmission of ZEBOV in Europe arising from the handling and preparation prior to consumption, as well as from consumption, of bushmeat.

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