Zoonosis risks along bushmeat supply chains in Central Africa. The case of the Salonga Landscape as source, Kinshasa and other major urban centres as destination, Democratic Republic of Congo

Key findings and recommendations - July 2024

Project led by the *World Wildlife Fund* (WWF), in partnership with the *Institut National de Recherche Biomédicale* (INRB) and the Helmholtz Institute for One Health (HIOH).

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[1] The International Alliance against Health Risks in Wildlife Trade: International Alliance against Health Risks in Wildlife Trade (alliance-health-wildlife.org)

SUMMARY

- From May to August 2022, WWF and its partners the *Institut National de Recherche Biomédicale* (INRB) and the Helmholtz Institute for One Health (HIOH) visited 159 outlets, interviewed 1,288 people involved in the bushmeat trade, and collected and analysed 656 samples of nine orders of wild animals found for sale, between the landscape of the Salonga National Park and several urban centres in the Democratic Republic of Congo (DRC), to describe the bushmeat trade and assess the risks of zoonoses along the supply chain.
- 69% of the 159 outlets visited represented a high or very high risk of zoonosis.
- Artiodactyls, Primates and Rodents made up the vast majority of taxa found at the outlets and were those most often cited by those involved in the bushmeat trade. Carnivores and Pholidots (pangolins), other taxa with a high pathogenic risk, were also sometimes cited or found at outlets.
- More than half of those questioned said that they hunt, transport, sell or consume bushmeat several times a month, or even every week.
- Bushmeat is transported and stored with other food products, outside refrigerated compartments (e.g. coolers), sometimes over several hundred kilometres, passing through several intermediaries (hunters, transporters, vendors), mainly from territories bordering the Salonga National Park to urban centres, in the form of fresh or dried/smoked whole dead animals and pieces of dried or smoked meat.
- Fresh whole animals are butchered at the outlets, so that in most territories, and for most taxa, fresh meat can be found in comparable or even higher proportions than smoked/dried meat. Wild meat is generally sold in contact with other food products.
- Bushmeat is handled under limited hygiene conditions (little or no running water, soap, cleaning of hands and surfaces in contact with the meat).
- Bushmeat consumption is mainly motivated by a taste preference for wild rather than domestic meat, but also by the idea that it is healthier.
- Less than 10% of those questioned said they had been directly or indirectly exposed to a disease linked to the consumption or handling of bushmeat, and less than 15% of them knew what zoonoses were.
- The samples came from 303 Artiodactyls, 179 Primates, 120 Rodents, 19 Carnivores, 24 Reptiles (Squamates, Testudines, Crocodilians), 10 Pholidots and one Tubulidendata. We detected viruses and bacteria with zoonotic potential in eleven samples (around 1.7% of samples) in the territories of Lomela, Kole, Dekese, Oshwe, Bulungu/Kikwit, and Kinshasa, in primates (4.5% of primate samples) and Artiodactyls (1% of Artiodactyl samples).
- We detected the *Primate* T-Lymphotropic Virus *(PTLV*), the Taterapox virus, an Orthopoxvirus, the bacterium Bacillus Cereus Biovar. Anthracis (BCBVA), responsible for anthrax, all of which are transmissible or potentially transmissible to humans.
- The PTLV-positive samples came from roadside stalls and rural markets which we classified as medium risk (two outlets concerned) or high risk (five outlets concerned). The level of risk at these seven outlets was mainly due to the presence of high-risk taxa within them, in particular primates. The samples positive for Taterapox and BCBVA came from two outlets classified as very high-risk. These very high-risk outlets were urban markets selling high-risk taxa.
- The results of this project call for:
    - the introduction of monitoring with more in-depth and regular sampling, particularly at outlets where taxonomic groups known to be transporters of zoonotic pathogens with epidemic or pandemic potential are sold (e.g. Primates and Rodents, but also Artiodactyls as highlighted in our study), outlets frequented by many vendors and customers, and those where sanitary and hygiene conditions are limited or non-existent;
    - developing awareness campaigns on the risks of zoonoses and the behaviours to adopt to reduce these risks;
    - the development of a participatory early warning system for zoonotic diseases;

- the development of policies and practices to strictly regulate and control the bushmeat trade.

CONTEXT

- There is growing concern about the link between the bushmeat trade and diseases or infections transmissible from vertebrate animals to humans (zoonoses). Particularly in Central Africa, where some 60 million tonnes of bushmeat are consumed every year. Bushmeat contributes 20-70% of the total protein intake of human populations, for a trade estimated at 1-3 billion US dollars, and which involves many animal species carrying pathogens with zoonotic potential.

- And this bushmeat from the Congo Basin is not only consumed in the region. For example, around 15 tonnes of bushmeat are smuggled out of Africa every week from the DRC, much of it ending up in major European cities such as London, Paris and Brussels, and in American cities such as Washington.

- Many factors can facilitate and trigger the emergence and spread of zoonoses along bushmeat supply chains, for example:

    - the lack of hygiene at outlets and overcrowding facilitate the spread of pathogens between animals, which increases the number of infected animals, the viral load in these animals, the chances of mutations, and therefore the risk of transmission between animal species;

    - situations where large numbers of people come into close contact with wild animals on a regular basis, lack of hygiene when handling wild animals, the proximity of slaughtering or butchering sites to places where animals are traded or consumed, all increase the risk of transmission of a pathogenic agent from animals to humans;

    - crowded markets in well-served urban areas, and chains where those involved in the bushmeat trade - transporters, vendors and customers - travel long distances by public transport, all increase the risk of rapid, large-scale spread of infection among human populations.

- It is essential to monitor these factors and assess these risks to inform policies for monitoring, regulating and controlling bushmeat consumption and trade in order to mitigate the risks of potentially dramatic zoonoses for both the public health sector and the economy.

- In the landscape of the Salonga National Park in the DRC, recent socio-economic surveys of the local population revealed that 40% of households consumed bushmeat at least four times a week, and that over 60% of them bartered or sold more than half their bushmeat, fuelling growing demand from neighbouring urban centres as far away as Kinshasa.

- It is against this backdrop that the present project has been carried out, in the landscape of the Salonga National Park, the largest tropical forest park in Africa, along the main bushmeat transport routes, roads and rivers, to several of the country's urban centres, including Kinshasa.

PROJECT OBJECTIVES AND KEY ISSUES

- The overall aim of this project was to identify and map the risks of zoonosis transmission in the bushmeat trade along the routes between the Salonga National Park and several urban centres.

- In particular, the project aimed to i) identify and assess the scale of the bushmeat trade, the species consumed and traded, and the modes and drivers of trade and consumption; ii) detect the presence of pathogens in bushmeat samples at different nodal points in the chains; iii) identify the risks triggering the presence of pathogens, transmission to humans, and the spread of infections to human populations along the chains.

METHODS

To answer the study questions, we conducted:

1. Documentary research to collate existing data and pre-identify the main areas where bushmeat is hunted, traded and consumed.

2. Visits to bushmeat outlets to identify and map the risks of zoonoses using the approach and tool developed by the WWF[2] (Figure 1), in order to assess:
    - The risks of zoonoses linked to the characteristics of outlets, i.e. their size, hygiene conditions, volume of vendors and customers;
    - The risk associated with taxonomic groups sold at these outlets and known to be transporters of zoonotic pathogens with epidemic or pandemic potential.

3. Interviews of hunters, transporters, vendors, and consumers to gather detailed information on the movement of bushmeat along the supply chains, and on consumption and trade behaviour, including harvesting, handling, storage, transport and consumption practices.

4. Sampling of bushmeat at outlets for detailed identification of zoonotic pathogens: (i) complete families or genera of viruses, including Coronaviruses, Filoviruses (e.g. Ebola and Marburg viruses), Orthopoxviruses (e.g. Monkeypox virus) and various Retroviruses (e.g., Simian Immunodeficiency Virus/Human Immunodeficiency Virus and PTLV/Human T-Lymphotropic Virus), and ii) Bacillus anthracis and BCBVA anthrax bacteria.

   We selected these target pathogens because their transmission to humans can be linked to bushmeat, and their monitoring is relevant not only at Congo Basin level but also at global level. Coronaviruses contain important zoonotic pathogens responsible for respiratory diseases, including Middle East Coronavirus (MERS-CoV) and Severe Acute Respiratory Syndrome Coronaviruses 1 and 2 (SARS-COV-1 and SARS-CoV-2). Filoviruses include the Ebolavirus genus, members of which can cause Ebola virus disease, a serious illness with a high fatality rate that occurs sporadically in the DRC and other African countries. Retroviruses include the human immunodeficiency virus, responsible for AIDS, but also other important zoonotic viruses such as PTLV, which can cause leukaemia and tropical spastic paraparesis in humans. Anthrax, caused by Bacillus anthracis and BCBVA, is a serious, acute and often fatal disease which, depending on the route of infection, can affect the skin, lungs and intestinal tract. While Bacillus anthracis is present in savannah ecosystems, the BCBVA bacterium has been detected in a wide range of mammalian species in the tropical rainforests of West and Central Africa.

   We carried out laboratory analyses at the INRB in Kinshasa, and confirmatory analyses at the HIOH in Greifswald, Germany. We first extracted genetic material from the samples using commercially

[2] Dietrich, M., et al. 2020. Assessing risk factors for viral disease emergence within the wildlife trade. WWF Wildlife Practice.
Wikramanayake, E., Olson, D., Pfeiffer, D. A tool for rapid assessment of wildlife markets in the Asia-Pacific region for risk of future zoonotic disease outbreaks. One Health. 2021;13

available extraction kits. The extracts generated contained both host and pathogen genetic material, if the animal was infected. We then tested whether the genetic material contained the pathogens described above using polymerase chain reaction (PCR), a standard laboratory tool that amplifies and identifies genetic markers of interest. We used different PCR systems targeting the pathogens mentioned above, as well as a PCR to identify the mammalian species from which the sample was taken. If a PCR was positive, we proceeded to sequence the genetic material in order to confirm our results and obtain additional information about the pathogen in question (Figure 2).

From 15 May to 18 December 2022, our field teams[3] have:

- Travelled through the territories of Bulungu, Ilebo, Kinshasa, Kole, Lodja, Lomela and Oshwe;
- Carried out surveys at 159 outlets in four different categories: roadside stalls, restaurants, rural markets and urban markets, involving 357 bushmeat vendors (Photo 1);
- Interviewed 1,288 hunters, transporters, vendors and consumers[4] of bushmeat (Photo 2);
- Collected and analysed 656 samples from nine different orders of animals (Photo 3), 303 from Artiodactyla, 179 samples from Primates, 120 from Rodents, 19 from Carnivores, 24 from Reptiles (Squamates, Testudines, Crocodilians), 10 from Pholidots, and one Tubulidendata.

RESULTS

Results of outlet surveys

- Risk of zoonosis at outlets (Map 1):
    - 84% of the 159 outlets visited sold bushmeat.
    - 69% of these outlets represented a high or very high risk of zoonoses. These were outlets:
        - where whole wild animals, their parts and their meat are piled up, mixed with other food products, under limited hygiene conditions (Photo 4);
        - where wild animals are handled, butchered and cut up without hygiene measures or protection, in contact with customers and other food products (Photo 5);
        - which are hubs of the bushmeat trade, supplied by vendors who bring in bushmeat from distant territories, and frequented by numerous customers who sometimes travel very long distances to get their supplies;
        - where taxa known to be transporters of zoonotic pathogens with epidemic or pandemic potential are sold, e.g. monkeys, rats, pangolins, viverrids (high risk), squirrels, suids, artiodactyls, porcupines (medium risk) (Photo 6).
    - This is particularly true of the urban markets in Kinshasa, Ilebo and Kole, as well as numerous small stalls in the territories of Oshwe, Dekese, Kole and Lomela along the roads between the Salonga National Park and the main urban centres, and along the Lukenie river between Dekese and Oshwe.

- Wild animal products for sale (Map 2) :
    - Of the 651 products (meat, legs, viscera, etc.) that our teams found at the outlets visited:
        - 51% came from species of the order Artiodactyla, mainly Bay duiker (*Cephalophus dorsalis*), Yellow-backed duiker (*Cephalophus silvicultor*), Black-fronted duiker

[3] WWF DRC employees and local civil society organisations such as APPACOL and RAIFORCO.

[4] 99% of hunters were men (out of 217), 36% of transporters were women (out of 424), 66% of sales staff were women (out of 422), and 50% of consumers were of each sex (out of 225).

(*Cephalophus nigrifrons*), Blue duiker (*Philantomba monticola*), Weyns' duiker (*Cephalophus weynsi*) and Red River Hog (*Potamochoerus porcus*).
        - 30% came from the order of Primates, mainly from Red-tailed Monkey (*Cercopithecus Ascanius*), De Brazza's Monkey (*Cercopithecus neglectus*), Tshuapa Red Colobus (*Piliocolobus tholloni*), Angolan Colobus (*Colobus angolensis*), Golden-bellied Mangabey (*Cercocebus chrysogaster*) and Black Mangabey (*Lophocebus aterrimus*).
        - 15% were rodents, mainly African Brush-tailed Porcupine (*Atherurus africanus*) and Southern Giant Pouched Rat (*Cricetomys ansorgei*).
    - Of the 357 vendors inspected, 64% sold Artiodactyls, 40% Primates and 21% Rodents.
    - Depending on the area, between 40% and 80% of the products found at outlets were fresh (not smoked or dried), sold mainly as meat (57% of products) but also as whole and dead animals (35%).

***Results of surveys of hunters, transporters, vendors and consumers of bushmeat* (Figure 3:)**

What are your favourite species?

- Artiodactyls (41-46% of responses), Primates (26-35%) and Rodents (14-30%) were the taxa most frequently cited by respondents.
- For these taxa, the species most often cited are duikers, Red River Hog, Cercopithecus, Colobus, Mangabeys, Bonobo, African Brush-tailed Porcupine and Southern Giant Pouched Rat.
- Carnivores, such as civets and mongooses, are the fourth most frequently cited taxon (5% or less).
- These results corroborate the observations made at outlets.

How often is it traded and consumed?

- More than half of those questioned said they hunt, transport, sell or consume bushmeat several times a month, or even every week.
- Among consumers, 95% had eaten bushmeat in the 12 months prior to the study.
- What drives consumption ?
    - For 50% of consumers surveyed, bushmeat tastes better than domestic meat, and 31% say it is healthier.
    - less than 20% say they eat bushmeat because it is cheaper or easier to find than domestic meat.

How is bushmeat transported, handled and stored, and in what forms?

Hunters

Transport

- 90% of the hunters questioned transport animals (whole or in pieces) without changing territory, i.e. the destinations of their hunting products are in the same territories as their hunting areas.
- They cover short distances, averaging 24 ± 22 kilometres. On foot or by bicycle on the roads, and by pirogue on the rivers.
- Their products are intended for personal consumption as well as for sale or barter in the villages and communities in their territory.

Forms

- Among hunters, bushmeat is mainly handled in the form of whole dead animals: this form accounted for 73% of responses for Artiodactyls, 83% for Primates and 81% for Rodents.

- The remainder is handled in the form of fresh or smoked/dried pieces (equivalent % of responses) (Photo 7).

Storage

- The majority of animals killed are eaten or sold immediately or within 24 hours (62 - 75% of responses for Artiodactyls, Primates and Rodents).
- The remainder is stored for several days or even weeks (24 - 37% of responses for Artiodactyls, Primates and Rodents).
- Storage is mainly at the hunter's home, without the use of a cool box or refrigerated compartment.
- Fresh meat is generally stored here for a few hours or even a few days, while dried/smoked meat is stored for a few days or even a few months.

Transporters

Transport (Map 3)

- Transporters and vendors collect hunting products in the Salonga Landscape territories, mainly Oshwe, Monkoto, Dekese Lomela and Kole.
- The towns of Oshwe, Lodja, Lomela and Dekese appear to be the main hubs of the bushmeat trade in the Salonga Landscape, with buyers travelling up to several hundred kilometres for supplies.
- From these areas and towns, bushmeat is then transported to be (re)sold in the same areas, in neighbouring areas, but also as far away as Kinshasa, Kamoni and Kabinda[5] .
- The distances covered can therefore reach several hundred or even thousands of kilometres.
- The main modes of locomotion mentioned are walking, cycling and motorbikes on the road, and pirogues and *baleinières* on the river route to Kinshasa.
- For longer journeys, this suggests transport in several sections involving several intermediaries.
- Over 50% of the transporters questioned said that they transported bushmeat with other food products and/or domestic animals, and did not use any form of refrigeration (e.g. coolers).

Forms

- Artiodactyls are mainly transported in the form of smoked/dried pieces (68% of responses), followed to a lesser extent by whole dead animals (28% of responses).
- Primates and rodents are transported in the form of whole dead animals or smoked/dried pieces in similar proportions (40-50% of responses) (Photo 8).

Vendors

Forms

- Artiodactyls and Primates are mainly sold as dried/smoked meat (around 50% of responses), as whole dead animals (around 30% of responses), then as fresh meat (around 12% of responses).
- Rodents are sold as dried/smoked meat or as whole dead animals in equal proportions (approx. 40% of responses for each), then as fresh meat (approx. 12% of responses).
- For the majority of vendors interviewed, animals are butchered, cut up and displayed on the same stall as other food products (e.g. beef, sheep, pork, fish).
- The waste from butchering and cutting (entrails, viscera, blood, etc.) is left on the ground at the foot of the sales stalls.

Storage

- When they are not sold immediately, animals (whole dead or in pieces) are stored mainly in dried/smoked form (83% of responses), for a period of a few days (61% of responses) or even a few months (24% of responses). Fresh animals or cuts are stored for shorter periods, a few hours (51% of responses) or even a few days (41% of responses).
- These products are stored in the same room/compartment where other food products are stored, and never in coolers or refrigerated compartments.

[5] This study only covers areas in the south/south-western part of the Salonga Landscape. The areas to the north and east of the landscape are certainly the source of other bushmeat trade routes.

Consumers (customers)

Forms

- Artiodactyls are mainly bought in the form of whole dead animals (46% of responses), then in the form of smoked/dried cuts (35% of responses), and to a lesser extent in the form of fresh cuts (18% of responses).
- Primates are mainly purchased in the form of whole dead animals (64% of responses), smoked/dried cuts (22% of responses), and to a lesser extent in the form of fresh cuts (13% of responses).
- Rodents are mainly purchased as whole animals (58% of responses), followed by smoked/dried or fresh cuts in equal proportions (18 and 16% of responses).
- Equivalent percentages of responses were also observed for Carnivores and Pholidots, which were the fourth and fifth most frequently mentioned taxa by respondents, far behind Artiodactyls, Primates and Rodents.

Storage

- For most taxa, purchased bushmeat is usually consumed within 24 hours of purchase.
- If not consumed within 24 hours:
    - Fresh meat is stored for a few days (24% of responses).
    - Smoked/dried meat is stored for a few days (16% of responses) or even a few months (17% of responses).
- Coolers and refrigerated compartments are rarely, if ever, used.

<u>What basic hygiene measures are followed?</u>

- Most people questioned, and hunters in particular, say they regularly come into direct contact with the blood, organs, skin or body fluids of wild animals that are transported, handled or eaten.
- Up to 50% of those questioned said that they had been injured a few times, or even regularly, when handling wild animals.
- However, very few, if any, of the people questioned use protection (e.g. gloves) when handling meat. Similarly, a minority of respondents said that they washed their hands, knives, cutting boards, vehicles or storage areas after handling, transporting or storing bushmeat (Photo 9).

<u>What knowledge or experience do you have of animal diseases?</u>

- Very few (less than 5%) of those questioned said they had fallen ill after handling or eating bushmeat.
- And less than 10% say they know someone who has been ill after handling or eating bushmeat. Among hunters, however, the percentage is higher, at around 16%.
- Around 10% of those questioned said they were aware of illnesses/symptoms linked to eating wild animals, and 23% of hunters.
- The diseases or symptoms most frequently cited are scabies, the appearance of pimples on the skin, Monkeypox and Ebola.
- Monkeys, rodents, squirrels, bats, pangolins and duikers are the taxonomic groups most often cited as being responsible for these diseases or symptoms.

Laboratory analysis results

- We detected viruses and bacteria with zoonotic potential in eleven samples (around 1.7% of samples taken and analysed), in Primates (4.5% of primate samples) and in Artiodactyls (1% of Artiodactyls samples).

- Zoonotic pathogens were detected in various areas close to the Salonga National Park, Dekese (in Dekese and Yoso), Oshwe (in Oshwe and Bombole), Lomela (in Looto/Omandja), Kole (in Kole), as well as in the towns of Kikwit and Kinshasa (Map 5).
- We detected the PTLV virus in seven Primate samples (i.e. 3.9% of Primate samples) from six different outlets, three in Dekese territory (one sample in Dekese and two in Yoso from the same vendor), two in Oshwe territory (in Oshwe and Bombole), one in Lomela territory (in Looto/Omandja) and one in Kinshasa. PTLV is a zoonotic retrovirus known to infect Primates and cause disease in humans. It is the only pathogen that we have also detected in a smoked/dried sample. PTLV is specific to Primates, which is why animals of other orders were not tested for this pathogen.
- We detected a Taterapox virus, an Orthopoxvirus, in a sample from a duiker in Kikwit, in the Sonas town market (0.15% of samples taken and tested). Orthopoxviruses are a viral genus that includes zoonotic viruses and viruses that infect humans, such as the Variola virus (responsible for Smallpox, a virus that has now been eradicated), the virus responsible for monkeypox and the Cowpox virus. The Taterapox virus has not yet been detected in humans. Due to its close genetic relationship with other zoonotic Orthopoxviruses infecting humans, Taterapox virus must be considered as potentially zoonotic. The sample was taken from fresh bushmeat.
- Finally, we detected the bacterium Bacillus Cereus Biovar. Anthracis (BCBVA), responsible for anthrax, in three samples from a Primate and two Artiodactyls (a duiker and a Red River Hog) in the Kole area. The positive samples came from the same town market in Kole (0.5% of all animals sampled and 15.8% of animals sampled at the market), from three different vendors and from pieces of fresh meat.
- The PTLV-positive samples came from roadside stalls and rural markets which we classified as medium risk (two outlets concerned) or high risk (five outlets concerned). The level of risk at these seven outlets was mainly due to the presence of high-risk taxa within them, in particular Primates. The samples positive for Taterapox and BCBVA came from two outlets classified as very high-risk. These very high-risk outlets were urban markets selling high-risk taxa.

CONCLUSIONS

The aim of this project was to identify and map the risks of transmission of zoonoses in the bushmeat trade along the routes from the Salonga National Park to several urban centres, including Kinshasa.

Extent of the bushmeat trade along the routes from the Salonga National Park to several urban centres in the DRC

- The bushmeat trade between the Salonga landscape and the urban centres of the landscape, as far as the towns of Ilebo, Kikwit and Kinshasa, is a dynamic trade fuelled by sustained flows of bushmeat, with a high monthly or even weekly frequency of hunting, transport, sale and consumption.

- It is also a structured and extensive trade with, from the source to the customers, long chains of several hundreds or even thousands of kilometres, involving a large portion of the population, several intermediaries, stages and destinations[6] .

- Several taxonomic groups are involved in this trade, which is mainly made up of Artiodactyls, Primates and Rodents, followed to a lesser extent by Carnivores and Pholidots. These are

[6] This project only covered the southern and south-western parts of the Salonga landscape. Other trade routes originate in the north and north-east of the landscape and supply other destinations.

taxonomic groups known to be transporters of zoonotic pathogens with epidemic or pandemic potential.

- The main reasons why respondents eat bushmeat are a preference for its taste and the belief that it is healthier than domestic meat. For a smaller proportion of the population surveyed, bushmeat consumption is motivated by the scarcity and/or higher price of domestic meat.

Presence of pathogens in bushmeat samples at various nodal points in the supply chain

- We found more pathogens causing chronic infections than acute infections. PTLV causes a chronic infection in Primates, while BCBVA and Taterapox cause acute infections in different Mammalian hosts. The time taken to hunt an animal suffering from an acute infection is much shorter than for a chronic infection. In fact, animals infected with BCBVA generally die so quickly that it seems more likely that the positive cases come from found carcasses rather than from hunted animals. We detected PTLV in its Primate hosts, while Taterapox virus and BCBVA were detected in accidental hosts (the reservoirs of Taterapox virus are Rodents, and BCBVA can survive for long periods in the environment). Taken together, these factors may help to explain why the majority of pathogens detected in this study were PTLV (64%, compared with around 27% BCBVA and only 9% Taterapox).
- Pathogens causing acute infections were detected in urban markets classified as very high risk. BCBVA and the Taterapox virus were detected in urban markets classified as very high risk. PTLV, which causes chronic infections, was detected in roadside stalls and rural markets classified as medium to high risk. Although this is a very interesting observation, the data are not sufficient to draw any conclusions about the link between the level of risk at the point of sale and the chronic or acute nature of the infection transmitted by the pathogen found there.
- Bushmeat can potentially serve as an amplifying host for pathogens acquired from other reservoir species. We detected Taterapox virus in a Artiodactyl, although rodents are the natural hosts of the virus. Artiodactyls are known to be susceptible to certain Orthopoxviruses, in particular the various cowpox viruses (which also have rodent reservoirs). Our results suggest that Taterapox can pass from its rodent reservoir to Artiodactyls. Since Artiodactyls are the most frequently consumed bushmeat, they could be a source of human infection by Taterapox. Although no human cases have been reported to date, it cannot be ruled out that the virus could also be transmitted to humans, who are susceptible to closely related Orthopoxviruses (responsible for Smallpox, Monkeypox and Cowpox).
- Bushmeat can contain extremely dangerous acute pathogens. BCBVA was detected in three animals from the same urban market in the town of Kole. The very localised detection of BCBVA in bushmeat sampled on the same day is probably a snapshot of an acute epidemic in wildlife. Although a rare event, it also demonstrates that extremely dangerous pathogens can be found in the bushmeat trade. BCBVA causes anthrax, an acute and often fatal disease.
- The detection of BCBVA suggests that carcasses (i.e. animals found dead, not hunted) are sold as bushmeat. For Primates infected with BCBVA, it has been observed that the animals die within hours of the onset of the disease. For classical anthrax (caused by Bacillus anthracis), ungulates (Artiodactyls) are known to die rapidly once infected. It is therefore very likely that BCBVA-positive bushmeat samples come from found carcasses rather than from hunted animals. Contact with found carcasses is riskier than contact with hunted bushmeat, as many animals die from an infectious disease that can also be harmful to humans. The use of found carcasses as bushmeat should be avoided.
- All but one of the positive samples were taken from fresh bushmeat. PTLV was the only pathogen also detected in a dried/smoked sample. Smoking/drying can inactivate certain pathogens. The fact that we were able to detect PTLV in a dried/smoked sample may be linked to the biology of the virus; as a Retrovirus, PTLV integrates a copy of its genome into the host's genetic material.

Although PTLV genetic material can still be detected in this form, we were unable to determine whether the dried/smoked bushmeat was still infectious at the time of collection.

- Bushmeat containing pathogens can reach Kinshasa and other major urban centres. In the capital Kinshasa, only one dried/smoked sample was positive for a pathogen (PTLV). However, fresh samples are also transported to Kinshasa, and although in this study no pathogens were detected in fresh bushmeat sampled in Kinshasa, their detection in other regions shows that infected bushmeat can potentially reach Kinshasa and other major population centres. The detection of the Taterapox virus in Kikwit is another example.
- Primates can be considered a high-risk taxon. Due to the close relationship between Humans and Non-Human Primates, these species are susceptible to a similar range of pathogens. In this study, around 72% of pathogen-positive samples were from Primates (although this figure is also influenced by our selection of target pathogens). Beyond Primates, it is not possible to draw conclusions from our study on the risk of diseases associated with different taxa. However, it is clear that species that are not considered high-risk may also be transporters of important zoonotic pathogens, such as Artiodactyls.
- The detection of pathogens with zoonotic potential in around 1.7% of bushmeat samples is quite striking. Given the huge amount of bushmeat consumed and traded in the region, this suggests that local populations are regularly exposed to these pathogens. The detection of BCBVA is particularly worrying, not only because BCBVA can cause fatal disease in humans, but also because it indicates that animals found dead are ending up on bushmeat markets. The risk of carcasses containing pathogens is higher than for hunted animals. For example, the butchering and consumption of infected primate carcasses has led to the spread of the Ebola virus in the past. Fortunately, none of the pathogens detected has the epidemic potential of the Ebola viruses. Infections with human T-lymphotropic virus (the human equivalent of PTLV) occur in the DRC but do not spread rapidly among humans. No human cases of the Taterapox virus are known to date, but the related Monkeypox virus is endemic in the DRC. BCBVA is not easily transmitted between humans, so potential cases of transmission of BCBVA to humans would probably involve only one or a few individuals and could therefore go undetected.

Factors favouring the presence of pathogens, animal-to-human transmission and the spread of infections to human populations along value chains from the Salonga landscape to urban centres

A multitude of factors, including the structural conditions of the outlets, the length of the supply chains, the taxa marketed and certain behaviours, all contribute to increasing the risk of transmission of infection from wild animals to humans and the spread of infection in human populations along the bushmeat supply chains from the Salonga landscape:

- **Risk of transmission between animals:**
    - Presence of taxa known to carry zoonotic pathogens with epidemic or pandemic potential.
    - Wild animals and meat transported over long distances, stored and sold whole and fresh, in unrefrigerated conditions and in contact with other food products, including domestic animals and meat.

- **Risk of transmission to humans:**
    - People who are regularly exposed and soiled by the bodily fluids of the wild animals they hunt, transport, sell and consume.
    - People who often injure themselves handling animals and wild meat.
    - A lack of running water, soap and sanitary facilities at outlets.
    - They rarely clean the knives, stalls, vehicles or compartments used to cut, sell, transport or store wild animals and meat.

- A lack of knowledge about zoonoses does not encourage the adoption of healthy and hygienic behaviours that could reduce the risk.
- Handling the carcasses of animals found dead (and not hunted).

- **Risk of human-to-human transmission:**
  - Chains involving several intermediaries, along which wild animals and meat, but also potentially infected people, travel long distances.
  - These markets are veritable hubs of the bushmeat trade, with bushmeat, vendors and customers regularly flocking to them in large quantities.

RECOMMENDATIONS

1. For a more exhaustive understanding of the conditions that favour the emergence of pathogens, their spread between animals, transmission to humans, and then their spread within human populations in bushmeat chains, we recommend further study, in particular by:
   a. Carrying out targeted sampling of at-risk taxa at the start of the chain with hunters, once they have hunted, and on fresh animal tissues.
   b. Sampling the environmental DNA of surfaces with which animals and wild meat have come into contact, for example in baskets and vehicles used to transport animals and meat, on stalls and cutting boards, on knives, and also on floors soiled with animal entrails, viscera and blood.
   c. Carrying out serological monitoring in communities where zoonotic diseases are suspected (after obtaining their prior consent). Sampling could be carried out in central hospitals and local clinics.
   d. By sampling at different times of the year, to take account of potential seasonal effects.
   e. Testing samples to detect a greater number of known zoonotic pathogens and using pathogen discovery to detect unknown pathogens.
   f. Identifying other methods of trade for species not present at the outlets in this study but which are known to be consumed, in particular chiropterans.
   g. By investigating hunting and animal acquisition practices, in particular whether and under what conditions animals found dead are collected and eaten or sold.

2. To inform the public about zoonoses and the factors that encourage their appearance and spread: run a campaign to raise awareness of the risks among hunters and trade hubs.

3. To limit risky behaviour, e.g. lack of hygiene, consumption and trade in species potentially carrying pathogens: run a campaign to change behaviour, aimed in particular at improving hygiene conditions at outlets and avoiding wild animal carcasses.

4. To detect the emergence of zoonoses more quickly and systematically, and improve surveillance, and to do so:
   a. Design and implement sampling strategies and routine tests:
      - With the participation of the *Institut Congolais pour la Conservation de la Nature* (ICCN), and under the supervision of INRB, hire and train ecoguards to collect samples in the Salonga landscape.
      - Do the same in the most at-risk markets with agents from health institutions, and with the collaboration of civil society organisations.
      - Set up routine tests in health centres along the supply chains and analyse all samples collected within a short timeframe.

b. Set up mobile clinics in communities[7] and train them in disease prevention, symptom identification, healthy hunting practices, hygiene practices, proper treatment of bushmeat to reduce the presence of pathogens (e.g. cooking it).
c. Develop a participatory early warning system for zoonotic diseases requiring:
    - Improvement of existing communication protocols for rapid notification of health authorities.
    - Standard operating procedures specific to the surveillance sites in order to respond appropriately to any positive cases of zoonotic diseases in humans or animals.
    - Engagement with national authorities and other relevant organisations (World Health Organisation, World Organisation for Animal Health) to understand the chain of communication of positive laboratory results, and identify any gaps.

5. Develop policies and practices to rigorously regulate and control the bushmeat trade, identify the conditions for moving from an unsustainable, unsafe and illegal bushmeat trade to a sustainable, safe and legal trade in the DRC[8] .

ACKNOWLEDGEMENTS

The partners in this project would like to warmly thank all the institutions and individuals who have contributed to its success. In particular, we would like to thank the International Alliance against Health Risks in Wildlife Trade, the *Institut Congolais pour la Conservation de la Nature*, the Ministry of the Environment and Sustainable Development, the Ministry of Scientific Research, the CITES Coordination in the DRC, Mr. Papy Lolatui, Mr. Ménard Mbende, Mr. Christian Mpassi, the APPACOL and RAIFORCO teams, and all the local communities approached by the field teams.

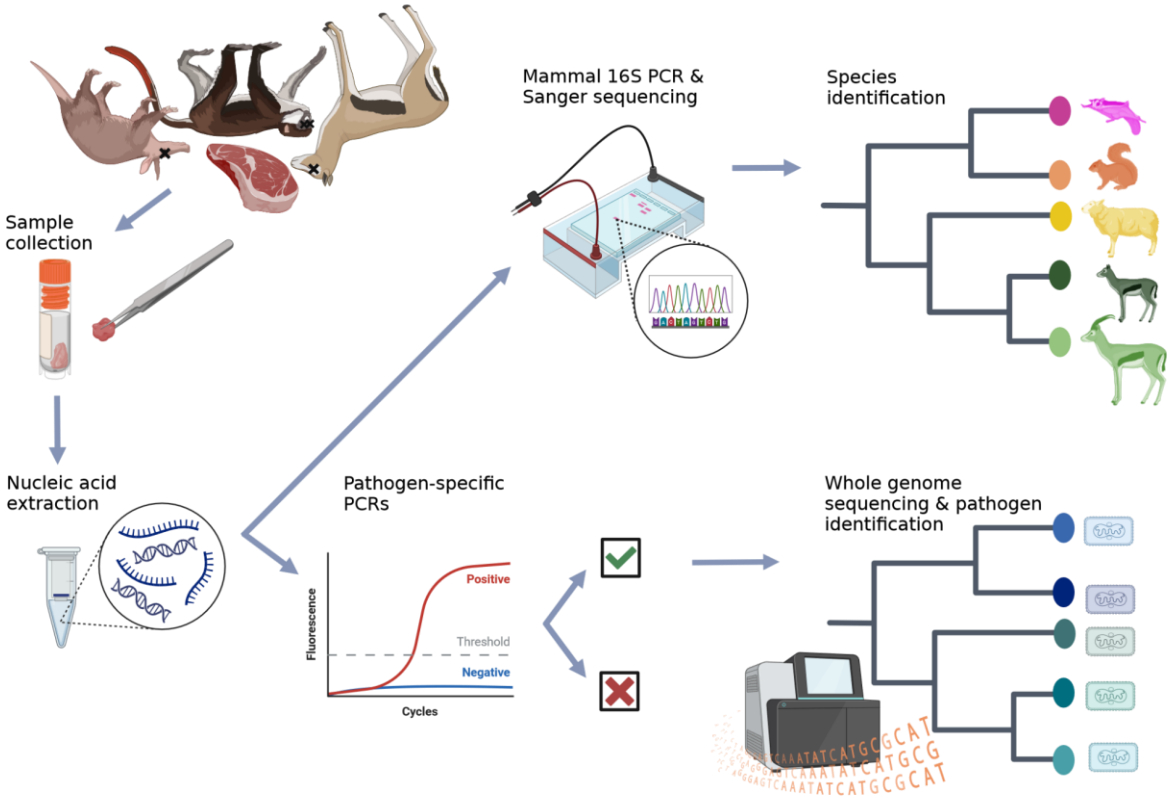
[7] For example, the Bwanga Project, https://bwanga.org/

[8] See the *Sustainable Wildlife Management* Project (https://www.swm-programme.info/) for an example.

Figure 1: Matrix of risks associated with types of outlet and the taxa sold there (Adapted from Wikramanayake et al., 2021).[2]
LW: Low Risk; MR: Medium Risk; HR: High Risk; VHR: Very High Risk.

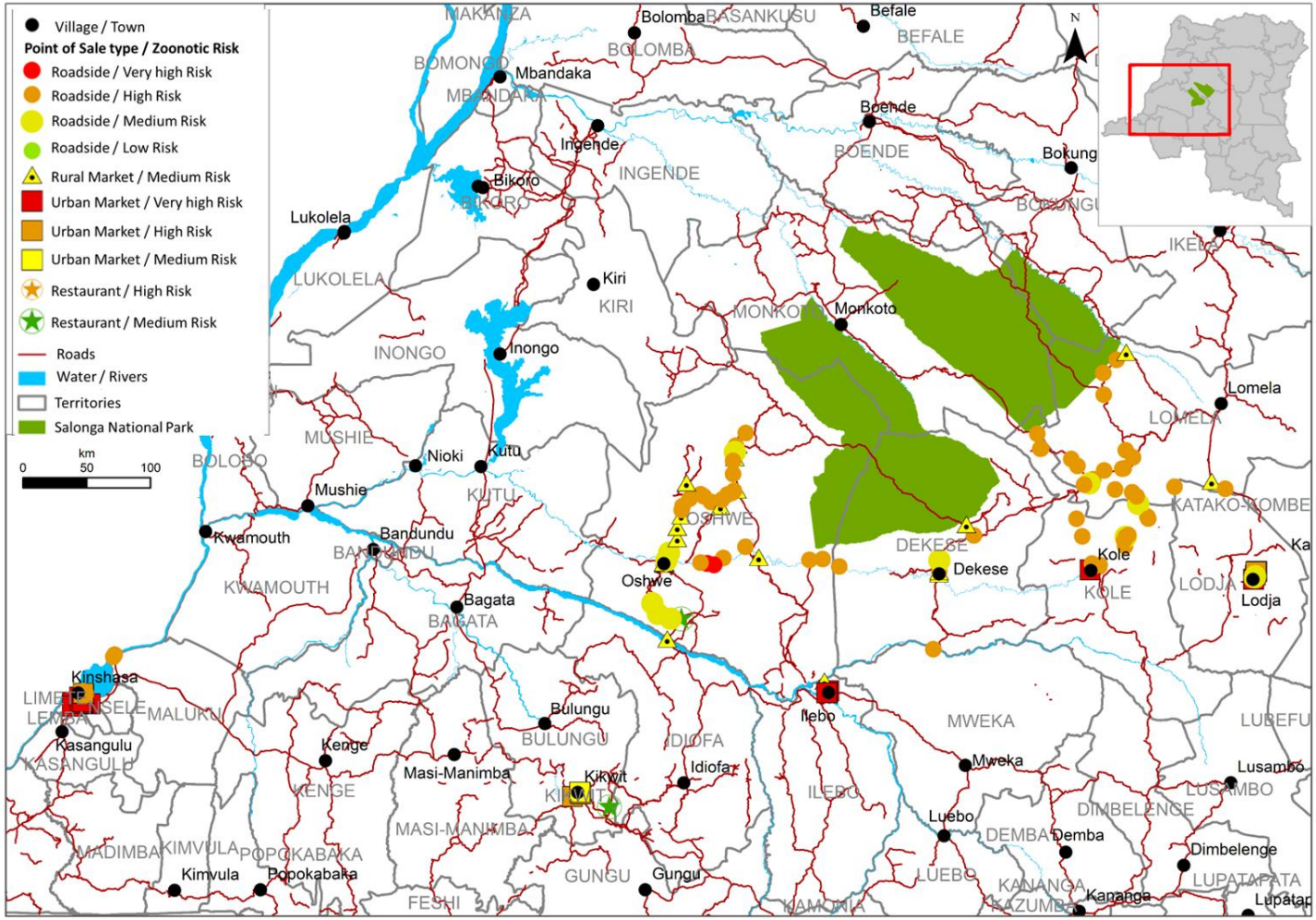
Combined risk of taxon on sale and type of outlet						
High risk taxa: Primates, Rodents, Manids, Viverrids (civets)	High Risk (HR)	**Risk associated with taxa present at points of sale**	MR	HR	VHR	VHR
Medium risk taxa: Artiodactyla (Bovidae, Suidae), Hystricidae (porcupine)	Medium Risk (MR)		MR	MR	HR	HR
Taxa at low risk: Reptiles, Amphibians, Fish	Low Risk (LR)		LR	MR	MR	MR
			Risk linked to the type of outlet			
			LR	MR	HR	HR
			Rural bushmeat market	**Roadside or riverside stalls**	**Bushmeat restaurant**	**Urban bushmeat market**

Figure 2: Laboratory workflow for pathogen analysis and species identification (graphic created with Biorender.com, Credit JS).

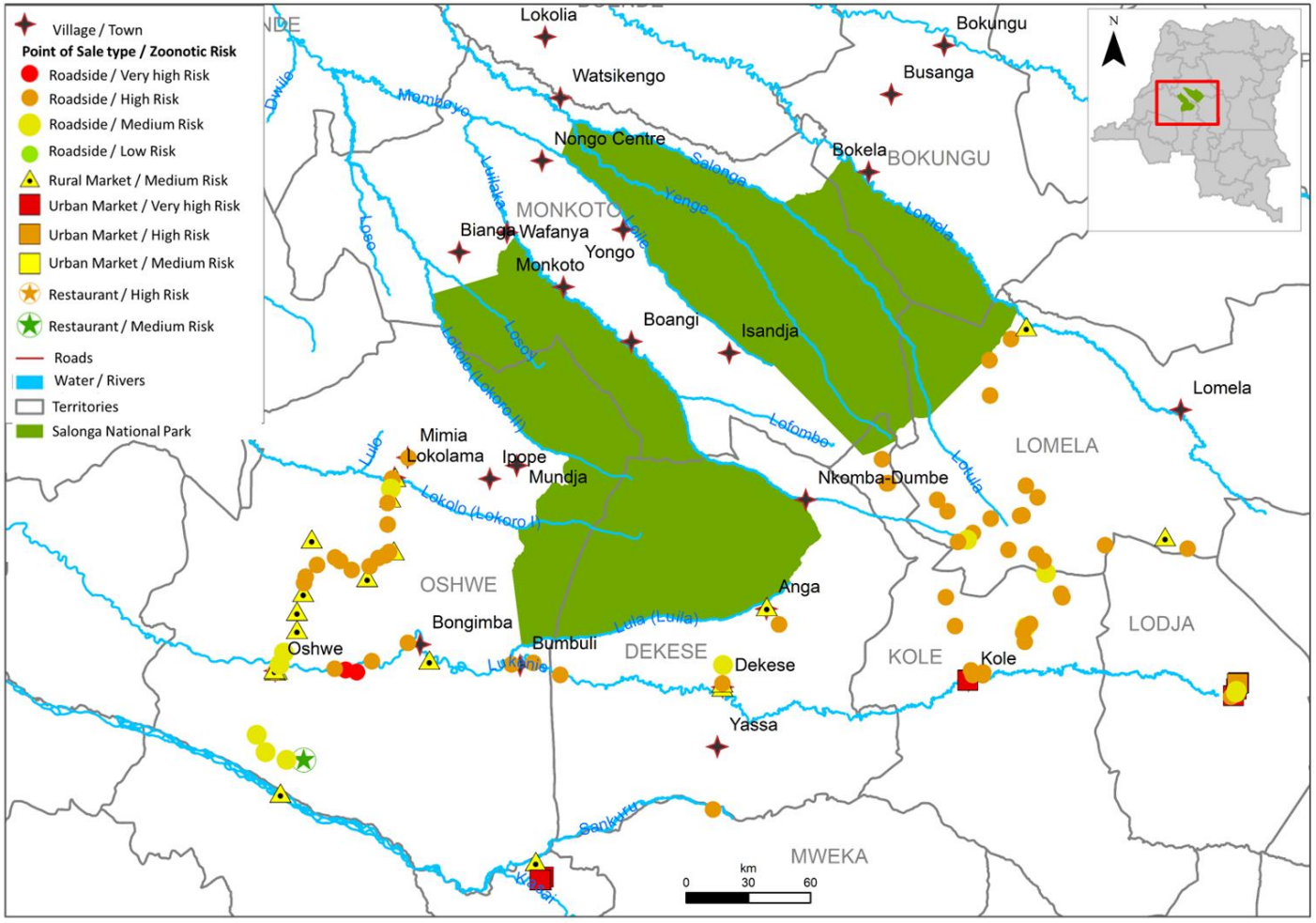


Map 1: Risks of zoonoses at outlets visited between May and August 2022, a) over the entire study area, b) in the Salonga landscape.

a)



b)


Village / Town
Point of Sale type / Zoonotic Risk
Roadside / Very high Risk
Roadside / High Risk
Roadside / Medium Risk
Roadside / Low Risk
Rural Market / Medium Risk
Urban Market / Very high Risk
Urban Market / High Risk
Urban Market / Medium Risk
Restaurant / High Risk
Restaurant / Medium Risk
Roads
Water / Rivers
Territories
Salonga National Park
Lokolia
Bokungu
Watsikengo
Busanga
Nongo Centre
Bokela
BOKUNGU
MONKOTO
Biangi
Wafanya
Yongo
Monkoto
Boangi
Isandja
Lomela
LOMELA
Mimia
Lokolama
Ipope
Mundja
Nkomba-Dumbe
OSHWE
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Map 2: Proportions of taxonomic groups found on vendors' stalls at outlets visited between May and August 2022 (out of 651 products).

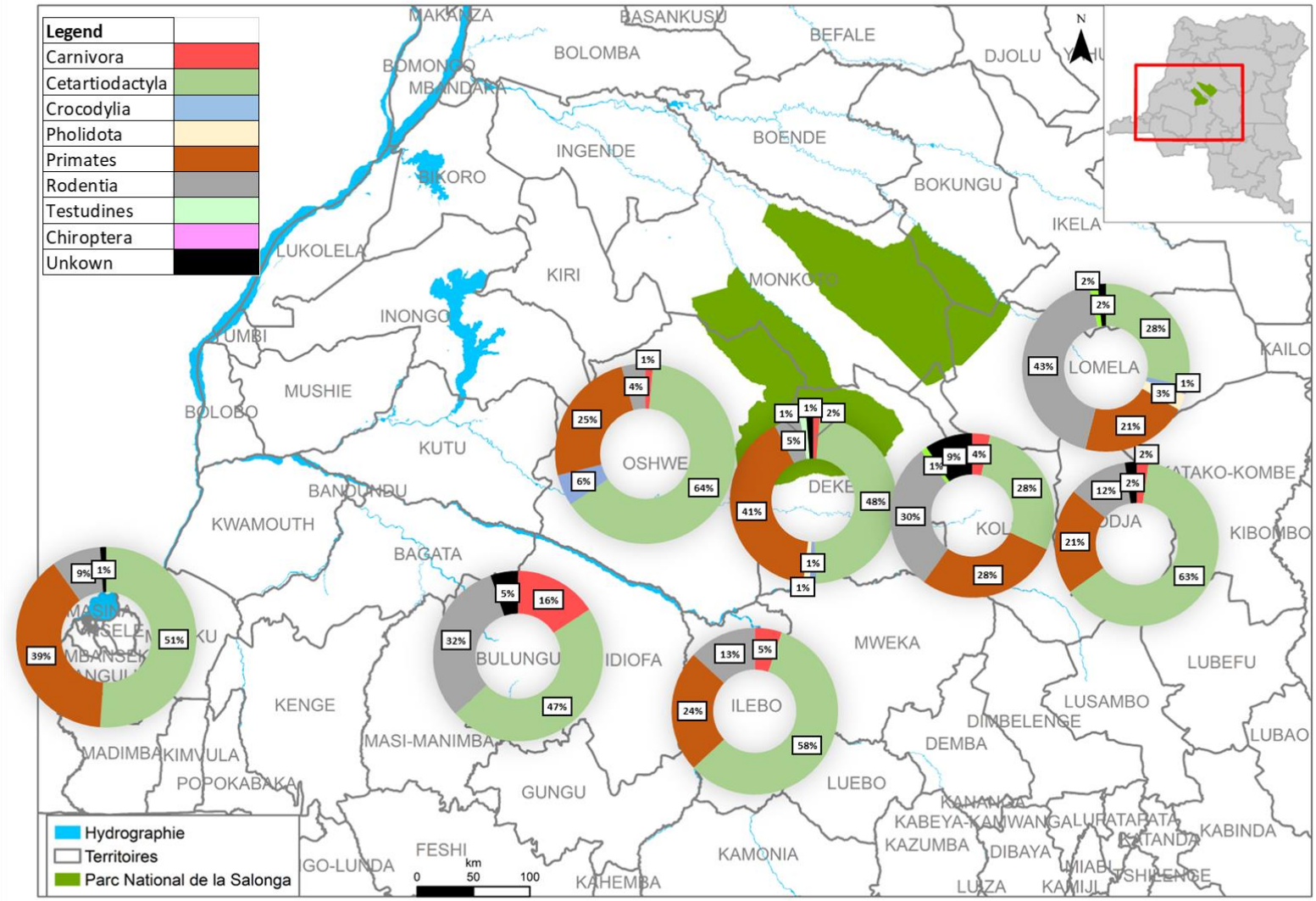
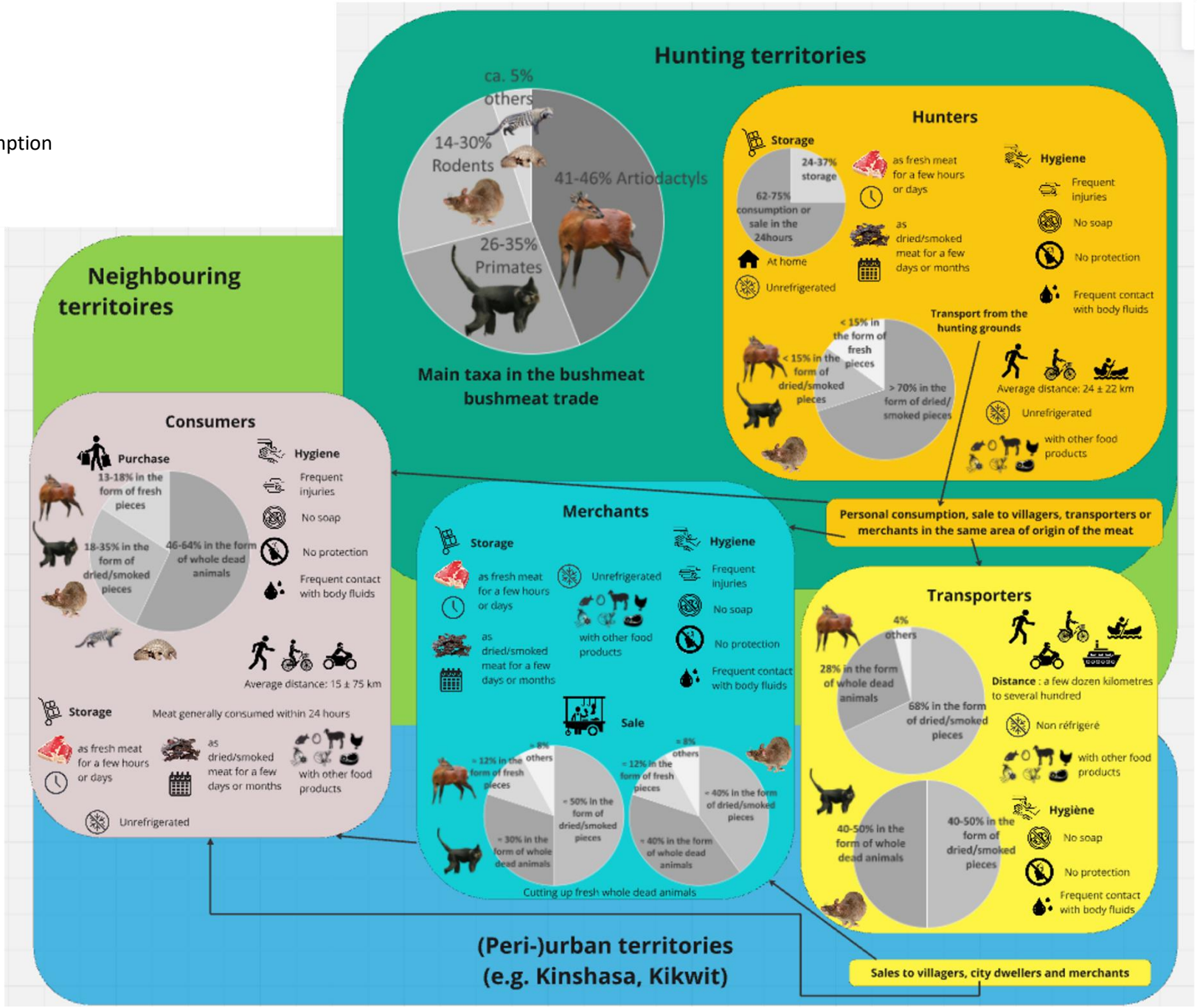
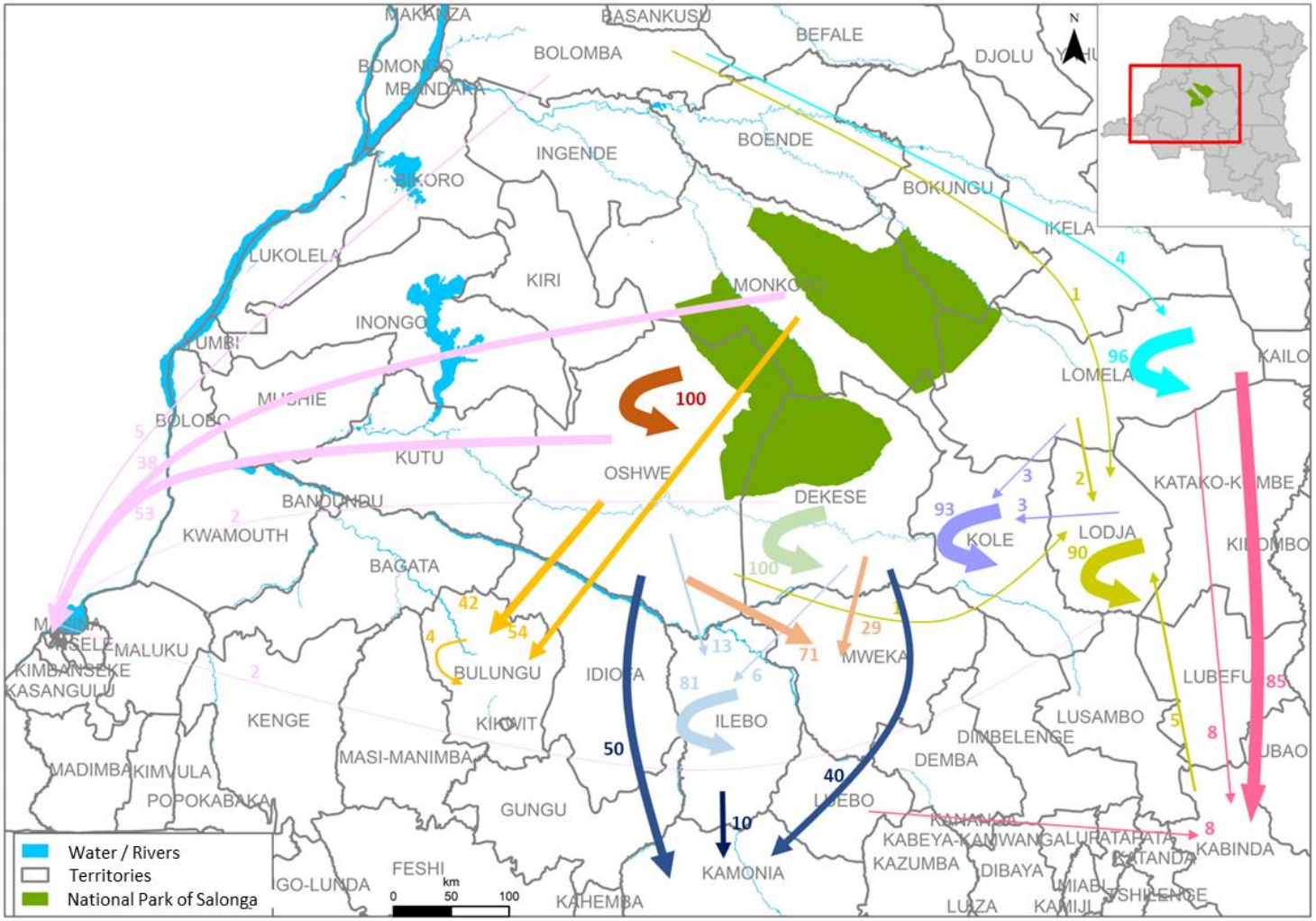


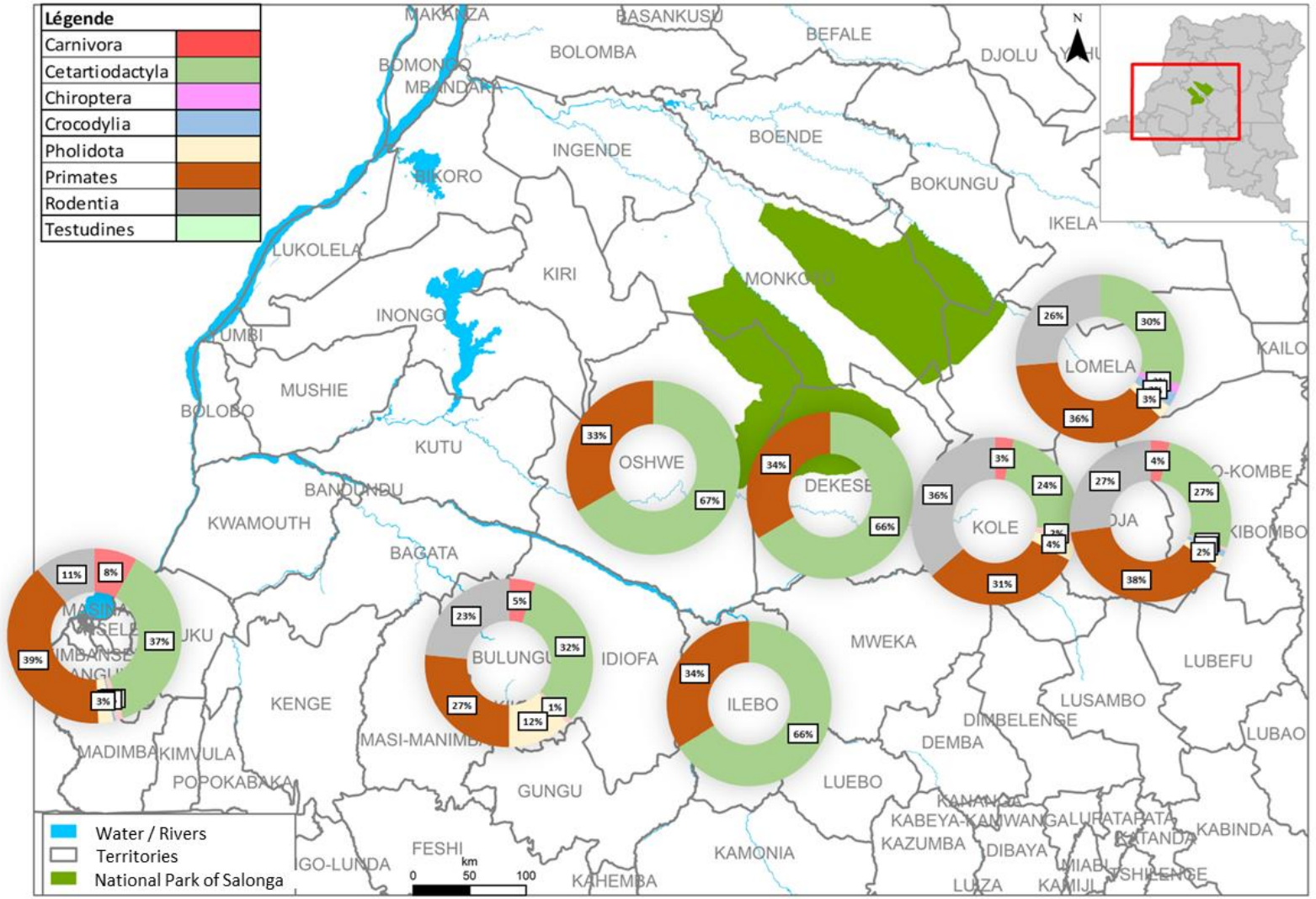
Figure 3: Illustration of bushmeat trade and consumption along value chains between the Salonga landscape and urban markets (e.g. Kinshasa and Kikwit).



Map 3: Map illustrating the territories of origin and destination of bushmeat. Each destination territory cited by the transporters surveyed is linked to the territory of origin by an arrow. The numbers and thickness of the arrows reflect the % of transporters attributing a given territory of origin to a given territory of destination (out of 424 transporters surveyed). For example, 53% of the transporters citing Kinshasa as their destination attributed the territory of Oshwe as their origin.



Map 4: Proportion of taxonomic groups cited by consumers surveyed (out of 225 consumers surveyed).



Map 5: Location of samples tested positive for the presence of pathogens.

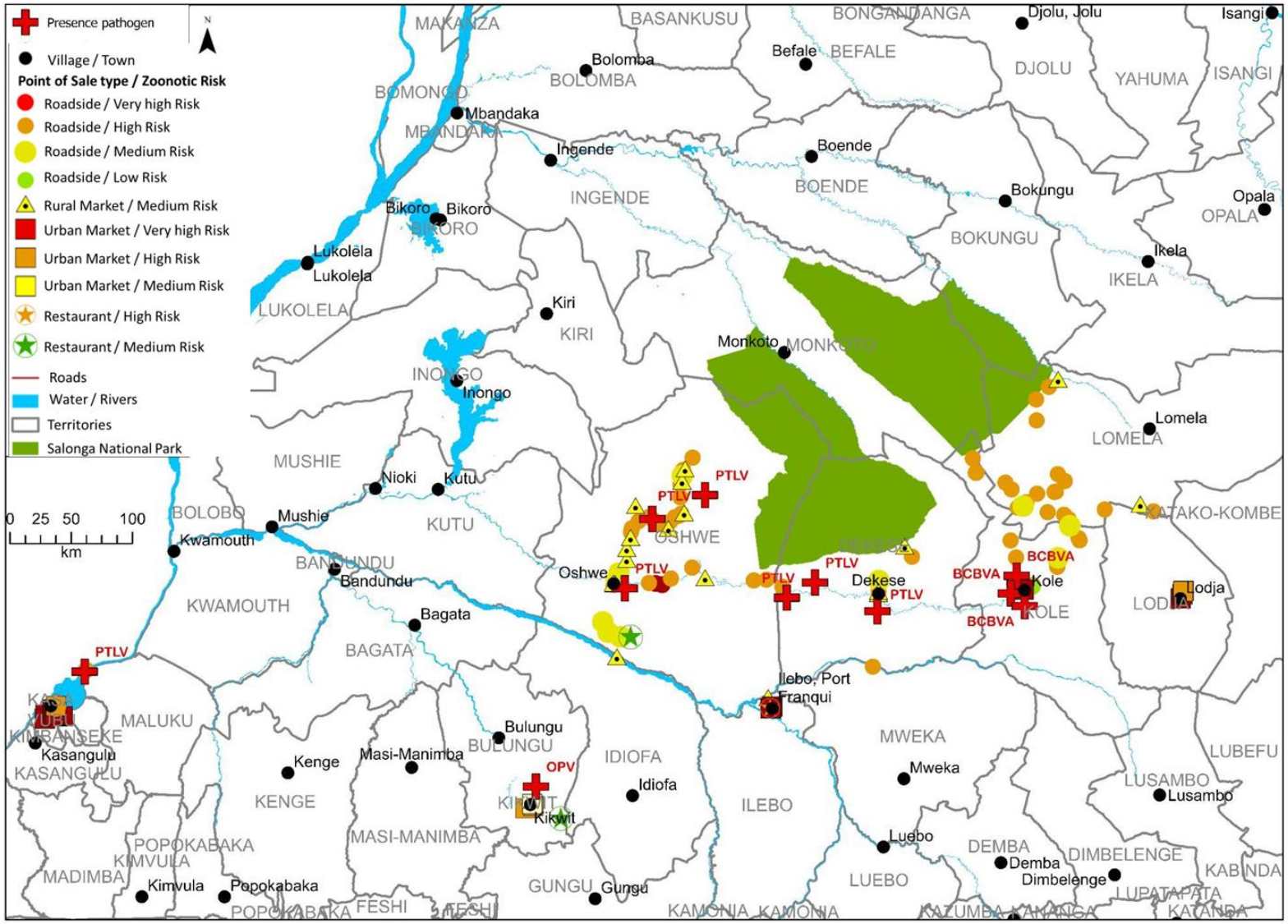


Photo 1: A field team visiting a sales outlet (Credit: WWF)

Photo 2: Field team interviewing vendors (Credit: WWF)

Photo 3: Field team taking bushmeat tissue samples (Credit: WWF)

Photo 4: Several species in contact on the same stalls (Credit: WWF)

Photo 5: Saleswoman in direct contact with the blood of a common genet (*Genetta genetta*) (Credit: WWF**)**

Photo 6: Taxa known to carry zoonotic pathogens with epidemic or pandemic potential found at outlets, e.g. primate, pangolin, rodent (Credit: WWF)

Photo 7: Primate smoking (Credit: WWF)

Photo 8: Transport of whole, dried wild animals (Credit: WWF)

Photo 9: Knife and chopping board not cleaned after butchering (Credit: WWF).