

Microbiological safety of the main unconventional animal resources consumed as meat in Benin

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Unconventional animal resources such as bushmeat, amphibians, reptiles, rodents, and wild birds represent an important part of local diets in Benin. This study aimed to assess the microbiological safety of the main unconventional animal resources consumed as meat in Benin. Therefore, 5 fresh composite samples of deer, grasscutter, partridge, monitor lizard (*Varanus exanthematicus*), frog (*Hoplobatrachus occipitalis*), shea caterpillars (*Cirina butyrospermi*) and grasshoppers (*Zonoceros variegatus*) were collected for microbiological analysis according to the specific ISO Standards. The results showed that in mammalian meat, total aerobic mesophiles were comparable between grasscutter (4.93×10^6 CFU/g) and deer (4.59×10^6 CFU/g), as were total coliforms (3.93×10^2 vs. 3.59×10^2 CFU/g) and fecal coliforms (2.38×10^2 vs. 2.30×10^2 CFU/g). In insects, shea caterpillars showed higher total coliforms (3.70×10^2 CFU/g) compared to grasshoppers (1.78×10^2 CFU/g), while other microbial counts were similar. Among amphibians and reptiles, microbial loads varied significantly according to the species with total aerobic mesophiles ranged from 1.68×10^6 CFU/g in frog to 5.03×10^6 CFU/g in monitor lizard, total coliforms ranged from 1.05×10^2 to 3.99×10^2 CFU/g, and fecal coliforms from 0.48×10^2 to 2.53×10^2 CFU/g. *Staphylococcus aureus* and *E. coli* counts followed similar patterns. No *Salmonella* spp. was detected in any sample. Effective hygiene practices are essential to ensure microbiological safety of these unconventional animal resources consumed as meat.

Keywords: Benin, game meat, insect, frog, monitor lizard, microbial safety

INTRODUCTION

In many regions of the world, including sub-Saharan Africa, unconventional animal resources such as bushmeat, amphibians, reptiles, rodents, and wild birds are an important part of local diets. Nowadays, consumer nutritional trends are increasingly oriented toward quality, safety, and traceability, particularly with respect to foods of animal origin. When properly processed, game meat can effectively meet all of these requirements. In Benin, these unconventional resources are consumed both for cultural reasons and as alternative sources of animal protein in contexts of food insecurity. In this West Africa country, unconventional foods such as bushmeat and edible insects

have attracted growing attention due to their nutritional value, sustainability, and potential contribution to food security. According to Tohozin et al. (2025), the main unconventional animal resources consumed include deer and cane rat (or grasscutter) among mammals, the partridge (*Francolinus bicalcaratus*) as a wild avian resource, the monitor lizard (*Varanus exanthematicus*) as a reptile, the frog (*Hoplobatrachus occipitalis*) as an amphibian, and insects such as shea caterpillars (*Cirina butyrospermi*) and grasshoppers (*Zonoceros variegatus*). These non-conventional meats provide essential nutrients and hold promise for addressing food insecurity and malnutrition in local communities (Main et al., 2024; Koffi et al., 2019).

However, as with conventional livestock, their consumption can represent a major risk for public health, since meat from wild and non-traditional animals is often a potential vehicle for foodborne pathogens and toxins (Atanassova et al., 2008; El-Ghareeb et al., 2009; Gill, 2007; Paulsen and Winkelmayr, 2004; Paulsen et al., 2012). The microbiological safety and hygienic quality of these unconventional meats are closely linked to a number of critical factors, including hunting or slaughtering practices, the ecological environment at the time of capture, delays before evisceration, as well as temperature and sanitary measures during carcass transport, processing, and storage (Gill, 2007; Sauvala et al., 2019; Orsoni et al., 2020). Several studies carried out in Europe have highlighted the frequent contamination of game meat by pathogens such as *Salmonella* spp., *Escherichia coli* (including Shiga toxin-producing strains), *Yersinia* spp., and parasites such as *Toxoplasma gondii* and *Sarcocystis* spp. (Miko et al., 2009; Obwegeser et al., 2012; Soriano et al., 2016; Plaza et al., 2020; Skorpikova et al., 2018). Viral agents, including hepatitis E virus (HEV), have also been detected in wild animals consumed as food, raising additional concerns for zoonotic transmission (Ryll et al., 2018; Abravanel et al., 2017; De Sabato et al., 2018).

In contrast to Europe, where legal frameworks such as Regulations (EC) No 853/2004 and No 2073/2005 provide specific hygiene and microbiological criteria for foodstuffs (European Commission, 2004, 2007), in many African countries including Benin, the microbiological safety of unconventional meats remains poorly documented. The absence of standardized sanitary controls, combined with informal hunting and slaughtering practices, increases the risk of consumer exposure to zoonotic pathogens (Gomes-Neves et al., 2021). However, the increasing demand for wild and alternative meats, driven by perceptions of naturalness, cultural heritage, and nutritional value (Tohozin et al., 2025), highlights the urgent need for evidence-based microbiological risk assessment (Orsoni et al., 2020; Sauvala et al., 2019).

The aim of this study is to evaluate the microbiological safety of the main unconventional animal resources consumed as meat in Benin.

MATERIALS AND METHODS

Study area

The study was conducted with the hunters, game meat and insect traders of Parakou in the Department of Borgou, Partogo (Djougou) in the Department of Donga and Zogbodomey in the Department of Zou (Figure 1). The lab analyses were done conjointly at the Quality and at the Food Safety Unit/Laraeq of the University of Parakou and the laboratory of National Agency of Food Safety of Benin.

The Department of Borgou is located in northeastern Benin, between 8°52' and 10°25' N latitude and 2°36' and 3°41' E longitude, covering an area of 25,856 km². The climate is Sudanese type, characterized by a dry season (November to May) and a rainy season (June to October) with annual rainfall ranging from 900 to 1200 mm (Adam and Boko, 1993).

With a Sudanian-Guinean type of climate, the Department of Donga is characterized by a rainy

season (mid-April to mid-October) and a dry season (mid-October to mid-April). This climatic pattern is similar to that of the Atacora Department. However, the average annual rainfall, ranging from 1,200 mm to 1,300 mm, is higher than in Atacora. The month of August records the heaviest rainfall.

Zou Department borders Collines Department to the north, Plateau Department to the east, Ouémé Department and Atlantique Department to the south, Kouffo Department to the south-west, and Togo to the west. The department is characterized by plateaus, ranging from 20 to 200 m (66 to 656 ft) above the mean sea level, which are split by valleys running from north to south, created by the Zou River and Kauoffo River. The southern regions of Benin receive two spells of rain from March to July and September to November, while the northern regions of the country receive one season of rainfall from May to September. The country receives an average annual rainfall of around 1,200 mm.

Meat and insect sampling

Samples from seven species of freshly shot unconventional animal resources consumed as meat were taken from 3 different hunting grounds in 3 municipalities of Benin. In most cases, the hunts lasted more than six hours. After the end of the hunt, all harvested animals were transported to a central marketing point for processing and sampling. Approximately four hours after hunting, the animals were processed for about 45–60 minutes to allow for carcass evisceration and sampling.

In accordance with the requirement of the laboratory of National Agency of Food Safety of Benin, a composite sample of 500 g of Deer meat, 500 g of grasscutter meat (*Thryonomys swinderianus*), 500 g of monitor lizard meat, 500 g of partridge and 500 g of frog meat (*Hoplobatrachus occipitalis*) samples (Figures 2 and 3) were aseptically sampled from the hunted animals purchased on the game meat markets of Zogbodomey, Parakou and Partago in Benin (Figure 1).

Insect sampling were also done aseptically into a sterile plastic bag with the support of women traders of edible insects of Parakou. A total of 500 g of alive shea caterpillars (*Cirina butyrospermi*) and 500 g of grasshoppers (*Zonoceros variegatus*) were also sampled for microbial analysis. The samples were kept under +4°C until laboratory for analysis according to the recommended ISO standards.

Microbiological analysis of the samples

The microbiological quality of the samples was assessed through enumeration of Total Aerobic Flora (TAF), coliforms, fecal coliforms, *Escherichia coli*, *Staphylococcus aureus*, yeasts, moulds, and detection of *Salmonella* spp according to the methods described by Asakura et al. (2017) for game meat and Pal et al. (2024) for edible insects. Total Aerobic Flora (TAF) was enumerated using the standard NF IN ISO 6222:1999 method. Samples were plated on Plate Count Agar (PCA) and incubated at 30°C for 72 hours to determine the total viable bacterial count (AOAC, 2016). Coliforms and fecal coliforms were enumerated according to NF V08-053 (2012). Each unconventional animal resource sample was homogenized and serially diluted, then plated on Violet Red Bile Lactose (VRBL) agar and incubated at 37°C for 24 hours for total coliforms. Fecal coliforms were determined by incubation at 44°C for 24 hours. Colonies showing typical morphology were counted and expressed as CFU/g (AOAC, 2016; Asakura et al., 2017; Pal et al., 2024). *Escherichia coli* enumeration followed ISO 16649-2:2001. Samples were plated on TBX (Tryptone Bile X-glucuronide) agar and incubated at 44°C for 24 hours. Typical blue-green colonies were confirmed as *E. coli* and expressed as CFU/g of the unconventional animal resource sample (ISO, 2001; Asakura et al., 2017; Pal et al., 2024). Yeasts and moulds were enumerated according to ISO 7954:1987. Serial dilutions of the different unconventional animal resource samples were plated on Sabouraud Dextrose Agar supplemented with chloramphenicol and incubated at 25°C for five days to inhibit bacterial growth while allowing fungal development (ISO, 1987; Asakura et al., 2017; Pal et al., 2024). *Staphylococcus aureus* enumeration was performed following NF IN ISO

6888-1:1999 using Baird-Parker agar. Plates were incubated at 37°C for 24 - 48 hours, and typical colonies were confirmed morphologically and biochemically (Asakura et al., 2017; Pal et al., 2024; AOAC, 2016). Detection of *Salmonella* spp. was conducted according to NF IN ISO 6579:2002, involving pre-enrichment in non-selective broth, selective enrichment in Rappaport-Vassiliadis and Muller-Kauffmann tetrathionate broths, followed by plating on selective agar and biochemical confirmation of presumptive colonies (Sessou et al., 2016; AOAC, 2016). All analyses were performed in triplicate, and results were expressed in colony-forming units per gram (CFU/g). Given the absence of specific standards for assessing the microbiological quality of game in Benin, the AFNOR standard (AFNOR, 1980) for microbial safety assessment of beef meat and fish was adopted as the reference for product classification.

Statistical analysis

Statistical analysis was conducted using Statistical Analysis System software (SAS, 2006). Means were calculated using the PROC MEANS procedure. The procedure Proc glm of SAS was used for variance analysis. The variation source used was the animal species. The student T test was used to compare the means.

RESULTS

Variation of microbiological quality of the wild mammal's meat (deer and grasscutter)

The microbiological profile of grasscutter and deer meat is summarized in Table 1. The loads of total aerobic mesophiles were similar in both species (4.93×10^6 CFU/g vs. 4.59×10^6 CFU/g). Likewise, total coliforms (3.93×10^2 CFU/g vs. 3.59×10^2 CFU/g) and fecal coliforms (2.38×10^2 CFU/g vs. 2.30×10^2 CFU/g) did not differ significantly. No *Salmonella* spp. was detected in any of the samples (0 CFU/25 g). The counts of *Staphylococcus aureus*, *Escherichia coli*, and yeast/moulds also showed no significant variation between grasscutter and deer meat.

Variation of microbiological quality of the insects consumed as food (shea caterpillars and grasshoppers)

The microbiological characteristics of shea caterpillars and grasshoppers are presented in Table 2. Total aerobic mesophile counts were comparable (3.20×10^6 CFU/g vs. 3.05×10^6 CFU/g). However, total coliforms were significantly higher in shea caterpillars (3.70×10^2 CFU/g) than in grasshoppers (1.78×10^2 CFU/g). Fecal coliforms, *Staphylococcus aureus*, *Escherichia coli*, and yeast/moulds showed no significant differences between the two insect groups. No *Salmonella* spp. was detected (0 CFU/25 g).

Variation of microbiological quality of the main species of wild bird, reptile and frog consumed as meat

The microbiological quality of frog, partridge, and monitor lizard meat is given in Table 3. The load of total aerobic mesophiles was lowest in frog (1.68×10^6 CFU/g), intermediate in partridge (2.59×10^6 CFU/g), and highest in monitor lizard (5.03×10^6 CFU/g). Similarly, total and fecal coliform counts were significantly lower in frog (1.05×10^2 and 0.48×10^2 CFU/g, respectively) compared to partridge (3.09×10^2 and 2.30×10^2 CFU/g) and monitor lizard (3.99×10^2 and 2.53×10^2 CFU/g). *Staphylococcus aureus* and *E. coli* counts followed the same pattern, with minimal levels in frog (0.18×10^2 and 0.30×10^2 CFU/g) and highest in monitor lizard (3.35×10^2 and 1.88×10^2 CFU/g). Yeast and mould loads varied from 3.00×10^1 to 4.43×10^1 CFU/g, but the differences were not statistically significant. No *Salmonella* spp. was detected in any of the tested species.

Microbiological quality comparison of the seven leading non-conventional animal food resources in Benin

The microbiological quality of unconventional animal food resources showed significant variation according to species (Table 4). The total aerobic mesophilic flora loads were highest in monitor lizard (5.03×10^6 CFU/g) and shea caterpillars (5.20×10^6 CFU/g), while the lowest counts were observed in frog (1.68×10^6 CFU/g). Similarly, total coliform levels were significantly higher in shea caterpillars (4.20×10^2 CFU/g), grasscutter (3.93×10^2 CFU/g), and monitor lizard (3.99×10^2 CFU/g) compared to frog (1.05×10^2 CFU/g) and grasshoppers (1.78×10^2 CFU/g). Fecal coliforms followed the same pattern, with the highest load in monitor lizard (2.53×10^2 CFU/g) and the lowest in frog (0.48×10^2 CFU/g).

No *Salmonella* spp. was detected in any of the investigated species (0 CFU/25 g), indicating an absence of this major foodborne pathogen in the tested samples. In contrast, *Staphylococcus aureus* counts varied strongly among species, ranging from 3.35×10^2 CFU/g in monitor lizard and 3.40×10^2 CFU/g in shea caterpillars to as low as 0.18×10^2 CFU/g in frog. Likewise, *Escherichia coli* loads were highest in shea caterpillars (1.90×10^2 CFU/g) and monitor lizard (1.88×10^2 CFU/g), while frog presented the lowest values (0.30×10^2 CFU/g). Yeast and mould loads varied from 2.45×10^1 CFU/g in deer to 4.73×10^1 CFU/g in grasshoppers, with no significant differences among species.

Overall, these results indicate that microbial contamination levels are highly dependent on the species, with frogs showing the lowest bacterial loads while monitor lizard and shea caterpillars exhibited the highest counts for several microbial groups. Although no *Salmonella* was found, the elevated presence of coliforms, *S. aureus*, and *E. coli* in some species suggests potential public health risks if these foods are consumed without adequate cooking or hygienic processing.

DISCUSSION

The present study revealed that the different unconventional animal resources consumed as meat investigated herein exhibited important microbial loads including aerobic mesophiles, coliforms, *E. coli*, and *Staphylococcus aureus*. These results are consistent with previous reports indicating that the microbial status of game meat is largely influenced by post-harvest handling rather than intrinsic species differences (Atanassova et al., 2008; Gill, 2007). The absence of *Salmonella* spp. in both species aligns with several European investigations, which similarly reported low or negligible prevalence in hunted ruminant carcasses (Paulsen et al., 2012; Obwegeser et al., 2012; Sauvala et al., 2019). However, occasional outbreaks linked to contaminated game products highlight that *Salmonella* remains a relevant hazard under poor hygiene conditions (Gil Molino et al., 2019; Sannö et al., 2018).

Overall, the microbiological quality of unconventional animal found in the current study varied significantly by species, reflecting differences in ecology, physiology, and post-harvest handling practices. Total aerobic mesophilic flora (TAMF) loads were highest in monitor lizards (5.03×10^6 CFU/g) and shea caterpillars (5.20×10^6 CFU/g), while frogs exhibited the lowest counts (1.68×10^6 CFU/g). TAMF is a general indicator of microbial contamination and potential spoilage; elevated levels in reptiles and insects may reflect greater exposure to environmental microbes during capture, storage, and transport (Atanassova et al., 2008; Gill, 2007). Similar trends have been reported for large game and wild birds, where handling practices and environmental exposure influence microbial loads (El-Ghareeb et al., 2009; Avagnina et al., 2012; Orsoni et al., 2020). According to Abrantes et al. (2023), the microbiological contamination of wild animal meat depends on the hygiene practices that hunters apply during its preparation, from the point of collection to its refrigeration.

Total coliforms and fecal coliforms, indicators of fecal contamination and hygiene quality, were significantly higher in shea caterpillars (4.20×10^2 CFU/g) and monitor lizards (3.99×10^2 CFU/g) than in frogs (1.05×10^2 CFU/g) and grasshoppers (1.78×10^2 CFU/g). This finding is consistent with observations in game meat, where species exposed to soil, water, or decaying matter show

higher enteric bacterial loads (Haindongo et al., 2018; Miko et al., 2009; Obwegeser et al., 2012). The absence of *Salmonella* spp. in all species (0 CFU/25 g) is reassuring and aligns with previous European studies on hunted game, which report low prevalence when hygienic practices are observed (Paulsen and Winkelmayer, 2004; Paulsen et al., 2012; Gil Molino et al., 2019).

Importantly, although *Salmonella* spp. was not detected in any sample, the presence of fecal coliforms and *E. coli* in several species remains a concern, as these organisms are widely used as indicators of fecal contamination and potential pathogen presence (European Commission, 2007; EFSA, 2019). Moreover, wild game and unconventional meats have been recognized as reservoirs of zoonotic agents such as shiga toxin-producing *E. coli* (Miko et al., 2009; Haindongo et al., 2018), *Toxoplasma gondii* (Skorpikova et al., 2018; Plaza et al., 2020), and even viral pathogens such as hepatitis E virus (Ryll et al., 2018; Abravanel et al., 2017). These risks highlight the public health importance of monitoring unconventional food resources in addition to traditional livestock products. Sporadic outbreaks linked to wildlife highlight that even low-prevalence pathogens remain a concern. (Sannö et al., 2018).

Staphylococcus aureus loads varied significantly, with the highest counts in monitor lizards (3.35×10^2 CFU/g) and shea caterpillars (3.40×10^2 CFU/g), and the lowest in frogs (0.18×10^2 CFU/g). *S. aureus* is commonly introduced during handling, suggesting that post-harvest hygiene is a key factor influencing contamination levels (European Commission, 2004; Gomes-Neves et al., 2021). Similarly, *Escherichia coli* loads were highest in shea caterpillars (1.90×10^2 CFU/g) and monitor lizards (1.88×10^2 CFU/g), and lowest in frogs (0.30×10^2 CFU/g). These patterns reflect potential fecal contamination, cross-contamination during processing, or intrinsic microbial carriage, as reported for game species in Europe and Africa (Mateus-Vargas et al., 2017; Díaz-Sánchez et al., 2012; Sauvala et al., 2019).

Yeast and mould loads, ranging from 2.45×10^1 CFU/g in deer to 4.73×10^1 CFU/g in grasshoppers, did not differ significantly among species, suggesting that fungal contamination is more influenced by storage conditions and moisture rather than species-specific factors, in agreement with prior studies on wildlife meat and insects (Peruzy et al., 2019).

These findings indicate that microbial contamination is highly species-dependent. Frogs consistently exhibited the lowest microbial loads across all tested groups, suggesting that their habitat, rapid capture, or minimal handling may limit bacterial proliferation. Conversely, monitor lizards and shea caterpillars harboured the highest bacterial loads, reflecting both environmental exposure and potential deficiencies in post-harvest handling (Atanassova et al., 2008; Gill, 2007; El-Ghareeb et al., 2009). Although *Salmonella* spp. was absent, the elevated presence of total coliforms, *S. aureus*, and *E. coli* in several species represents a potential public health risk if these foods are consumed without adequate cooking or hygienic processing (EFSA, 2019; Gomes-Neves et al., 2021).

Birds such as partridge are known to carry enteropathogens including *E. coli*, *Salmonella*, and *Campylobacter* (Díaz-Sánchez et al., 2012; El-Ghareeb et al., 2009), and wild reptiles are frequently associated with higher microbial loads due to their contact with soil and contaminated water sources. Such interspecies variability is well documented in wild game studies, where handling, evisceration timing, and environmental exposure strongly influence carcass contamination (Paulsen and Winkelmayer, 2004; Soriano et al., 2016; Orsoni et al., 2020).

The results underscore the importance of implementing good harvesting and post-harvest practices for unconventional animal food resources, including proper evisceration, rapid cooling, hygienic handling, and thorough cooking before consumption (Soriano et al., 2016; European Commission, 2007; Horigan et al., 2014). Furthermore, continued monitoring of microbial loads in these species is essential to ensure food safety, particularly in regions where these resources constitute an important dietary component and where traditional preparation methods may not always meet safety standards (Plaza et al., 2020; Ryll et al., 2018). The hygienic quality and safety of game meat

are strongly influenced by factors such as hunting practices, environmental conditions at the time of shooting, as well as temperature management and sanitary measures during handling and transport (Gill, 2007; Paulsen et al., 2012).

CONCLUSION

The present study demonstrates that the microbiological quality of unconventional animal foods consumed in Benin varies significantly according to species. Total aerobic mesophiles, coliforms, fecal coliforms, *Staphylococcus aureus*, and *E. coli* showed species-dependent differences, with monitor lizard and shea caterpillars generally presenting higher microbial loads, while frog and grasshoppers exhibited lower levels. Notably, *Salmonella* spp. was absent in all samples, suggesting a minimal risk of salmonellosis from these products. Despite this, the presence of indicator microorganisms highlights potential hygiene deficiencies during slaughtering, harvesting, or processing, which could pose public health risks if proper handling is not ensured. Overall, the findings emphasize the need for targeted hygiene and safety measures in the collection, processing, and consumption of unconventional animal foods to reduce microbial contamination and protect consumers' health.

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