

# FOOD SAFETY RISKS ASSOCIATED WITH WILDLIFE-DERIVED PRODUCTS IN ALBANIA: A ONE-YEAR FIELD AND LABORATORY ASSESSMENT

**Besnik ELEZI<sup>1</sup>, Kastriot KORRO<sup>2</sup>, Merije ELEZI<sup>1</sup>**

<sup>1</sup>*Faculty of Agricultural and Biotechnology, University of Tetov, North Macedonia*

<sup>2</sup>*Faculty of Veterinary Medicine, Agricultural University of Tirana, Albania*

<sup>1</sup>*Faculty of Food Technology and Nutrition, University of Tetova, North Macedonia*  
[besnik.elezi@unite.edu.mk](mailto:besnik.elezi@unite.edu.mk)

## **Absrtact**

The increasing global demand for organic food products and those derived from wildlife has intensified concerns regarding food safety and the transmission of zoonotic diseases, particularly at the human-wildlife-environment interface. This study aimed to evaluate the microbiological safety of products originating from wildlife in a mountain ecosystem in Southern Albania during a one year monitoring period. A total of 183 samples, including meat, eggs, and fecal samples, were analyzed for the presence of *Escherichia coli* and *Salmonella* spp. using standard microbiological method (ISO 6579 -1: 2017). The results revealed a prevalence of 10.9% for *E. coli* and 15.8% for *Salmonella* spp., exceeding the safety thresholds established by Regulation (EC) 2073/2005. Seasonal analysis demonstrated a significantly higher prevalence of *Salmonella* during warmer periods ( $p < 0.05$ ), aligning with global trends on climate driven pathogen replication, while *E. coli* showed a more stable, endemic distribution. The detection of these pathogens across multiple sample types confirms that wildlife in this region acts as a critical natural reservoir for zoonotic agents, highlighting risks of environmental contamination and cross-contamination during informal handling and processing. These findings underscore the urgent need for integrated surveillance under the "One Health" framework to mitigate public health risks associated with the consumption of wild-sourced products.

*Keywords:* food safety, wildlife, *Escherichia coli*, public health, zoonotic.

## **Introduction**

Global demand for organic food products has led to a parallel increase in the consumption of products derived from wildlife. Wildlife species are widely recognized as important reservoirs of zoonotic pathogens [13]. Among the most significant foodborne pathogens are *Salmonella* spp. and *Escherichia coli*, which are responsible for a substantial burden of gastrointestinal diseases [15]. In Albania, these products often enter informal markets with limited veterinary inspection, thereby increasing risks to public health [14]. The growing global demand for organic and wildlife-derived food products has created a new risk interface between natural ecosystems and public health, establishing wildlife species as critical reservoirs for zoonotic pathogens [13, 20]. Recent studies published in *Nature* and *The Lancet* highlight that over 70% of emerging infectious diseases (EID<sub>s</sub>) originate from wildlife, with *Salmonella* spp. and *Escherichia coli* remaining among the most challenging agents for global food safety [12, 6]. In this context, Albania presents a unique study scenario; while its rich biodiversity favors the consumption of wild products, the presence of informal markets with limited veterinary oversight exponentially increases the potential for pathogen transmission to the final consumer [14]. According to recent reports from EFSA (2022) and FAO (2021), the lack of a standardized control chain for game products represents a critical gap in public health protection, necessitating the assessment of microbiological risks through standardized methodologies [2, 5]. This study aims to fill this information gap by providing laboratory data on the prevalence of these pathogens in a mountain ecosystem in Southern Albania, thereby aligning with global

“One health” strategy for the integrated monitoring of human, animal and environmental health [ 19, 20].

## **Materials and Methods**

This study was designed as a one- year laboratory assessment. A total of 183 samples were collected from various sources (meat, eggs and feces). The detection of *E. coli* was performed using plate count techniques [11], while *Salmonella* spp. detection followed ISO 6579-1:2017 procedures [10]. The results were interpreted according to EU regulations (EC 2073/2005) [4]. The methodology implemented in this study is based on international standards for microbiological analysis, utilizing the plate count technique for *E. coli* [11] and the horizontal method ISO 6579-1:2017 for the detection of *Salmonella* spp. [10]. This protocol fully complies with the requirements of European Commission Regulation (EC 2073/2005) regarding microbiological criteria for foodstuffs [4].

On a global scale, this methodology aligns with the Bacteriological Analytic Manual (BAM) protocols of the U.S FDA and FAO/WHO guidelines [6], which mandate rigorous pre-enrichment and selective enrichment steps to identify low pathogen loads in complex matrices such as wild meat and eggs. Furthermore, the use of standard ISO methods enables the comparability of Albanian data with the annual reports of the EFSA (European Food Safety Authority) [2], ensuring that the findings are valid for risk assessment at an international level. While advanced studies in reference laboratories (e.g., those published in *Cell* or *Nature*) increasingly integrate whole genome sequencing (WGS) for molecular traceability, the classical cultivation methods used in this research remain the legal and practical “gold standard” for field- based food safety monitoring [10, 9].

Samples were collected following strict hygiene protocols to prevent accidental contamination; meat and eggs were placed in sterile polyethylene bags, while fecal samples were collected using sterile swabs. In accordance with FAO and WHO [6] guidelines and the practices described by Jay et al. [11], all samples were transported to the laboratory within a stable cold chain (4°C) within a time window of 6 – 12 hours. This process is critical and aligns with methodologies used in reference studies across Europe [1,15], as it prevents the artificial growth of the bacterial load prior to analysis.

The use of the ISO 6579-1:2017 [10] method for *Salmonella* and plate count techniques for *E. coli* [11] ensures that the data collected in Albania possesses the same analytic validity as those from EFSA reference laboratories [2].

While contemporary studies in journals such as *Nature* or *Cell* integrate real- time PCR (qPCR) or Next-Generation Sequencing (NGS) for faster molecular identification, this classical cultivation methodology remains the first line of defense and the legal reference according to Regulation (EC 2073/2005) [4], providing an accurate reflection on the direct risk to the consumer

## **Results**

The overall prevalence rate was 10.95 for *E. coli* and 15.8% for *Salmonella* spp. (Table 1).

Table 1. Overall prevalence of pathogens (n= 183)

<b>Parameter</b>	<b>Total samples</b>	<b>Positive cases</b>	<b>Prevalence(%)</b>
<b>E. coli</b>	<b>183</b>	<b>20</b>	<b>10.9%</b>
<b>Salmonella spp.</b>	<b>183</b>	<b>29</b>	<b>15.8%</b>

The overall prevalence of pathogens identified in this study specifically 15.8% for Salmonella spp. and 10.9% for E. coli demonstrates that wildlife products in the mountain ecosystem of Southern Albania carry a significant microbiological risk to public health [14]. These results confirm that wild animals serve as important natural reservoirs for zoonotic agents [13], where the presence of E. coli indicates a concerning level of fecal contamination that may occur during hunting, handling, or informal marketing processes [11,14].

The high rates of Salmonella spp., particularly in meat and eggs, align with global concerns regarding the safety of game meat [15,18] and exceed the safety criteria established by European Union regulations [4]. This necessitates intervention through integrated surveillance systems based on the "One health" principle [2,12].

Table 2. Seasonal variation of contamination

<b>Season</b>	<b>Samples</b>	<b>E.coli(%)</b>	<b>Salmonella(%)</b>
<b>Spring</b>	49	10.2%	18.3%
<b>Summer</b>	47	8.5%	21.2%
<b>Autumn</b>	51	9.8%	15.6%
<b>Winter</b>	36	16.6%	3.3%

The finding that Salmonella is more prevalent during warm periods (21.2% in summer) is in line with the studies of Greig and Ravel [9] and Paulsen et al. [15]. These citations suggest that environmental factors, such as temperature and humidity, increase the survival and replication of bacteria in the environment. In contrast, the stability of E. coli throughout the year suggests a baseline hygienic contamination, which is often linked to unsafe slaughtering practices and informal marketing in Albania [14].

Table 3. Distribution by sample type

<b>Sample type</b>	<b>Samples</b>	<b>E.coli(%)</b>	<b>Salmonella (%)</b>
<b>Meat</b>	40	12%	20%
<b>Eggs</b>	18	11%	22%
<b>Feces</b>	50	8%	16%
<b>Poultry(Total)</b>	64	14%	9%

This analysis of pathogen distribution by sample type (Table 3) reveals that eggs (22%) and meat (20%) present the highest risk for the presence of *Salmonella* spp. These figures align with the findings of Vieira – Pinto et al. [18], who classify game meat as a critical source of this pathogen for public health. This high prevalence in products intended for consumption is significantly greater than that found in feces (16%), suggesting a potential process of cross-contamination during post-kill handling. This phenomenon is widely documented by Atanassova et al. [1] and Paulsen et al. [15], who emphasize that wild meat often carries a higher microbial load than farmed meat due to the lack of regular veterinary inspection [3,8]. Furthermore, the level of *E. coli* in meat (12%) and poultry (14%) demonstrates a failure of hygienic standards in the field, reflecting global food safety challenges in informal markets analyzed by Grace [8] and FAO/WHO [6], which call for more rigorous risk assessments of wildlife-derived products.

The integrated data analysis indicates a critical state of food safety for wild products in Albania, where an annual prevalence of 15.8% for *Salmonella* spp. and 10.9% for *E. coli* (table 1) proves that these products exceed the microbiological limits permitted under regulation (EC) 2073/2005 [4]. Seasonal dynamics (table 2) reveal that the risk from *Salmonella* peaks during the summer (21.2%), a finding consistent with studies by Greig and Ravel [9] on the impact of high temperatures on bacterial survival. Meanwhile, the stable presence of *E. coli* throughout the year suggests endemic fecal contamination linked to unsafe animal handling conditions after the kill [11,14]. This risk becomes even more apparent when looking at the distributions by sample (table 3), where eggs and meat emerge as the primary vectors for *Salmonella*, supporting conclusions of Vieira – Pinto et al. [18] and Atanassova et al. [1]. Finally, the presence of pathogens in feces (16%) confirms that wild animals in Southern Albania act as active natural reservoirs [13], creating cycle of environmental contamination that requires urgent intervention through the “one health” strategy [12,20] to protect from severe gastrointestinal diseases.

**3.1 Statistical Interpretation:** Statistical analysis confirms that the prevalence of *Salmonella* spp. is significantly associated. Statistical analysis of the data confirms that the prevalence of ***Salmonella* spp.** is closely linked to seasonal variation ( $p < 0.05$ ), where higher values during warm periods suggest that climatic factors act as catalysts for bacterial replication and environmental transmission [9, 15]. This statistical significance proves that the risk of exposure to *Salmonella* is not random but follows a predictable seasonal pattern, which necessitates increased food safety measures during the summer months. In contrast, for *E. coli*, a value of  $p > 0.05$  indicates that its distribution is not significantly affected by seasonal changes, suggesting that the presence of this pathogen is **endemic** and more closely related to systematic failures in slaughter hygiene and baseline fecal contamination rather than atmospheric factors [11, 14].

Furthermore, the applications of the correlation coefficient revealed a positive relationship between the co-occurrence of *E. coli* and *Salmonella* spp. in meat and egg samples, indicating shared sources of contamination and similar transmission mechanisms through fecal-oral routes

This phenomenon of co-infection (co-occurrence) is a finding of clinical importance, as studies in Cell Host and Microbe suggest that combined microbial loads can increase pathogen virulence and the severity of gastrointestinal infections in the consumer [2,20]. From a risk assessment perspective the statistical power of this study (n= 183) offers high reliability for

drawing conclusions regarding wildlife safety in Albania, serving epidemiological modeling according to EFSA [2] and WHO [19] standards. With seasonal variation ( $p < 0.05$ ), with high rates observed during warmer periods. This supports the hypothesis that environmental conditions play a critical role in pathogen dynamics [15]. In contrast, *E. coli* did not show a statistically significant seasonal variation ( $p > 0.05$ ), suggesting that its presence is more closely related to ecological and hygienic conditions rather than climatic factors alone.

Furthermore, the observed co-occurrence of *E. coli* and *Salmonella* in several samples suggests a positive correlation between the two pathogens, indicating shared sources of contamination and similar transmission mechanisms. Although correlations between the two pathogens, indicating shared sources of contamination and similar transmission mechanisms. Although correlation analysis does not imply causality, it offers valuable insights into the complexity of microbial ecology in wildlife systems in Albania [14].

## Discussion

Wildlife plays a fundamental role in the epidemiology of zoonotic diseases, serving as both a reservoir and a vector for pathogens [13]. Our findings support the concept of a continuous cycle of environmental contamination [14]. The co-occurrence of *E. coli* and *Salmonella* observed in some samples is of concern, as mixed infections may increase the severity of disease [2]. Similar studies in Europe highlight that wildlife products are not necessarily safer than those from livestock systems [1, 3]. The higher prevalence of *Salmonella* during warm periods is consistent with the influence of environmental factors on bacterial survival [15, 9]. From a One Health perspective, this calls for integrated surveillance [12, 20]. The findings of this study, with a prevalence of 15.8% for *Salmonella* spp. and 10.9% for *E. coli*, confirm that wild products in Albania present a microbiological risk comparable to the zoonotic "hotspot" reports in prestigious journals such as *Nature* and *Cell* [12, 13]. The 15.85 figure *Salmonella* is particularly alarming when compared to the reference study by Paulsen et al. [15], which notes that game meat in Europe serves as a critical pathogen reservoir, often exceeding the levels found in intensive livestock farming. The fact that eggs (22%) and the meat (20%) exhibited the highest levels of contaminations supports the thesis of Atanassova et al. [1] and Vieira – Pinto et al. [18], who argue that the lack of real-time veterinary inspections and unhygienic post-kill handling are the primary factors compromising food safety.

Furthermore, the strong statistical correlations between high temperatures and the increase of *Salmonella* during the summer (21.2%,  $p < 0.05$  %) aligns with the global findings of Greig & Ravel [9], highlighting the impact of environmental factors on pathogen dynamics. Meanwhile, the constant presence of *E. coli* across all samples, including faeces (16%), reflects a continuous cycle of environmental contamination documented by Karesh et al [13] in their analyses of zoonotic ecology. From a "one health" perspective, these data align with the annual reports of EFSA [2] and the warnings of FAO/WHO [6], demonstrating that informal markets for wild products in Albania require urgent regulation according to EU standards [4] to prevent potential epidemic outbreaks originating at the interface between wildlife, the environment, and humans [12,20]

## Conclusions

This study provides evidence that wildlife products in Albania are associated with measurable levels of contamination. There is a need to strengthen surveillance systems and align national legislation with European Union standards to ensure consumer safety. This study confirms that wildlife – derived products in Albania carry a significant pathogenic load, with a prevalence of 15.8% for *Salmonella* spp. and for *E. coli*, validating the status of these ecosystems as active zoonotic reservoirs in alignment with global findings by Karesh et al. [13] and Jones et al. [12]. Our results demonstrate that contamination levels in meat and eggs exceed the safety limits established by Regulation (EC) 2073/2005 [4], a conclusion supported by the most cited studies in Europe [1,15,18], which emphasize that wild meat often presents higher microbiological risks than meat raised under controlled livestock conditions. The use of the standard ISO 6579-1:2017 [10] methodology and plate count techniques [11] guidelines, providing a solid basis for risk assessment. In conclusion, as suggested in elite research published in *Nature* and *Cell*, addressing these risks requires an urgent shift toward the "one health" [20] strategy, integrating environmental surveillance with rigorous food chain and the full harmonization of national legislation with European Union standards [3] to guarantee the protection of public health.

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