A.D. 1308 unipg
UNIVERSITÀ DEGLI STUDI DI PERUGIA

A.D. 1308 unipg
DIPARTIMENTO DI MEDICINA VETERINARIA

UNIVERSITÀ DEGLI STUDI DI PERUGIA

Department of Veterinary Medicine

DOTTORATO DI RICERCA in *Sanità e Scienze Sperimentali Veterinarie*

DOCTORAL PROGRAM in *Health and Experimental Veterinary Science*

XXXVII CICLO SSD MVET-02/B

Accademic year 2023/2024

Use of agro-food industry by-products to improve the hygienic characteristics of hunted wild boar meat

Impiego di sottoprodotti dell'industria agro-alimentare per migliorare le caratteristiche igieniche delle carni di cinghiale cacciato

Ph.D. Candidate

Dr. Caterina Altissimi

Supervisor

Prof. David Ranucci

Ph.D. Coordinator

Prof. Beniamino Terzo Cenci Goga

SIRI UNIONE EUROPEA
Fondo Sociale Europeo
Fondo Europeo di Sviluppo Regionale

Summary

The present doctoral project falls within the framework of the "Programma Operativo Nazionale Ricerca e Innovazione 2014-2020" (PON R&I), an initiative of the Italian Ministry of University and Research aimed at promoting and supporting research projects focused on themes related to environmental sustainability and technological innovation. In this context, green topics play a central role, representing not only a response to global challenges such as climate change and environmental protection but also an opportunity to develop sustainable and circular approaches in production sectors.

One particularly significant aspect is the management of wild game meat, especially wild boar, which, as reported worldwide, is an issue expected to intensify due to the continuous growth in wild animal populations. However, this situation also presents an opportunity to develop sustainable supply chains that add value to this food resource, turning a management challenge into an asset for the territory and for human nutrition, as an integrated protein source.

Simultaneously, the management of agro-industrial by-products, often viewed as critical from an environmental standpoint due to the amount of waste produced and its high pollutant potential, offers opportunities for innovative reuse. Some of these by-products contain bioactive compounds of great interest, such as polyphenols, which could be used to improve meat quality. Applying these substances could enhance hygienic characteristics and extend the shelf life of game meat. Furthermore, it would promote environmental sustainability by integrating the reuse of by-products into a circular economy approach and reducing the impacts associated with their disposal, transforming them into useful technological resources for the food sector. In this perspective, the project combines the importance of green topics with a

practical and innovative approach to managing wild boar meat quality, demonstrating how environmental and ecological issues can be addressed through sustainable and integrated solutions.

As a first step, it was necessary to study and select the by-products to be used based on their availability and accessibility, evaluating their potential antimicrobial activity *in vitro* (paper: Roila, R., Branciari, R., Primavilla, S., Altissimi, C., Perioli, L., Valiani, A., Pagano, C., Veneziani, G., Ranucci, D. (2023). *Revalorization of agrifood industry by-products: natural extracts as a sustainable strategy to enhance food safety*. In XXIII CONGRESSO NAZIONALE CIRIAF-Sviluppo Sostenibile, Tutela dell'Ambiente e della Salute Umana. Morlacchi Editore University Press.; paper: Roila, R., Primavilla, S., Ranucci, D., Galarini, R., Codini, M., Giusepponi, D., Altissimi, C., Valiani, A., Casagrande-Proietti, P., Branciari, R. (2024). *Measuring the antimicrobial activity of natural extracts against food spoilage bacteria to enhance food hygiene: preliminary in vitro results*. Acta IMEKO, *13*(2), 1-5). Simultaneously, it was essential to explore the potential risks associated with wild boar, particularly concerning foodborne bacteria (paper: Altissimi, C., Noé-Nordberg, C., Ranucci, D., Paulsen, P. (2023). *Presence of foodborne bacteria in wild boar and wild boar meat—a literature survey for the period 2012–2022*. Foods, *12*(8), 1689).

Following the *in vitro* evaluations, studies on wild boar carcasses were performed, using both lactic acid, currently permitted in the European Union only for bovine carcasses, to assess its effects on hygienic characteristics (paper: Roila, R., Altissimi, C., Branciari, R., Primavilla, S., Valiani, A., Cambiotti, F., Cardinali, L., Cioffi, A., Ranucci, D. (2022). *Effects of spray application of lactic acid solution and aromatic vinegar on the microbial loads of wild boar carcasses obtained under optimal harvest conditions*. Applied Sciences, *12*(20), 10419), and an *in situ* model to simulate experimentally contaminated carcass surfaces,

evaluating the effect of the polyphenolic extract deemed most promising based on the *in vitro* studies (paper: Altissimi, C., Roila, R., Primavilla, S., Branciari, R., Valiani, A., Ranucci, D. (2024). *Surface carcass treatment with olive mill wastewater polyphenolic extract against Salmonella enteritidis and Listeria monocytogenes: in vitro and in situ assessment*. Italian Journal of Food Safety, 13:12403).

Finally, tests were carried out on the application of polyphenolic extracts from olive mill wastewater to game meat, evaluating various application methods and their effects on hygienic, physico-chemical, and qualitative characteristics (paper: Altissimi, C., Roila, R., Ranucci, D., Branciari, R., Cai, D., Paulsen, P. (2024). *Preventing microbial growth in game meat by applying polyphenolic extracts from olive mill vegetation water*. Foods, *13*(5), 658; paper: Altissimi, C., Ranucci, D., Bauer, S., Branciari, R., Paulsen, P. (2024). *Physico-chemical quality traits and microbiological condition of burger patties from wild boar meat with added polyphenolic extracts from olive mill vegetation water*. *Submitted article*).

Among the various extracts tested, the most promising was the powder polyphenolic extract derived from olive mill wastewater, as it demonstrated moderate antimicrobial efficacy and good antioxidant activity on meat, showing significant potential for industrial applications on wild boar meat.

Further studies are needed to assess the effects of polyphenolic extracts on specific pathogenic microorganisms on the meat and to evaluate the applicability of this approach on carcasses as a method for decontamination or enhancing hygienic standards.

Table of content

Chapter 1 - General introduction 1

1.1 Food industry by-products 3

1.1.1 Agro-industrial by products and their applications 5

1.1.2 Application of bioactive compounds in the meat industry 8

1.2 Wild boar: population and meat characteristics 11

1.2.1 The rise of wild boar populations: ecological, economic, and public health implications 11

1.2.2 Wild boar meat: composition and nutritional characteristics 14

1.2.3 Game meat harvesting: methods and hygienic characteristics 15

Chapter 2 – Research activities 36

Aims 36

Presence of foodborne bacteria in wild boar and wild boar meat — a literature survey for the period 2012–2022 39

Revalorization of agrifood industry by-products: natural extracts as a sustainable strategy to enhance food safety 128

Measuring the antimicrobial activity of natural extracts against food spoilage bacteria to enhance food hygiene: preliminary *in vitro* results 136

Surface carcass treatment with olive mill wastewater polyphenolic extract against *Salmonella* Enteritidis and *Listeria monocytogenes*: *in vitro* and *in situ* assessment 154

Effects of spray application of lactic acid solution and aromatic vinegar on the microbial loads of wild boar carcasses obtained under optimal harvest conditions 172

Preventing microbial growth in game meat by applying polyphenolic extracts from olive mill vegetation water 198

Physico-chemical quality traits and microbiological condition of burger patties from wild boar meat with added polyphenolic extracts from olive mill vegetation water 224

Chapter 3 – General conclusions 251

Chapter 1 - General introduction

The transition towards sustainability is a strategic priority across all sectors, driven by increasing consumer awareness of environmental issues and supported by significant investments at both national and European levels. Programs such as React-EU and the "Programma Operativo Nazionale Ricerca e Innovazione 2014-2020" (PON R&I) have mobilized substantial resources to promote green innovation, circular practices, and digital transformation. These initiatives not only aim to reduce environmental impact but also to foster research and development, including the financing of doctoral programs focused on innovative sustainability approaches. Such investments highlight the critical role of research and education in addressing global challenges and advancing the transition to a circular economy.

The application of the circular economy model in the food industry involves concerted efforts from companies, governments, and consumers. Transforming by-products into value-added resources offers tangible economic benefits, reduces pollution, and meets the growing demand for sustainable products. Modern consumers are increasingly inclined towards green products and are more conscious of the environmental impacts of their purchasing choices, prioritizing items that are environmentally friendly, including recyclable packaging materials, and produced with ethical and sustainable practices (Boz et al., 2020; Wunderlich & Smoller, 2019). As a result, the food industry must address these demands by adopting practices that minimize environmental impact across the entire production and distribution chain. However, this approach demands substantial investments in research, development, and the implementation of appropriate technologies.

The transition to a circular economy also requires regulatory frameworks that support sustainability initiatives, particularly in the food sector. For example, the European Union's Circular Economy Action Plan aims to accelerate the transition to a regenerative growth model that benefits both the economy and the environment (European Commission., 2020). This plan emphasizes waste prevention, improved resources use, and support for the valorization of by-products across various industries, including the food sector. By promoting innovation and encouraging the adoption of sustainable practices, such frameworks can play a key role in achieving a more resilient and environmentally friendly food system.

Aligning with these initiatives enables the food industry to not only meet regulatory expectations but also respond effectively to the growing consumer preference for ethical and eco-friendly products. The valorization of bioactive compounds, for instance, provides health and nutritional benefits, enhancing production efficiency and creates new markets as well. Achieving this transformation requires both public policy support and the adoption of innovative technologies that optimize the extraction and transformation processes of by-products. Such transformations rely on a collaborative approach involving governments, private enterprises, and academic institutions to build a resilient, green, and sustainable food system that addresses both environmental and socio-economic challenges.

In this context, the objective of this doctoral project (PON R&I action IV.5 on green topics) has been to explore two emerging and environmentally impactful issues: the management of food industry by-products and the increasing population of wild game. Through a circular economy approach, the aim was to transform these challenges

into valuable resources, demonstrating how environmental problems can be addressed while generating new opportunities.

1.1 Food industry by-products

The management of food industry by-products has become one of the foremost environmental challenges of the modern age. Each year, millions of tons of waste are generated during food production and processing. Traditionally, these residues have been disposed of in landfills or incinerated, leading to high costs and significant environmental impacts, including greenhouse gas emissions (Narasimmalu & Ramasamy, 2020). On the contrary, the circular economy model offers a sustainable and innovative alternative, repurposing by-products and reintegrating them into the value chain. This approach intend to minimize waste production by extending the life cycle of resources (Blomsma & Brennan, 2017). The valorization of food by-products, by transforming waste into valuable resources through innovative and biotechnological processes, enables to mitigate both environmental and economic impacts while generating new source of revenue (Rajković et al., 2020).

Food industry residues, such as peels, seeds, and other by-products, often contain valuable bioactive compounds like polyphenols, terpenes, aldehydes, carotenoids, and flavonoids that can be recovered and utilized in various industrial sectors (Gómez-García et al., 2021; Reguengo et al., 2022). If not managed properly, these agro-industrial by-products can cause significant environmental issues due to their composition. Organic decomposition in landfills produces methane, a greenhouse gas, while incineration releases toxic substances into the air, further contributing to climate change (Narasimmalu & Ramasamy,

2020). Moreover, wastewater rich in organic compounds and nutrients can cause eutrophication in waterways and marine environments, promoting harmful algae growth and reducing biodiversity (Smith & Schindler, 2009). Additionally, the accumulation of organic residues in soil can alter its structure and negatively affect biodiversity by impacting both flora and fauna (Bardgett & Van Der Putten, 2014). Therefore, conventional methods of managing food waste through landfilling or incineration are unsustainable, leading to harmful emissions with negative impacts on public health, air quality, and climate change (Gómez-García et al., 2021). Implementing a circular economy model in the food industry encourages to progressively transform by-products from previous production cycles into valuable resources, thus reducing environmental impacts and adding value. This approach not only reduces the amount of generated waste but also promotes the production of resources in a more sustainable and responsible manner. For instance, by-products rich in polyphenols, essential oils, carotenoids, and peptides can be used in high-value sectors like cosmetics, dietary supplements, and pharmaceuticals (Reguengo et al., 2022).

The circular economy represents an innovative paradigm, as opposed to the traditional linear economic model based on extraction, production, consumption, and disposal towards a sustainable system where resources are reused and regenerated trough the principles of “reducing, reusing and recycling” materials to minimize waste and maximize resource efficiency (Kirchherr et al., 2017).

1.1.1 Agro-industrial by products and their applications

Food by-products, such as peels, seeds, and pulp residues, are rich in valuable bioactive compounds like polyphenols, flavonoids, and carotenoids, which possess antioxidant, antimicrobial, and health-promoting properties (Faustino et al., 2019). These compounds help counteract free radicals and reduce inflammation and therefore they could find application in the pharmaceutical and nutraceutical sectors (Radha et al., 2024). For instance, polyphenols extracted from citrus and grape peels have been studied for their ability to reduce inflammation and protect cells from oxidative stress, which is a major cause of chronic diseases (Reguengo et al., 2022).

Agro-industrial by-products have significant potential for energy recovery and resource reuse. Food waste can be processed through anaerobic digestion or composting, transforming organic waste into biogas or organic fertilizers. These processes contribute to waste reduction and nutrient recycling, providing ecological solutions for waste management and supporting more sustainable agricultural production (Narasimmalu & Ramasamy, 2020).

A promising application for agro-industrial by-products is their use as natural additives and preservatives in the food industry (Bouarab Chibane et al., 2019). The olive oil industry generates a significant amount of by-products, depending on the production technique and they can be found in solid or liquid form, such as olive leaves, olive pomace, olive pit and wastewaters. These by-products are recognized for their high concentrations of various phenolic compounds, along with other organic components like pectins, insoluble dietary fibers, proteins, sugars, and nitrogenous substances (Galanakis, 2018). The main phenolic compounds of olive industry by-products are 3,4-

dihydroxyphenylethanol (3,4-DHPEA or hydroxytyrosol), p-hydroxyphenylethanol (p-HPEA or tyrosol) and secoiridoids derivatives, in particular, the dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA or p-HPEA (3,4DHPEA-EDA or p-HPEA-EDA, respectively), widely studied for their antioxidant and antibacterial activity. Due to their potential antioxidant and antimicrobial activities, these by-products can serve as a valuable strategy in the food industry as natural preservatives. The growing interest in these substances has encouraged scientific research and the study of these by-products and their bioactive compounds in meat (Balzan et al., 2017; Barbieri et al., 2021; Munekata et al., 2020), fish (Cedola et al., 2017; Khemakhem et al., 2019; Martínez et al., 2019), and dairy products (Ferguous et al., 2023; Palmeri et al., 2019; Roila et al., 2019). Several studies have also explored the use of bioactive compounds in active packaging to delay lipid oxidation and extend shelf life of foods (Amaro-Blanco et al., 2018; Moudache et al., 2016, 2017).

Another industry with a significant impact on by-product generation is the tomato processing sector. In this case, the by-products, mainly peels and seeds, are rich in bioactive substances such as lycopene, β-carotene, glycoalkaloids and phenolic compounds. These by-products have been studied as colorants, natural preservative, or as functional ingredients to enhance texture and nutritional value or to delay lipid oxidation and extend the shelf life of food products (Domínguez et al., 2020; Faustino et al., 2019). Especially in the meat industry, tomato by-products can be used in different forms such as powders, pastes or oleoresins as natural alternative to synthetic antioxidants and colorants. Tomato powder or paste added to meat patties or sausages has been shown to improve redness while delaying oxidation and discolouration

during storage (Domínguez et al., 2020; Kim et al., 2013; Savadkoohi et al., 2014). This is especially valuable because oxidation can lead to off-flavors and reduced nutritional value in meat. Moreover, lycopene is heat-stable, meaning that it retains its antioxidant and colorant properties even during the cooking process (Domínguez et al., 2020).

Other agro-industrial by-products worth mentioning are those generated from the coffee production chain. These by-products include pulp, husks, parchment, and spent coffee grounds, and they contain valuable bioactive compounds such as chlorogenic acids, alkaloids, diterpens and other secondary metabolites (de Melo Pereira et al., 2020). These by-products can be repurposed as an excellent natural fertilizer or as a supplement for animal feed (Iriondo-DeHond et al., 2020). Spent coffee grounds, a common widely available by-product of coffee preparation can be used in the production of biofuels, bioplastics, and as adsorbent materials (Iriondo-DeHond et al., 2020). Additionally, spent coffee grounds have applications in cosmetics as natural exfoliants and in skincare products due to their antioxidant properties (Campos-Vega et al., 2015).

Recent *in vitro* studies evaluated the use of spent coffee grounds in the food sector as a natural antimicrobial, although the results have been mixed (Díaz-Hernández et al., 2022; Jiménez-Zamora et al., 2015; Sousa et al., 2015). A common challenge across all by-products is the difficulty in comparing studies and their outcomes due to variables such as extraction methods, the types and quantities of bioactive compounds present, and their interactions with other substances.

1.1.2 Application of bioactive compounds in the meat industry

The meat industry faces ongoing challenges related to quality preservation, safety, and consumer demand for natural and sustainable solutions. Agro-industrial by-products can be used as natural additives, preservatives, or functional ingredients, helping to reduce the use of synthetic additives while enhancing product quality and shelf life. Plant-derived bioactive compounds can contribute to microbial control in meat products, offering an alternative to synthetic preservatives such as nitrates and nitrites. In this chapter, we will explore some practical examples.

Rosemary is commonly used as a spice and flavouring agent in meat products. Its essential oil contains around 15 bioactive compounds and research highlights its antimicrobial properties. Rosemary ethanol extracts have been shown to reduce *L. monocytogenes* in beef by 2 log CFU/g after 9 days of refrigeration at 4 °C (Soyer et al., 2020). In chicken meat, 5 mg/mL of rosemary essential oil has reduced the growth of coliform, aerobic, lactic acid, and anaerobic bacteria by 0.87–1.75 log CFU/g after 24 hours at 18 °C, while decreasing *S. Enteritidis* by over 2 log CFU/g at 18 °C and less than 1 log CFU/g at 4 °C (Stojanović-Radić et al., 2018). Additionally, applying 0.2% rosemary essential oil with modified atmosphere packaging has inhibited the growth of *S. Typhimurium* and *L. monocytogenes* in poultry filets under refrigeration for 7 days, with minimal impact on sensory properties. However, its antibacterial effects on *L. monocytogenes* were limited, with only a 0.1 log CFU/g reduction on the first day of storage (Kahraman et al., 2015).

Oregano is another plant widely used in Mediterranean foods and researches highlight its potential in food preservation. Oregano

essential oil, containing carvacrol (42.94%) and thymol (17.40%), was tested on black wildebeest *Biceps femoris* muscles stored at 2.6 ± 0.6 °C, showing slower bacterial growth for total viable count, lactic acid bacteria and coliform counts throughout storage in the treated group compared to control (Shange et al., 2019). In vacuum-packed minced beef, a combination of 0.2% oregano essential oil, 0.5% caprylic acid, and 0.1% citric acid reduced lactic acid bacteria by 1.5 log CFU/g and psychrotrophic bacteria and *L. monocytogenes* by over 2.5 log CFU/g stored at 3 °C over 10 days (Hulankova et al., 2013).

Tomato, melon and carrot by-products were investigated by Ricci et al., (2021) for their antimicrobial activity against spoilage microorganisms in minced meat and ready-to-eat vegetables products. Notably, the fermented tomato by-product extract was tested in minced pork meat, where it allowed to effectively maintained a lower total microbial lad compared to the control. Moreover, at concentrations of 1.6% and 2.4%, its antimicrobial activity was comparable to that of sodium lactate/sodium diacetate, a widely used preservative in meat preservation (Ricci et al., 2021).

In addition to antimicrobial properties, bioactive compounds derived from agro-industrial by-products demonstrate remarkable antioxidant activity, which plays a critical role in preserving meat quality. For example, pomegranate by-products, including juice and rind powder extract, showed superior antioxidant performance compared to synthetic butylated hydroxytoluene (BHT) in cooked chicken patties, protecting against oxidative rancidity and lipid degradation (Naveena et al., 2008). Similarly, grape pomace was successfully incorporated into raw beef patties, significantly inhibiting lipid oxidation due to its high polyphenolic content (García-Lomillo et al., 2017). Another study highlighted the use of peanut skin extract, rich in phenolic acids and

flavonoids, which effectively delayed lipid oxidation in cooked chicken patties (Munekata et al., 2015) and preserved the sensory attributes of salami (Larrauri et al., 2013).

Olive oil industry by-products have been studied for their potential as functional ingredients in meat products. Hayes et al. (2010) reported that an extract derived from olive leaves effectively inhibited lipid oxidation in beef patties stored under both aerobic and modified atmosphere packaging, significantly delaying the development of rancid odours (Hayes et al., 2010). Furthermore, studies have highlighted the efficacy of olive leaf extract in reducing lipid oxidation during storage in various meat products, such as frankfurter type sausages (Alirezalu et al., 2018), cooked pork sausages (Hayes et al., 2011), and pork patties (Hayes et al., 2010). Research has also explored the use of olive industry extracts in meat products for their antimicrobial activity. Veneziani et al. (2017) demonstrated the antimicrobial potential of a purified extract from olive mill wastewater in fermented salami, where the addition of 0.15% of the extract significantly inhibited *L. monocytogenes* growth after 45 days (Veneziani et al., 2017). Fasolato et al. (2015) investigated the use of a crude phenolic concentrate from olive mill wastewater as a preservative for chicken breast. Samples dipped in the solution for 60 seconds before packaging showed delayed growth of *Enterobacteriaceae* and *Pseudomonas* spp., extending shelf life by at least two days. Additionally, treated samples exhibited significantly lower lipid oxidation (TBAR values), improved odour, and a slight yellowing of the surface color compared to controls (Fasolato et al., 2015).

These examples underscore the practical use of agro-industrial by-products as sustainable, natural alternatives to synthetic antioxidants in meat products.

1.2 Wild boar: population and meat characteristics

1.2.1 The rise of wild boar populations: ecological, economic, and public health implications

The global population of wild ungulates, especially wild boars, has increased dramatically during the last decade leading to a range of environmental, economic, public health, and social challenges (Massei et al., 2015; Tack, 2018).

Several factors may affect wild boar population dynamics and growth. Firstly, it is important to consider the species-specific traits that distinguish these ungulates, particularly their high level of adaptability. This characteristic allows wild boars to thrive in a wide range of habitats, from semi-arid environments to marshes, forests, alpine grasslands and more recently even in urban environments, where they have access to anthropogenic wastes (Cahill et al., 2012; Castillo-Contreras et al., 2021; Colomer et al., 2024).

Another key factor that has significantly contributed to wild boar spread worldwide is their high reproductive output, due to their high fertility and prolific rate (Fonseca et al., 2011; Massei et al., 2015).

Animal-related factors, combined with the phenomenon of rural depopulation, reforestation and climate change resulting in increasingly mild winters, have facilitated the exponential growth of wild boar populations, leading them, in some cases, to venture into urban areas in search of food.

Especially in regions where natural predators of wild boars are absent, wildlife management plans have become the primary means of controlling these expanding populations. Historically, hunting has been the main factor in regulating wild boar numbers and remains the most

significant cause of mortality for this species (Keuling et al., 2013). However, practices like animal releases for hunting purposes, along with baiting and supplementary feeding, have contributed to the demographic growth of these animals. Despite this, the number of hunters has either declined or remained steady in several countries, making recreational hunting insufficient to curb the growth of wild boars and mitigate their impact (Massei et al., 2015).

The uncontrolled growth of wild boar populations has led to significant damage to agricultural crops, generating economic losses, while also negatively affecting ecosystems and biodiversity (Amici et al., 2012). Additionally, wild boars pose a public safety risk, particularly as they increasingly cause road accidents, adding further concerns for communities affected by their expanding presence (Sáenz-de-Santa-María & Tellería, 2015).

Wildlife can transmit or act as reservoirs for various pathogens harmful to humans, livestock and domestic animals via direct contact with wild animals, through contaminated food or indirectly through contaminated environment (Fredriksson-Ahomaa, 2019). Regarding wild boars, their potential role in spreading several bacterial (e.g., *Mycobacterium* spp., *Salmonella* spp., *Brucella* spp.) (Fredriksson-Ahomaa et al., 2020; Varela-Castro et al., 2020), viral (e.g., Hepatitis E virus, African swine fever virus) (Fanelli et al., 2022; Ruiz-Fons et al., 2008), and parasitic (e.g., *Trichinella* spp., *Toxoplasma gondii*) (Fichi et al., 2015; Lizana et al., 2021; Rostami et al., 2017) diseases has been documented. Their increasing numbers and geographical spread pose a significant health risk, particularly for zoonotic diseases. Moreover, they also serve as carriers for several important infectious diseases affecting livestock such as African swine fever, classical swine fever, and Aujeszky's disease, with substantial economic repercussions for the swine industry

in several countries (Meier et al., 2015; Postel et al., 2018; Schulz et al., 2017).

In 2023, African swine fever (ASF) spread to new regions within the EU and non-EU countries. It was reported for the first time in Croatia, Sweden, Bosnia and Herzegovina, and Kosovo, and re-emerged in Greece and Italy. The same year saw the highest number of ASF outbreaks in domestic pigs since the disease entered the EU in 2014, with 1,929 outbreaks in the EU and 2,528 in non-EU countries, particularly in Croatia, Romania, Bosnia and Herzegovina, and Serbia. ASF outbreaks in wild boar increased by 9% compared to 2022, with 7,855 cases reported (European Food Safety Authority (EFSA) et al., 2024). Outbreaks in wild boars can quickly spill over into domestic pig populations, posing a continuous threat to pig farms and leading to severe economic issues in the pork industry due to the high mortality rate and the trade restrictions that follow ASF outbreaks.

This underscores the need to manage and properly control wild boar population, which will naturally result in a greater availability of wild boar meat. For example, in Italy, a comprehensive plan for the period 2023-2028 has been developed to mitigate the risk of African swine fever spread. This extraordinary eradication program involves the capture and culling of approximately 612,000 wild boars per year across the national territory. The plan is regionally distributed based on categories of criticality (Commissario Straordinario alla Peste suina africana, 2024).

The upcoming challenge will be to develop commercial strategies to effectively enhance the value of this meat and its related products, for example establishing local supply chains that deliver high-quality and traceable products to consumers.

1.2.2 Wild boar meat: composition and nutritional characteristics

Wild boar meat is traditionally associated with the culinary heritage of certain regions or specific territories, albeit due to its nutritional and quality characteristics, it could also satisfy the demands of the modern consumer (Niewiadomska et al., 2020).

The nutritional profile and chemical composition of wild boar meat possess peculiar qualities that distinguish it from other meats, especially when compared to domestic pork. Wild boar meat is characterized by high protein content, ranging from 22% to 26%, and low total lipid content, which is typically between 2% and 5% depending on cut, season and animal's diet (Sales & Kotrba, 2013). In addition to a low overall lipid content, the fats present are predominantly unsaturated fatty acids, known for their beneficial and positive impact on health (Di Bella et al., 2024; Viganò et al., 2019). In particular, wild boar meat contains high levels of omega-3 and omega-6 polyunsaturated fatty acids (PUFAs), which play a crucial role in cardiovascular health by reducing blood lipid levels and modulating inflammatory responses. Moreover, the meat exhibits a favorable ratio of monounsaturated fats, which are known to positively influence cholesterol levels, thereby reducing the risk of heart disease (Ciobanu et al., 2022; Di Bella et al., 2024). The polyunsaturated/saturated fatty acids (PUFA/SFA) ratio in wild boar meat is notably more favorable compared to that of conventionally farmed pork, primarily due to the animal's natural diet, which includes acorns, roots, and other vegetation. Studies have shown that wild boars consuming acorns-rich diets, especially in Mediterranean regions, show higher levels of oleic acid and PUFAs, further improving the PUFA/SFA ratio (Di Bella et al., 2024; Sales & Kotrba, 2013; Schley & Roper, 2003).

Furthermore, wild boar meat is rich in essential minerals such as iron, zinc, and phosphorus, which are crucial for various physiological processes, including oxygen transport, immune function, and bone health. It also contains notable amounts of vitamins, particularly B-complex vitamins like B12, B6, niacin, and riboflavin, which play key roles in energy metabolism and maintaining nervous system function (Sales & Kotrba, 2013). Additionally, wild boar meat offers a higher concentration of micronutrients compared to pork, which enhances its value as a nutrient-dense food source (Sales & Kotrba, 2013). This composition makes it a suitable choice for consumers seeking natural and nutrient-rich dietary options.

In addition to nutritional and quality aspects, modern consumers' opinions are increasingly becoming more favorable towards game meat, particularly due to its ethical characteristics since it comes from animals born and raised in their natural habitats, free from any pharmacological treatments (Niewiadomska et al., 2020).

1.2.3 Game meat harvesting: methods and hygienic characteristics

Game meat, particularly wild boar, presents highly variable hygienic, sanitary, and quality characteristics that are closely related to pre-harvest and post-harvest procedures.

Game meat production, typically carried out in the wild, encounters challenges in upholding strict hygiene standards due to possible suboptimal environmental conditions, where animals are more prone to contamination during hunting and following related practices (Gomes-Neves et al., 2021). The production chain itself is unique, involving several stages where different individuals are responsible for ensuring the safety and quality of the meat. Several factors such as hunting

method, hunter's expertise, environmental conditions and the evisceration process can significantly affect the hygiene of the carcass and, as a result, the overall quality of the meat.

1.2.3.1 Hunting methods

In Europe, especially in Italy, the most common methods for hunting wild boar include driven hunts, stalking, and still hunting. The most traditional and widely practiced method is drive hunting, a collective hunting performed during hunting season, where wild boar is chased by dogs and run towards shooters in fixed positions. This type of hunt involves a large number of participants and typically lasts for several hours. During the chase, the boar experiences considerable stress, and multiple shots are frequently needed to bring the animal down.

Stalking is a more targeted and scaled-down version of drive hunting, typically involving fewer hunters and a single leashed dog used to guide wild boars toward designated positions.

Still hunting, on the other hand, is a completely different approach, characterized by a quieter and less invasive method that could be performed all year round as control management. The hunter positioned in a strategic location, often a blind or elevated spots, waits for the wild boar to pass, usually during the twilight hours. Only few hunters without dogs are involved. This method significantly reduces the animal’s stress, as it involves no pursuit, and usually, one-shot ethical killing can be performed.

Trapping is another effective population management tool, complementing traditional hunting (Torres-Blas et al., 2020). It entails the use of various types and sizes of traps, such as cages, enclosures, drop-net and corral traps, designed to lure wild boars inside with bait.

1.2.3.2 Factors affecting the hygienic quality of wild boar carcasses and meat

In Italy, wild game carcasses or meat may be used for personal consumption by the hunter, sold directly in small quantities to final consumers or local retailers, or commercialized through an authorized game-handling facility (*Conferenza Stato Regioni 25 marzo 2021 - Linee guida in materia di igiene delle carni di selvaggina selvatica*, s.d.).

Different regulations apply depending on the final use, with various roles along the supply chain responsible for ensuring the hygienic quality of the product. In case of personal consumption and direct supply, the hunter is primarily responsible. For personal consumption, no specific regulations apply; however, for direct supply, Regulation (EC) 178/2002 and Regulation (EU) 1375/2015, regarding *Trichinella* monitoring are required (Regulation (EC) 178/2002, s.d.; Regulation (EU) 2015/1375, s.d.). Wild game intended for commercial sale must be processed and inspected by veterinarian officers at an authorized game-handling establishment, in compliance with Regulation (Regulation (EC) 853/2004, s.d.). In this case, the carcasses may first be taken to a collection center, where they are weighted, eviscerated, and refrigerated without skinning, before being transfered to a game-handling establishment.

The timing and environmental conditions under which bleeding and evisceration procedures occur are critical to achieving carcasses with high hygienic quality. The environmental temperature during harvesting is a critical factor that significantly impacts hygiene parameters of wild boar carcasses. Even when carcasses are transported

to the collection centers within five hours from shooting, higher ambient temperatures can accelerate microbial growth (Ranucci et al., 2021). Studies have shown that lower environmental temperatures, particularly those below 15 °C, enable to rapidly lower the body temperature of the animal, thereby limiting microbial proliferation (Ranucci et al., 2021; Stella et al., 2018). Conversely, when harvesting occurs in warmer conditions, the slower cooling rate can contribute to higher bacterial loads of the carcass if neither immediate nor rapid refrigeration is applied. For this reason, proximity to a collection center, where carcasses can be promptly cooled, is essential for maintaining a high hygienic standard, especially in warmer climates or for population control activities, which can occur nearly year-round.

Research highlights that inadequate hygiene practices, such as eviscerating wild boar carcasses directly on the ground or washing the carcass skin and interior surfaces post-evisceration, can significantly influence and compromise the microbiological quality of the final product (Mirceta et al., 2017).

It is therefore crucial that evisceration and skinning procedures must be performed by properly trained operators, since there are critical steps to avoid the rupture of the gastrointestinal trait and to prevent contamination from the skin or any other source of contamination (Orsoni et al., 2020).

Additionally, both the time elapsed between shooting and evisceration and the handling methods performed can substantially affect the microbial load present on game meat carcasses (Avagnina et al., 2012; Orsoni et al., 2020). Moreover, Orsoni et al., 2020 identified a correlation between the microbiological contamination of wild boar carcasses and the animal's weight, suggesting that, since weight and age are strictly related, older boars may carry a higher contamination risk

and thus require even more care during handling to minimize contamination.

The skill level of hunters also significantly impacts the final quality of the meat. Inexperienced hunters may require multiple shots to bring down the animal, increasing the risk of abdominal shots and gastrointestinal rupture, which can lead to contamination and associated hygienic issues. However, the hunting method itself can contribute to these risks; certain hunting techniques such as drive hunting, make such situations more likely, regardless of the hunter's expertise or accuracy.

In the European Union, there are no specific microbiological standards set for wild boar carcasses. Consequently, the pork carcass standards outlined in Regulation (EC) 2073/2005 on food safety are often used voluntarily to evaluate microbiological quality in wild boar meat (Regulation (EC) 2073/2005, s.d.). According to this regulation, testing for aerobic colony count, *Enterobacteriaceae* counts and the presence of *Salmonella* spp. on different sampling sites of pork carcasses is required to monitor the processing hygiene. Several studies have assessed the hygienic quality of wild boar carcasses, highlighting a wide variability in results influenced by all the factors discussed earlier. Using Regulation (EC) 2073/2005 as a reference, specifically the limits for pork carcasses regarding aerobic colony count and *Enterobacteriaceae* count, some studies report low microbial levels on wild boar carcasses that would be considered acceptable under these standards (Atanassova et al., 2008; Avagnina et al., 2012; Orsoni et al., 2020; Ranucci et al., 2021; Stella et al., 2018). Conversely, other research has found microbial counts that would be deemed unacceptable due to high levels of contamination (Mirceta et al., 2017; Peruzy et al., 2019, 2022).

References

Alirezalu, K., Hesari, J., Nemati, Z., & Farmani, B. (2018). *Effects of Selected Plant-Derived Nutraceuticals on the Quality and Shelf-Life Stability of Frankfurter Type Sausages during Storage*. *12*(9).

Amaro-Blanco, G., Delgado-Adámez, J., Martín, M. J., & Ramírez, R. (2018). Active packaging using an olive leaf extract and high pressure processing for the preservation of sliced dry-cured shoulders from Iberian pigs. *Innovative Food Science & Emerging Technologies*, *45*, 1–9. https://doi.org/10.1016/j.ifset.2017.09.017

Amici, A., Serrani, F., Rossi, C. M., & Primi, R. (2012). Increase in crop damage caused by wild boar (Sus scrofa L.): The "refuge effect". *Agronomy for Sustainable Development*, *32*(3), 683–692. https://doi.org/10.1007/s13593-011-0057-6

Atanassova, V., Apelt, J., Reich, F., & Klein, G. (2008). Microbiological quality of freshly shot game in Germany. *Meat Science*, *78*(4), 414–419. https://doi.org/10.1016/j.meatsci.2007.07.004

Avagnina, A., Nucera, D., Grassi, M. A., Ferroglio, E., Dalmasso, A., & Civera, T. (2012). The microbiological conditions of carcasses from large game animals in Italy. *Meat Science*, *91*(3), 266–271. https://doi.org/10.1016/j.meatsci.2012.01.025

Balzan, S., Taticchi, A., Cardazzo, B., Urbani, S., Servili, M., Di Lecce, G., Zabalza, I. B., Rodriguez-Estrada, M. T., Novelli, E., & Fasolato, L. (2017). Effect of phenols extracted from a by-product of the oil mill on the shelf-life of raw and cooked fresh pork sausages in the absence of chemical additives. *LWT - Food*

Science and Technology, *85*, 89–95. https://doi.org/10.1016/j.lwt.2017.07.001

Barbieri, S., Mercatante, D., Balzan, S., Esposto, S., Cardenia, V., Servili, M., Novelli, E., Taticchi, A., & Rodriguez-Estrada, M. T. (2021). Improved Oxidative Stability and Sensory Quality of Beef Hamburgers Enriched with a Phenolic Extract from Olive Vegetation Water. *Antioxidants*, *10*(12), 1969. https://doi.org/10.3390/antiox10121969

Bardgett, R. D., & Van Der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature*, *515*(7528), 505–511. https://doi.org/10.1038/nature13855

Blomsma, F., & Brennan, G. (2017). The Emergence of Circular Economy: A New Framing Around Prolonging Resource Productivity. *Journal of Industrial Ecology*, *21*(3), 603–614. https://doi.org/10.1111/jiec.12603

Bouarab Chibane, L., Degraeve, P., Ferhout, H., Bouajila, J., & Oulahal, N. (2019). Plant antimicrobial polyphenols as potential natural food preservatives. *Journal of the Science of Food and Agriculture*, *99*(4), 1457–1474. https://doi.org/10.1002/jsfa.9357

Boz, Z., Korhonen, V., & Koelsch Sand, C. (2020). Consumer Considerations for the Implementation of Sustainable Packaging: A Review. *Sustainability*, *12*(6), Articolo 6. https://doi.org/10.3390/su12062192

Cahill, S., Llimona, F., Cabañeros, L., & Calomardo, F. (2012). Characteristics of wild boar (Sus scrofa) habituation to urban areas in the Collserola Natural Park (Barcelona) and comparison with other locations. *Animal Biodiversity and Conservation*, *35*(2), Articolo 2.

Campos-Vega, R., Loarca-Piña, G., Vergara-Castañeda, H. A., & Oomah, B. D. (2015). Spent coffee grounds: A review on current research and future prospects. *Trends in Food Science & Technology*, *45*(1), 24–36. https://doi.org/10.1016/j.tifs.2015.04.012

Castillo-Contreras, R., Mentaberre, G., Fernandez Aguilar, X., Conejero, C., Colom-Cadena, A., Ráez-Bravo, A., González-Crespo, C., Espunyes, J., Lavín, S., & López-Olvera, J. R. (2021). Wild boar in the city: Phenotypic responses to urbanisation. *Science of The Total Environment*, *773*, 145593. https://doi.org/10.1016/j.scitotenv.2021.145593

Cedola, A., Cardinali, A., Del Nobile, M. A., & Conte, A. (2017). Fish burger enriched by olive oil industrial by-product. *Food Science & Nutrition*, *5*(4), 837–844. https://doi.org/10.1002/fsn3.461

Ciobanu, M. M., Postolache, A. N., Lipşa, F. D., Munteanu, M., Rațu, R. N., Murariu, O. C., & Boișteanu, P. C. (2022). Meat Fatty Acid Composition of Wild Boars Hunted in Romania in Relationship to Gender and Age-Class. *Animals*, *12*(7), 810. https://doi.org/10.3390/ani12070810

Colomer, J., Massei, G., Roos, D., Rosell, C., & Rodríguez-Teijeiro, J. D. (2024). What drives wild boar density and population growth in Mediterranean environments? *Science of The Total Environment*, *931*, 172739. https://doi.org/10.1016/j.scitotenv.2024.172739

Commissario Straordinario alla Peste suina africana. (2024). *Piano Straordinario di catture, abbattimento e smaltimento dei cinghiali (Sus scrofa) e Azioni Strategiche per l'Elaborazione dei Piani di Eradicazione nelle Zone di Restrizione da Peste Suina Africana (PSA).*

Conferenza Stato Regioni 25 marzo 2021—Linee guida in materia di igiene delle carni di selvaggina selvatica. (s.d.). Recuperato 3 giugno 2024, da https://www.anmvioggi.it/images/banners/LINEE_GUIDA_IGIENE_DELLE_CARNI_DI_SELVAGGINA.pdf

de Melo Pereira, G. V., de Carvalho Neto, D. P., Magalhães Júnior, A. I., do Prado, F. G., Pagnoncelli, M. G. B., Karp, S. G., & Soccol, C. R. (2020). Chapter Three—Chemical composition and health properties of coffee and coffee by-products. In F. Toldrá (A c. Di), *Advances in Food and Nutrition Research* (Vol. 91, pp. 65–96). Academic Press. https://doi.org/10.1016/bs.afnr.2019.10.002

Di Bella, S., Branciari, R., Haouet, N. M., Framboas, M., Mercuri, M. L., Codini, M., Roila, R., Malimpensa, A., & Ranucci, D. (2024). Does hunted wild boar meat meet modern consumer nutritional expectations? *Italian Journal of Food Safety*. https://doi.org/10.4081/ijfs.2024.11608

Díaz-Hernández, G. C., Alvarez-Fitz, P., Maldonado-Astudillo, Y. I., Jiménez-Hernández, J., Parra-Rojas, I., Flores-Alfaro, E., Salazar, R., & Ramírez, M. (2022). Antibacterial, Antiradical and Antiproliferative Potential of Green, Roasted, and Spent Coffee Extracts. *Applied Sciences*, *12*(4), Articolo 4. https://doi.org/10.3390/app12041938

Domínguez, R., Gullón, P., Pateiro, M., Munekata, P. E. S., Zhang, W., & Lorenzo, J. M. (2020). Tomato as Potential Source of Natural Additives for Meat Industry. A Review. *Antioxidants*, *9*(1), Articolo 1. https://doi.org/10.3390/antiox9010073

European Commission. (2020). *COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE*

COUNCIL, THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE AND THE COMMITTEE OF THE REGIONS. A new Circular Economy Action Plan.

European Food Safety Authority (EFSA), Ståhl, K., Boklund, A. E., Podgórski, T., Vergne, T., Abrahantes, J. C., Cattaneo, E., Papanikolaou, A., & Mur, L. (2024). Epidemiological analysis of African swine fever in the European Union during 2023. *EFSA Journal*, *22*(5). https://doi.org/10.2903/j.efsa.2024.8809

Fanelli, A., Tizzani, P., & Buonavoglia, D. (2022). A systematic review and meta-analysis of hepatitis E virus (HEV) in wild boars. *Research in Veterinary Science*, *142*, 54–69. https://doi.org/10.1016/j.rvsc.2021.11.015

Fasolato, L., Cardazzo, B., Balzan, S., Carraro, L., Taticchi, A., Montemurro, F., & Novelli, E. (2015). Minimum Bactericidal Concentration of Phenols Extracted from Oil Vegetation Water on Spoilers, Starters and Food-Borne Bacteria. *Italian Journal of Food Safety*, *4*(2), 4519. https://doi.org/10.4081/ijfs.2015.4519

Faustino, M., Veiga, M., Sousa, P., Costa, E. M., Silva, S., & Pintado, M. (2019). Agro-Food Byproducts as a New Source of Natural Food Additives. *Molecules*, *24*(6), 1056. https://doi.org/10.3390/molecules24061056

Ferguous, H., Mehyar, G., & Ibrahim, S. A. (2023). The Antimicrobial and Antioxidation Activities of Olive Pomace Extract in Pasteurized White Cheese. *Jordan Journal of Agricultural Sciences*, *19*(4), Articolo 4. https://doi.org/10.35516/jjas.v19i4.432

Fichi, G., Stefanelli, S., Pagani, A., Luchi, S., De Gennaro, M., Gómez-Morales, M. A., Selmi, M., Rovai, D., Mari, M., Fischetti, R.,

& Pozio, E. (2015). Trichinellosis Outbreak Caused by Meat from a Wild Boar Hunted in an Italian Region Considered to be at Negligible Risk for *Trichinella*. *Zoonoses and Public Health*, *62*(4), 285–291. https://doi.org/10.1111/zph.12148

Fonseca, C., da Silva, A. A., Alves, J., Vingada, J., & Soares, A. M. V. M. (2011). Reproductive performance of wild boar females in Portugal. *European Journal of Wildlife Research*, *57*(2), 363–371. https://doi.org/10.1007/s10344-010-0441-6

Fredriksson-Ahomaa, M. (2019). Wild Boar: A Reservoir of Foodborne Zoonoses. *Foodborne Pathogens and Disease*, *16*(3), 153–165. https://doi.org/10.1089/fpd.2018.2512

Fredriksson-Ahomaa, M., London, L., Skrzypczak, T., Kantala, T., Laamanen, I., Biström, M., Maunula, L., & Gadd, T. (2020). Foodborne Zoonoses Common in Hunted Wild Boars. *EcoHealth*, *17*(4), 512–522. https://doi.org/10.1007/s10393-020-01509-5

Galanakis, C. M. (2018). Phenols recovered from olive mill wastewater as additives in meat products. *Trends in Food Science & Technology*, *79*, 98–105. https://doi.org/10.1016/j.tifs.2018.07.010

García-Lomillo, J., Gonzalez-SanJose, M. L., Del Pino-García, R., Ortega-Heras, M., & Muñiz-Rodríguez, P. (2017). Antioxidant effect of seasonings derived from wine pomace on lipid oxidation in refrigerated and frozen beef patties. *LWT*, *77*, 85–91. https://doi.org/10.1016/j.lwt.2016.11.038

Gomes-Neves, E., Abrantes, A. C., Vieira-Pinto, M., & Müller, A. (2021). Wild Game Meat—A Microbiological Safety and Hygiene Challenge? *Current Clinical Microbiology Reports*, *8*(2), 31–39. https://doi.org/10.1007/s40588-021-00158-8

Gómez-García, R., Campos, D. A., Aguilar, C. N., Madureira, A. R., & Pintado, M. (2021). Valorisation of food agro-industrial by-products: From the past to the present and perspectives. *Journal of Environmental Management*, *299*, 113571. https://doi.org/10.1016/j.jenvman.2021.113571

Hayes, J. E., Stepanyan, V., Allen, P., O'Grady, M. N., & Kerry, J. P. (2010). Effect of lutein, sesamol, ellagic acid and olive leaf extract on the quality and shelf-life stability of packaged raw minced beef patties. *Meat Science*, *84*(4), 613–620. https://doi.org/10.1016/j.meatsci.2009.10.020

Hayes, J. E., Stepanyan, V., Allen, P., O'Grady, M. N., & Kerry, J. P. (2011). Evaluation of the effects of selected plant-derived nutraceuticals on the quality and shelf-life stability of raw and cooked pork sausages. *LWT - Food Science and Technology*, *44*(1), 164–172. https://doi.org/10.1016/j.lwt.2010.05.020

Hulankova, R., Borilova, G., & Steinhauserova, I. (2013). Combined antimicrobial effect of oregano essential oil and caprylic acid in minced beef. *Meat Science*, *95*(2), 190–194. https://doi.org/10.1016/j.meatsci.2013.05.003

Iriondo-DeHond, A., Iriondo-DeHond, M., & del Castillo, M. D. (2020). Applications of Compounds from Coffee Processing By-Products. *Biomolecules*, *10*(9), Articolo 9. https://doi.org/10.3390/biom10091219

Jiménez-Zamora, A., Pastoriza, S., & Rufián-Henares, J. A. (2015). Revalorization of coffee by-products. Prebiotic, antimicrobial and antioxidant properties. *LWT - Food Science and Technology*, *61*(1), 12–18. https://doi.org/10.1016/j.lwt.2014.11.031

Kahraman, T., Issa, G., Bingol, E. B., Kahraman, B. B., & Dumen, E. (2015). Effect of rosemary essential oil and modified-atmosphere packaging (MAP) on meat quality and survival of pathogens in poultry fillets. *Brazilian Journal of Microbiology*, *46*(2), 591–599. https://doi.org/10.1590/S1517-838246220131201

Keuling, O., Baubet, E., Duscher, A., Ebert, C., Fischer, C., Monaco, A., Podgórski, T., Prevot, C., Ronnenberg, K., Sodeikat, G., Stier, N., & Thurfjell, H. (2013). Mortality rates of wild boar Sus scrofa L. in central Europe. *European Journal of Wildlife Research*, *59*(6), 805–814. https://doi.org/10.1007/s10344-013-0733-8

Khemakhem, I., Fuentes, A., Lerma-García, M. J., Ayadi, M. A., Bouaziz, M., & Barat, J. M. (2019). Olive leaf extracts for shelf life extension of salmon burgers. *Food Science and Technology International*, *25*(2), 91–100. https://doi.org/10.1177/1082013218795816

Kim, I. S., Jin, S. K., Yang, M. R., Chu, G. M., Park, J. H., Rashid, R. H. I., Kim, J. Y., & Kang, S. N. (2013). Efficacy of Tomato Powder as Antioxidant in Cooked Pork Patties. *Asian-Australasian Journal of Animal Sciences*, *26*(9), 1339–1346. https://doi.org/10.5713/ajas.2013.13079

Kirchherr, J., Reike, D., & Hekkert, M. (2017). Conceptualizing the circular economy: An analysis of 114 definitions. *Resources, Conservation and Recycling*, *127*, 221–232. https://doi.org/10.1016/j.resconrec.2017.09.005

Larrauri, M., Barrionuevo, M. G., Riveros, C., Mestrallet, M. G., Zunino, M. P., Zygadlo, J. A., Grosso, N. R., & Nepote, V. (2013). Effect of peanut skin extract on chemical stability and

sensory properties of salami during storage. *Journal of the Science of Food and Agriculture*, *93*(7), 1751–1757. https://doi.org/10.1002/jsfa.5965

Lizana, V., Gortázar, C., Muniesa, A., Cabezón, Ó., Martí-Marco, A., López-Ramon, J., & Cardells, J. (2021). Human and environmental factors driving Toxoplasma gondii prevalence in wild boar (Sus scrofa). *Research in Veterinary Science*, *141*, 56–62. https://doi.org/10.1016/j.rvsc.2021.10.007

Martínez, L., Castillo, J., Ros, G., & Nieto, G. (2019). Antioxidant and Antimicrobial Activity of Rosemary, Pomegranate and Olive Extracts in Fish Patties. *Antioxidants*, *8*(4), Articolo 4. https://doi.org/10.3390/antiox8040086

Massei, G., Kindberg, J., Licoppe, A., Gačić, D., Šprem, N., Kamler, J., Baubet, E., Hohmann, U., Monaco, A., Ozoliņš, J., Cellina, S., Podgórski, T., Fonseca, C., Markov, N., Pokorny, B., Rosell, C., & Náhlik, A. (2015). Wild boar populations up, numbers of hunters down? A review of trends and implications for Europe. *Pest Management Science*, *71*(4), 492–500. https://doi.org/10.1002/ps.3965

Meier, R. K., Ruiz-Fons, F., & Ryser-Degiorgis, M.-P. (2015). A picture of trends in Aujeszky's disease virus exposure in wild boar in the Swiss and European contexts. *BMC Veterinary Research*, *11*(1), 277. https://doi.org/10.1186/s12917-015-0592-5

Mirceta, J., Petrovic, J., Malesevic, M., Blagojevic, B., & Antic, D. (2017). Assessment of microbial carcass contamination of hunted wild boars. *European Journal of Wildlife Research*, *63*(2), 37. https://doi.org/10.1007/s10344-017-1096-3

Moudache, M., Colon, M., Nerín, C., & Zaidi, F. (2016). Phenolic content and antioxidant activity of olive by-products and

antioxidant film containing olive leaf extract. *Food Chemistry*, *212*, 521–527. https://doi.org/10.1016/j.foodchem.2016.06.001

Moudache, M., Nerín, C., Colon, M., & Zaidi, F. (2017). Antioxidant effect of an innovative active plastic film containing olive leaves extract on fresh pork meat and its evaluation by Raman spectroscopy. *Food Chemistry*, *229*, 98–103. https://doi.org/10.1016/j.foodchem.2017.02.023

Munekata, P. E. S., Calomeni, A. V., Rodrigues, C. E. C., Fávaro-Trindade, C. S., Alencar, S. M., & Trindade, M. A. (2015). Peanut skin extract reduces lipid oxidation in cooked chicken patties. *Poultry Science*, *94*(3), 442–446. https://doi.org/10.3382/ps/pev005

Munekata, P. E. S., Nieto, G., Pateiro, M., & Lorenzo, J. M. (2020). Phenolic Compounds Obtained from Olea europaea By-Products and Their Use to Improve the Quality and Shelf Life of Meat and Meat Products—A Review. *Antioxidants*, *9*(11), Articolo 11. https://doi.org/10.3390/antiox9111061

Narasimmalu, A., & Ramasamy, R. (2020). Food Processing Industry Waste and Circular Economy. *IOP Conference Series: Materials Science and Engineering*, *955*(1), 012089. https://doi.org/10.1088/1757-899X/955/1/012089

Naveena, B. M., Sen, A. R., Vaithiyanathan, S., Babji, Y., & Kondaiah, N. (2008). Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. *Meat Science*, *80*(4), 1304–1308. https://doi.org/10.1016/j.meatsci.2008.06.005

Niewiadomska, K., Kosicka-Gębska, M., Gębski, J., Gutkowska, K., Jeżewska-Zychowicz, M., & Sułek, M. (2020). Game Meat

Consumption—Conscious Choice or Just a Game? *Foods*, *9*(10), Articolo 10. https://doi.org/10.3390/foods9101357

Orsoni, F., Romeo, C., Ferrari, N., Bardasi, L., Merialdi, G., & Barbani, R. (2020). Factors affecting the microbiological load of Italian hunted wild boar meat (Sus scrofa). *Meat Science*, *160*, 107967. https://doi.org/10.1016/j.meatsci.2019.107967

Palmeri, R., Parafati, L., Trippa, D., Siracusa, L., Arena, E., Restuccia, C., & Fallico, B. (2019). Addition of Olive Leaf Extract (OLE) for Producing Fortified Fresh Pasteurized Milk with an Extended Shelf Life. *Antioxidants*, *8*(8), Articolo 8. https://doi.org/10.3390/antiox8080255

Peruzy, M. F., Murru, N., Smaldone, G., Proroga, Y. T. R., Cristiano, D., Fioretti, A., & Anastasio, A. (2022). Hygiene evaluation and microbiological hazards of hunted wild boar carcasses. *Food Control*, *135*, 108782. https://doi.org/10.1016/j.foodcont.2021.108782

Peruzy, M. F., Murru, N., Yu, Z., Kerkhof, P.-J., Neola, B., Joossens, M., Proroga, Y. T. R., & Houf, K. (2019). Assessment of microbial communities on freshly killed wild boar meat by MALDI-TOF MS and 16S rRNA amplicon sequencing. *International Journal of Food Microbiology*, *301*, 51–60. https://doi.org/10.1016/j.ijfoodmicro.2019.05.005

Postel, A., Austermann-Busch, S., Petrov, A., Moennig, V., & Becher, P. (2018). Epidemiology, diagnosis and control of classical swine fever: Recent developments and future challenges. *Transboundary and Emerging Diseases*, *65*(S1), 248–261. https://doi.org/10.1111/tbed.12676

Radha, Prakash, S., Kumari, N., Sharma, N., Puri, S., Singh, J., Thakur, M., Pundir, A., & Kumar, M. (2024). Bioactives and

Bioactivities from Food Byproducts. *Current Food Science and Technology Reports*, *2*(3), 297–308. https://doi.org/10.1007/s43555-024-00039-3

Rajković, M., Popović-Minić, D., Milinčić, D., & Zdravković, M. (2020). Circular economy in food industry. *Zastita Materijala*, *61*(3), 229–250. https://doi.org/10.5937/zasmat2003229R6

Ranucci, D., Roila, R., Onofri, A., Cambiotti, F., Primavilla, S., Miraglia, D., Andoni, E., Di Cerbo, A., & Branciari, R. (2021). Improving Hunted Wild Boar Carcass Hygiene: Roles of Different Factors Involved in the Harvest Phase. *Foods*, *10*(7), 1548. https://doi.org/10.3390/foods10071548

Reguengo, L. M., Salgaço, M. K., Sivieri, K., & Maróstica Júnior, M. R. (2022). Agro-industrial by-products: Valuable sources of bioactive compounds. *Food Research International*, *152*, 110871. https://doi.org/10.1016/j.foodres.2021.110871

Regulation (EC) 178/2002. (s.d.). *REGULATION (EC) No 178/2002 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 28 January2002 laying down the general principles and requirements of food law, establishing the European Food SafetyAuthorityand laying down procedures in matters of food safety*.

Regulation (EC) 853/2004. (s.d.). *REGULATION (EC) No 853/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs.* Recuperato 21 dicembre 2024, da https://eur-lex.europa.eu/eli/reg/2004/853/oj/eng

Regulation (EC) 2073/2005. (s.d.). *Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria*

for foodstuffs. Recuperato 21 dicembre 2024, da http://data.europa.eu/eli/reg/2005/2073/oj/eng

Regulation (EU) 2015/1375. (s.d.). *COMMISSION IMPLEMENTING REGULATION (EU) 2015/1375 of 10 August 2015 laying down specific rules on official controls for Trichinella in meat.*

Ricci, A., Bertani, G., Maoloni, A., Bernini, V., Levante, A., Neviani, E., & Lazzi, C. (2021). Antimicrobial Activity of Fermented Vegetable Byproduct Extracts for Food Applications. *Foods*, *10*(5), 1092. https://doi.org/10.3390/foods10051092

Roila, R., Valiani, A., Ranucci, D., Ortenzi, R., Servili, M., Veneziani, G., & Branciari, R. (2019). Antimicrobial efficacy of a polyphenolic extract from olive oil by-product against "Fior di latte" cheese spoilage bacteria. *International Journal of Food Microbiology*, *295*, 49–53. https://doi.org/10.1016/j.ijfoodmicro.2019.02.013

Rostami, A., Riahi, S. M., Fakhri, Y., Saber, V., Hanifehpour, H., Valizadeh, S., Gholizadeh, M., Pouya, R. H., & Gamble, H. R. (2017). The global seroprevalence of Toxoplasma gondii among wild boars: A systematic review and meta-analysis. *Veterinary Parasitology*, *244*, 12–20. https://doi.org/10.1016/j.vetpar.2017.07.013

Ruiz-Fons, F., Segalés, J., & Gortázar, C. (2008). A review of viral diseases of the European wild boar: Effects of population dynamics and reservoir rôle. *The Veterinary Journal*, *176*(2), 158–169. https://doi.org/10.1016/j.tvjl.2007.02.017

Sáenz-de-Santa-María, A., & Tellería, J. L. (2015). Wildlife-vehicle collisions in Spain. *European Journal of Wildlife Research*, *61*(3), 399–406. https://doi.org/10.1007/s10344-015-0907-7

Sales, J., & Kotrba, R. (2013). Meat from wild boar (Sus scrofa L.): A review. *Meat Science*, *94*(2), 187–201. https://doi.org/10.1016/j.meatsci.2013.01.012

Savadkoohi, S., Hoogenkamp, H., Shamsi, K., & Farahnaky, A. (2014). Color, sensory and textural attributes of beef frankfurter, beef ham and meat-free sausage containing tomato pomace. *Meat Science*, *97*(4), 410–418. https://doi.org/10.1016/j.meatsci.2014.03.017

Schley, L., & Roper, T. J. (2003). Diet of wild boar Sus scrofa in Western Europe, with particular reference to consumption of agricultural crops. *Mammal Review*, *33*(1), 43–56. https://doi.org/10.1046/j.1365-2907.2003.00010.x

Schulz, K., Staubach, C., & Blome, S. (2017). African and classical swine fever: Similarities, differences and epidemiological consequences. *Veterinary Research*, *48*(1), 84. https://doi.org/10.1186/s13567-017-0490-x

Shange, N., Makasi, T., Gouws, P., & Hoffman, L. C. (2019). Preservation of previously frozen black wildebeest meat (Connochaetes gnou) using oregano (Oreganum vulgare) essential oil. *Meat Science*, *148*, 88–95. https://doi.org/10.1016/j.meatsci.2018.10.012

Smith, V. H., & Schindler, D. W. (2009). Eutrophication science: Where do we go from here? *Trends in Ecology & Evolution*, *24*(4), 201–207. https://doi.org/10.1016/j.tree.2008.11.009

Sousa, C., Gabriel, C., Cerqueira, F., Manso, M. C., & Vinha, A. F. (2015). *Coffee industrial waste as a natural source of bioactive compounds with antibacterial and antifungal activities*.

Soyer, F., Keman, D., Eroğlu, E., & Türe, H. (2020). Synergistic antimicrobial effects of activated lactoferrin and rosemary

extract in vitro and potential application in meat storage. *Journal of Food Science and Technology*, *57*(12), 4395–4403. https://doi.org/10.1007/s13197-020-04476-5

Stella, S., Tirloni, E., Castelli, E., Colombo, F., & Bernardi, C. (2018). Microbiological Evaluation of Carcasses of Wild Boar Hunted in a Hill Area of Northern Italy. *Journal of Food Protection*, *81*(9), 1519–1525. https://doi.org/10.4315/0362-028X.JFP-18-077

Stojanović-Radić, Z., Pejčić, M., Joković, N., Jokanović, M., Ivić, M., Šojić, B., Škaljac, S., Stojanović, P., & Mihajilov-Krstev, T. (2018). Inhibition of Salmonella Enteritidis growth and storage stability in chicken meat treated with basil and rosemary essential oils alone or in combination. *Food Control*, *90*, 332–343. https://doi.org/10.1016/j.foodcont.2018.03.013

Tack, J. (2018). *Wild Boar (Sus scrofa) populations in Europe: A scientific review of population trends and implications for management. European Landowners' Organization, Brussels, 56 pp.* https://wildbeimwild.com/wp-content/uploads/2023/08/12-Tack-J-Wild-Boar-Population-Trends-in-Europe-2018.pdf

Torres-Blas, I., Mentaberre, G., Castillo-Contreras, R., Fernández-Aguilar, X., Conejero, C., Valldeperes, M., González-Crespo, C., Colom-Cadena, A., Lavín, S., & López-Olvera, J. R. (2020). Assessing methods to live-capture wild boars (Sus scrofa) in urban and peri-urban environments. *Veterinary Record*, *187*(10), e85–e85. https://doi.org/10.1136/vr.105766

Varela-Castro, L., Alvarez, V., Sevilla, I. A., & Barral, M. (2020). Risk factors associated to a high Mycobacterium tuberculosis complex seroprevalence in wild boar (Sus scrofa) from a low

bovine tuberculosis prevalence area. *PLOS ONE*, *15*(4), e0231559. https://doi.org/10.1371/journal.pone.0231559

Veneziani, G., Novelli, E., Esposto, S., Taticchi, A., & Servili, M. (2017). Chapter 11—Applications of recovered bioactive compounds in food products. In C. M. Galanakis (A c. Di), *Olive Mill Waste* (pp. 231–253). Academic Press. https://doi.org/10.1016/B978-0-12-805314-0.00011-X

Viganò, R., Demartini, E., Riccardi, F., Corradini, A., Besozzi, M., Lanfranchi, P., Chiappini, P. L., Cottini, A., & Gaviglio, A. (2019). Quality parameters of hunted game meat: Sensory analysis and pH monitoring. *Italian Journal of Food Safety*, *8*(1), 7724. https://doi.org/10.4081/ijfs.2019.7724

Wunderlich, S., & Smoller, M. (2019). Consumer awareness and knowledge about food sources and possible environmental impact. *International Journal of Environmental Impacts: Management, Mitigation and Recovery*, *2*(1), 85–96. https://doi.org/10.2495/EI-V2-N1-85-96

Chapter 2 – Research activities

Aims

The aim of this thesis is to enhance the hygienic quality of wild boar carcasses and meat through the use of bioactive compounds derived from food industry by-products. The need to control wild boar population density, either through hunting or population management activities, will result in an increased availability of wild boar meat. This surplus could be utilized effectively by establishing local supply chains. The use of extracts from food industry by-products could be a strategy to improve the value of this meat, promoting sustainability and circular economy principles, while enhancing hygienic and quality traits.

As a first step, it was necessary to assess the main microorganisms regarding wild boar meat. A review of the literature and field sampling allowed the evaluation of the hygienic quality of wild boar meat and the identification of key pathogens, with particular focus on zoonotic agents that may pose a health risk to humans.

Simultaneously, *in vitro* studies were conducted to evaluate the antimicrobial efficacy of various extracts derived from food industry by-products by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The most promising extract, among those studied, turned out to be a polyphenolic extract obtained from olive mill vegetation waters from the olive processing industry and therefore it was then used to assess its potential application on carcasses against pathogenic bacteria. Since direct experimental contamination of carcasses with pathogens is not feasible in a slaughterhouse environment, the evaluation was conducted with an

in situ model to simulate the carcass surface. The samples were experimentally contaminated with two pathogens, one Gram-positive (*Listeria monocytogenes*) and one Gram-negative (*Salmonella* Enteritidis), and the bactericidal or bacteriostatic effect of the polyphenolic extract was evaluated.

After this initial phase of *in vitro* studies, the research shifted its focus to carcasses and meat.

According to European legislation, post-slaughter treatments with substances other than water are allowed only after specific approval from the European Commission. Currently, the use of 2-5% of lactic acid solution sprayed on bovine carcasses is the only approved treatment. In this context, an assessment of the effect of spraying lactic acid and aromatic vinegar solutions on the surface of wild boar carcasses was performed to evaluate the possible improvement of the hygienic level.

The project initially aimed to apply the most promising polyphenolic extract, among those tested, to the surface of wild boar carcasses to evaluate its potential effectiveness in improving hygienic characteristics, with a view toward possible regulatory approval. However, due to organizational reasons and supply chain considerations, it was not possible to conduct these trials. Instead, the research focused on applying these extracts directly to the meat to assess their impact on hygienic and qualitative characteristics.

Finally, studies regarding the evaluation of the potential application of polyphenolic extracts on game meat were carried out, exploring different application methods, formulations, and concentrations. In particular, research focused on the use of microencapsulated and non-encapsulated polyphenolic extracts obtained from olive mill vegetation water on game meat (wild boar, roe deer, red deer) with varying

microbial contamination levels. The impact of the concentration and application method of polyphenolic extracts on hygiene indicators and spoilage microorganisms was assessed. Moreover, the application of polyphenolic extracts from olive mill wastewater was also studied on minced meat products, such as wild boar patties. The aim of the study was not only to evaluate the antimicrobial effect of the extract but also to assess its impact on physico-chemical properties, lipid oxidation and the product's shelf life.

Presence of foodborne bacteria in wild boar and wild boar meat — a literature survey for the period 2012–2022

Caterina Altissimi[1], Clara Noè-Nordberg[2], David Ranucci[1] and Peter Paulsen[3]

[1] Department of Veterinary Medicine, University of Perugia, Via San Costanzo 4, 06121 Perugia, Italy
[2] Esterhazy Betriebe GmbH, Esterházyplatz 5, 7000 Eisenstadt, Austria
[3] Unit of Food Hygiene and Technology, Institute of Food Safety, Food Technology and Veterinary Public Health, University of Veterinary Medicine Vienna, Veterinärplatz 1, 1210 Vienna, Austria

Foods, DOI: 10.3390/foods12081689

Abstract

The wild boar is an abundant game species with high reproduction rates. The management of the wild boar population by hunting contributes to the meat supply and can help to avoid a spillover of transmissible animal diseases to domestic pigs, thus compromising food security. By the same token, wild boar can carry foodborne zoonotic pathogens, impacting food safety. We reviewed literature from 2012–2022 on biological hazards, which are considered in European Union legislation and in international standards on animal health. We identified 15 viral, 10 bacterial, and 5 parasitic agents and selected those nine bacteria that are zoonotic and can be transmitted to humans via food. The prevalence of *Campylobacter*, *Listeria monocytogenes*, *Salmonella*, Shiga toxin-producing *E. coli*, and *Yersinia enterocolitica* on muscle surfaces or in muscle tissues of wild boar varied from 0 to

ca. 70%. One experimental study reported the transmission and survival of *Mycobacterium* on wild boar meat. *Brucella*, *Coxiella burnetii*, *Listeria monocytogenes*, and Mycobacteria have been isolated from the liver and spleen. For *Brucella*, studies stressed the occupational exposure risk, but no indication of meat-borne transmission was evident. Furthermore, the transmission of *C. burnetii* is most likely via vectors (i.e., ticks). In the absence of more detailed data for the European Union, it is advisable to focus on the efficacy of current game meat inspection and food safety management systems.

1. *Introduction*

During the last decade, numbers of wild ungulates, in particular wild boars, have been rising significantly worldwide, generating environmental, economic, public health, and social concerns. Wild boar is the most widespread species due to its high adaptability and fertility rate, and its spread has been facilitated by climate change, the abandonment of rural areas, reforestation, a lack of predators, animal introductions, and supplementary feeding for hunting purposes [1–4]. The high density of this expanding species is causing, in particular, in Europe, not only relevant damages to agriculture and ecosystems and an increase in road accidents but also increases the risk of transmission of pathogens from wild boar to humans, livestock, and domestic animals [5,6]. The synanthropic behavior of wild boars is an important co-factor in creating disease-transmission scenarios [7]. Furthermore, the attention being paid to wild boar population control is leading to an increase in the availability of game meat. Additionally, the market has to face different harvesting practices, the wider distribution of this product, and, simultaneously, guarantee its safety aspects. In this context, it is of the utmost importance to understand the

epidemiological situation and the major hazards due to the consumption of such meat.

Indeed, it has been highlighted by several authors how wild boar could act as a reservoir, playing an important role in the maintenance, circulation, and diffusion of certain pathogens for humans and animals [8–12]. In particular, the same authors focused their attention on the most relevant bacterial food hazards that: cause disease to wild boar and can be present in the meat (e.g., *Brucella* spp., *Mycobacterium tuberculosis* complex); are harbored in the gut or other tissues and then transferred to the meat during processing (e.g., *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Yersinia enterocolitica*); contaminate the carcass due to their presence on animal skin and in the environment (e.g., *Listeria* spp., *Staphylococcus aureus*).

In a framework of global health, it is essential to consider not only zoonotic diseases but also animal diseases with an impact on food security. The aim of this review is to give an overview of publications from the period 2012–2022 on the presence of biological hazards in the wild boar population. In particular, foodborne zoonotic bacteria commonly reported in meat from domestic animals will be the focus, and their presence in wild boars will be reviewed.

2. *Materials and Methods*

A list of infectious agents was compiled, combining zoonotic agents included in compulsory monitoring in the European Union (Directive 2003/99/EC List A) [13], zoonotic agents monitored according to the epidemiological situation (Directive 2003/99/EC List B) [13], swine and multiple species diseases, infections, and infestations listed by the World Organisation for Animal Health (OIE), and the most common agents responsible for foodborne outbreaks reported from the European

Food Safety Authority (EFSA) during the period 2015–2020 and in the EU Rapid Alert System for Food and Feed (RASFF).

For each agent on the list, a literature search was conducted on SCOPUS using the name of the selected pathogen or the related disease combined with the search string: "wild" AND "boar" OR "feral AND pig" OR "warthog". During the literature search, biological hazards that do not concern wild boars were excluded. The search was then adjusted for (i) the time period 2012–2022, (ii) document type as article or review, and (iii) English as the selected language. Papers about the prevalence and control strategy of selected diseases were considered, whereas articles reporting solely detection methods were included only if relevant for the interpretation of results. Although our work focuses on the relevance of wild boar (meat) in the European Union, we included references from other countries in view of imports of wild boar meat from third countries in the EU; similarly, studies on feral pigs and warthogs were included.

We also report the number of publications per agent and year as a proxy for the relevance of the agent and the interest and effort of the scientific community in this topic [14]. From this long list of biological hazards specifically addressed in national legislation or by international organizations, we selected those with evidence that they are actually transmitted via the handling, processing, and consumption of porcine meat and meat products.

3. *Results*

3.1. Overview of Biological Hazards in Wild Boar and Their Impact on Food Safety and Security

The array of biological agents addressed in EU legislation and international organizations such as the OIE is displayed in Table 1.

Information on zoonotic potential and mode of transmission was taken from OIE, EFSA, and ECDC documentation. Notably, not all agents are zoonotic, and not all zoonotic agents are transmitted by meat. Among the pre-selected (i.e., taken from EU and OIE documents) infectious agents, no scientific literature was retrieved for two viruses and one bacterial genus. A clear increase (i.e., more than one doubling) in the average number of publications per year in the period 2017–2022 compared with that from 2012–2017 was noted for the viral diseases African swine fever, West Nile fever, and Japan encephalitis; the bacterium *Listeria*; and the parasite genera *Cryptosporidium*, *Cysticercus*, and *Echinococcus*.

Table 1. Agents or diseases of wild boar covered in the literature survey (2012–2022), their coverage in legislation, and the number of pertinent publications.

Agent/Disease	Type	Zoonotic	EU Zoonoses Directive	OIE listed	n, period 2012–2022	n, period 2012–2016	n, period 2017–2022	Average /year, period 2012–2016	Average/year, period 2017–2022	ratio of averages
African Swine Fever	V	n		y	499	58	441	11.6	73.5	6.3
Aujeszky´s Disease	V	n		y	108	43	65	8.6	10.8	1.3
CSF	V	n		y	158	54	104	10.8	17.3	1.6
Foot and Mouth Disease	V	n		y	35	13	22	2.6	3.7	1.4
Porcine Respiratory and Reproductive Syndrome	V	n		y	62	27	35	5.4	5.8	1.1
West Nile Fever	V	n		y	17	4	13	0.8	2.2	2.7
Hepatitis A	V	y	f		0	0	0			

Influenza	V	y	f		0	0	0			
Japan Encephalitis	V	y		y	21	6	15	1.2	2.5	2.1
Rabies	V	y	f	y	19	6	13	1.2	2.2	1.8
Paratuberculosis	B	n		y	9	7	2	1.4	0.3	0.2
Bacillus anthracis	B	y		y	3	2	1	0.4	0.2	0.4
Borrelia	B	y	f		30	9	21	1.8	3.5	1.9
Brucella	B	y	m	y	95	36	59	7.2	9.8	1.4
Campylobacter	B	y	m		22	7	15	1.4	2.5	1.8
Clostridium	B	y	f (*C. botulinum*)		0	0	0			
Francisella	B	y		y	12	6	6	1.2	1.0	0.8
Leptospira	B	y	f		55	17	36	3.4	6.0	1.8
Listeria	B	y	m		12	3	9	0.6	1.5	2.5
Q-Fever	B	y		y	23	7	16	1.4	2.7	1.9
Salmonella	B	y	m		80	25	55	5.0	9.2	1.8
St. aureus	B	y	*		27	10	17	2.0	2.8	1.4
Tuberculosis	B	y	m (*M. bovis*), f (others)		214	97	117	19.4	19.5	1.0
Verotoxinogenic *E. coli*	B	y	m		27	10	17	2.0	2.8	1.4
Yersinia	B	y	f		40	13	27	2.6	4.5	1.7
Cryptosporidium	P	y	f		18	5	13	1.0	2.2	2.2
Cysticercus	P	y	f	y	9	2	7	0.4	1.2	2.9
Echinococcus	P	y	m	y	47	12	35	2.4	5.8	2.4
Toxoplasma	P	y	f		90	35	55	7.0	9.2	1.3
Trichinella	P	y	m	y	167	67	100	13.4	16.7	1.2

V=virus; B=bacterium; P=parasite; f=facultative, according to the epidemiological situation; m=mandatory; * = multi-resistant *St. aureus*.

For a detailed review of the occurrence and significance of biological hazards, we focused on bacteria since these are the main causative agents for foodborne diseases reported in the EU [15].

3.2. Occurrence and Prevalence of Selected Zoonotic Bacteria in Wild Boar

3.2.1. Brucella

Brucella (B.) are gram-negative, nonsporeforming, aerobic, short-rod bacteria that include several pathogenic species. In the EU, monitoring of brucellosis is compulsory (Directive 2003/99/EC List A) [13]. In ruminants, swine, and dogs, infection with the agents causes diseases of the reproductive system, e.g., abortion or epididymitis. Symptomless carriers can excrete the pathogen, e.g., via milk. Small ruminants with mastitis caused by Brucella-melitensis can excrete the pathogen via milk. Ingestion of raw milk, inhalation, or close contact with infected animals or parts thereof (e.g., when dressing hunted wild game) can lead to human infections. These may resemble a feverish flu, whereas more severe courses involve splenomegaly and splenic or hepatic abscesses. In 2021, cattle livestock in 21 EU member states was officially free from brucellosis (*B. abortus*, *B. melitensis*, and *B. suis*), and as regards small ruminant livestock, 20 member states were officially free from the pathogen. In 2021, 162 human cases were reported, two of them foodborne. In 2020, there were also 2 cases linked to the consumption of sheep meat products, with *B. melitensis* being the causative species [15]. In the EU rapid alert system for food and feed (RASFF), no notification of the presence of Brucella in food was found.

As regards wild boar and Brucella, 96 documents were retrieved. Those reporting prevalence data were included in Table 2 (seropositivity) and Table 3 (DNA or viable bacteria). With respect to serological testing, the cross-reactivity with the Yersinia enterocolitica O9 antigen is a well-known issue. More recent methods may overcome this problem [16]. Some authors present seroprevalences corrected for cross-

reactivity [17]. When tissues/organs of the animal were tested by bacteriological culturing, or PCR, blood, lymphatic organs, genital organs, and fetuses were examined. There was no study on Brucella in muscle tissue or commonly consumed organs, e.g., liver, from wild boar. When Brucella species and biovars are explicitly reported, it is mainly B. suis biovar 2.

While no documented cases of meat-borne brucellosis could be retrieved, several cases of brucellosis in humans hunting wild boar and dressing wild boar carcasses have been published; most reports are from the USA [18–21], but also from France [22] and Australia [23]. In two cases, neurological disorders [18,23] were reported, and in one case, arterial and venous thromboses were reported [20], which are otherwise rarely observed [24]. Similarly, dogs frequently in contact with wild boar are at risk of seropositivity to Brucella [25–27].

Table 2. Prevalence of Brucella spp. antibodies in wild boars (2012–2022), by country and continent.

Prevalence/ frequency	Species	Matrix	Country	Comment	Ref
15.6% (15/96)	*B.* spp.	sera	Italy (Tuscany)	serology	[28]
5.74% (16/287)	*B.* spp.	sera	Italy (Tuscany)	RBT, CFT	[29]
5.1% (22/434) 13.5% (58/434)	*B.* spp.	sera	Italy (Campania)	RBT ELISA	[30]
0.53% (2/374)	*B.* spp.	sera	Italy (Tuscany)	RBT, CFT	[31]
6.2% (35/570)	*B.* spp.	sera	Italy (Sardinina)	ELISA	[32]
15% (19/126)	*B. suis*	sera	Italy (Central)	serology	[33]
59.3% (121/204)	*B.* spp.	sera	Spain (Extremadura)	ELISA	[34]

9.4% (45/480)	*B. suis* biovar 2	sera	Serbia	RBT, ELISA	[35]
1.3% (42/3230)	*B*. spp.	sera	Croatia	RBT; CFT; ELISA	[36]
6.4% (131/2057)	*B*. spp.	sera	Netherlands	ELISA	[37]
0% (0/286)	*B. suis*	blood	Sweden	ELISA	[38]
9% (8/87)	*B*. spp.	blood	Finland	RBT, ELISA; visceral organs from 5 seropos. animals available, in 4 of which *B. suis* biovar 2 was detected	[39]
13.3% (139/1044)	*B. suis*	sera	Latvia	RBT, CFT, ELISA, data corrected for O9-cross-reactivity	[17]
0% (0/100)	*B*. spp.	sera	South Africa	Warthog	[40]
12.5% (1/8)	*B*. spp.	sera	Kenya	Warthog; Antibody-ELISA	[41]
0% (0/86)	*B*. spp.	sera	Brazil	Agglutination, 2MET	[42]
0% (0/61)	*B*. spp.	sera	Brazil (Santa Catarina)		[43]
0.49% (1/205)	*B*. spp.	blood	Brazil	Feral pigs; serology (BAPA, FPT)	[44]
0% (0/15)	*B*. spp.	blood	Colombia	Feral pigs	[45]
2.2% (1/46)	*B*. spp.	blood	Guam	Feral pigs; FPT	[46]
0.7% (2/282)	*B. abortus*	sera	USA (Oklahoma)	BAPA, RIV, FPT	[47]
2.95% (7/238)	*B. suis*	sera	Australia (NSW)	RBT, CFT	[48]
9.6% (8/83)	*B. suis*	blood	Australia (Queensland)	RBT, CFT	[49]
0% (0/303)	*B*. spp.	sera	Finland	RBT	[50]
54.9% (641/1168)	*B*. spp.	sera	Belgium	ELISA	[51]

BAPA = Buffered Acidified Plate Antigen, CFT = Complement Fixation Test, RBT = Rose-Bengal-Test, RIV = Rivanol Agglutination, 2MET = 2-Mercapto-Ethanol.

Table 3. Prevalence of *Brucella* spp. (viable bacteria or DNA) in wild boar (2012–2022), by country and continent.

Prevalence/ frequency	Species	Matrix	Country	Comment	Ref
12.5% (1/8)	*B.* spp.	sera	Kenya	Warthog; PCR	[41]
1.4% (4/287) 1.7% (5/287) 2.2% 0% (0/287)	*B. suis* biovar 2	lymph nodes epididymides fetuses pooled livers, spleens	Italy (Tuscany)	DNA	[29]
0.83% (2/240)	*B.* spp.	inner organs	Denmark	culture	[52]
3.8% (7/180) 10.5% (19/180)	*B.* spp.	tonsils	Netherlands	culture PCR; confirmed as *B. suis* biovar 2	[37]
22% (19/87)	*B. suis*	faeces	USA (Georgia)	Feral pigs, PCR	[53]
1.3% (5/389)	*B. suis* biovar 2	retropharyngeal lymph nodes	Italy	culture	[54]
3.7% (7/188)	*B. suis* biovar 2	reproductive organs	Spain (Extremadura)	culture, PCR	[34]
0% (0/238)	*B.* spp.	blood	Australia (NSW)	culture	[48]

3.2.2. Campylobacter

Campylobacter is a genus of gram-negative, nonsporeforming, microaerophilic, motile spiral-shaped bacteria, with *C. jejuni* and *C. coli* as the main species involved in Campylobacteriosis. The principal symptoms of *Campylobacter* infections are diarrhea, abdominal pain, fever, headache, nausea, and vomiting. The disease is usually self-limiting, and death is rare except in severe cases in elderly people, very young children, or immunocompromised patients [55]. In 2021, campylobacteriosis was the zoonosis with the highest number of human

cases reported in the EU, with 127,840 cases of illness and 10,469 hospitalizations. With respect to foodborne outbreaks, it was the fourth most frequently reported agent with 249 outbreaks, 1051 cases, and 134 hospitalizations [15]. *Campylobacter* is common in food animals such as poultry, pigs, and cattle, and the main transmission route is via meat and meat products, as well as raw milk and milk products.

Twenty-two articles have been published from 2012 to 2022 regarding the prevalence of *Campylobacter* in wild boars, five of which were excluded as not relevant. The main matrix considered for the isolation of *Campylobacter* is feces, as reported in Table 4. The references highlighted the role of wild boars as a possible source of *Campylobacter* infection due to the prevalence of *Campylobacter* spp. in feces samples, albeit in a variable range from 12.5% [56] to 66% [57]. Several species have been isolated from fecal samples in varying prevalence ranges, e.g., *C. lanienae* from 1.2% [56] to 69% [58], *C. hyointestinalis* from 0.8% [59] to 22.1% [60], *C. coli* from 0.8% [56] to 16.3% [58], and *C. jejuni* from 0% [61] to 4.1% [58] of samples. As suggested by [59], the degree of urbanization of some areas populated by wild boars could have a relationship with the detection frequency of some *Campylobacter* species; in particular, *C. lanienae* was more frequently isolated in low urbanizations areas, suggesting that this pathogen could be interconnected with the kind of diet available.

During the period considered, only two studies were conducted on carcasses, and they presented similar results, with a prevalence of *Campylobacter* spp. of 11.1% [62] and 16.7% [63]. Peruzy et al. [64] investigated the presence of *Campylobacter* in wild boar meat samples, but the pathogen was not detected.

To date, the EU has set food processing hygiene criteria for *Campylobacter* only for poultry [65].

Table 4. Prevalence of Campylobacter spp. in wild boar (2012–2022) feces or on carcasses or meat.

Prevalence/ frequency	Species	Matrix	Country	Comment	Ref.
51.8% (29/56)	*Campylobacter* spp.	faeces	Italy		[63]
50% (38/76) 40.8% (31/76)	*Campylobacter* spp. *C. lanienae*	faeces	Italy	Campylobacter spp. with levels up to 10^3 CFU/g was detected in 39.5% animals.	[66]
66% (188/287)	*Campylobacter* spp.	faeces	Spain	One isolate was identified as *C. jejuni*	[57]
60.8%(79/130) 46.2%(60/130) 16.9%(22/130) 0.8% (1/130) 0% (0/130)	*Campylobacter* spp. *C. lanienae* *C. coli* *C. hyointestinalis* *C. jejuni*	faeces	Spain	4% WB had both *C. lanienae* and *C. coli*, and 1% had both *C. lanienae* and *C. hyointestinalis.* All the isolates were resistant to at least one antimicrobial agent considered	[59]
38.9%(49/126) 69.4% (34/49) 16.3% (8/49) 4.1% (2/49)	*Campylobacter* spp. *C. lanienae* *C. coli* *C. jejuni*	faeces	Spain		[58]
19.51% (8/41) 4.88% (2/41) 0% (0/41)	*Campylobacter* spp. *C. coli* *C. jejuni*	faeces	Spain		[61]
43.8%(53/121) 25.6%(31/121) 17.4%(21/121) 0.8%(1/121)	*Campylobacter* spp. *C. lanienae* *C. hyointestinalis* *C. jejuni*	faeces	Japan	Five (16%) and 6 (29%) isolates of *C. lanienae* and *C. hyointestinalis*, respectively, were resistant to enrofloxacin	[67]
22.1%(71/321)	*C. hyointestinalis*	faeces	Japan		[60]

12.5%(31/248) 9.7% (25/248) 1.2% (3/248) 0.8% (2/248)	*Campylobacter* spp. *C. hyointestinalis* *C. lanienae* *C. coli*	faeces	Japan		[56]
3.5% (13/370) 1.6% (6/370)	*C. coli* *C. jejuni*	faeces	USA	*C. coli* was significantly more frequent (*P* =.008) in female feral pigs	[68]
0% (0/87)	*C. jejuni*	faeces	USA		[53]
16.7% (5/30)	*Campylobacter spp.*	carcass	Italy		[63]
11.1% (4/36)	*Campylobacter spp.*	carcass	Italy		[62]
0% (0/28)	*Campylobacter spp.*	meat	Italy		[64]

WB = wild boars.

3.2.3. Coxiella burnetii—Q-Fever

Coxiella burnetii is a gram-positive short-rod bacterium that grows aerobically within but also outside of host cells. It can form spores and persist under dry and acidic conditions. The bacterium is not only excreted via effluents, but several tick species can act as vectors for the pathogen. Infection of humans can occur via contact with effluents, ingestion of contaminated food, and inhalation of aerosolized pathogens, but also by tick bites. Infection causes a feverish disease (Q-fever) with pneumonia, followed by affections of the heart, liver, and spleen. In the EU, human cases are notifiable. Data indicate that the number of human cases as well as prevalence in animals is declining. However, monitoring of farm and wild animals is not harmonized in the EU [15]. At least 347 of the 460 confirmed human cases of Q-fever in 2021 were acquired within the EU, and the pathogen was prevalent in 5.2%, 5.9%, and 16.5% of samples from cattle, goats, and sheep, respectively. Since not all member states submitted data, the reported

percentages are not necessarily representative of the EU [15]. Studies conducted on *C. burnetii* and wild boar can be grouped into three categories: (i) those on ticks collected from wild boars or from hunters or dogs in frequent contact with wild boars; (ii) those on serum or spleen samples from wild boar; and (iii) studies on the genetic diversity of *C. burnetii*.

Within Europe, studies originated in Spain and Italy (Table 5). DNA from *C. burnetii* was detected in 1.9% of spleen samples [69], and antibodies were found in 5.5% of serum samples [70] from wild boar in Spain. In studies from Italy, the pathogen was not recovered from wild boar samples but from ticks feeding on wild boars (0.5%; [71]) and from dogs in contact with wild boars (5.1%; [72]). Wild boar is not a specific or primary host for the pathogen [73], but since the agent is occasionally detected in tissues from wild boar, hunters and consumers handling and processing wild boar (meat) are both occupationally and dietary exposed. Similarly, hunters and dogs often in contact with wild boars are at risk of exposure to tick-borne pathogens, among them *C. burnetii* [71].

Table 5. Presence of *Coxiella burnetii* or antibodies in wild boar or in vectors associated with wild boar, according to country and continent, 2012–2022.

Prevalence/frequency	Matrix	Country	Comment	Ref.
0% (0/100)	spleen	Italy (Central)	PCR	[73]
0% (0/93) 0% (0/176)	spleen ticks	Italy	PCR	[74]
5.1% (6/117)	blood of dogs	Italy (Central)	PCR	[72]
0.48% (2/411)	ticks	Italy (South)	Ticks collected from hunters and dogs	[71]

0% (0/40) feeding ticks 0% (0/489) questing ticks	ticks	Spain (Northwest)	PCR	[75]
5.5% (4/73)	serum	Spain (Northwest)	antibodies	[70]
1.9% (9/484)	spleen	Spain (North)	PCR	[69]
0% (0/2256) 0% (0/167)	ticks spleen	Spain	Near to Barcelona, a highly populated area	[76]
0% (0/8)	serum	Kenya	antibodies Serology (ELISA)	[41]
0% (0/67)	blood	Brazil		[77]
5% (4/79)	ticks	Thailand	PCR	[78]
18.3% (19/104)	serum of dogs	Australia	Queensland	[79]

No notifications regarding the presence of *C. burnetii* in foods were listed in the EU rapid alarm system (RASFF).

3.2.4. *Listeria monocytogenes*

Listeriosis is a zoonotic disease caused by *Listeria monocytogenes*, a gram-positive, nonsporeforming, facultatively anaerobic bacterium. Foodborne listeriosis is one of the most severe diseases, causing septicemia, neurologic disorders, and reproductive disorders. Pregnant women, elderly people, and individuals with weakened immune systems are at risk for severe courses of the disease. *Listeria* is a ubiquitous microorganism that thrives in soil, water, vegetables, and the digestive tracts of animals. It can survive and proliferate in different environmental conditions since it is tolerating a wide range of pH and temperatures [80]. The main transmission route of *Listeria* is through the ingestion of contaminated food [15].

Twelve studies have been found from 2012 to 2022 regarding the presence of *Listeria* spp. in wild boar carcasses, meat, and related

products, two of which were excluded as not relevant (Table 6). *Listeria monocytogenes* was detected by many authors in tonsil samples, highlighting this organ as the preferred matrix for the presence and detection of *Listeria* [63,81,82]. Fredriksson-Ahomaa et al. [39] found *L. monocytogenes* in 48% of spleen and kidney samples from wild boars. Almost all isolates belonged to serotype 2a, except for two isolates identified as serotype 4b. The presence of *Listeria* in tonsils and in visceral organs underlines the necessity of particular attention during handling and evisceration of wild boar carcasses.

Regarding the presence of *Listeria* in wild boar meat products, Roila et al. [83] did not detect the pathogen in wild boar salami, whereas Lucchini et al. [84] isolated *Listeria* spp. in 65% of cured game meat sausages. Three species were identified: *L. monocytogenes,* 24%*; L. innocua*, 32% and *L. welshimeri,* 8%. Counts of *L. monocytogenes* were, however, always below the legal limit of 100 cfu/g set by Regulation (EC) 2073/2005 [65].

In the years 2020–2022, 340 notifications regarding the presence of *L. monocytogenes* in foods were listed in the EU rapid alarm system RASFF, of which 82 implicated meat and meat products; there was no explicit mention of game meat or wild boar meat in particular.

Table 6. Presence of *Listeria* sp. in wild boar, 2012–2022.

Prevalence/ frequency	Species	Matrix	Country	Comment	Ref.
0. 35% (1/287)	*L. monocytogenes*	rectal swabs	Italy	*L.m.* serogroup IVb, serovar 4b; resistant to cefoxitin, cefotaxime and nalidixic acid	[85]

68.5% (37/54) 35.3% (18/51) 26.7% (8/30) 0% (0/30)	*Listeria* spp. *L. monocytogenes* *Listeria* spp. *L. monocytogenes*	tonsils tonsils carcass carcass	Italy	prevalence influenced by animal age and environmental temperature	[63]
48% (63/130)	*L. monocytogenes*	spleen and kidneys	Finland		[39]
24.5% (12/49)	*L. monocytogenes*	Liver or tonsils or faeces or intestinal lymph nodes, caecum content	Germany	Positive in at least one of the different matrices studied	[81]
14.3% (7/49)	*L. monocytogenes*	tonsils	Germany		[81]
2% (1/49)	*L. monocytogenes*	Liver and intestinal lymph nodes and caecum content and faeces	Germany	The same animal resulted positive for *L.m.* in all the matrices analyzed	[81]
51.8% (14/27) 40.7% (11/27) 0% (0/27)	*Listeria* spp. *L. monocytogenes* *L. monocytogenes*	tonsils tonsils faeces	Spain		[82]
37.3% (28/75) 0% (0/75)	*Listeria* spp. *L. monocytogenes*	faeces	Japan		[67]
0% (0/72)	*L. monocytogenes*	carcass	Italy		[86]
65% (24/37) 24% (9/37) 32% (12/37) 8% (3/37)	*Listeria* spp. *L. monocytogenes* *L. innocua* *L. welshimeri*	game meat cured sausages	Italy	*L.m.* <10 cfu/g	[84]
0% (0/40)	*L. monocytogenes*	wild boar salami	Italy		[83]

3.2.5 Mycobacterium tuberculosis Complex

Mycobacterium tuberculosis complex is a group of *mycobacteria* that include *M. tuberculosis,* the major cause of human tuberculosis (TB), and other genetically related species that affect livestock and wild animals but are also implicated in human disease [87,88]. Among these species, in the last decade, *M. bovis* [89–115], *M. caprae* [89,104,111,116,117], and *M. microti* [118–124] have been frequently reported from wild boar, feral pigs, and warthogs in different countries. The MTC bacteria can cause localized granulomas (primary complex) after enter- ing the host through the respiratory or digestive tract, and when the organism´s immune system cannot contain it (which can be the case in the elderly, children, and in people with compromised immune systems), it may be followed by primary or secondary- reactivated TB. Meningitis, extrapulmonary granulomas, miliary tuberculosis, and other disseminated/generalized forms are only a few examples of the various manifestations, along with a variety of clinical symptoms [125]. *M. bovis* is usually transmitted through oral ingestion, and therefore the extrapulmonary lesions in humans are more frequent than for *M. tuberculosis* [126]. In wild boar, the main primary complex is usually located in the submandibular and retropharyngeal lymph nodes, where the MTC is most fre- quently isolated [89,98,105,117,122,127,128]. Lesions were also reported in the tonsils, lung, mediastinal lymph nodes, spleen, liver, and kidney [106,117,127,128]. The lesion in the lymph nodes is characterized by caseous or necrotic-calcified tubercles that are defined as tuberculosis-like lesions (TBLL), as other *mycobacteria* different from MTC (e.g., *M. avium subsp. hominissuis*) could cause the same lesion [119,129–131]. *M. bovis* and *M. caprae* could also be detected (isolated/PCR) in

lymph nodes without visible lesions [94,105,128,131]. Wild boar is reported for MTC shedding through the oral, nasal, and fecal routes [132], and therefore animal aggregation areas could result in contaminated water and soil and the maintenance of the infection in wildlife and livestock [118,133,134].

In addition, 214 studies regarding MTC and non-MTC in wild *Suidae* species have been found in the literature over the considered period, but only 35 were related to prevalence studies of MTC and were therefore considered. These studies were performed both by serol- ogy (Table 7) and by isolation or direct identification of *mycobacteria* in organs and tissues (Table 8). The prevalence of MTC varies between countries and between regions/counties inside each nation (e.g., Spain), but also due to the investigated matrix and the diagnostic methods adopted [94,98,135]. In this context, some studies were performed to define the sensitivity of different diagnostic tools on sera and on organs and tissues [94,96,119,136]. The serological prevalence of MTC in wild boar is generally conducted over multi-year stud- ies and ranged from 87.7% in Montes de Toledo and Doñana National Park (Spain) [132] to near 0% in the USA [137]. The prevalence of MTC isolation in tissue and organs, considering studies conducted on more than 100 subjects, ranges from 64.2% for *M. microti* in the Lombardia region (Italy) [123] to 1.1% for *M. bovis* in the Basque Country (Spain) [89].

The presence of MTC in wild boar is still recognized as one of the main barriers to the eradication of the disease in livestock and, subsequently, in humans, particularly when extensive pastoral systems are implemented and there is an interface between farmed and wild animals [93,100,101,104,111,133,138,139]. Although the disease is notifiable in many countries (such as Europe and the United States), its

control in wild boar is primarily restricted to standard visual game meat inspection, which is thought to be insufficient to find primary complex and small lesions [117], especially as post-mortem inspection could be carried out also by trained hunters [EC Regulation 853/2004 [140]]. Even the cultural method for bacterial isolation is less effective than other diagnostic tools (e.g., screening PCR directly performed on target tissues, such as head lymph nodes, even when no TBLL are detected) [94,136]. Another topic to be considered is the free movement of wildlife that could spread the disease in different geographic areas. The identification and long- term monitoring of the genotype/spoligotype existing in a territory may aid in specific surveillance plans and control actions [100,141].

Despite the role of wild boar as a reservoir for MTC and the possible transmission through food [11], wild boar meat and meat products as a source for human infection are reported only by Clausi et al. [142]. In this study, PCR tests revealed the presence of MTC DNA on the carcass surface of wild boar without TBLL, but no *Mycobacterium* spp. could be isolated. Clausi et al. [142] added lymph nodes with active TBLL (*M. bovis*) to meat batter during sausage processing. Although live bacteria could be isolated only at day 23 after the contamination of the sausages (neither before nor after), bacterial DNA was detected (PCR) throughout the entire study period (end of sampling at day 41). When *M. bovis* (10^5 CFU/g) was directly added during sausage manufacturing, it was isolated for up to 22 days of ripening. When meat surfaces were experimentally contaminated with *M. bovis,* the bacterium could be recovered after frozen storage for over 5 months [142]. The role of wild boar meat and derived raw meat products could therefore be

further investigated, even if other authors consider meat a negligible source of human infection [117].

Table 7. Seroprevalence of MTC in wild boar, feral pigs, and warthogs, 2012–2022.

Prevalence/ frequency	Species	Country	Area	Comment	Ref.
16.7% (5/30)	*MTC*	Malaysia	Selangor	Sampling in 2019-2020 Test used: bovine purified protein derivative (bPPD)-based indirect in-house ELISA	[127]
17% (326/1902)	*MTC*	Spain	Basque Country	Sampling in 2010-2016 Test used: in house validated enzyme-linked immunosorbent assay (ELISA)	[139]
10.6% (46/434)	*MTC*	Italy	Campania Region	Sampling in 2012-2017 Test Used: Indirect ELISA INgezim Tuberculosis DR kit based on recombinant M. bovis protein (MPB83)	[92]
2.4% (16/278)	*MTC*	Portugal	Several County	Sampling in 2006-2013 Test used: bPPD-based indirect in-house ELISA	[95]
49.0% (49/100)	*M. bovis*	South Africa	uMhkuze Nature Reserve in Kwa-Zulu	Sampling in 2013-2015 Test used: Indirect PPD	[96]

			Natal, Marloth Park on the southern border of Kruger National Park in Mpumalanga	ELISA and TB ELISA-VK®	
87.7% (36/41)	*MTC*	Spain	Montes de Toledo and Doñana National Park	Sampling in 2011-2013 Test used: bPPD-based indirect in-house ELISA Prevalence was obtained adding the number of animals with lesions at necroscopy to the number of positive serological samples	[132]
0.0003% (1/2735)	*MTC*	USA	National survey	Sampling in 2007-2015 Test used: bPPD-based indirect ELISA	[137]
2.4% (18/743)	*MTC*	Switzerland	Geneva, Mittelland, Jura, Thurgau, Tessin	Sampling in 2008-2013 Test used: bPPD-based indirect in-house ELISA	[109]
5.9% (123/2080)	*MTC*	France	58 Departments	Sampling in 2000-2004/2009-2010 Test used: bPPD-based indirect ELISA	[144]
2.1% (22/1057)	*MTC*	Spain	Asturias and Galicia	Sampling in 2010-2012	[111]

				Test used: bPPD-based indirect ELISA	
67.7% (87/130)	*MTC*	Spain	Andalusia	Sampling in 2006-2010 Test used: MPB83-ELISA	[115]

Table 8. Prevalence of *Mycobacterium* spp. in wild boar, feral pigs and warthog organs and tissues, 2012–2022.

Prevalence/ frequency	Species	Country	Area	Comment	Ref
37.7% (29/77)	*M. bovis*	Brasil	Rio Grande do Sul	Sampling in 2013-2019 Test used: DNA extraction from lungs, lymph nodes, liver, spleen and kidney followed by PCR	[91]
1.1% (10/894)	*MTC*	Spain	Basque County	Sampling in 2010-2019 Test used: isolation from lymph nodes followed by real time PCR and spoligotyping of the isolates Positive cultures were detected only form head lymph nodes	[89]
2.8% (5/176)	*MTC* (mainly *M. microti)*	Switzerla nd	Canton of Ticino	Sampling in 2017-2018 Test used: isolation from lymph nodes + direct PCR followed by MALDI-TOF MS identification	[119]

				High prevalence of N-MTC identification (57.4%)	
38.2% (21/55)	*M. caprae*	Poland	Bieszczady Mountains region	Sampling in 2011-2017 Test used: isolation form lymph nodes followed by PCR and spoligotyping of the isolates	[116]
76.7% (946/1235)	*Mycobacterium spp.*	Spain	Doñana National Park	Sampling in 2006-2018 Test used: Visual inspection for TBLL.	[133]
1.6% (8/495) Culture 4.4% (17/386) PCR	*M. bovis*	France	Aquitaine, Côte d'Or and Corsica	Samplimg 2014-2016 Test used: isolation or direct PCR form lymph nodes followed by spoligotyping of the isolates	[94]
47.1% (16/34)	*M. bovis*	South Africa	Greater Kruger National Park	Sampling in 2015 Test used: Intradermal Tuberculin Test (ITT) on captured warthog. Lymph nodes bacterial culture followed by PCR identification	[97]
2.4% (180/7634)	*M. bovis*	France	National scale (11 at-risk areas)	Sampling in 2011- 2017 Test used: Lymph nodes bacterial culture followed by PCR identification	[98]

				Detected in 7 of the 11 at-risk areas	
37.0% (25/67)	*M. bovis*	South Africa	uMhkuze Nature Reserve in Kwa-Zulu Natal, Marloth Park on the southern border of Kruger National Park in Mpumalanga	Sampling in 2013-2015 Test used: Lymph nodes bacterial culture followed by PCR identification	[96]
6.8% (19/280)	*Mycobacterium spp.*	Italy	Sicily	Sampling in 2004-2014 Test used: Visual inspection for TBLL. Tissue samples with TBLs were cultures followed by PCR identification. *M. bovis* was isolated from one sample	[100]
16.2% (647/3963)	*Mycobacterium spp.*	Portugal	Idanha-a-Nova	Sampling in 2006-2016 Test used: Visual inspection for tuberculosis-like lesions (TBLL). Considered positive when at least in one organ or lymph node showed TBLs	[129]

4,3% (329/7729)	*MTC*	Spain	Castilla y León	Sampling in 2011-2015 Test used: Lymph nodes bacterial culture followed by PCR identification	[134]
2,5% (3/118)	*M. bovis*	South Korea	Gyeongg i Province	Sampling in 2011-2015 Test used: Lymph nodes and lung bacterial culture followed by PCR identification	[102]
38.3% (16/41)	*M. bovis*	Portugal	Castelo Branco	Sampling in 2009-2013 Test used: first screening by visual inspection for TBLL (41/192 had lesions). Tissue samples with TBLs were cultures followed by PCR identification.	[105]
18.2% (8/44)	*Mycoba cterium* spp.	Slovenia	Different areas	Sampling in 2010-2013 Test used: Lymph nodes and liver bacterial culture followed by PCR identification. No MTC were isolated	[130]
13.5% (36/267)	*M. caprae*	Hungary	South-Western Hungary	Sampling in 2008-2013 Test used: bacterial culture followed by PCR identification.	[117]
33.9% (18/58)	*M. bovis*	Spain	Sevilla province	Sampling in 2012-2013 Test used: Lymph nodes bacterial	[108]

				culture followed by PCR identification and spoligotyping. The study was performed on wild boar piglets	
0% (0/9)	*M. bovis*	Brasil	Pantanal area	Test used: bacterial culture of unspecified feral pigs tissues followed by PCR identification	[145]
8.5% (51/602) PCR 5.8% (35/602) RFPL	*M. microti*	Italy	Lombardia Region	Sampling in 2006 Test used: Lymph nodes histology, bacterial culture, direct PCR, direct RFLP	[123]
7.5% (23/307) Culture 64.2% (197/307) PCR 55.0% (169/307) RFPL	*M. microti*	Italy	Lombardia Region	Sampling in 2007-2011 (only wild boar with TBLL) Test used: Lymph nodes histology, bacterial culture, direct PCR, direct RFLP	[123]
59% (1512/2562)	*Mycobacterium* spp.	Spain	Ciudad Real province	Sampling in 2008-2012 Test used: Visual inspection for TBLL in lymph nodes and organs. Generalised TBLs were detected in 51% of the subjects	[146]
2.59% (33/1275)	*MTC*	Spain	Asturias and Galicia	Sampling in 2008-2012 Test used: lymph nodes and organs	[111]

				culture followed by PCR identification and spoligotyping of the isolates Number of *M. bovis* isolates = 19 and *M. caprae* isolates = 14	
3.64% (6/165)	*MTC*	Switzerland and Liechtenstein	Geneva, Thurgovia, Saint Gall, Grisons, Tessin, Liechtenstein	Sampling in 2009-2011 Test used: lymph nodes and tonsil culture followed by PCR identification and spoligotyping of the isolates	[124]
37.3% (293/785)	*M. bovis*	New Zealand	Different areas	Sampling in 1997-2007 Test used: Lymph nodes culture followed by PCR identification	[114]
88.9% (16/18)	*M. bovis*	Spain	Andalusia	Sampling in 2006-2010 Test used: Culture of pool homogeneate of lymph nodes and lungs followed by PCR and spoligotyping of the isolates	[115]
13.3% (2/15)	*M. bovis*	Italy		Test used: Culture and PCR of swab samples on muscle surface of wild boar without TBLL	[143]
8.7 R_0	*Mycobacterium* spp.	Spain and Portugal	29 sites	Metadata analyses from 2010-2019. Test used: gross pathology and culture	[138]

	Reproduction number (R_0) defined considering prevalence in the host species, MTC excretion in infected host species, abundance of the host species, transmission rate to host species

3.2.6 Salmonella

Salmonellosis is an enteric infection caused by species of the *Salmonella* genus other than *Salmonella* Typhi and *Salmonella* Paratyphi. Salmonellae are gram-negative bacteria belonging to the Enterobacteriaceae family. They are motile, nonsporeforming, aerobic, or facultatively anaerobic. The transmission of this infection occurs principally by the fecal-oral route: the ingestion of contaminated food or water, contact with infected animals, feces or contaminated environments. The main symptoms of salmonellosis are diarrhea, abdominal cramps, vomiting, and fever. The severity and course of the disease are related to the serotype, the number of microorganisms ingested, and the individual's immune system [147]. *Salmonella* spp. is widely spread for its ability to infect several animal species and survive in different environmental conditions with a wide range of temperatures (2–54 °C) and pH values (3.7–9.4) [148]. Salmonellosis is a public health issue, and it was the second zoonosis reported in the EU in 2021, with 60,050 confirmed human cases, 11,785 hospitalisations, and 71 fatalities [15]. The *Salmonella* genus consists of

two species: *Salmonella bongori* and *Salmonella enterica*, the latter divided into six subspecies and several serotypes [149]. The main *Salmonella* serovars implicated in human infections in 2020 and 2021 were *S.* Enteritidis, *S.* Typhimurium, monophasic *S.* Typhimurium (1,4,[5],12:i:-), *S.* Infantis, and *S.* Derby [15,150].

Overall, 80 articles regarding *Salmonella* in wild boars have been found in the literature from 2012 to 2022, seven of which are reviews [10,11,150–155], and 28 articles were not considered relevant for this study. The prevalence of *Salmonella* in the wild boar population has been studied through the analysis of different matrices. Some authors investigated the seroprevalence from blood serum, diaphragm, or muscle samples, achieving different percentages: 1.27% (141/1103) [156], 3.6% (14/393) [157], 4.3% (4/94) [158], 5% (1/20) [159], 17% (21/126) [160], 19.3% (52/269) [161], 38% (69/181) [39], and 66.5% (255/383) [162].

Testing of serum samples can reveal the presence of antibodies against *Salmonella* spp. in wild boars but not the presence of the microorganism on carcass surfaces or meat. The prevalence of *Salmonella* spp. in other matrices such as feces, spleen, kidney, submandibular lymph nodes, ileocecal lymph nodes, mesenteric lymph nodes, and tonsils is reported in Table 9, which shows that feces are the main investigated samples with a prevalence range of 0% to 43%. As shown in Table 10, the prevalence of *Salmonella* spp. in wild boar carcasses is between 0% and 2.5%, while in meat samples it ranges from 0% to 35.7%. This wide variability could be due to different geographic sampling areas, sampling methods, and the hygienic level of process procedures and the environment. The presence of *Salmonella* in wild boar cured meat products was investigated only by Roila et al. [83] in wild boar salami. *Salmonella enterica* serovar

typhimurium and *Salmonella enterica* serovar Rissen were found in different batches of meat batter and salami after 7 days of curing, but in the final product after 60 days of aging, *Salmonella* spp. were not detected. However, it was not possible to specify if wild boar had been the source of *Salmonella* since the salami were made with 50% wild boar meat and 50% pork meat.

Table 9. Prevalence of *Salmonella* spp. in wild boar, feces, lymphatic tissues, and inner organs, 2012–2022.

Prevalence/ frequency	Species	Matrix	Country	Comment	Ref.
3.1% (13/425) 0.2% (1/425)	*Salmonella* spp. *Salmonella* spp.	Feces Mesenteric lymph nodes	Serbia	*S. Enteritidis* was the main serotype identified	[163]
3.1% (4/130)	*S. enterica*	Feces	Spain	Serotype identified were monophasic *S. Typhimurium*, *S. Bardo*, *S. Enteritidis*	[59]
35.6% (32/90) 17.8% (16/90)	*Salmonella* spp. *Salmonella* spp.	Faeces Lymph nodes	Italy	46.7% (42/90) animals were positive in faeces or lymph nodes, of which 11.9% (5/42) were positive at the same time in both matrices. *S. Abony*, S. Newport, *S. Agona*, *S. Derby*, *S. Hermannswerder*, *S. Saintpaul*, *S. Elomrane*, *S.*	[164]

				Salamae were identified.	
7.8% (5/64) 4.7% (3/64)	*Salmonella* spp. *Salmonella* spp.	Mesenteric lymph nodes Carcass	Italy	Campionamenti da game-handling establishment, game collection point, mattatoio	[165]
6% (260/4335)	*Salmonella* spp.	Liver	Italy	Sampling in 2013-2017. Isolated strains belonged to all six *Salmonella enterica* subspecies and the main serotype was *S. enteritidis*.	[166]
4.18% (12/287)	*Salmonella* spp.	Liver or spleen or rectal swab	Italy	*S. diarizonae*, *S. houtenae*, *S. Newport*, *S. Kottbus*, *S. London*, *S. Infantis*, *S. Rubislaw* were identified.	[85]
2.4% (13/552)	*Salmonella* spp.	faeces	Germany	*S. Enteritidis*, *S. Typhimurium*, *S. Stanleyville*, were identified.	[167]
5% (6/130)	*Salmonella* spp.	Spleen and kidney	Finland		[39]
0% (0/115)	*Salmonella* spp.	faeces	Denmark		[52]
15.9% (30/189) 3.2% (6/189)	*Salmonella* spp. *Salmonella* spp.	mesenteric lymph nodes Faeces	Italy	Three animals were positive in both samples.	[168]
18.69% (40/214) 5.06% (21/415)	*Salmonella* spp. *Salmonella* spp.	Tonsils Submandibular lymph nodes Faeces	Spain	Sampling in 2010-2015 From 148 wild boars the 3 matrices were collected in the same animals and	[169]

2.98% (25/838)	*Salmonella* spp.			27.02% (40/148) of them were positive to *Salmonella* spp. (31/148 tonsils, 12/148 lymph nodes, 2/148 faeces) but none of them were positive in the three samples simultaneously.	
7% (4/57) 3.5% (2/57)	*S. enterica* *S. enterica*	Faeces Mesenteric lymph glands	Italy	*S. Thompson* and *S. Braenderup* were identified.	[63]
43.9% (194/442)	*Salmonella* spp.	Faeces	USA	Sampling from 2013 to 2015. Main serovars identified were *S. Montevideo*, *S. Newport* and *S. Give*.	[170]
5% (1/21)	*Salmonella* spp.	Faeces	Portugal		[171]
5.1% (9/175) 1.8% (1/56) 1.1% (1/88)	*Salmonella* spp. *Salmonella* spp. *Salmonella* spp.	Tonsils Ileocaecal lymph nodes Faeces	Sweden	*S. enterica* and *S. diarizone* were identified.	[172]
33.3% (1/3) 33.3% (1/3)	*Salmonella* spp. *Salmonella* spp.	Tonsils Tongue	Argentina	Tonsils carried both *S. Gaminara* and *S. Newport*, while only *S. Gaminara* were isolated from tongue.	[173]
5% 2/40	*S. enterica*	Faeces	Spain	*Salmonella enterica* serotype *Anatum* and *Corvallis* were isolated.	[61]
7.4% (9/121)	*Salmonella* spp.	Faeces	Japan	*S. enterica* subsp. *enterica* serovar *Agona* (3), *S.*	[67]

				Narashino (2), *S. Enteritidis* (1), *S. Havana* (1), *S.* Infantis (1), and *S. Thompson* (1) were obtained.	
0.3% (1/333)	*Salmonella* spp.	Faeces	Spain	One animal was positive in both carcass and faeces samples. *S. Bardo*, S. Montevideo, *S. Arizonae III* (16:i,v:1,5,7) and *S. Typhimurium* were identified.	[57]
10.8% (54/499)	*Salmonella* spp.	Faeces	Italy	*S. enterica* subsp. *salamae II*, *S. enterica* subsp. *Diarizonae III b*, *S. enterica* subsp. *houtenae IV* and *S. Fischerhuette* were the most common isolated.	[162]
24.82% (326/1313)	*Salmonella* spp.	Faeces	Italy	Sampling from 2007 to 2010 *S. enterica* subsp. *enterica* was the main serovar isolated (79.5%)	[174]
15.4% (33/214)	*Salmonella* spp.	Faeces	Spain		[175]

Table 10. Prevalence of *Salmonella* spp. in wild boar meat and carcasses, 2012–2022.

Prevalence/ frequency	Species	Matrix	Country	Comment	Ref.
2.7% (1/36) 0% (0/36)	*Salmonella* spp. *Salmonella* spp.	Meat Carcass	Italy		[62]

35.7% (10/28)	*Salmonella* spp.	Meat	Italy	*S. Veneziana*, *S. Kasenyi*, *S. Coeln*, *S. Manhattan*, *S. Thompson*, and *S. Stanleyville* were identified	[64]
2.5% (3/121)	*Salmonella* spp.	Carcass	Italy	Two *S. Stanleyville* and one *S. Typhimurium* were identified.	[176]
1.1% (1/90)	*Salmonella* spp.	Carcass	Italy		[164]
0% (0/37)	*Salmonella* spp.	Meat	Italy	Meat cut sampled were fillet and legquarter.	[177]
31.82% (7/22)	*Salmonella* spp.	Meat	Italy	*S. Stanleyville*, *monophasic S. Typhimurium*, and *S. Kasenyi* were identified	[178]
0% (0/30)	*S. enterica*	Carcass	Italy		[63]
0% (0/128)	*Salmonella* spp.	Meat	Japan		[179]
1.4% (3/210) 1.9% (4/210)	*Salmonella* spp. *Salmonella* spp.	Skin Carcass	Serbia		[180]
4.55% (1/22)	*Salmonella* spp.	Meat	Italy	Meat cut sampled was *Longissimus dorsi* muscle.	[181]
1.2% (4/333)	*Salmonella* spp.	Carcass	Spain	One animal was positive in both carcass and faeces samples.	[57]
0% (0/72)	*Salmonella* spp.	Carcass	Italy		[86]

In order to reduce the risk of infection, it is recommended to pay particular attention to the skinning and evisceration processes, maintain the cool chain, have a good hygienic level during meat cutting, and to cook the final product.

3.2.7 *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive, spherical, nonsporeforming, coagulase-positive, aerobic or anaerobic, facultative, halophilic bacterium with the tendency to aggregate in "grape-like" clusters. The usual habitat of this commensal microorganism is the skin and nose of healthy humans and animals, but in some cases, it could lead to a wide range of clinical infections such as bacteremia, endocarditis, pneumonia, infections of the skin and soft tissues, mastitis, and bone and joint infections [182,183]. Some *S. aureus* strains may develop resistance to beta-lactam antibiotics, which are widely used to treat infections, and these strains are termed methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA used to be associated mainly with hospital-related infections, but recently this strain has been found also in people without any contact with hospitals and, in companion animals, livestock, and wild animals [184]. There is an increasing interest in understanding the role of wild boars as possible reservoirs of *S. aureus* and MRSA in particular. About this topic, it has been found in 27 articles from 2012 to 2022, 14 of which were relevant for this study. The majority of studies performed nasal swabs for the detection of *S. aureus*, with a variable prevalence as shown in Table 11. Sousa et al. [185] considered both oral and nasal swabs, with a prevalence of *S. aureus* of 33%. Both studies from Porrero et al. [186,187] considered skin and nasal swabs; in the first study, they found 0.86% of animals positive for MRSA, of which 62.5% were detected from skin swabs and 37.5% from nasal swabs, and only one wild boar was positive in both the skin and nasal samples. Instead, Porrero et al. [187] noticed a higher percentage of positives for *S. aureus* in the nasal sample rather than in skin swabs, but without skin swabs, 18.25% of positives for wild boars would

not have been detected. Only Traversa et al. [188] considered lymph nodes for the detection of *S. aureus* in wild boar and revealed a prevalence of 3.2%. No studies on the presence of *S. aureus* in carcasses, raw meat, or processed meat were retrieved in our literature survey.

Table 11. Prevalence of MRSA on wild boar mucosal membranes and in lymphatic organs, 2012–2022.

Prevalence/ frequency	Species	Matrix	Country	Comment	Ref.
36.9% (41/111)	*S. aureus*	nasal swab	Germany	MRSA were not detected	[189]
33% (30/90)	*S. aureus*	oral and nasal swab	Portugal	7 isolates showed resistance to at least one of the antibiotics tested; 1 MRSA CC398 (spa-type t899) was identified	[185]
32.2% (57/177)	*S. aureus*	nasal swab	Portugal	Isolates were resistant to all antimicrobials tested, with the exception of trimethoprim-sulfamethoxazole and vancomycin	[190]
17.8% (66/371) 13.7% (51/371) 1.96% (1/51)	CoPS *S. aureus* MRSA	nasal swab	Spain	74.5% isolates were susceptible to all the antimicrobials analyzed, 19.6% were resistant to penicillin and 9.8% were resistant to streptomycin	[191]
17.67% (126/713)	MSSA	Skin and/or nasal swabs	Spain		[187]
6.8% (8/117)	*S. aureus*	nasal swabs	Germany	No antibiotic resistance were detected	[192]
3.2% (23/697)	*S. aureus*	lymph nodes	Italy	MRSA were not detected	[188]

0.87% (5/577)	MRSA	nasal swab	Germany		[167]
0.86% (7/817)	MRSA	skin and nasal swabs	Spain	8 isolates were identified from 7 positive animals: 3 from nasal swabs and 5 from skin swabs. One animal was MRSA positive for both skin and nasal swabs	[186]
0% (0/90)	MRSA	nasal swab	Spain		[193]
0% (0/439)	MRSA	nasal swab	Germany		[194]
0% (0/244)	MRSA	nasal swab	Denmark		[52]

MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-susceptible *Staphylococcus aureus* (MSSA); CoPS: coagulase positive *Staphylococcus.*

3.2.8 Verotoxinogenic/Shigatoxinogenic E. coli

Verotoxinogenic/Shigatoxinogenic *E. coli* (VTEC/STEC) form a group of pathogenic *E. coli* (gram-positive short-rods) that elaborate Shiga-like toxins together with other vir- ulence factors. Infections in humans can range from bloody diarrhea to life threatening coagulopathy and renal failure/hemolytic-uremic syndrome. Originally associated with the presence of the O157 antigen, a number of strains with other O-serotypes have been identified as STEC. It has been proposed to use *stx*-gene typing to assess the pathogenicity of STEC (EFSA 2020). In particular, *E. coli* with genes encoding for the stx-2 gene and the virulence factor intimin (*eae*) are associated with severe courses of the disease [15]. In 2021, 6084 confirmed cases were reported in the EU, with 901 hospitalizations and 18 fatalities. From the 5 strong evidence outbreaks, 3 were attributable to meat or meat products [15]. In many animal species, asymptomatic STEC carriers are the rule. In

particular, ruminants do not show symptoms since they lack vascular receptors for the Shiga-toxins [195]. A survey of notifications in the RASFF revealed no cases of wild boar meat contamination with STEC.

As regards wild boar, the literature search retrieved 27 documents. The definitions for pathogenic *E. coli* were not consistent between the studies. In 12 studies, the prevalence of STEC was reported, ranging from 0 to 28.3% (Table 12). Data on meat were reported in merely four studies, with a prevalence ranging from 0 to nearly 43% (Table 13). A more detailed view of other isolates with pathogenic potential and antimicrobial resistance described in the studies is outside the scope of our review. E.g., one study reported the isolation of STEC from wild boars with the additional feature of producing enterotoxins (*sta1* and *stb* genes), causing oedema disease [196].

Three studies reported the transmission of STEC from the feces of wild boar to fresh produce [197,198] or to recreational waters [199]. Although not the primary focus of this review, the studies highlight indirect transmission routes of pathogenic bacteria to humans.

Table 12. Prevalence of Shiga toxin-forming *E. coli* in wild boar, fecal samples, lymphatic organs, 2012–2022.

Prevalence/ frequency	Species	Matrix	Country	Comment	Ref.
14% (8/56)	*STEC (stx2)*	faeces	Portugal	Culture and PCR, WGS	[200]
6.9% (37/536)	*STEC*	faeces	Germany	Culture, PCR	[167]
1.9% (9/474)	*STEC* O157	Faeces	Japan	Culture, PCR	[201]
6.5% (13/200)	*STEC*	Faeces	Italy (Tuscany)	Culture, PCR	[202]
1.2% (3/248)	*STEC*	Faeces	Japan	Culture, PCR	[56]

28.3% (43/152)	*STEC*	Faeces	Poland	Culture, PCR; includes STEC and AE-STEC	[203]
4.8% (1/21)	*STEC*	Faeces	Portugal	Culture, PCR	[204]
3.33% (3/90)	*STEC*	faeces	Spain	Culture, PCR	[205]
3.4% (4/117)	*E. coli* O157	faeces	Spain	Culture	[206]
0% (0/88)	*E. coli* O157:H7	Tonsils, lymphnodes, faeces	Finland	Culture, PCR	[172]
0% (0/121)	*STEC* O157, O26	faeces	Japan	Culture, PCR	[67]
0% (0/301)	*STEC* O157	faeces	Spain	Culture, PCR	[57]

Table 13. Prevalence of Shiga toxin-forming *E. coli* in wild boar meat and carcasses.

Prevalence/ frequency	**Species**	**Matrix**	**Country**	**Comment**	**Ref.**
42.9% (12/28)	*STEC(stx1+ stx2+eae)*	Meat (foreleg)	Italy (Campania)	Culture, PCR (27/28 *eae* positive)	[64]
0% (0/128)	*STEC*	Meat	Japan	Culture	[179]
0% (0/310)	*STEC* O157	Meat	Spain	Culture, PCR	[57]
5.3% (3/57)	*STEC*	Meat and meat products	Spain	Culture, PCR	[207]

3.2.9 Yersinia

The Enterobacteriaceae family includes the food-borne pathogen *Yersinia enterocolitica,* responsible for yersiniosis in humans, a gastrointestinal disease that could simulate appen- dicitis and can cause mesenteric lymphadenitis, reactive arthritis, erythema nodosum, and conjunctivitis [208,209]. The disease appears to be widespread, with

ca. 6800 cases in Eu- rope in 2020 and 100,000 illnesses every year in the USA [EFSA, 2022; CDC, 2016] [15,210].

The epidemiological situation could be even more severe, as the role of biotype 1A in human infection and disease symptoms (considered non-pathogenic compared to biotypes 1B, 2, 3, 4 and 5) is still debated and therefore underestimated [211].

Ready-to-eat foods are the major sources of human infection, especially as *Y. enteroco- litica* can resist cold environments and even replicate at refrigeration temperatures [211]. Animals, especially pigs, are considered the main reservoir of the bacteria, which could be found mainly in the intestine and tonsils [212]. Nevertheless, the outbreaks reported in 2021 are related to prepared dishes and ready-to-eat vegetables [15], and no reports are available on wild boar meat as an outbreak source.

The database research retrieved 39 studies regarding *Y. enterocolitica* in wild boars and feral pigs between 2012–2022. The articles that reported studies on the prevalence of the microorganism in animal tissue, feces, or carcasses/muscles of wild boars were 21. Only two articles describe the prevalence of antibodies against *Y. enterocolitica* in animal blood samples. Papers on *Yersinia pseudotuberculosis* were not considered. Most of the studies were conducted in Europe (19 out of 21), especially in Italy (10 articles). Samples of different matrices were considered: eight studies on fecal samples, nine on organs different from muscles, four on carcass surfaces (external or internal), and four in muscles (Table 14).

The seroprevalence in wild boar was above 50% (in Finland and the Czech Republic), proving that the microorganism is widespread in this species. Fecal material is consid- ered the main source of contamination of the carcass and, ultimately, of the meat. This contamination could

happen during hunting (the precision of the shot), evisceration, or carcass processing and cutting [176,180]. Fecal sample positivity for *Y. enterocolitica* ranges from 0% (different Italian regions) to 74% (Japan). Thus, as for other genus belonging to the Enterobacteriaceae family, the fecal shedding could be intermittent [213]. Regarding organs and tissues that could harbour the microorganism in *Suidae*, the prevalence of the microorganism in the tonsils of wild boar ranges from 14% (Sweden) to 64% (Campania Region, Italy), with a higher percentage than in lymph nodes (ranging from 0% to 4.4%). The presence of the pathogen in such tissues could be considered during carcass processing to avoid the spread of the microorganism to the meat. Nonetheless, in wild boar, in contrast to the domestic pig, the head is removed during carcass dressing at cervical vertebrae level, thus the laryngeal and pharyngeal area is removed from the carcass at an early stage of the processing chain.

The presence of *Y. enterocolitica* on carcass surfaces ranges from 0% to 85.7%. Such a wide range could be due to different sampling methods and areas, but also to differences in the hygienic level of the process. The same might hold true for muscles, where the prevalence ranges from 0% to 71%. The wide range of prevalence denotes that, although wild boar can harbour microorganisms in the intestines and tonsils, the procedures to obtain the meat are relevant to prevent contamination of muscles. In this perspective, the training of the personnel, the presence of suitable structure and equipment, the correct hygienic procedure implementation, and standard sanitation operating procedures are of paramount importance.

Another important aspect that emerged from the literature survey is that the bio- type most frequently observed in wild boar is 1A, the least

pathogenic but also the most underrated of the *Y. enterocolitica* biotypes.

Table 14. Prevalence of *Yersinia enterocolitica* in wild boar, feral pigs and warthog.

Prevalence/ frequency	Country	Area	Matrix	Comment	Ref
0% (0/107)	Italy	Valle d'Aosta Region	Faeces	Sampling in 2015-2018 Test used: PCR	[214]
85.7% (12/36)	Italy	Campania Region	Carcass	Sampling in 2019 Test used: bacterial isolation and SYBR green PCR-assay for ystA and ystB genes. 12 animals carried ystB gene, and 3 animals both ystA and ystB genes	[62]
64.3% (9/36)			Tonsils		
71.4% (10//36)			Muscle		
0.01% (1/110)	Tunisia	Ariana, Bizerte, Manouba, Nabeul and Siliana	Faeces	Sampling in 2018-2020 Test used: bacterial isolation and biochemical identification	[215]
0% (0/64)	Italy	Parma and Bologna province	Carcass and Mesenteric lymph nodes	Sampling in 2020 Test used: bacterial isolation and biochemical identification	[165]
2.6% (126/4890)	Italy	Liguria Region	Liver	Sampling in 2013-2018 Test used: bacterial isolation, Serotyping and Real Time PCR for virulence genes. Biotype 1A was the most isolated (92.9%), then	[216]

				biotype 1B (6.3%) and 2 (0.8%).	
18.8% (54/287)	Italy	Tuscany Region	Rectal swab	Sampling in 2018-2020 Test used: bacterial isolation, biochemical identification. and Real Time PCR for virulence Genes. Identification of gene *ystA* in 14 out of 54 isolates, *inv* in 13, *ail* in 12, *ystB* in 10 and *virF* in 8.	[85]
56.4% (102/181)	Finland	12 out of 19 regions	Blood	Sampling in 2016 Test used: seroprevalence ELISA test.	[39]
16.9% (22/130)			Spleen and kidneys	Test used: Organs: real-time PCR based on SYBRGreen for *ail* gene.	
6.2% (19/305)	Italy	Parma and Piacenza provinces	Faeces	Sampling in 2017-2019 Test used: bacterial isolation, biochemical identification and Real Time PCR for virulence Genes. All isolates belonged to biotype 1A	[217]
3.3% (10/305)			Mesenteric lymph nodes		
74.1% (40/54)	Japan	Not specified	Faeces	Sampling in 2014-2016 Test used: bacterial isolation, biochemical identification.	[218]

				Prevalence is reported for Yersinia spp. 97.4% of the *Y. enterocolitica* isolates belonged to biotype 1A	
13.6% (3/22)	Italy	Campania region	Muscle	Sampling in 2017 Test used: bacterial isolation, biochemical identification, and Real Time PCR for virulence Genes. All isolates present only *ystB* gene.	[178]
6.7% (6/90)	Sweden	13 counties in southern Sweden	Faeces	Sampling in 2014–2016 Test used: bacterial isolation, and Real Time PCR for ail gene.	[219]
14.0% (19/136)			Tonsils		
4.4% (4/90)			Mesenteric lymph nodes		
25.3% (110/434)	Poland	12 out of 16 Polish regions	Rectal swab	Sampling in 2013–2014 Test used: bacterial isolation, and multiplex PCR for ail, ystA and ystB genes. 92.5% of the isolates belong to biotype 1A	[220]
0% (0/42)	Italy	Tuscany Region	Muscle	Sampling in 2013–2014 Test used: bacterial isolation, and biochemical identification	[181]
65.9% (89/135)	Czech Republic	Moravian Regions	Blood	Sampling in 2013–2014 Test used: ELISA	[221]

55.5% (11/20)	Poland	North-East Poland	Swab samples from tonsils area, peritoneum and perineum	Sampling in 2013 Test used: bacterial isolation, and biochemical identification biotyping, serotyping and molecular characterisation. 90.5% of the isolates belong to biotype 1A	[222]
33.3% (24/72)	Spain	Basque Country	Tonsils	Sampling in 2001–2012 Test used: bacterial isolation, biochemical identification and molecular characterisation.	[223]
15.3% (17/111)	German y	Lower saxony	Tonsils	Sampling in 2013–2014 Test used: bacterial isolation, MALDI-TOF identification, Real Time PCR for virulence Genes. 89.55% of the isolates belong to biotype 1A	[224]
20.5% (18/88)	Sweden	Central Sweden	Faeces and Ileocaecal lymph nodes and Tonsils	Sampling in 2010–2011 Test used: bacterial isolation, and multiplex PCR for *ail* gene.	[219]
27.3% (18/66)	Spain	Basque Country	Tonsils	Sampling in 2010–2012 Test used: bacterial isolation, and biochemical identification and direct real time PCR with new	[225]

				enrichment protocol.	
0% (0/3)	Argentin a	San Luis city	Tonsils and tongue	Sampling in 2008–2012 Test used: bacterial isolation and biochemical identification	[173]
14.8% (34/230)	Italy	Viterbo Province	Muscle	Sampling in 2012–2013 Test used: bacterial isolation, and multiplex PCR for *ail* gene.	[157]
4.2% (3/72)	Italy	Upper Susa valley Piedmont Region	Carcass	Sampling in Test used: bacterial isolation, biochemical identification and molecular characterisation for *inv, ail* and *yst* genes. *ail* and *yst* genes were not detected	[86]

4. *Conclusions*

The increasing popularity of meat from wild game is observed in many countries. Diseases in wildlife have often been seen as an issue or spill-over or spill-back of infection agents from farm animals, and exposure of humans and animals in frequent and close contact with wild animals has been studied to some extent. Additionally, while the presence of antibodies against a specific pathogen may be useful for epidemiological purposes, its value for the assessment of meat safety is primarily that the given pathogen must be considered a potential hazard. Similarly, the presence of pathogens in the feces and even in the lymph nodes of the digestive tract mainly indicates that the host organism can keep the pathogen under control. Similar to farm

animals, it can be expected that stress, but also the dressing procedures after killing, can cause the spread of the pathogen on/in edible organs. Since these scenarios do not result in any typical lesion, the routine ante- and post-mortem examinations [226] will not give an indication of the presence of a certain pathogen, and minimizing the spread of the agent is a matter of good hygienic practice. However, if serological or other testing has demonstrated the presence of a certain pathogen in wildlife in a certain region, it would be wise to adopt hygienic precautions (i.e., no admittance of carcasses with "gut shots" in the food chain; or disinfecting knives after cutting in the tonsillar area).

For five (*Campylobacter*, *Listeria monocytogenes*, *Salmonella*, Shiga toxin-forming *E. coli,* and *Yersinia enterocolitica*) of the nine agents we reviewed, one or more studies dealt with the presence of the pathogen on muscle surfaces or muscle tissues of wild boar, with prevalences ranging from 0 to ca. 70%. One experimental study was retrieved on the transmission and survival of *Mycobacterium* on wild boar meat. As regards edible inner organs, the liver and spleen have been examined for the presence of *Brucella*, *Coxiella burnetii*, *Listeria monocytogenes,* and *Mycobacteria*, and the latter four agents have actually been recovered, albeit with varying percentages. For *Brucella,* human case reports and epidemiological studies in (hunting) dogs stressed the occupational exposure risk, but no indication of meat-borne transmission to humans was evidenced. Similarly, the mode of transmission of *C. burnetii* is more likely via vectors (i.e., ticks). In most studies, animals without specific histories or pathologies had been examined.

In essence, the literature we reviewed confirmed that food-borne pathogenic bacteria present in meat from domestic animals [15] and

implicated in food-borne disease can also be found in wild boars, with varying prevalence and regional differences. It is unclear to what extent such differences are biased by sampling and analytical procedures. In the absence of more detailed data for the European Union, it might be advisable to focus on the efficacy of current game meat inspection [226] and handling practices [140] to minimize introduction in the game meat chain. Similarly, the implementation of HACCP-based food safety management systems [227] needs to be stressed.

With respect to the placing on the market of meat from wild hunted game, European Union legislation distinguishes an "approved" chain (i.e., the hunted game specimens are collected, post-mortem inspected, and processed in approved establishments) from an unapproved chain, which is largely subject to national regulation (for primary products, i.e., the eviscerated carcass, see Recital 10 and Article 1 of EC Regulation 852/2004 [228]; for processed or unprocessed products, see Recital 11 and Article 1 of EC Regulation 853/2004 [140]). This unapproved chain represents the supply of small quantities of wild game or wild game meat directly from the hunter to the final consumer or to local retail establishments directly supplying the final consumer [140].

Currently, there is no uniform way in which this unapproved sector is regulated in the member states; there is even no consistent definition of "small quantities of wild game or wild game meat" [140]. Admittedly, all national legislation has a common baseline represented by EC Regulation 178/2002 (in particular, Articles 14, 16–19; "safe food", traceability, identification of hazards, and management of risks) [229,230]. An in-depth and comprehensive consideration of said regulation should, in fact, be sufficient to

warrant food safety. European Union member states have chosen different approaches [231,232], but there are no real metrics to assess how the systems actually perform in managing the consumers´ risk posed by the presence of foodborne pathogens in game meat.

References

1. Bieber, C.; Ruf, T. Population Dynamics in Wild Boar *Sus scrofa*: Ecology, Elasticity of Growth Rate and Implications for the Management of Pulsed Resource Consumers: Population Dynamics in Wild Boar. *J. Appl. Ecol.* **2005**, *42*, 1203–1213.
2. Keuling, O.; Baubet, E.; Duscher, A.; Ebert, C.; Fischer, C.; Monaco, A.; Podgórski, T.; Prevot, C.; Ronnenberg, K.; Sodeikat, G.; et al. Mortality Rates of Wild Boar *Sus scrofa* L. in Central Europe. *Eur. J. Wildl. Res.* **2013**, *59*, 805–814.
3. Massei, G.; Kindberg, J.; Licoppe, A.; Gacˇic´, D.; Šprem, N.; Kamler, J.; Baubet, E.; Hohmann, U.; Monaco, A.; Ozolin, š, J.; et al. Wild Boar Populations up, Numbers of Hunters down? A Review of Trends and Implications for Europe: Wild Boar and Hunter Trends in Europe. *Pest. Manag. Sci.* **2015**, *71*, 492–500.
4. Johann, F.; Handschuh, M.; Linderoth, P.; Dormann, C.F.; Arnold, J. Adaptation of Wild Boar (*Sus scrofa*) Activity in a Human- Dominated Landscape. *BMC Ecol.* **2020**, *20*, 4.
5. Sáenz-de-Santa-María, A.; Tellería, J.L. Wildlife-Vehicle Collisions in Spain. *Eur. J. Wildl. Res.* **2015**, *61*, 399–406.
6. Jori, F.; Massei, G.; Licoppe, A.; Ruiz-Fons, F.; Linden, A.; Václavek, P.; Chenais, E.; Rosell, C. Management of Wild Boar Populations in the European Union before and during the ASF Crisis. In *Understanding and Combatting African Swine Fever*; Iacolina, L., Penrith, M.-L., Bellini, S., Chenais, E., Jori, F., Montoya, M., Ståhl, K., Gavier-Widén,

D., Eds.; Wageningen Academic Publishers: Wageningen, The Netherlands, 2021; pp. 197–228.
7. Bacigalupo, S.A.; Dixon, L.K.; Gubbins, S.; Kucharski, A.J.; Drewe, J.A. Wild Boar Visits to Commercial Pig Farms in Southwest England: Implications for Disease Transmission. *Eur. J. Wildl. Res.* **2022**, *68*, 69.
8. Ruiz-Fons, F.; Segalés, J.; Gortázar, C. A Review of Viral Diseases of the European Wild Boar: Effects of Population Dynamics and Reservoir Rôle. *Vet. J.* **2008**, *176*, 158–169.
9. Meng, X.J.; Lindsay, D.S.; Sriranganathan, N. Wild Boars as Sources for Infectious Diseases in Livestock and Humans. *Philos. Trans. R. Soc. B.* **2009**, *364*, 2697–2707.
10. Brown, V.R.; Bowen, R.A.; Bosco-Lauth, A.M. Zoonotic Pathogens from Feral Swine That Pose a Significant Threat to Public Health. *Transbound. Emerg. Dis.* **2018**, *65*, 649–659.
11. Fredriksson-Ahomaa, M. Wild Boar: A Reservoir of Foodborne Zoonoses. *Foodborne Pathog. Dis.* **2019**, *16*, 153–165.
12. Gomes-Neves, E.; Abrantes, A.C.; Vieira-Pinto, M.; Müller, A. Wild Game Meat—A Microbiological Safety and Hygiene Challenge? *Curr. Clin. Microbiol. Rep.* **2021**, *8*, 31–39.
13. Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. *Off. J. Eur. Union* **2003**, *L325*, 31–40.
14. Trimmel, N.E.; Walzer, C. Infectious Wildlife Diseases in Austria-A Literature Review From 1980 Until 2017. *Front. Vet. Sci.* **2020**, *7*, 3.

15. European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC). The European Union One Health 2021 Zoonoses Report. *EFSA J.* **2022**, *20*, 7666.
16. Touloudi, A.; McGiven, J.; Cawthraw, S.; Valiakos, G.; Kostoulas, P.; Duncombe, L.; Gortázar, C.; Boadella, M.; Sofia, M.; Athanasakopoulou, Z.; et al. Development of a Multiplex Bead Assay to Detect Serological Responses to *Brucella* Species in Domestic Pigs and Wild Boar with the Potential to Overcome Cross-Reactivity with *Yersinia enterocolitica* O:9. *Microorganisms* **2022**, *10*, 1362.
17. Grantina-Ievina, L.; Avsejenko, J.; Cvetkova, S.; Krastina, D.; Streikisa, M.; Steingolde, Z.; Vevere, I.; Rodze, I. Seroprevalence of *Brucella suis* in eastern Latvian wild boars (*Sus scrofa*). *Acta Vet. Scand.* **2018**, *60*, 19.
18. Powers, H.R.; Nelson, J.R.; Alvarez, S.; Mendez, J.C. Neurobrucellosis associated with feral swine hunting in the southern United States. *BMJ Case Rep.* **2020**, *13*, 238216.
19. Landis, M.; Rogovskyy, A.S. The Brief Case: *Brucella suis* Infection in a Household of Dogs. *J. Clin. Microbiol.* **2022**, *60*, e00984-21.
20. Gowe, I.; Parsons, C.; Vickery, S.; Best, M.; Prechter, S.; Haskell, M.G.; Parsons, E. Venous thrombosis, peripheral aneurysm formation, and fever in a feral pig hunter with Brucellosis. *IDCases* **2022**, *27*, e01449.
21. Franco-Paredes, C.; Chastain, D.; Taylor, P.; Stocking, S.; Sellers, B. Boar hunting and brucellosis caused by *Brucella suis*. *Travel Med. Infect. Dis.* **2017**, *16*, 18–22.

22. Mailles, A.; Ogielska, M.; Kemiche, F.; Garin-Bastuji, B.; Brieu, N.; Burnusus, Z.; Creuwels, A.; Danjean, M.P.; Guiet, P.; Nasser, V.; et al. *Brucella suis* biovar 2 infection in humans in France: Emerging infection or better recognition? *Epidemiol. Infect.* **2017**, *145*, 2711–2716.
23. Munckhof, W.J.; Jennison, A.V.; Bates, J.R.; Gassiep, I. First report of probable neurobrucellosis in Australia. *Med. J. Aust.* **2013**, *199*, 423–425.
24. Mor, S.M.; Wiethoelter, A.K.; Massey, P.D.; Robson, J.; Wilks, K.; Hutchinson, P. Pigs, pooches and pasteurisation: The changing face of brucellosis in Australia. *Aust. J. Gen. Pract.* **2018**, *47*, 99–103.
25. Orr, B.; Malik, R.; Norris, J.; Westman, M. The Welfare of Pig-Hunting Dogs in Australia. *Animals* **2019**, *9*, 853.
26. Orr, B.; Westman, M.E.; Norris, J.M.; Repousis, S.; Ma, G.; Malik, R. Detection of *Brucella* spp. during a serosurvey of pig-hunting and regional pet dogs in eastern Australia. *Aust. Vet. J.* **2022**, *100*, 360–366.
27. Kneipp, C.C.; Sawford, K.; Wingett, K.; Malik, R.; Stevenson, M.A.; Mor, S.M.; Wiethoelter, A.K. *Brucella suis* Seroprevalence and Associated Risk Factors in Dogs in Eastern Australia, 2016 to 2019. *Front. Vet. Sci.* **2021**, *8*, 727641.
28. Fabbri, M.C.; Crovetti, A.; Tinacci, L.; Bertelloni, F.; Armani, A.; Mazzei, M.; Fratini, F.; Bozzi, R.; Cecchi, F. Identification of candidate genes associated with bacterial and viral infections in wild boars hunted in Tuscany (Italy). *Sci. Rep.* **2022**, *12*, 8145.
29. Cilia, G.; Fratini, F.; Turchi, B.; Ebani, V.V.; Turini, L.; Bilei, S.; Bossù, T.; De Marchis, M.L.; Cerri, D.; Bertelloni, F. Presence and Characterization of Zoonotic Bacterial Pathogens in Wild

Boar Hunting Dogs (Canis Lupus Familiaris) in Tuscany (Italy). *Animals* **2021**, *11*, 1139.

30. Montagnaro, S.; D'ambrosi, F.; Petruccelli, A.; Ferrara, G.; D'alessio, N.; Iovane, V.; Veneziano, V.; Fioretti, A.; Pagnini, U. A serological survey of brucellosis in Eurasian wild boar (*Sus scrofa*) in Campania region, Italy. *J. Wildl. Dis.* **2020**, *56*, 424–428.
31. Bertelloni, F.; Mazzei, M.; Cilia, G.; Forzan, M.; Felicioli, A.; Sagona, S.; Bandecchi, P.; Turchi, B.; Cerri, D.; Fratini, F. Serological Survey on Bacterial and Viral Pathogens in Wild Boars Hunted in Tuscany. *EcoHealth* **2020**, *17*, 85–93.
32. Pilo, C.; Addis, G.; Deidda, M.; Tedde, M.T.; Liciardi, M. A serosurvey for brucellosis in wild boar (*Sus scrofa*) in Sardinia, Italy. *J. Wildl. Dis.* **2015**, *51*, 885–888.
33. Di Nicola, U.; Scacchia, M.; Marruchella, G. Pathological and serological findings in wild boars (*Sus scrofa*) from Gran Sasso and Monti della Laga National Park (Central Italy). *Large Anim. Rev.* **2015**, *21*, 167–171.
34. Risco, D.; García, A.; Serrano, E.; Fernandez-Llario, P.; Benítez, J.M.; Martínez, R.; García, W.L.; de Mendoza, J.H. High-Density dependence but low impact on selected reproduction parameters of *Brucella suis* biovar 2 in wild boar hunting estates from south-western Spain. *Transbound. Emerg. Dis.* **2014**, *61*, 555–562.
35. Zurovac Sapundzic, Z.; Zutic, J.; Stevic, N.; Milicevic, V.; Radojicic, M.; Stanojevic, S.; Radojicic, S. First Report of *Brucella* Seroprevalence in Wild Boar Population in Serbia. *Vet. Sci.* **2022**, *9*, 575.

36. Cvetnic, Ž.; Duvnjak, S.; Zdelar-Tuk, M.; Reil, I.; Mikulic´, M.; Cvetnic´, M.; Špicˇic´, S. Swine brucellosis caused by *Brucella suis* biovar 2 in Croatia. *Slov. Vet. Res.* **2017**, *54*, 149–154.
37. van Tulden, P.; Gonzales, J.L.; Kroese, M.; Engelsma, M.; de Zwart, F.; Szot, D.; Bisselink, Y.; van Setten, M.; Koene, M.; Willemsen, P.; et al. Monitoring results of wild boar (*Sus scrofa*) in The Netherlands: Analyses of serological results and the first identification of *Brucella suis* biovar 2. *Infect. Ecol. Epidemiol.* **2020**, *10*, 1794668.
38. Malmsten, A.; Magnusson, U.; Ruiz-Fons, F.; González-Barrio, D.; Dalin, A.-M. A serologic survey of pathogens in wild boar (*Sus scrofa*) in Sweden. *J. Wildl. Dis.* **2018**, *54*, 229–237.
39. Fredriksson-Ahomaa, M.; London, L.; Skrzypczak, T.; Kantala, T.; Laamanen, I.; Biström, M.; Maunula, L.; Gadd, T. Foodborne Zoonoses Common in Hunted Wild Boars. *EcoHealth* **2020**, *17*, 512–522.
40. Neiffer, D.; Hewlett, J.; Buss, P.; Rossouw, L.; Hausler, G.; Deklerk-Lorist, L.-M.; Roos, E.; Olea-Popelka, F.; Lubisi, B.; Heath, L.; et al. Antibody Prevalence to African Swine Fever Virus, Mycobacterium bovis, Foot-and-Mouth Disease Virus, Rift Valley Fever Virus, Influenza A Virus, and *Brucella* and *Leptospira* spp. in Free-Ranging Warthog (*Phacochoerus africanus*) Populations in South Africa. *J. Wildl. Dis.* **2021**, *57*, 60–70.
41. Gakuya, F.; Akoko, J.; Wambua, L.; Nyamota, R.; Ronoh, B.; Lekolool, I.; Mwatondo, A.; Muturi, M.; Ouma, C.; Nthiwa, D.; et al. Evidence of co-exposure with *Brucella* spp., *Coxiella burnetii*, and Rift Valley fever virus among

various species of wildlife in Kenya. *PLoS Negl. Trop. Dis.* **2022**, *16*, e0010596.

42. Kmetiuk, L.B.; Paulin, L.M.S.; Villalobos, E.M.C.; Lara, M.D.C.C.S.H.; Filho, I.R.B.; Pereira, M.S.; Bach, R.W.; Lipinski, L.C.; Fávero, G.M.; Dos Santos, A.P.; et al. Seroprevalence of anti-brucella spp. Antibodies in wild boars (*Sus scrofa*), hunting dogs, and hunters of Brazil. *J. Wildl. Dis.* **2021**, *57*, 974–976.
43. Severo, D.R.T.; Werlang, R.A.; Mori, A.P.; Baldi, K.R.A.; Mendes, R.E.; Surian, S.R.S.; Coldebella, A.; Kramer, B.; Trevisol, I.M.; Gomes, T.M.A.; et al. Health profile of free-range wild boar (*Sus scrofa*) subpopulations hunted in Santa Catarina State, Brazil. *Transbound. Emerg. Dis.* **2021**, *68*, 857–869.
44. Zimmermann, N.P.; Schabib Peres, I.A.H.F.; Braz, P.H.; Juliano, R.S.; Mathias, L.A.; Pellegrin, A.O. Serological prevalence of *Brucella* spp. in feral pigs and sympatric cattle in the pantanal of mato grosso do sul, Brazil [Prevalência sorológica de *Brucella* spp. em porcos ferais e bovinos em simpatria no pantanal do mato grosso do sul, brasil]. *Semin. Cienc. Agrar.* **2018**, *39*, 2437–2442.
45. Montenegro, O.L.; Roncancio, N.; Soler-Tovar, D.; Cortés-Duque, J.; Contreras-Herrera, J.; Sabogal, S.; Acevedo, L.D.; Navas- Suárez, P.E. Serologic survey for selected viral and bacterial swine pathogens in colombian collared peccaries (*Pecari tajacu*) and feral pigs (*Sus scrofa*). *J. Wildl. Dis.* **2018**, *54*, 700–707.
46. Cleveland, C.A.; DeNicola, A.; Dubey, J.P.; Hill, D.E.; Berghaus, R.D.; Yabsley, M.J. Survey for selected pathogens

in wild pigs (*Sus scrofa*) from Guam, Marianna Islands, USA. *Vet. Microbiol.* **2017**, *205*, 22–25.
47. Gaskamp, J.A.; Gee, K.L.; Campbell, T.A.; Silvy, N.J.; Webb, S.L. Pseudorabies virus and brucella abortus from an expanding wild pig (*Sus scrofa*) population in southern Oklahoma, USA. *J. Wildl. Dis.* **2016**, *52*, 383–386.
48. Ridoutt, C.; Lee, A.; Moloney, B.; Massey, P.; Charman, N.; Jordan, D. Detection of brucellosis and leptospirosis in feral pigs in New South Wales. *Aust. Vet. J.* **2014**, *92*, 343–347.
49. Pearson, H.E.; Toribio, J.-A.L.M.L.; Hernandez-Jover, M.; Marshall, D.; Lapidge, S.J. Pathogen presence in feral pigs and their movement around two commercial piggeries in Queensland, Australia. *Vet. Rec.* **2014**, *174*, 325.
50. Hälli, O.; Ala-Kurikka, E.; Nokireki, T.; Skrzypczak, T.; Raunio-Saarnisto, M.; Peltoniemi, O.A.T.; Heinonen, M. Prevalence of and risk factors associated with viral and bacterial pathogens in farmed European wild boar. *Vet. J.* **2012**, *194*, 98–101.
51. Grégoire, F.; Mousset, B.; Hanrez, D.; Michaux, C.; Walravens, K.; Linden, A. A serological and bacteriological survey of brucellosis in wild boar (*Sus scrofa*) in Belgium. *BMC Vet. Res.* **2012**, *8*, 80.
52. Petersen, H.H.; Takeuchi-Storm, N.; Enemark, H.L.; Nielsen, S.T.; Larsen, G.; Chriél, M. Surveillance of Important Bacterial and Parasitic Infections in Danish Wild Boars (*Sus scrofa*). *Acta Vet. Scand.* **2020**, *62*, 41.
53. Lama, J.K.; Bachoon, D.S. Detection of *Brucella Suis*, *Campylobacter Jejuni*, and *Escherichia coli* Strains in Feral Pig (*Sus scrofa*) Communities of Georgia. *Vector-Borne*

Zoonotic Dis. **2018**, *18*, 350–355.

54. Di Sabatino, D.; Garofolo, G.; Di Provvido, A.; Zilli, K.; Foschi, G.; Di Giannatale, E.; Ciuffetelli, M.; De Massis, F. *Brucella suis* biovar 2 multi locus sequence type ST16 in wild boars (*Sus scrofa*) from Abruzzi region, Italy. Introduction from Central-Eastern Europe? *Infection, Genet. Evol.* **2017**, *55*, 63–67.
55. WHO. Available online: https://www.who.int/news-room/fact-sheets/detail/campylobacter (accessed on 1 March 2023).
56. Tomino, Y.; Andoh, M.; Horiuchi, Y.; Shin, J.; Ai, R.; Nakamura, T.; Toda, M.; Yonemitsu, K.; Takano, A.; Shimoda, H.; et al. Surveillance of Shiga Toxin-Producing *Escherichia coli* and *Campylobacter* spp. in Wild Japanese Deer (*Cervus nippon*) and Boar (*Sus scrofa*). *J. Vet. Med. Sci.* **2020**, *82*, 1287–1294.
57. Díaz-Sánchez, S.; Sánchez, S.; Herrera-León, S.; Porrero, C.; Blanco, J.; Dahbi, G.; Blanco, J.E.; Mora, A.; Mateo, R.; Hanning, I.; et al. Prevalence of Shiga Toxin-Producing *Escherichia coli*, *Salmonella* spp. and *Campylobacter* spp. in Large Game Animals Intended for Consumption: Relationship with Management Practices and Livestock Influence. *Vet. Microbiol.* **2013**, *163*, 274–281.
58. Carbonero, A.; Paniagua, J.; Torralbo, A.; Arenas-Montes, A.; Borge, C.; García-Bocanegra, I. Campylobacter Infection in Wild Artiodactyl Species from Southern Spain: Occurrence, Risk Factors and Antimicrobial Susceptibility. *Comp. Immunol. Microbiol. Infect. Dis.* **2014**, *37*, 115–121.
59. Castillo-Contreras, R.; Marín, M.; López-Olvera, J.R.; Ayats,

T.; Fernandez Aguilar, X.; Lavín, S.; Mentaberre, G.; Cerdà-Cuéllar, M. Zoonotic Campylobacter Spp. and Salmonella Spp. Carried by Wild Boars in a Metropolitan Area: Occurrence, Antimicrobial Susceptibility and Public Health Relevance. *Sci. Total Environ.* **2022**, *822*, 153444.

60. Morita, S.; Sato, S.; Maruyama, S.; Miyagawa, A.; Nakamura, K.; Nakamura, M.; Asakura, H.; Sugiyama, H.; Takai, S.; Maeda, K.; et al. Prevalence and Whole-Genome Sequence Analysis of Campylobacter Spp. Strains Isolated from Wild Deer and Boar in Japan. *Comp. Immunol. Microbiol. Infect. Dis.* **2022**, *82*, 101766.
61. Navarro-Gonzalez, N.; Casas-Díaz, E.; Porrero, C.M.; Mateos, A.; Domínguez, L.; Lavín, S.; Serrano, E. Food-Borne Zoonotic Pathogens and Antimicrobial Resistance of Indicator Bacteria in Urban Wild Boars in Barcelona, Spain. *Vet. Microbiol.* **2013**, *167*, 686–689.
62. Peruzy, M.F.; Murru, N.; Smaldone, G.; Proroga, Y.T.R.; Cristiano, D.; Fioretti, A.; Anastasio, A. Hygiene Evaluation and Microbiological Hazards of Hunted Wild Boar Carcasses. *Food Control* **2022**, *135*, 108782.
63. Stella, S.; Tirloni, E.; Castelli, E.; Colombo, F.; Bernardi, C. Microbiological Evaluation of Carcasses of Wild Boar Hunted in a Hill Area of Northern Italy. *J. Food Prot.* **2018**, *81*, 1519–1525.
64. Peruzy, M.F.; Cristiano, D.; Delibato, E.; D'Alessio, N.; Proroga, Y.T.R.; Capozza, R.L.; Rippa, A.; Murru, N. Presence of Enteric Bacterial Pathogens in Meat Samples of Wild Boar Hunted in Campania Region, Southern Italy. *Ital. J. Food Saf.* **2022**, *11*, 9967.

65. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on Microbiological Criteria for Foodstuffs. *Off. J. Eur. Union* **2005**, *L338*, 1–26.
66. Kerkhof, P.J.; Peruzy, M.F.; Murru, N.; Houf, K. Wild boars as reservoir for Campylobacter and Arcobacter. *Vet. Microbiol.* **2022**,
270, 109462.
67. Sasaki, Y.; Goshima, T.; Mori, T.; Murakami, M.; Haruna, M.; Ito, K.; Yamada, Y. Prevalence and Antimicrobial Susceptibility of Foodborne Bacteria in Wild Boars (*Sus scrofa*) and Wild Deer (*Cervus Nippon*) in Japan. *Foodborne Pathog. Dis.* **2013**, *10*, 985–991.
68. Cummings, K.J.; Rodriguez-Rivera, L.D.; McNeely, I.; Suchodolski, J.S.; Mesenbrink, B.T.; Leland, B.R.; Bodenchuk, M.J. Fecal Shedding of Campylobacter Jejuni and Campylobacter Coli among Feral Pigs in Texas. *Zoonoses Public Health* **2018**, *65*, 215–217.
69. Zendoia, I.I.; Cevidanes, A.; Hurtado, A.; Vázquez, P.; Barral, M.; Barandika, J.F.; García-Pérez, A.L. Stable prevalence of *Coxiella burnetii* in wildlife after a decade of surveillance in northern Spain. *Vet. Microbiol.* **2022**, *268*, 109422.
70. Espí, A.; Del Cerro, A.; Oleaga, Á.; Rodríguez-Pérez, M.; López, C.M.; Hurtado, A.; Rodríguez-Martínez, L.D.; Barandika, J.F.; García-Pérez, A.L. One health approach: An overview of q fever in livestock, wildlife and humans in Asturias (northwestern spain). *Animals* **2021**, *11*, 1395.
71. Sgroi, G.; Iatta, R.; Lia, R.P.; Napoli, E.; Buono, F.; Bezerra-Santos, M.A.; Veneziano, V.; Otranto, D. Tick exposure and risk of tick-borne pathogens infection in hunters and hunting

dogs: A citizen science approach. *Transbound. Emerg. Dis.* **2022**, *69*, e386–e393.

72. Ebani, V.V.; Nardoni, S.; Fognani, G.; Mugnaini, L.; Bertelloni, F.; Rocchigiani, G.; Papini, R.A.; Stefani, F.; Mancianti, F. Molecular detection of vector-borne bacteria and protozoa in healthy hunting dogs from Central Italy. *Asian Pac. J. Trop. Biomed.* **2015**, *5*, 108–112.
73. Ebani, V.; Bertelloni, F.; Cecconi, G.; Sgorbini, M.; Cerri, D. Zoonotic tick-borne bacteria among wild boars (Sus scrofa) in Central Italy. *Asian Pac. J. Trop. Dis.* **2017**, *7*, 141–143.
74. Sgroi, G.; Iatta, R.; Lia, R.P.; D'Alessio, N.; Manoj, R.R.S.; Veneziano, V.; Otranto, D. Spotted fever group rickettsiae in *Dermacentor marginatus* from wild boars in Italy. *Transbound. Emerg. Dis.* **2021**, *68*, 2111–2120.
75. Del Cerro, A.; Oleaga, A.; Somoano, A.; Barandika, J.F.; García-Pérez, A.L.; Espí, A. Molecular identification of tick-borne pathogens (*Rickettsia* spp., *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato, *Coxiella burnetii* and piroplasms) in questing and feeding hard ticks from North-Western Spain. *Ticks Tick-Borne Dis.* **2022**, *13*, 101961.
76. Castillo-Contreras, R.; Magen, L.; Birtles, R.; Varela-Castro, L.; Hall, J.L.; Conejero, C.; Aguilar, X.F.; Colom-Cadena, A.; Lavín, S.; Mentaberre, G.; et al. Ticks on wild boar in the metropolitan area of Barcelona (Spain) are infected with spotted fever group rickettsiae. *Transbound. Emerg. Dis.* **2022**, *69*, e82–e95.

77. Santana, M.D.S.; Hoppe, E.G.L.; Carraro, P.E.; Calchi, A.C.; de Oliveira, L.B.; do Amaral, R.B.; Mongruel, A.C.B.; Machado, D.M.R.; Burger, K.P.; Barros-Batestti, D.M.; et al. Molecular detection of vector-borne agents in wild boars (Sus scrofa) and associated ticks from Brazil, with evidence of putative new genotypes of *Ehrlichia*, *Anaplasma*, and haemoplasmas. *Transbound. Emerg. Dis.* **2022**, *69*, e2808–e2831.
78. Sumrandee, C.; Baimai, V.; Trinachartvanit, W.; Ahantarig, A. Molecular detection of *Rickettsia*, *Anaplasma*, *Coxiella* and *Francisella* bacteria in ticks collected from Artiodactyla in Thailand. *Ticks Tick-Borne Dis.* **2016**, *7*, 678–689.
79. Orr, B.; Malik, R.; Westman, M.E.; Norris, J.M. Seroprevalence of *Coxiella burnetii* in pig-hunting dogs from north Queensland, Australia. *Aust. Vet. J.* **2022**, *100*, 230–235.
80. WOAH. Available online: https://www.woah.org/app/uploads/2022/02/listeria-monocytogenes-infection-with.pdf (accessed on 1 March 2023).
81. Weindl, L.; Frank, E.; Ullrich, U.; Heurich, M.; Kleta, S.; Ellerbroek, L.; Gareis, M. *Listeria Monocytogenes* in Different Specimens from Healthy Red Deer and Wild Boars. *Foodborne Pathog. Dis.* **2016**, *13*, 391–397.
82. Palacios-Gorba, C.; Moura, A.; Leclercq, A.; Gómez-Martín, Á.; Gomis, J.; Jiménez-Trigos, E.; Mocé, M.L.; Lecuit, M.; Quereda,

J.J. *Listeria* spp. Isolated from Tonsils of Wild Deer and Boars: Genomic Characterization. *Appl. Environ. Microbiol.* **2021**, *87*, e02651-20.

83. Roila, R.; Branciari, R.; Primavilla, S.; Miraglia, D.; Vercillo, F.; Ranucci, D. Microbial, Physicochemical and Sensory Characteristics of Salami Produced from Wild Boar (*Sus scrofa*). *Slovak J. Food Sci.* **2021**, *15*, 475–483.
84. Lucchini, R.; Armani, M.; Novelli, E.; Rodas, S.; Masiero, A.; Minenna, J.; Bacchin, C.; Drigo, I.; Piovesana, A.; Favretti, M.; et al. Listeria monocytogenes in game meat cured sausages. In *Trends in Game Meat Hygiene: From Forest to Fork*; Paulsen, P., Bauer, A., Smulders, F.J.M., Eds.; Wageningen Academic Publishers: Wageningen, The Netherlands, 2014; pp. 167–174.
85. Cilia, G.; Turchi, B.; Fratini, F.; Bilei, S.; Bossù, T.; De Marchis, M.L.; Cerri, D.; Pacini, M.I.; Bertelloni, F. Prevalence, Virulence and
Antimicrobial Susceptibility of Salmonella Spp., Yersinia Enterocolitica and Listeria Monocytogenes in European Wild Boar (*Sus scrofa*) Hunted in Tuscany (Central Italy). *Pathogens* **2021**, *10*, 93.
86. Avagnina, A.; Nucera, D.; Grassi, M.A.; Ferroglio, E.; Dalmasso, A.; Civera, T. The Microbiological Conditions of Carcasses from Large Game Animals in Italy. *Meat Sci.* **2012**, *91*, 266–271.
87. Kanabalan, R.D.; Lee, L.J.; Lee, T.Y.; Chong, P.P.; Hassan, L.; Ismail, R.; Chin, V.K. Human Tuberculosis and Mycobacterium Tuberculosis Complex: A Review on

Genetic Diversity, Pathogenesis and Omics Approaches in Host Biomarkers Discovery. *Microbiol. Res.* **2021**, *246*, 126674.
88. Prodinger, W.M.; Indra, A.; Koksalan, O.K.; Kilicaslan, Z.; Richter, E. Mycobacterium Caprae Infection in Humans. *Expert Rev. Anti-Infect. Ther.* **2014**, *12*, 1501–1513.
89. Varela-Castro, L.; Gerrikagoitia, X.; Alvarez, V.; Geijo, M.V.; Barral, M.; Sevilla, I.A. A Long-Term Survey on Mycobacterium Tuberculosis Complex in Wild Animals from a Bovine Tuberculosis Low Prevalence Area. *Eur. J. Wildl. Res.* **2021**, *67*, 43.
90. Reis, A.C.; Ramos, B.; Pereira, A.C.; Cunha, M.V. The Hard Numbers of Tuberculosis Epidemiology in Wildlife: A Meta-Regression and Systematic Review. *Transbound. Emerg. Dis.* **2021**, *68*, 3257–3276.
91. Lopes, B.C.; Vidaletti, M.R.; Loiko, M.R.; da Andrade, J.S.; Maciel, A.L.G.; Doyle, R.L.; Bertagnolli, A.C.; Rodrigues, R.O.; Driemeier, D.; Mayer, F.Q. Investigation of Mycobacterium Bovis and Metastrongylus Sp. Co-Infection and Its Relationship to Tuberculosis Lesions' Occurrence in Wild Boars. *Comp. Immunol. Microbiol. Infect. Dis.* **2021**, *77*, 101674.
92. Iovane, V.; Ferrara, G.; Petruccelli, A.; Veneziano, V.; D'Alessio, N.; Ciarcia, R.; Fioretti, A.; Pagnini, U.; Montagnaro, S. Prevalence of Serum Antibodies against the Mycobacterium Tuberculosis Complex in Wild Boar in Campania Region, Italy. *Eur. J. Wildl. Res.* **2020**, *66*, 20.
93. Jamin, C.; Rivière, J. Assessment of Bovine Tuberculosis Surveillance Effectiveness in French Wildlife: An Operational

Approach.
Prev. Vet. Med. **2020**, *175*, 104881.

94. Richomme, C.; Courcoul, A.; Moyen, J.-L.; Reveillaud, É.; Maestrini, O.; de Cruz, K.; Drapeau, A.; Boschiroli, M.L. Tuberculosis in the Wild Boar: Frequentist and Bayesian Estimations of Diagnostic Test Parameters When Mycobacterium Bovis Is Present in Wild Boars but at Low Prevalence. *PLoS ONE* **2019**, *14*, e0222661.
95. Santos, N.; Nunes, T.; Fonseca, C.; Vieira-Pinto, M.; Almeida, V.; Gortázar, C.; Correia-Neves, M. Spatial Analysis of Wildlife Tuberculosis Based on a Serologic Survey Using Dried Blood Spots, Portugal. *Emerg. Infect. Dis.* **2018**, *24*, 2169–2175.
96. Roos, E.O.; Olea-Popelka, F.; Buss, P.; Hausler, G.A.; Warren, R.; Van Helden, P.D.; Parsons, S.D.C.; De Klerk-Lorist, L.-M.; Miller, M.A. Measuring antigen-specific responses in Mycobacterium bovis-infected warthogs (Phacochoerus africanus) using the intradermal tuberculin test. *BMC Vet. Res.* **2018**, *14*, 360.
97. Roos, E.O.; Olea-Popelka, F.; Buss, P.; de Klerk-Lorist, L.-M.; Cooper, D.; van Helden, P.D.; Parsons, S.D.C.; Miller, M.A. Seroprevalence of Mycobacterium bovis infection in warthogs (Phacochoerus africanus) in bovine tuberculosis-endemic regions of South Africa. *Transbound. Emerg. Dis.* **2018**, *65*, 1182–1189.
98. Réveillaud, É.; Desvaux, S.; Boschiroli, M.-L.; Hars, J.; Faure, É.; Fediaevsky, A.; Cavalerie, L.; Chevalier, F.; Jabert, P.; Poliak, S.; et al. Infection of Wildlife by Mycobacterium

Bovis in France Assessment Through a National Surveillance System, Sylvatub. *Front. Vet. Sci.* **2018**, *5*, 262.

99. Maciel, A.L.G.; Loiko, M.R.; Bueno, T.S.; Moreira, J.G.; Coppola, M.; Dalla Costa, E.R.; Schmid, K.B.; Rodrigues, R.O.; Cibulski, S.P.; Bertagnolli, A.C.; et al. Tuberculosis in Southern Brazilian Wild Boars (*Sus scrofa*): First Epidemiological Findings. *Transbound. Emerg. Dis.* **2018**, *65*, 518–526.
100. Amato, B.; Di Marco Lo Presti, V.; Gerace, E.; Capucchio, M.T.; Vitale, M.; Zanghì, P.; Pacciarini, M.L.; Marianelli, C.; Boniotti, M.B. Molecular Epidemiology of Mycobacterium Tuberculosis Complex Strains Isolated from Livestock and Wild Animals in Italy Suggests the Need for a Different Eradication Strategy for Bovine Tuberculosis. *Transbound. Emerg. Dis.* **2018**, *65*, e416–e424.
101. Madeira, S.; Manteigas, A.; Ribeiro, R.; Otte, J.; Fonseca, A.P.; Caetano, P.; Abernethy, D.; Boinas, F. Factors That Influence Mycobacterium Bovis Infection in Red Deer and Wild Boar in an Epidemiological Risk Area for Tuberculosis of Game Species in Portugal. *Transbound. Emerg. Dis.* **2017**, *64*, 793–804.
102. Jang, Y.; Ryoo, S.; Lee, H.; Kim, N.; Lee, H.; Park, S.; Song, W.; Kim, J.-T.; Lee, H.S.; Myung Kim, J. Isolation of Mycobacterium Bovis from Free-Ranging Wildlife in South Korea. *J. Wildl. Dis.* **2017**, *53*, 181–185.
103. Che'Amat, A.; Armenteros, J.A.; González-Barrio, D.; Lima, J.F.; Díez-Delgado, I.; Barasona, J.A.; Romero, B.; Lyashchenko, K.P.; Ortiz, J.A.; Gortázar, C. Is Targeted Removal a Suitable Means for Tuberculosis Control in Wild

Boar? *Prev. Vet. Med.* **2016**, *135*,132–135.

104. García-Jiménez, W.L.; Cortés, M.; Benítez-Medina, J.M.; Hurtado, I.; Martínez, R.; García-Sánchez, A.; Risco, D.; Cerrato, R.; Sanz, C.; Hermoso-de-Mendoza, M.; et al. Spoligotype Diversity and 5-Year Trends of Bovine Tuberculosis in Extremadura, Southern Spain. *Trop. Anim. Health Prod.* **2016**, *48*, 1533–1540.
105. Matos, A.C.; Figueira, L.; Martins, M.H.; Pinto, M.L.; Matos, M.; Coelho, A.C. New Insights into Mycobacterium Bovis Prevalence in Wild Mammals in Portugal. *Transbound. Emerg. Dis.* **2016**, *63*, e313–e322.
106. El Mrini, M.; Kichou, F.; Kadiri, A.; Berrada, J.; Bouslikhane, M.; Cordonnier, N.; Romero, B.; Gortázar, C. Animal Tuberculosis Due to Mycobacterium Bovis in Eurasian Wild Boar from Morocco. *Eur. J. Wildl. Res.* **2016**, *62*, 479–482.
107. Miller, M.; Buss, P.; de Klerk-Lorist, L.-M.; Hofmeyr, J.; Hausler, G.; Lyashchenko, K.; Lane, E.P.; Botha, L.; Parsons, S.; van Helden, P. Application of Rapid Serologic Tests for Detection of Mycobacterium Bovis Infection in Free-Ranging Warthogs (Phacochoerus Africanus) - Implications for Antemortem Disease Screening. *J. Wildl. Dis.* **2015**, *52*, 180–182.
108. Che' Amat, A.; González-Barrio, D.; Ortiz, J.A.; Díez-Delgado, I.; Boadella, M.; Barasona, J.A.; Bezos, J.; Romero, B.; Armenteros, J.A.; Lyashchenko, K.P.; et al. Testing Eurasian Wild Boar Piglets for Serum Antibodies against Mycobacterium Bovis. *Prev. Vet. Med.* **2015**, *121*, 93–98.
109. Beerli, O.; Blatter, S.; Boadella, M.; Schöning, J.; Schmitt,

S.; Ryser-Degiorgis, M.-P. Towards Harmonised Procedures in Wildlife Epidemiological Investigations: A Serosurvey of Infection with Mycobacterium Bovis and Closely Related Agents in Wild Boar (*Sus scrofa*) in Switzerland. *Vet. J.* **2015**, *203*, 131–133.

110. Broughan, J.M.; Downs, S.H.; Crawshaw, T.R.; Upton, P.A.; Brewer, J.; Clifton-Hadley, R.S. Mycobacterium Bovis Infections in Domesticated Non-Bovine Mammalian Species. Part 1: Review of Epidemiology and Laboratory Submissions in Great Britain 2004–2010. *Vet. J.* **2013**, *198*, 339–345.

111. Muñoz-Mendoza, M.; Marreros, N.; Boadella, M.; Gortázar, C.; Menéndez, S.; de Juan, L.; Bezos, J.; Romero, B.; Copano, M.F.;
Amado, J.; et al. Wild boar tuberculosis in Iberian Atlantic Spain: A different picture from Mediterranean habitats. *BMC Vet. Res.* **2013**, *9*, 176.

112. Boadella, M.; Vicente, J.; Ruiz-Fons, F.; de la Fuente, J.; Gortázar, C. Effects of Culling Eurasian Wild Boar on the Prevalence of Mycobacterium Bovis and Aujeszky's Disease Virus. *Prev. Vet. Med.* **2012**, *107*, 214–221.

113. Zanella, G.; Bar-Hen, A.; Boschiroli, M.-L.; Hars, J.; Moutou, F.; Garin-Bastuji, B.; Durand, B. Modelling Transmission of Bovine Tuberculosis in Red Deer and Wild Boar in Normandy, France. *Zoonoses Public Health* **2012**, *59*, 170–178.

114. Nugent, G.; Whitford, J.; Yockney, I.J.; Cross, M.L. Reduced Spillover Transmission of Mycobacterium Bovis to Feral Pigs (*Sus scofa*) Following Population Control of Brushtail Possums

(Trichosurus Vulpecula). *Epidemiol. Infect.* **2012**, *140*, 1036–1047.

115. García-Bocanegra, I.; de Val, B.P.; Arenas-Montes, A.; Paniagua, J.; Boadella, M.; Gortázar, C.; Arenas, A. Seroprevalence and Risk Factors Associated to Mycobacterium Bovis in Wild Artiodactyl Species from Southern Spain, 2006–2010. *PLoS ONE* **2012**, *7*, e34908.
116. Orłowska, B.; Krajewska-Wędzina, M.; Augustynowicz-Kopeć, E.; Kozińska, M.; Brzezińska, S.; Zabost, A.; Didkowska, A.; Welz, M.; Kaczor, S.; Żmuda, P.; et al. Epidemiological Characterization of Mycobacterium Caprae Strains Isolated from Wildlife in the Bieszczady Mountains, on the Border of Southeast Poland. *BMC Vet. Res.* **2020**, *16*, 362.
117. Csivincsik, Á.; Rónai, Z.; Nagy, G.; Svéda, G.; Halász, T. Surveillance of Mycobacterium Caprae Infection in a Wild Boar (*Sus scrofa*) Population in Southwestern Hungary. *Vet. Arh.* **2016**, *86*, 767–775.
118. Tagliapietra, V.; Boniotti, M.B.; Mangeli, A.; Karaman, I.; Alborali, G.; Chiari, M.; D'Incau, M.; Zanoni, M.; Rizzoli, A.; Pacciarini, M.L. Mycobacterium Microti at the Environment and Wildlife Interface. *Microorganisms* **2021**, *9*, 2084.
119. Ghielmetti, G.; Hilbe, M.; Friedel, U.; Menegatti, C.; Bacciarini, L.; Stephan, R.; Bloemberg, G. Mycobacterial Infections in Wild Boars (*Sus scrofa*) from Southern Switzerland: Diagnostic Improvements, Epidemiological Situation and Zoonotic Potential. *Transbound. Emerg. Dis.* **2021**, *68*, 573–586.

120. De Val, B.; Sanz, A.; Soler, M.; Allepuz, A.; Michelet, L.; Boschiroli, M.L.; Vidal, E. Mycobacterium Microti Infection in Free- Ranging Wild Boar, Spain, 2017–2019. *Emerg. Infect. Dis.* **2019**, *25*, 2152–2154.
121. Bona, M.C.; Mignone, W.; Ballardini, M.; Dondo, A.; Mignone, G.; Ru, G. Tuberculosis in wild boar (Sus scrofa) in the Western Liguria Region. *Épidémiologie Et St. Anim.* **2018**, *74*, 151–158.
122. Chiari, M.; Ferrari, N.; Giardiello, D.; Avisani, D.; Pacciarini, M.L.; Alborali, L.; Zanoni, M.; Boniotti, M.B. Spatiotemporal and Ecological Patterns of Mycobacterium Microti Infection in Wild Boar (*Sus scrofa*). *Transbound. Emerg. Dis.* **2016**, *63*, e381–e388.
123. Boniotti, M.B.; Gaffuri, A.; Gelmetti, D.; Tagliabue, S.; Chiari, M.; Mangeli, A.; Spisani, M.; Nassuato, C.; Gibelli, L.; Sacchi, C.; et al. Detection and molecular characterization of Mycobacterium microti isolates in wild boar from northern Italy. *J. Clin. Microbiol.* **2014**, *52*, 2834–2843.
124. Schöning, J.M.; Cerny, N.; Prohaska, S.; Wittenbrink, M.M.; Smith, N.H.; Bloemberg, G.; Pewsner, M.; Schiller, I.; Origgi, F.C.; Ryser-Degiorgis, M.-P. Surveillance of Bovine Tuberculosis and Risk Estimation of a Future Reservoir Formation in Wildlife in Switzerland and Liechtenstein. *PLoS ONE* **2013**, *8*, e54253.
125. Natarajan, A.; Beena, P.M.; Devnikar, A.V.; Mali, S. A Systemic Review on Tuberculosis. *Indian J. Tuberc.* **2020**, *67*, 295–311.
126. Torres-Gonzalez, P.; Cervera-Hernandez, M.E.; Martinez-

Gamboa, A.; Garcia-Garcia, L.; Cruz-Hervert, L.P.; Bobadilla-del Valle, M.; Ponce-de Leon, A.; Sifuentes-Osornio, J. Human Tuberculosis Caused by Mycobacterium Bovis: A Retrospective Comparison with Mycobacterium Tuberculosis in a Mexican Tertiary Care Centre, 2000–2015. *BMC Infect. Dis.* **2016**, *16*, 657.

127. Lekko, Y.M.; Che-Amat, A.; Ooi, P.T.; Omar, S.; Ramanoon, S.Z.; Mazlan, M.; Jesse, F.F.A.; Jasni, S.; Ariff Abdul-Razak, M.F. Mycobacterium Tuberculosis and Avium Complex Investigation among Malaysian Free-Ranging Wild Boar and Wild Macaques at Wildlife-Livestock-Human Interface. *Animals* **2021**, *11*, 3252.
128. Mentaberre, G.; Romero, B.; de Juan, L.; Navarro-González, N.; Velarde, R.; Mateos, A.; Marco, I.; Olivé-Boix, X.; Domínguez, L.; Lavín, S.; et al. Long-Term Assessment of Wild Boar Harvesting and Cattle Removal for Bovine Tuberculosis Control in Free Ranging Populations. *PLoS ONE* **2014**, *9*, e88824.
129. Aranha, J.; Abrantes, A.C.; Gonçalves, R.; Miranda, R.; Serejo, J.; Vieira-Pinto, M. GIS as an Epidemiological Tool to Monitor the Spatial–Temporal Distribution of Tuberculosis in Large Game in a High-Risk Area in Portugal. *Animals* **2021**, *11*, 2374.
130. Pate, M.; Zajc, U.; Kušar, D.; Žele, D.; Vengušt, G.; Pirš, T.; Ocepek, M. *Mycobacterium* spp. in Wild Game in Slovenia. *Vet. J.* **2016**, *208*, 93–95.
131. García-Jiménez, W.L.; Benítez-Medina, J.M.; Martínez, R.; Carranza, J.; Cerrato, R.; García-Sánchez, A.; Risco, D.; Moreno, J.C.; Sequeda, M.; Gómez, L.; et al. Non-

Tuberculous Mycobacteria in Wild Boar (*Sus scrofa*) from Southern Spain: Epidemiological, Clinical and Diagnostic Concerns. *Transbound. Emerg. Dis.* **2015**, *62*, 72–80.

132. Barasona, J.A.; Torres, M.J.; Aznar, J.; Gortázar, C.; Vicente, J. DNA Detection Reveals Mycobacterium Tuberculosis Complex Shedding Routes in Its Wildlife Reservoir the Eurasian Wild Boar. *Transbound. Emerg. Dis.* **2017**, *64*, 906–915.
133. Barroso, P.; Barasona, J.A.; Acevedo, P.; Palencia, P.; Carro, F.; Negro, J.J.; Torres, M.J.; Gortázar, C.; Soriguer, R.C.; Vicente, J. Long-Term Determinants of Tuberculosis in the Ungulate Host Community of Doñana National Park. *Pathogens* **2020**, *9*, 445.
134. Gortázar, C.; Fernández-Calle, L.M.; Collazos-Martínez, J.A.; Mínguez-González, O.; Acevedo, P. Animal Tuberculosis Main- tenance at Low Abundance of Suitable Wildlife Reservoir Hosts: A Case Study in Northern Spain. *Prev. Vet. Med.* **2017**, *146*, 150–157.
135. Ramos, B.; Pereira, A.C.; Reis, A.C.; Cunha, M.V. Estimates of the global and continental burden of animal tuberculosis in key livestock species worldwide: A meta-analysis study. *One Health* **2020**, *10*, 100169.
136. Costa, P.; Ferreira, A.S.; Amaro, A.; Albuquerque, T.; Botelho, A.; Couto, I.; Cunha, M.V.; Viveiros, M.; Inácio, J. Enhanced detection of tuberculous mycobacteria in animal tissues using a semi-nested probe-based real-time PCR. *PLoS ONE* **2013**, *8*, e81337.
137. Pedersen, K.; Miller, R.S.; Anderson, T.D.; Pabilonia, K.L.; Lewis, J.R.; Mihalco, R.L.; Gortazar, C.; Gidlewski, T.

Limited antibody evidence of exposure to Mycobacterium bovis in feral swine (*Sus scrofa*) in the USA. *J. Wildl. Dis.* **2017**, *53*, 30–36.

138. Santos, N.; Colino, E.F.; Arnal, M.C.; de Luco, D.F.; Sevilla, I.; Garrido, J.M.; Fonseca, E.; Valente, A.M.; Balseiro, A.; Queirós, J.; et al. Complementary Roles of Wild Boar and Red Deer to Animal Tuberculosis Maintenance in Multi-Host Communities. *Epidemics* **2022**, *41*, 100633.
139. Palmer, M.V. Mycobacterium bovis: Characteristics of wildlife reservoir hosts. *Transb. Emerg. Dis.* **2013**, *60* (Suppl. 1), 1–13.
140. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 Laying Down Specific Hygiene Rules for Food of Animal Origin. *Off. J. Eur. Union* **2003**, *L139*, 55.
141. Reis, A.C.; Tenreiro, R.; Albuquerque, T.; Botelho, A.; Cunha, M.V. Long-term molecular surveillance provides clues on a cattle origin for Mycobacterium bovis in Portugal. *Sci. Rep.* **2020**, *10*, 20856.
142. Clausi, M.T.; Ciambrone, L.; Zanoni, M.; Costanzo, N.; Pacciarini, M.; Casalinuovo, F. Evaluation of the Presence and Viability of Mycobacterium bovis in Wild Boar Meat and Meat-Based Preparations. *Foods* **2021**, *10*, 2410.
143. Varela-Castro, L.; Alvarez, V.; Sevilla, I.A.; Barral, M. Risk factors associated to a high Mycobacterium tuberculosis complex seroprevalence in wild boar (*Sus scrofa*) from a low bovine tuberculosis prevalence area. *PLoS ONE* **2020**, *15*, e0231559.

144. Richomme, C.; Boadella, M.; Courcoul, A.; Durand, B.; Drapeau, A.; Corde, Y.; Hars, J.; Payne, A.; Fediaevsky, A.; Boschiroli, M.L. Exposure of Wild Boar to Mycobacterium Tuberculosis Complex in France since 2000 Is Consistent with the Distribution of Bovine Tuberculosis Outbreaks in Cattle. *PLoS ONE* **2013**, *8*, e77842.
145. Albertti, L.A.G.; Souza-Filho, A.F.; Fonseca-Júnior, A.A.; Freitas, M.E.; de Oliveira-Pellegrin, A.; Zimmermann, N.P.; Tomás, W.M.; Péres, I.A.H.F.S.; Fontana, I.; Osório, A.L.A.R. Mycobacteria Species in Wild Mammals of the Pantanal of Central South America. *Eur. J. Wildl. Res.* **2015**, *61*, 163–166.
146. Vicente, J.; Barasona, J.A.; Acevedo, P.; Ruiz-Fons, J.F.; Boadella, M.; Diez-Delgado, I.; Beltran-Beck, B.; González-Barrio, D.; Queirós, J.; Montoro, V.; et al. Temporal trend of tuberculosis in wild ungulates from Mediterranean Spain. *Transb. Emerg. Dis.* **2013**, *60*, 92–103.
147. WOAH. Available online: https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.09.08_SALMONELLOSIS. pdf (accessed on 1 March 2023).
148. WOAH. Available online: https://www.woah.org/app/uploads/2021/05/salmonella-enterica-all-serovarsinfection-with.pdf (accessed on 1 March 2023).
149. Grimont, P.A.; Weill, F.X. Antigenic formulae of the Salmonella serovars. *WHO Collab. Cent. Ref. Res. Salmonella* **2007**, *9*, 1–166.
150. European Food Safety Authority (EFSA) and European

Centre for Disease Prevention and Control (ECDC). The European Union One Health 2020 Zoonoses Report. *EFSA J.* **2021**, *19*, 6971.

151. Torres, R.T.; Fernandes, J.; Carvalho, J.; Cunha, M.V.; Caetano, T.; Mendo, S.; Serrano, E.; Fonseca, C. Wild Boar as a Reservoir of Antimicrobial Resistance. *Sci. Total Environ.* **2020**, *717*, 135001.
152. Shinoda, S.; Mizuno, T.; Miyoshi, S. General Review on Hog Cholera (Classical Swine Fever), African Swine Fever, and *Salmonella Enterica* Serovar Choleraesuis Infection. *J. Disaster Res.* **2019**, *14*, 1105–1114.
153. Greig, J.; Rajic´, A.; Young, I.; Mascarenhas, M.; Waddell, L.; LeJeune, J. A Scoping Review of the Role of Wildlife in the Transmission of Bacterial Pathogens and Antimicrobial Resistance to the Food Chain. *Zoonoses Public Health* **2015**, *62*, 269–284.
154. Billinis, C. Wildlife Diseases That Pose a Risk to Small Ruminants and Their Farmers. *Small Rum. Res.* **2013**, *110*, 67–70.
155. Paulsen, P.; Smulders, F.J.M.; Hilbert, F. Salmonella in Meat from Hunted Game: A Central European Perspective. *Food Res. Int.* **2012**, *45*, 609–616.
156. Cano-Manuel, F.J.; López-Olvera, J.; Fandos, P.; Soriguer, R.C.; Pérez, J.M.; Granados, J.E. Long-Term Monitoring of 10 Selected Pathogens in Wild Boar (*Sus scrofa*) in Sierra Nevada National Park, Southern Spain. *Vet. Microbiol.* **2014**, *174*, 148–154.

157. Flores Rodas, E.M.; Bogdanova, T.; Bossù, T.; Pecchi, S.; Tomassetti, F.; De Santis, P.; Tolli, R.; Condoleo, R.; Greco, S.; Brozzi, A.; et al. Microbiological Assessment of Freshly-Shot Wild Boars Meat in Lazio Region, Viterbo Territory: A Preliminary Study. *Ital. J. Food Saf.* **2014**, *3*, 1711.
158. Touloudi, A.; Valiakos, G.; Athanasiou, L.V.; Birtsas, P.; Giannakopoulos, A.; Papaspyropoulos, K.; Kalaitzis, C.; Sokos, C.; Tsokana, C.N.; Spyrou, V.; et al. A Serosurvey for Selected Pathogens in Greek European Wild Boar. *Vet. Rec. Open* **2015**, *2*, e000077.
159. McGregor, G.F.; Gottschalk, M.; Godson, D.L.; Wilkins, W.; Bollinger, T.K. Disease Risks Associated with Free-Ranging Wild Boar in Saskatchewan. *Can. Vet. J.* **2015**, *56*, 839–844.
160. Bassi, A.M.G.; Steiner, J.C.; Stephan, R.; Nüesch-Inderbinen, M. Seroprevalence of Toxoplasma Gondii and Salmonella in Hunted Wild Boars from Two Different Regions in Switzerland. *Animals* **2021**, *11*, 2227.
161. Ortega, N.; Fanelli, A.; Serrano, A.; Martínez-Carrasco, C.; Escribano, F.; Tizzani, P.; Candela, M.G. Salmonella Seroprevalence in Wild Boar from Southeast Spain Depends on Host Population Density. *Res. Vet. Sci.* **2020**, *132*, 400–403.
162. Zottola, T.; Montagnaro, S.; Magnapera, C.; Sasso, S.; De Martino, L.; Bragagnolo, A.; D'Amici, L.; Condoleo, R.; Pisanelli, G.; Iovane, G.; et al. Prevalence and Antimicrobial Susceptibility of Salmonella in European Wild Boar (*Sus scrofa*); Latium Region—Italy. *Comp. Immunol. Microbiol. Infect. Dis.* **2013**, *36*, 161–168.
163. Petrovic´, J.; Mircˇeta, J.; Babic´, J.; Maleševic´, M.;

Blagojevic´, B.; Radulovic´, J.P.; Antic´, D. *Salmonella* in Wild Boars *(Sus scrofa)*: Characterization and Epidemiology. *Acta Vet.* **2022**, *72*, 184–194.

164. Piras, F.; Spanu, V.; Siddi, G.; Gymoese, P.; Spanu, C.; Cibin, V.; Schjørring, S.; De Santis, E.P.L.; Scarano, C. Whole-Genome Sequencing Analysis of Highly Prevalent Salmonella Serovars in Wild Boars from a National Park in Sardinia. *Food Control* **2021**, *130*, 108247.
165. Bonardi, S.; Tansini, C.; Cacchioli, A.; Soliani, L.; Poli, L.; Lamperti, L.; Corradi, M.; Gilioli, S. Enterobacteriaceae and Salmonella Contamination of Wild Boar (*Sus scrofa*) Carcasses: Comparison between Different Sampling Strategies. *Eur. J. Wildl. Res.* **2021**, *67*, 88.
166. Razzuoli, E.; Listorti, V.; Martini, I.; Migone, L.; Decastelli, L.; Mignone, W.; Berio, E.; Battistini, R.; Ercolini, C.; Serracca, L.; et al. Prevalence and Antimicrobial Resistances of Salmonella Spp. Isolated from Wild Boars in Liguria Region, Italy. *Pathogens* **2021**, *10*, 568.
167. Plaza-Rodríguez, C.; Alt, K.; Grobbel, M.; Hammerl, J.A.; Irrgang, A.; Szabo, I.; Stingl, K.; Schuh, E.; Wiehle, L.; Pfefferkorn, B.; et al. Wildlife as Sentinels of Antimicrobial Resistance in Germany? *Front. Vet. Sci.* **2021**, *7*, 627821.
168. Bonardi, S.; Bolzoni, L.; Zanoni, R.G.; Morganti, M.; Corradi, M.; Gilioli, S.; Pongolini, S. Limited Exchange of Salmonella Among Domestic Pigs and Wild Boars in Italy. *EcoHealth* **2019**, *16*, 420–428.
169. Gil Molino, M.; García Sánchez, A.; Risco Pérez, D.; Gonçalves Blanco, P.; Quesada Molina, A.; Rey Pérez, J.; Martín Cano, F.E.; Cerrato Horrillo, R.; Hermoso-de-

Mendoza Salcedo, J.; Fernández Llario, P. Prevalence of Salmonella Spp. in Tonsils, Mandibular Lymph Nodes and Faeces of Wild Boar from Spain and Genetic Relationship between Isolates. *Transbound. Emerg. Dis.* **2019**, *66*, 1218–1226.

170. Cummings, K.J.; Rodriguez-Rivera, L.D.; Grigar, M.K.; Rankin, S.C.; Mesenbrink, B.T.; Leland, B.R.; Bodenchuk, M.J. Prevalence and Characterization of *Salmonella* Isolated from Feral Pigs Throughout Texas. *Zoonoses Public Health* **2016**, *63*, 436–441.
171. Dias, D.; Torres, R.T.; Kronvall, G.; Fonseca, C.; Mendo, S.; Caetano, T. Assessment of Antibiotic Resistance of *Escherichia coli* Isolates and Screening of Salmonella Spp. in Wild Ungulates from Portugal. *Res. Microbiol.* **2015**, *166*, 584–593.
172. Sannö, A.; Aspán, A.; Hestvik, G.; Jacobson, M. Presence of *Salmonella* Spp., *Yersinia Enterocolitica*, *Yersinia Pseudotuberculosis* and *Escherichia coli* O157:H7 in Wild Boars. *Epidemiol. Infect.* **2014**, *142*, 2542–2547.
173. Favier, G.I.; Lucero Estrada, C.; Cortiñas, T.I.; Escudero, M.E. Detection and Characterization of Shiga Toxin Producing *Escherichia coli*, *Salmonella* spp., and *Yersinia* Strains from Human, Animal, and Food Samples in San Luis, Argentina. *Int. J. Microbiol.* **2014**, *2014*, 284649.
174. Chiari, M.; Zanoni, M.; Tagliabue, S.; Lavazza, A.; Alborali, L.G. Salmonella Serotypes in Wild Boars (*Sus scrofa*) Hunted in Northern Italy. *Acta Vet. Scand.* **2013**, *55*, 42.
175. Navarro-Gonzalez, N.; Mentaberre, G.; Porrero, C.M.; Serrano, E.; Mateos, A.; López-Martín, J.M.; Lavín, S.;

Domínguez, L. Effect of Cattle on Salmonella Carriage, Diversity and Antimicrobial Resistance in Free-Ranging Wild Boar (*Sus scrofa*) in Northeastern Spain. *PLoS ONE* **2012**, *7*, e51614.

176. Ranucci, D.; Roila, R.; Onofri, A.; Cambiotti, F.; Primavilla, S.; Miraglia, D.; Andoni, E.; Di Cerbo, A.; Branciari, R. Improving Hunted Wild Boar Carcass Hygiene: Roles of Different Factors Involved in the Harvest Phase. *Foods* **2021**, *10*, 1548.
177. Orsoni, F.; Romeo, C.; Ferrari, N.; Bardasi, L.; Merialdi, G.; Barbani, R. Factors Affecting the Microbiological Load of Italian Hunted Wild Boar Meat (*Sus scrofa*). *Meat Sci.* **2020**, *160*, 107967.
178. Peruzy, M.F.; Murru, N.; Yu, Z.; Kerkhof, P.-J.; Neola, B.; Joossens, M.; Proroga, Y.T.R.; Houf, K. Assessment of Microbial Communities on Freshly Killed Wild Boar Meat by MALDI-TOF MS and 16S RRNA Amplicon Sequencing. *Int. J. Food Microbiol.* **2019**, *301*, 51–60.
179. Asakura, H.; Kawase, J.; Ikeda, T.; Honda, M.; Sasaki, Y.; Uema, M.; Kabeya, H.; Sugiyama, H.; Igimi, S.; Takai, S. Microbiological Quality Assessment of Game Meats at Retail in Japan. *J. Food Prot.* **2017**, *80*, 2119–2126.
180. Mirceta, J.; Petrovic, J.; Malesevic, M.; Blagojevic, B.; Antic, D. Assessment of Microbial Carcass Contamination of Hunted Wild Boars. *Eur. J. Wildl. Res.* **2017**, *63*, 37.
181. Russo, C.; Balloni, S.; Altomonte, I.; Martini, M.; Nuvoloni, R.; Cecchi, F.; Pedonese, F.; Salari, F.; Sant'ana Da Silva, A.M.; Torracca, B.; et al. Fatty Acid and Microbiological Profile of the Meat (Longissimus Dorsi Muscle) of Wild Boar

(*Sus Scropha Scropha*) Hunted in Tuscany. *Ital. J. Anim. Sci.* **2017**, *16*, 1–8.

182. Holmes, M.A.; Zadoks, R.N. Methicillin resistant S. aureus in human and bovine mastitis. *J. Mammary Gland. Biol. Neoplasia* **2011**, *16*, 373–382.
183. Tong, S.Y.C.; Davis, J.S.; Eichenberger, E.; Holland, T.L.; Fowler, V.G. Staphylococcus Aureus Infections: Epidemiology, Patho- physiology, Clinical Manifestations, and Management. *Clin. Microbiol. Rev.* **2015**, *28*, 603–661.
184. European Food Safety Authority (EFSA). Joint scientific report of ECDC, EFSA and EMEA on meticillin resistant Staphylococcus aureus (MRSA) in livestock, companion animals and food. *EFSA Sci. Rep.* **2009**, *301*, 1–10.
185. Sousa, M.; Silva, N.; Manageiro, V.; Ramos, S.; Coelho, A.; Gonçalves, D.; Caniça, M.; Torres, C.; Igrejas, G.; Poeta, P. First Report on MRSA CC398 Recovered from Wild Boars in the North of Portugal. Are We Facing a Problem? *Sci. Total Environ.* **2017**, *596–597*, 26–31.
186. Porrero, M.C.; Mentaberre, G.; Sánchez, S.; Fernández-Llario, P.; Gómez-Barrero, S.; Navarro-Gonzalez, N.; Serrano, E.; Casas- Díaz, E.; Marco, I.; Fernández-Garayzabal, J.-F.; et al. Methicillin Resistant Staphylococcus Aureus (MRSA) Carriage in Different Free-Living Wild Animal Species in Spain. *Vet. J.* **2013**, *198*, 127–130.
187. Porrero, M.C.; Mentaberre, G.; Sánchez, S.; Fernández-Llario, P.; Casas-Díaz, E.; Mateos, A.; Vidal, D.; Lavín, S.; Fernández- Garayzábal, J.-F.; Domínguez, L. Carriage of Staphylococcus Aureus by Free-Living Wild Animals in Spain. *Appl Environ. Microbiol.* **2014**, *80*, 4865–4870.

188. Traversa, A.; Gariano, G.R.; Gallina, S.; Bianchi, D.M.; Orusa, R.; Domenis, L.; Cavallerio, P.; Fossati, L.; Serra, R.; Decastelli, L. Methicillin Resistance in Staphylococcus Aureus Strains Isolated from Food and Wild Animal Carcasses in Italy. *Food Microbiol.* **2015**, *52*, 154–158.
189. Seinige, D.; Von Altrock, A.; Kehrenberg, C. Genetic Diversity and Antibiotic Susceptibility of Staphylococcus Aureus Isolates from Wild Boars. *Comp. Immunol. Microbiol. Infect. Dis.* **2017**, *54*, 7–12.
190. Ramos, B.; Rosalino, L.M.; Palmeira, J.D.; Torres, R.T.; Cunha, M.V. Antimicrobial Resistance in Commensal Staphylococcus Aureus from Wild Ungulates Is Driven by Agricultural Land Cover and Livestock Farming. *Environ. Pollut.* **2022**, *303*, 119116.
191. Mama, O.M.; Ruiz-Ripa, L.; Fernández-Fernández, R.; González-Barrio, D.; Ruiz-Fons, J.F.; Torres, C. High Frequency of Coagulase-Positive Staphylococci Carriage in Healthy Wild Boar with Detection of MRSA of Lineage ST398-T011. *FEMS Microbiol. Lett.* **2019**, *366*, fny292.
192. Meemken, D.; Blaha, T.; Hotzel, H.; Strommenger, B.; Klein, G.; Ehricht, R.; Monecke, S.; Kehrenberg, C. Genotypic and Phenotypic Characterization of Staphylococcus Aureus Isolates from Wild Boars. *Appl. Environ. Microbiol.* **2013**, *79*, 1739–1742.
193. Rey Pérez, J.; Zálama Rosa, L.; García Sánchez, A.; Hermoso de Mendoza Salcedo, J.; Alonso Rodríguez, J.M.; Cerrato Horrillo, R.; Zurita, S.G.; Gil Molino, M. Multiple Antimicrobial Resistance in Methicillin-Resistant Staphylococcus Sciuri Group Isolates from Wild Ungulates in

Spain. *Antibiotics* **2021**, *10*, 920.

194. Holtmann, A.R.; Meemken, D.; Müller, A.; Seinige, D.; Büttner, K.; Failing, K.; Kehrenberg, C. Wild Boars Carry Extended- Spectrum β-Lactamase- and AmpC-Producing *Escherichia coli*. *Microorganisms* **2021**, *9*, 367.
195. EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards); Koutsoumanis, K.; Allende, A.; Alvarez-Ordonez, A.; Bover-Cid, S.; Chemaly, M.; Davies, R.; De Cesare, A.; Herman, L.; Hilbert, F.; et al. Pathogenicity assessment of Shiga toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC. *EFSA J.* **2020**, *18*, e05967.
196. Perrat, A.; Branchu, P.; Decors, A.; Turci, S.; Bayon-Auboyer, M.-H.; Petit, G.; Grosbois, V.; Brugère, H.; Auvray, F.; Oswald, E. Wild Boars as Reservoir of Highly Virulent Clone of Hybrid Shiga Toxigenic and Enterotoxigenic Escherichia coli Responsible for Edema Disease, France. *Emerg. Infect. Dis.* **2022**, *28*, 382–393.
197. Topalcengiz, Z.; Danyluk, M.D. Assessment of Contamination Risk from Fecal Matter Presence on Fruit and Mulch in the tomato fields based on generic *Escherichia coli* population. *Food Microbiol.* **2022**, *103*, 103956.
198. Topalcengiz, Z.; Jeamsripong, S.; Spanninger, P.M.; Persad, A.K.; Wang, F.; Buchanan, R.L.; Lejeune, J.; Kniel, K.E.; Jay-Russell, M.T.; Danyluk, M.D. Survival of shiga toxin–producing *Escherichia coli* in various wild animal feces that may contaminate produce. *J. Food Prot.* **2020**, *83*, 1420–1429.
199. McKee, A.M.; Bradley, P.M.; Shelley, D.; McCarthy, S.; Molina, M. Feral swine as sources of fecal contamination in

recreational waters. *Sci. Rep.* **2021**, *11*, 4212.

200. Dias, D.; Costa, S.; Fonseca, C.; Baraúna, R.; Caetano, T.; Mendo, S. Pathogenicity of Shiga toxin-producing *Escherichia coli* (STEC) from wildlife: Should we care? *Sci. Total Environ.* **2022**, *812*, 152324.
201. Morita, S.; Sato, S.; Maruyama, S.; Nagasaka, M.; Murakami, K.; Inada, K.; Uchiumi, M.; Yokoyama, E.; Asakura, H.; Sugiyama, H.; et al. Whole-genome sequence analysis of shiga toxin-producing escherichia coli O157 strains isolated from wild deer and boar in Japan. *J. Vet. Med. Sci.* **2021**, *83*, 1860–1868.
202. Bertelloni, F.; Cilia, G.; Bogi, S.; Ebani, V.V.; Turini, L.; Nuvoloni, R.; Cerri, D.; Fratini, F.; Turchi, B. Pathotypes and antimicrobial susceptibility of *Escherichia coli* isolated from wild boar (*Sus scrofa*) in Tuscany. *Animals* **2020**, *10*, 744.
203. Szczerba-Turek, A.; Socha, P.; Bancerz-Kisiel, A.; Platt-Samoraj, A.; Lipczynska-Ilczuk, K.; Siemionek, J.; Kończyk, K.; Terech- Majewska, E.; Szweda, W. Pathogenic potential to humans of Shiga toxin-producing *Escherichia coli* isolated from wild boars in Poland. *Int. J. Food Microbiol.* **2019**, *300*, 8–13.
204. Dias, D.; Caetano, T.; Torres, R.T.; Fonseca, C.; Mendo, S. Shiga toxin-producing *Escherichia coli* in wild ungulates. *Sci. Total Environ.* **2019**, *651*, 203–209.
205. Alonso, C.A.; Mora, A.; Díaz, D.; Blanco, M.; González-Barrio, D.; Ruiz-Fons, F.; Simón, C.; Blanco, J.; Torres, C. Occurrence and characterization of stx and/or eae-positive *Escherichia coli* isolated from wildlife, including a typical EPEC strain from a wild boar. *Vet. Microbiol.* **2017**, *207*, 69–73.

206. Navarro-Gonzalez, N.; Porrero, M.C.; Mentaberre, G.; Serrano, E.; Mateos, A.; Cabal, A.; Domínguez, L.; Lavín, S. *Escherichia coli* O157:H7 in Wild Boars (*Sus scrofa*) and Iberian Ibex (*Capra pyrenaica*) Sharing Pastures with Free-Ranging Livestock in a Natural Environment in Spain. *Vet. Q.* **2015**, *35*, 102–106.
207. Díaz-Sánchez, S.; Sánchez, S.; Sánchez, M.; Herrera-León, S.; Hanning, I.; Vidal, D. Detection and characterization of Shiga toxin-producing *Escherichia coli* in game meat and ready-to-eat meat products. *Int. J. Food Microbiol.* **2012**, *160*, 179–182.
208. Rosner, B.M.; Werber, D.; Höhle, M.; Stark, K. Clinical Aspects and Self-Reported Symptoms of Sequelae of Yersinia Enterocolitica Infections in a Population-Based Study, Germany 2009–2010. *BMC Infect. Dis.* **2013**, *13*, 236.
209. Fonnes, S.; Rasmussen, T.; Brunchmann, A.; Holzknecht, B.J.; Rosenberg, J. Mesenteric Lymphadenitis and Terminal Ileitis Is Associated With *Yersinia* Infection: A Meta-Analysis. *J. Surg. Res.* **2022**, *270*, 12–21.
210. CDC. Yersinia Enterocolitica (Yersiniosis). Available online: www.cdc.gov/yersinia/index.html (accessed on 24 October 2016).
211. Platt-Samoraj, A. Toxigenic Properties of Yersinia Enterocolitica Biotype 1A. *Toxins* **2022**, *14*, 118.
212. Fois, F.; Piras, F.; Torpdahl, M.; Mazza, R.; Ladu, D.; Consolati, S.G.; Spanu, C.; Scarano, C.; De Santis, E.P.L. Prevalence, Bioserotyping and Antibiotic Resistance of Pathogenic *Yersinia Enterocolitica* Detected in Pigs at Slaughter in Sardinia. *Int. J. Food Microbiol.* **2018**, *283*, 1–6.

213. Argüello, H.; Estellé, J.; Leonard, F.C.; Crispie, F.; Cotter, P.D.; O'Sullivan, O.; Lynch, H.; Walia, K.; Duffy, G.; Lawlor, P.G.; et al. Influence of the Intestinal Microbiota on Colonization Resistance to *Salmonella* and the Shedding Pattern of Naturally Exposed Pigs. *mSystems* **2019**, *4*, e00021-19.
214. Carella, E.; Romano, A.; Domenis, L.; Robetto, S.; Spedicato, R.; Guidetti, C.; Pitti, M.; Orusa, R. Characterisation of *Yersinia Enterocolitica* Strains Isolated from Wildlife in the Northwestern Italian Alps. *J. Vet. Res.* **2022**, *66*, 141–149.
215. Selmi, R.; Tayh, G.; Srairi, S.; Mamlouk, A.; Ben Chehida, F.; Lahmar, S.; Bouslama, M.; Daaloul-Jedidi, M.; Messadi, L. Prevalence, Risk Factors and Emergence of Extended-Spectrum β-Lactamase Producing-, Carbapenem- and Colistin-Resistant Enterobacterales Isolated from Wild Boar (*Sus scrofa*) in Tunisia. *Microb. Pathog.* **2022**, *163*, 105385.
216. Modesto, P.; De Ciucis, C.G.; Vencia, W.; Pugliano, M.C.; Mignone, W.; Berio, E.; Masotti, C.; Ercolini, C.; Serracca, L.; Andreoli, T.; et al. Evidence of Antimicrobial Resistance and Presence of Pathogenicity Genes in Yersinia Enterocolitica Isolate from Wild Boars. *Pathogens* **2021**, *10*, 398.
217. Bonardi, S.; Brémont, S.; Vismarra, A.; Poli, I.; Diegoli, G.; Bolzoni, L.; Corradi, M.; Gilioli, S.; Le Guern, A.S. Is Yersinia Bercovieri Surpassing Yersinia Enterocolitica in Wild Boars (*Sus scrofa*)? *EcoHealth* **2020**, *17*, 388–392.
218. Takahashi, T.; Kabeya, H.; Sato, S.; Yamazaki, A.; Kamata, Y.; Taira, K.; Asakura, H.; Sugiyama, H.; Takai, S.;

Maruyama, S. Prevalence of *Yersinia* among Wild Sika Deer (*Cervus nippon*) and Boars (*Sus scrofa*) in Japan. *J. Wildl. Dis.* **2020**, *56*, 270.

219. Sannö, A.; Rosendal, T.; Aspán, A.; Backhans, A.; Jacobson, M. Distribution of Enteropathogenic *Yersinia* spp. and *Salmonella* spp. in the Swedish Wild Boar Population, and Assessment of Risk Factors That May Affect Their Prevalence. *Acta Vet. Scand.* **2018**, *60*, 40.

220. Syczyło, K.; Platt-Samoraj, A.; Bancerz-Kisiel, A.; Szczerba-Turek, A.; Pajdak-Czaus, J.; Łabuc´, S.; Procajło, Z.; Socha, P.; Chuzhebayeva, G.; Szweda, W. The Prevalence of Yersinia Enterocolitica in Game Animals in Poland. *PLoS ONE* **2018**, *13*, e0195136.

221. Lorencova, A.; Babak, V.; Lamka, J. Serological Prevalence of Enteropathogenic *Yersinia* Spp. in Pigs and Wild Boars from Different Production Systems in the Moravian Region, Czech Republic. *Foodborne Pathog. Dis.* **2016**, *13*, 275–279.

222. Bancerz-Kisiel, A.; Socha, P.; Szweda, W. Detection and Characterisation of Yersinia Enterocolitica Strains in Cold-Stored Carcasses of Large Game Animals in Poland. *Vet. J.* **2016**, *208*, 102–103.

223. Arrausi-Subiza, M.; Gerrikagoitia, X.; Alvarez, V.; Ibabe, J.C.; Barral, M. Prevalence of *Yersinia Enterocolitica* and *Yersinia Pseudotuberculosis* in Wild Boars in the Basque Country, Northern Spain. *Acta Vet. Scand.* **2016**, *58*, 4.

224. Von Altrock, A.; Seinige, D.; Kehrenberg, C. *Yersinia enterocolitica* isolates from wild boars hunted in Lower Saxony, Germany. *Appl. Environ. Microbiol.* **2015**, *81*, 4835–4840.

225. Arrausi-Subiza, M.; Ibabe, J.C.; Atxaerandio, R.; Juste, R.A.; Barral, M. Evaluation of different enrichment methods for pathogenic *Yersinia* species detection by real time PCR. *BMC Vet. Res.* **2014**, *10*, 192.
226. Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying Down Uniform Practical Arrangements for the Performance of Official Controls on Products of Animal Origin Intended for Human Consumption in Accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No 2074/2005 as Regards Official Controls. *Off. J. Eur. Union* **2004**, *L131*, 51.
227. Commission Notice on the Implementation of Food Safety Management Systems Covering Good Hygiene Practices and Proce- dures Based on the HACCP Principles, Including the Facilitation/Flexibility of the Implementation in Certain Food Businesses 2022/C 355/01—C/2022/5307. *Off. J. Eur. Union* **2002**, *C355*, 1–58.
228. Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the Hygiene of Foodstuffs. *Off. J. Eur. Union* **2004**, *L139*, 1.
229. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 Laying Down the General Principles and Requirements of Food Law, Establishing the European Food Safety Authority and Laying Down Procedures in Matters of Food Safety. *Off. J. Eur. Union* **2002**, *L131*, 1–24.
230. Bandick, N.; Hensel, A. Zoonotic diseases and direct marketing of game meat: Aspects of consumer safety in

Germany. In *Game Meat Hygiene in Focus: Microbiology, Epidemiology, Risk Analysis and Quality Assurance*; Paulsen, P., Bauer, A., Vodnansky, M., Winkelmayer, R., Smulders, F.J.M., Eds.; Wageningen Academic Publishers: Wageningen, The Netherlands, 2011; pp. 93–100.

231. Winkelmayer, R.; Stangl, P.-V.; Paulsen, P. Assurance of food safety along the game meat production chain: Inspection of meat from wild game and education of official veterinarians and "trained persons" in Austria. In *Game Meat Hygiene in Focus: Microbiology, Epidemiology, Risk Analysis and Quality Assurance*; Paulsen, P., Bauer, A., Vodnansky, M., Winkelmayer, R., Smulders, F.J.M., Eds.; Wageningen Academic Publishers: Wageningen, The Netherlands, 2011; pp. 245–258.
232. Citterio, C.V.; Bragagna, P.; Novelli, E.; Giaccone, V. Approaches to game hygine in the province Belluno (Italy): From training to meat microbiology. In *Game Meat Hygiene in focus: Microbiology, Epidemiology, Risk Analysis and Quality Assurance*; Paulsen, P., Bauer, A., Vodnansky, M., Winkelmayer, R., Smulders, F.J.M., Eds.; Wageningen Academic Publishers: Wageningen, The Netherlands, 2011; pp. 267–270.

Revalorization of agrifood industry by-products: natural extracts as a sustainable strategy to enhance food safety

Rossana Roila[1], Raffaella Branciari[1], Sara Primavilla[2], Caterina Altissimi[1], Luana Perioli[3], Andrea Valiani[2], Cinzia Pagano[3], Gianluca Veneziani [4], David Ranucci[1]

[1] Department of Veterinary Medicine, University of Perugia, via San Costanzo 4, 06126 Perugia, Italy
[2] Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", via Salvemini 1,
06126 Perugia, Italy.
[3] Department of Pharmaceutical Sciences, University of Perugia, Via del Liceo 1, 06123 Perugia, Italy.
[4] Department of Agricultural, Food and Environmental Sciences, University of Perugia, Via S. Costanzo,
06126 Perugia, Italy

Atti XXXIII Congresso Nazionale CIRIAF, *Sviluppo Sostenibile, Tutela dell'Ambiente e della Salute Umana*. Perugia, 13-14 aprile 2023, Università degli Studi di Perugia.

Abstract

Recently, there has been a growing interest in the use of natural antimicrobial agents able to limit the growth of food-borne bacteria, and therefore enhance food safety. The aim of this work was to investigate the antibacterial properties of different natural extracts from different natural matrices and by-products such as saffron petals, olive mill wastewater, spent coffee grounds, *M. oleifera, G. simplicifolia.*

The antibacterial activity was assessed determining the MICs and MBCs against two microorganisms crucially involved in food safety: *S. aureus* and *E. coli*. The results suggest that olive mill wastewater, *M. oleifera* and saffron petals extracts could represent a valuable natural alternative to conventional preservatives largely adopted in food production. Further studies are needed to define the most suitable applications in the food industry.

1. *Introduction*

The growth of undesired microbial population such as pathogenic and spoilage microorganisms affects the safety, the quality and the shelf life of food products impacting on consumer health and thus on the economic and public health service. Particularly, according to the European Center for Disease Control -European Food Safety Authority report on zoonoses, during 2019–2020, 8261 food- borne outbreaks occurred leading to 69,480 cases of illness, 5534 hospitalizations and 94 deaths [1, 2]. Moreover, microbial food spoilage is an issue of global concern as it means the original nutritional value, texture and flavour of the food are damaged and therefore it becomes undesirable or unacceptable for human consumption due to changes in sensory characteristics. It has been estimated that not less than 25% of all food produced is lost in post-harvest or post-slaughter phases due to undesired microbial activity [3].

Chemical antimicrobials have widely been employed at an industrial level to inhibit the microbial development in foods in order to improve its safety and shelf-life [4]. The scientific community and food industry have recently been urged to look for viable alternatives to the chemical antibacterial frequently employed in food preservation as a result of a greater understanding of the effects of diet on human health [5]. The

use of these compounds indeed, although strictly regulated [6], is considered with mistrust by consumers, because of the potential long-term harm to their health they could cause [7, 8]. However, the worries about the safety of synthetic preservatives led the food industry to search for new additives from natural sources.

In this perspective, vegetable extracts are worthy of investigation as rich in bioactive molecules, such as polyphenols, terpenes, aldehydes and flavonoids as alternatives to synthetic preservatives. More recently high interest has been also oriented towards agri-food by-products that can still provide a high amount of bioactive compounds, which could be exploited in several productive sectors, including food industries. The circular economy model in the agri-food sector, with particular attention to the reuse and valorization of wastes and by-products, is particularly relevant for the scientific community and this good practice is in continuous evolution [9]. Europe plays a leading role in this research field, thanks to the primary involvement of the Member States, policy makers and stakeholders [9]. An improved science based understanding of the effects of natural derived bioactive compounds on the growth and activity of spoilage microorganisms in foods is crucial for the development of safe and eco-friendly preservation strategies and subsequent improvement of food hygiene and reduction of losses due to spoilage. This study aims to report an overview of the preliminary results obtained through the *in vitro* antimicrobial testing of different natural and by- products extracts on two microorganisms crucially involved in food safety namely *S. aureus* and *E. coli*. The extracts considered in this study were obtained from: saffron petals, olive mill wastewater, spent coffee grounds, *M. oleifera, G. simplicifolia.*

2. *Materials and Methods*

2.1 Extracts

Saffron petals are a rich source of bioactive compounds such as crocins and safranal, which have been shown to possess significant antimicrobial activity against a range of pathogenic microorganisms. Olive mill wastewater contains high levels of phenolic compounds such as hydroxytyrosol and oleuropein, which have been found to inhibit the growth of a variety of microorganisms. Coffee grounds contain various compounds such as caffeine, chlorogenic acid, and trigonelline, which have shown potential as natural preservatives against spoilage bacteria. *M. oleifera*, commonly known as the horseradish tree, contains several bioactive compounds such as niazimicin and quercetin, which have been found to possess antimicrobial activity against pathogenic microorganisms. *G. simplicifolia*, also known as the African wild mango, contains a compound called Irvingia gabonensis, which has been shown to have antimicrobial properties against various bacteria and fungi.

2.2 Microorganisms

Reference strains *Staphylococcus aureus* (WDCM 00034) and *Escherichia coli* (WDCM 00013) were bought from Microbiologics, St. Cloud, MN, USA.

2.3 Broth microdilution method

Considering the results of the agar well diffusion test, to quantitatively determine the antibacterial activity of phenolic extract the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. MICs/MBCs were measured using a standard broth microdilution technique, according to Clinical

Laboratory Standards Institute (CLSI) guidelines [33]. The bacterial suspensions used for the assay were prepared adjusting the number of bacteria to 10^5 CFU/mL with fresh Mueller-Hinton broth (Biolife Italiana s.r.l., Milan, Italy). Aliquots of each suspension were added to 96-well microplates (Starlab International GmbH, Hamburg, Germany) containing the same volumes of two-fold serial dilution of the extracts. Moreover, three controls were set up: these included antibiotic control (with benzylpenicillin sodium salt; Sigma-Aldrich, St. Louis, MO, USA), organism control (with culture medium and bacterial suspension) and negative control (with culture broth and the extract solution at the same concentration tested). The plates were incubated for 24 h at 37 °C under aerobic conditions. MIC was defined as the lowest concentration of extract that produced no bacterial growth when compared to time 0 wells. The MBC was determined by subculturing the broths used for MIC determination. A quantity of 10 µL of broths culture of the wells, corresponding to the MIC and to the higher MIC concentrations, was plated onto fresh 5% Sheep Blood agar dishes (Microbiol s.r.l., Cagliari, Italy) and then incubated for 24 h at 37 °C, under aerobic conditions. The MBC was represented as the smallest amount of extract that was capable of killing the bacterial inoculum, demonstrated by the total absence of growth.

3. *Results*

As shown in Table 1 olive mill wastewater is the extract that shows higher antimicrobial efficacy towards the two microorganisms tested. *M. oleifera* showed an even lower MIC and MBC values albei only against *S.aureus c*orroborating the outcome reported by other authors in literature according to which gram positive bacteria can be more sensitive to polyphenolic compounds [10]. Saffron petals extract, like

olive mill wastewater, shows its effects against both bacteria but at much higher MIC and MBC values. Concerning spent coffee grounds and *G. simplicifolia* the results show that ant the tested concentrations this two extract did not show any antibacterial activity.

Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of different extracts against *S.aureus* and *E.coli*.

	Staphylococcus aureus		*Escherichia coli*	
Extract	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Olive mill wastewater	15.6	31.3	15.6	15.6
Saffron petals	250	250	250	250
M. oleifera	6.25	12.5	-	-
Spent coffee grounds	-	-	-	-
G. simplicifolia	-	-	-	-

4. *Conclusion*

These preliminary results reported in this study suggest that olive mill wastewater and *M. oleifera* extracts have the potential to be exploited in food industry for their antimicrobial activity. Despite the higher MIC/MBC values, saffron petals extract could represents valuable source of active compounds, to be used as alternatives to conventional preservatives. It is crucial to consider the green and eco- friendly nature of the tested extracts that confer to this approach high sustainability value. Future studies are needed to evaluate possible applications in the food industry, aiming to improve the quality of foodstuff and to protect consumers' health.

References

1. European Food Safety Authority and European Centre for Disease Prevention and Control. The European Union One Health 2019 Zoonoses Report. *EFSA J.* **2021**, 19, 6406.
2. European Food Safety Authority and European Centre for Disease Prevention and Control. The European Union One Health 2020 Zoonoses Report. *EFSA J.* **2021**, 19, 6971.
3. Abbas, K. A., Saleh, A. M., Mohamed, A., & Lasekan, O. The relationship between water activity and fish spoilage during cold storage: A review. *J. Food Agric. Environ*, **2009**, 7(3/4), 86-90.
4. Tropea, A. Microbial Contamination and Public Health: An Overview. *Int. J. Environ. Res. Public. Health*, **2022**, 19, 7441
5. Antolak, H.; Czyzowska, A.; Kregiel, D. Antibacterial and Antiadhesive Activities of Extracts fromEdible Plants against Soft Drink Spoilage by Asaia spp. *J. Food. Prot*. **2017**, 80, 25–34.
6. EC (European Community). Commission regulation (EC) No. 1333/2008: On food additives. *Off.J.* **2008**, 354, 16.
7. Etienne, J.; Chirico, S.; McEntaggart, K.; Papoutsis, S.; Millstone, E. EU Insights–Consumer perceptions of emerging risks in the food chain. *EFSA Support. Publ*. **2018**, 15, 1394.
8. Roila, R.; Sordini, B.; Esposto, S.; Ranucci, D.; Primavilla, S.; Valiani, A.; Taticchi, A.; Branciari, R.; Servili, M. Effect of the Application of a Green Preservative Strategy on Minced Meat Products: Antimicrobial Efficacy of Olive Mill Wastewater Polyphenolic Extract in Improving Beef Burger Shelf-Life. *Foods,* **2022**, 11, 2447.
9. Chiaraluce, G., Bentivoglio, D., & Finco, A. Circular economy for a sustainable agri-food supply chain: A review for current

trends and future pathways. *Sustainability*, **2021**, 13(16), 9294.

10. Álvarez-Martínez, F. J., Barrajón-Catalán, E., Encinar, J. A., Rodríguez-Díaz, J. C., & Micol, V. Antimicrobial capacity of plant polyphenols against gram-positive bacteria: A comprehensive review. *Current medicinal chemistry*, **2020**, 27(15), 2576-2606.

Measuring the antimicrobial activity of natural extracts against food spoilage bacteria to enhance food hygiene: preliminary *in vitro* results

Rossana Roila[1], Sara Primavilla[2], David Ranucci[1], Roberta Galarini[2], Michela Codini[3], Danilo Giusepponi[2], Caterina Altissimi[1], Andrea Valiani[2], Patrizia Casagrande-Proietti[1], Raffaella Branciari[1],

[1] Department of Veterinary Medicine, University of Perugia, via San Costanzo 4, 06126 Perugia, Italy

[2] Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", via Salvemini 1, 06126 Perugia, Italy

[3] Department of Pharmaceutical Sciences, University of Perugia, Via del Liceo 1, 06123 Perugia, Italy

Acta Imeko, DOI: 10.21014/actaimeko.v13i2.1669

Abstract

Food products are prone to microbial contamination able to affect their safety and quality attributes and their nutritional value. The interest in the potential use of bioactive compounds deriving from natural matrices, especially agro-industrial wastes, as alternatives to classical food preservatives has rapidly increased. In the present study a food grade olive mill wastewater polyphenolic extract and a commercial mix were characterised and their antioxidant and antimicrobial capacity were assessed. The antimicrobial activity was preliminary assessed *in vitro* by agar well diffusion, subsequently by microdilution method to define the minimum inhibitory and bactericidal concentration. The olive mill wastewater polyphenolic extract registered a higher

antioxidant capacity [(13.3 ± 1.0) 102 µgTE/(100 g)] and antimicrobial efficacy (max MBC value 0.2500 g/mL) compared to commercial mix with wide potential application in food industry.

1. *Introduction*

Food products are characterised by high susceptibility to microbial contamination with several potential consequences on safety and quality attributes as well as on the reduction of the nutritional aspects [1]. To counteract this phenomenon, chemical additives with different activities such as antimicrobial and antioxidant, are commonly added to food stuff. Synthetic preservatives, indeed, are widely employed in the industry during food production to decrease or eradicate the unwanted presence of microorganisms and so extend the shelf life of food products [2], [3]. Despite the fact that food additives are strictly regulated (Regulation (EC) No.1333/2008, s.m.i.) [4], consumers are sceptical of chemical substances that are purposefully added to foodstuff due to the possibility of long-term negative effects on human health [5].

Recently, however, the interest in bioactive substances derived from natural matrices, particularly agro-industrial wastes, as substitutes for conventional food preservatives has remarkably increased. These substances may represent a novel approach to prevent Foodborne illnesses and limiting food waste[6]-[8].

Fruit and vegetable processing does produce a high amount of waste, but reusing those wastes in the food business could help to solve the environmental, economic and social issues [6], [9]. Also, since these bioactive chemicals have the capability to limit the growth of microorganisms that cause food spoiling, their use in food manufacturing may have a favourable impact on food safety [7]. To

effectively contain microbial contamination along the food production chain, for instance, the use of natural antibacterial agents defines a valuable and sustainable alternative method [10], [11]. For instance, olive oil by-products can be exploited as a source of bioactive molecules that might be suitable for improving food hygiene [1], [12]. Olive oil by-products are characterised by several hydrophilic phenols, particularly represented by secoiridoids, whose precence has been revealed only for plants belonging to *Oleaceae* family, that seem to be able to inhibit the growth of several Gram-positive and Gram-negative bacteria and to express high antioxidant properties as well [12]. The recovery of the high-value bioactive compounds from the olive mill wastewater could enable the possible exploitation of this agro-industrial waste, enhancing the economic and environmental sustainability of the agro-industrial sector, especially considering its high generation rate (49 % of total mass) [1], [11]. In order to protect public health and reduce the significant economic and social effects of food waste, it is crucial that competent authorities and the scientific community continue to focus on ensuring food hygiene and safety for consumers [1].

The present study aimed to compare the antibacterial activity of a commercial mix used as an ingredient in meat product formulations with a food grade polyphenolic extract from olive mill wastewater. To determine the antibacterial activity the agar well diffusion was preliminarily applied. Then the microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The study targeted spoilage microorganisms strongly related to food hygiene.

2. Material and Methods

2.1 Extracts

The crude olive mill wastewater phenolic extract (PE) used was obtained by means of a membrane filtration process using fresh olive mill wastewaters. To obtain a stable powder formulation, the extract was subjected to spray drying after their combination with maltodextrins as carrier matrix (1:1dw) [13].

The beetroot commercial mix (CM) is represented by a fine powder intended to be added to hamburger meat batter functioning as stabilizing agent (MecImport GroupSrl, Perugia, Italy).

2.2 Polyphenols determination by LC-QTOF

Two aliquots of the extract were collected and diluted 50 and 500-fold, respectively, with a mixture of acetic acid 0.025 %/methanol 90/10 (v/v). After filtration, both aliquots were injected. Liquid-Chromatography Quadrupole Time-Of Flight spectrometry (LC-QTOF) was applied to determine twelve polyphenols. The equipment consisted of an ExionLC™ coupled to a 6600+TripleTOF™ (ABSciex, Foster, CA, USA) equipped with an electrospray ionization source operating in negative mode (ESI-). Chromatographic separation was carried out on an Acquity BEH C18 (150 mm × 2.1 mm, 1.7 µm, Waters, Milford, MA, USA).

Water with 0.025 % aceticacid (A) and methanol/ACN 90/10 v/v % (B) were used as mobile phases. The gradient started with 0 % of B (1min); the percentage of B was increased to 20 % in 10 min, followed by an increase to 50 % B in 4 min and another one to 100 % in 1 min. After 4 min, B percentage was reported to initial conditions (0 %) in 1 min. Finally, the system was re-equilibrated for 5 min (run time: 26 min). The column temperature was set at 40 °C and the autosampler

temperature was kept at 25 °C. Flow rate and injection volume were 0.25 mL/min and 10 µL, respectively. Compressed air was used as GS1 (55 arbitrary units) and GS2 (55 arbitrary units), whereas nitrogen was the curtain gas (40 arbitrary units). The spray voltage was set at −4.5 kV and interface source temperature at 450°C. Single infusions of each analyte were carried out to optimize declustering potential (DP) and collision energy (CE). Theprecursor ([M-H+]) and fragmentions acquired in MRMHR mode are listed in Table 1. Mass error was ≤ 5 ppm.

Table 1. Analyte Retention Times and monitored ions.

Analyte	RT (min)	Molecular formula	Precursor (m/z)	Fragment (m/z)	DP (V)	CE (V)
Hydroxytyrosol	9.2	$C_8H_{10}O_3$	153.0557	123.0455	-80	-14
Hydroxytyrosol-D4	9.2	$C_8H_6D_4O_3$	157.0808	125.0588	-80	-15
Tyrosol	11.9	$C_8H_{10}O_2$	137.0608	119.0520	-90	-18
Vanillic acid	13.3	$C_8H_8O_4$	167.0350	152.0111	-70	-15
Vanillin	14.9	$C_8H_8O_3$	151.0401	136.0166	-60	-14
p-Coumaric acid	15.4	$C_9H_8O_3$	163.0401	119.0500	-60	-14
Verbascoside	16.4	$C_{29}H_{36}O_{15}$	623.1981	161.0251	-90	-38
Oleuropein	17.3	$C_{25}H_{32}O_{13}$	539.1770	307.0824	-100	-27
Pinoresinol	17.4	$C_{20}H_{22}O_6$	357.1344	151.0410	-80	-20
Luteolin	17.5	$C_{15}H_{10}O_6$	285.0405	133.0293	-110	-36
Oleuropeinaglygone	17.6	$C_{19}H_{22}O_8$	377.1242	307.0824	-80	-14
Apigenin	17.7	$C_{15}H_{10}O_5$	269.0456	117.0343	-110	-35

2.3 Antioxidant capacity of extracts

The antioxidant capacity was evaluated using the Oxygen Radical Absorbance Capacity method (ORACFL). To do this, one gram of each extract was mixed separately with a buffer solution with a pH of 7.2, containing 13.19 g of K_2HPO_4 and 10.26 g of KH_2PO_4 dissolved in 900 mL of deionized water. This mixture was homogenised using an

Ultra-Turrax homogenizer (Ultra Turrax T25 Basic, IKA Labortechnik Janke & Kunkel GmbH, Stavfen, Germany) for one minute, followed by two minutes of vortexing. After homogenization, the samples were centrifuged at 6000 rpm for 20 minutes at a temperature of 4°C. The resulting supernatant was then used to determine the antioxidant capacity through the ORACFL method. The ORACFL method measures antioxidant capacity by comparing it to a reference standard, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, obtained from Sigma-Aldrich, Steinheim, Germany). This comparison is based on the rate of fluorescence decay of a probe when exposed to a radical oxygen species (ROO). The ORACFL assays were carried out through a FLUO-star OPTIMA microplate fluorescence reader (BMGLABTECH, Offenburg, Germany) with an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The results are reported as micrograms of Trolox equivalents (TE) per 100 g of the sample.

2.4 In vitro measurement of antibacterial activity – Agar well diffusion

Extracts' antibacterial activity was determined by the agar well-diffusion method against different food spoilage bacteria. In particular *Escherichia coli*, *Pseudomonas fluorescens, Pseudomonas aeruginosa, Lactobacillus plantarum subsp. plantarum*, *Lactobacillus sakei subsp. sakei, Lactococcus lactis* strains were bought from Microbiologics, St.Cloud, MN, USA, while *Shewanella putrefaciens* and *Brochothrix thermosphacta* derived from Istituto Zooprofilattico Sperimentale dell' Umbria and Marche "Togo Rosati" (IZSUM) collection isolated from meat samples.

For each organism, a suspension with a turbidity of 0.5 McFarland in a 0.9 % sterile saline solution was prepared. Subsequently, 100 µL of this suspension were evenly spread onto each quadrant of Mueller-Hinton agar (MHA) or Mueller-Hinton agar supplemented with 5 % defibrinated sheep blood (MHAB) plates (manufactured by Oxoid Limited, Basingstoke, UK) using a swab [1]. Circular holes with a diameter of 7 mm were created in the agar plates by removing a portion of the medium with a sterilized cork borer. Subsequently, 50 µL of an extract solution in sterile demineralised water (with a concentration of 1000 mg/mL) were introduced into these holes. The plates were then incubated under conditions suitable for the growth of each specific bacterial strain. After the designated incubation period, the presence and size of the inhibition zone were assessed using a measuring gauge in millimeters [14], [15].

2.5 Minimum Inihibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determination

The investigation into the antibacterial properties of the extracts was extended through the determination of MICs/MBCs for the specific microorganisms of interest. MICs/MBCs were assessed using the standard broth microdilution method, following the guidelines established by the Clinical Laboratory Standards Institute (CLSI) [16]. To carry out this procedure, bacterial suspensions were prepared, adjusting the bacterial count to 105 CFU/mL by diluting them in fresh Mueller-Hinton broth with 5 % blood (supplied by Biolife Italiana s.r.l., Milan, Italy). Aliquots of each suspension were added to 96-well microplates (manufactured by Starlab International GmbH, Hamburg, Germany) containing equal volumes of two-fold serial dilutions of the extract, ranging from concentrations of 0.5 to 0.0039 g/mL. Three

control groups were established: an antibiotic control (using benzylpenicillin sodium salt from Sigma-Aldrich, St. Louis, MO, USA), an organism control (comprising culture medium and bacterial suspension), and a negative control. Subsequently, the plates were incubated under the best growth conditions of each bacterial strain. The MIC was defined as the lowest concentration of the extract that exhibited no bacterial growth when compared to the wells at the start of the incubation period [17]. The MBC was determined by transferring samples from the broths utilised for MIC determination onto culture media.

2.6 Statystical analysis

The data obtained from the agar well-diffusion test underwent statistical analysis using an analysis of variance (ANOVA) model as implemented in SAS software (SAS Institute Inc., Cary, NC, USA, 2001). In order to elucidate any noteworthy differences in means (with a significance level of $p < 0.05$), Tukey's *post-hoc* analysis was applied.

3. Results and Discussion

3.1 Polyphenols determination by LC-QTOF

Table 1 reports the Analyte Retention Times and monitored ions assessed by means of LC-QTOF. The chemical composition of this olive mill wastewater extract is in line with that of other extracts obtained from similar products already reported in the literature [17]-[19].

The most representative bioactive compounds belong to the class of phenols. Specifically, hydroxytyrosol (3,4-DHPEA) and tyrosol (p-HPEA) are phenolic alcohols, verbascoside is a hydroxycinnamic acid’s derivative, the caffeic acid, the p-coumaric acid, and the vanillic

acid are phenolic acids and derivatives. The luteolin is a flavone and the pinoresinol a lignans, verbascoside is a secoiridoid.

Due to their properties, these phenolic compounds can be adopted by the pharmaceutical sector as well as in cosmetics and medicine and as nutraceutical products and antioxidants in foods [20], [21]. The specific content of the major phenolic compounds in olive mill wastewater spray dry extract was 13.0±1.0, 2.2±0.3 and 0.59±0.01 mg/g for hydroxytyrosol, tyrosol, and vanillic acid, respectively. Notably, hydroxytyrosol and tyrosol are known to have numerous biological activities, proved both *in vitro* and *in vivo* [22].

3.2 Evaluation of antioxidant capacity of extracts

A higher antioxidant activity was found in the PE in comparison with CM containing ascorbic acid and beetroot (532 ± 4) and (13.3 ± 0.1) 102 µgTE/100g in CMandPE, respectively). The high antioxidant activity registered in the PE is in accordance with previous studies that refer to the powerful antioxidant activity of olive phenolic compounds [21], [23].

Oxidation has been demonstrated as the main non-microbial cause of food quality deterioration. For instance, oxidative deterioration is capable of limiting food acceptability and shortening its shelf-life by causing discoloration, the development of off-flavours and the formation of toxic compounds. Recently, great interest has been addressed to natural antioxidants that can be used as technological strategies applying antioxidants directly into food products or by coating packaging materials with natural extracts to improve the oxidative stability of the products, therefore avoiding or reducing the use of chemical compounds [23]. Another approach is represented by the dietary manipulations in which antioxidant compounds, or their

metabolites, are introduced into the food (milk, muscle or egg) via feed [24].

3.3 Measurements of the in vitro antibacterial activity – Agar well diffusion

PE and CM were evaluated qualitatively and quantitatively for their in vitro antibacterial activity against the chosen bacteria based on the presence or absence of inhibition zones (Figure 1).

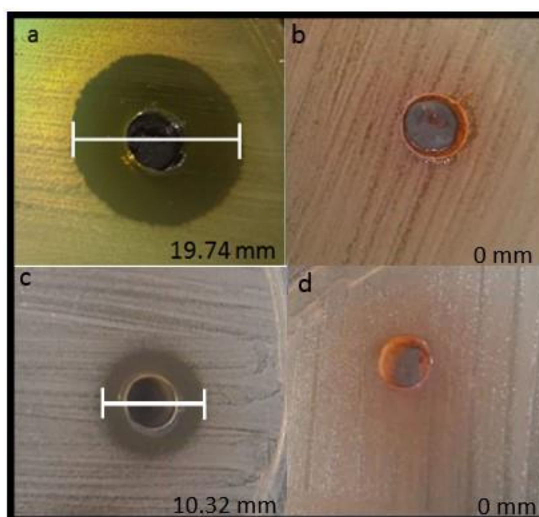


Figure 1. Example of inhibition halos obtained on Mueller-Hinton agar in the screening test for *B. Termosphacta* exposed to crude olive mill wastewater phenolic extract (a) and commercial beetroot mix (b) *L. Plantarum* exposed to crude olive mill waste-water phenolic extract (c) and commercial beetroot mix (d).

A preliminary assessment utilized the agar well-diffusion technique to conduct a screening test. Table 2 displays the measurement of inhibition zones (in millimeters) for each microorganism. It demonstrates that the

polyphenolic extract displays antimicrobial activity against all the tested microorganisms. As shown in Table 2 at the highest extract concentration (1 g/mL), the greater effect was registered for *P. fluorescens* (halo of 23.80 mm), while the lowest was registered for *L. Sakei* with a halo of 8.15 mm ($p < 0.05$).

Totally absent antibacterial activity was observed, instead, for beetroot commercial extract against Gram-negative and positive bacteria targeted in the study as no inhibition halos were measured (Figure 1 and Table 2).

Table 2. Inhibition halos obtained for PE and CM for the different strains tested. Values are expressed as means ± standard deviation.

Olive mill wastewater extract (PE)					Positive Control
Concentration	**1 g/mL**	**0.5 g/mL**	**0.25g/mL**	**0.13g/mL**	**Tetracycline 30 µg/disc**
E. coli	17.7 ± 0.6	13.0 ± 0.7	9.0 ± 1	-	33.2 ± 0.5
S. putrefaciens	16 ± 1.0	8.7 ± 0.6	-	-	20.6 ± 0.6
P. fluorescens	24.0 ± 1.0	17.8 ± 0.5	14.0 ± 1	11.5 ± 0.2	34.7 ± 0.1
P. aeruginosa	16.5 ± 0.3	12.4 ± 0.5	8.2 ± 0.1	-	30.0 ± 1.0
B.termosphacta	19.7 ± 0.6	15.0 ± 0.0	11.6 ± 0.6	-	28.0 ± 1.0
L. plantarum	10.3 ± 0.6	-	-	-	20.2 ± 0.6
L. sakei	8.1 ± 0.5	-	-	-	19.0 ± 3.0
L. lactis	19.0 ± 1.0	10.0 ± 1.0	-	-	26.1 ± 0.4
Commercial mix (CM)					
E. coli	-	-	-	-	
S. putrefaciens	-	-	-	-	
P. fluorescens	-	-	-	-	
P. aeruginosa	-	-	-	-	
B.termosphacta	-	-	-	-	
L. plantarum	-	-	-	-	
L. sakei	-	-	-	-	
L. lactis	-	-	-	-	

3.4 *Minimum Inihibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determination measured on PE and CM extract*

The obtained results, reported in Table 3, highlight MIC/MBC values for PE ranging from 0.0156 g/ml to 0.2500 g/ml suggesting an antibacterial activity against the assayed strains, that seem to be exerted more efficaciously towards Gram-positive bacteria. These preliminary results confirm the outcome of previous studies testing the antimicrobial activity of different microorganisms relevant in food production where, on average, Gram-positive bacteria show lower MIC/MBC [25]. Furthermore, as expected, the CM extract didn't show relevant antimicrobial activity at the concentrations tested in the study. In particular, attention has been oriented towards the microbial food spoiling process, in fact, despite chill chains, chemical preservatives, and a more in-depth understanding of microbial food spoilage, it has been estimated that 25 % of all foods produced globally is lost *post harvest* or *post slaughter* due to microbial spoilage [26].

Consequently, both consumers and producers of food products are looking for natural ingredients and efficient formulation strategies to improve the shelf life of final products [27]. It is important to consider, however, that the efficacy of a natural extract against microbial growth depends not only on its chemical composition and the extraction technique, but it is also strongly related to the specific sensitivity of microbial species and further studies are needed to better explore this aspect [1], [19].

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the olive mill wastewater extract (PE) and of commercial mix (CM).

	PE (mg/mL)		**CM (mg/mL)**	
	MIC	MBC	MIC	MBC
E. coli	15.6	15.6	> 500	> 500
S. putrefaciens	15.6	15.6	> 500	> 500
P. fluorescens	15.6	31.3	> 500	> 500
P. aeruginosa	15.6	31.3	> 500	> 500
B. termosphacta	15.6	15.6	> 500	> 500
L. plantarum	125	250	> 500	> 500
L. sakei	62.5	62.5	> 500	> 500
L. lactis	62.5	125	> 500	> 500

4. *Conclusions*

The results demonstrate the *in vitro* efficacy of the tested polyphenolic extract against the growth of both Gram positive and negative undesired spoilage microorganisms, defining preliminary threshold values for future application on food models. Further studies are needed to address the main challenges in the use of natural antimicrobial such as its low stability, adverse effects on sensory properties, low solubility, high needed doses.

References

1. S. Primavilla, C. Pagano, R. Roila, R. Branciari, D. Ranucci, A. Valiani, L. Perioli, Antibacterial Activity of Crocus sativus L. Petals Extracts against Foodborne Pathogenic and Spoilage Microorganisms, with a Special Focus on Clostridia, Life, 13 (2023) 1, 60. DOI: 10.3390/life13010060
2. R. Roila, B. Sordini, S. Esposto, D. Ranucci, S. Primavilla, A. Valiani, A. Taticchi, R. Branciari, M. Servili, Effect of the Application of a Green Preservative Strategy on Minced Meat Products: Antimicrobial Efficacy of Olive Mill Wastewater Polyphenolic Extract in Improving Beef Burger Shelf-Life, Foods,11 (2022) 16, 2447. DOI:10.3390/foods11162447
3. A. Tropea, Microbial Contamination and Public Health: An Overview, Int. J. Environ. Res. Public. Health, 19 (2022), 7441. DOI: 10.3390/ijerph19127441
4. EC (European Community). Commission regulation (EC) No.1333/2008: On food additives. Off. J.2008,354,16.
5. J. Etienne, S. Chirico, K. McEntaggart, S. Papoutsis, E. Millstone, EU Insights–Consumer perceptions of emerging risks in the food chain. EFSA Support. Publ.,15 (2018),1394. DOI:10.2903/sp.efsa.2018.EN-1394
6. L. Martínez, P. Bastida, J. Castillo, G. Ros, G. Nieto, Green Alternatives to Synthetic Antioxidants, Antimicrobials, Nitrates, and Nitrites in Clean Label Spanish Chorizo. Antioxidants,8 (2019),184. DOI:10.3390/antiox8060184
7. R. Gyawali, S. A. Ibrahim, Natural Products As Antimicrobial Agents. FoodControl, 46 (2014),412. DOI: 10.1016/j.foodcont.2014.05.047

8. P. S. Negi, Plant Extracts For The Control Of Bacterial Growth: Efficacy, Stability And Safety Issues For Food Application. Int. J. Food Microbiol.,156 (2012),7. DOI: 10.1016/j.ijfoodmicro.2012.03.006
9. H. Kowalska, K. Czajkowska, J. Cichowska, A. Lenart, What's New In Biopotential Of Fruit And Vegetable By-Products Applied In The Food Processing Industry. Trends Food Sci.Tech., 67 (2017),150. DOI: 10.1016/j.tifs.2017.06.016
10. J. Jiang, Y. L. Xiong, Natural Antioxidants As Food And Feed Additives To Promote Health Benefits And Quality Of Meat Products: A Review. MeatSci.,120, (2016), 107. DOI: 10.1016/j.meatsci.2016.04.005
11. L. A. Gonçalves, J. M. Lorenzo, M. A. Trindade, Fruit and Agro-Industrial Waste Extracts as Potential Antimicrobials in Meat Products: A Brief Review. Foods, 10, (2021),1469. DOI: 10.3390/foods10071469
12. M. Servili, B. Sordini, S. Esposto, S. Urbani, G. Veneziani, I. D. Maio, R. Selvaggini, A. Taticchi, Biological Activities Of Phenolic Compounds Of Extra Virgin Olive Oil. Antioxidants, 3, (2013) 1. DOI: 10.3390/antiox3010001
13. I. Zbicinski, A. Delag, C. Strumillo, J. Adamiec, Advanced Experimental Analysis Of Drying Kinetics In Spray Drying. Chem. Eng. J.2002,86,207–216. DOI: 10.1016/S1385-8947(01)00291-1
14. M. Balouiri, M. Sadiki, S. K. Ibnsouda, Methods For In Vitro Evaluating Antimicrobial Activity: A Review. J. Pharm. Anal., 6, (2016), 71. DOI: 10.1016/j.jpha.2015.11.005
15. C. Pagano, M. R. Ceccarini, P. Calarco, S. Scuota, C. Conte, S. Primavilla, , M. Ricci, L. Perioli, Bioadhesive Polymeric Films

Based On Usnic Acid For Burn Wound Treatment: Antibacterial And Cytotoxicity Studies, Colloids and Surfaces B: Biointerfaces, 178, (2019), 488. DOI: 10.1016/j.colsurfb.2019.03.001

16. Clinical and Laboratory Standards Institute. Methods for Determining Bactericidal Activity of Antimicrobial Agents; Approved Guideline; CLSI Document M26-A; CLSI: Wayne, PA,USA, 1998.
17. R. Roila, R. Branciari, D. Ranucci, R. Ortenzi, S. Urbani, M. Servili, A. Valiani, Antimicrobial Activity Of Olive Mill Waste Water Extract Against *Pseudomonas Fluorescens* Isolated From Mozzarella Cheese. Ital. J. Food.Saf.,5 (2016),111. DOI: 10.4081/ijfs.2016.5760
18. G. La Scalia, R. Micale, L. Cannizzaro, F. P. Marra, A Sustainable Phenolic Compound Extraction System From Olive Oil Mill Wastewater. J Clean Prod, 142 (2017), 3782. DOI: 10.1016/j.jclepro.2016.10.086
19. M. Aggoun, R. Arhab, A. Cornu, J. Portelli, M. Barkat, B. Graulet, Olive Mill Wastewater Microconstituents Composition According To Olive Variety And Extraction Process. FoodChem 209 (2016), 72. DOI: 10.1016/j.foodchem.2016.04.034
20. A. De Toffoli, E. Monteleone, G. Bucalossi, G. Veneziani, G. Fia, M. Servili, C. Dinnella, Sensory And Chemical Profile Of A Phenolic Extract From Olive Mill Waste Waters In Plant-Base Food With Varied Macro-Composition. Food ResInt, 119 (2019), 236. DOI: 10.1016/j.foodres.2019.02.005
21. R. Branciari, R. Galarini, D. Giusepponi, M. Trabalza-Marinucci, C. Forte, R. Roila, D. Miraglia, M. Servili, G. Acuti,

A. Valiani, Oxidative Status And Presence Of Bioactive Compounds In Meat From Chickens Fed Polyphenols Extracted From Olive Oil Industry Waste. Sustainability, (2017), 1566. DOI: 10.3390/su9091566

22. M. Servili, B. Sordini, S. Esposto, S. Urbani, G. Veneziani, I.D. Maio, R. Selvaggini, A. Taticchi, Biological Activities Of Phenolic Compounds Of Extra Virgin Olive Oil. Antioxidants, 3 (2013), 1. DOI: 10.3390/antiox3010001
23. E. M. Insani, A. Eyherabide, G. Grigioni, A. M. Sancho, N. A. Pensel, A. M. Descalzo, Oxidative Stability And Its Relationship With Natural Antioxidants During Refrigerated Retail Display Of Beef Produced In Argentina. MeatSci, 79 (2008), 444. DOI: 10.1016/j.meatsci.2007.10.017
24. A. Dal Bosco, E. Mourvaki, R. Cardinali, M. Servili, B. Sebastiani, S. Ruggeri, C. Castellini, Effect Of Dietary Supple Mentation With Olive Pomaces On The Performance And Meat Quality Of Growing Rabbits. MeatSci, 92 (2012), 783. DOI: 10.1016/j.meatsci.2012.07.001
25. L. Fasolato, B. Cardazzo, S. Balzan, L. Carraro, A. Taticchi, F. Montemurro, E. Novelli, Minimum Bactericidal Concentration Of Phenols Extracted From Oil Vegetation Water On Spoilers, Starters and food-Borne Bacteria. ItalJ of Food Saf, 4, (2015). DOI: 10.4081/ijfs.2015.4519
26. L. Gram, L. Ravn, M. Rasch, J. B. Bruhn, A. B. Christensen, M. Givskov, Food Spoilage—Interactions Between Food Spoilage Bacteria. Int JFood Microbiol, 78 (2002), 79. DOI: 10.1016/S0168-1605(02)00233-7
27. S. N. Jamali, E. Assadpour, J. Feng, S. M. Jafari, Natural Antimicrobial-Loaded Nano Emulsions For The Control Of

Food Spoilage/Pathogenic Microorganisms. Adv Colloid InterfaceSci, 295 (2021), 102504. DOI: 10.1016/j.cis.2021.102504

Surface carcass treatment with olive mill wastewater polyphenolic extract against *Salmonella* Enteritidis and *Listeria monocytogenes*: *in vitro* and *in situ* assessment

Caterina Altissimi[1], Rossana Roila[1], Sara Primavilla[2], Raffaella Branciari[1], Andrea Valiani[2], David Ranucci[1]

[1] Department of Veterinary Medicine, University of Perugia, via San Costanzo 4, 06126 Perugia, Italy
[2] Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", via Salvemini 1, 06126 Perugia, Italy

Italian Journal of Food Safety, DOI:10.4081/ijfs.2024.12403

Abstract
In recent years, there has been an increased interest in substances that could inhibit or reduce microbial growth in food products. Olive oil industry by-products, due to bioactive compounds with potential antimicrobial properties such as polyphenols, could be used in carcass treatment to enhance hygienic and quality traits. The assessment of the antimicrobial efficacy of bioactive molecules against pathogens should be determined with *in vitro* and *in situ* models since it is not possible to evaluate it directly on carcasses at the slaughterhouse. This study aimed to evaluate the effect of an olive mill wastewater polyphenolic extract against *Salmonella* Enteritidis and *Listeria monocytogenes*, simulating carcass surfaces using bovine dermis samples that were experimentally contaminated with the selected pathogens. The minimum inhibitory concentration and minimum bactericidal concentration were first determined for *S*. Enteritidis and *L. monocytogenes*. *In situ,* bactericidal

activity assessment was performed using 20 cm2 derma samples contaminated with 5 Log CFU/20 cm2 of *S.* Enteritidis and *L. monocytogenes* in separate trials. Treatment with the polyphenolic extract was not effective for either microorganism. In order to establish the bacteriostatic activity of the polyphenolic extract, suspensions of about 2 Log CFU/20 cm2 of *S*. Enteritidis and *L. monocytogenes* were used. Polyphenolic extract treatment was not effective against *Salmonella*, while for *Listeria* it allowed microbial growth to delay (around 1 Log CFU/cm2 difference at 3, 7, and 14 days between treated and con- trol groups). Further investigations are needed to evaluate the application of polyphenolic compounds on carcass surfaces and their effects on sensory traits.

1. Introduction

Regarding meat safety, the European Union promotes a preventive approach based on the “Hazard Analysis and Critical Control Points” (HACCP) system and good hygiene practices to avoid potential contamination and microbial growth (European Parliament and Council of the European Union, 2004a). Food business operators are not permitted to use any substance other than potable water to remove surface contamination from products of animal origin unless specifically authorized for that purpose (European Parliament and Council of the European Union, 2004b). Currently, only lactic acid can be used to reduce microbial surface contamination on bovine carcasses (European Commission, 2013). Regardless, its use should not be considered a substitute for good hygienic practices during slaughtering procedures (EFSA, 2011). Several authors have highlighted the potential of various substances and compounds to limit microbial development (Dakheli, 2020; Gonzalez-Fandos *et al*., 2020; Han *et al*.,

2020; Roila *et al*., 2022). Olive oil industry by-products are regarded as a source of bioactive molecules such as polyphenols that, due to their antimicrobial and antioxidant capacity (Foti *et al*., 2021), might be used to treat carcasses to enhance hygienic and quality traits.

Furthermore, it is crucial to evaluate the effectiveness of these compounds not only against hygiene parameters but also against pathogenic microorganisms. Friedman *et al*. (2013) investigated the effect of ten nutraceutical powders derived from the food industry, including olive pomace and olive juice powder, against four major foodborne pathogens (*Salmonella enterica*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus*) using a quantitative bactericidal activity assay, highlighting the possible use of these substances due to their inhibitory activity. Tomalok *et al.* (2022) pointed out the potential use of organic acids such as lactic, acetic, and glycolic acids in swine slaughterhouses due to the reduction of *S. enterica* serotype Choleraesuis and *L. monocytogenes* artificially inoculated in pork jowl fat. The assessment of the antimicrobial effect of bioactive compounds against pathogens cannot be carried out directly on carcasses at the slaughterhouse through their experimental contamination, and therefore *in vitro* and *in situ* models must be used.

This work aimed to evaluate, through an *in situ* model consisting of portions of bovine dermis experimentally contaminated with *S.* Enteritidis and *L. monocytogenes*, the bactericidal and bacteriostatic efficacy of a polyphenolic extract from olive mill wastewaters.

2. *Materials and Methods*

Olive mill wastewater polyphenolic extract

The polyphenolic extract used in this study is a commercial extract derived from olive oil mill wastewater. It originates from pressed olive

pulps (*Olea europaea* L.), and the final product is obtained in powder form through freeze-drying and pulverization processes. The extract consists of 15.7 mg/g total polyphenols, of which hydroxytyrosol (12.9 mg/g), tyrosol (2.22 mg/g), and vanil- lic acid (0.59 mg/g) are the main compounds.

Minimum inhibitory concentration and minimum bactericidal concentration

The evaluation of the antibacterial activity of the olive mill wastewater polyphenol extract was assessed by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) on *S. enterica* subsp. *enterica* serovar Enteritidis and *L. monocytogenes*, using both ATCC strains (*S*. Enteritidis WDCM 00030 and *L. monocytogenes* WDCM 00021) and a *S.* Enteritidis wild strain isolated from food samples. MICs/MBCs were deter- mined using a standard broth microdilution method (Clinical and Laboratory Standards Institute, 1999) as in the procedure described by Primavilla *et al*. (2022), properly modified.

The MIC was defined as the compound's lowest concentration at which no visible bacterial growth occurred. The broths used for MIC determination were subcultured to determine the MBC, as the lowest concentration of extract resulted in a reduction of 99.9% of the bacterial inoculum. MIC/MBC values of the most resistant bac- terial strain were used as a reference to establish the concentration of extract to be applied in the *in situ* tests.

Sampling preparation

Fresh bovine skin was obtained from a local slaughterhouse, and samples of about 20 cm2 (4×5 cm) were collected. For each sample,

the surface layer was removed, and derma samples were exposed to UV light under a UV cabinet for 2 hours (1 hour for each side). UV treatment efficacy was determined through aerobic colony count using Plate Count Agar (Biolife Italiana, Milano, Italy) incubated at 30°C for 72 hours.

In situ assessment of bactericidal activity

Bovine skin dermis samples were placed into sterile petri dish- es and experimentally contaminated with *Salmonella enterica* subsp. *enterica* serovar Enteritidis WDCM 00030 (Figure 1).

A bacterial suspension of 0.5 McFarland (108 CFU/mL) in 0.9% sterile saline solution was prepared, and serial decimal dilu- tions were performed to achieve a final concentration of 5 Log CFU/mL. The suspension was inoculated on the surface of each sample in an amount as to obtain a final concentration of about 5 Log CFU/20 cm2, evenly distributed through a disposable L-shape spreader and left for 30 minutes under aseptic conditions to allow the attachment of bacteria. Samples were then divided into two experimental groups: a group treated with the polyphenolic extract and a control group. A solution containing 0.25 g/mL of olive mill wastewater polyphenol extract was prepared and 0.5 mL was inoc- ulated on the surface of the samples, while for the control group sterile demineralized water was used. Polyphenolic extract solution and water were both evenly distributed over the respective samples with disposable L-shape spreaders and left for 30 minutes under aseptic conditions. Samples were then stored at 4°C and ana- lyzed after 0, 3, 6, 12, and 24 hours from the treatment. The experiment was repeated three times and performed in triplicate. The same procedure was adopted to assess the bactericidal activity of the polyphenolic ectract towards *L. monocytogenes* WDCM 0021.

In situ assessment of bacteriostatic activity

The evaluation of bacteriostatic activity was conducted similarly to that of bactericidal activity. In this case, dermis samples were artificially contaminated with a suspension of *S.* Enteritidis to obtain a final concentration of about 2 Log CFU/20 cm2. A solution consisting of 0.125 g/mL of olive mill wastewater polyphenolic extract was set up, and 0.5 mL was inoculated onto the sample sur- face. Samples were stored at 4°C, and microbiological analyses were determined after 0, 3, 7, 14, and 21 days from the treatment. The experiment was repeated three times and performed in triplicate. The same methodology was used with *L. monocytogenes* WDCM 0021.

Microbiological analyses

Salmonella spp. enumeration was performed on Chromogenic Salmonella Agar Base with Salmonella selective supplement (Biolife Italiana, Milano, Italy) and incubated at 37°C for 18-24 hours, while *L. monocytogenes* enumeration was determined according to the ISO 11290-2:2017 (ISO, 2017) procedure, using Agar *Listeria* according to Ottoviani and Agosti (ALOA, Biolife Italiana, Milano, Italy) with ALOA Enrichment Supplement and ALOA selective supplement and incubated at 37°C for 24-48 hours. Counting results were reported in terms of Log CFU/cm^2.

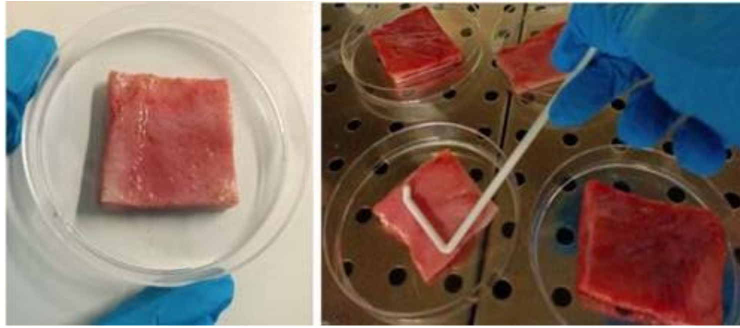


Figure 1. Bovine dermis samples

Statistical analysis

Data were analyzed using the general linear model procedure of SAS (SAS Institute, Inc., Cary, NC, USA, 2001) by an analysis of variance (ANOVA), with treatment and time as fixed effects. Tukey's test was used to identify the differences in the means and was deemed significant at $p<0.05$. The growth parameters of the two pathogens considered were evaluated using the predictive microbiology software Combase (https://browser.combase.cc/DMFit.aspx, accessed on June 2023) with the DMFit tool, by which parameters such as lag phase duration and maximum growth rate were determined using Baranyi and Roberts (1994) model. A one-way ANOVA model with treatment as a fixed effect and Tukey's test ($p<0.05$) were used to analyze the fitted data.

3. *Results*

Determinations of minimum inhibitory concentra- tion and minimum bactericidal concentration

MIC and MBC results are shown in Table 1. *Salmonella* strain isolated from food samples (wild strain) was more resistant compared to ATCC strains.

Table 1. Minimum inhibitory concentration and minimum bactericidal concentration against *Salmonella* Enteritidis and *Listeria monocytogenes*.

Microorganism	**MIC (mg/mL)**	**MBC (mg/mL)**
Salmonella Enteritidis WDCM 0030	15.6	31.3
Salmonella Enteritidis (*Wild strain*)	62.5	125
Listeria monocytogenes WDCM 0021	15.6	15.6

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

Evaluation of the bactericidal activity

Results from the *in situ* bactericidal assessment showed no bactericidal action of the polyphenolic extract against the two pathogens considered in this study. The initial microbial load of *S*. Enteritidis was 4.07 Log CFU/cm^2 and the final load at 24 hours was 3.68 Log CFU/cm^2 for the control group and 3.98 Log CFU/cm^2 for the treated group, with treatment and time factors not statistically significant ($p>0.05$). Similarly, *L. monocytogenes* had an initial microbial concentration of 3.76 Log CFU/cm^2 and final values at 24 hours of 4.17 Log CFU/cm^2 and 3.59 Log CFU/cm^2 for the control and treated groups, respectively, with treatment and time factors not statistically significant ($p>0.05$).

Evaluation of the bacteriostatic activity

Similar to the assessment of bactericidal activity, the polyphenolic extract did not determine a bacteriostatic effect against *S*. Enteritidis. The starting microbial load was 1.22 Log CFU/cm^2 and the final values at 21 days were 1.29 Log CFU/cm^2 and 1.46 Log CFU/cm^2 for the

control and treated groups, respectively, with no significant differences ($p>0.05$).

Regarding *L. monocytogenes*, the outcomes of the polyphenolic extract's bacteriostatic efficacy are illustrated in Figure 2 and *Supplementary Table 1*.

Statistical analysis showed significant differences between treated and control groups at 3, 7 and 14 days after treatment. More specifically, a difference of 1.19 Log CFU/cm^2, 1.25 Log CFU/cm^2 and 1.31 Log CFU/cm^2 was observed between the groups at T1, T2, and T3 days from treatment, respectively. Furthermore, the polyphenolic extract application allowed a delay in microbial growth, maintaining the same microbial load at both T0 and T1 day from treatment ($p<0.05$). No significant difference was reported between the groups at T4.

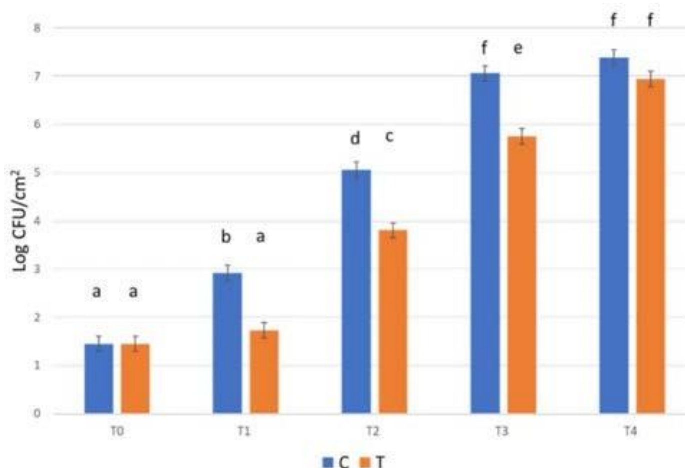


Figure 2. Effect of olive mill wastewater polyphenolic extract application against *Listeria monocytogenes* (initial inoculums 2 Log CFU/20 cm^2). C, control; T, treated with polyphenolic extract; T0, T1, T2, T3, T4, 0, 3, 7, 14, 21 days from treatment, respectively. Different letters indicate statistically different mean values ($p<0.05$)

Data regarding *L. monocytogenes* were analyzed with the ComBase software, and the resultant estimated growth curves are presented in Figure 3. The DMFit tool was used to calculate the growth parameters, reported in Table 2. Results showed a significant difference in the lag phase (ʎ), which was longer in treated samples compared to the control group. Over time, the growth curves tend to approximate the same microbial load, although a statistically significant difference was obtained between the final values.

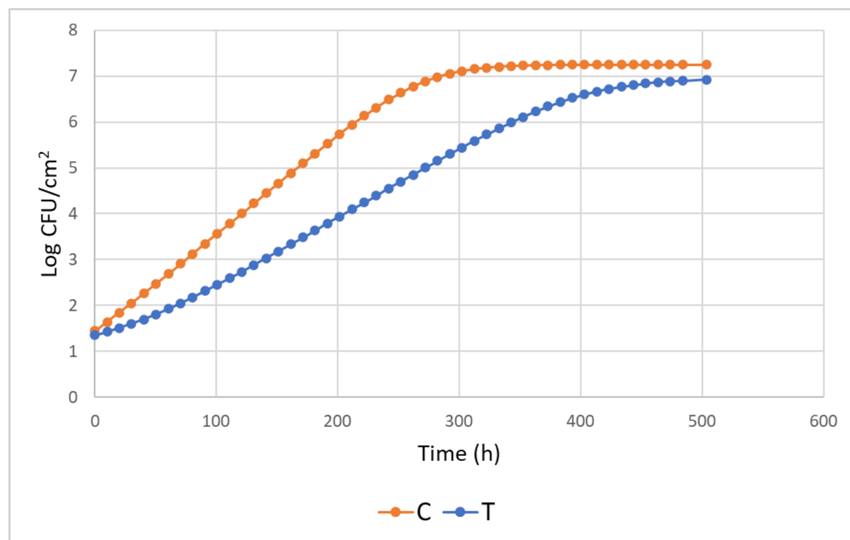


Figure 3. Estimated growth curves of Listeria monocytogenes using the DMFit tool. C, control; T, treated with polyphenolic extract.

Table 2. Output parameters estimated by the DMFit program for *Listeria monocytogenes* growth rate (initial inoculums 2 Log CFU/ 20cm^2).

	C	T
Initial value (Log CFU/cm^2)	1.48 ± 0.22	1.39 ± 0.17
ʎ (h)	9.70 ± 15.03^a	55.57 ± 26.65^b
μ_max (Log CFU/cm^2/h)	0.0230 ± 0.0046	0.0216 ± 0.0108
Final value (Log CFU/cm^2)	7.26 ± 0.25^b	6.81 ± 0.27^a
R^2	0.995 ± 0.002	0.974 ± 0.003
SE of fit	0.184 ± 0.023	0.370 ± 0.021

C, control; T, treated with polyphenolic extract; μ _max, specific maximum growth rate; ʎ, lag phase; R^2, adjusted R-square statistics of the fit; SE, standard error of fit. Different letters in the same row denote significant differences ($p<0.05$).

4. *Discussion*

Results regarding *in vitro* antibacterial activity against *Salmonella* are in line with those achieved by Liu *et al*. (2017) using an olive leaf extract, while, concerning *L. monocytogenes*, the same authors reported higher values compared to the present study. Guo *et al.* (2019) obtained a MIC of 1.25 mg/mL of an olive oil polyphenol extract against *L. monocytogenes* and demonstrated the ability of the extract to inhibit microbial growth by reducing intracellular adenosine triphosphate, cell membrane depolarization, protein and DNA decrease, and cell fluid leakage due to cell morphology destruction.

Regarding *Salmonella*, our findings differ from those of Guo *et al*. (2020), who reported MIC of 0.625 mg/mL of an olive oil polyphenol

extract, although it is not feasible to compare MIC values of different extracts with diverse phenolic compounds and concentrations. Even though the polyphenolic extract used in this study demonstrated a bactericidal and bacteriostatic effect *in vitro* towards the two pathogens considered, this has not been confirmed *in situ*.

Regarding *S.* Enteritidis, the polyphenolic extract did not exert any bactericidal or bacteriostatic effect *in situ*. Different authors report an *in vitro* bactericidal and bacteriostatic effect of polyphenols from olive mill vegetation water or leaves against *Salmonella*, much higher than other foodborne pathogens, including *Listeria* (Fasolato *et al*., 2015; Liu *et al*., 2017). Furthermore, *Salmonella* is growing slowly under refrigeration conditions (4°C) in meat (Pradhan *et al.*, 2012), not allowing a full evaluation of bacteriostatic effects. Nonetheless, a bacteriostatic effect against *S. enterica* was reported for activated coating with tyrosol on sliced tofu, experimentally contaminated and stored at 10°C, mainly when other antimicrobial substances were added (bacteriocins and benzoic acid) (Viedma *et al.*, 2016).

Concerning *L. monocytogenes*, the polyphenolic extract did not allow the death of the microorganism; however, bacteriostatic activity was detected. As a matter of fact, the olive mill wastewater extract seems to slow down the growth of *L. monocytogenes,* especially in the early stage of growth. The polyphenolic extract concentration used in this study enabled the delay of microbial growth by about 1 Log CFU/cm^2 up to 14 days from treatment. Furthermore, after 21 days from treatment, the treated group achieved the same *Listeria* concentration that the control group reached after 14 days.

This study confirmed both *in vitro* and *in situ* that Gram-positive bacteria are more sensitive to polyphenolic compounds com- pared to

Gram-negative bacteria, as widely highlighted in the literature (Seow *et al*., 2014; Fasolato *et al*., 2015; Oulahal and Degraeve, 2022).
Several compounds, such as weak acids, phenols, and essential oils, have been studied to evaluate their ability to reduce the microbial load of carcasses (Dakheli, 2020; Gonzalez-Fandos *et al*., 2020; Sallam *et al*., 2020; Kannan *et al*., 2021; Roila *et al*., 2022). Due to their potential bacteriostatic capacity to slow down and delay the growth of *L. monocytogenes*, olive mill wastewater polyphenolic extracts might be applied to carcasses as a preventive approach to reduce surface contamination and microbial growth. The use of bioactive compounds such as polyphenols may be considered a valid strategy to prevent microbial growth on carcasses and could be considered in the HACCP system and critical control point definition. Furthermore, the use of these substances to prevent possible microbial development is part of the perspective endorsed by the European Union due to their bacteriostatic action, which therefore does not disregard the use of good hygienic slaughtering practices and a proper starting hygienic level of carcasses.

5. *Conclusions*

Olive mill wastewater polyphenolic extract revealed a bacteriostatic ability against L. monocytogenes by limiting microbial growth over time under refrigerating conditions. These findings point out the possible application of polyphenolic extract to delay carcass surface contamination. In addition, the use of natural extracts derived from food industry by-products represents a sustainable approach and allows the valorization of waste materials.
In this study, the polyphenolic extract treatment was ineffective regarding S. Enteritidis, either a bacteriostatic or bactericidal effect.

Nevertheless, further studies are needed to investigate the efficacy of this by-product against these pathogens, considering different concentrations of polyphenols. However, studies regarding the application of polyphenolic extracts to carcass surfaces are needed to assess their effects on quality and sensory traits.

References

Baranyi J, Roberts TA, 1994. A dynamic approach to predicting bacterial growth in food. Int J Food Microbiol 23:277-94.

Clinical and Laboratory Standards Institute, 1999. M26-A. Methods for determining bactericidal activity of antimicrobial agents; approved guideline. Available from: https://clsi.org/media/1462/m26a_sample.pdf.

Dakheli MJ, 2020. Effects of grape and pomegranate waste extracts on poultry carcasses microbial, chemical, and sensory attributes in slaughterhouse. Food Sci Nutr 8:5622-30.

EFSA, 2011. Scientific Opinion on the evaluation of the safety and efficacy of lactic acid for the removal of microbial surface contamination of beef carcasses, cuts and trimmings. EFSA J 9:2317.

European Commission, 2013. Regulations of the Commission Regulation (EU) of 4 February 2013 concerning the use of lactic acid to reduce microbiological surface contamination on bovine carcasses, 101/2013. In: Official Journal, L 34/1, 5/2/2013.

European Parliament, Council of the European Union, 2004a. Regulation of the European Parliament and of the Council of 29 April 2004 laying down on the hygiene of foodstuffs, 852/2004/CE. In: Official Journal, L 139/1, 30/04/2004.

European Parliament, Council of the European Union, 2004b. Regulation of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs, 853/2004/CE. In: Official Journal, L 139/55, 30/04/2004.

Fasolato L, Cardazzo B, Balzan S, Carraro L, Taticchi A, Montemurro F, Novelli E, 2015. Minimum bactericidal con- centration of phenols extracted from oil vegetation water on spoilers, starters and food-borne bacteria. Ital J Food Saf 4:4519.

Foti P, Romeo FV, Russo N, Pino A, Vaccalluzzo A, Caggia C, Randazzo CL, 2021. Olive mill wastewater as renewable raw materials to generate high added-value ingredients for agro-food industries. Appl Sci 11:7511.

Friedman M, Henika PR, Levin CE, 2013. Bactericidal activities of health-promoting, food-derived powders against the food-borne pathogens Escherichia coli, Listeria monocytogenes, Salmonella enterica, and Staphylococcus aureus. J Food Sci 78:M270-5.

Gonzalez-Fandos E, Martinez-Laorden A, Perez-Arnedo I, 2020. Effect of decontamination treatments on Campylobacter jejuni in chicken. Foods 9:1453.

Guo L, Gong S, Wang Y, Sun Q, Duo K, Fei P, 2020. Antibacterial activity of olive oil polyphenol extract against Salmonella Typhimurium and Staphylococcus aureus: possible mechanisms. Foodborne Pathog Dis 17:396-403.

Guo L, Sun Q, Gong S, Bi X, Jiang W, Xue W, Fei P, 2019. Antimicrobial activity and action approach of the olive oil polyphenol extract against Listeria monocytogenes. Front Microbiol 10:1586.

Han J, Luo X, Zhang Y, Zhu L, Mao Y, Dong P, Yang X, Liang R, Hopkins DL, Zhang Y, 2020. Effects of spraying lactic acid and peroxyacetic acid on the bacterial decontamination and bacterial composition of beef carcasses. Meat Sci 164:108104.

ISO, 2017. Microbiology of the food chain. Horizontal method

for the detection and enumeration of Listeria monocytogenes and of Listeria spp. – Part 2: enumeration method. ISO Norm 11290-2:2017. International Standardization Organization ed., Geneva, Switzerland.

Kannan G, Mahapatra AK, Degala HL, 2021. Preharvest manage- ment and postharvest intervention strategies to reduce Escherichia coli contamination in goat meat: a review. Animals 11:2943.

Liu Y, McKeever LC, Malik NSA, 2017. Assessment of the antimicrobial activity of olive leaf extract against foodborne bacterial pathogens. Front Microbiol 8-113.

Oulahal N, Degraeve P, 2022. Phenolic-rich plant extracts with antimicrobial activity: an alternative to food preservatives and biocides?. Front Microbiol 12:753518.

Pradhan AK, Li M, Li Y, Kelso LC, Costello TA, Johnson MG, 2012. A modified Weibull model for growth and survival of Listeria innocua and Salmonella Typhimurium in chicken breasts during refrigerated and frozen storage. Poultry Sci 91:1482-8.

Primavilla S, Pagano C, Roila R, Branciari R, Ranucci D, Valiani A, Ricci M, Perioli L, 2022. Antibacterial activity of Crocus sativus l. petals extracts against foodborne pathogenic and spoilage microorganisms, with a special focus on Clostridia. Life 13:60.

Roila R, Altissimi C, Branciari R, Primavilla S, Valiani A, Cambiotti F, Cardinali L, Cioffi A, Ranucci D, 2022. Effects of spray application of lactic acid solution and aromatic vinegar on the microbial loads of wild boar carcasses obtained under optimal harvest conditions. Appl Sci 12:10419.

Sallam KI, Abd-Elghany SM, Hussein MA, Imre K, Morar A, Morshdy AE, Sayed-Ahmed MZ, 2020. Microbial decontami- nation of

beef carcass surfaces by lactic acid, acetic acid, and trisodium phosphate sprays. BioMed Res Int 2020:2324358.
Seow YX, Yeo CR, Chung HL, Yuk H-G, 2014. Plant essential oils as active antimicrobial agents. Crit Rev Food Sci Nutr 54:625- 44.
Tomalok C de CG, Wlodarkievicz ME, Puton BMS, Colet R, Zeni J, Steffens C, Backes GT, Cansian RL, 2022. Organic acids as an alternative method to control Salmonella enterica serotype Choleraesuis and Listeria monocytogenes in pork jowl fat. J Food Saf 42:e12999.
Viedma PM, Burgos MJ., Pulido RP, Soriano B, Gálvez A, Lucas R, 2016. Inhibition of Salmonella enterica and Listeria mono-cytogenes in tofu by activated plastic films. IJRSMB 2:25-9.

Effects of spray application of lactic acid solution and aromatic vinegar on the microbial loads of wild boar carcasses obtained under optimal harvest conditions

Rossana Roila[1], Caterina Altissimi[1], Raffaella Branciari[1], Sara Primavilla[2], Andrea Valiani[2], Fausto Cambiotti[3], Lorenzo Cardinali[1], Attilia Cioffi[4], and David Ranucci[1]

[1] Department of Veterinary Medicine, University of Perugia, via San Costanzo 4, 06126 Perugia, Italy

[2] Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", via Salvemini 1, 06126 Perugia, Italy

[3] Health Department Umbria 1-Alto Chiascio, Via Cavour 38, 06024 Gubbio, Italy

[4] Health Department Umbria 1-Perugino, Via XIV Settembre 79, 06121 Perugia, Italy

Applied Sciences, DOI: 10.3390/app122010419

Abstract

Solutions of lactic acid 2% and aromatic vinegar were investigated for microbial load reduction on the surfaces of wild boar carcasses. The animals were hunted and processed according to production specifications to obtain the best hygiene for carcasses. The solutions were sprayed on carcass surfaces after skinning, and sites of 5 × 5 cm were sampled 2 h and 48 h post-treatment, with the carcasses under refrigeration conditions. The values of the microbial loads were lower for lactic-acid-treated sites, compared with control, after both 2 and 48 h. Nonetheless, the differences in the microbial loads were only higher

than 1 Log/CFU 25 cm^2 for the aerobic colony count. The aromatic vinegar resulted in lower values than in controls only after 48 h for the aerobic colony count, *Staphylococcus* count, and *Lactobacillus* count, with values always below 1 Log/CFU 25 cm^2. The implementation of a lactic acid solution could represent a valuable strategy to reduce microbial growth on wild boar carcasses, thus becoming a relevant critical control point in this peculiar and niche meat production process.

1. *Introduction*

The European Union policy on meat safety is based on the implementation of good hygienic practices and the HACCP system to prevent the contamination of meat and, therefore, microbial growth. According to EC regulation, indeed, if the procedures during slaughtering are correctly adopted, the hygienic level of the carcasses would be high, and there would be no need for decontamination strategies [1].

It is clear that the EU approach, reflected in the regulatory framework, is based on the prevention of meat contamination limiting further treatments. EC Regulation 853/2004 states that food business operators (FBOs) shall not use any substance other than potable water on food surfaces and, therefore, also on carcasses [1]. Nonetheless, the European legislation establishes that post-slaughtering treatments with substances other than water could be performed only after EU Commission's specific approval. The use of an approved substance shall not affect the FBOs' duty to comply with hygienic production requirements [1].

In this context, the EFSA indicates that weak acid solutions could be considered for carcass treatment after slaughter without safety issues for consumers [2,3]. The opinions about lactic acid solutions

specifically referred to bovine [2], pigs [4], and recently also to goat, sheep, kangaroo, and wild pigs carcasses and meat [5]. The EFSA also recommends that FBOs should validate the antimicrobial efficacy of such treatments under their specific processing conditions [6].

Only the use of 2–5% of lactic acid solution sprayed on bovine carcasses was approved by the Commission, following the EFSA opinion, and is now admitted in Europe [6]. This treatment is not permitted if it causes irreversible physical modification of the meat as well as in carcasses with visible fecal contamination. The lactic acid treatment could be, therefore, considered in the HACCP system as a relevant CCP to be monitored. The possible use of weak acid solutions in other species is not yet allowed, even if the EFSA provides a positive opinion on the use of peroxyacetic acids in poultry and red meat [3]. There are no indications and recommendations on hunted game meat so far.

Game meat in industrialized countries is considered a niche production of highly valu- able food [7,8]. The interest of consumers in this type of meat is debatable: There are ethical doubts due to hunting practices or meat consumption [9], while there is also increasing awareness of the nutritional quality of game meat and the sustainability that characterizes its production, compared with meat from intensively farmed animals [10–12]. In Europe, the number of some wild species has been rising dramatically in recent decades due to several anthropic and non-anthropic factors, such as the availability of widely abandoned or marginally rural areas; the increase in protected areas lacking in monitoring and managing invasive species; the introduction of both typical and alien species for recreational hunting purposes, and other environmental and animal-related factors [13–17]. The rise in wild populations, in particular large ungulates, is generating impacts on

agronomic, economic, environmental (i.e., biodiversity), and public health (i.e., the spread of infectious disease) aspects. Wild boar is probably the best example of this phenomenon in Europe, and it became a relevant issue to be managed by different proponents (i.e., politicians, hunters, and animal rights activists) [18]. In the Umbria Region, central Italy, the population of wild boar is enormously increased, with more than 70,000 subjects and over 20,000 hunted animals in 2021, over a surface of 8500 km2 [19]. In this region, hunting is the main strategy for population control, but to date, more than 95% of the meat obtained is intended for self-consumption by hunters and/or partially directly sold in small quantities to local restaurants. Both these conditions do not provide the implementation of specific hygienic rules set by EU regulation [1]. This generates poor attention to meat hygiene and quality and a black market for meat without a comprehensive control procedure by local authorities. For this reason, there is a strong interest in creating specific certificated production chains that could increase consumer demand for high-quality meat, give economic incentives to hunters, and guarantee proper controls by official authorities during the pre-harvest and post-harvest phases [7]. These certified chains are fully respondent to EC regulation [1] and are based on specific procedures designed to obtain good hygienic levels of the carcasses. These procedures must be implemented by hunters, in the harvest phase; operators of the collection centers where animals are eviscerated and refrigerated without skinning; and operators of game meat establishments where wild boars are properly dressed and refrigerated. In these certified chains, further improvement to wild boar carcass hygiene could be obtained with the use of weak acid solutions applied by the operators of game meat establishments under the supervision of competent

authorities. No studies are yet available, to the best of our knowledge, on the use of organic acid on wild-hunted ungulate carcasses in general and on wild boar in particular.

The aim of this study is to evaluate, in a real production environment, the effects of spraying selected organic acids solutions on the surfaces of wild boar carcasses. The hypothesis of this study is that the use of a low dose of weak organic acids will improve the hygienic level of wild boar carcasses, without any irreversible physical modification of the carcass surface. To comply with the FBOs' duty in terms of hygienic procedures, the trial was conducted exclusively on the carcasses obtained under best hunting and post-harvest management procedures.

2. *Materials and methods*

2.1 Sampling of the carcasses

Sampling was performed on the external surfaces of wild boar carcasses obtained from animals hunted with the "still hunting" method [20] during the winter of 2020–2021 and 2021–2022 in the Umbria Region (central Italy). The hunted animals were selected according to specific pre-harvest and post-harvest procedures to obtain the best hygienic level of the carcasses. Specifically, the factors considered were animal weight between 45 and 55 kg (non-eviscerated); environmental temperature during hunting below 15 °C; the absence of rainfall during hunting; shot-to-kill position in head or heart, without damaging the intestine and the carcass; the time between killing, bleeding, and evisceration less than 1 h; the time before the refrigeration of the carcasses less than 2 h; refrigeration temperature in the collection center below 7 °C in the deep muscle; and maximum 2 days of refrigerated storage under the skin in the collection center. The carcasses were then transferred under refrigerated conditions to a local

slaughterhouse, which serves as a game meat establishment, for further skinning and storage. A total of 54 carcasses were transferred to the game meat establishment during hunting seasons but only 10 of them (6 male and 4 female) fitted with all the defined and aforementioned factors and were, therefore, taken into consideration. After skinning, for each carcass, 6 sites were defined for microbial surface sampling purposes (the lateral part of the thigh, the flank, and the thorax, on the left and the right sides of the carcasses; Figure 1).

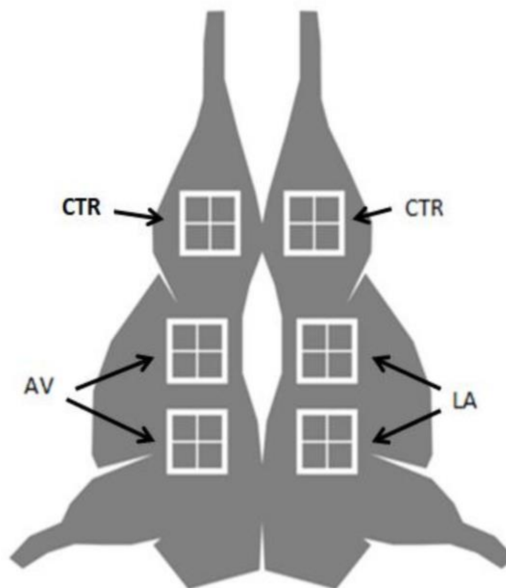


Figure 1. Sampling areas of wild boar external carcass surfaces sprayed with 2% lactic acid solution (LA), aromatic vinegar (AV), or untreated (CTR).

These sampling sites were chosen to avoid different levels of contamination, as preliminary studies proved no difference in their microbial loads (Table S1).

The carcasses were already refrigerated at 7 °C before skinning and maintained at the same temperature until sampling was performed. Two sites (the lateral surface of the tights of both the right and left sides of the carcass) were considered as a control (CTR) and not treated with solutions or sterile water; two sites (the lateral surface of the thorax of the right side of the carcass) were treated with 2 mL of fine sprayed 2% lactic acid solution at 15 ℃ (LA-Todini chemicals, Milan, Italy, pH 2.4); two sites (the lateral surface of the thorax of the left side of the carcass) were treated with 2 mL fine sprayed food grade aromatic vinegar at 15 °C (aromatic vinegar GPI 6.2—Lazzari Equipment and Packaging, Settimo di Pescantina, VR. Italy, characterized by a pH of 6.02, acetic acid 5%, and a total phenolic count of 2.6 mg gallic acid equivalent/mL). Fine spraying was performed using a one-hand sprayer (hand nebulizer ECON Stocker s.r.l., Lana (BZ), Italy—1 bar pressure) at 50 cm from the surface. The number of solutions, as well as the pressure adopted, were not sufficient to define it as a rinse or a wash; for this reason, no sprayed sterile water was considered as a control. Other authors adopted the same approach for control samples when antimicrobial solutions were tested [21].

From each of the sites, 4 sampling areas of 5 × 5 cm were randomly sampled after 2 h (T2), and 4 other sampling areas of the same dimensions were sampled after 48 h (T48). The sampling time was chosen to allow for surface drying after the treatment (T2) and when the carcasses were still available in the game meat establishment (T48), before their transfer to cutting plants. The total number of samples was, therefore, 240 (10 carcasses, 3 treatments, 4 sampling areas of 25 cm^2, 2 sampling times). This sampling protocol was defined in order to have a high number of samples from a limited number of wild boar carcasses obtained in the same optimal conditions, as the consistency of pre-

harvest and harvest conditions represents a general problem in game meat sampling [8,13]. After the first treatments and sampling, the carcasses were kept under refrigerated conditions (7 ± 1 ℃). The samples were obtained with the wet and dry swab method [22], and both swabs, belonging to one sampling site, were put in a vial containing 9 mL of sterile solutions (NaCl 0.9% solution, Oxoid, Basingstoke, UK) and kept under refrigerated conditions (isothermal box) until microbiological determination.

2.2 Microbiological determination

The samples were, therefore, vortexed, andserial decimal dilutions (NaCl 0.9% solution—Oxoid) were performed. The dilutions were used for the following determinations:

- Aerobic colony count (ACC) [23] on plate count agar (PCA-Bio-RAD Laboratories, Marnes-la-Coquette, France) aerobically incubated at 30 ℃ for 48 h;
- Psychotropic colony count (PCC) [24] on PCA (Bio-RAD Laboratories) aerobically incubated at 7 ◦C for 10 days;
- *Enterobacteriaceae* count (EC) [25] on Vilet Red Bile Glucose Agar (VRBGA-Bio-RAD Laboratories) aerobically incubated at 37 ℃ for 24 h;
- *Staphylococcus* spp. count (SC) on Mannitol Salt Agar (MSA-Biolife Italiana s.r.l., Milan, Italy) incubated at 37 ◦C for 48 h;
- *Lactobacillus* spp. count (LABC) on De Man Rogosa Sharp Agar (MRSA-Oxoid) anaero- bically incubated at 30 °C for 48 h;

The colonies were, therefore, counted, and the results were converted into Log colony forming units (CFUs)/25 cm^2. When no colonies were counted in the lower dilution plates, a middle bound level of detection (LOD) approach was used to manage the left censored data, taking into

account the lowest sample dilution (1:10) and the quantity of the sample (1 mL or 0.1 mL) included or spread onto the plates. In particular, for ACC, PCC, and *Enterobacteriaceae* counts, a value of 0.7 Log CFU/25 cm^2 was assigned for non- detectable colonies in the sample, while for *Staphylococcus* and *Lactobacillus* counts, 1.7 Log CFU/25 cm^2 were assigned. Foodborne pathogens were not investigated because no experimental contamination could be performed in the game meat establishment.

2.3 Same-different test analysis

To evaluate if the treatments irreversibly affected the surface characteristics, the same 10 carcasses sampled after 24 h underwent visual examination by 8 trained assessors to confirm if there was a perceivable difference between the treated and untreated surfaces. Each of the 8 judges performed 4 same–different tests over 10 experimental sessions (1 for each carcass) according to the following scheme: A = control, B = LA, or AV. The differences between the samples were recorded considering modification of odor, discoloration, and surface appearance. The same–different test was conducted as follows: Each assessor was asked to analyze a pair of different square surfaces of the carcass and was asked to determine if there was a perceptible similarity or difference with the following sample sequence: <AA>, <BB>, <AB>, and <BA>, in random order. For each pair of samples, the judges were asked to answer the question if the samples were the "same" (S) or "different" (D). The same–different test was separately conducted both for LA and AV treatment versus control.

1.2 Statistical analyses

Data were analyzed using the GLM procedure of SAS [26] to define descriptive statis- tics (mean and standard errors). Furthermore, the effect of treatment and time on the different microbial populations considered was determined with an ANOVA model with treatments (CTR, LA, and AV) and time (T2 and T48) as fixed variables. Post hoc Tukey tests were, therefore, used to compare the least square means, and the significance was set at $p < 0.05$. For the same–different test performed on the carcasses, an χ^2 test was carried out [27]. A type I error of 5% with $\alpha = 0.05$ was chosen. The critical value to define the similarity of the treatment was calculated with chi-squared distribution with a one-tailed test and one degree of freedom (n 1) and fixed to 3.84.

3. Results

The results of the microbial loads (MLs) are presented in Figure 2. Some samples had ML values below the LOD, in particular LA samples (Table 1).

Table 1. Number of samples below the limit of detection for the microbial load considered in treated and untreated surfaces of wild boar carcasses at different storage times.

	CTR		**LA**		**AV**	
Treatments	Negative/Total samples		Negative/Total samples		Negative/Total samples	
	T2	T48	T2	T48	T2	T48
ACC	8/40	5/40	15/40	18/40	5/40	6/40
PCC	15/40	19/40	33/40	29/40	26/40	21/40
EC	27/40	29/40	31/40	35/40	33/40	29/40
SC	15/40	14/40	32/40	35/40	25/40	26/40
LABC	17/40	20/40	29/40	34/40	19/40	29/40

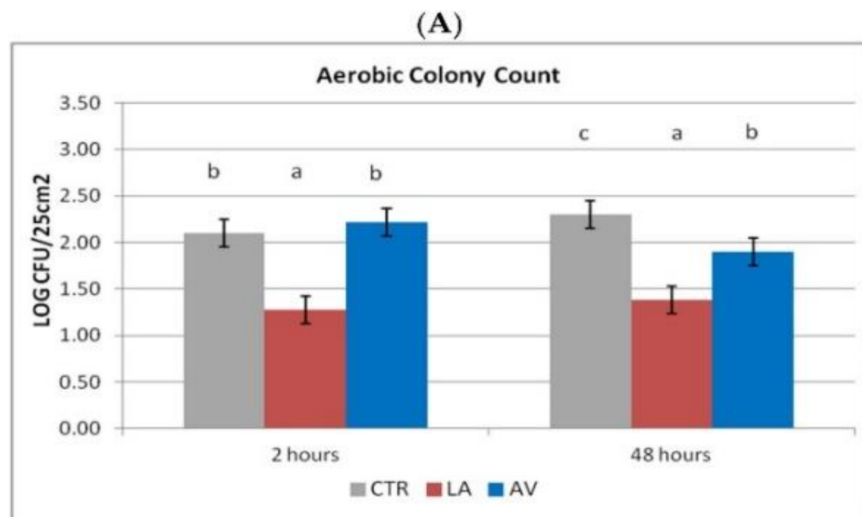
ACC = aerobic colony count; PCC = psychrophilic colony count; EC = Enterobacteriaceae count; SC = Staphylococcus count; LABC = Lactobacillus count; LA = 2% of lactic acid solution; AV = aromatic vinegar; CTR = control samples; T2 = 2 h; T48 = 48 h, SEM = standard error of the mean; negative sample = MLs < LOD.

The ACC counts at T2 reveal lower average values for LA than the other groups, and at T48 the highest values were detected for CTR, followed by AV and LA samples. The PCC values were lower for LA than CTR in both of the times considered, without differences with AV. Regarding EC, the LA group differed from the other two groups both at T2 and T48. Similar results were detected for SC and LABC, with the main difference between CTR and LA groups at T2 and CTR and LA and AV at T48. In general, the treatment effect was always statistically significant, while the time effect was not detected. Only ACC increased in CTR samples during the storage time.

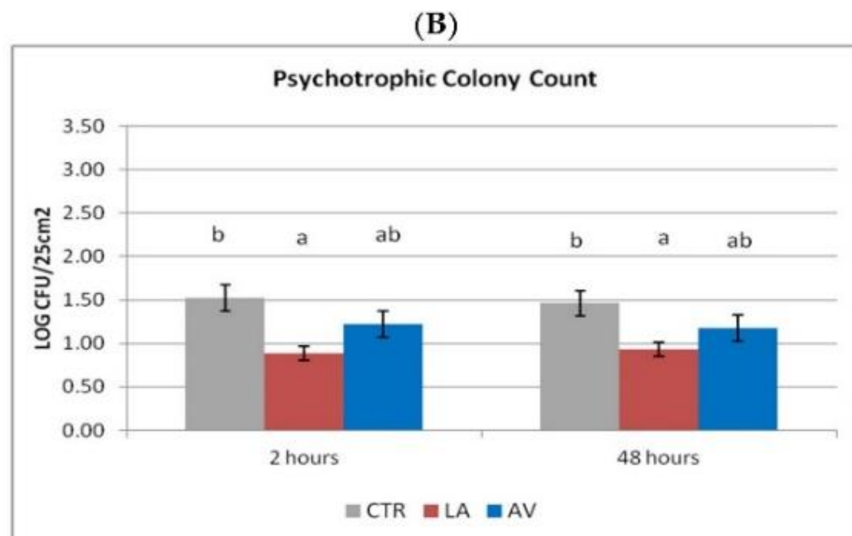
Regarding the results of the same–different test, the calculated χ^2 statistic was below 3.84, indicating that no significant differences between the compared two surfaces (CTR versus AV and CTR versus LA, respectively) were recorded by assessors.

Figure 2. Aerobic colony count (**A**), *Enterobacteriaceae* count (**B**), psychotrophic colony counts (**C**), *Staphylococcus* count (**D**), and *Lactobacillus* count (**E**) on external surfaces of wild boar carcasses sprayed with 2% Lactic acid solution (LA), aromatic vinegar (AV), and untreated (CTR) after 2 and 48 h from the treatments. Different letters on the bars describe statistically different mean values ($p < 0.05$).

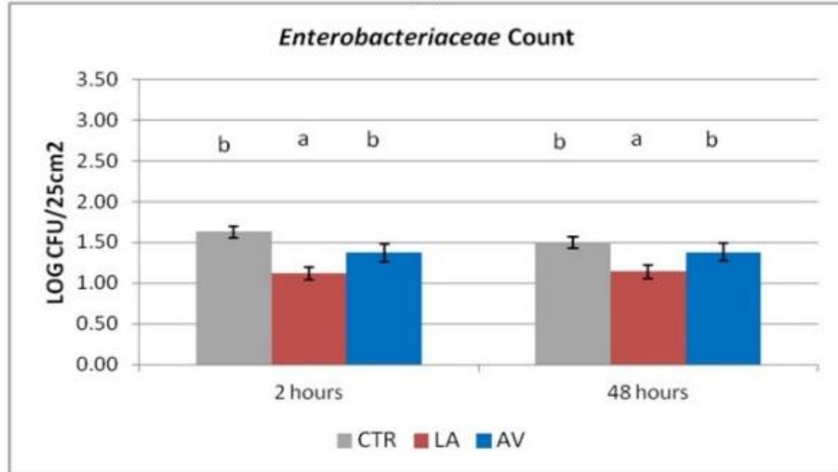
(A)



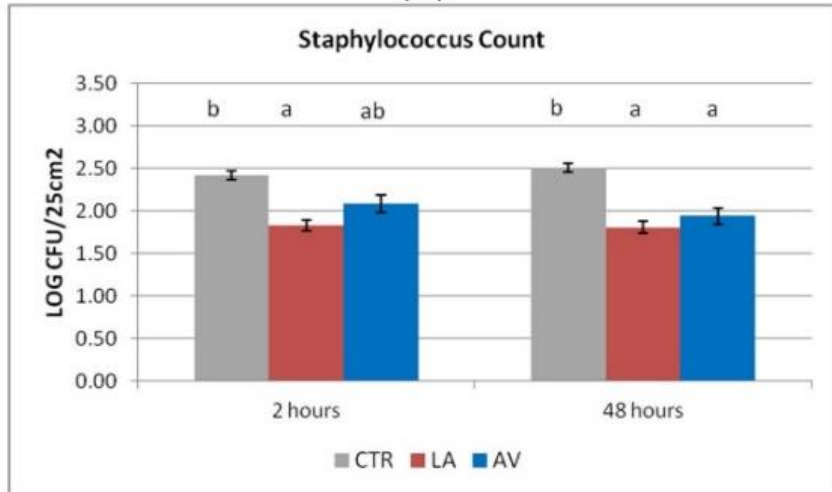
(B)



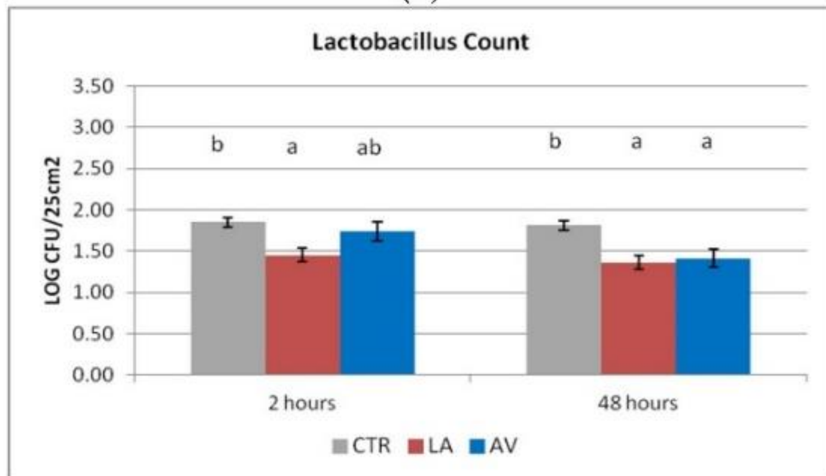
(C)



(D)



(E)



4. *Discussion*

The effect of lactic acid applied on the surfaces of wild boar carcasses was evident on all the microbial loads, compared with untreated carcasses. Aromatic vinegar showed less effective activity because it exerted effects only 48 h after the treatment in some of the microbial parameters tested (ACC, SC, and LABC).

The results of the activity of lactic acid solutions against the microorganisms that grow on the carcasses are in line with other authors' findings, even if several factors of those studies differ from the present study and, therefore, only an attempt of comparison could be made: the percentage and temperature of the solution used; the application procedures of the solution on the carcasses (or on the meat); the time and site of sampling; sampling methods; the microorganism investigated; experimentally contaminated samples; and animal species [21,28,29]. Lactic acid is active against microbial loads and specific populations of both Gram-negative and Gram-positive bacteria, in vitro and on meat [30,31]. This outcome has been registered also in the present study, where the application of lactic acid solution affected both EC and SC. The effects of lactic acid on LAB highlighted in this study depend on the specific LAB population and on different factors, such as the concentration of lactic acid and the presence of other organic acids. For instance, Vermuelen et al. (2007) [32] observed higher effects of lactic acid addition to sauces on *L. plantarum* than on *L. fructivorans*, and the growth probability for both strains is related to the buffering capacity of the media after the treatment (pKa 3.8).

The reduction in the MLs between CTR and LA samples was higher than 1 Log only for ACC at T48, while it was lower than 1 Log in the other parameters and times tested (Figure 2). The high hygienic level of the carcasses detected in the present study could not allow for an

evaluation of higher ML reductions. Similar effects on MLs (ACC and EC) are reported in beef carcasses by Han et al. (2020) [33] with lactic acid solution up to 4%, even though samples were collected only after 45 min. Nonetheless, there is no consensus on the microbial quantitative reduction values when lactic acid solutions are applied on carcass surfaces. A reduction of 1 Log was recorded at 24 h by Rodriguez-Melcon et al. [34] in ACC and PCC on beef carcasses using from 2% to 5% LA solution. Reduction values decreased only after 72 h. Moreover, Castiglio et al. [35] reported a higher efficiency of the treatment on beef carcasses, with a reduction in ACC of up to 3 Log CFU but using a larger amount of a 4% solution of LA (500 mL), while lower effects (mainly < 1 Log) were reported for coliforms. Residual growth inhibitions were even reported by Carpenter et al. [36] that could explain the similar level recorded for MLs at T2 and T48.

The antimicrobial activity of organic acids can be exerted by different biochemical pathways, even though the specific mechanisms are still not entirely understood [37]. Many authors indicated that organic acids tend to modify the pH of the surface to an unacceptable level for most microorganisms [28]. Some studies showed that lactic acid is more effective than other organic acids probably due to its higher acidity; in fact, it is documented that acids with lower pH values usually have higher antimicrobial efficacy [38,39]. Week organic acids have a lower pKa value than the pH of the cell cytoplasm, and when the undissociated acid enters into the cell and dissociates, with the release of protons (H+), a consequent reduction in intracellular pH value is produced [33,40]. This dissociation of the acids also produces and accumulates anions, which determine homeostatic stress and metabolic perturbation of bacteria [21,39]. Furthermore, organic acids are hydrophilic, and this trait enhances their antimicrobial activity since

bacteria also tend to suspend in the water phase [41]. Another way to exert the antimicrobial action of weak acids is the promotion of oxidative stress, which changes and disrupts cell regulation and produces free radicals, leading to cell death [21,40].

Regarding AV, the effects of acetic or peroxyacetic acid solutions sprayed on beef carcasses were reported by different authors on ACC, coliforms, and some staphylococcus strains [33,41,42]. As previously reported, in the present study, the values of MLs differed between CTR and AV mainly after T48 for some of the parameters considered. However, when compared with LA at the same sampling time, the AV values were similar to LA ones only for PCC, SC, and LABC. Sallam et al. [42] recorded comparable ML values between the carcasses treated with 2% lactic acid solution and 2% acetic acid solution. The similar values of microbial growth registered at T2 and T48 for the carcasses treated with AV suggest a bacteriostatic activity of this solution, as already reported on different bacterial populations in other types of food [43]. Furthermore, the effect of AV on the microbial population depends not only on organic acids (acetic acid) but also on polyphenols [44,45]. These latter compounds could affect microbial growth by increasing the duration of the lag phase (λ, h) and decreasing the maximum growth rate (μmax, Log CFU/mL/h) values [46]. Indeed, the antimicrobial activity of polyphenols is influenced by the compounds' structure, their concentration, and how they enter and modify the bacterial cell membrane [45,47]. The use of organic acids in decontamination strategies could represent a useful tool to reduce the level of MLs and also to inhibit several pathogens that could grow on wild boar carcasses [36].

Some authors in the literature assume that acids would have the potential to accelerate the oxidation of myoglobin and impart acidic

odors or flavors to meat, but the literature abounds with disparity, and this could be attributable to the extent of variability in treatments [48]. Smulder et al. [48], in agreement with this study, reported that the decontamination of red meat carcasses with 1% to 2% of lactic or acetic acid had no impact on the sensory characteristics. The absence of sensory modification on the treated surface was also attributed to the fact that the lactic acid solution temporarily reduces the pH of the meat surface. However, due to the buffering capacity of the meat, the pH quickly returns to near-previous levels [5,49].

Taking into account the definition of CCP as “a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level” [50], in small slaughterhouses, such as the game handling establishments considered in trials, there are still controversies on the presence of production steps that would prevent, eliminate, or reduce the likely occurrence of a biological hazard to an acceptable level [51,52]. For this reason, the use of 2% LA solution on carcass surfaces should be considered in game meat establishments, as it could represent the only real CCP in this certified chain. The validation of this process, according to EC regulations, must be performed by adopting the aerobic colony count and *Enterobacteriaceae* count as microbiological hygiene criteria [53]. Both of these counts were considered in this study and were reduced by treating carcasses with a 2% LA solution. Furthermore, the positive effects of LA solutions on food-borne pathogens have already been reported [54,55].

5. *Conclusion*

The results of the present study suggest that the adoption of LA could be considered a valuable strategy to improve the hygienic level of

carcasses in hunted game meat production and could represent a suitable candidate for EU Commission approval. By contrast, AV was proven less effective than LA for carcass treatment.

The treatment with LA, as well as the other procedures adopted in the present study for pre-harvest and post-harvest phases, could lay down the basis for the definition and implementation of wild boar meat certified production chain able to ensure high quality and good hygienic standards to consumers.

Further studies could investigate their potential use on game meat food-borne pathogens in situ, as well as the effects on game meat shelf-life.

References

1. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 Laying Down Specific Hygiene Rules for Food of Animal Origin. Available online: https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32004R0853 (accessed on 1 September 2022).
2. European Food Safety Authority. Scientific Opinion on the Evaluation of the Safety and Efficacy of Lactic Acid for the Removal of Microbial Surface Contamination of Beef Carcasses, Cuts and Trimmings. *EFSA J.* **2011**, *9*, 2317.
3. European Food Safety Authority. Scientific Opinion on the evaluation of the safety and efficacy of peroxyacetic acid solutions for reduction of pathogens on poultry carcasses and meat. *EFSA J.* **2014**, *12*, 3599.
4. European Food Safety Authority. Scientific Opinion on the evaluation of the safety and efficacy of lactic and acetic acids to reduce microbiological surface contamination on pork carcasses and pork cuts. *EFSA J.* **2018**, *16*, 5482.
5. European Food Safety Authority. Scientific Opinion on the evaluation of the safety and efficacy of lactic acid to reduce microbiological surface contamination on carcases from kangaroos, wild pigs, goats and sheep. *EFSA J.* **2022**, *20*, 7265.
6. Commission Regulation (EU) No 101/2013 of 4 February 2013 Concerning the Use of Lactic Acid to Reduce Microbiological Surface Contamination on Bovine Carcases. Available online: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32013R0101 (accessed on 1 September 2022).

7. Marescotti, M.E.; Caputo, V.; Demartini, E.; Gaviglio, A. Discovering market segments for hunted wild game meat. *Meat Sci.* **2019**, *149*, 163–176.
8. Niewiadomska, K.; Kosicka-Gębska, M.; Gębski, J.; Gutkowska, K.; Jeżewska-Zychowicz, M.; Sułek, M. Game Meat Consumption— Conscious Choice or Just a Game? *Foods* **2020**, *9*, 1357.
9. Czarniecka-Skubina, E.; Stasiak, D.M.; Latoch, A.; Owczarek, T.; Hamulka, J. Consumers' Perception and Preference for the Consumption of Wild Game Meat among Adults in Poland. *Foods* **2022**, *11*, 830.
10. Demartini, E.; Vecchiato, D.; Tempesta, T.; Gaviglio, A.; Viganò, R. Consumer preferences for red deer meat: A discrete choice analysis considering attitudes towards wild game meat and hunting. *Meat Sci.* **2018**, *146*, 168–179.
11. Hoffman, L.C.; Wiklund, E. Game and venison—Meat for the modern consumer. *Meat Sci.* **2006**, *74*, 197–208.
12. Soriano, A.; Sánchez-García, C. Nutritional Composition of Game Meat from Wild Species Harvested in Europe. In *Meat and Nutrition*; Ranabhat, C.L., Ed.; IntechOpen: London, UK, 2021; pp. 77–100.
13. Branciari, R.; Onofri, A.; Cambiotti, F.; Ranucci, D. Effects of Animal, Climatic, Hunting and Handling Conditions on the Hygienic Characteristics of Hunted Roe Doer (*Capreolus capreolus* L.). *Foods* **2020**, *9*, 1076.
14. Orsoni, F.; Romeo, C.; Ferrari, N.; Bardasi, L.; Merialdi, G.; Barbani, R. Factors affecting the microbiological load of Italian hunted wild boar meat (*Sus scrofa*). *Meat Sci.* **2020**, *160*, 107967.

15. Roila, R.; Branciari, R.; Primavilla, S.; Miraglia, D.; Vercillo, F.; Ranucci, D. Microbial, physico-chemical and sensory characteristics of salami produced from wild boar (*Sus scrofa*). *Potravin. J. Food Sci.* **2021**, *15*, 475–483.
16. Sales, J.; Kotrba, R. Meat from wild boar (*Sus scrofa* L.): A review. *Meat Sci.* **2013**, *94*, 187–201.
17. Stella, S.; Tirloni, E.; Castelli, E.; Colombo, F.; Bernardi, C. Microbiological evaluation of carcasses of wild boar hunted in a hill area of northern Italy. *J. Food Prot.* **2018**, *81*, 1519–1525.
18. Massei, G.; Kindberg, J.; Licoppe, A.; Gacˇic´, D.; Šprem, N.; Kamler, J.; Baubet, E.; Hohmann, U.; Monaco, A.; Ozolinš, J.; et al. Wild boar populations up, numbers of hunters down? A review of trends and implications for Europe. *Pest. Manag. Sci.* **2015**, *71*, 492–500.
19. Regione Umbria. Caccia al Cinghiale 2021. Available online: https://www.regione.umbria.it/turismo-attivita-sportive/caccia/caccia-al-cinghiale (accessed on 1 September 2022).
20. Ranucci, D.; Roila, R.; Onofri, A.; Cambiotti, F.; Primavilla, S.; Miraglia, D.; Andoni, E.; Di Cerbo, A.; Branciari, R. Improving hunted wild boar carcass hygiene: Roles of different factors involved in the harvest phase. *Foods* **2021**, *10*, 1548.
21. Manzoor, A.; Jaspal, M.H.; Yaqub, T.; Haq, A.U.; Nasir, J.; Avais, M.; Asghar, B.; Badar, I.H.; Ahmad, S.; Yar, M.K. Effect of lactic acid spray on microbial and quality parameters of buffalo meat. *Meat Sci* **2020**, *159*, 107923.
22. International Organization for Standardization. *ISO 17604:2014*; Microbiology of the Food Chain—Carcass Sampling for

Microbiological Analysis. International Organization for Standardization: Geneva, Switzerland, 2014.

23. International Organization for Standardization. *ISO 4833-1:2013*; Microbiology of the Food Chain—Horizontal Method for the Enumeration of Microorganisms—Part 1: Colony Count at 30 ◦C by the Pour Plate Technique. International Organization for Standardization: Geneva, Switzerland, 2013.
24. International Organization for Standardization. *ISO 17410:2001*; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Psychrotrophic Microorganisms. International Organization for Standardization: Geneva, Switzerland, 2001.
25. International Organization for Standardization. *ISO 21528-2:2017*; Microbiology of the Food Chain—Horizontal Method for the Detection and Enumeration of Enterobacteriaceae—Part 2: Colony-Count Technique. International Organization for Standardiza- tion: Geneva, Switzerland, 2017.
26. Statistical Analysis System. *JMP Statistics and Graphics Guide*; Version 4; SAS Institute Inc.: Cary, NC, USA, 2001.
27. Kim, I.A.; Yoon, J.Y.; Lee, H.S. Measurement of consumers' sensory discrimination and preference: Efficiency of preference-difference test utilizing the 3-point preference test precedes the same-different test. *Food Sci. Biotechnol.* **2015**, *24*, 1355–1362.
28. Nkosi, D.V.; Bekker, J.L.; Hoffman, L.C. The Use of Organic Acids (Lactic and Acetic) as a Microbial Decontaminant during the Slaughter of Meat Animal Species: A Review. *Foods* **2021**, *10*, 2293.

29. Loretz, M.; Stephan, R.; Zweifel, C. Antibacterial activity of decontamination treatments for cattle hides and beef carcasses. *Food Control* **2011**, *22*, 347–359.
30. King, D.A.; Lucia, L.M.; Castillo, A.; Acuff, G.R.; Harris, K.B.; Savell, J.W. Evaluation of peroxyacetic acid as a post-chilling intervention for control of Escherichia coli O157: H7 and Salmonella Typhimurium on beef carcass surfaces. *Meat Sci.* **2005**, *69*, 401–407.
31. Saad, S.M.; Hassanin, F.S.; Salem, A.M.; Saleh, E.A.E. Efficiency of some organic acids as decontaminants in sheep carcasses. *Benha Med. J.* **2020**, *38*, 116–119.
32. Vermeulen, A.; Devlieghere, F.; Bernaerts, K.; Van Impe, J.; Debevere, J. Growth/no growth models describing the influence of pH, lactic and acetic acid on lactic acid bacteria developed to determine the stability of acidified sauces. *Int. J. Food Microbiol.* **2007**, *119*, 258–269.
33. Han, J.; Luo, X.; Zhang, Y.; Zhu, L.; Mao, Y.; Dong, P.; Yang, X.; Liang, R.; Hopkins, D.L.; Zhang, Y. Effects of spraying lactic acid and peroxyacetic acid on the bacterial decontamination and bacterial composition of beef carcasses. *Meat Sci.* **2020**, *164*, 108104.
34. Rodríguez-Melcón, C.; Alonso-Calleja, C.; Capita, R. Lactic acid concentrations that reduce microbial load yet minimally impact colour and sensory characteristics of beef. *Meat Sci.* **2017**, *129*, 169–175.
35. Castillo, A.; Lucia, L.M.; Mercado, I.; Acuff, G.R. In-plant evaluation of a lactic acid treatment for reduction of bacteria on chilled beef carcasses. *J. Food Prot.* **2001**, *64*, 738–740.

36. Carpenter, C.E.; Smith, J.V.; Broadbent, J.R. Efficacy of washing meat surfaces with 2% levulinic, acetic, or lactic acid for pathogen decontamination and residual growth inhibition. *Meat Sci.* **2011**, *88*, 256–260.
37. Ricke, S.C. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poult. Sci. J.* **2003**, *82*, 632–639.
38. Yagnik, D.; Serafin, V.; Shah, A.J. Antimicrobial activity of apple cider vinegar against Escherichia coli, Staphylococcus aureus and Candida albicans; downregulating cytokine and microbial protein expression. *Sci. Rep.* **2018**, *8*, 1732.
39. Hirshfield, I.N.; Terzulli, S.; O'Byrne, C. Weak organic acids: A panoply of effects on bacteria. *Sci. Prog.* **2003**, *86*, 245–270.
40. Boomsma, B.; Bikker, E.; Lansdaal, E.; Stuut, P. L-Lactic Acid-A Safe Antimicrobial for Home-and Personal Care Formulations. *Sofw. J.* **2015**, *141*, 2–5.
41. Van Ba, H.; Seo, H.W.; Pil-Nam, S.; Kim, Y.S.; Park, B.Y.; Moon, S.S.; Kang, S.J.; Choi, Y.M.; Kim, J.H. The effects of pre-and post-slaughter spray application with organic acids on microbial population reductions on beef carcasses. *Meat Sci.* **2018**, *137*, 16–23.
42. Sallam, K.I.; Abd-Elghany, S.M.; Hussein, M.A.; Imre, K.; Morar, A.; Morshdy, A.E.; Sayed-Ahmed, M.Z. Microbial decontamina- tion of beef carcass surfaces by lactic acid, acetic acid, and trisodium phosphate sprays. *Biomed. Res. Int.* **2020**, *2020*, 2324358.
43. Di Toro, J.; Branciari, R.; Roila, R.; Altissimi, S.; Jang, H.; Zhou, K.; Perucci, S.; Codini, M.; Ranucci, D. Eficacy of an aromatic vinegar in reducing psychrotrophic bacteria and biogenic amines

in salmon fillets (Salmo salar) stored in modified atmosphere packaging. *Pol. J. Food Nutr. Sci.* **2019**, *69*, 397–405.
44. Bakir, S.; Devecioglu, D.; Kayacan, S.; Toydemir, G.; Korbancioglu-Guler, F.; Capanoglu, E. Investigating the antioxidant and antimicrobial activities of different vinegars. *Eur. Food Res. Technol.* **2017**, *243*, 2083–2094.
45. Chen, H.; Chen, T.; Giudici, P.; Chen, F. Vinegar function on health: Constituents, sources, and formation mechanisms. *Compr. Rev. Food Sci.* **2016**, *15*, 1124–1138.
46. Roila, R.; Valiani, A.; Ranucci, D.; Ortenzi, R.; Servili, M.; Veneziani, G.; Branciari, R. Antimicrobial efficacy of a polyphenolic extract from olive oil by-product against "Fior di latte" cheese spoilage bacteria. *Int. J. Food Microbiol.* **2019**, *295*, 49–53.
47. Daglia, M. Polyphenols as antimicrobial agents. *Curr. Opin. Biotechnol.* **2012**, *23*, 174–181.
48. Smulders, F.J.M.; Greer, G.G. Integrating microbial decontamination with organic acids in HACCP programmes for muscle foods: Prospects and controversies. *Int. J. Food Microbiol.* **1998**, *44*, 149–169.
49. Grajales-Lagunes, A.; Rivera-Bautista, C.; Ruiz-Cabrera, M.; Gonzalez-Garcia, R.; Ramirez-Telles, J.; Abud-Archila, M. Effect of lactic acid on the meat quality properties and the taste of pork Serratus ventralis muscle. *Agric. Food Sci.* **2012**, *21*, 171–181.
50. Codex Alimentarius Commission. *Recommended International Code of Practice—General Principles of Food Hygiene—CAC/RCP 1-1969, Rev.4-2003*; FAO: Rome, Italy, 1969.
51. European Food Safety Authority. Scientific opinion on hazard analysis approaches for certain small retail establishments in view

of the application of their food safety management systems. *EFSA J.* **2017**, *15*, 4697.

52. Howlett, B.; Bolton, D.J.; O'Sullivan, C. *Development of Pre-Requisite Programmes and HACCP Principles for Irish Beef SLAUGHTER- HOUSES*; Teagasc—The National Food Centre: Wexford, PA, USA, 2005.
53. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on Microbiological Criteria for Foodstuffs. Available online:https://eurlex.europa.eu/legalcontent/EN/ALL/?uri=CELEX%3A32005R2073 (accessed on 1 September 2022).
54. Wang, C.; Chang, T.; Yang, H.; Cui, M. Antibacterial mechanism of lactic acid on physiological and morphological properties of Salmonella Enteritidis, *Escherichia coli* and Listeria monocytogenes. *Food Control* **2015**, *47*, 231–236.
55. Epling, L.K.; Carpenter, J.A.; Blankenship, L.C. Prevalence of Campylobacter spp. and Salmonella spp. on pork carcasses and the reduction effected by spraying with lactic acid. *J. Food Prot.* **1993**, *56*, 536–537.

Preventing microbial growth in game meat by applying polyphenolic extracts from olive mill vegetation water

Caterina Altissimi[1], Rossana Roila[1], David Ranucci[1], Raffaella Branciari[1], Dongjie Cai[2] and Peter Paulsen[3],

[1]Department of Veterinary Medicine, University of Perugia, 06121 Perugia, Italy
[2]College of Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, China
[3]Centre for Food Science and Veterinary Public Health, Clinical Department for Farm Animals and Food System Science, University of Veterinary Medicine Vienna, 1012 Vienna, Austria

Foods, DOI:10.3390/foods13050658

Abstract

We studied the efficacy of different formulations of polyphenol extracts (mainly containing hydroxytyrosol and tyrosol) from olive mill vegetation water on the microflora on the surfaces of game meat cuts with high or low initial bacterial loads. Meat with a high microbial load (>5 Log cfu/g; mean value = 6.83 ± 0.45 standard deviation) was immersed for 10 or 60 sec into 25% and 10% solutions of microencapsulated freeze-dried and non-encapsulated polyphenolic extracts. Aerobic colony, *Enterobacteriaceae*, *Pseudomonas* spp., and lactic acid bacteria counts were determined on treated samples compared to controls after 7 days of storage (in vacuum-packed conditions at +3 ◦C). Significant differences were registered only for aerobic colony count for a 10% liquid extract treatment (0.64 log

reduction). In contrast, the dipping or immersion of game meat with low initial microbial loads (<5 Log cfu/g; mean value = 3.58 ± 0.72 standard deviation) in 10% solutions of the polyphenol extracts effectuated significant reductions in all bacteria counts ($p < 0.002$) at 7 and 14 days of storage for different extracts, independently from the application methods. The use of the extracts to inhibit bacterial growth in game meat should only be considered if a good hygienic baseline is guaranteed.

1. *Introduction*

The interest in natural preservatives in meat and meat product manufacturing has increased, especially regarding those derived from food industry by-products [1]. Because of their antioxidant and anti-inflammatory qualities, polyphenols are plant secondary metabolites that are increasingly being utilized in food, beverages, and innovative cosmetic formulations [2,3]. They are also employed in nutraceutical supplements [4]. Furthermore, in the last decade, numerous studies have described the effects of polyphenols obtained from plant extracts on foodborne pathogens and spoilage bacteria [5–8], both in vitro and in meat models. A few studies have also reported on the antimicrobial effects of specific polyphenols, preventing bacterial growth when directly applied to fresh meat or added to minced meat preparations [9,10]. Furthermore, the synergistic or antagonistic interactions of phenolic compounds with multiple other chemicals may influence the antibacterial properties of plant extracts [11]. Polyphenols are also evaluated after being mixed with other molecules or adsorbed onto coating agents [12,13]. Different polyphenols are present in olive leaves and fruits, and their amounts change according to geographical localization, cultivar, and season [14,15]. Although the olive fruit

contains a high concentration of phenolic compounds, only 2% of them are found in the oil phase. The majority are lost in the solid pomace residue (about 45%; approximately 2–8 g of polyphenols/kg depending on processing) and the aqueous phase (approximately 53%) [16]. Large amounts of water (0.6–1.3 m3/1000 kg of processed olives) are added during the widely used three-phase extraction systems' olive oil production process [17], which leads to the production of over 30 million m3 of oil mill vegetation water (OMVW) worldwide [18]. OMVW is a dark, mildly acidic liquid with high conductivity that is obtained from mechanically processing olives during the production of olive oil and contains a wide range of polyphenols, such as secroiridoids (oleuropein), simple phenols (hydroxytyrosol; tyrosol, 4-hydroxyphenyl acetate), phenolic acids (chlorogenic acid; vanillic acid; caffeic acid; p-coumaric acid; ferulic acid; and verbascoside), and flavones (luteolin, apigenin), in concentrations of 1–10 g/L [18]. Therefore, it can be used for polyphenol extraction.

Polyphenol extracts from olive mill vegetation water (OMVW) have been investigated for their potential applications and effects, both in vitro [19,20] and in fresh meats, mainly in pork sausages and beef patties [19,21–23] but also chicken [24,25]. Despite having the same by-product origin, different compounds can be extracted, with potentially different antioxidant and antimicrobial activities [26]. Recently, specific preparations obtained from OMVW containing mainly hydroxytyrosol and tyrosol (without secroiridoids) are available on the market for application in food production. However, to date, no studies have reported on the antimicrobial effects of such compounds on game meat.

Due to its ability to meet a growing number of demands from conscious consumers, hunted wild game meat is becoming more and more

popular in the last decades. Consumer requirements include positive nutritional aspects regarding fat and protein content and quality [27], the careful consideration of consumer health aspects such as the avoidance of antibiotics and pharmaceuticals [28], and the ethical treatment of animals during the entire manufacturing process [29]. Indeed, game meat is sourced from animals free to live in a natural setting, not reared in intensive farming systems, and not subject to the stress of live transport or slaughtering [27,29]. Nevertheless, some concerns may arise regarding the hygiene level and safety of game meat [30]. Game meat is peculiar with respect to the way that it undergoes a different production process and is more prone to microbial contamination than meat from slaughtered farm animals [31,32]. This may be due to the mode of killing, i.e., a more or less accurate shot (e.g., involving the rupture of the gut or the exposure of damaged parts to the environment); an improperly performed in-field bleeding and evisceration process; delayed evisceration or a late onset of cooling [33–35]. The level of bacterial contamination is very important for fresh meat as it could affect its quality. The growth of spoilage microorganisms, together with endogenous enzymes and oxidation, can degrade various nutrients in meat and generate off-odors and off-flavors, as well as discoloration and slime, making the meat unfit for human consumption and generating waste [36]. For these reasons, the chemical antimicrobial treatment of hunted game carcasses, such as with lactic acid, has been suggested by different authors to be able to prevent bacterial growth during storage [32,37].

The aim of the study was to define the antimicrobial effect of different formulations of microencapsulated and non-encapsulated polyphenols obtained from OMVW on fresh game meat with different levels of contamination. Two series of trials were performed to assess the effects

of the concentration and application method of polyphenolic extracts on meat hygiene indicators and spoilage microorganisms.

2. *Materials and Methods*

Two formulations of food-grade OMVW Polyphenolic Extract (PEs) already available on the market were considered in the trials. One was not encapsulated (in liquid state, LPE; Stymonphen liquid, Stymon, Patras, Greece; polyphenol content: 50,000 mg/kg; hydroxy-tyrosol/tyrosol ratio of 5:1 *w*/*w*), and one freeze-dried and encapsulated in maltodextrins (FPE; Stymonphen W50, Stymon, Patras, Greece; polyphenol content: 50,000 mg/kg; hy- droxytyrosol/tyrosol ratio of 6:1 *w*/*w*).

Fresh game meat (mainly from shoulder cuts) from wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*), and red deer (*Cervus elaphus*) was obtained from retailers in Austria.

According to their records, the meat was from free-living wild game originating from Austria. The meat was kept vacuum-packaged at 3 ± 1 ◦C until the start of the trial. The muscles were cut into cubes measuring 2.5 × 2.5 × 2.5 cm.

Microbial counts were determined to assess the initial microbial loads. To this end, the samples were placed in sterile bags, and nine parts of Maximum Recovery Diluent (MRD) (Oxoid, Basingstoke, UK) were added. Homogenization of the sample was achieved using a Stomacher-type blender (Interscience, St. Nom, France); subsequently, serial tenfold dilutions were prepared in MRD. Samples were subjected to the following analyses: an aerobic colony count (ACC) performed according to ISO 4833-1:2013 [38] on Plate Count Agar (from Merck, Darmstadt, Germany) incubated for 72 h at 30 ◦C; enterobacteriaceae count (ENT) determined according to ISO 21528-2:2017 [39] on Violet

Red Bile Glucose Agar (Merck) incubated for 24 h at 37 ◦C; *Pseudomonas* spp. (PSE) (Glutamate–Starch–Penicillin Agar (Merck) with Penicillin G supplement (Sandoz, Kundl, Austria) incubated for 72 h at 25 °C; and lactic acid bacteria (LAB) (on de Man Rogosa Sharpe Agar (Biolife Italiana, Milan, Italy) incubated for 48 h at 37 °C. The number of colony-forming units (cfu) per gram was converted to Log cfu/g. Two experiments were conducted.

2.1 Treatment of Game Meat with Initial High Microbial Loads (Experiment 1)

An initial trial was designed to determine the effect of the two different PEs on game meat with a high ACC load after 7 days of storage under refrigerated conditions.

The pre-trial microbiological condition (T0) of the meat cubes was determined in 18 samples (6 from wild boar, 6 from red deer, and 6 from roe deer). The average values were 6.83 Log cfu/g (±0.45 standard deviation—sd) for ACC, 4.48 Log cfu/g (±0.44 sd) for ENT, 6.66 Log cfu/g (±0.43 sd) for PSE, and 4.44 Log cfu/g (±1.22 sd) for LAB.

The other 45 samples from the same muscles of the same subjects (15 from wild boar, 15 from red deer, and 15 from roe deer) were divided into five groups, with three replicates each: a control group (C) without any treatment, a group immersed for 1 min in a solution of 10% LPE (LPE10), a group immersed for 1 min in a solution of 25% LPE (LPE25), a group immersed for 1 min in a solution of 10% FPE (FPE10), and a group immersed for 1 min in a solution of 25% FPE (FPE25). After treatment, samples were allowed to dry for 5 min; then, they were vacuum-packaged (PA/PE film, Combivac90; Felzmann, Linz, Austria), and the packages were stored in refrigerated conditions

(3 ± 1 ◦C) for 7 days, after which period the ACC, ENT, PSE, and LAB were determined as described previously.

Statistical analyses were performed using GLM SAS (SAS Institute, Cary, NY, USA) [40]. An ANOVA model was used to evaluate differences between C at T0 and T7. Another ANOVA model included PE formulation (C, LPE, and FPE) and concentration (10% and 25%) as fixed variables without a time effect, since that was available only for the C group. Animal species were not considered in the model as previous analyses had shown that species did not have a statistically significant effect. Tukey's post hoc test was performed to evaluate the difference of the means. Statistical significance was established at $p < 0.05$.

2.2 Treatment of Game Meat with Initial Low Microbial Loads (Experiment 2)

A second trial was designed to determine the effect of application methods of 10% solutions of the two different PEs on game meat with low ACC load.

The microbial loads of 6 samples of wild boar meat were determined before the treatments (T0).

A total of 45 samples were obtained from the same muscle, and the samples were randomly assigned to five groups: a control group (C) to which no treatments were applied; a group immersed for 1 min in a solution of 10% LPE (LPE I); a group dipped for 10 s in a solution of 10% LPE (LPE D); a group immersed for 1 min in a solution of 10% FPE (FPEI); and a group dipped for 10 s in a solution of 10% FPE (FPED). This trial was replicated three times. The average values of microbial counts at T0 (three replicates) were 3.58 Log cfu/g (±0.72 sd) for ACC, 1.88 Log cfu/g (±0.54 sd) for ENT, 2.20 Log cfu/g (±0.81 sd)

for PSE, and 2.54 Log cfu/g (±0.72 sd). No differences were detected between replicates.

Samples were stored under vacuum under refrigerated conditions (3 ± 1 ◦C) for 7, 14, and 21 after which period, ACC, ENT, PSE and LAB counts were determined.

Statistical analyses were performed using the abovementioned software, and an ANOVA model was used to evaluate differences between C at T0 and T7, T14, and T21 and between C at T0 and PE groups at T7. Furthermore, another ANOVA model was used, with PE formulation (C, LPE, and FPE), method of application (I and D), and time (7, 14, and 21 days) serving as fixed variables. Replicates were not included as a factor in the model as no statistical differences were detected. Post hoc Tukey tests were therefore performed to evaluate the difference of the mean, and the difference was considered to be significant when p was <0.05.

3. *Results*

3.1 Treatment of Game Meat with Initial High Microbial Loads (Experiment 1)

In the C samples with high initial microbial loads, an increase in the ACC, ENT, and LAB was observed during the 7 days of observation. No difference was detected regarding the PSE counts.

The results obtained after 7 days of storage are reported in Table 1 for ACC, ENT, PSE, and LAB. For the ACCs, differences were recorded only between C and LPE10 and were below 1 Log cfu/g (Table 1). No differences were recorded for ENT, PSE, and LAB with values over 6 Log cfu/g for PSE and LAB and over 4.5 Log cfu/g for ENT.

Table 1. Microbial counts (Log cfu/g) of game meat samples from experiment 1 (high initial ACC loads) after 7 days of storage.

Group		**ACC**	**ENT**	**PSE**	**LAB**
C		7.84 b	5.12	6.97	7.05
LPE10		7.20 a	4.84	6.53	6.69
LPE25		7.66 ab	4.91	6.59	6.97
FPE10		7.44 ab	4.77	6.47	6.60
FPE25		7.73 ab	4.65	6.62	6.95
SEM		0.140	0.199	0.152	0.166
p value	PE	0.010	0.568	0.632	0.266
	Concentration	0.082	0.573	0.212	0.917
	PE × Con	0.328	0.250	0.766	0.456

n = 9 per experimental group. ACC = aerobic colony count; ENT = Enterobacteriaceae count; PSE = Pseudomonas count; LAB = Lactic acid bacteria count; C = control group; LPE10 = 10% solution of liquid polyphenolic extract; LPE25 = 25% solution of liquid polyphenolic extract; FPE10 = 10% solution of freeze-dried polyphenolic extract; FPE25 = 25% solution of freeze-dried polyphenolic extract. Different letters in the same column (a,b) describe difference in the mean values ($p < 0.05$).

3.2 Treatment of Game Meat with Initial Low Microbial Loads (Experiment 2)

The results regarding the microbial loads of the C samples at T0 and T7 reveal an increase in all the parameters considered except for ENT. Indeed, no differences were recorded between C0 and FPEs and LPEs

after 7 days of storage under vacuum-packaged and refrigerated conditions (Figure 1).

The statistical analyses performed on the microbial loads (Table 2) showed significant effects of PE application and time on the microbial growth of all the bacteria populations considered. No significant difference was detected between the average microbial load values according to the method used for PE application (immersion or dipping), as the *p* values were always over 0.05. Time significantly affected the ACC and LAB microbial loads independently from the PE type and methods used. Regarding ENT, a significant increase was registered only between the C and FPE I groups, while for PSE, a significant difference was only detected between the C with the FPE D group (Table 2).

Figure 1. Difference of microbial loads (mean and standard error) between C samples before treatment and C, FPE I, FPE D, LPE I, and LPE D after 7 days of storage (Log cfu/g). Different letters for each microbial count (a,b) describe differences in the mean values ($p < 0.05$).

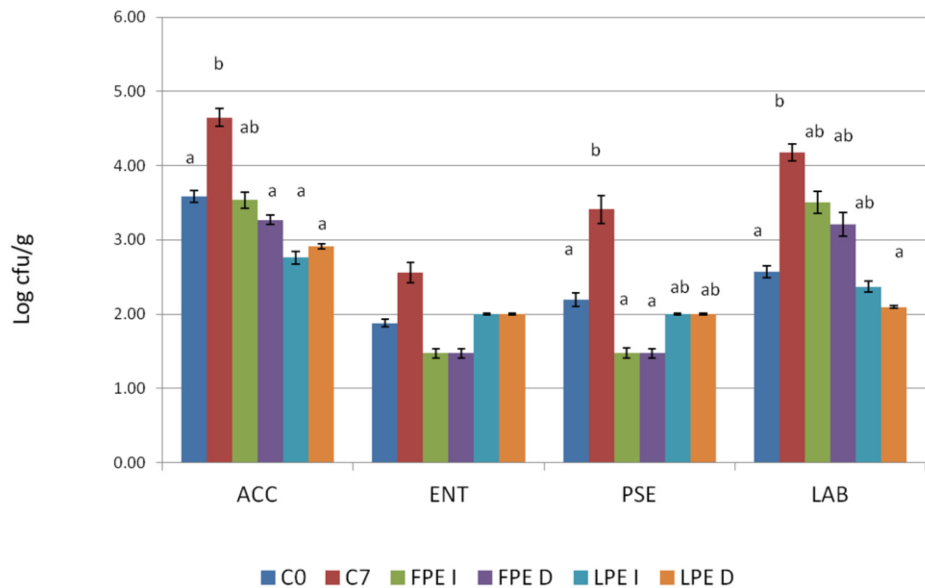


Table 2. Microbial loads (Log cfu/g) of game meat samples from experiment 2 (low initial ACC loads) after 7, 14, and 21 days of storage.

Group	Time	ACC	ENT	PSE	LAB
C	7 days	4.65 aV	2.56 a	3.29 W	4.18 aW
	14 days	6.55 bY	3.31 bV	3.71 X	6.07 bX
	21 days	7.16 b	5.33 bY	4.54	6.78 b
FPE I	7 days	3.53 aVW	1.47	1.48 V	3.51 aVW
	14 days	5.90 bXY	1.47 W	1.65 Y	4.96 bXY
	21 days	6.41 b	2.93 XY	2.76	6.07 b
FPE D	7 days	3.27 aW	1.47 a	1.48 aV	3.21 aVW
	14 days	4.75 bX	1.47 aW	1.47 aY	4.41 bY
	21 days	7.23 c	4.19 bXY	3.72 b	7.04 c
LPE I	7 days	2.76 aW	1.99	2.00 VW	2.37 aVW
	14 days	5.43 bXY	1.99 VW	2.00 XY	4.87 bXY
	21 days	6.63 b	2.58 X	2.58	6.54 c
LPE D	7 days	2.91 aW	1.99	1.99 VW	2.10 aV
	14 days	5.20 bXY	2.10 VW	1.99 XY	4.75 bXY
	21 days	6.22 b	3.11 XY	2.36	6.15 b
SEM		0.411	0.494	0.529	0.451
p value	PE	<0.001	<0.001	<0.001	0.002
	Method	0.471	0.160	0.651	0.689
	Time	<0.001	<0.001	<0.001	<0.001
	PE × T	0.238	0.023	0.014	0.203
	PE × M	0.946	0.572	0.417	0.588
	M × T	0.320	0.151	0.595	0.554
	PE × T × M	0.160	0.671	0.302	0.346

n = 9 per experimental group for each replicate. ACC = aerobic colony count; ENT = Enterobacteriaceae count; PSE = Pseudomonas count;

LAB = lactic acid bacteria count; C = control group; LPEI = sample immersed for 1 min in a 10% solution of liquid polyphenolic extract; LPE D = sample dipped for 10 s in a 10% solution of liquid polyphenolic extract; FPE I = sample immersed for 1 min in a 10% solution of freeze-dried polyphenolic extract; FPE D = sample dipped for 10 s in a 10% solution of freeze-dried polyphenolic extract; PE = polyphenolic extract; T = time; M = method of application of the polyphenolic extract. In each column, different small letters (a,b,c) within the same group denote statistically significant differences in the mean values between times of storage ($p < 0.05$); likewise, different capital letters (V,W,X,Y) indicate significant differences in the mean values between groups ($p < 0.05$) for the same storage time.

The effect of the PEs on the C samples was significant for ACC after 7 days, with the exception of FPE I, and after 14 days only for FPE D. For ENT, differences were registered after 14 days between C and FPE I and FPE D and after 21 days between C and LPE I. The PSE counts were different between C and FPE I and FPE D at 7 and 14 days of observation. The LAB count was higher in C than LPE D after 7 days and in FPE D after 14 days. No differences were detected at 21 days between the C and PE groups with respect to ACC, PSE, and LAB. ENT at 21 days was lower in the LPE I group than in the C group.

4. *Discussion*

The first trial confirmed that game meat can be highly contaminated just after the handling and butchering processes. In this case, values even higher than 7 log per g or cm^2 could be found [33]. The microbial loads in these meats increased under cold- storage conditions of +3 ◦C

and under vacuum to final concentrations in the same order of magnitude as in meat cuts with low initial contamination but stored for 21 days.

For meat cuts with high initial bacterial loads, the effect of PEs on the microflora is limited or absent, even when high concentrations of PEs (25%) are used, and no decontamination of the meat is observed. Indeed, polyphenols are more likely to exert bacteriostatic rather than bactericidal effects, reducing the growth of some microbial populations during storage. The possible mechanisms of action proposed for similar compounds are the depletion of ATP inside the bacteria due to polyphenols binding ATP synthetase and altering the microbial metabolism [41]. Furthermore, polyphenols also cause the depolarization of bacterial cells with cell morphology modification, resulting in damage to the cell membrane and the leakage of cytoplasm [42,43]. According to other studies, tyrosol suppresses the activity of cyclooxygenase enzymes, and hydroxytyrosol can cause protein denaturation [44]. All these mechanisms could be responsible for the increase in the bacterial lag phase and the reduction in the exponential growth phase (log phase) detected in different bacterial populations [10,23]. It is possible that when a high number of bacteria were present in the meat, all the mechanisms that interfere with bacterial adaptation to the environment could not be overcome. The effectiveness of compounds with bacteriostatic activity may be restricted in these circumstances.

For an improvement of PE efficacy in food preservation, other possible technologies or antimicrobial substances could be combined in the food industry (i.e., vacuum packaging, high-pressure processing, bacteriocins, polysaccharides, and additives) [45–47]. PEs, as additional hurdles, combined with traditional and innovative

preservation technologies require further investigation with respect to game meat.

When the initial microbial loads were below 4 Log cfu/g, the 10% PE solutions seemed to affect microbial growth. Indeed, for some of the groups tested, a delay in ACC, PSE, and LAB growth was registered after 7 days and remained to some extent until 14 days (i.e., the ACC in groups C and FPE D). At 21 days, when the microbial load reached high concentrations, the effects were no longer apparent. Other authors have reported a delay in microbial growth when OMVW polyphenols were used, but comparing data from the literature is challenging due to differences in the compounds present in the extracts, their concentrations and the application method used, and the meat matrix under study (e.g., minced meat preparations). The inhibition of bacterial growth was induced by the incorporation of polyphenol extracts from olive leaves and various by-products from the olive oil process into raw or cooked ground meats [10,23,48]. Fasolato et al. [24] reported a reduction in *Enterobacteriaceae* and *Pseudomonas* counts in chicken breast fillets dipped in a crude extract containing a total concentration of phenols of about 22 g/kg, whose main compounds were oleuropein aglycone, hydroxytyrosol, tyrosol, and verbascoside.

The results of experiment 2 reveal that a delay in ENT growth was more evident after 14 and 21 days of storage, differing from the results concerning chicken meat reported by Fasolato et al. [24]. This could be due to differences in the meat considered and the PEs used, particularly when the extracted compounds exerted synergic effects. This phenomenon is still under debate. Some authors suggest that antimicrobial in vitro activity is best assessed by using purified molecules [49,50] with dose-dependent inhibition effects, also depending on the culture media adopted [51]. Other authors mention

potential synergic effects between phenolic compounds or with other molecules [41,52], but further studies are needed on raw meat.
Dipping for a short time (10 s), adopted by Fasolato et al. [24], was still sufficient for achieving bacteriostatic effects, regardless of whether PEs were in liquid or powder form. Encapsulation in maltodextrin after freeze-drying does not seem to affect the efficacy of the extract.
The results confirm the possible use of these PEs on game meat to prevent microbial growth, with a double impact on food sector sustainability, either via reducing food wastage due to microbial spoilage or due to meat oxidation or via reusing an olive-oil-by-product, which is an environmental pollutant necessitating specific disposal strategies [53]. Nonetheless, the relevance of the results should be discussed with respect to other aspects such as the effects on meat quality and regulatory considerations. Since polyphenol extracts are already available on the market as food-grade "flavouring", their application, depending on the concentration, should have an impact on the sensorial characteristics of the products. Furthermore, the antioxidant activity of these compounds has to be taken into consideration when sensory attributes are investigated since they could be used to prevent the production of an off-color, -odor, and -taste due to the oxidation of meat during storage [54]. Other authors reported that acceptable sensory traits could be obtained by using 7% hydroxytyrosol formulations from OMVW in chicken frankfurters [55]. No sensory effects were reported for PEs from olive leaves (100 and 150 μg of phenols/g meat) in raw minced beef [56], and an improvement in the visual quality of cooked beef burgers was obtained with the use of OMVW PEs (87.5 mg of phenols/kg of meat) [57]. Furthermore, undesired side-effects of PEs on quality attributes could be mitigated by incorporating PEs in coating materials [58]. For this reason,

polyphenols from olive byproducts have been used in different types of food packaging, such as polyethylene terephthalate/polypropylen (PET/PT); chitosan + glycerol; alginate + gelatin + glycerol; pectin-fish gelatin + glutaraldehyde +glycerol; κ-carrageenan + glycerol; multilayer polyethylene; and polyvinyl alcohol [59]. This is probably the most promising application of these PEs in the food industry, but it has been reported at the laboratory scale only, for limited kinds of foods, and not for game meat.

Indeed, a deeper analysis of all the conditions affecting PEs' efficacy with respect to the microbial spoilage of game meat, but also the effects on game meat quality attributes, is still needed.

5. *Conclusions*

The use of OMVW PE to inhibit bacterial growth in game meat should be considered only if a good hygienic baseline is guaranteed. When treatment was applied to highly contaminated meat, no consistent effect was found. This underpins EU considerations, where the prevention of microbial contamination and growth is of primary importance, whereas decontaminating treatments are only adjunct measures [60]. When low bacterial contamination occurred, a beneficial effect was evident in game meat stored under vacuum and refrigerated conditions. In these circumstances, the use of PEs could be favorable. Further studies are needed to consider the application of PEs to carcasses after evisceration, with the skin on (i.e., to treat body cavities), or to freshly exposed meat surfaces after the skinning of a carcass as an early-stage treatment with potential beneficial effects for the meat cuts taken from the so-treated carcasses. In addition, it would be appropriate to evaluate the effects of treatment on meat quality characteristics, particularly their antioxidant effect in meats that are more prone to oxidation than

those of other animals. Furthermore, combinations with other preservatives or treatments need further evaluation.

References

1. Efenberger-Szmechtyk, M.; Nowak, A.; Czyzowska, A. Plant extracts rich in polyphenols: Antibacterial agents and natural preservatives for meat and meat products. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 149–178.
2. Ciriminna, R.; Meneguzzo, F.; Fidalgo, A.; Ilharco, L.M.; Pagliaro, M. Extraction, benefits and valorization of olive polyphenols. *Eur. J. Lipid Sci. Technol.* **2016**, *118*, 503–511.
3. Ranucci, D.; Roila, R.; Andoni, E.; Braconi, P.; Branciari, R. Punica granatum and Citrus spp. extract mix affects spoilage microorganisms growth rate in vacuum-packaged cooked sausages made from pork meat, emmer wheat (*Triticum dicoccum* Schübler), almond (*Prunus dulcis* Mill.) and hazelnut (*Corylus avellana* L.). *Foods* **2021**, *8*, 664.
4. Martelli, A.; Flori, L.; Gorica, E.; Piragine, E.; Saviano, A.; Annunziata, G.; Di Minno, M.N.D.; Ciampaglia, R.; Calcaterra, I.; Maione, F.; et al. Vascular effects of the polyphenolic nutraceutical supplement taurisolo®: Focus on the protection of the endothelial function. *Nutrients* **2021**, *13*, 1540.
5. Oulahal, N.; Degraeve, P. Phenolic-rich plant extracts with antimicrobial activity: An alternative to food preservatives and biocides? *Front. Microbiol.* **2022**, *12*, 753518.
6. Wang, X.; Li, Y.; Liu, S.; Wang, H.; Chang, X.; Zhang, J. Chestnut Shell Polyphenols Inhibit the Growth of Three Food-Spoilage Bacteria by Regulating Key Enzymes of Metabolism. *Foods* **2023**, *12*, 3312.
7. Rodriguez Vaquero, M.J.; Aredes Fernandez, P.A.; Manca De Nadra, M.C.; Strasser De Saad, A.M. Effect of phenolic

compounds from argentinean red wines on pathogenic bacteria in a meat model system. *J. Food Biochem.* **2013**, *37*, 425–431.

8. Lobiuc, A.; Pavăl, N.E.; Mangalagiu, I.I.; Gheorghiț ă, R.; Teliban, G.C.; Amăriucăi-Mantu, D.; Stoleru, V. Future antimicrobials: Natural and functionalized phenolics. *Molecules* **2023**, *28*, 1114.
9. Difonzo, G.; Squeo, G.; Pasqualone, A.; Summo, C.; Paradiso, V.M.; Caponio, F. The challenge of exploiting polyphenols from olive leaves: Addition to foods to improve their shelf-life and nutritional value. *J. Sci. Food. Agric.* **2021**, *101*, 3099–3116.
10. Roila, R.; Sordini, B.; Esposto, S.; Ranucci, D.; Primavilla, S.; Valiani, A.; Taticchi, A.; Branciari, R.; Servili, M. Effect of the Application of a Green Preservative Strategy on Minced Meat Products: Antimicrobial Efficacy of Olive Mill Wastewater Polyphenolic Extract in Improving Beef Burger Shelf-Life. *Foods* **2022**, *11*, 2447.
11. Kalogianni, A.I.; Lazou, T.; Bossis, I.; Gelasakis, A.I. Natural phenolic compounds for the control of oxidation, bacterial spoilage, and foodborne pathogens in meat. *Foods* **2020**, *9*, 794.
12. Zhao, S.; Li, N.; Li, Z.; He, H.; Zhao, Y.; Zhu, M.; Wang, Z.; Kang, Z.; Ma, H. Shelf life of fresh chilled pork as affected by antimicrobial intervention with nisin, tea polyphenols, chitosan, and their combination. *Int. J. Food. Prop.* **2019**, *22*, 1047–1063.
13. Zhou, Y.; Xu, T.; Zhang, Y.; Zhang, C.; Lu, Z.; Lu, F.; Zhao, H. Effect of tea polyphenols on curdlan/chitosan blending film properties and its application to chilled meat preservation. *Coatings* **2019**, *9*, 262.
14. Zakraoui, M.; Hannachi, H.; Paskovic´, I.; Vidovic´, N.; Polic´ Paskovic´, M.; Palcˇic´, I.; Major, N.; Ban, S.G.; Hamrouni, L.

Effect of geographical location on the phenolic and mineral composition of Chetoui olive leaves. *Foods* **2023**, *12*, 2565.

15. Palmeri, R.; Siracusa, L.; Carrubba, M.; Parafati, L.; Proetto, I.; Pesce, F.; Fallico, B. Olive leaves, a promising byproduct of olive oil industry: Assessment of metabolic profiles and antioxidant capacity as a function of cultivar and seasonal change. *Agronomy* **2022**, *12*, 2007.
16. Rodis, P.S.; Karathanos, V.T.; Mantzavinou, A. Partitioning of olive oil antioxidants between oil and water phases. *J. Agric. Food Chem.* **2012**, *50*, 596–601.
17. Roig, A.; Cayuela, M.L.; Sanchez-Monedero, M. An overview on olive mill wastes and their valorisation methods. *Waste Manag.* **2006**, *26*, 960–969.
18. Davies, L.C.; Vilhena, A.M.; Novais, J.M.; Martins-Dias, S. Olive mill wastewater characteristics: Modelling and statistical analysis. *Grasas Aceites* **2004**, *55*, 233–241.
19. Caballero-Guerrero, B.; Garrido-Fernandez, A.; Fermoso, F.G.; Rodriguez-Gutierrez, G.; Fernández-Prior, M.Á.; Reinhard, C.; Nyström, B.; BEnitez-Cabello, A.; Arroyo-Lopez, F.N. Antimicrobial effects of treated olive mill waste on foodborne pathogens. *LWT* **2022**, *164*, 113628.
20. Sar, T.; Akbas, M.Y. Antimicrobial Activities of Olive Oil Mill Wastewater Extracts against Selected Microorganisms. *Sustainability* **2023**, *15*, 8179.
21. Hayes, J.E.; Stepanyan, V.; Allen, P.; O'Grady, M.N.; Kerry, J.P. Effect of lutein, sesamol, ellagic acid and olive leaf extract on the quality and shelf-life stability of packaged raw minced beef patties. *Meat Sci.* **2010**, *84*, 613–620.
22. Fasolato, L.; Carraro, L.; Facco, P.; Cardazzo, B.; Balzan,

S.; Taticchi, A.; Andrea, N.; Montemurro, F.; Martino, M.E.; Elena, M.; et al. Agricultural by-products with bioactive extracts: A multivariate approach to evaluate microbial and physicochemical changes in a fresh pork sausage enriched with phenolic compounds from olive vegetation water. *Int. J. Food Microbiol.* **2016**, *228*, 34–43.
23. Djenane, D.; Gómez, D.; Yangüela, J.; Roncalés, P.; Ariño, A. Olive leaves extract from algerian oleaster (*Olea europaea* var. Sylvestris) on microbiological safety and shelf-life stability of raw halal minced beef during display. *Foods* **2019**, *8*, 10.
24. Fasolato, L.; Cardazzo, B.; Balzan, S.; Carraro, L.; Andreani, A.N.; Taticchi, A.; Novelli, E. Using a concentrate of phenols obtained from olive vegetation water to preserve chilled food: Two case studies. *Ital. J. Food Saf.* **2016**, *5*, 5651.
25. Veneziani, G.; Novelli, E.; Esposto, S.; Taticchi, A.; Servili, M. Applications of recovered bioactive compounds in food products. In *Olive Mill Waste*; Academic Press: Cambridge, MA, USA, 2017; pp. 231–253.
26. D'Antuono, I.; Kontogianni, V.G.; Kotsiou, K.; Linsalata, V.; Logrieco, A.F.; Tasioula-Margari, M.; Cardinali, A. Polyphenolic characterization of olive mill wastewaters, coming from Italian and Greek olive cultivars, after membrane technology. *Food Res. Int.* **2014**, *65*, 301–310.
27. Hoffman, L.C.; Wiklund, E. Game and venison–meat for the modern consumer. *Meat Sci.* **2006**, *74*, 197–208.
28. Needham, T.; Bureš, D.; Cˇ erný, J.; Hoffman, L.C. Overview of game meat utilisation challenges and opportunities: A European perspective. *Meat Sci.* **2023**, *204*, 109284.

29. Marescotti, M.E.; Caputo, V.; Demartini, E.; Gaviglio, A. Discovering market segments for hunted wild game meat. *Meat Sci.* **2019**, *149*, 163–176.
30. Corradini, A.; Marescotti, M.E.; Demartini, E.; Gaviglio, A. Consumers' perceptions and attitudes toward hunted wild game meat in the modern world: A literature review. *Meat Sci.* **2022**, *194*, 108955.
31. Branciari, R.; Onofri, A.; Cambiotti, F.; Ranucci, D. Effects of animal, climatic, hunting and handling conditions on the hygienic characteristics of hunted roe doer (*Caprelous capreolus* L.). *Foods* **2020**, *9*, 1076.
32. Nkosi, D.V.; Bekker, J.L.; Hoffman, L.C. The Use of Organic Acids (Lactic and Acetic) as a Microbial Decontaminant during the Slaughter of Meat Animal Species: A Review. *Foods* **2021**, *10*, 2293.
33. Paulsen, P.; Winkelmayer, R. Seasonal variation in the microbial contamination of game carcasses in an Austrian hunting area. *Eur. J. Wildl. Res.* **2004**, *50*, 157–159.
34. Mirceta, J.; Petrovic, J.; Malesevic, M.; Blagojevic, B.; Antic, D. Assessment of microbial carcass contamination of hunted wild boars. *Eur. J. Wildl. Res.* **2017**, *63*, 37.
35. Ranucci, D.; Roila, R.; Onofri, A.; Cambiotti, F.; Primavilla, S.; Miraglia, D.; Andoni, E.; Di Cerbo, A.; Branciari, R. Improving hunted wild boar carcass hygiene: Roles of different factors involved in the harvest phase. *Foods* **2021**, *10*, 1548.
36. Roila, R.; Altissimi, C.; Branciari, R.; Primavilla, S.; Valiani, A.; Cambiotti, F.; Cardinali, L.; Cioffi, A.; Ranucci, D. Effects of Spray Application of Lactic Acid

Solution and Aromatic Vinegar on the Microbial Loads of Wild Boar Carcasses Obtained under Optimal Harvest Conditions. *Appl. Sci.* **2022**, *12*, 10419.
37. Dave, D.; Ghaly, A.E. Meat spoilage mechanisms and preservation techniques: A critical review. *Am. J. Agric. Biol. Sci.* **2011**, *6*, 486–510.
38. *ISO 4833-1:2013*; Microbiology of the Food Chain. Horizontal Method for the Enumeration of Microorganisms Part 1: Colony Count at 30 °C by the Pour Plate Technique. International Organization for Standardization: Geneve, Switzerland, 2013.
39. *ISO 21528-2:2017*; Microbiology of the Food Chain. Horizontal Method for the Detection and Enumeration of Enterobacteriaceae. Part 2: Colony-Count Technique. International Organization for Standardization: Geneve, Switzerland, 2017.
40. *SAS/STAT User's Guide, Version 8*, 2nd ed.; SAS Institute Inc.: Cary, NC, USA, 2000; Volume 2.
41. Amini, A.; Liu, M.; Ahmad, Z. Understanding the link between antimicrobial properties of dietary olive phenolics and bacterial ATP synthase. *Int. J. Biol. Macromol.* **2017**, *101*, 153–164.
42. Guo, L.; Sun, Q.; Gong, S.; Bi, X.; Jiang, W.; Xue, W.; Fei, P. Antimicrobial activity and action approach of the olive oil polyphenol extract against Listeria monocytogenes. *Front. Microbiol.* **2019**, *10*, 1586.
43. Guo, L.; Gong, S.; Wang, Y.; Sun, Q.; Duo, K.; Fei, P. Antibacterial activity of olive oil polyphenol extract against

Salmonella Typhimurium and Staphylococcus aureus: Possible Mechanisms. *Foodborne Path. Dis.* **2020**, *17*, 396–403.

44. Medina, E.; Brenes, M.; Garcia, A.; Romero, C.; de Castro, A. Bactericidal activity of glutaraldehyde-like compounds from olive products. *J. Food Prot.* **2009**, *72*, 2611–2614.
45. Ju, J.; Liao, L.; Qiao, Y.; Xiong, G.; Li, D.; Wang, C.; Wu, W.; Ding, A.; Shi, L.; Li, X. The effects of vacuum package combined with tea polyphenols (V+ TP) treatment on quality enhancement of weever (*Micropterus salmoides*) stored at 0 °C and 4 °C. *LWT* **2018**, *91*, 484–490.
46. de Oliveira, T.L.C.; Ramos, A.L.; Ramos, E.M.; Piccoli, R.H.; Cristianini, M. Natural antimicrobials as additional hurdles to preservation of foods by high pressure processing. *Trends Food Sci. Technol.* **2015**, *45*, 60–85.
47. Dai, Z.; Han, L.; Li, Z.; Gu, M.; Xiao, Z.; Lu, F. Combination of chitosan, tea polyphenols, and nisin on the bacterial inhibition and quality maintenance of plant-based meat. *Foods* **2022**, *11*, 1524.
48. Hawashin, M.D.; Al-Juhaimi, F.; Ahmed, I.A.M.; Ghafoor, K.; Babiker, E.E. Physicochemical, microbiological and sensory evaluation of beef patties incorporated with destoned olive cake powder. *Meat Sci.* **2016**, *122*, 32–39.
49. Friedman, M.; Rasooly, R.; Do, P.M.; Henika, P.R. The olive compound 4-hydroxytyrosol inactivates Staphylococcus aureus bacteria and staphylococcal enterotoxin A (SEA). *J. Food Sci.* **2011**, *76*, M558–M563.
50. Peng, M.; Zhao, X.; Biswas, D. Polyphenols and tri-

terpenoids from *Olea europaea* L. in alleviation of enteric pathogen infections through limiting bacterial virulence and attenuating inflammation. *J. Funct. Foods* **2017**, *36*, 132–143.
51. Medina-Martínez, M.S.; Truchado, P.; Castro-Ibáñez, I.; Allende, A. Antimicrobial activity of hydroxytyrosol: A current contro- versy. *Biosci. Biotechnol. Biochem.* **2016**, *80*, 801–810.
52. Tafesh, A.; Najami, N.; Jadoun, J.; Halahlih, F.; Riepl, H.; Azaizeh, H. Synergistic antibacterial effects of polyphenolic compounds from olive mill wastewater. *eCAM* **2011**, *2011*, 431021.
53. Kavvadias, V.; Doula, M.; Theocharopoulos, S. Long-term effects on soil of the disposal of olive mill waste waters (OMW). *Environ. Forensics* **2014**, *15*, 37–51.
54. Falowo, A.B.; Fayemi, P.O.; Muchenje, V. Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review. *Food Res. Int.* **2014**, *64*, 171–181.
55. Nieto, G.; Martínez, L.; Ros, G. Hydroxytyrosol extracts, olive oil and walnuts as functional components in chicken sausages. *J. Sci. Food Agric.* **2017**, *97*, 3761–3771.
56. Aouidi, F.; Okba, A.; Hamdi, M. Valorization of functional properties of extract and powder of olive leaves in raw and cooked minced beef meat. *J. Sci. Food Agricul.* **2017**, *97*, 3195–3203.
57. Barbieri, S.; Mercatante, D.; Balzan, S.; Esposto, S.; Cardenia, V.; Servili, M.; Novelli, E.; Taticchi, A.; Rodriguez-Estrada, M.T. Improved oxidative stability and

sensory quality of beef hamburgers enriched with a phenolic extract from olive vegetation water. *Antioxidants* **2021**, *10*, 1969.

58. Bermúdez-Oria, A.; Rodríguez-Gutiérrez, G.; Rubio-Senent, F.; Fernández-Prior, Á.; Fernández-Bolaños, J. Effect of edible pectin-fish gelatin films containing the olive antioxidants hydroxytyrosol and 3, 4-dihydroxyphenylglycol on beef meat during refrigerated storage. *Meat Sci.* **2019**, *148*, 213–218.
59. Khwaldia, K.; Attour, N.; Matthes, J.; Beck, L.; Schmid, M. Olive byproducts and their bioactive compounds as a valuable source for food packaging applications. *Compreh. Rev. Food Sci. Food Saf.* **2022**, *21*, 1218–1253.
60. Commission Regulation (EU) No 101/2013 of 4 February 2013 concerning the use of lactic acid to reduce microbiological surface contamination on bovine carcases. *Off. J. Europ. Union* **2013**, *L34*, 1–3.

Physico-chemical quality traits and microbiological condition of burger patties from wild boar meat with added polyphenolic extracts from olive mill vegetation water

Caterina Altissimi[1], David Ranucci[1], Susanne Bauer[2], Raffaella Branciari[1], Peter Paulsen[2]

[1]Department of Veterinary Medicine, University of Perugia, Perugia, Italy
[2]Centre for Food Science and Veterinary Public Health, Clinical Department for Farm Animals and Food System Science, University of Veterinary Medicine Vienna, Vienna, Austria

Ongoing submission process

Abstract
The significant increase in wild boar population and the associated risks require proper management and control strategies, which result in a greater availability of wild boar meat. This study explores the application of a polyphenolic extract from olive mill vegetation water (PPE) on physico-chemical traits, oxidative stability, and microbiological quality of wild boar patties during storage. PPE effectively reduced lipid oxidation, achieving significant antioxidant effects even at concentrations as low as 0.05%, as indicated by Thiobarbituric Acid Reactive Substances (TBARS). Microbiological analysis revealed a limited reduction in bacterial growth at 2% PPE, though synergistic effects were observed when combined with sodium chloride (NaCl). The inclusion of 2% PPE into wild boar burgers led to changes in physico-chemical properties, such as reduced moisture

content and color alterations, though these tended to diminish during storage and were in a magnitude not noticeable to consumers. These findings highlight the potential of the olive mill wastewater polyphenoic extract as a natural preservative, aligning with consumer demand for sustainable and additive-free food products.

1. *Introduction*

In recent years, several countries have been dealing with the exponential increase in wild ungulate populations, particularly that of wild boar, which is responsible for significant damages and repercussions in agriculture, society, and public health. It is therefore essential to carry out a proper management and control strategies to contain these animals, which will consequently lead to a greater availability of wild boar meat [1]. Wild boar meat is usually associated with regional culinary traditions of specific territories, although due to its nutritional value and representing a viable sustainable alternative to intensive livestock farming, it satisfies the needs and requests of the modern consumer, who is becoming more conscious of nutritional, environmental, and ethical aspects [2]. Wild boar meat is characterized by a high nutritional value, with a lower fat content and a better fatty-acid profile in comparison to pork, with a higher amount of polyunsaturated fatty acids (PUFA), mostly omega 3, thereby a better polyunsaturated fatty acids/saturated fatty acids (PUFA/SFA) ratio [3]. Furthermore, consumers' opinions on game meat are becoming more positive, especially for its ethical trait, since it is derived from animals born and lived in their natural wild setting without any pharmacological treatment [2].

Meanwhile, consumers' demand for food products without any synthetic additives as food preservatives has promoting interest and

research towards natural substances. Food industry by-products are often rich in bioactive compounds, such as polyphenols, known for their antioxidant and antibacterial activity, which could be used to enhance food quality and shelf life, in a circular economy perspective [4,5]. Several authors investigated the effects of polyphenols applied to meat products to improve quality traits during storage [6–9]. In particular, the use of polyphenolic extracts could be favourable in minced meat products, which are particularly prone to oxidation and hygienic issues.

The aim of this study was to explore how the addition of different concentrations of polyphenolic extract from olive mill vegetation water (OMVW) into wild boar meat patties would enhance microbiological and chemical shelf life, and how it would influence physico-chemical traits, such as colour, water activity and texture properties.

2. Material and Methods

2.1 Experiments

We studied the effect of varying concentrations of a polyphenol extract (PPE) from OMVW and salt (NaCl) on pH, water activity, colour, microflora and Thiobarbituric-Acid-Reactive Substances (TBARS) of burger patties made from wild boar meat. The polyphenol extract was a commercial, food-grade preparation (PPE; Stymonphen W50, Stymon, Greece, a freeze-dried product with polyphenols encapsulated in maltodextrins; polyphenol content = 50.000 mg/kg; hydroxytyrosol/tyrosol ratio = 6:1). As regards TBARS, samples were tested after heating and subsequent cold storage. All tests were done in triplicate, unless stated otherwise.

In the first set of experiments, we studied proximate composition, water activity and pH of freshly prepared wild boar meat patties with 0–2 %

PPE added. Raw patties were stored vacuum-packaged for 1 or 3 days at 3 ± 1 °C, whereafter colour was measured. Then patties were heated and stored refrigerated for one day. After this period, TBARS were determined in order to study the course of fat oxidation. Two trials were studied, trial 1 with 0–1–2 % PPE, and trial 2, with 0–0.5–1 and 2 % PPE.

In the second set of experiments (corresponding to trial 3), raw patties were stored vacuum-packaged for 3 or 5 days, and then subjected to colour measurement and microbiological examination, in order to establish to what extent the PPE concentration would retard growth of contaminant bacteria and extend shelf life. Stored raw patties were subjected to heat treatment and TBARS determination as described for set 1.

The third set of experiments (trial 4) aimed to detect the minimum PPE concentration exhibiting a statistically significant effect on fat oxidation. For the minimum concentration with significant antioxidant effect, pH and water activity (a_W) were studied.

In the last set of experiments (trial 5), we studied colour, TBARS, pH, a_W and the evolution of the microflora and textural properties (Texture Profile Analysis, TPA) in wild boar meat patties manufactured with a combination of PPE (0 or 2 %) and sodium chloride (0 or 1.5 %).

2.2 Sample preparation

We obtained vacuum-packed meat cuts (in packages of 4–5 kg) of wild boar shoulder from an approved game-handling establishment. Packs arrived within 5 days post cutting and were used at the day of arrival. Storage was at 0–2 °C. The meat cuts were divided in appropriate portions (ca. 1.5 kg) per trial. The portions were minced (MaDo Primus, Dornhan, Germany) through a 3 mm plate. Then, a 10 g aliquot was

taken for determination of Total Mesophilic Aerobic Bacteria and *Enterobacteriaceae*.

A number of trials was performed, see section 2.1. Minced meat was divided into one control group (pure wild boar meat) and treatment groups (addition of NaCl or/and PPE, see Table 1). Both control and treatment groups were mixed manually for ca. 3 min and then, portions of 140 g were formed to burger patties ("Burgerpresse", Gintersdorfer, Saxen, Austria).

For storage, raw patties were vacuum-packed in plastic bags (Combivac 90µm; Felzmann, Linz, Austria) and kept in this condition for 1, 3 or 5 days at 3 °C. We also studied changes in TBARS of such stored patties after heating and subsequent storage for 1 day at 3°C under aerobic conditions, to simulate cold storage of leftover patties to consume them the next day. Patties were heated to an internal temperature of 75 °C on a plate grill (Turbo Super Quick Grill II GG; J. Zimmermann, Oberlienz, Austria) and temperature was measured with a testo 105 thermometer (Testo AG, Titisee-Neustadt, Germany).

2.3 Analyses

2.3.1 Measurement of pH and water activity

Determination of pH and water activity (a_w) were performed as described previously [10]. In brief, three measurements per sample were taken with a penetrating electrode (Testo 205; Testo AG, Titisee-Neustadt, Germany) and with a capacitance sensor device (Lab-Swift, Novasina, Lachen, Switzerland). Per sample, the average was reported. Water activity data refer to 22.0 °C. Only non-heated samples were tested.

2.3.2 Colour Measurement

Colour (CIE L*, a*, b*) was measured in non-heated samples after 'blooming', i.e. keeping the meat patties wrapped in one layer of cling foil for 1 h at 3 °C to allow oxygenation of the haemoglobin. Per sample, measurements were taken on 5 spots and 3–6 patties were tested per treatment condition. We used a double-beam spectrophotometer with the following settings: aperture size: 8 mm, illumination: 6500 K and obverver angle: 10° (Phyma Codec 400, Phyma, Gießhübl, Austria).

Delta-E [$(\Delta E = (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{0.5}$] [11] was calculated to describe the 'distance' between two colours. A ΔE value of 1 is the smallest colour difference distinguishable to the human eye [12]. In practice, ΔE <2 is indicative for differences perceivable only for trained assessors, and consumers would identify a difference in colours when ΔE exceeds 3 [13], whereas a ΔE above 5 equals two different colours [14]. Only non-heated samples were tested.

2.3.3 Texture Measurement

Texture profile analysis was conducted using a CT3 texture analyser, equipped with a 50 N load cell (Ametek Brookfield, Middleboro, USA). From the heated patties, samples with 30 mm diameter were punched out with a cork borer.

Samples were placed between the plate of the texture analyser and a 36 mm diameter cylindrical probe. Device settings were: speed 2 mm/sec., 50 % compression, and 2 seconds between the two cycles.

The following parameters were calculated: hardness (N), toughness (mJ), springiness, chewiness (N). Number of replicates was 4 or 8 (see Tables), and the average ± standard deviation are reported.

2.3.4 *Determination of proximate composition and of Thiobarbituric-Acid-Reactive Substances (TBARS)*

Moisture (drying at 102 °C for 3 h), crude protein (nitrogen content as determined by Kjeldahl method multiplied by 6.25), crude fat (ether extract), ash (wet ashing method) were determined according to German official methods [15–18]. TBARS were determined according to Witte, Krause, & Bailey [19].

Reagents were obtained from Merck (Darmstadt, Germany). Analyses were done in duplicate and the average reported.

2.3.5 *Microbiological Analyses*

A 10-g aliquot of the sample was placed in sterile bags, and nine parts of Maximum Recovery Diluent (MRD) (Oxoid, Basingstoke, UK) were added. Homogenisation of the sample was achieved by a Stomacher-type blender (Interscience, St. Nom, France) and subsequently, serial tenfold dilutions were prepared in MRD. Samples were subjected to the following analyses: Total Mesophilic Aerobic Bacteria (TMAB) (according to ISO 4833-1:2013 [20] on Plate Count Agar, Merck, Darmstadt, Germany; incubated for 72 h at 30 °C), *Enterobacteriaceae* count (EB) (according to ISO 21528-2:2017 [21] on Violet Red Bile Glucose Agar, Merck; incubated for 24 h at 37 °C) and for *Pseudomonas* (PS) (GSP agar, Merck; incubated for 72 h at 25 °C) [22]. The number of colony-forming-units (cfu) per gram was converted to Log cfu/g.

2.4 *Statistical Analyses*

Depending on the experimental design, T-test (pairwise comparison) or ANOVA (with PPE or NaCl concentration as independent factors) with Tukey´s post-hoc test to discriminate among means were used

(Statgraphics 3.0, Statgraphics Technologies, The Plains, USA). Statistical significance was established at $p<0.05$.

3. *Results*

3.1 Characteristics of wild boar meat patties with 0.5, 1 and 2 % PPE added, when stored for 1 or 3 days and then subjected to heat treatment

3.1.1 Proximate composition

Proximate composition of fresh meat patties was studied in trial 2 and results are given in Table 1. Addition of PPE resulted in a decrease of moisture, with significantly lower moisture in samples with 2 % PPE. Crude protein, crude fat and ash were not significantly affected.

Table 1. Proximate composition of raw wild boar meat patties, trial 2, with no or 0.5, 1 and 2 % polyphenol-extract (PPE) added.

PPE (g/100g)	Moisture (g/100g)	Crude protein (g/100g)	Crude fat (g/100g)	Ash (g/100g)
0	$72.2^{c} \pm 0.3$	21.4 ± 0.3	4.2 ± 0.3	1.2 ± 0.2
0.5	$72.1^{c} \pm 0.3$	21.4 ± 0.2	4.3 ± 0.3	1.2 ± 0.2
1	$71.6^{b} \pm 0.3$	21.3 ± 0.2	4.3 ± 0.2	1.1 ± 0.1
2	$70.9^{a} \pm 0.3$	21.0 ± 0.2	4.2 ± 0.3	1.2 ± 0.1

Samples were tested after manufacture, before storage. Each value represents the average of 3 measurements. Within columns, different superscripts indicate statistically significant differences, $p<0.05$.

3.1.2 Colour

Raw patties with 1 and 2 % PPE (trial 1) were tested after 24 h cold storage and demonstrated somewhat lower lightness (L*), and lower redness (a*) and yellowness (b*) than the control prepared without addition of PPE (Table 2). A $\Delta E \geq 2$ was observed only when 2% PPE

had been added. Similar results were obtained when the experiment was repeated with 0, 0.5, 1 and 2 % PPE (trial 2), see Table 3. In no case, ΔE was ≥5. In both experiments, the colour parameters L*, a* and b* in patties with 2 % PPE were significantly different from those of controls ($p<0.05$).

Table 2. Colour of raw wild boar meat patties, trial 2, with no or 2 % polyphenol-extract (PPE) added, after 24 h storage at 3 °C (vacuum-package).

PPE (g/100g)	L*	a*	b*	ΔE (compared to 0 %)
0	39.24^{b} ± 3.77	15.44^{b} ± 2.00	11.90^{b} ± 0.94	-
1	38.65^{b} ± 2.37	15.07^{b} ± 1.19	11.50^{b} ± 0.94	0.80
2	37.40^{a} ± 2.41	14.14^{a} ± 1.27	10.52^{a} ± 0.94	2.58

Samples were tested after manufacture, before heating. Each value represents the average of 20 measurements. Within columns, different superscripts indicate statistically significant differences, $p<0.05$.

Table 3. Colour of raw wild boar meat patties, trial 1, with different amounts of polyphenol-extract (PPE) added, after 24 h storage at 3 °C (vacuum-package).

PPE (g/100g)	L*	a*	b*	ΔE (compared to 0 %)
0	41.17^{b} ± 3.11	14.19^{c} ± 1.56	12.70^{b} ± 1.43	-
0.5	40.82^{b} ± 3.36	13.68^{bc} ± 1.46	12.69^{b} ± 0.88	0.61
1	41.43^{b} ± 3.64	12.90^{ab} ± 1.63	12.11^{ab} ± 1.18	1.45
2	38.00^{a} ± 2,49	12.78^{a} ± 0.96	11.97^{a} ± 0.69	3.55

Samples were tested after manufacture, before heating. Each value represents the average of 20 measurements. Within columns, different superscripts indicate statistically significant differences, $p<0.05$.

3.1.3 *pH and water activity*

Water activity was 0.97 ± 0.00 in the control group (0 % PPE) and the same results were found in the group with 2 % PPE added. The pH in the control group (5.45 ± 0.02) was significantly lower than in in the group with 2 % PPE added (5.60 ± 0.01), $p<0.05$. Measurements were done after 24 h storage at 3 °C (vacuum-package).

3.1.4 *TBARS*

TBARS were determined in patties after 1 day or 3 days vac.-storage at 3 °C, followed by heating and subsequent cold storage for 1 day (Tables 4, 5). Addition of 0.5 or more per cent PPE significantly retarded fat oxidation, as assessed by TBARS.

Table 4. TBARS in wild boar meat patties, batch 1, with 1 or 2 % of polyphenol-extract (PPE) added.

PPE (g/100g)	TBARS (mg MDA/kg)	TBARS (mg MDA/kg), 1 day vac.- storage at 3 °C, then heated and stored for 1 day at 3 °C	TBARS (mg MDA/kg), 3 days vac.- storage at 3 °C, then heated and stored for 1 day at 3 °C
0	0.09 ± 0.02	0.78^{b} ± 0.10	7.65^{b} ± 0.65
1	Nd	0.11^{a} ± 0.04	0.22^{a} ± 0.01
2	Nd	0.19^{a} ± 0.05	0.15^{a} ± 0.02

Each value represents the average of 3 measurements. Nd = not tested. Within columns, different superscripts indicate statistically significant differences, $p<0.05$.

Table 5. TBARS in wild boar meat patties, trial 2, with 0.5 to 2 % of polyphenol-extract (PPE) added.

PPE (g/100g)	TBARS (mg MDA/kg)	TBARS (mg MDA/kg), 1 day vac.- storage at 3 °C, then heated and stored for 1 day at 3 °C	TBARS (mg MDA/kg), 3 days vac.- storage at 3 °C, then heated and stored for 1 day at 3 °C
0	0.12 ± 0.04	1.00^{b} ± 0.16	3.26^{c} ± 0.65
0.5	Nd	0.14^{a} ± 0.02	0.28^{b} ± 0.02
1	Nd	0.15^{a} ± 0.03	0.14^{a} ± 0.02
2	Nd	0.17^{a} ± 0.05	0.19^{a} ± 0.04

Each value represents the average of 3 measurements. Nd = not tested. Within columns, different superscripts indicate statistically significant differences, $p<0.05$.

3.2 Characteristics of raw wild boar meat patties with 0.5, 1 and 2 % PPE added, when stored for 3 or 5 days

3.2.1 Colour

Colour data of raw patties stored for 3- or 5-days vacuum-packaged at 3 °C (trial 3) are shown in Table 6. Samples with PPE demonstrated lower redness (a*) and yellowness (b*) than the control prepared without addition of PPE. In stored samples, some statistically significant changes in colour parameters were observed. However, ΔE values were consistently below 2.

1.1.1 Microbiology

Development of the microflora of raw patties stored for 3- or 5-days vacuum-packaged at 3 °C is shown in Table 7. Generally, samples with 2 % PPE added demonstrated significantly ($p<0.05$) lower numbers of Total Mesophilic Aerobic Bacteria and *Enterobacteriaceae* than controls (0 % PPE), but the differences were in the order of magnitude of merely 0.7 log cycles. Similar results were observed for *Pseudomonas* in samples stored 3 days. The results indicate that even an addition of 2 % PPE would have only limited antimicrobial effects.

Table 6. Colour of raw wild boar meat patties (trial 3), with different amounts of polyphenol-extract (PPE) added, stored for 3 or 5 days vacuum-packaged at 3 °C.

PPE (g/100g)	Day 0				Day 3				Day 5			
	L*	a*	b*	ΔE^1	L*	a*	b*	ΔE^1	L*	a*	b*	ΔE^1
0	41.56 ± 2.65	14.59^{c} ± 1.15	13.65^{c} ± 0.75		42.49^{b} ± 1.91	13.78 ± 1.56	13.50 ± 0.94		42.88 ± 3.06	15.09^{ab} ± 1.19	14.94^{b} ± 0.49	
0.5	41.15 ± 1.23	13.08^{b} ± 0.75	13.05^{b} ± 0.56	1.67	42.37^{b} ± 1.69	13.29 ± 1.12	13.27 ± 0.62	0.56	42.66 ± 0.97	14.55^{a} ± 0.69	14.33^{ab} ± 0.74	0.85
1	40.69 ± 1.54	12.83^{b} ± 0.68	13.18^{b} ± 0.65	2.02	42.13^{ab} ± 1.85	14.16 ± 1.46	13.61 ± 0.64	0.53	42.77 ± 1.90	15.27^{b} ± 0.91	14.52^{a} ± 0.69	0.47
2	40.91 ± 1.42	11.67^{a} ± 0.61	12.45^{a} ± 0.59	3.20	40.91^{a} ± 2.24	13.73 ± 0.84	13.30 ± 0.65	1.60	42.49 ± 2.16	15.13^{ab} ± 0.68	14.03^{a} ± 0.84	1.66

Each value represents the average of 15 (3 patties with 5 measurements each) measurements. [1] = compared to the 0 % PPE group. Within columns, different superscripts indicate statistically significant differences, $p<0.05$.

Table 7. Microflora of raw wild boar meat patties, trial 3, with different amounts of polyphenol-extract (PPE) added, stored for 3 for 3 days vacuum-packaged at 3 °C.

PPE (g/100g)	TMAB (log cfu/g)			*Enterobacteriaceae* (log cfu/g)			*Pseudomonas* (log cfu/g)		
	fresh	3 days vac.-storage, 3 °C	5 days vac.-storage, 3 °C	fresh	3 days vac.-storage, 3 °C	5 days vac.-storage, 3 °C	fresh	3 days vac.-storage, 3 °C	5 days vac.-storage, 3 °C
0	6.12 ± 0.24	$6.96^{b} \pm 0.09$	$7.20^{b} \pm 0.11$	2.91 ± 0.41	$3.02^{a} \pm 0.29$	$3.43^{b} \pm 0.10$	3.20 ± 0.35	$3.68^{b} \pm 0.13$	3.61 ± 1.56
0.5	Nd	$6.46^{ab} \pm 0.42$	$6.91^{a} \pm 0.16$	Nd	$2.64^{ab} \pm 0.57$	$2.98^{ab} \pm 0.19$	Nd	$3.34^{ab} \pm 0.23$	3.39 ± 1.12
1	Nd	$6.45^{ab} \pm 0.29$	$6.89^{a} \pm 0.15$	Nd	$2.92^{ab} \pm 0.28$	$3.06^{ab} \pm 1.09$	Nd	$3.37^{ab} \pm 02.0$	3.61 ± 1.46
2	Nd	$6.25^{a} \pm 0.25$	$6.78^{a} \pm 0.11$	Nd	$2.30^{a} \pm 0.02$	$2.33^{a} \pm 0.35$	Nd	$2.96^{a} \pm 0.42$	3.18 ± 0.84

Samples were tested after manufacture, before heating. Nd = not determined. Each value represents the average of 3 measurements. Within columns, different superscripts indicate statistically significant differences, $p<0.05$. TMAB = Total Mesophilic Aerobic Bacteria

1.1.2 TBARS

Development of TBARS of raw patties stored for 3 or 5 days vacuum-packaged at 3 °C is shown in Table 8. Addition of 0.5 or more per cent PPE effectuated a significant reduction of TBARS, more pronounced in heated-stored samples than in non-heated samples.

Table 8. TBARS in wild boar meat patties, trial 3, with 0.5 to 2 % of polyphenol-extract (PPE) added.

PPE (g/100g)	TBARS (mg MDA/kg)	TBARS (mg MDA/kg), vac.-storage at 3 °C		TBARS (mg MDA/kg), vac.-storage for 3 or 5 days at 3 °C, then heated and stored for 1 day at 3 °C	
	fresh	3 days	5 days	3 days vac.	5 days vac
0	0.10 ± 0.04	0.26^{b} ± 0.01	0.31^{b} ± 0.02	2.01^{b} ± 0.18	1.44^{b} ± 0.29
0.5	Nd	0.22^{a} ± 0.01	0.29^{ab} ± 0.01	0.57^{a} ± 0.05	0.58^{a} ± 0.05
1	Nd	0.22^{a} ± 0.01	0.27^{a} ± 0.02	0.46^{a} ± 0.09	0.45^{a} ± 0.04
2	Nd	0.23^{a} ± 0.01	0.25^{a} ± 0.02	0.58^{a} ± 0.06	0.53^{a} ± 0.05

Each value represents the average of 3 measurements. Nd = not tested. Within columns, different superscripts indicate statistically significant differences, $p<0.05$.

1.2 Assessment of the minimum PPE concentration exhibiting antioxidant effects

Although concentrations of 0.1 % PPE exhibited a statistically significant antioxidant effect on heated-stored patties (Table 9), a marked reduction was observed when 0.25 or 0.5 % had been added.

Table 9. TBARS in wild boar meat patties, trials 4 & 5, with 0.01 to 2 % of polyphenol-extract (PPE) added.

	TBARS (mg MDA/kg)	TBARS (mg MDA/kg), vac.-storage for 3 or 5 days at 3 °C, then heated and stored for 1 day at 3 °C	
% PPE	Day 0	3 days vac.	5 days vac.
0	0.083 ± 0.02	1.82^{c} ± 0.18	1.94^{c} ± 0.16
0.01	Nd	1.64^{c} ± 0.09	1.65^{bc} ± 0.07
0.05	Nd	1.20^{b} ± 0.15	1.34^{ab} ± 0.17
0.1	Nd	0.91^{a} ± 0.12	1.14^{a} ± 0.32
% PPE	Day 0	3 days vac.	5 days vac.
0	Nd	1.31^{c} ± 0.08	1.26^{c} ± 0.12
0.1	Nd	0.83^{b} ± 0.06	0.90^{b} ± 0.08
0.25	Nd	0.52^{a} ± 0.03	0.64^{a} ± 0.01
0.5	Nd	0.45^{a} ± 0.02	0.56^{a} ± 0.02

Each value represents the average of 3 measurements. Nd = not tested. Within columns, different superscripts indicate statistically significant differences, $p<0.05$.

For 0.25 % PPE, we studied pH and water activity compared to a control (0 % PPE). The pH of freshly prepared burgers was 5.45 ± 0.03 when no PPE had been added and 5.44 ± 0.03 when 0.25 % PPE had been added; corresponding figures for water activity were 0.98 ± 0.00 and 0.97 ± 0.00, with no statistically significant difference ($p > 0.05$).

1.3 Characteristics of raw wild boar meat patties with PPE and NaCl added

1.3.1 pH and water activity

Addition of PPE and NaCl effectuated a rise in pH, and lowered the water activity to a limited extent (0.01 units). Rise in pH was more pronounced (0.15 units) in samples stored for 3 days, see Table 10.

Table 10. pH and water activity wild boar meat patties, trial 6, with various amounts of polyphenol-extract (PPE) and NaCl added, vac.-storage for 3 or 5 days at 3 °C.

NaCl (g/100g)	PPE (g/100g)	3 days		5 days	
		pH	a_w	pH	a_w
0	0	$5.45^{a} \pm 0.02$	$0.97^{b} \pm 0.01$	$5.49^{a} \pm 0.01$	$0.97^{b} \pm 0.01$
1.5	0	$5.59^{b} \pm 0.01$	$0.96^{a} \pm 0.01$	$5.59^{d} \pm 0.01$	$0.96^{a} \pm 0.01$
0	2	$5.60^{b} \pm 0.01$	$0.96^{a} \pm 0.01$	$5.51^{b} \pm 0.02$	$0.96^{a} \pm 0.01$
1.5	2	$5.61^{b} \pm 0.01$	$0.96^{a} \pm 0.01$	$5.54^{c} \pm 0.01$	$0.96^{a} \pm 0.01$

Each value represents the average of 3 measurements. Within columns, different superscripts indicate statistically significant differences, $p<0.05$.

1.3.2 Microbiology

Development of the microflora of raw patties stored for 3- or 5-days vacuum-packaged at 3 °C is shown in Table 11. Generally, samples with 2 % PPE or 2 % PPE and 1.5 % NaCl added demonstrated significantly ($p<0.05$) lower numbers of Total Mesophilic Aerobic Bacteria, *Pseudomonas* and Enterobacteriaceae than controls (0 % PPE, 0 % NaCl), with differences were in the order of magnitude of 1 log cycle in samples stored for 5 days.

Table 11. Microbiota in wild boar meat patties, trial 6, with various amounts of polyphenol-extract (PPE) and NaCl added, vac.-storage for 3 and 5 days at 3 °C.

NaCl g/100g	PPE g/100g	3 days			5 days		
		TMAB log cfu/g	PS log cfu/g	EB log cfu/g	TMAB log cfu/g	PS log cfu/g	EB log cfu/g
0	0	7.03^{c} ± 0.39	4.07^{b} ± 0.13	3.63^{b} ± 0.34	8.25^{c} ± 0.12	4.53^{b} ± 0.09	4.02^{c}± 0.02
1.5	0	7.03^{c} ± 0.25	4.07^{b} ± 0.05	3.63^{b} ± 0.10	7.53^{b} ±0.14	4.28^{b} ± 0.14	3.86^{c} ± 0.04
0	2	6.66^{b} ± 0.51	3.64^{a} ± 0.11	3.33^{a} ± 0.05	7.34^{b} ±0.04	3.61^{a} ± 0.10	3.42^{b} ± 0.05
1.5	2	6.47^{a} ± 0.06	3.55^{a} ± 0.02	3.10^{a} ± 0.22	7.00^{a} ±0.07	3.53^{a} ± 0.07	3.04^{a} ± 0.07

TMAB = Total Mesophilic Aerobic Bacteria, PS = *Pseudomonas*, EB = *Enterobacteriaceae*; each value represents the average of 3 measurements. Within columns, different superscripts indicate statistically significant differences, $p<0.05$.

1.3.3 Texture Profyle Analyses

Addition of 1.5 % NaCl effectuated significantly higher results for hardness, toughness and chewiness, whereas addition of 2 % PPE had no significant effect on textural properties (Table 12).

Table 12. Textural characteristics of wild boar meat patties, trial 6, with various amounts of polyphenol-extract (PPE) and NaCl added, vac.-storage for 5 days at 3 °C, then heated and stored for 1 day at 3 °C.

NaCl (g/100g)	PPE (g/100g)	Hardness (N)	Toughness (mJ)	Springiness	Chewiness (N)
0	0	$99.6^{a} \pm 4.4$	$350.0^{a} \pm 29.6$	$0.7^{a} \pm 0.1$	$37.5^{a} \pm 3.0$
1.5	0	$116.3^{b} \pm 4.6$	$547.2^{b} \pm 15.5$	$0.9^{b} \pm 0.1$	$47.3^{b} \pm 3.6$
0	2	$82.1^{a} \pm 4.1$	$329.8^{a} \pm 22.6$	$0.8^{a} \pm 0.1$	$30.9^{a} \pm 4.8$
1.5	2	$131.7^{b} \pm 2.7$	$688.6^{b} \pm 50.8$	$0.8^{a} \pm 0.1$	$50.6^{b} \pm 2.4$

Each value represents the average of 8 measurements. Within columns, different superscripts indicate statistically significant differences, $p<0.05$.

2. *Discussion*

Our study demonstrated a significant reduction in lipid oxidation in wild boar patties with the addition of polyphenolic extract, especially at concentrations of 0.25% or higher, as indicated by reduced TBARS levels. However, even lower concentrations, specifically as low as 0.05% PPE, also achieved significant reduction in oxidative activity, though not as pronounced as at higher concentrations. This effect highlights the potent antioxidant capacity of PPE, which is crucial for extending shelf life and maintaining meat quality. The reduction in TBARS suggests that PPE efficiently inhibited lipid peroxidation, a primary factor in the quality deterioration of meat during storage. The polyphenolic compounds in PPE, particularly hydroxytyrosol, play a significant role in the antioxidant activity. Hydroxytyrosol and other phenols are known for their ability to scavenge free radicals, interrupting the oxidative chain reaction that leads to lipid degradation [8,23]. Our findings align with those reported by Roila et al. [24], where

beef patties treated with olive mill wastewater polyphenolic extract showed up to 62% lower TBARS levels over seven days compared to untreated controls, highlighting the effectiveness of polyphenolic extracts in reducing lipid oxidation. Furthermore, Martínez-Zamora et al. [25] observed a similar reduction in lipid oxidation when hydroxytyrosol was applied to lamb patties, achieving a 35% decrease in oxidation compared to control samples. Moreover, our data suggest that the antioxidant properties of PPE were effective after heating and subsequent cold storage, simulating realistic consumption scenarios for processed meat products.

The addition of 2% PPE led to differences in the physicochemical properties of wild boar patties, particularly a slight reduction in moisture content and colour changes. Specifically, these changes included decreased lightness, redness, and yellowness in patties with 2% PPE compared to the control group. These variations were perceptible to consumers ($\Delta E > 3$) only on the day of production and after 24 hours of refrigerated storage. Textural properties were not significantly affected by the addition of 2 % PPE. Martínez-Zamora et al. [25] also evaluated the effects of polyphenolic extracts in lamb patties, specifically rosemary extract and hydroxytyrosol extracted from olive tree vegetation water. They found that these extracts did not affect the chemical composition of the patties but did result in a reduction in both redness and yellowness indices for up to three days of storage.

The use of polyphenolic extracts appears to be a promising strategy for the production of meat products, aiming to reduce lipid oxidation and preserve quality traits. This can be achieved either by incorporating polyphenols directly into the product, such as patties, or by developing packaging materials that contain polyphenols. For instance, Moudache

et al. [26] demonstrated the positive effects of a plastic film material containing olive leaf extract when applied to fresh minced meat.

Regarding antimicrobial activity, the inclusion of PPE showed a limited effect. The use of a 2% concentration of PPE in wild boar patties led to a slight reduction in TMAB and EB compared to the control, observed at both 3 and 5 days of refrigerated storage, while the reduction of PS was noted only after 3 days of storage. Similar limited antimicrobial effects of polyphenols were noted in a previous study on game meat by Altissimi et al. [9], despite variations in concentrations, application methods, and storage times. While the antimicrobial effects of PPE were modest, this aligns with previous findings indicating that polyphenols generally exert mild antibacterial activity in beef meat products [24]. Notably, some studies suggest that using different concentrations of polyphenols or combining them with other natural antimicrobials, such as essential oils or organic acids, may offer a strategic approach to enhance the antimicrobial efficacy [4,27].

The combination of polyphenolic extract (PPE) with sodium chloride (NaCl) further improved the microbiological stability of the patties, effectively reducing microbial spoilage. The higher values for hardness and other textural properties in patties manufactured with NaCl are in agreement with sensory assessments ("firmness") of coarse meat patties manufactured varying amounts of NaCl [28].

NaCl is known to have antimicrobial effects due to osmotic pressure, and its combination with PPE appears to create a synergistic effect that enhances bacterial inhibition. The synergistic action of salt and natural antimicrobials has been studied and appears to be a promising strategy to enhance the preservative effect [29]. This approach allows for a reduction in the concentration of plant-derived extracts, which at high levels could negatively affect the sensory characteristics of some

products, while also decreasing the use of salt in certain meat products, thereby ensuring the safety and quality of meat products [29].

3. *Conclusion*

The use of polyphenolic extract derived from olive mill vegetation water improved the oxidative stability of wild boar patties, confirming their potent antioxidant activity even at low concentrations. The incorporation of polyphenols in meat appears to be a promising approach to reduce the use of synthetic additives and extend the shelf life of these products. However, further research is needed to determine the optimal concentrations or combinations with other natural substances to enhance the microbiological and physico-chemical quality effectively.

References

1. Needham, T.; Bureš, D.; Černý, J.; Hoffman, L.C. Overview of Game Meat Utilisation Challenges and Opportunities: A European Perspective. *Meat Sci.* **2023**, *204*, 109284, doi:10.1016/j.meatsci.2023.109284.
2. Niewiadomska, K.; Kosicka-Gębska, M.; Gębski, J.; Gutkowska, K.; Jeżewska-Zychowicz, M.; Sułek, M. Game Meat Consumption—Conscious Choice or Just a Game? *Foods* **2020**, *9*, 1357, doi:10.3390/foods9101357.
3. Di Bella, S.; Branciari, R.; Haouet, N.M.; Framboas, M.; Mercuri, M.L.; Codini, M.; Roila, R.; Malimpensa, A.; Ranucci, D. Does Hunted Wild Boar Meat Meet Modern Consumer Nutritional Expectations? *Ital. J. Food Saf.* **2024**, doi:10.4081/ijfs.2024.11608.
4. Bouarab Chibane, L.; Degraeve, P.; Ferhout, H.; Bouajila, J.; Oulahal, N. Plant Antimicrobial Polyphenols as Potential Natural Food Preservatives. *J. Sci. Food Agric.* **2019**, *99*, 1457–1474, doi:10.1002/jsfa.9357.
5. Stagos, D. Antioxidant Activity of Polyphenolic Plant Extracts. *Antioxidants* **2020**, *9*, 19, doi:10.3390/antiox9010019.
6. Martínez, L.; Ros, G.; Nieto, G. Hydroxytyrosol: Health Benefits and Use as Functional Ingredient in Meat. *Medicines* **2018**, *5*, 13, doi:10.3390/medicines5010013.
7. Balzan, S.; Taticchi, A.; Cardazzo, B.; Urbani, S.; Servili, M.; Di Lecce, G.; Zabalza, I.B.; Rodriguez-Estrada, M.T.; Novelli, E.; Fasolato, L. Effect of Phenols Extracted from a By-Product of the Oil Mill on the Shelf-Life of Raw and Cooked Fresh Pork Sausages in the Absence of Chemical Additives. *LWT - Food Sci. Technol.* **2017**, *85*, 89–95, doi:10.1016/j.lwt.2017.07.001.

8. Galanakis, C.M. Phenols Recovered from Olive Mill Wastewater as Additives in Meat Products. *Trends Food Sci. Technol.* **2018**, *79*, 98–105, doi:10.1016/j.tifs.2018.07.010.
9. Altissimi, C.; Roila, R.; Ranucci, D.; Branciari, R.; Cai, D.; Paulsen, P. Preventing Microbial Growth in Game Meat by Applying Polyphenolic Extracts from Olive Mill Vegetation Water. *Foods* **2024**, *13*, 658, doi:10.3390/foods13050658.
10. Csadek, I.; Vankat, U.; Schrei, J.; Graf, M.; Bauer, S.; Pilz, B.; Schwaiger, K.; Smulders, F.J.M.; Paulsen, P. Treatment of Ready-To-Eat Cooked Meat Products with Cold Atmospheric Plasma to Inactivate Listeria and Escherichia Coli. *Foods* **2023**, *12*, 685, doi:10.3390/foods12040685.
11. CIE (Commission Internationale de L'Éclairage (International Commission on Illumination) Recommendations on Uniform Color Spaces, Color-Difference Equations, Psychometric Color Terms; Commission Internationale de l'Eclairage: Paris, France, 1978.
12. Upton, S. Delta E: The Color Difference. Available online: https://www.colorwiki.com/wiki/Delta_E:_The_Color_Difference.
13. Upton, S. Delta E: The Color Difference. Available online: http://www.chromix.com/ColorNews/ (accessed on 21 November 2024).
14. Altmann, B.A.; Gertheiss, J.; Tomasevic, I.; Engelkes, C.; Glaesener, T.; Meyer, J.; Schäfer, A.; Wiesen, R.; Mörlein, D. Human Perception of Color Differences Using Computer Vision System Measurements of Raw Pork Loin. *Meat Sci.* **2022**, *188*, 108766, doi:10.1016/j.meatsci.2022.108766.

15. Amtliche Sammlung von Untersuchungsverfahren Nach §64 LFGB, Untersuchung von Lebensmitteln - Bestimmung Der Asche in Fleisch, Fleischerzeugnissen Und Wurstwaren - Gravimetrisches Verfahren (Referenzverfahren), 2017, L06.00-4; Beuth: Berlin.
16. Amtliche Sammlung von Untersuchungsverfahren Nach §64 LFGB, Untersuchung von Lebensmitteln - Bestimmung Des Gesamtfettgehaltes in Fleisch Und Fleischerzeugnissen - Gravimetrisches Verfahren Nach Weibull-Stoldt – Referenzverfahren, 2014, L06.00-6; Beuth: Berlin.
17. Amtliche Sammlung von Untersuchungsverfahren Nach §64 LFGB, Untersuchung von Lebensmitteln - Bestimmung Des Rohproteingehaltes in Fleisch Und Fleischerzeugnissen; Titrimetrisches Verfahren Nach Kjeldahl; Referenzverfahren, 2014, L. 06.00-7; Beuth: Berlin.
18. Amtliche Sammlung von Untersuchungsverfahren Nach §64 LFGB, Untersuchung von Lebensmitteln - Bestimmung Des Wassergehaltes in Fleisch Und Fleischerzeugnissen - Gravimetrisches Verfahren – Referenzverfahren, 2014, L06.00-3; Beuth, Berlin.
19. Witte, V.C.; Krause, G.F.; Bailey, M.E. A New Extraction Method for Determining 2-Thiobarbituric Acid Values of Pork and Beef During Storage. *J. Food Sci.* **1970**, *35*, 582–585, doi:10.1111/j.1365-2621.1970.tb04815.x.
20. Microbiology of the Food Chain — Horizontal Method for the Enumeration of Microorganisms — Part 2: Colony Count at 30 °C by the Surface Plating Technique. ISO 4833-2:2013.

21. Microbiology of the Food Chain — Horizontal Method for the Detection and Enumeration of Enterobacteriaceae — Part 2: Colony-Count Technique. ISO 21528-2:2017.
22. Kielwein, G. Ein Nährboden Zur Selektiven Züchtung von Pseudomonaden Und Aeromonaden. *Archiv für Lebensmittelhygiene* **1969**, *20*, 131–133.
23. Papuc, C.; Goran, G.V.; Predescu, C.N.; Nicorescu, V.; Stefan, G. Plant Polyphenols as Antioxidant and Antibacterial Agents for Shelf-Life Extension of Meat and Meat Products: Classification, Structures, Sources, and Action Mechanisms. *Compr. Rev. Food Sci. Food Saf.* **2017**, *16*, 1243–1268, doi:10.1111/1541-4337.12298.
24. Roila, R.; Sordini, B.; Esposto, S.; Ranucci, D.; Primavilla, S.; Valiani, A.; Taticchi, A.; Branciari, R.; Servili, M. Effect of the Application of a Green Preservative Strategy on Minced Meat Products: Antimicrobial Efficacy of Olive Mill Wastewater Polyphenolic Extract in Improving Beef Burger Shelf-Life. *Foods* **2022**, *11*, 2447, doi:10.3390/foods11162447.
25. Martínez-Zamora, L.; Ros, G.; Nieto, G. Synthetic vs. Natural Hydroxytyrosol for Clean Label Lamb Burgers. *Antioxidants* **2020**, *9*, 851, doi:10.3390/antiox9090851.
26. Moudache, M.; Nerín, C.; Colon, M.; Zaidi, F. Antioxidant Effect of an Innovative Active Plastic Film Containing Olive Leaves Extract on Fresh Pork Meat and Its Evaluation by Raman Spectroscopy. *Food Chem.* **2017**, *229*, 98–103, doi:10.1016/j.foodchem.2017.02.023.
27. Rodríguez Vaquero, M.J.; Aredes Fernández, P.A.; Manca de Nadra, M.C.; Strasser de Saad, A.M. Phenolic Compound Combinations on Escherichia Coli Viability in a Meat System.

J. Agric. Food Chem. **2010**, *58*, 6048–6052, doi:10.1021/jf903966p.

28. Ruusunen, M.; Vainionpää, J.; Lyly, M.; Lähteenmäki, L.; Niemistö, M.; Ahvenainen, R.; Puolanne, E. Reducing the sodium content in meat products: The effect of the formulation in low-sodium ground meat patties. *Meat Science* **2005,** *69(1)*, 53-60. doi: 10.1016/j.meatsci.2004.06.005.
29. Santiesteban-López, N.A.; Gómez-Salazar, J.A.; Santos, E.M.; Campagnol, P.C.B.; Teixeira, A.; Lorenzo, J.M.; Sosa-Morales, M.E.; Domínguez, R. Natural Antimicrobials: A Clean Label Strategy to Improve the Shelf Life and Safety of Reformulated Meat Products. *Foods* **2022**, *11*, 2613, doi:10.3390/foods11172613.

Chapter 3 – General conclusions

Wild boars pose a potential risk to both public health and the livestock sector. This population serves as a reservoir for pathogens, including zoonoses, which can represent a serious threat to the entire national swine industry. Population control relies almost exclusively on hunting, and hunting methods significantly affect the quality of the meat. From a regulatory standpoint, there are notable gaps in the safety and hygienic criteria for game carcasses and meat, making it difficult to establish quality standards. These criteria could both ensure consumer safety and support the development of local supply chains based on high-quality products.

The application of bioactive compounds from agro-industrial by-products to game meat could improve its quality characteristics, enhancing its value while simultaneously addressing the issue of managing and reducing the disposal of potentially polluting by-products.

The studies conducted in this thesis demonstrated that extracts derived from food industry by-products, particularly those from the olive oil industry, could be effectively used in the meat production chain as natural preservatives. These extracts offer a promising alternative to synthetic additives due to their antioxidant and antimicrobial properties. However, the efficacy of such extracts is strongly influenced by several factors, including the extraction techniques for bioactive compounds, the concentration used, the application method, and the type of matrix on which they are applied.

Polyphenols exhibited highly variable antimicrobial effects. *In vitro*, they showed bactericidal activity, whereas their application to meat

products generally resulted in a limited reduction of the growth of some microbial populations, particularly in meat with a high initial hygienic level.

Polyphenolic extracts from the olive oil industry also proved to be excellent antioxidants, even at low concentrations. Therefore, they have a significant potential for a widespread use in the food industry as natural preservatives, aligning with modern consumer demands and market trends for sustainable solutions.

Further research could explore the application of polyphenolic extracts derived from the olive oil industry directly on wild boar carcasses. These studies could assess their impact on the hygienic quality of the carcasses and, subsequently, on the shelf life of the meat. If positive effects are observed, these extracts could be further evaluated and potentially approved for application on carcasses through methods such as spray treatments or washing. Such applications could help prevent or reduce microbial contamination effectively.

A deeper understanding of these polyphenolic extracts could also involve investigating their effects on a broader range of bacterial populations, as well as their potential activity against specific pathogens such as Hepatitis E virus, *Toxoplasma gondii*, and *Trichinella*.

Beyond their antimicrobial and antioxidant efficacy, the choice of extracts to be tested is primarily influenced and driven by the availability and accessibility of the by-products. Indeed, a significant challenge associated with the use of by-products is their seasonality, as large quantities are typically produced within specific and limited timeframes. Advanced extraction and stabilization techniques could address this issue by preserving these by-products and ensuring their

availability throughout the year.

The research activities performed during this doctoral study primarily focused on the wild boar, as it represents an emerging issue of global concern. However, these studies should be expanded to include other species of wild ungulates, such as roe deer, red deer, and fallow deer, to gain a more comprehensive understanding and address broader challenges in wildlife management and meat production.

This work has been funded by the European Union – PON REACT-EU. However, the views and opinions expressed are those of the authors alone and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the European Commission can be held responsible for them.