UNIVERSITÀ DI PARMA

UNIVERSITA’ DEGLI STUDI DI PARMA

DOTTORATO DI RICERCA IN
SCIENZE MEDICO - VETERINARIE

CICLO XXXVII

The missing process hygiene criteria in the game meat chain: challenges in wild boar meat production

Coordinatore:
Chiar.mo Prof. Gaetano Donofrio

UNIVERSITÀ DI PARMA
Firmato digitalmente da Gaetano Donofrio
Data: 14.03.2025 11:34:34 CET

Tutori:
Chiar.ma Prof.ssa Cristina Bacci
Chiar.ma Prof.ssa Silvia Bonardi

UNIVERSITÀ DI PARMA
Firmato digitalmente da Cristina Bacci
Data: 14.03.2025 09:11:52 CET

UNIVERSITÀ DI PARMA
Firmato digitalmente da Silvia Bonardi
Data: 14.03.2025 09:16:06 CET

Dottoranda: Laura Andriani

Anni Accademici 2021/2022 – 2023/2024

Index

Abstract

Wild boars are one of the most common large wild mammals globally. Their remarkable adaptive capacity and ecological plasticity, as well as human-related factors enable them to achieve high population densities, result in significant environmental, economic, social, and public health challenges. This human-wild boar conflict underscores the need for effective management strategies. Hunting is the most effective control method; it has increased culling rates and meat yields, presenting an opportunity to develop a game meat supply chain with numerous potential benefits. However, wild boars are reservoirs of foodborne pathogens and antimicrobial-resistant bacteria, while the hunting process itself involves variables that can affect carcass contamination. Despite these risks, process hygiene criteria for game meat are not currently defined in Regulation (EC) No 2073/2005.

This study aimed to evaluate the hygiene criteria set for carcasses of livestock species, *i.e.* Aerobic Colony Count (ACC), *Enterobacteriaceae* Count (EntC), and *Salmonell*a, in wild boars hunted in Emilia-Romagna region (Italy). Furthermore, it included *Escherichia coli* as a hygiene indicator by assessing its prevalence, ESβL and carbapenemen- resistance, and pathotype profiles.

During the 2022/2023 and 2023/2024 hunting seasons, a total of 115 sponge samples from wild boar carcasses and 78 diaphragm muscle samples from a proportion of the same animals were collected across eight game-handling establishments (GHEs). Biometric data of the wild boars, along with hunting process variables, including the killing methods, the time between killing, evisceration, and skinning, as well as the carcass storage temperatures, were recorded.

The mean values of ACC and EntC were 4.22 $\log_{10}$ CFU/cm^2 and 2.27 $\log_{10}$ CFU/cm^2, respectively. The prevalence of *Salmonella* was 3.48%, with isolates identified as *Salmonella* Coeln, *Salmonella* Typhimurium, and *Salmonella diarizonae* O:50 (z). Resistance in *Salmonella* was detected only against sulfamethoxazole in one *Salmonella diarizonae* O:50 (z) isolate. Hunting-related factors included the different GHEs, and the time between killing, evisceration, and skinning significantly influenced carcass contamination. The prevalence of *E. coli* was 97.43%. No resistance to third-generation cephalosporins or carbapenem was observed. Concerning, *E. coli* pathotypes, 3.33% were classified as atypical enteropathogenic *E. coli* (aEPEC), 3.33% as enteroaggregative *E. coli* (EAEC), and 93.3% as atypical enteroaggregative *E. coli* (aEAEC). Even in absence of criteria for wild game species, the overall values for ACC, EntC, and the prevalence of *Salmonella* could be deemed “acceptable” within the Regulation (EC) No 2073/2005. Nevertheless, the results of the study emphasize that the interval between killing and evisceration should ideally be kept less than 3 hours, with evisceration to skinning not exceeding 10 hours. Completing the entire process within 4 hours, however, is preferable. Furthermore, our results confirm that wild boars serve as reservoirs for pathogens such as *Salmonella* and pathogenic *E. coli*. This study underscores the need for standardized

microbiological hygiene criteria at the EU level for game meat, as well as integrated control measures throughout the hunting process.

1. Introduction

Pigs are one of the few domesticated species whose wild counterparts, wild boars (*Sus scrofa*), continue to thrive in natural environments. Wild boars are one of the most widespread large mammals globally. Native to Eurasia, they are now present in every continent except Antarctica, due to numerous human introductions as well as their remarkable adaptive capacity (Keuling et al., 2017). The ecological plasticity and high reproductive potential enable them to reach high population densities within a very short period of time, resulted in numerous economic, environmental, and social problems. Wild ungulates are key components of biological communities and can influence biodiversity through effects in all trophic levels, as well as regulating of carnivore abundance (Calosi et al., 2024).

Wild boars are identified as one of the "100 World's Worst Invasive Alien Species" as they have highly destructive behaviours which broadly impact ecosystems (Risch et al., 2021). Depending on the habitat type, wild boars may carry out different trophic functions, acting as crop pests, frugivores, predators, destroyers of seed banks and plant dispersers (Ballari et al., 2014).

It is expected that as wild boar populations continue to grow and spread, threats to native wildlife will also increase. Nevertheless, in Europe, increasing numbers of wild boar sightings were reported also in urban and suburban areas, for instance in Berlin, Barcelona, Rome, Vilnius, Budapest, Genoa, and Warsaw (Massei at al., 2015).

Wild boars pose significant conflicts with human activities and well-being, including the risk of disease transmission to humans, livestock, and other domestic animals, as well as road collisions with vehicles and damage to crops and infrastructure. Furthermore, they contribute to the reduction of plant and animal abundance and richness. For these reasons, wild boars are considered environmental pests in many countries. The mitigation of the ecological and economic impacts of wild boars presents one of the most challenging issues in wildlife management worldwide (Fattorini et al., 2020).

Management strategies for wild boar populations are diverse, including fencing, traps, chemical sterilization, and hunting, the latter being the most widely implemented approach worldwide (Massei et al., 2011). Furthermore, hunting techniques vary significantly across different regions and cultural contexts. In Italy, the wild boar population has rapidly expanded in recent years, with an estimated 1.5 million individuals. Driven hunting is the most commonly implemented management strategy (ISPRA, 2023).

As a result, the availability of meat from culled wild ungulates is increasing rapidly. Wild boar meat provides a renewable and locally sourced protein option, serving as a sustainable alternative to pork (Macháčková et al., 2021). Game meat is often considered organic, free from antibiotics, and environmentally sustainable. Consequently, the growing demand for sustainable food sources, along with an increasing awareness of the health benefits associated with game meat, emphasizes the need to establish commercial game meat markets (von Essen, 2018).

Wild boar meat is characterized by favourable nutritional properties, primarily due to its low fat content and beneficial fatty acid composition (Lestingi, 2023). Although the organoleptic and microbiological quality of wild boar meat can be excellent, it varies significantly based on the conditions under which the animals are hunted, handled, and processed. The European regulations, including the Hygiene Package 2004 and Regulation (EC) No 178/2002, establish proper hygiene practices and delineate the responsibilities of hunters concerning meat safety. These regulations also mandate official veterinary inspections at designated game-handling establishments (GHE) and ensure the traceability of game meat (European Commission, 2004a; 2004b; 2019; 2002). However, considering the natural behaviour of wild boars and the numerous variables associated with the hunting process, the risk of foodborne diseases linked to the consumption of wild ungulate meat cannot be entirely eliminated. Furthermore, Regulation (EC) No 2073/2005, which establishes microbiological criteria for certain microorganisms in foodstuffs, does not specify criteria for hunted wild game carcasses and meat (Lestingi, 2023). Notably, wild boars act as reservoirs for foodborne zoonotic pathogens-as for *Salmonella* spp. and pathogenic strains of *Escherichia coli* (Swift et al., 2019). Furthermore, the indiscriminate use of antimicrobials in both human and veterinary medicine, combined with widespread urbanization and human activities, contributes to the rising development of antimicrobial resistance in wildlife. Consequently, wild boars can also harbour antimicrobial-resistant bacteria. For all these reasons, implementing surveillance and monitoring programs is essential to effectively adopt a One Health approach (Torres et al., 2020).

2. Wild boars: biology, distribution, and ecological and human impact

2.1 Characteristics, habitat and behaviours of wild boars

The term ‘wild swine’ refers to a group of biotypes classified under the species *Sus scrofa*, which includes: various pure biotypes of Eurasian wild boar, the original form; hybrid biotypes resulting from cross-breeding between domestic pigs and pure Eurasian wild boar, and feral biotypes that have originated from domestic pig escapes (Ruiz-Fons, 2017).

Wild boars are ungulates belonging to the family of *Suidae* and order of *Artyodactyla*, placental mammals with hooves (Figure 1). The natural range of wild boars extends from Western Europe and the Mediterranean basin to Eastern Russia, Japan, and Southeast Asia (Massei et al., 2015). Their introduced range includes the Americas, Africa, and Oceania (Barrios-Garcia et al., 2012). European, Asian, and north African populations predominantly consist of pure biotypes of the Eurasian wild boar, whereas populations in the Americas, southern Africa, Oceania, and various islands across the Pacific, Atlantic, and Indian Oceans are primarily composed of feral domestic pig variants, a result of historical introductions (Ruiz- Fons, 2017).

Wild boars appeared in western Eurasia about 1 million years ago. The initial domestication of this species took place in the Near East around 8,500 to 8,000 years ago. Subsequently, in Europe, these pigs underwent continuous human-mediated selection and were intentionally interbred with local wild populations. Eventually, many pig populations reverted to the wild, becoming feral. This process of selective breeding and crossbreeding for specific traits led to a remarkable diversity of wild, domestic, and feral forms, showcasing a wide range of both naturally and artificially selected phenotypic patterns (Fulgione et al., 2022).

Presently, the species has developed more subspecies divided in four regional groups: Western, Indian, Eastern, and Indonesian. These animals have massive and robust build, thin legs, short and massive trunk and large head (one third of the body’s length). The wild boar is a dimorphic species, males are generally bigger, heavier and with protruding canine teeth than females, which have about 60% of the male body mass. Furthermore, wild boar adult size and weight are affected by environmental factors. For instance, in Europe males average 75-100 kg, 75-80 cm in shoulder height and 150 cm in body length, whereas females average 60-80 kg, 70 cm in shoulder height and 140 cm in body length (Baskin et al., 2003).

Wild boars have a well-developed sense of smell, which is used in social communication, navigation, and predator avoidance, and good sense of hearing, using in communication together with vocal signals. Wild boars have about 20 different types of calls, such as grunts, squeals, and trumpets, which vary in amplitude, frequency, and modulation depending on the context. Otherwise wild boars have limited visual ability (Keuling et al., 2017).

The body shape and the optimal olfactory and auditory ability characterize them as a species living in dense vegetation. Their main habitats are forests, scrublands, and river valleys (Fernández-Llario et al., 2003). Wild boars' distribution and density in the environment are due to the presence of shelter, food, and water. These ungulates are sensitive to low temperatures and prefer milder climates, but they do not have sweat glands, and therefore need to regulate body temperature behaviourally to avoid overheating. However, the broad distribution and wide range of occupied environment is explained by their flexibility and adaptive capacity together with their high reproductive rate (Frauendorf et al., 2016). Additionally, the difference in social dynamics and reproductive responsibilities, between males and females, may produce differences in the dependence on and selection of the habitat (Clontz et al., 2022).

They are a multiparous species and, in relation to their body mass, have the highest reproductive potential and fecundity of all ungulate species worldwide. The mass and age of female wild boars significantly impact foetal development; consequently, larger and older females are deemed key individuals for population growth. Additionally, female weight, along with external conditions and food availability, influences litter size, which averages five to six offspring per birth, after approximately 114 days of gestation (Chinn et al., 2022).

Within the first three to four months, piglets are striped, afterwards became unicoloured brown. Both males and females may reach puberty within twelve months, the time of mating is seasonal and depending on photoperiodicity, with a peak in November- December when most of the reproductively active females come into oestrus. Males actively compete for access to oestrous sows, which perceive pheromones in the boar saliva. In Europe, farrowing has been observed all year long and specially in late winter-spring time (Fonseca et al., 2011). The pregnant sow, shortly before giving birth, begins to build the nest away from the group. Indeed wild boars are organized in matrilineal societies: a family group of five-ten individuals, made up to one to several adult females, participating in cooperative nursing, and their offspring. Otherwise males are solitary; they leave maternal groups early, and only in the breeding season rejoin a group (Jensen, 1986).

Wild boars are not territorial animals and interactions between different groups are frequent (Podgórski et al., 2014). They move daily, influenced by resource availability, predator avoidance, interactions with other groups and weather conditions. Usually wild boar do not move faster than 1 km/h, covering less than 10 km/h, per day for feeding, exploring, wallowing and marking. For excursions and escape behaviours, they use fast movements like trotting and galloping, and reach speeds of 10–40 km/h (Morelle et al., 2015). Wild pigs are also good and strong swimmers, and can easily traverse medium- to large-sized rivers and channels, being able to swim across open waters up to 6-7 kilometres in width (Russo et al., 1997).

Despite exhibiting remarkable variations in home range size and considerable plasticity in spatial behaviour, across diverse geographic locations, these ungulates have an average annual home range size of approximately 8 km² (Keuling et al., 2008). Wild boars can skilfully cope with human infrastructure, crossing bridges, motorways, culverts, and overpasses (Morelle et al., 2015).

Scent marks seem to function as landmarks, assisting in navigation within the home range by creating internal maps or route systems. Like other animals, wild pigs can use idiothetic cues (information from their vestibular system, muscle, and joint receptors) to orient themselves in space and find their way back home (Morelle et al., 2015). The presence of hunters and hunting dogs can induce longer movements, cause temporary emigration from their home range, or lead to a reduction in activity and the adoption of hiding behaviour. Moreover, in anthropized landscapes, wild boars activity, which is usually diurnal or evenly spread throughout day and night, becomes largely nocturnal. Wild boars usually alternate periods of resting and activity, moving for feeding, both during daylight and night hours. The duration of activity, in different seasons, is similar (6-12 h a day) except for autumn, when wild boars have to increase fat reserves before winter (Podgorski et al., 2013).

These mammals are opportunistic omnivores and their food habits vary greatly depending on the geographical region, season, and food availability. They have four main feeding behaviours: browsing and grazing, foraging on the ground, rooting, and predation. Dietary differences between ages and sexes of wild boars were reported, and they can be attributed to different nutritional requirements. Moreover, the tendency of piglets to readily accept foods with flavours encountered through the maternal diet enables wild boars to swiftly adapt and expand into new environments within just a few generations. Indeed, the understanding of the environment by young animals, and the enhancement of their fitness are influenced by chemical molecules that pass through the amniotic fluid, and represent pre-birth knowledge (Fulgione et al., 2022). Plant food, including seeds, fruits, leaves, stems, shoots, bulbs, tubers, and roots, forms the 96% of their diet (Schley et al., 2003). As monogastric mammal, wild boars have a limited ability to digest cellulose, making their survival and reproductive success heavily reliant on the availability of high-energy foods. For this reason, acorns and beechnuts are highly favoured over other food resources. Acorns are rich in fats, carbohydrates, vitamins, and minerals and they also provide lysine, an essential amino acid, crucial for lactation performance in domestic pig females. The type of mast production significantly affects growth and reproduction (Gamelon et al., 2017). They have also innate immunity to some poisonous plants. However, in Europe, when supplementary food, provided by hunters or crops is available, wild boars may modify their behaviour and their regular diet. Agricultural crops such as cereals, vegetables, legumes, and fruits are available throughout the year and are predominant in the diet of wild boars residing in certain European regions, including the western coast of the Mediterranean Sea. Wild boars also feed on both invertebrates, like earthworms, insects, snails, and slugs, and vertebrates like rodents, fish, amphibians, reptiles, and birds. Animal-based food sources are an important protein component of wild boars' diets, particularly influencing growth rates in females. Additionally, it is not uncommon for wild boars to ingest garbage, plastic, rubber, and stones (Ballari et al., 2014).

In terms of age structure, juvenile wild boars (< 1 year) comprise 35.5% of the population, yearlings 31.9%, and adults 37.6%. They exhibit high natality rates, coupled with a very high mortality rate during their first year of life, estimated at around 80% (Mayer, 2009). These mammals live about 10 years. Starvation, due to weather conditions, and predation are the major causes of natural mortality. The primary diseases affecting wild boars include foot-and-mouth disease, classical swine fever, Aujeszky's disease, African swine fever, brucellosis, leptospirosis, melioidosis, and pathogenic parasites. In Europe, these diseases are associated with an estimated mortality rate of 30% (Cowled, et al., 2008). The natural predators of wild boars vary depending on their geographical range. They include wolves (*Canis lupus*), bears (*Ursus sp*.), leopards (*Panthera pardus*), striped hyenas (*Hyaena hyaena*), Eurasian lynx (*Lynx lynx*), bobcats *(Lynx rufus*), mountain lions (*Puma concolor*), and eagles (*Aquila chrysaetos*), which primarily prey on piglets (Fulgione et al., 2022). In Europe, the primary natural predator of wild boars is the wolf, which can kill between 50 and 80 wild boars per year. However, deaths due to disease and starvation tend to exceed those caused by wolf predation (Nores et al., 2008). Nonetheless, the greatest contributors to wild boar mortality are human activities. Despite the mortality rate, wild boar population can double and triple in size within 4 and 12 months, respectively. In Europe, they have systematically increased over the past decade, likely due to a combination of human and environmental factors, as well as their remarkable adaptive capabilities (Tack, 2018).

*Figure 1: A female wild boar (*Sus scrofa*) with her piglets in a forested environment (Casamitjana, 2022).*

2.2 Wild boars’ spread and influence on ecosystems

2.2.1 Wild boars’ distribution and spreading causes

Mapping the geographical distribution of species and variability in population density is essential for the conservation and management of biodiversity and landscapes. Understanding how animals navigate landscapes in response to weather changes and their interactions with land resources and other species is crucial (Clontz et al., 2022). Species-habitat relationships can be defined by the ecological niche which provides a useful framework to understand the range and abundance of species in relation to biotic and abiotic factors (Lewis et al., 2017).

Wild boars exhibit substantive plasticity in their movements and behaviour, which has been one of the factors contributing to their widespread expansion (Clontz et al., 2022). The motivations driving exploration of the surrounding environment may stem from physiological needs, such as energy acquisition, or cognitive abilities, like resource assessment. Wild boars can adopt an anti-starvation strategy by increasing visits to high-energy food sources and enhancing their movement within their territory to fulfil their needs (Morelle et al., 2015).

In several European countries a simultaneous increase in wild boar populations was initially detected between 1960 and 1980. The range expansion of wild boars in northern and eastern areas of Eurasia (natural dispersal from nearby areas or countries), and America and Australia (where they were introduced) began towards the end of the 20th century (Markov et al., 2022) (Figure 2). In Europe, the wild boar population is estimated at approximately 10 million individuals (Bevins et al., 2014). These animals inhabit 64% of the continent, spanning an area of over 3 million km² (Linnell et al., 2020).

Wild boars inhabit a wide range of environments, from semi-arid regions to marshes, forests, and alpine grasslands (Massei et al., 2015). They often spread along landscape features such as hedges, ditches, stone walls, rows of trees, bushes, and streams. Consequently, monitoring wild boar populations is challenging; a wide range of resources, various methodological choices, and diverse analytical approaches must be available (Engeman et al., 2013). Some studies indicate that fluctuations in hunting bags and road accidents involving ungulates may reflect changes in wild boar population size (Vetter et al., 2015). In Europe, approximately 864,000 wild boars were harvested annually in the 1990s (Massei et al., 2015); today, this number has risen to nearly 7 million (Linnell et al., 2020). The average density of wild boars in Europe is 4.8 individuals/km², with significant regional variations. Densities in southern Europe can reach 6 wild boars/km², while western regions average 5.8/km². In contrast, the east and north areas show lower densities of 2.4 and 1.0 wild boars/km², respectively (ENETWILD et al., 2022).

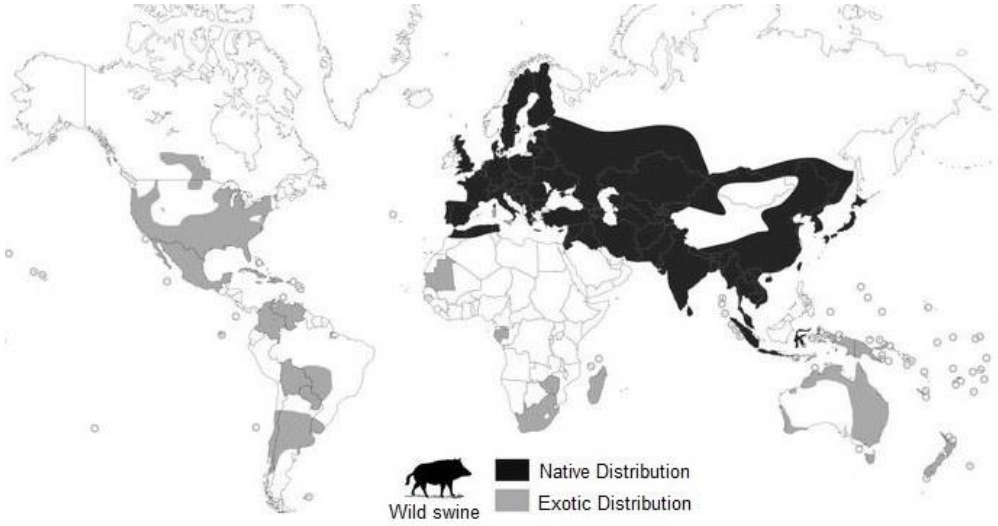


Figure 2: Global distribution of wild swine*. The species' native range is outlined in black, while the introduced range is shaded in gray. The circles represent the islands where* wild swine *has been introduced (Barrios-Garcia et al., 2012).*

The increase in wild boar numbers and their spread can be attributed to a combination of species-specific biological traits, such as vigorous reproduction and a highly adaptable diet, along with various environmental and socio-economic factors. These include climate change leading to higher temperatures, a lack of large predators, low hunting pressure, reforestation, changes in agricultural crops, deliberate releases in previously unoccupied areas, supplementary feeding, and habitat alterations due to human activities (Tack, 2018). Climate change significantly affects the distribution, population structure, and demographics of various species. According to experts, the harshness of the climate is the most important factor influencing the distribution of wild boars, as harsh winter conditions affect their ability to find food and survive. In this context, supplementary feeding has been as impactful as climate warming in facilitating further expansion of wild boar populations (Markov et al., 2022). Climate change is likely to impact the population and phenotypes of wild boars, affecting both reproduction and juvenile mortality (Vetter et al., 2020). The size of wild boar populations is positively correlated with milder winters, and this positive relationship is observed across a broad climatic range, not just in the coldest regions. Despite local climate differences, rising average winter temperatures have led to nearly simultaneous increases in wild boar populations throughout Europe. Wild boars in cooler climates tend to have larger body sizes to minimize heat loss, whereas those in warmer regions are smaller. Additionally, adverse weather conditions, such as snow, can limit their geographic expansion and affect their movement and activity levels (Morelle et al., 2015). Climate change also indirectly influences their population growth by affecting food availability. Warm and dry spring weather can enhance pollination conditions, whereas severe late spring frosts may hinder flower development and seed production across vast geographic areas. Climate projection models forecast a continued rise in temperatures over the next century, which may lead to an increased occurrence of weather patterns that benefit both oak pollination and fruit production (Touzot et al., 2020). In recent decades, an increase in the frequency of tree seed masting has been observed, which can lead to a corresponding rise in the average of wild boar population size. These food sources enable juvenile boars to cope with high thermoregulatory costs in winter, and allow adults to accumulate substantial body energy reserves for reproduction (Vetter et al., 2015). Some studies have found that the probability of pregnancy and piglet survival is greater in years following spring precipitation and abundant mast crops (Chinn et al., 2022). However, due to global warming, reduced precipitation in drier areas may constrain wild boar populations, potentially driving a shift towards higher and wetter areas within the region (Colomer et al., 2024).

While predation can influence wild boars' population density, it is not a decisive factor in their overall distribution. Large carnivores are generally unlikely to prevent the expansion of wild boar populations into new habitats. In Europe wolves are the primary predators of wild boars, with predation-related mortality estimated at 13.9% (ENETWILD et al., 2022). Despite a decline in wolf populations between the late 19th and early 20th centuries, they have then recovered and now are present in various European countries, with an estimated 17,000 grey wolves. Studies suggest that as the number of large carnivore species increases, wild boar density tends to decrease (Melis et al., 2006). However, non-human predation of wild boars is limited in the native and introduced range, because of low abundance of large carnivores. Carnivore predation accounts for relatively low mortality in wild boar populations compared to the mortality caused by hunters, which is nearly double than all other predators combined (Barrios-Garcia et al., 2012).

An Italian study highlighted that each year, hunters harvest 65.8% of the wild boars, while wolf packs account for only 8.9% (Bassi et al., 2020). Hunting affects different age classes of wild boars, leading to significant implications for population dynamics. In most forest ecosystems in Europe human hunting activities have altered the natural predator-prey dynamics. In this regard, several studies concur that hunting harvest is the primary cause of ungulate mortality, serving as a more significant limiting factor than predation, habitat quality, disease, winter severity, or accidents (Bassi et al., 2020).

Predators like wolves tend to prey on younger wild boars, whereas hunters target older individuals, which generally have higher survival rates. As a result, hunters have a greater capacity than wolves to regulate population size and structure directly and indirectly. Numerous studies have demonstrated that hunting can affect wildlife in terms of their spatial, social, and temporal behaviours, as well as their overall population structure (Keuling et al., 2021). An increase in the mortality of juvenile wild boars, especially females, due to hunting, has a strongly negative effect on population growth. Furthermore, there are also some evidences suggesting that human disturbance, particularly from hunting pressure, can induce stress, alter reproductive hormone production, and lead to earlier sexual maturity and reproductive activity in juvenile female wild boars. This, in turn, may shorten generation times and result in accelerated population growth (Fulgione et al., 2022).

Moreover, differences in hunting techniques and frequency, use of dogs, season, hunter numbers, wild boar density, as well as the availability and size of nearby refuge areas, collectively shape wild boar behaviour and distribution patterns (Keuling et al., 2021). During the hunting season, family groups and solitary males exhibit distinct habitat selection behaviours. They make slight adjustments in their use of various types of forest areas within their typical home ranges. Males also demonstrate a partial shift in habitat preferences, while groups led by sows display a more pronounced response. This difference in habitat selection between the sexes, during the high-risk season, may be interpreted as a potential difference in anti-predation strategies. Flight responses to human disturbance in ungulates are strongly influenced by the reproductive status of females. In fact, females with offspring display the most pronounced flight responses, likely due to their heightened sensitivity to predation risks. These ungulates often exhibit a consistent preference for selecting dense vegetative cover as resting sites. However, hunting pressure can force them to alter their resting locations more unpredictably, increasing the likelihood of relocating to different habitats (Saïd et al. 2012).

Over the years, humans have implemented various strategies to maintain wild boars as a hunting resource. These efforts include breeding and releasing individuals into areas where they had disappeared, supplementary feeding, and conservation hunting, such as sparing reproductive males and dominant sows. The demand for wild boar meat has driven population expansion, with boar farms now established in regions where they were once hunted to extinction, due to their high economic value as game species (Linnell et al., 2020). Furthermore, illegal translocations for hunting purposes have contributed to the spread of invasive feral pig populations across several continents, and they are now a major source of new populations worldwide (Lewis et al., 2017).

In Europe, the growth of wild boar population has occurred, also, alongside the expansion of urbanization, as well as changes in land use and wildlife management, which have led to a different perception of rural areas, and a decrease in hunting activity (Massei et al., 2015). Hunting, especially recreational hunting, is increasingly viewed by the expanding urban population as an unsustainable and morally questionable extractive activity (Fischer et al. 2013). This perception plays a significant role in stabilizing or even reducing the number of hunters and leads to a low recruitment of new participants. Consequently, hunters may now find it difficult to manage the substantial growth observed in many wild ungulate populations (Quirós-Fernández et al., 2017). Moreover, the abandonment of mountain pastures and agricultural lands, along with the creation of expansive national parks and regulatory exploitation activities,

have significantly influenced landscape patterns and the composition of animal communities (Fulgione et al., 2022).
New urban habitats can attract wild boars, particularly during times of food scarcity or extreme temperatures in their natural environments. In many regions, wild boar populations depend heavily on agricultural products, making their diet largely influenced by human activity. Direct and indirect human food sources, such as garbage, which may contain edible waste (e.g. food scraps) as well as non-food items (e.g. diapers, paper, plastics, and rubber), and leftover pet food, encourage wild boar habituation. In summer, wild boars are more frequently encountered in urban areas than in autumn and winter, when food availability is higher in environments like the Mediterranean area (Nàhlik et al., 2017). Reports indicate that urban wild boars frequently incorporate discarded human waste in their diet when it is more accessible or preferable than natural options. Consequently, wild pigs foraging in landfills, or more generally using anthropogenic food sources, show an increase in body mass. This, in turn, leads to larger litter size, higher survival rates, and therefore an increase in population size. Wildlife research on supplementary feeding has demonstrated several issues, including dependence on supplemented food, habituation to humans, disruption of normal behaviours, and nutritional problems such as metabolic diseases and obesity, as well as exposing urban wild boars to pollutants, poisons, or toxins from human waste. Thus, it is important to consider that human-related risks may lead to higher mortality rates compared to those in natural areas (Castillo-Contreras et al., 2021). Moreover, pig foraging disrupts and uncovers hidden decomposing waste, which then becomes exposed and dispersed. This scattered waste attracts local scavengers, further spreading the waste in the area (Mayer et al., 2021).
Wild boars are increasingly linked to human activities and adapt readily to landscapes modified by human presence. This adaptability allows them to thrive in proximity to cities, where they benefit from the increased accessibility of anthropogenic resources. Moreover, urban areas can create fragmented habitats for wild boars, resulting in subpopulations with potentially altered demography, health, and genetics. This supports the idea that urban wild boars may exhibit personality traits different from their rural counterparts (Zsolnai et al., 2022). Indeed, in areas with constant hunting pressure and human presence, the animals may respond by hiding or remaining still until the hunters leave. On the other hand, inexperienced animals or those living in regions where hunting is less predictable might move farther in response to culling, potentially increasing their impact on surrounding areas (Keuling et al., 2021).

Another critical factor influencing wild boar populations is the presence of agricultural crops, which significantly enhances resource availability. Studies indicate a positive correlation between higher litter sizes and reproductive output in wild boars, and the presence of crops, often supplemented by food provided by hunters (Massei et al., 2015). It has also been shown that, abundant food results in an earlier onset of oestrus and first reproduction, increasing fertility (Geisser et al., 2005).

However, the forest remains the preferred habitat type by wild boars, with vegetation playing a strong role in their density. The growth rate of wild boar populations is strongly linked to the availability of deciduous trees like oaks and beeches. The intensity of both past and present mast production affects the timing of oestrus and the proportion of breeding females each year (Gamelon et al., 2017). Instead, the extent of bare areas across the landscape leads to a decline in population density. In the last 20 years, in all European regions, the forest area has expanded (Massei et al., 2015). Reforestation in many regions worldwide, coupled with the shift of human activities to lower valleys and coastal areas, has created less disturbed environments that are favourable to wild boars. These areas provide suitable habitats for reproduction and availability of trophic resources (Fulgione et al., 2022).

Wild boars' habitat is often influenced by the abundance and suitability of resources in their environment, leading them to adapt their behaviour based on what is available. This behaviour reflects what is known as a "functional response", a concept describing how animals alter their resource use according to environmental factors (Mysterud et al., 1998).

2.2.2 Ecological impacts of wild boars on wildlife

In forests, their primary habitat, wild boars can disrupt plant diversity, vegetation composition, and regeneration patterns. Due to their capacity to naturally expand into new areas, or because they are introduced by humans, wild boars are regarded as an invasive species. The invasion of exotic species and/or the overabundance of native ones disrupt the dynamic equilibrium of ecosystems and alter species composition. This occurs through increased predation pressure, displacement of native species, hybridization, and disease spread, leading to reduced biodiversity (Geisser et al., 2005). These native invaders typically emerge due to human activities and environmental changes, which allow them to exploit new niches or fill vacancies left by the loss or decline of other native species (Carey et al., 2012).

Wild boars are among the most successful mammalian invaders wherever they have introduced themselves or have been introduced. They can significantly alter habitat characteristics and are considered ecosystem engineers, as their rooting behaviour can disrupt soil structure and processes, thereby affecting the entire food chain. However, the limited number of studies shows conflicting results regarding their impact. Some studies have found no significant differences between disturbed and undisturbed soils, while others have reported a reduction in mineral salts and nutrient concentrations, alterations in nitrogen processes, and potential effects on the structure of bacterial communities (Siemann et al., 2009). The most apparent direct effect of wild boar rooting is the reduction of plant cover, which can decrease by as much as

80% in some cases. This leads to a decline in species diversity and alterations in ecosystem composition. These last aspects are also influenced by consumption of fruits and seeds. Furthermore, wild boars can affect native animal communities through predation, destruction of nests and habitats, and competition for resources. Indeed, the diet of wild boar can overlap with that of small canids, felids, herbivores, and omnivores, as they compete for herbaceous plants, fruits, invertebrates, as well as the carcasses of animals (O'Brien et al., 2019).

The rooting activity of wild boars can also influence aquatic communities, altering the composition of aquatic animal and plant ecosystems, changing water quality and chemistry, and spreading diseases or pathogens (Barrios-Garcia et al., 2012). In addition, the wallowing and tree-rubbing behaviours of wild boars have been identified as important vectors for the passive dispersal of invertebrates and seeds. It is established that long-distance seed dispersal by vertebrates is an important mechanism for preservation of plant populations. However, vertebrates that are long-distance seed dispersers can serve as dispersal agents of new invasive species, which damage natural protected areas (Dovrat et al., 2012).

Wild boars are recognized as reservoirs of viruses, bacteria and parasites which can pose a risk for humans, livestock and wildlife. For wildlife, the consequences of disease transmission by wild boars are not well known. However, they have been shown to influence the spread of dieback disease, caused by the soil-borne plant pathogen *Phytophthora cinnamomi* (Li et al., 2010), and to transmit Aujeszky's disease, caused by an endemic wild boar herpes virus. Wild boars may also indirectly influence disease transmission through mosquito populations, which serve as disease vectors. Studies have found correlations between wild boar activity and both the abundance and distribution of mosquitoes. Furthermore, evidence suggests that wild boars may serve as potential vectors for the dissemination of pathogenic microorganisms in the environment (Fulgione et al., 2022).

The International Union for the Conservation of Nature's (IUCN) Red List of threatened species database allows evaluating the threat of invasive species to global biodiversity. Currently global biodiversity is decreasing at an alarming rate. Wild pigs are identified as one of the worst invasive species, and the only terrestrial invader with omnivorous behaviour that preys on vertebrate and invertebrate species. They threaten 672 taxa in 54 different countries in the world: 345 are plants (59 families), 123 herpetofauna (25 families), 96 birds (38 families), 84 invertebrates (22 families), and 24 mammals. 14 species have been driven to extinction as a direct consequence of wild boar impacts including disturbing nest sites and plant assemblages, hybridizing with other endangered *Suidae*, competing with native fauna, and acting as vectors for disease transmission (Risch et al., 2021).

The continental regions facing the highest threat rates from feral pigs are primarily Australia, and then Europe and North America. Although wild boars are a native species in Europe, they are considered pests in many regions (Barrios-Garcia et al., 2012). Indeed, in Europe, 0.7% of birds (7 taxa), 0.2% of invertebrates (6 taxa), 0.5% of plants (16 taxa), and 0.9% of herpetofauna (4 taxa) are threatened by wild boars (Risch et al., 2021).

In all the places where wild boars have been introduced negative impacts to the ecosystems like changes on species composition, local extinctions of plants, reduction of species diversity and promotion of exotic plant invasion by soil disturbance were reported (Sanguinetti et al., 2010). On the other hand, a sustainable wild boar population can positively impact the preservation of native wildlife by serving as an alternative hunting target, thereby alleviating over-harvesting pressures on native species (Barrios-Garcia et al., 2012). Additionally, wild boars can enhance plant diversity by reducing competition intensity and improving soil properties through their foraging behaviour (Bevins et al., 2014).

Additionally, as wild boars invade urban areas, they pose risks to humans, leading to conflicts in various regions worldwide (Simberloff et al., 2011).

2.3 Wild boar-human conflict and population management strategies

2.3.1 Impact of wild boars on agricultural and livestock economies

Habituation is defined as the reduction of a wildlife species' fear response to humans following repeated, non-threatening, encounters. Wild boars may enter peri-urban areas in search of human-provided food sources during summer, especially if food is scarce in nearby woodland. The growing habituation of wild boars to humans allows the species to colonize flat cropland areas, peri-urban and even urban sites, probably influencing the growth of the conflict, as the road network is denser in these areas than in forested mountain habitats (Fulgione et al., 2022). Conflicts arise when the presence of these animals overlaps both in time and space with human activities, leading to disturbances for residents (Cahill et al., 2012). In recent years, higher densities of mammal species have been detected in urban areas compared to surrounding rural environments (Stillfried et al., 2017). Wild boar tolerance to human proximity appears therefore to be a behavioural adaptation to the urban environment, to the extent that the species is now considered as an ‘urban exploiter’. However, human professional and recreational rural activities, such as jogging, hunting, biking, and walking dogs, have consistently disturbed wildlife over time (Saïd et al., 2011). This disturbance has significant consequences for the socio-biology of the species and has led to movements beyond their normal distribution range (Fulgione et al., 2022). Population expansion of wild boars has impacted human societies through crop damage, vehicle collisions, and disease transmission, leading to human-wildlife conflicts (Massei et al., 2015). The invasion and the spread of these animals can result in significant economic losses in agriculture, animal husbandry, and forestry (Geisser et al., 2005). Wild boars of all ages have been found roaming around towns at all hours, causing extensive damage to private gardens, public parks, sport grounds and cemeteries (Fulgione et al., 2022).

The main economic concern, associated with wild boars, is the damage to agricultural crops, which in many different countries amounts to several million annually. Agricultural crops comprise almost 90% of the volume of the stomach contents of wild boars. Agricultural plants, in the Mediterranean area, are consumed by wild boars year-round, but primarily during summer and autumn, when their nutritional value is highest, or when the availability of natural foods becomes unpredictable (Amici et al., 2012).

These ungulates cause damage to a variety of crops, including maize, acorns, beechnuts, chestnuts, pine seeds, olives, cereal grains, sunflower seeds, wheat, barley, oil palm fruit, sugarcane, grapes, and potatoes. Notably, crop damage is positively correlated with the abundance of wild boars in the area and negatively correlated with high acorn yields and hunting effort (Barrios-Garcia et al., 2012). Human disturbances, field location, and specific characteristics significantly influence the likelihood of wild boar crop damage. The risk of damage decreases in fields situated near major roads, rail networks, and buildings. Although wild boars can adapt to peri-urban environments, they show a marked preference for wooded habitats (Cappa et al., 2021). Indeed, damages are predominantly found in fields adjacent to woodlands. The proximity of mixed and coniferous forests to agricultural fields contributes to increased crop damage. This supports the "refuge effect" suggesting that when wildlife can find shelter in nearby forests, they may be more likely to move into adjacent agricultural fields, leading to increased crop damage (Amici et al., 2012).

The impacts on forest plantations are also well-documented. Wild boars frequently uproot seedlings or feed on the roots of newly planted trees. Such activities can lead to regeneration failures and significantly hinder recruitment within forest ecosystems (Bevins et al., 2014). The role of rivers, in crop damage, is also significant, as they function as ecological corridors that pass through forests and extend into cultivated floodplains, providing a continuous source of water for wild boars. Consequently, the presence of a permanent river near intensively farmed land can contribute to heightened crop damage. Therefore, wild boar damage is shaped by topographical and landscape features, making these important factors to consider when assessing damage risk (Amici et al., 2012).

In Europe, wild boars cause agricultural damage that is estimated to result in annual costs of €80 million (Massei et al., 2011). This economic impact is primarily linked to crop destruction but also includes losses of livestock due to predation by wild boars.

Nevertheless, documenting predation by wild boars can be challenging since the entire carcass is usually consumed, leaving minimal evidence. Moreover, feral swine often scavenge dead animals, including the carcasses of other swine (Seward et al., 2004).

2.3.2 Wild boar as reservoir of pathogens

Despite the presence of numerous pathogens in wild swine, only a limited number demonstrate a moderate to high transmissibility potential (Ruiz-Fons et al., 2017). Given these considerations, wild boars can serve as effective surveillance tools for the early detection of diseases, enabling proactive management strategies in wildlife health and public safety (Seward et al., 2004).

The interactions between wild boars and domestic livestock present substantial risks and cost for producers, including losses due to diseases, increased veterinary care expenses, and the costs of control measures to minimize contact. Actually, the major concern associated with wild boars is their role as reservoirs for a broad spectrum of bacterial and viral pathogens and parasites, which establish long-term cycles within wild species. These pathogens present significant risks to both human and livestock health. They can spread to livestock through direct interaction with wild boars or their faeces, as well as through the consumption of contaminated wild boar meat (Barrios-Garcia et al., 2012).

The dynamics of wildlife diseases are often based on the assumption that transmission is density-dependent, meaning that there is a population density threshold below which the disease cannot persist. Wild boar populations currently exhibit high density in many countries around the world (Keuling et al., 2021).

A notable example of disease transmission between wild boars and domestic pigs is Aujeszky's disease, also known as pseudorabies, which is endemic in swine populations and can be transmitted by close contact including mating. This disease results in high mortality rates in piglets, making it a significant concern for domestic swine producers (Bevins et al., 2014). However, in countries with strict segregation measures, different strains of the virus have been identified in wild and domestic swine populations (Charrieret al., 2018).

Wild pigs also play a role in the transmission of foot-and-mouth disease, a viral infection. The economic impact of an outbreak in livestock is estimated to be in the millions (Miller et al., 2017). These ungulates also been documented as being infected by *Mycobacterium bovis* in various European countries (Brown et al., 2018). Additionally, in Italy, genetic similarities between *M. bovis* strains from wild boars and cattle have been demonstrated, suggesting possible inter-species transmission (Serraino et al., 1999). Other significant diseases, which wild boars can transmit to livestock, include classical swine fever and African swine fever (ASF), both of which have the potential for serious socio-economic consequences. These transboundary animal diseases can lead to high morbidity and mortality in susceptible animal populations, thereby threatening national economies and international trades. In particular, the ASF is a serious viral disease that presents a significant threat to the swine industry (Ruiz-Fons et al., 2017). Currently, ASF is present in several European countries, including Poland, Germany, Italy, Estonia, Latvia, Slovakia, Greece, Lithuania, Romania, Hungary, and Bulgaria. The number of reported wild boars testing positive for ASF in the EU Member States in 2024 has risen compared to previous years, indicating a concerning trend in the disease spread among wild boar populations (EFSA, 2024). Commission Implementing Regulation (EU) 2023/594 of 16 March 2023, laying down special disease control measures for African swine fever. The Regulation establishes rules regarding the zones of restriction and the movements of swine as well as pork fresh meat and derivate products (Reg. EU 2023/594). Moreover, the European Commission's Notice on guidelines for ASF within the Union (C/2023/1504) outlines the measures to be taken to prevent, control, and eradicate ASF in wild boar populations. Wild boar density is regarded as the most influential risk factor for the occurence of the disease in these animals. Biosafety measures, epidemiological data collection, passive surveillance, restricted access to infected areas, limitations on supplemental feeding, and cooperation between competent authorities, environmental agencies, and hunters are emphasized. Hunters should receive training to raise awareness about the risks posed by the disease and the best practices to adopt, with the adaptation of hunting practices to the evolving epidemiology of the disease (EU Commission Notice, 2023).

Wild boars can harbour also *Brucella* spp., which can lead to significant morbidity and mortality in various livestock species and in humans (Ruiz-Fons et al., 2017).

In this respect, besides their agricultural and livestock impacts, wild boars are linked to a wide range of public health concerns, and the prevalence of zoonotic pathogens in wild swine correlates directly with the potential for human exposure to these pathogens. This prevalence is influenced by population dynamics, which can be affected by human practices such as hunting and farming, and environmental disturbances, as well as natural changes (Ruiz- Fons et al., 2017).

The disturbance caused by hunting events may induce the movement of infectious individuals and modulate pathogen spread between contiguous populations, increasing the likelihood of interactions with livestock and humans. Moreover, movements of wild swine into new environments, for game management or farming restocking, can lead to the introduction of pathogens to previously unexposed populations or increase the proportion of susceptible individuals to endemic pathogens in receiving areas, thereby altering the local ecological dynamics of pathogens (Gortázar et al., 2006). Human and livestock interactions with wildlife increase the exposure to sylvatic disease cycles, thereby heightening the risk of pathogen spillover. Livestock may act as intermediate or amplifier hosts, while humans can also be infected directly from wildlife or their vectors (Jones et al., 2013).

Human exposure to infected animals and carcasses constitutes the primary route of direct transmission of zoonotic pathogens from wild boars. Depending on the pathogen, transmission can occur via oral, respiratory, conjunctival, transdermal routes, or through skin lesions. The majority of clinical cases of diseases contracted from wild swine are diagnosed among professionals in fields such as game biology, wild swine farming, hunting, veterinary medicine, wildlife health, and slaughterhouse work (Ruiz- Fons et al., 2017).

As a matter of fact, direct exposure to wild boar carcasses has been linked to human cases of *Streptococcus suis* meningitis (Bensaid et al., 2003). A significant association between swine and the evolution and transmission of influenza viruses has also been demonstrated, raising substantial concerns about the role of wild swine in influenza dynamics (Clavijo et al., 2013). Humans can also contract zoonotic pathogens from wild boars, including through aerosol transmission and bites from arthropod vectors. Wild boars significantly influence arthropod vector-borne diseases, particularly through their interactions with ticks and mosquitoes (Ruiz-Fons et al., 2017).

Another major transmission route for zoonotic pathogens such as Hepatitis E virus (HEV), enterobacteria, mycobacteria, and macroparasites is the consumption of inadequately processed or undercooked wild boar meat (Bevins et al., 2014). These pathways highlight the need for careful handling and preparation of wild swine products to prevent disease transmission (Ruiz-Fons et al., 2017).

Trichinella spp. is a nematode parasite found across various mammalian species, with human infections commonly linked to the consumption of undercooked pork. Significant outbreaks of trichinosis have been connected to the ingestion of contaminated meat from wild boars (Gottstein et al., 2009).

The risks of transmission of zoonotic bacteria such as *Salmonella* spp., pathogenic *Escherichia coli*, *Campylobacter* spp., and *Leptospira* spp. arise from their ability to be transmitted both directly, through exposure to wild swine and their carcasses, and indirectly, via contaminated food or water. In this context, the role of wild boars in foodborne illnesses has gained increased attention (Bevins et al., 2014).

Wild boars pose also a significant risk of spreading pathogens into the environment. They have been identified as a major source of *E. coli* contamination in watersheds, including pathogenic strains such as Shiga toxin-producing *E. coli* (STEC) which is associated with severe gastrointestinal illness and can lead to haemolytic uremic syndrome (HUS) in humans. This contamination not only affects water quality but also poses serious public health risks. In urban areas, where wild boar populations are prevalent water contamination has been found, posing a high risk of infections from pathogens like *E. coli* and *Leptospira* (Cahill et al., 2012).

Risk assessment for pathogen transmission has been qualitatively evaluated on geographic distribution, prevalence and transmissibility of the pathogens in wild boar populations, as well as on human susceptibility to infections and documented evidence of transmission from wild pigs to humans. Currently, the zoonotic risk is primarily limited to local disease outbreaks, mainly affecting hunters and professionals involved in game management. Additionally, it has been demonstrated that adopting biosafety measures, such as wearing latex gloves, facial masks, protective eyewear, and wound covers when skinning wild boar carcasses, can reduce the risk of infection by wild swine pathogens (Ruiz-Fons et al., 2017).

However, the rate of human-wild boar contact is elevated and seems to be increasing due to changes in the population dynamics. The rise in outdoor tourism, recreational activities, and the expansion of human settlements into peri-urban and semi-natural areas leads to more frequent opportunities for the exchange of zoonotic pathogens between humans and wild swine worldwide (Cahill et al., 2012).

2.3.3 Wild troublemakers

The landscape changes induced by human actions, particularly in developed countries, consist also in the expansion of the road and railway networks. In Europe, an estimated 7.3 million kilometres of public roads traverse the landscape, sometime even crossing the forests. This, together with increasing vehicle speeds and the high animal densities in the respective areas, has led to some traffic issues, such as wildlife-vehicle collision (Fulgione et al., 2022). In some studies, it has been detected that the frequency of wildlife-vehicle collisions increased with the growing number of forest patches and water bodies along roads. Indeed, the higher likelihood of existing migration corridors, connecting fragmented areas intersecting with roads, elevates the risk of collisions. Ungulate species are often the primary victims of vehicle collisions due to their widespread presence and abundance in many regions. They are considered the most problematic species for traffic safety in Europe, being involved in the highest number of collisions and causing the most damage (Jakubas et al., 2018). Wild boar-vehicle collisions occur more frequently at night and during the months from October to January, coinciding with the main rutting period and hunting season. These seasonal factors contribute to behavioural changes in wild boars, which are associated with an increased incidence of collisions. Each year, more than half a million collisions between vehicles and ungulates are reported. These accidents lead to vehicle damage, human injuries, and fatalities, making them a significant source of ungulate mortality. Over 73,000 wild boars have been involved in road accidents in Europe (Linnell et al., 2020). In southern Europe, wild boar causes a major conflict, being responsible for up to 85 % of all accidents involving animals (Nahlik et al., 2017). The rising number of wild boars throughout Europe led to expectations of a growing conflict in transport networks. Wild boar-vehicle collisions have significantly increased across various European countries, posing serious social and economic challenges. These accidents result in significant financial costs per incident, and can also result in human injuries or fatalities (Massei et al., 2015; Beasley et al., 2014). For these reasons, the management of invasive wild boars is crucial for safeguarding biodiversity, minimizing damage to forestry and agriculture, and enhancing human, animal, and ecosystem health (Didero et al., 2023).

2.3.4 Management strategies of wild boar populations

The management of game species is a multifaceted process influenced by the interaction of natural system dynamics, and the choices and actions of various stakeholders (Quirós-Fernández et al., 2017). Various techniques are utilized to manage, control, and eradicate wild boar populations. These methods encompass hunting and culling, baiting and shooting, poisoning, trapping, and the implementation of boar-proof fencing (Barrios-Garcia et al., 2012).

The most effective approach to mitigate damage caused by wild boars in areas where they are not yet present is to prevent their introduction and establishment. However, implementing this approach has proven challenging and has achieved limited success (Barrios-Garcia et al., 2012). The primary issue, in several European countries, is their tendency to adopt a reactive approach to managing ungulate populations, where interventions are implemented only after conflicts or sanitary issues have already arisen (Nàhlik et al., 2017).

Some potential solutions for reducing wildlife-vehicle collision risks involve the implementation of physical infrastructure, such as overpasses and underpasses, along with measures like roadside fencing, wildlife crossing signs, and sonic deterrents. The selection of these strategies should be tailored to the specific ecological and socio-economic conditions of the area. Indeed, these structures are commonly utilized for a variety of vertebrate species and have demonstrated their effectiveness. However, the substantial installation costs and the impracticality of widespread implementation pose ongoing challenges (Beasley et al., 2014). Fencing serves as a preventive measure to protect agricultural lands, natural reserves, and urban areas from the destructive activities of wild boars, as well as to reduce ungulate-vehicle collisions (Massei et al., 2011). Nevertheless, some studies indicate that fences do not significantly reduce wild boar-vehicle collisions and, in some cases, their presence has been associated with an increase in such incidents. These findings suggest that fences may be ineffective if not properly designed, installed, or maintained (Zuberogoitia et al., 2014). Electric fences have demonstrated effectiveness in mitigating the damage caused by wild boars to crops. However, since the maintenance costs for electric fencing are elevated, it is crucial to secure funding for these structures and provide technical support for their installation, particularly for small business owners and local farmers (Fulgione et al., 2022).

Electric fencing, wire barriers, chemical sterilization, and repellents are also among the preventive measures utilized in management strategies to reduce the impact of wild boars in protected natural areas where year-round hunting is prohibited (Frank et al., 2015). A diverse range of olfactory, acoustic, and gustatory deterrents, including electronic sound emitters, and wild boar alarm calls, have been developed to mitigate the impact of wild boars on human

activities. However, it has been shown that wild boars rapidly become accustomed to all deterrents within a few days, which diminishes their overall effectiveness (Massei et al., 2011). Additionally, enhancing predator conservation efforts could be another management approach, as it may help restore their natural role in regulating wild boar populations. For example, wolves can be regarded as a significant natural resource in this context (Fulgione et al., 2022).
Another strategy used to divert wild boars from seasonally available resources is baiting, which remains a crucial component of management strategies. The actual effectiveness of supplementary feeding in reducing crop damage remains contentious. However, it has been cautioned that this practice may enhance the reproductive success and survival rates of wild boars, potentially leading to a long-term increase in crop damage (Massei et al., 2011).

Traps are widely used in managing wild boar populations, as they can efficiently remove significant numbers of animals and are particularly useful for mitigating issues in residential areas. However, maintaining and regularly monitoring traps can be costly in terms of staff time, and their application is generally limited to relatively small areas. The success of trapping is influenced by several factors, including topography, season, trap type, trap density and location, duration of trap deployment, and the type of bait used (Massei et al., 2011).

The most significant factor limiting wild boar distribution is hunting pressure, highlighting the necessity for effective hunting management to control their populations (Markov et al., 2022). Hunting regulation employs adaptive management, which involves continuous population monitoring to enable managers to improve their strategies and gradually modify their actions (Quirós-Fernández et al., 2017). Recreational hunting is widely practiced across many European countries, with hunters operating individually, in small teams, or as part of larger groups. In the driven hunting method, hunting posts are evenly distributed around the designated area. Packs of trained dogs flush wild boars from their cover and drive them toward the perimeter, where hunters are stationed at fixed positions, waiting for opportunities to shoot the animals as they emerge. Driven hunting is commonly practiced in countries like Spain, Germany, France, and Italy, although they are prohibited in others, such as the Netherlands. Alternative hunting techniques commonly practiced in European traditions include the "turning hunt" (also known as "girata"). The "girata" is a quieter method that relies on the use of a specialized tracking dog, known as the "limiere". This dog has excellent tracking skills, allowing hunters to approach their target stealthily, thus avoiding any alarm for the wild boars. The "limiere" locates the wild boars, flushes them out, and gently directs them toward designated positions without applying excessive pressure. Instead of scattering wildly in front of the dogs, as occurs in traditional driven hunting, the wild boars tend to follow their usual pathways that they normally use to move in the forest, emerging near the designated positions (Fernandez-de-Simon et al., 2023).

However, the use of dogs introduces additional considerations in wildlife management. While dogs in driven hunting methods significantly improve hunting success by enhancing the detection of active game, their use can reduce harvest selectivity and cause considerable disturbances to wildlife. This may affect the population dynamics, behaviour, genetic structure, and life history traits of both target and non-target species (Varruzza et al., 2023).

Driven hunting is regarded as the most efficient technique for managing wild boar density, demonstrating effectiveness in areas with high populations, where a significant number of animals can be culled in a relatively short time frame (Massei et al., 2011). However, this method can not allow for the targeted removal of specific age or gender categories. The sex ratio of piglets taken by hunters appears to exhibit no significant bias, likely due to the hunters' difficulty in accurately identifying the sex at this early stage. Conversely, the mortality rate of male yearlings is markedly higher than that of females, which may be attributed to increased movement during migration. Additionally, hunters frequently avoid targeting females accompanied by piglets. The mortality of adult males is also relatively substantial, especially in regions where trophy hunting is a major focus, as is commonly seen in numerous European countries. In these instances, males are usually hunted at a more mature age. It is particularly crucial to protect male yearlings and potentially younger adult males (Scillitani et al., 2010).

Some studies have proved that in areas where hunting pressure is consistently high, wild swine may learn to adapt to the disturbance by hiding or remaining inactive until the hunters have left. Indeed, it has been demonstrated that hunting is also more effective in mitigating damage to crops than fencing or supplementary feeding (Massei et al. 2011).

In spite of this, recreational hunting seems insufficient to control wild boar densities, as shown by current trends in Europe. In the last years, recreational hunting has been supplemented by selective hunting, a more effective strategy for managing wild boar populations that, however, requires enhanced training for hunters (Keuling et al., 2021).

To effectively regulate wild boar hunting, it is crucial to study the biology of the species, particularly its behavioural and ecological adaptability, as well as to select the most appropriate hunting methods and evaluate both the attitudes and availability of hunters (Keuling et al., 2021). Some studies suggest that targeting specific sex or age classes could improve hunting efficiency. Increasing hunting pressure on adult females appears to be the most effective strategy for controlling population size (Bieber et al., 2005).

Consequently, hunters should receive appropriate training to enhance their awareness of issues related to wild boar and wildlife management. They should be encouraged to foster collaboration with neighbouring hunters, implement supplementary hunting and other management practices, and selectively target animals in accordance with expert guidance. Specialists, drawing on field studies and scientific research, will recommend the appropriate number, sex, and age class of animals to be culled, considering factors such as population structure, local densities, and the specific impacts of the population within the region.

Furthermore, efforts should be made to educate hunters on optimizing hunting practices, such as ensuring the proper disposal of offal and remains from culled animals to prevent improper burial or dispersal in the environment. Additionally, they should report any wild boar carcasses to enable health authorities to conduct necessary investigations. Passive surveillance by citizens, in fact, could provide a valuable contribution to reducing the uncontrolled spread of wild boars and mitigating their negative impacts by facilitating the early detection of infection hotspots, allowing for rapid and effective interventions (Fulgione et al., 2022).
However, currently, hunting seems insufficient for mitigating population growth. Therefore, it is essential to integrate hunting with alternative methods, such as trapping or fencing. Employing reliable techniques to assess wild boar density before and after control measures is crucial. Furthermore, monitoring the impact of hunting pressure on population size, along with coordinating efforts with hunting organizations and stakeholders to establish collaborative management strategies, can enhance overall effectiveness (Massei et al., 2011). Thus, regulating wild boar populations may be achievable if public awareness is raised and the concept of "hunting management" is broadened into a more comprehensive "wildlife management" approach. This new strategy would encompass not only private and recreational hunting but also other human influences on wildlife and efforts toward nature conservation (Keuling et al., 2021).

In recent years, fertility control has emerged as a non-lethal alternative or a complementary option to culling, especially in areas where public support for hunting or lethal control is limited. Chemical sterilization method utilizes immunocontraceptives, which work by inducing the production of antibodies against hormones or proteins crucial for reproduction. These compounds have been formulated as single-shot vaccines capable of inducing long-term infertility after a single injection. For instance, the Gonadotropin-Releasing Hormone (GnRH) vaccine stimulates the production of antibodies against GnRH, which is responsible for the production of sex hormones that lead to ovulation and spermatogenesis. Animals injected with this vaccine can be rendered infertile for 1 to 5 years and have been found to be safe and efficient for many years without side effects on the animals' behaviour, welfare, or physiology (Massei et al. 2008). Fertility control garners a substantial level of public approval and could be employed to reduce wild boar populations. However, managing wild pig populations with injectable contraceptives is significantly more expensive than trapping methods and this strategy is likely to be confined to small-scale applications, such as in urban environments. Furthermore, when compared to trapping or shooting, fertility control generally operates more slowly in decreasing local populations. Actually, ongoing research aims to develop oral contraceptives. For instance, the Boar Operated System (BOS™) is an efficient device designed to administer contraceptives, and other pharmaceuticals through wild boar-specific bait dispensers. This system has been successfully utilized for this purpose in Europe (Massei et al., 2011).

In urban and residential settings where hunting is challenging, the application of chemical sterilization, repellents, and capture cages serves as complementary strategies for managing wild boar populations. Nevertheless, these methods present significant logistical difficulties due to their non-selective characteristics. Major concerns include safeguarding non-target wildlife and establishing protocols for managing captured animals (Fulgione et al., 2022).

Furthermore, there has been a recent increase in community opposition to lethal wildlife management strategies, arising from concerns regarding animal welfare, human safety in urban areas, and the environmental repercussions of toxic substances. This opposition is driven by concerns over the existence value of wild boars, animal rights issues, and a general distrust in the management of population control within protected areas (Massei et al., 2011).

Government agencies, academic institutions, and local communities should collaborate to analyze the complexities of the issue at both local and potentially broader scales. Addressing the wild boar crisis poses challenges in identifying practical, sustainable, and context-specific solutions. This necessitates integrated strategies that involve the coordinated efforts of various stakeholders, including territorial management institutions, municipalities, researchers, farmers, and breeders. The effectiveness of these strategies depends on the environmental, political, and social context, as well as the availability of economic resources, often requiring ongoing adjustments (Fulgione et al., 2022).

Reducing wild boar population densities is critically important, particularly to interrupt infection chains and minimize ecological, agricultural, and human damage (Keuling et al., 2016). On the other hand, the eradication of wild boar populations in their native range is not desirable, as they fulfil important ecological functions within their ecosystems. Through their rooting behaviour, wild boars overturn the soil and bury seeds from various forest species. This process not only facilitates seed germination and the growth of new plants but also improves soil aeration, which enhances root health and contributes to the overall vitality of forest ecosystems (Fernandez-de-Simon et al., 2023).

2.4 Wild boars in Italy: impact of overpopulation and management strategies

In Italy, wild boars have rapidly expanded their range over the past 50 years. In the early 1950s, wild boar population had been reduced to a few small, fragmented groups. However, starting in the 1960s, the species began to colonize hilly and mountainous regions of the Italian peninsula, and more recently, it has been observed in various areas of the Alps and intensively cultivated plains. Currently, the Italian Institute for Environmental Protection and Research (ISPRA) estimates the wild boar population in Italy to be approximately 1.5 million individuals (ISPRA, 2023). Wild boars now inhabit around 64% of the national territory, increasingly encroaching on suburban and urban areas (Amici et al., 2012). Population densities throughout the country range from 0.01–0.05 to 2.32–10.5 individuals/km^2 (Pittiglio et al., 2018).

This widespread presence results in adverse effects on both the environment and human socioeconomic well-being. For instance, in the Northern Apennine forests, the rooting activity of wild boars adversely affects natural forest regeneration. Specifically, the rooting and trampling behaviours of wild boars differentially impact the chemical, physical, biochemical, and structural properties of the soil, depending on the level of animal pressure. When this pressure is high, it can alter the texture of the soil surface, resulting in changes to water flow and erosion processes. On the other hand, the quantity of fruits was not significantly impacted by wild boar activity, despite evidence indicating that they consume substantial amounts of fruits and seeds as part of their diet. Interestingly, wild boar pressure appears to exert a more pronounced impact in cultivated environments, which are generally more susceptible, compared to woodland areas (Bongi et al., 2017).

Wild boars are responsible for most of the agricultural damage, estimated at just under € 120 million, resulting from over 105,000 documented instances of damages to crops and other agricultural resources (Amici et al., 2012). Overall, approximately € 30 million in damages caused by wild boars were associated with national and regional protected areas, while around € 89 million were attributed to unprotected areas. Cereals, including maize, wheat, oats, and barley, were the crops most frequently subjected to damage, accounting for 46.48% of the recorded incidents. Within this category, maize emerged as the most impacted, representing 22.98% of cases. Permanent crops, particularly vineyards and hazelnuts, experienced significant damage, with additional impact noted in grassland, sunflowers, chestnuts, grain legumes, and vegetables. Specifically, the destruction of cereal crops was primarily caused by wild boar trampling and the removal of grains, whereas damage to permanent crops predominantly resulted from rooting behaviour, which disrupts the mechanical harvesting of fallen fruits (Amici et al., 2011).

The regions of Piedmont and Abruzzo are the most significantly impacted, with annual damages estimated at around €17 million for each region (ISPRA, 2023). Nonetheless, all regions of Italy are adversely affected by the substantial presence of wild boars. In Calabria, where the population is estimated to be approximately 300,000 wild boars, these animals not only devastate agricultural crops but also contribute to car accidents and serve as vectors for the transmission of African swine fever. Their uncontrolled proliferation is exacerbated by the existence of three major national parks: Sila, Pollino, and Aspromonte. In Puglia, the devastation caused by this uncontrolled wildlife population is also considerable, with an estimated 250,000 wild boars damaging crops and posing risks to both humans and domesticated animals. In Molise, the population surpasses 40,000 animals, significantly exceeding the recommended threshold of two wild boars per square kilometre necessary for maintaining ecological balance. Meanwhile, in Campania, the wild boar population has escalated to nearly 60,000 individuals. In the Marche region, the cumulative costs associated with compensating farmers and addressing road accidents amount to approximately € 2 million annually, with an estimated 40,000 animals in the area. The situation remains critical in Sardinia and Sicily, particularly near protected areas, where approximately 100,000 wild boars are present. In Basilicata, Umbria, and Veneto, the populations also exceed 100,000. In Lazio, the situation has escalated to an unsustainable level for residents, as evidenced by the increasing incidence of fatal accidents on roadways. Tuscany is similarly affected, with an estimated population of around 200,000 wild boars. In Liguria, 55,000 wild boars are estimated, impacting primarily mountainous and inland areas, although they have recently started to invade coastal areas. In Friuli-Venezia Giulia, wild boars are present in an estimated population of around 20,000. In Trentino-Alto Adige region they have also starting inflicting damage. Additionally, some reports indicate their presence in Valle d’Aosta, where they have colonized habitats outside their typical range, including agricultural and cultivated lands, even at altitudes exceeding 2,000 meters (Ricci, 2024).

In Lombardy, the estimated population of approximately 70,000 wild boars inflicts extensive damage on agriculture and poses a significant risk to pig farms as carriers of ASF. Concerns regarding the spread of ASF infection are particularly pronounced in Emilia-Romagna, which is currently facing a critical situation with an estimated wild boar population of 80,000 individuals (Ricci, 2024) and a density ranging from 1.37 to 2.31 wild boars/km² (Pittiglio et al., 2018). Moreover, the Tosco-Emiliano-Romagnolo Apennine has proven to be a highly suitable habitat for wild boars, where intraspecific interactions between wild boars and domesticated pigs can readily occur, particularly within extensive pig farming systems. In the Emilia-Romagna region, there are 2,986 farms rearing a total of 1,024,627 pigs. This zootechnical sector is well-established and holds significant economic importance due to the production of various high-quality foods in the Italian Food Valley, including Parma ham and an array of cured pork products, which are generating revenue of € 1,778 billion in 2019 (Rossi et al., 2023).

In 2022, the first ASF cases were reported in northern Italy, following a surge of infections that had already been observed in several European countries in preceding years. The supply chain associated with this industry generates approximately € 20 billion annually and provides employment for 100,000 individuals. According to ISPRA the ASF virus demonstrates considerable resilience in the environment, enabling the disease to circulate for years, even within wild boar populations characterized by extremely low densities (e.g., approximately 0.5/km^2) (ISPRA, 2023). In response, immediate measures to mitigate the spread of ASF have been implemented through a legislative decree, which encompasses the deployment of armed forces and the activation of volunteer organizations and civil protection agencies to contain and prevent this threat (Decreto Legge n. 63/2024).

Management measures, such as fencing and trapping, are being implemented to regulate wild boar populations and mitigate their impact. Nevertheless, ecological and economic considerations render the widespread use of permanent mechanical fences impractical. While these structures, when properly constructed, can effectively protect crops, they also significantly reduce bio-permeability, restricting wildlife movement across the landscape (Monaco et al. 2023).

In the Apennine region, the recent increase in the wolf population corresponds with the expansion of wild boars. Recent studies, confirm the importance of wild boars in the wolf's diet. Specifically, in the central-northern Apennine areas, wild boars can comprise up to 52% of the wolf's diet, often serving as the most frequent prey among various wild ungulates in terms of percentage (Monaco et al. 2023).

The extensive geographical and demographic expansion of the wild boar population in recent decades has led to a rapid and progressive increase in recreational hunting activities. In Italy, hunting has predominantly served recreational purposes, focusing on maximizing game yields rather than regulating wildlife populations to maintain ecological balance with human activities. The rising conflicts between wild boars and humans have prompted public authorities to implement population control measures. In this context, hunting remains the most commonly employed method for managing wild boar populations in Italy. Law No. 157/92, together with regional regulations, establishes areas, methods, timelines, and techniques for wild boar hunting, with careful consideration of the environmental impacts associated with these practices. The guidelines specify the autumn-winter period for driven hunting and “girata”, as well as the spring-winter period designated for selective hunting, while also precisely defining the specific hunting days within each week (Monaco et al., 2003). Furthermore, it is mandatory for hunters to undergo training, obtain the appropriate licenses and necessary equipment, demonstrate proficiency with firearms, join an accredited hunting squad, and hold valid insurance from one of the several specialist companies in Italy (Hearn et al., 2014). The predominant hunting technique employed in Italy is driven hunting, which is favoured for its perceived efficacy in maximizing game yield and its deep-rooted traditions within rural communities (Varruzza et al., 2023).

The ISPRA reported that from 2015 to 2021, the wild boar harvest increased of 45%, with an average of approximately 300,000 wild boars culled annually (257,000 during regular hunting and 42,000 during wildlife management interventions), resulting in a cumulative total exceeding 1.8 million over the seven-year period. The majority of hunting activities (94%) were conducted on public land, while only 6% occurred within private hunting reserves (ISPRA, 2023). Indeed, the Italian territory is divided into more wildlife management areas, with differing objectives, including those where hunting is permitted such as hunting areas (ATC), alpine zones, wildlife-hunting enterprises, and agri-tourism-hunting enterprises; and those where hunting is entirely prohibited, such as National and Regional Parks. Nevertheless, in accordance with national regulations, protected areas may be designated for the execution of population control activities for wild boars through hunting, utilizing either internal personnel (Protected Area Surveillance Service, State Forestry Corps) or external personnel specifically authorized by the park authorities. However, the current situation is characterized by a significant lack of standardized and consistent wildlife management criteria, which are essential for the planned and responsible control of the species. This issue is further exacerbated by the persistent absence of coordinated strategies between hunting areas and protected zones, impeding effective planning for wild boar population management and limiting efforts to mitigate their impact on human activities (Monaco et al. 2023). In Italy, provincial wildlife management plans must be developed by territorial hunting areas (Ambiti Territoriali di Caccia - ATC), outlining the objectives for wild boar control and specifying the methods to achieve them based on the analysis of the territories under their jurisdiction (Monaco et al., 2003). Additionally, in the last several years, the Italian ATC has allocated a portion of the registration fees from wild boar hunters to support compensation for agricultural damages and to fund preventive measures. Consequently, this has resulted in an increase in the cost of wild boars of approximately 29%, with the average cost per culled wild boar being around € 180 (Monaco et al,. 2003).

3. The supply chain of wild boar meat: strengths and weaknesses

The rising population of wild boars has led to an increase in culling, with hunting remaining the primary management strategy employed. Ungulate hunting, while historically serving as a primary source of protein, has evolved into a predominantly recreational activity in modern times. This change carries significant economic and social implications, and only recently hunting has been recognized as a wildlife control strategy (Fernandez-de-Simon et al., 2023). Across Europe, various hunting methods, traditions, and cultures are practiced, each holding substantial national or regional importance. These traditions are vital for maintaining local knowledge and continue to provide recreational benefits for communities (FACE, 2021). A central element of local hunting culture is also expressed through food, such as wild boar fresh and cured meat, which has become a significant part of local culinary traditions, as an important nutritional source (Hearn et al., 2014). In Central and Mediterranean Europe, wild boar meat is commonly featured in local restaurants and fairs, reflecting a well-established gastronomic interest in this type of meat (Lestingi, 2023).

3.1 Sustainability and animal welfare

Wild boar meat serves as a renewable and locally source of proteins, providing a sustainable alternative to farmed pork, particularly when quality control measures are enforced (Macháčková et al., 2021).

Sustainability refers to the ability of ecosystems to maintain ecological processes, biodiversity, and productivity over extended periods. A report by the Food and Agriculture Organization (FAO) indicates that the global livestock sector is responsible for 18% of global greenhouse gas emissions, with pig farming contributing 9% of the sector's total emissions. It has also been reported that the cumulative greenhouse gas emissions, associated with pork production and consumption, amount to 12.1 kg of CO_2 for each kilogram of pork. Indeed, intensive meat production systems often depend on feed grains and other inputs sourced from distant regions, rendering these practices both economically inefficient and environmentally unsustainable. Moreover, vast areas of land have been transformed for intensive monoculture farming, resulting in the contamination of aquatic ecosystems using of pesticides and fertilizers. The European Union (EU), as the world's second-largest producer of pork and a major exporter of pork products, plays a significant role in contributing to these environmental challenges (Lestingi, 2023).

In contrast to pork, wild boar meat exhibits a significantly lower carbon footprint, as it is considered a natural product. The primary contributor to greenhouse gas emissions, in this context, arises from the travel of hunters over considerable distances to reach hunting locations.

Notably, wild boars do not require breeding facilities, heating systems, waste management for excrement, or feed production. Thus, wild boar meat can be regarded as organic, free from antibiotics, and environmentally friendly, as wild animals are free roaming. Additionally, it reflects the quality characteristics associated with local food products, produced and consumed "from forest to fork" (Marescotti et al., 2019).

Another important aspect of wild game meat is animal welfare. The assessment of animal welfare can be grounded in biochemical and physiological indicators, which reflect the internal equilibrium of an organism as influenced by various external environmental factors (Tajchman et al., 2018). In hunting practices, animal welfare should be evaluated based on the duration and intensity of the animal suffering after being shot. According to "Best Practice" guidelines in most countries, hunters target the thoracic region to induce fatal haemorrhaging, by aiming for the highly vascularised lungs, heart, or major thoracic blood vessels. Nevertheless, death from blood loss is not instantaneous, and the time from bullet impact to incapacitation largely depends on the rate of haemorrhage (Stokke et al. 2018). Shots to the head, heart, neck, and spine result in rapid death, causing minimal damage to the meat, a low level of stress, and a short fleeing distance, which together create optimal conditions for maintaining good microbiological quality of the carcasses (Borilova et al., 2016). Wild game meat can, thus, achieve the highest standards of animal welfare among meat products, when harvested under strict and regulated hunting practices, as wild animals live according to their natural grazing behaviours. Furthermore, hunting eliminates the need for transportation and the confinement of animals at slaughterhouses, which are common sources of stress and suffering in traditional livestock farming systems (Macháčková et al., 2021).

Severe stress can disrupt homeostasis, leading to an imbalance in protein levels and the release of proteins from the liver and other organs into the bloodstream, which negatively affects the animal welfare. Factors occurring both prior to and after the shot, including the length of pursuit before the shooting, the location of the injury, the duration of trauma, and any disturbances present after the shot, are significant. These elements can influence the outcome of hunting activities and the welfare of the targeted animal. The method of hunting is a significant variable influencing the stress responses of ungulates. Specifically, driven hunting that involves dogs and a large number of hunters are associated with elevated stress levels. Additionally, reducing the duration of pre-trauma disturbances and stress proves beneficial for the quality of game meat. In this context, it should be noted that wild animals are consistently exposed to natural stressors more frequently than their domesticated counterparts (Gentsch et al., 2018). Some studies, indeed, show that vehicle collisions, as well as severe illnesses and injuries, tend to cause greater distress in animals compared to hunting-related injuries (Tajchman et al., 2018).

3.2 Nutritional properties and organoleptic characteristics of wild boar meat

In light of all these considerations, wild boar meat presents a promising alternative to pork from multiple perspectives. Notably, it offers also high nutritional value. The average chemical composition reveals high protein content (22–26%), and relatively low total lipid levels (2–5%). The lipid fraction mainly consists of oleic (36–43%), linoleic (13–19%), linolenic (0.6–1%), and palmitic (20–21%) acids. From a nutritional perspective, oleic acid plays a significant role in the human diet by reducing both LDL (low-density lipoprotein) cholesterol and triglycerides. Wild boar meat contains also lower lipid and cholesterol levels (55–59 mg/100 g) compared to other meat (Lestingi, 2023). The ratios of polyunsaturated to saturated fatty acids in meat from hunted wild boars exceed the minimum recommended ratio of 0.40, which contributes to a reduced risk of coronary diseases in humans (Sales et al., 2013). It is known that atherogenic and thrombogenic indices, which serve as measurements of lipid quality and potential markers of cardiovascular disease risk, are better in wild boar meat compared to commercially farmed pork (Marsico et al., 2007).

Several factors, including live weight, sex, hunting season, and region, can significantly influence the meat's fatty acid profile. The primary determinant of these variations appears to be the dietary intake. The nutritional status of wild animals, influenced by seasonal fluctuations and the availability of vegetation, can significantly impact both the fat content and the overall quality of the meat. As reported in the former chapters, the dietary habits of wild boars vary across geographic regions, reflecting local feed availability. Conversely, compared to pasture-based feeding, the inclusion of concentrates in the diets of intensive farmed animals tends to elevate overall fat and saturated fatty acid levels, while simultaneously reducing the content of polyunsaturated fatty acids (PUFAs) (Ramanzin et al., 2010).

Additionally, wild boar meat is a rich source of vitamins, particularly B and E, along with minerals, fibres, and carotenoids (Macháčková et al., 2021). While vitamin E is often supplemented in the diet of domestic pigs, wild boar muscles naturally contain higher levels of this vitamin E. In particular, wild boars roam free-range in forested areas, where they consume larger quantities of hard mast, show elevated concentrations of the γ-tocopherol isoform of vitamin E. This vitamin plays a crucial role in protecting against lipid oxidation in both fresh and derived pork products, thereby enhancing meat stability and shelf-life (Quaresma et al., 2011). Indeed, lipid oxidation is a leading factor in the decline of meat and meat product quality, resulting in discoloration, off-odors, unpleasant flavours, texture defects, and the formation of potentially harmful compounds (Cifuni et al., 2014).

Wild boar meat contains also lower levels of sodium and higher concentrations of iron, manganese, phosphorus, and zinc compared to pork (Sales et al., 2013). The macro and micro mineral contents in wild boar muscles often correlate with the mineral concentrations in the soil of the areas where the animals are hunted. Moreover, microstructural differences in muscle fibres between wild boar and domestic pig have been identified, largely due to the wild boar's natural behaviour and diet. Muscle fibres composition influences the physical and chemical characteristics of the meat, resulting in a darker, firmer, and leaner texture, generally making it less juicy and tender than domestic pork ones. Elevated pH, a darker appearance, and reduced drip loss in the meat are also attributed to the increased stress levels experienced by the animals before and during hunting. Some studies have demonstrated that hunting methods significantly affect lipid oxidation in the meat of wild ungulates, as physical activity raises concentrations of oxygen-containing free radicals in tissues, subsequently accelerating lipid oxidation (Cifuni et al., 2014).

The distinctive flavour of wild boar meat is largely attributed to potent odor-active compounds, such as methional and phenylacetaldehyde, while the pheromones androstenone, 3α-androstenol, and 3β-androstenol are responsible for the typical boar taint (Morán et al., 2019).

The quality of wild boar meat exhibits considerable variability, influenced by factors such as diet, sex, age, body fat, hunting season, and hunting method. Additionally, initial processing practices, including exsanguinations, evisceration, carcass storage temperature and meat conservation methods, play a crucial role in determining the overall quality and safety of the meat (Amici et al., 2015).

3.3 Consumer perspective and the development of the game meat supply chain

The reputation of wild game meat has significantly increased among consumers in recent years, attributed to its positive characteristics and growing popularity. Notable, attributes include its nutritional quality and the fact that the animals live entirely in the wild, differentiating it markedly from livestock meat (Fantechi et al., 2022). Indeed, game meat obtained through hunting is considered one of the most responsible and sustainable ways to consume meat today. Claiming to hunt one’s own meat, suggests a practice perceived as significantly more humane than conventional meat production methods (von Essen, 2018). European consumers, including non-hunters, generally hold a favourable attitude toward hunting, when it is conducted for food production, game management, and environmental conservation, provided that these activities are carried out sustainably. Over the past year, game meat consumption has risen, a trend correlated with the growing population density of wild game, resulting in greater availability of this meat. Additionally, the increasing demand for wild boar meat is particularly driven by consumer awareness of its health benefits, distinctive sensory attributes, and reduced environmental impact (Needham et al., 2023).

Consumer awareness regarding game meat significantly influences purchasing decisions, highlighting the importance of education programs aimed at informing the public about its origin, safety, and nutritional benefits (Corradini et al., 2022). A significant hesitation towards consuming wild boar meat is likely linked to apprehensions about its safety. Consequently, appropriate labelling and traceability are essential also to guarantee safety and quality (Fantechi et al., 2022). Certain consumers have negative attitudes and perceptions regarding hunted wild game meat. This scepticism often arises from their views on animal rights and the perception of hunters, who are frequently seen as people with no respect for the preservation of the environment (Marescotti et al., 2021). To address these concerns, it is crucial for consumers to be informed about European hunting regulations, the role of hunting in managing the overpopulation of large wild animals, and the effects that high animal densities can have on ecosystems and human activities. Hunted wild game meat can effectively address the ethical, health, and environmental concerns tied to intensive livestock farming. Wild boars, having lived freely until harvested, can be generally regarded as experiencing a higher standard of welfare compared to animals reared in conventional livestock systems (Marescotti et al., 2020).

The increasing interest in sustainable food sources, along with growing awareness of the health benefits associated with game meat, underlines the importance of developing commercial game meat markets (von Essen, 2018). The meat obtained from hunting can contribute to the local supply of proteins and generates income through hunting tourism and meat exports (Massei et al., 2011). Considering the volume of wild boars harvested, there is considerable potential for the growth of sustainable commercial game meat markets (Needham et al., 2023). Moreover, wild boar meat can be effectively integrated into processed products, such as sausages and both cooked and raw hams, where optimal muscle acidification enhances the ripening process. In Italy, one of the products made from wild boar is salami, where wild boar meat is present in proportions ranging from 5% to 60%, combined with pork, and subjected to a curing process lasting from 30 to 60 days. However, it is essential for consumer preferences to shift towards this type of product, to support the development of a national supply chain for wild game meat. Indeed, the economic sustainability of a game meat supply chain depends on the demand for these products, which, in turn, is influenced by consumer perceptions (Marescotti et al., 2019). Limited market availability and the seasonal nature of the products are key challenges for the expansion of the hunted wild game meat market. However, embracing the consumption of seasonal products can support a change toward more environmentally sustainable and health-conscious eating habits, particularly when the food is locally sourced (Corradini et al., 2022).

In Italy, recent estimates indicate that the annual consumption of meat from harvested ungulates per capita is very low, ranging from 0.1 to 0.3 kg. However, this figure becomes more significant when considering the number of hunters, who represent the most engaged consumer category. In this context, annual per capita consumption increases to between 1.0 and 4.0 kg. Additionally, consumption is expected to increase with higher culling rates (Ramanzin et al., 2010). Interestingly, local hunting activities do not meet domestic demand in terms of both product quality and quantity, despite the increasing populations of wild ungulates, particularly in the Alpine and Apennine regions. As a result, the needs of Italian consumers are met through a significant volume of imported meat. Indeed, the sale of local wild game is restricted to specialized shops and butchers, while meat sold in supermarkets is predominantly of foreign origin (Fantechi et al., 2022).

The development of a comprehensive wild ungulate meat supply chain should be considered a positive advancement for any region, particularly in mountainous areas, due to its potential for economic recovery. Research indicates that local communities could benefit from such a supply chain through increased tourism and the reduction of overpopulation among wild animals (Gaviglio et al., 2017).

3.4 Challenges in quality and hygiene assurance of game meat

While hunting offers certain benefits, it lacks the process standardization seen in conventional livestock meat production. The consumption of meat from hunted wild ungulates raises concerns regarding quality and hygiene assurance, as key processes from field shooting to final marketing are difficult to regulate effectively (Ramanzin et al., 2010). Meat quality parameters are influenced by various factors, including the stress experienced by the animal before shooting. The intense stress and activity contribute to reduced glycogen levels in muscle tissues, leading to an elevated pH that impacts colour, texture, and water retention capacity of the so-called “dark, firm and dry” (DFD) meat (Morán et al., 2019).

The consumption of game meat can pose risks if proper guidelines for slaughter, handling, storage, and cooking are not strictly followed. Inadequate practices may result in microbial contamination, zoonotic disease transmission, or spoilage, ultimately jeopardizing food safety and public health. Despite the nutritional benefits linked to wild boar meat, implementing effective hygienic and technological measures is crucial to mitigate the risks of zoonotic infections and foodborne illnesses (Lestingi, 2023). The microbiological quality of game meat exhibits greater variability being influenced by several factors, including the presence of microorganisms on the skin, the location of the shot, and the hygiene standards applied during evisceration, transportation, skinning, and cutting processes (Borilova et al., 2016). Notably, when animals are accurately shot and properly processed, microbial contamination of fresh carcasses can remain very low. Muscle tissue of healthy animals is generally considered virtually sterile, making shot accuracy essential (Giuggioli et al., 2018). A poorly executed shot can lead to undesirable consequences, particularly if the animal is wounded in the field, causing damage to internal organs. Any shot that penetrates the gut will rapidly result in microbial contamination of the meat. If death is delayed after wounding, microbial infections may proliferate due to gut spillage. Additionally, a wounded animal experiences significant stress before death, which not only raises ethical concerns but also severely compromises the quality of the meat (Ramanzin et al., 2010). Critical aspects concerning hygiene, safety, and the ultimate shelf life of wild boar meat include the immediate bleeding of the animal post-harvest, followed by the removal of the visceral organs. This prevents complications during evisceration and reduces the risk of perforating the gastrointestinal tract (Needham et al., 2023). Animals culled with a nonfatal shot and/or not properly bled exhibit a slower pH decrease in the first hours after death. The post-mortem pH decline, which results from lactic acid accumulation, is critical for the muscle-to-meat transformation process and significantly affects the resulting meat quality (Viganò et al., 2019). Wild ungulates are generally bled and eviscerated in the field, often in remote locations, which frequently causes delays in the evisceration process after the animal has been killed. Within a few hours, intestinal bacteria can penetrate the intestinal barrier and contaminate the surrounding muscle tissue. Even when evisceration is performed promptly, it remains a delicate procedure, as insufficient skill and poor sanitary conditions can greatly increase the likelihood of microbial contamination of the carcass (Ramanzin et al., 2010).

In addition to proper evisceration, rapid transportation of the carcass to a game-handling establishment (GHE) is crucial. The carcass must be cooled promptly to avoid spoilage, emphasizing the importance of the interval between killing and processing. To address safety concerns regarding game meat, key regulations are in force, including Regulation (EC) No 852/2004, Regulation (EC) No 853/2004, and Regulation (EU) No 2019/627, along with Regulation (EC) No 178/2002. The regulations mandate the training of hunters to ensure they are qualified to process game carcasses and meat for commercial purposes (European Commission, 2004a; 2004b; 2019; 2002). At least one member of the hunting group must have proper knowledge of the normal anatomical, physiological, and behavioural characteristics of wild game, as well as the ability to recognize abnormal behaviours and pathological changes due to diseases or environmental contamination that could affect human health after consumption. This individual is the so-called “trained person” among the group of hunters. Before being transferred to GHE, wild game must be inspected either in situ or at the collection centre by the trained person tasked with detecting potential health risks. If no irregularities are found during the examination, the trained person must sign a document detailing date, time of death, and location of hunting of each animal for tracking and traceability. In cases where anomalies are detected, it is mandatory for the trained person to inform the veterinary competent authority. In such cases, all internal organs, except for the stomach and intestines, must accompany the carcass to the GHE (European Commission, 2004a; 2004b; 2019; 2002). Moreover, proper hunting practices should follow the best guidelines to avoid environmental contamination by the burial or improper disposal of offal. Additionally, hunters should report any discovery of dead or dying wild boar to enable competent authorities to carry out necessary inspections and ensure the prevention of potential health risks (Giuggioli et al., 2018).

The transportation method must comply with sanitary regulations, ensuring that carcasses are not overcrowded and are maintained at a maximum temperature of 7°C (Giuggioli et al., 2018).

Upon arrival at the GHE, where skinning and butchering must take place in accordance with Regulation (EC) No 853/2004, the carcass undergoes further sanitary inspection by an official veterinarian, with additional analyses conducted in accordance with the Regulation (EC) No 2019/627 (European Commission, 2004b; 2019).

Although effective in removing diseased animals or visibly contaminated carcasses from the food chain, this approach is insufficient in detecting asymptomatic carriers of pathogens. Implementing Good Hygiene Practices (GHP) and Hazard Analysis and Critical Control Point (HACCP) principles is crucial for ensuring meat safety and quality (Paulsen et al., 2012). These practices are fundamental in minimizing contamination risks throughout the production process. Adhering to these standards not only reduces public health risks but also promotes sustainable and responsible hunting practices, thus enhancing food safety (Lestingi, 2023). Indeed, the meat is only submitted for market distribution after it has received approval in game-handling centres, sectioning facilities, and slaughterhouses under Regulation (EC) No 853/2004 (European Commission, 2004b).

However, hunting and game meat processing practices in Europe exhibit significant variation, leading to differences in the supply chains, quality control measures, market dynamics, and hygiene standards for game meat across different countries (Needham et al., 2023). As a result, coupled with the complexity of game meat production chains, the risk of contamination and disease outbreaks is significantly elevated (Needham et al., 2023).

In Italy, the State-Regions Conference has established hygiene guidelines for wild boar meat, recognizing the significant availability of wild game and consumer demand. These guidelines aim to facilitate the development of supply chains for wild game meat, ensuring quality and safety standards are met (Fantechi et al., 2022). However, a clear and applicable national or regional legal framework has yet to be established, which hampers the development of a robust game meat supply chain. Indeed, hunters, who utilize game meat for personal consumption or sell it directly to end consumers or local retailers, limited to 4 adult wild boars per hunter per year (Giunta Emilia-Romagna n.1319, 2024), are exempt from these regulations. “Local” is defined as the province or its neighbouring province, and the sale of game meat at fairs and events is expressly prohibited. Despite this, hunters are still required to adhere to traceability standards. Additionally, the traders who purchase game from hunters must keep records documenting the product's origin, in compliance with Regulation (EC) No 178/2002, which pertains to tracking and traceability (Giuggioli et al., 2018).

The legislation at all levels fails to designate an institutional authority responsible for ensuring the safety of game products intended for personal consumption or direct sale. This absence of comprehensive oversight throughout the production chain by a competent authority raises significant sanitary concerns. Moreover, there are currently no standardized surveillance programs for game meat in Europe, despite the importance of surveillance, monitoring strategies, and improved education in handling game carcasses (Olivastri et al., 2021).

The safety of food relies on several key elements: control at the source, proper product design, and effective safety process management. While these preventive measures improve oversight, it is still crucial to conduct tests for chemical and biological hazards to safeguard consumer health. This is especially relevant because wild animals, particularly wild boars, serve as bioindicators of environmental contamination. They reflect exposure to biological and chemical hazards, including bacteria, parasites, heavy metals, radioactive isotopes, pesticides, and synthetic organic compounds used in agriculture. Thus, wild boars are crucial indicators for evaluating ecosystem health and the potential risks to consumers of game meat.

The European Union has established regulatory limits for cadmium (Cd) and lead (Pb) levels in meat and offal under Regulation (EC) No 2023/915 (European Commission, 2023). Generally, the health risk associated with consuming wild boar meat is considered negligible, even in polluted regions, as the concentration of heavy metals in the wildlife food chain has decreased over recent decades (Ramanzin et al., 2010). On the other hand, a significant biological risk associated with the consumption of raw wild boar meat is the potential for infections from bacteria and parasites, such as *Trichinella* spp. Regulation (EC) No 1375/2015 mandates specific controls for detecting this parasite, particularly in 10 grams of diaphragm tissue (European Commission, 2015).

However, despite the presence of various European regulations, significant gaps remain, particularly within Regulation (EC) No 2073/2005 and its subsequent amendments. This regulation establishes microbiological criteria for certain microorganisms in foodstuffs, along with the implementation standards that food business operators must adhere to in their hygiene practices. It requires mandatory monitoring of specific microbiological criteria for process hygiene and food safety, wherein a "microbiological criterion" defines the acceptability of a product, a batch of food products, or a process based on the absence, presence, or number of microorganisms per unit of mass, volume, area, or batch. A microbiological criterion includes a description of the microorganisms of concern, along with their toxins or metabolites, and the reason for their relevance. It also specifies the analytical methods used for detecting or quantifying these microorganisms, as well as a sampling plan that defines how many field samples should be taken and the size of the analytical unit. Additionally, it establishes microbiological limits deemed appropriate for the food at a specific stage in the food chain, and determines the number of analytical units that must meet these limits (European Commission, 2005). To assess the hygiene of the process concerning farmed animal carcasses (such as equine, bovine, ovine, caprine and swine), the regulation mandates the enumeration of aerobic colony and *Enterobacteriaceae*, along with the detection of *Salmonella* spp., which also falls under the microbiological criteria for food safety (European Commission, 2005). The Aerobic Colony Count (ACC) is used as an indicator of the overall hygiene conditions of the facilities, workers, and environment, while *Enterobacteriaceae* Count (EntC) serves as indicators of faecal contamination. It is well established that the release of faecal matter into the body cavity greatly elevates surface contamination with *Enterobacteriaceae*, and typically increases bacterial presence within deeper tissues as well. Despite evidence confirming that the hunting activities significantly influence the contamination of wild boar meat, no specific process hygiene criteria are defined in Regulation (EC) No 2073/2005 for hunted wild game carcasses and meat, including wild boar (Paulsen et al., 2012).

4. One Health in wild boar meat: foodborne pathogens and antimicrobial resistance

4.1 *Salmonella* spp. and pathogenic *Escherichia coli*

The concept of One Health is increasingly gaining attention, integrating interdisciplinary values that connect human, animal, and ecosystem health. It aims to identify threats and foster sustainable coexistence between humans, animals, and the environment (Lerner et al., 2017). Interactions across these domains are mediated through the exchange of microbial communities among humans, animals, plants, and their environment, influencing the health of organisms, populations, and ecosystems (Mercato et al., 2022). One Health approaches should also be implemented in the context of game meat consumption, considering the impacts and mitigation strategies concerning animal health, ecosystems, and public health. Achieving this, requires coordinated efforts and communication across multiple sectors, which can be reinforced through capacity building in the primary processing sector of game meat (Abrantes et al., 2023).

As previously reported, wildlife, particularly wild boars, serves as a significant reservoir for pathogens that can be transmitted to domestic animals, livestock, and humans, potentially causing foodborne illnesses. Meat inspection procedures for wild game closely resemble those used for livestock. They play a role in reducing bacterial contamination by removing diseased animals and visibly contaminated carcasses from the food supply chain. However, the specific effectiveness of these measures in mitigating bacterial contamination, such as *Salmonella* spp, remains to be thoroughly evaluated (Paulsen et al., 2012). One of the risks linked to the consumption of wild boar meat is the potential infection with *Salmonella* spp., as wild boars can act as asymptomatic carriers of this pathogen (Altissimi et al., 2024). *Salmonella* is a Gram-negative, facultative anaerobic, rod-shaped bacterium, belonging to the *Enterobacteriaceae* family. The genus *Salmonella* is divided into two species: *Salmonella bongori* and *Salmonella enterica*, the latter comprising six subspecies: I *enterica*, II *salamae*, IIIa *arizonae*, IIIb *diarizonae*, IV *hountenae,* and VI *indica,* and more than 2,500 serovars. Most serovars of significance to humans and warm-blooded animals belong to *Salmonella enterica* subspecies *enterica*. The serovars are classified according to their antigenic formula based on the unique combination of somatic (O), and phase 1 and phase 2 flagella (H) antigens, as outlined in the White–Kauffmann–Le Minor scheme. This scheme is regularly updated by the World Health Organization’s Collaborating Centre for reference and research on *Salmonella* at the Pasteur Institute (Agbaje et al., 2011).

Salmonella spp. is one of the most frequently identified gastrointestinal pathogens in humans across the European Union (EU) (EFSA and ECDC, 2024a). This pathogen is common on livestock farms, especially linked to foods of animal origin such as eggs, pork, poultry, turkey, and game meat (Ortega et al., 2020). Transmission primarily occurs through the oral-faecal route, involving the consumption of contaminated food or water, direct contact with infected animals, or exposure to contaminated environments (WOAH, 2018). Salmonellosis is an enteric disease, and poses notable public health risks, manifesting in enteric fever, gastroenteritis, septicaemia, and focal infections. In 2023, a total of 77,486 cases of human illness were reported, along with 1,115 foodborne outbreaks resulting in 9,210 illness cases, 1,726 hospitalizations, and 16 fatalities. This made it the second-most frequently reported foodborne zoonosis in the EU. In Italy over 3,300 cases were documented (EFSA and ECDC, 2024a). The predominant *Salmonella* serovars responsible for human infections in 2023 were *S*. Enteritidis, *S*. Typhimurium, monophasic *S*. Typhimurium (1,4,[5],12:i:-), *S*. Infantis and *S*. Coeln (EFSA and ECDC, 2024). Notably, game meat was identified as a minor source during this period. However, the increase in the availability of wild boar meat in the market underscores the need for systematic and ongoing monitoring of *Salmonella* presence in this meat, employing a risk-based approach for its prevention and control (EFSA and ECDC, 2024a).

In Italy, the detection of *Salmonella* in the deep muscle tissues of wild boars hunted for consumption has been observed. Furthermore, its occurrence has also been documented on the carcasses and meat cuts of wild boars across several European countries (Razzuoli et al., 2021). Human health risks associated with wild boars infected with *Salmonella* can also arise from contamination related to agricultural practices, vegetable products, and watercourses. A more rigorous investigation into contamination dynamics is crucial to better understand the associated health risk. Moreover, updated data on the pathogen's prevalence, incidence, and the specific serovars found in different regions are essential for assessing the role of wild boars in its transmission. Such insights are critical for evaluating the effectiveness of control measures implemented by veterinary authorities (Altissimi et al., 2024). However, because of the variability and partial lack of standardization in hunting methods and post-mortem procedures, obtaining a clear quantitative knowledge of the prevalence and transmission of *Salmonella* spp. along the game meat production chain is challenging. According to Directive 2003/99/EC, enhancing monitoring of food-borne zoonotic pathogens is strongly recommended. In particular, the Committee has identified *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* (STEC) as public health priorities (European Commission, 2003).

Wild boars can serve also as significant reservoirs for *Escherichia coli*, including pathogenic strains. Like other members of the *Enterobacteriaceae* family, these bacteria primarily inhabit the digestive tracts of animals, and it is considered a Process Hygiene Indicator (Barco et al., 2014) by European legislation.

E. coli is a Gram-negative, rod-shaped bacterium that is ubiquitous in the environment and naturally inhabits the intestines of both animals and humans as a commensal organism. In humans, it coexists in a mutually beneficial relationship, being the most abundant facultative anaerobe within the intestinal microflora, and typically contributing to overall health. However, some strains of *E. coli* have acquired specific virulence factors, enhancing their ability to adapt to new environments and enabling them to cause a wide range of diseases. These virulence factors are often encoded on mobile genetic elements, which can be transferred between different strains, leading to novel combinations of pathogenic traits. In some cases, these genetic elements, have since integrated permanently into the bacterial genome, contributing to disease development (Kaper et al., 2004).

Pathogenic *E. coli* is currently divided into distinct pathotypes that are associated with gastrointestinal infections. These pathotypes are classified based on their virulence genes and mechanisms of pathogenicity. They include: enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC) or verocytotoxin-producing *E. coli* (VTEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), and atypical *E. coli* (EFSA and ECDC, 2024a). The pathotypes of *E. coli* are typically classified as clonal groups, characterized by shared O (lipopolysaccharide, LPS) and H (flagellar) antigens that determine their serotype. Pathogenic strains of *E. coli* utilize a multi-step pathogenesis strategy that is analogous to that of other mucosal pathogens. This process encompasses the colonization of mucosal surfaces, evasion of host defences, proliferation, and subsequent damage to the host (Kaper et al., 2004).

EPEC is a leading causative agent of infant diarrhoea, colonizing the small intestine and inducing characteristic attaching-and-effacing (A/E) lesions. These lesions are defined by the degeneration of microvilli, and the close adherence of the bacteria to the epithelial cell membranes. The virulence factors essential for pathogenicity are encoded on a 35-kb pathogenicity island, known as the locus of enterocyte effacement (LEE PAI). A marker for the presence of the LEE PAI is the *escV* gene, which encodes a protein component of the type III secretion apparatus that delivers virulence factors into host cells (Gauthier et al., 2003). The LEE PAI is found not only in the EPEC, but also in other pathotypes, such as STEC. Additionally, among the genes present in the LEE PAI, the *eae* gene encodes a 94-kDa outer membrane protein, known as intimin, which is responsible for the intimate attachment of the bacteria to epithelial cells and also triggers a mucosal immune response. Another key factor in this process is a 70–100 kb plasmid, known as the EPEC adherence factor (EAF) plasmid, which harbours the *bfp* gene responsible for encoding the bundle-forming pilus (BFP). This pilus mediates localized adherence and promotes the formation of bacterial microcolonies on host cells. Atypical enteropathogenic *E. coli* (aEPEC) lacks this plasmid (Müller et al., 2007).

In turn, the ETEC pathotype colonizes the mucosal surface of the intestine, and produces enterotoxins that induce intestinal secretion. These enterotoxins are classified into two groups:

heat-labile enterotoxins (LTs) and heat-stable enterotoxins (STs). ETEC strains can express either LT, ST, or both. The LTs activate the primary chloride channel in epithelial cells, resulting in diarrhoea. Additionally, LTs can stimulate prostaglandin synthesis and activate the enteric nervous system, both of which further promote secretion, and inhibit absorption. STs are small, single-peptide toxins that belong to two distinct classes (STa and STb), which differ in both structure and mechanism of action. The STa activates an intracellular cascade that leads to increased secretion. STb can increase cytosolic Ca^{2+} levels, stimulate the release of prostaglandin E_2, and promote the release of serotonin. These mechanisms collectively contribute to increased ion secretion (Kaper et al., 2004).

EAEC pathotype have become increasingly recognized as a significant cause of chronic diarrhoea in both children and adults. It adheres to host cells in an auto-aggregative manner, forming bacterial clusters that resemble a “stacked-brick” arrangement. Many EAEC strains secrete an enterotoxin called enteroaggregative *E. coli* heat-stable toxin 1 (EAST1), a 38-amino-acid protein that shares homology with the ETEC STa toxin and is encoded by the *astA* gene. Moreover, epidemiological studies consistently demonstrate a strong link between the transcriptional activator AggR regulon and diarrheal illness. AggR controls multiple EAEC virulence factors (Kaper et al., 2004). Additionally, also the gene *pic*, which encodes an extracellular serine protease, has been associated with diarrhoea caused by EAEC (Alhammadi et al., 2022).

EIEC pathotype can cause invasive inflammatory colitis and, in some cases, dysentery. A key virulence factor in this pathogen is the intermediary regulator protein encoded by *InvE* (invasion gene E), which controls the Ipa (invasion plasmid antigen) proteins. These proteins mediate epithelial signalling events, induce cytoskeletal rearrangements, facilitate cellular uptake, promote the lyses of the endocytic vacuole, and perform other critical functions (Müller et al., 2007).

STEC is one of the most common causes of gastrointestinal illness around the world. STEC infection can lead to a range of outcomes, from asymptomatic carriage and mild diarrhoea to more severe manifestations, including bloody diarrhoea, hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura. This pathotype is characterized by the production of Shiga toxins (Stx), which are also known as Verotoxins. There are two primary types of Stx: Stx1 and Stx2, with the *stx* genes carried by lambdoid bacteriophages integrated into the *E. coli* chromosome. The *stx1* gene comprises four subtypes (a, c, d, and e), whereas the *stx2* gene includes fifteen subtypes (a to o) (Lindsey et al., 2023), each contributing to varying degrees of virulence. Stx interferes with protein synthesis in target cells, and a single molecule can be sufficient to trigger cell death. Damage to the ribosome induced by Stx initiates a cellular response known as the “ribotoxic stress response,” which exhibits both proinflammatory and proapoptotic characteristics. Furthermore, Stxs are associated with an unfolded protein response that occurs due to stress on the endoplasmic reticulum (ER), potentially resulting in apoptosis as well (Melton-Celsa, 2014). The transmission of STEC strains predominantly occurs via the consumption of food and water contaminated with faecal material. Despite being associated with foodborne outbreaks, most STEC infections occur sporadically and are linked to the consumption of undercooked or raw meat and unpasteurized milk, as well as person-to-person transmission and contact with animals or their environments (Nüesch-Inderbinen et al., 2024). While ruminants, especially cattle, are the main reservoirs of STEC, wildlife, including game species, also plays a significant role as carriers of these pathogens (EFSA et al., 2020). In wild boars severe STEC infections can lead to oedema in various tissues, resulting in neurological disorders, as well as swelling of the eyelids, forehead, and ears (Perrat et al., 2022). However, many of these animals are asymptomatic carriers due to the lack of receptors for Shiga toxins. Consequently, they serve as healthy reservoirs for STEC, facilitating the spread of these pathogens (Dias et al., 2022). According to EFSA and ECDC (2024a), STEC is the fourth most frequently reported zoonosis in Europe, with 10,217 cases documented. This includes 66 foodborne outbreaks that resulted in 207 illness cases, 48 hospitalizations, and 1 fatality. The most commonly reported virulence gene combination in strains isolated from severe STEC cases is *stx2+/ eae+*.

In Europe, various studies indicate that wild boars serve as reservoirs for STEC and EPEC. The prevalence and pathotypes of these pathogens vary based on local conditions, hunting practices, and the type of sample collected (Bertelloni et al., 2020). Surveillance of these pathogens in wild boars is critically important due to their potential spread within wildlife, the risk of transmission to domestic pigs, and the associated foodborne risk to consumers of game meat (Perrat et al., 2022).

4.2 Antimicrobial resistance in wildlife and food-producing animals

Another potential risk associated with consuming contaminated game meat is the emergence of antimicrobial-resistant strains. Antimicrobial resistance (AMR) has existed for millions of years as an inevitable evolutionary consequence of microbial competition in natural environments. Globally, AMR poses a significant challenge in both human and veterinary medicine, obstructing the effective treatment of bacterial infections and is estimated to cause 25,000 deaths annually in Europe (Swift et al., 2019).

Antimicrobial-resistant bacteria can enter the food chain through antibiotic use in aquaculture, livestock, and crop farming. Environmental factors contribute as well, allowing resistant strains to spread throughout food production stages (Peruzy et al., 2020). Notably, AMR is no longer confined to livestock and human environments; it has become a wider environmental concern with substantial public health implications (Torres et al., 2020). Globally, AMR is increasing, with wildlife serving as a potential reservoir for clinically significant resistance determinants (Greig et

al., 2015). Indeed, antimicrobial-resistant bacteria can emerge in wildlife due to exposure to anthropogenic sources, such as agricultural practices, livestock, and human waste, which release antimicrobials into the environment. Some studies have shown that wildlife populations living near human or agricultural areas exhibit higher levels of antimicrobial resistance compared to those in more remote or natural settings. Wild boars constitute a reservoir for antimicrobial-resistant bacteria and may function as sentinel species for monitoring efforts (Li et al., 2024). Notably, due to their behaviours and close interactions with humans and livestock, wild boars can facilitate the spread of AMR into the wider environment (Torres et al., 2020).
A variety of antimicrobial-resistant pathogens, including *E. coli* and *Salmonella* spp., have been frequently isolated from wild boars (Razzuoli et al., 2021). Furthermore, a strong correlation has been identified between antimicrobial-resistant *Salmonella* spp. found in human cases and those originating from food-producing animals (Peruzy et al., 2020).

E. coli serves as a major reservoir for antibiotic resistance genes, raising risks of treatment failures in both human and veterinary settings. Over recent decades, the number of identified resistance genes in *E. coli* isolates has risen, often due to horizontal gene transfer between cells through mechanisms such as conjugation, transformation, or, in the case of bacteriophages, transduction. Within *Enterobacteriaceae*, *E. coli* functions both as a donor and a recipient of resistance genes enabling it also to transfer its own resistance genes to different bacterial species (Poirel et al., 2018). Indeed, resistance to major antibiotic classes in *E. coli* strains is frequently reported worldwide, with many strains exhibiting multidrug resistance. Both commensal and pathogenic strains can pose significant threats due to their antimicrobial resistance (Swift et al., 2019). In Europe, antimicrobial-resistant strains have also been isolated from wild boars (Castillo-Contreras et al., 2022).

The World Health Organization has endorsed a periodically updated list categorizing antimicrobials as “Currently Not Used in Humans”, “Important”, “Highly Important“, and “Critically Important”. To be classified as Critically Important Antimicrobials (CIA), these agents must meet two criteria: they should be the sole or one of the limited available treatments for severe human infections, caused by bacteria capable of acquiring resistance genes from nonhuman sources (WHO, 2019). The European Medicines Agency, aligning with the WHO list, classifies antimicrobials into four categories: Avoid (A), Restricted (B), Caution (C), and Prudence (D). Category A includes antimicrobial classes that are not authorized for use in veterinary medicine, while Category B encompasses antibiotic classes that should only be used in veterinary settings for the treatment of clinical conditions when no alternative antibiotics are available (EMA, 2019). Third and fourth-generation cephalosporins, along with carbapenems, are all recognized as CIA, however, they are classified in different EMA’s categories: cephalosporins are placed in Category B, while carbapenems in Category A. Notably, some of the most clinically significant mechanisms of AMR targeting these beta-lactam antimicrobials are extended-spectrum β-lactamases (ESβL) and carbapenemases (Torres et al., 2020). The β-lactamase enzymes hydrolyze the beta-lactam ring structure, which is essential for the activity of beta-lactam antibiotics, including penicillins, cephalosporins and their derivatives, as well as carbapenems (Queenan et al., 2007).

The β-lactamase classification is based on two key characteristics: functional and molecular properties. The molecular classification (Ambler classification), grounded in amino acid homology, identifies four major classes. Classes A, C, and D consist of β-lactamases that possess serine at their active site, while class B β-lactamases are metallo-enzymes, featuring zinc at the active site (Queenan et al., 2007). ESβLs are categorized as class A enzymes. These enzymes are biochemically distinguished by their capacity to hydrolyze extended-spectrum β-lactam antibiotics and their vulnerability to inhibition by β-lactamase inhibitors, especially clavulanate. ESβLs provide bacterial resistance to nearly all beta-lactam antibiotics, particularly third-generation cephalosporins, like ceftazidime and cefotaxime, while showing effectiveness against carbapenems and cephamycins. Although ESβLs share common biochemical characteristics, the genes that encode these enzymes exhibit considerable diversity and can be categorized into several families. The genes encoding ESβLs are located on mobile genetic elements, including plasmids, transposons, insertion sequences, integrons, and bacteriophages, which facilitate horizontal gene transfer (Castanheira et al., 2021).

Carbapenemases are β-lactamases that belong to enzyme classes A, B, and D. The major families of class A serine carbapenemases possess the ability to hydrolyze a wide range of β-lactam antibiotics, including carbapenems, cephalosporins, and penicillins. Metallo-β-lactamases (MBLs) belong to class B and are characterized by their ability to hydrolyze carbapenems and their resistance to commercially available β-lactamase inhibitors. However, they remain susceptible to inhibition by metal ion chelators, such as EDTA, due to the presence of zinc ions at their active site, which are essential for their enzymatic activity (Queenan et al., 2007).

ESβLs and carbapenemases are increasingly found in *Enterobacteriaceae*, particularly in *E. coli* but also in *Salmonella* (EFSA, 2011). In 2015, approximately 8,750 fatalities in the European Union were attributed to infections caused by *E. coli* strains resistant to third-generation cephalosporins. This alarming statistic underscores a notable rise in the isolation of ESβL-producing *E. coli* strains from non-human sources, especially from food-producing animals. Farm animals may act as reservoirs for ESβL-producing *E. coli*, with various studies identifying these strains in pigs, cattle, and poultry, particularly following treatment with third- and fourth-generation cephalosporins. This pattern suggests that these animals may contribute to the transmission of resistant strains to humans through direct contact or the consumption of animal-derived food products. Additionally, multiple investigations reveal that isolates from both human and animal sources, can exhibit identical ESβL types (Giufrè et al., 2021). Moreover, ESβL-producing *E. coli* were found also in game meat, as well as in the faeces and tissues of various wild animals, including wild boars (Bonardi et al., 2019a; Darwich et al., 2021). Additionally, the presence of carbapenem-resistant *E. coli* in food-producing animals, particularly in pigs, has been documented in several European countries (Bonardi et al., 2019b). Furthermore, carbapenemase-producing *E. coli* have been identified in wildlife, especially in wild birds (Kock et al., 2018; Ramírez-Castillo et al., 2023), and in wild boars (Banchiri et al., 2018).

Salmonella isolated from farmed pigs have shown low resistance to third-generation cephalosporins, with ESβL-producing strains rarely detected (EFSA and ECDC, 2024b). Although carbapenems are not permitted for use in treating farm animals, carbapenemase-producing *Salmonella* spp. have been identified in pigs (Bonardi et al., 2019b). On the other hand, the use of carbapenems in treating human salmonellosis is generally not recommended; however, in cases of extraintestinal manifestations or when patients are immunocompromised, treatment may be necessary. Consequently, *Salmonella* can serve as reservoirs for carbapenemases (EFSA and ECDC, 2019). Available data underscores the need to expand AMR surveillance in wild game to safeguard consumer health. Growing concerns over zoonotic pathogens and AMR in wild boars highlight the critical need for comprehensive oversight within a One Health framework (Torres et al., 2020).

5. Aim of the study

This study examines the microbiological contamination of wild boar carcasses hunted in the Emilia-Romagna region (Italy), with a focus on the process hygiene criteria, *i.e.* Aerobic Colony Count (ACC), *Enterobacteriaceae* Count (EntC), and the detection of *Salmonella* spp., as well as the antimicrobial resistance profiles of the isolates. Additionally, the study evaluates *E. coli* as a hygiene indicator in the diaphragm muscles of the same wild boars, analyzing its prevalence, antimicrobial resistance, and pathotypes. The influence of biometric data and specific hunting process variables on contamination was also investigated, providing meaningful insights into the potential health risk associated with the consumption of wild boar meat.

6. Materials and methods

6.1 Samples collection

During the 2022/2023 and 2023/2024 hunting seasons, a total of 115 sponge samples from wild boar carcasses and 78 diaphragmatic muscle samples from the same animals were collected across eight GHEs, designated as R1, R2, M1, M2, P1, P2, P3, and P4, in Emilia-Romagna, Italy. The wild boars sampled were harvested by local hunters in designated hunting areas (ATCs) within the provinces of Parma (ATC: PR04, PR07, PR08), Reggio-Emilia (ATC: RE03), Modena (ATC: MO02), Bologna (ATC: BO03), and Piacenza (ATC: PC06), and transported to the nearest GHE. R1 and R2 are located in ATC RE03, M1 and M2 in ATC MO02, P1 in ATC PR04, P2 in ATC PR07, P3 in ATC PR08, and P4 in ATC PC06 (Figure 3). According to the ISO 17604:2005 method for swine, the sponge procedure was conducted at four specific points (loin, jowl, medial surface of the leg, and belly) on the carcasses of wild boars, after evisceration and skinning, covering a total area of 400 cm^2. Diaphragmatic muscle samples were also collected, while the remaining parts of the carcasses were left at the disposal of hunters for subsequent consumption. However, it was not possible to collect diaphragmatic muscle samples from all wild boar carcasses. The biometric data of the wild boars, along with information on variables associated with the hunting process, were recorded. The evaluated variables encompassed:

- GHE of destination;
- sex;
- age (young or adult);
- hunting methods (drive hunting or “girata”);
- shot placement accuracy;
- presence of pathological alterations;
- time period between killing and evisceration;
- time period between evisceration and skinning;
- temperature of the refrigeration rooms at the GHEs.

Shot accuracy was deemed correct if it did not occur in the abdominal area, which could result in intestinal rupture.

The samples were transported as promptly as possible, under refrigerated conditions (4 ± 1°C), to the laboratory of Inspection of Food of Animal-Origin of the University of Parma (Parma, Italy). If not processed immediately, they were stored under refrigeration (4 ± 1°C) and processed within 24 hours.

Figure 3: Map of the Emilia-Romagna region with the corresponding ATCs. The ATCs highlighted in white indicate the locations of the eight GHEs (L.R. n.8, 1994).

6.2 Samples analysis

6.2.1 Aerobic Colony and *Enterobacteriaceae* Count

The Aerobic Colony Count (ACC) and *Enterobacteriaceae* Count (EntC) were initially performed according to ISO 6887-1:2017, with each sponge sample diluted 1:10 in 90 mL of Buffered Peptone Water (BPW) (Oxoid Ltd., Basingstoke, UK), followed by serial decimal dilutions in BPW up to 10^{-7} for ACC and 10^{-6} for EntC. For ACC, 1 mL of each dilution was plated, in duplicate, on Plate Count Agar (PCA) (Oxoid) plates and incubated at 30°C ± 1°C for 72 hours, in accordance with the ISO 4833-1:2022 method. For EntC, 1 mL of each dilution was plated, in duplicate, on Violet Red Bile Glucose Agar (VRBGA) (Oxoid) plates, and incubated at 37°C ± 1°C for 24 hours, in accordance with the ISO 21528-2:2017 method. Following the incubation period, colonies were counted on two plates per sample at two successive dilutions, along with their respective duplicates. Regarding EntC, five colonies were selected from each counted plate, and biochemical confirmations were performed using oxidase reaction and glucose fermentation tests. In accordance with the ISO 7218:2013 guidelines, count formula was applied for both ACC and EntC, with results expressed as colony-forming units per square centimetre (CFU/cm^2):

$$\frac{\sum C}{V*(1*n_1 + 0.1*n_2)*d}$$

Where:

$\sum C$ = summation of the counted colonies in the plates;

V = inoculum volume;

n_1= number of plates counted at the fist dilution;

n_2= number of plates counted at the second dilution;

d= dilution factor of the first plate counted.

The results were then adjusted to the measured area of 400 cm².

6.2.2 Detection and serotyping of *Salmonella* spp.

The same sponge samples, previously diluted in BPW, were incubated at 37 ± 1°C for 18 ± 1 h as a pre-enrichment step for the detection of *Salmonella* spp. in accordance with the ISO 6579-1:2017 method. The samples were then inoculated into selective enrichment broths: Muller-Kauffman Tetrationate Novobiocin Broth (Biolife, Milan, Italy) and Rappaport-Vassiliadis Broth (Oxoid), and incubated at 37 ± 1°C and 41 ± 1°C, respectively, for 24 h. 10 µL aliquots of the enrichment broths were plated onto selective and differential agar media, specifically Xylose Lysine Deoxycholate (XLD) agar (Oxoid) and Chromogenic *Salmonella* Agar Esterase (Neogen, Lansing, Michigan, USA), and incubated at 37 ± 1°C for 24 h. The colonies, exhibiting typical *Salmonella* spp. morphology, were subjected to biochemical and serological confirmation tests and subsequently identified using the API 20E microsubstrate system (bioMérieux, Lyon, France). *Salmonella* isolates were serotyped in accordance with the ISO/TR 6579–3:2014 method by the Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna.

6.2.3 *Salmonella* antimicrobial resistance evaluation

AMR of the *Salmonella* strains was evaluated by determining the Minimum Inhibitory Concentration (MIC) using the Sensititre™ EU Surveillance *Salmonella/E.coli* Plate (Thermo Fisher Scientific, Waltham, Massachusetts, USA), following the manufacturer's instructions. Bacterial suspensions in Mueller-Hinton Broth (Thermo Fisher Scientific) were prepared at a concentration of 5 × 10^5 CFU/mL, inoculated into the wells, and incubated at 35 ± 1 °C for 18 ± 1 h. Each plate was customized with the following antibiotics (µg/mL):

- β-lactams: ampicillin (AMP 1-32), cefotaxime (FOT 0.25-4), ceftazidime (TAZ 0.25-8), meropenem (MERO 0.03-16);
- macrolids: azithromycin (AZI 2-64);
- aminoglycosides: amikacin (AMI 4-128), gentamicin (GEN 0.5-16);
- quinolones/ fluoroquinolones: nalidixic acid (NAL 4-64), ciprofloxacin (CIP 0.015-8);
- polymixin: colistin (COL 1-16);

- glycylcycline: tigecycline (TGC 0.25-8);
- sulphonamides: trimethoprim (TMP 0.25-16), sulfamethoxazole (SMX 8- 512);
- tetracyclines (TET 2-32);
- chloramphenicol (CHL 8-64).

EUCAST (European Committee on Antimicrobial Susceptibility Testing, 2024) and CLSI (Clinical & Laboratory Standard Institute, 2024) guidelines were applied to classify MIC values as resistant, intermediate, or susceptible.

6.2.4 *E. coli* isolation and antimicrobial resistance evaluation

E. coli isolation was performed in 25 g of wild boar diaphragmatic muscle. Each sample was diluted 1:10 with 225 mL of BPW (Oxoid) and incubated at 37°C ± 1°C for 20 h. Following incubation, 20 µL of the enriched sample was plated onto Tryptone Bile X-glucuronide agar (TBX) (Oxoid) and incubated at 41°C ± 1°C for 24 h. A single isolated colony per sample exhibiting typical *E. coli* morphology was selected and subcultured on TBX under the same conditions to obtain a pure culture. The colonies were then seeded onto Tryptic Soy Agar (TSA) (Oxoid) and incubated at 37°C ± 1°C for 24 h for oxidase and indole testing. Biochemical identification was subsequently performed using the API 20E microsubstrate system (bioMérieux).

E. coli isolates were tested for AMR against cefotaxime (CTX), ceftazidime (CAZ), and meropenem (MEM) using the Kirby–Bauer disk diffusion method, following EUCAST (2024) guidelines. *E. coli* colonies were suspended in a saline solution (0.85% NaCl w/v, Oxoid) to achieve a turbidity equivalent to the McFarland 0.5 standard, corresponding to approximately 1–2 × 10^8 CFU/mL for *E. coli*. The suspension was then spread onto Mueller-Hinton agar plates (MHA, Oxoid) with a sterile cotton swab. The antimicrobial disks (CTX 5 µg, CAZ 10 µg, MEM 10 µg) (Oxoid) were placed on the MHA surface, and the plates were incubated at 35 ± 1°C for 16–20 h.

6.2.5 Detection of *E. coli* virulence factors

Two multiplex end-point PCR assays were used for the detection of *E. coli* typical pathotype genes. DNA from *E. coli* isolates was extracted using the heat-based lysis method. Specifically, three colonies grown on Tryptic Soy Agar (TSA) (Oxoid) were suspended in 1 mL of sterile distilled water, subjected to heating at 95 °C for 10 minutes, and the cellular debris was removed by centrifugation at 15,000 RCF (ALC microCentrifugette 4214) for 5 minutes. The DNA in the supernatant was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific) and subsequently used as a template for PCR amplification. The first multiplex PCR assay was used to detect the *stx1* and *stx2* genes, characteristic of STEC, and the *eae* gene, which is typically associated to EPEC and STEC. The primer sequences, PCR reaction mixture, and PCR cycling conditions were applied in accordance with Annex C of the ISO/TS 13136:2012 (Table 1). The PCR reaction mixture, prepared to a final volume of 50 µL per sample, consisted of 1x Green GoTaq Flexi Buffer, 2 U of GoTaq G2 Flexi DNA Polymerase, 1.2 mM of $MgCl_2$ (Promega, Wisconsin, USA), 0.2 mM of each dNTPs (Promega), primers at concentration of 0.25 µM, 10 µL of DNA sample template, and nuclease free water to reach the final volume.

Pathotype	Target gene	Primer Sequences	Size (bp)	PCR cycling conditions
STEC	*stx1*	**F** 5'-ATAAATCGCCATTCGTTGACTAC-3' **R** 5'-AGAACGCCCACTGAGATCATC-3'	180	35 PCR cycles, each consisting of 1 min of denaturation at 95°C; 2 min of annealing at 65°C for the first 10 cycles (decreasing by 1°C per cycle to 60°C from cycles 11 to 15); and 1.5 min of elongation at 72°C (extended to 2.5 min from cycles 25 to 35).
	stx2	**F** 5'-GGCACTGTCTGAAACTGCTCC-3' **R** 5'-TCGCCAGTTATCTGACATTCTG-3'	255	
EPEC, STEC	*eae*	**F** 5'-GACCCGGCACAAGCATAAGC-3' **R** 5'-CCACCTGCAGCAACAAGAGG-3'	384	

Table 1: Conditions for the first multiplex PCR assay used to detect E. coli *typical pathotype genes, as described in the ISO/TS 13136:2012.*

The second multiplex PCR assay targeted several genes: *escV*, characteristic of LEE-positive strains; *bfpB*, specific to EPEC; *elt*, *estIa*, and *estIb*, associated with ETEC; *invE,* a marker for EIEC pathotype; *astA*, *aggE*, and *pic* of EAEC; and the *uidA* gene, a housekeeping gene in *E. coli* encoding the beta-glucuronidase enzyme, which was used as an internal PCR control. The primer sequences, PCR reaction mixture, and PCR cycling conditions used were adapted from Müller et al. (2007) (Table 2). The PCR reaction mixture was prepared to a final volume of 25 µL per sample, including: 1x Green GoTaq Flexi Buffer; 2 U of GoTaq G2 Flexi DNA Polymerase; 2.1 mM of $MgCl_2$ (Promega); 0.3 mM of each dNTP (Promega); primers for *escV*, *estIa*, and *astA* at 0.4 µM; primers for *estIb*, *invE*, *aggR*, *pic*, and *uid* at 0.2 µM; primers for *elt* and *bfpB* at 0.1 µM; and 4.75 µL of DNA template.

The PCR amplicons were evaluated by electrophoresis on a 2% agarose gel stained with SYBR Safe DNA gel stain (Invitrogen, Carlsbad, CA, USA) and visualized using a UV transilluminator. A 100 base pairs DNA ladder (Promega) served as a molecular weight marker. *E. coli* ATCC 43894 served as positive control in the first PCR assay, while both negative and no-template controls were included in all PCR assays.

Pathotype	Target gene	Primer Sequences	Size (bp)	PCR cycling conditions
STEC, EPEC	*escV*	**F** 5’-ATTCTGGCTCTCTTCTTCTTTATGGCTG-3’ **R** 5’- CGTCCCCTTTTACAAACTTCATCGC -3’	544	5 min of initial denaturation, 30 cycles, each consisting of 30 sec of denaturation at 95°C; 30 sec of annealing at 63°C; and 1.5 min of elongation at 72°C; 5 min of final elongation at 72 °C.
EPEC	*bfpB*	**F** 5’-GACACCTCATTGCTGAAGTCG-3’ **R** 5’-CCAGAACACCTCCGTTATGC-3’	910	
ETEC	*elt*	**F** 5’-GAACAGGAGGTTTCTGCGTTAGGTG-3’ **R** 5’-CTTTCAATGGCTTTTTTTTGGGAGTC-3’	655	
	estIa	**F** 5’-CCTCTTTTAGYCAGACARCTGAATCASTTG-3’ **R** 5’-CAGGCAGGATTACAACAAAGTTCACAG -3’	157	
	estIb	**F** 5’-TGTCTTTTTCACCTTTCGCTC-3’ **R** 5’-CGGTACAAGCAGGATTACAACAC-3’	171	
EIEC	*invE*	**F** 5’-CGATAGATGGCGAGAAATTATATCCCG-3’ **R** 5’-CGATCAAGAATCCCTAACAGAAGAATCAC-3’	766	
EAEC	*astA*	**F** 5’-TGCCATCAACACAGTATATCCG-3’ **R** 5’-ACGGCTTTGTAGTCCTTCCAT-3’	102	
	aggR	**F** 5’-ACGCAGAGTTGCCTGATAAAG -3’ **R** 5’-AATACAGAATCGTCAGCATCAGC-3’	400	
	pic	**F** 5’-AGCCGTTTCCGCAGAAGCC-3’ **R** 5’-AAATGTCAGTGAACCGACGATTGG-3’	1,111	
Internal control	*uid*	**F** 5’-ATGCCAGTCCAGCGTTTTTGC-3’ **R** 5’-AAAGTGTGGGTCAATAATCAGGAAGTG-3’	1,487	

Table 2: Conditions for the second multiplex PCR assay used for the detection of E. coli *typical pathotype genes (Müller et al., 2007).*

6.3 Statistical analysis

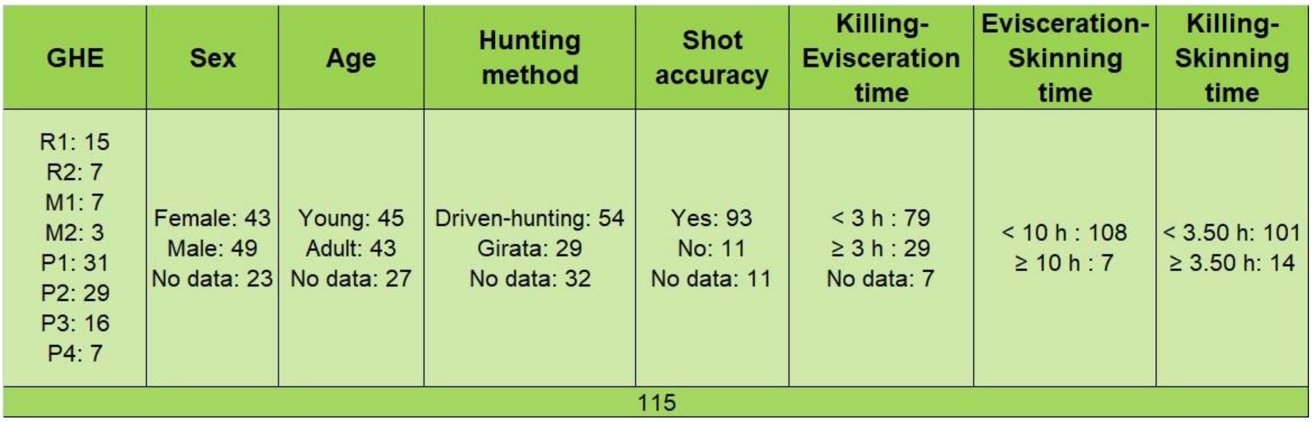
The ACC and EntC values were converted to $\log_{10}$ CFU/cm² for statistical analysis. Mean, standard deviation, median, quartiles (Q1, Q2, Q3, Q4), interquartile range (IQR), maximum, and minimum values were calculated for the ACC and EntC distributions. Data points outside the range defined by minimum > Q1 - 1.5 IQR and maximum < Q3 + 1.5 IQR were considered outliers. Normality of the ACC and EntC distributions was assessed using the z-score, calculated by dividing the skewness values by their standard errors (Hae-Young et al., 2013).
ACC and EntC values were analyzed separately, and statistically significant differences between count values and each recorded variable were assessed using one-way ANOVA. Tukey-Kramer's test was subsequently applied to identify significant differences between groups.
The prevalence of *Salmonella* spp. was evaluated. Statistically significant differences between samples with *Salmonella* detection and each recorded variable were assessed using the Chi-squared test or Fisher's exact test, depending on the number of observations in each category. A Mann-Whitney U test was then used to compare EntC values between samples with and without *Salmonella* detection.

The prevalence of *E. coli* was determined, along with the prevalence of virulence genes in the *E. coli* isolates, which were used to evaluate the probable associated pathotype.

7. Results

7.1 Wild boar samples and hunting variables

The 115 sponge samples collected from wild boar carcasses were analyzed for ACC, EntC, and *Salmonella* spp. The 78 diaphragmatic muscle samples were analyzed for the *E. coli* detection. No pathological alterations were observed in any of the wild boar carcasses. The temperature in the refrigeration rooms of the GHEs was always maintained below 7°C. Other variables considered in the study, including biometric data, hunting methods, shot placement accuracy, and the time intervals between killing, evisceration, and skinning, could not be fully recorded for every sample as detailed in Table 3. It has been reported that a 3-hour window between killing and evisceration is critical for minimizing bacterial spread from the gastrointestinal tract (Avagnina et al., 2012). Regarding the time between evisceration and skinning, the recommended timeframe is generally within a few days (Ranucci et al., 2021). However, in this study, the majority of the samples were killed, eviscerated, and skinned within 3.50 hours. A 10-hour window was selected for the interval between evisceration and skinning, based on the records obtained, to ensure a sufficient number of samples for statistical analysis.



GHE	Sex	Age	Hunting method	Shot accuracy	Killing-Evisceration time	Evisceration-Skinning time	Killing-Skinning time
R1: 15 R2: 7 M1: 7 M2: 3 P1: 31 P2: 29 P3: 16 P4: 7	Female: 43 Male: 49 No data: 23	Young: 45 Adult: 43 No data: 27	Driven-hunting: 54 Girata: 29 No data: 32	Yes: 93 No: 11 No data: 11	< 3 h : 79 ≥ 3 h : 29 No data: 7	< 10 h : 108 ≥ 10 h : 7	< 3.50 h: 101 ≥ 3.50 h: 14
115							

Table 3: Number of wild boars for each biometric data and associated hunting process variables.

7.2 ACC and EntC values in relation to the recorded variables

The ACC values ranged from a minimum of 0.7 $\log_{10}$ CFU/cm^2 to a maximum of 7.76 $\log_{10}$ CFU/cm^2, with a median of 3.97 $\log_{10}$ CFU/cm^2 and a mean of 4.22 $\log_{10}$ CFU/cm^2 (standard deviation of 1.27). The EntC values ranged from -0.9 $\log_{10}$ CFU/cm^2 to 6.13 $\log_{10}$ CFU/cm^2, with a median of 2.21 $\log_{10}$ CFU/cm^2, and a mean of 2.27 $\log_{10}$ CFU/cm^2 (standard deviation of 1.46). ACC values outside the range of 1.15 $\log_{10}$ CFU/cm^2 to 7.17 $\log_{10}$ CFU/cm^2 were identified as outliers. No outliers were observed in the EntC values (Figure 4). ACC and EntC values followed a normal distribution, as evidenced by z-score < 3.29 for both distributions, corresponding to $p < 0.05$. This result did not lead to the rejection of the null hypothesis, supporting the assumption of normality.

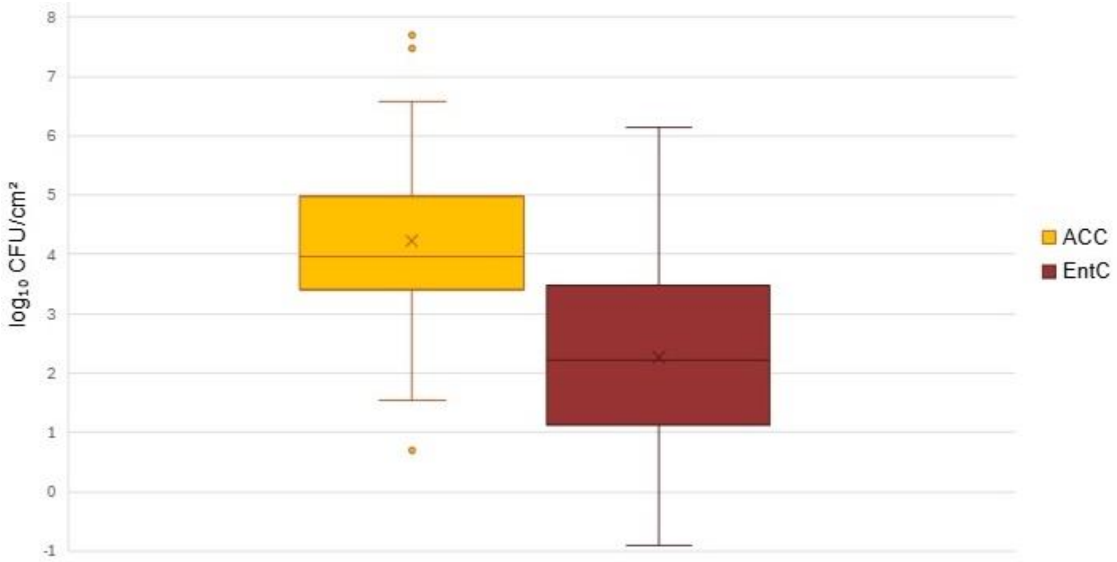


Figure 4: Distribution of ACC and EntC values, illustrating medians, means, interquartile ranges, extreme values and outliers.

The one-way ANOVA on ACC values revealed statistically significant differences across the eight GHEs (Figure 5). Tukey-Kramer’s test further identified the specific GHEs that exhibited significant differences. For a difference to be statistically significant, the calculated q-score must exceed the corresponding critical value of Tukey’s Q (Table 4).

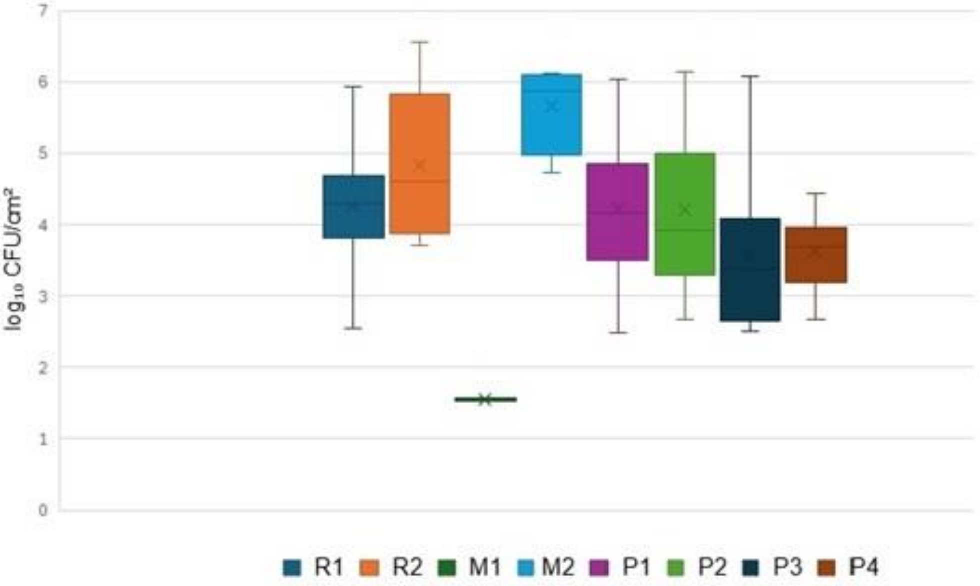


Figure 5: ACC values distribution among the eight GHEs (R1, R2, M1, M2, P1, P2, P3, and P4).

q-score	R1	R2	M1	M2	P1	P2	P3	P4
R1		1,72	5,41	3,66	0,23	0,35	3,04	2,12
R2	1,72		6,00	1,90	2,02	2,10	4,02	3,24
M1	5,41	6,00		7,08	5,48	5,42	3,97	3,87
M2	3,66	1,90	7,08		4,01	4,07	5,64	4,84
P1	0,23	2,02	5,48	4,01		0,16	3,32	2,15
P2	0,35	2,10	5,42	4,07	0,16		3,15	2,04
P3	3,04	4,02	3,97	5,64	3,32	3,15		0,27
P4	2,12	3,24	3,87	4,84	2,15	2,04	0,27	

Table 4: q-scores for ACC mean values among different GHEs. Yellow cells indicate the statistically significant differences (Tukey-Kramer's test; $p < 0.05$) between specific GHE pairs, where the q-scores exceeded the critical value (4.36).

Similarly, the analysis of EntC values in relation to the eight GHEs (Figure 6) also demonstrated significant variations, with Tukey-Kramer's test highlighting the specific GHEs exhibiting notable differences (Table 5).

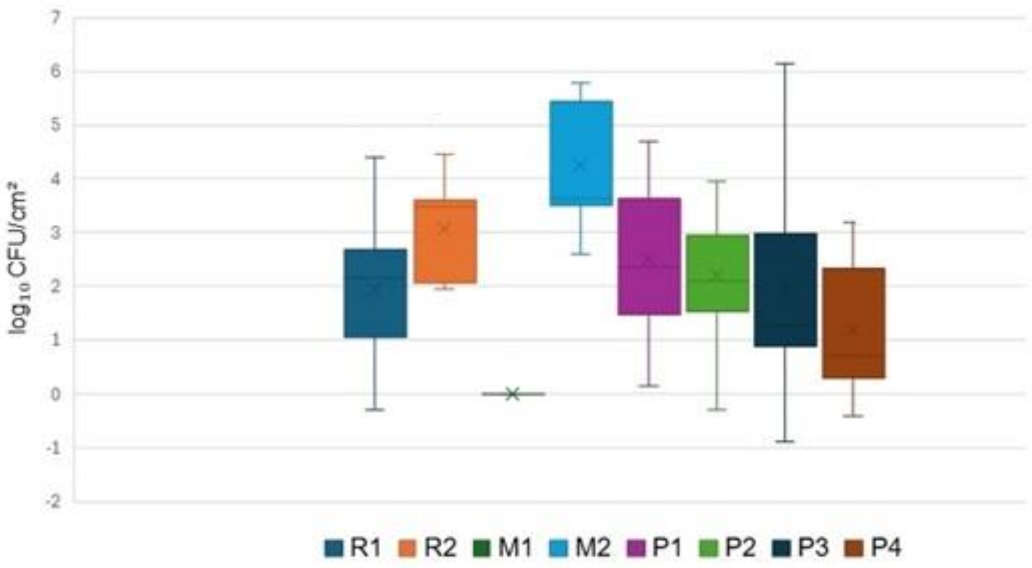


Figure 6: The EntC values distribution among the eight GHEs (R1, R2, M1, M2, P1, P2, P3, and P4).

q-score	R1	R2	M1	M2	P1	P2	P3	P4
R1		2,67	3,32	5,46	1,97	0,89	0,01	1,79
R2	2,67		4,82	2,39	1,44	2,23	2,69	3,82
M1	3,32	4,82		6,67	4,50	3,93	3,34	1,86
M2	5,46	2,39	6,67		4,49	5,26	5,51	6,21
P1	1,97	1,44	4,50	4,49		1,30	2,00	3,44
P2	0,89	2,23	3,93	5,26	1,30		0,89	2,62
P3	0,01	2,69	3,34	5,51	2,00	3,91		1,82
P4	1,79	3,82	1,86	6,21	3,44	2,62	1,82	

Table 5: q-scores for EntC mean values among different GHEs. Yellow cells indicate the statistically significant differences (Tukey-Kramer's test; $p < 0.05$) between specific GHE pairs, where the q-scores exceeded the critical value (4.36).

No statistically significant differences in ACC and EntC values were observed in relation to the sex and age of the wild boars, as well as the hunting method and shooting accuracy. Significant variations were observed for the following variables: the time interval between killing and evisceration, which showed a significant effect only for EntC (Figure 7); the time between evisceration and skinning, which was significant for both ACC and EntC (Figure 8); and the total time from killing to skinning, which was significant for both ACC and EntC (Figure 9).

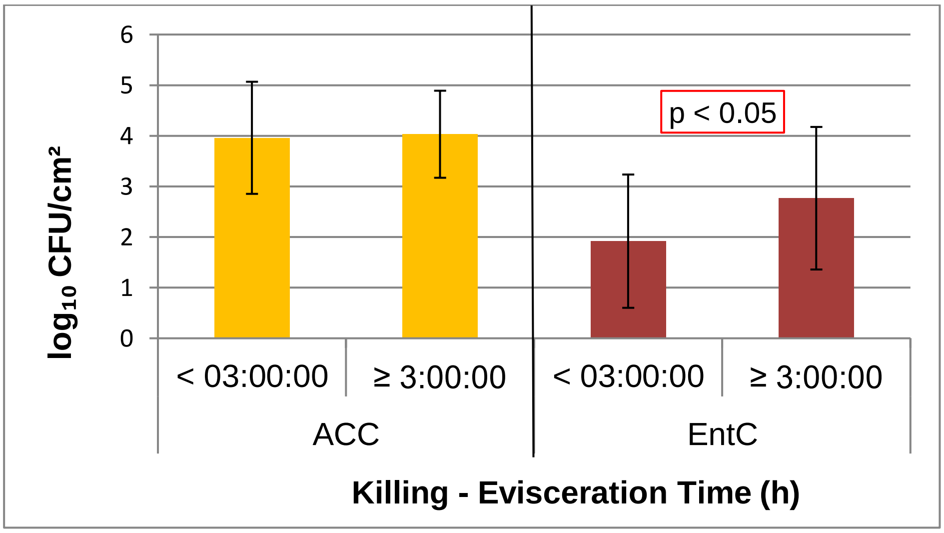


Figure 7: ACC and EntC mean values in wild boar samples eviscerated within 3 hours of killing and more than 3 hours after killing. ACC and EntC were examined independently. A statistically significant difference was observed for EntC (one-way ANOVA; $p < 0.05$).

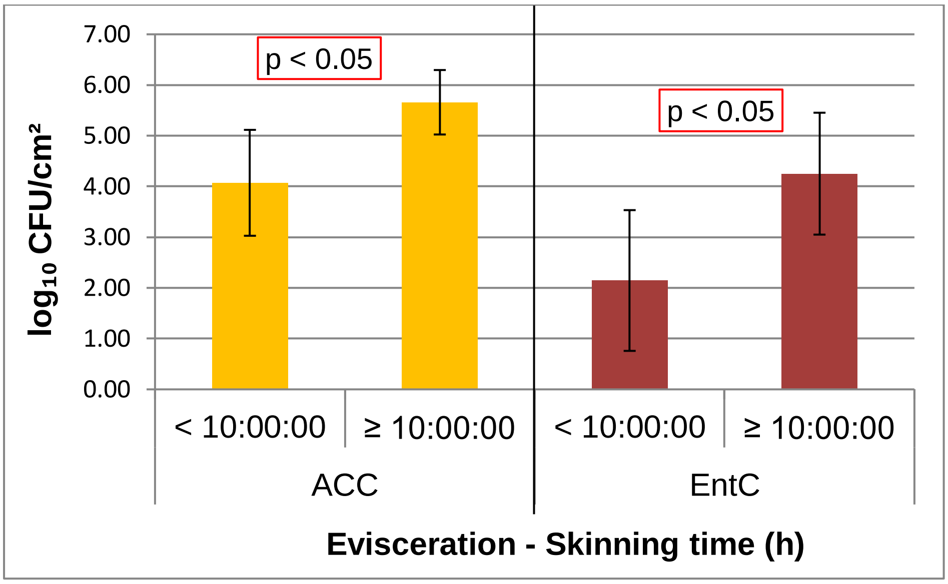


Figure 8: ACC and EntC mean values in wild boar samples skinned within 10 hours of evisceration and more than 10 hours after evisceration. Statistically significant differences were observed for both ACC and EntC (one-way ANOVA; $p < 0.05$).

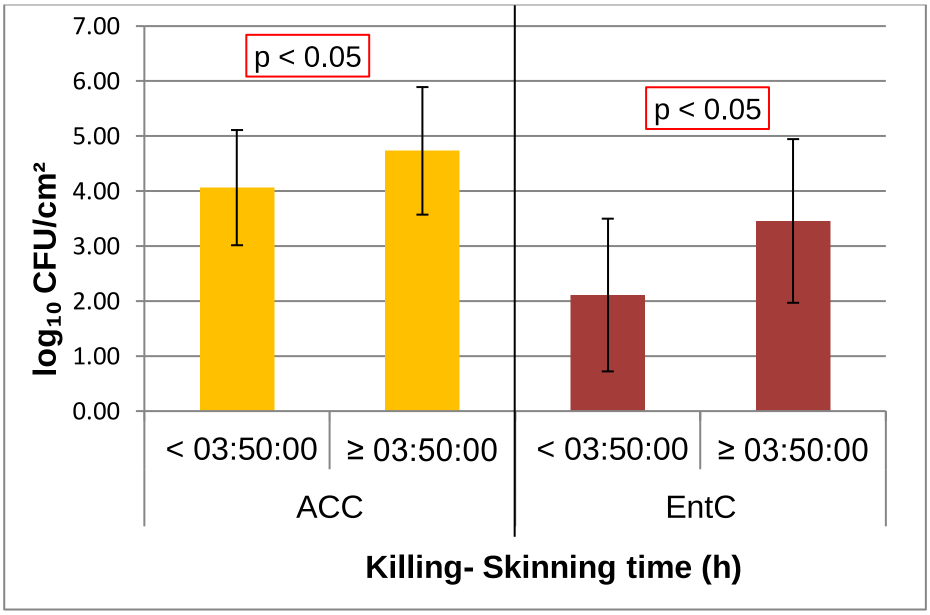
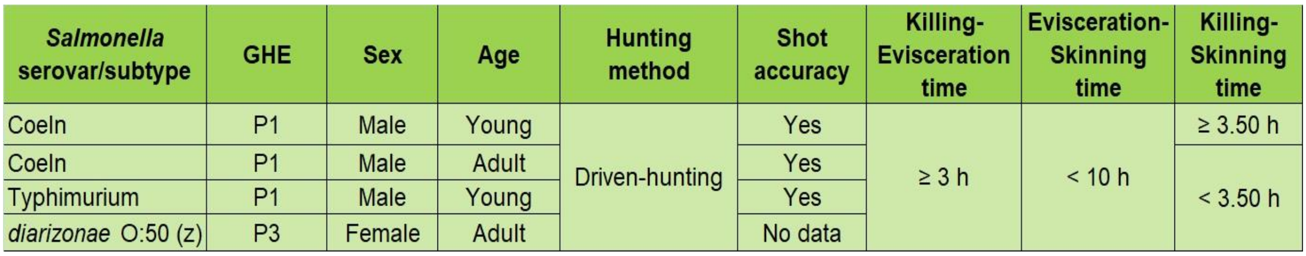


Figure 9: ACC and EntC mean values in wild boar samples skinned within 3.5 hours and more than 3.5 hours post-killing. Statistically significant differences were observed for both ACC and EntC (one-way ANOVA; $p < 0.05$).

7.3 *Salmonella* spp.

7.3.1 Prevalence and association with hunting variables

Salmonella isolates were detected in 4 out of 115 wild boar carcass sponges, representing a prevalence of 3.48% (95% CI = 0.13 - 6.83%). Among these isolates, three were identified as *S. enterica* subsp. *enterica,* i.e. two *S.* Coeln and one *S.* Typhimurium, and one was identified as *S. enterica* subsp. *diarizonae* O:50 (z). The variables associated with the *Salmonella* strains are shown in Table 6.



***Salmonella* serovar/subtype**	**GHE**	**Sex**	**Age**	**Hunting method**	**Shot accuracy**	**Killing-Evisceration time**	**Evisceration-Skinning time**	**Killing-Skinning time**
Coeln	P1	Male	Young	Driven-hunting	Yes	≥ 3 h	< 10 h	≥ 3.50 h
Coeln	P1	Male	Adult		Yes			< 3.50 h
Typhimurium	P1	Male	Young		Yes			
diarizonae O:50 (z)	P3	Female	Adult		No data			

Table 6: Salmonella *isolates and their associated recorded variables.*

A statistically significant difference was observed between positive or negative samples in relation to the time interval between killing and evisceration. Notably, all positive carcasses showed an interval ≥ 3 hours (Fisher's exact test; $p < 0.05$) (Figure 10). No significant differences were observed in any of the other variables analyzed. However, it is noteworthy that three out of the four *Salmonella* isolates were recovered from wild boars skinned in the same GHE.

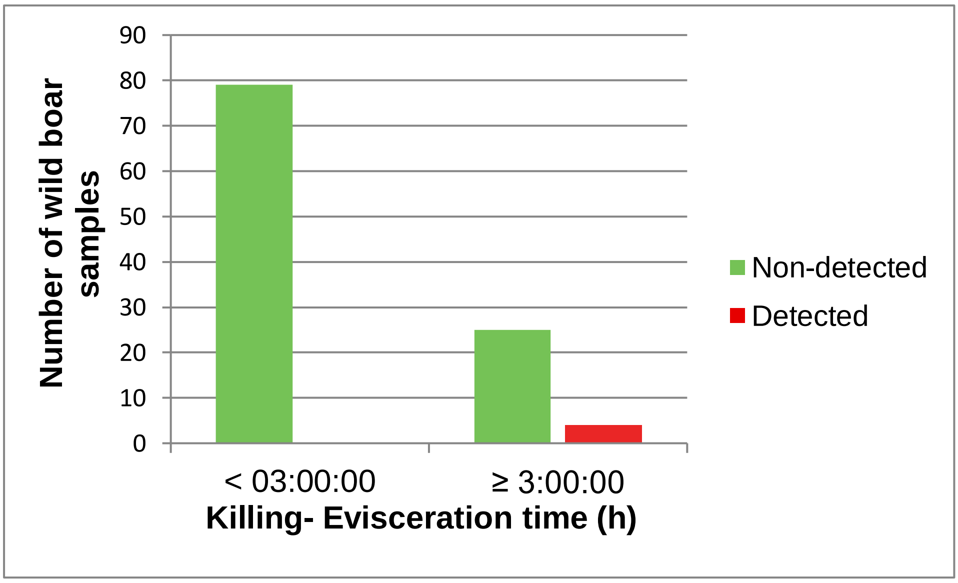


Figure 10: Number of wild boar carcasses in which Salmonella *was detected or not detected, comparing those eviscerated within 3 hours versus those eviscerated after more than 3 hours.*

A statistically significant difference was also observed in the EntC values between wild boar carcasses positive or negative for *Salmonella* spp., as determined by the Mann-Whitney U Test ($p < 0.05$) (Figure 11).

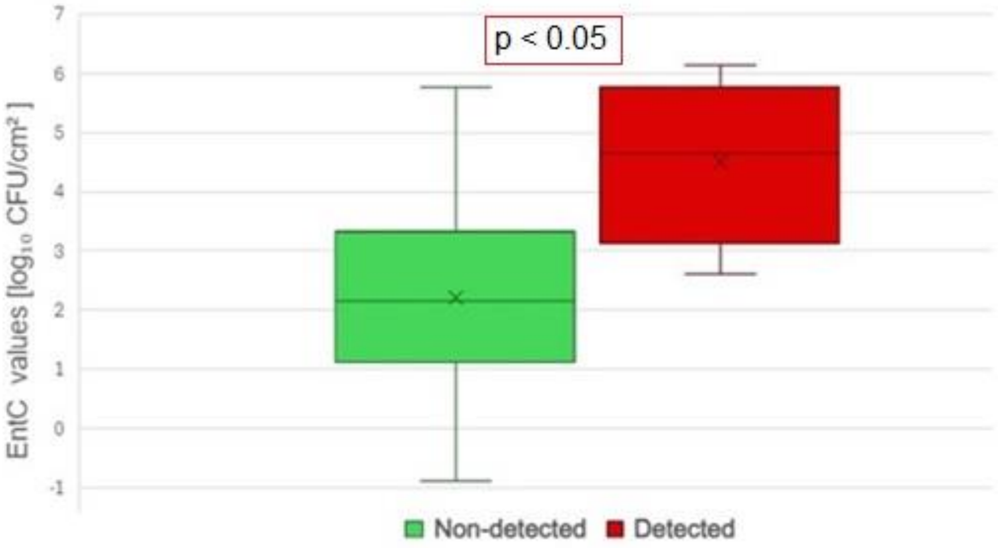
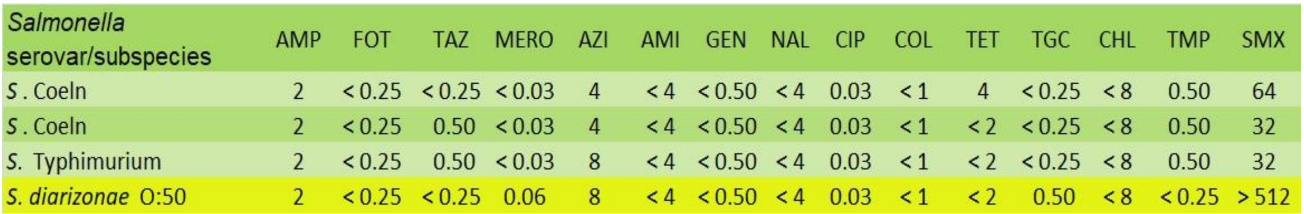


Figure 11: Distribution of EntC values in wild boar carcasses based on Salmonella *occurrence. A statistically significant difference was observed (Mann-Whitney U Test; $p < 0.05$).*

7.3.2 Antimicrobial resistance in *Salmonella* isolates

The four *Salmonella* isolates were tested for AMR. *S.* Coeln and *S.* Typhimurium were susceptible to all the antimicrobials tested, while *S. diarizonae* O:50 (z) exhibited resistance to sulfamethoxazole, as determined by EUCAST (2024) and CLSI (2024) guidelines. The MIC values (µg/mL) are reported in Table 7*.*



Salmonella serovar/subspecies	AMP	FOT	TAZ	MERO	AZI	AMI	GEN	NAL	CIP	COL	TET	TGC	CHL	TMP	SMX
S . Coeln	2	< 0.25	< 0.25	< 0.03	4	< 4	< 0.50	< 4	0.03	< 1	4	< 0.25	< 8	0.50	64
S . Coeln	2	< 0.25	0.50	< 0.03	4	< 4	< 0.50	< 4	0.03	< 1	< 2	< 0.25	< 8	0.50	32
S. Typhimurium	2	< 0.25	0.50	< 0.03	8	< 4	< 0.50	< 4	0.03	< 1	< 2	< 0.25	< 8	0.50	32
S. diarizonae O:50	2	< 0.25	< 0.25	0.06	8	< 4	< 0.50	< 4	0.03	< 1	< 2	0.50	< 8	< 0.25	> 512

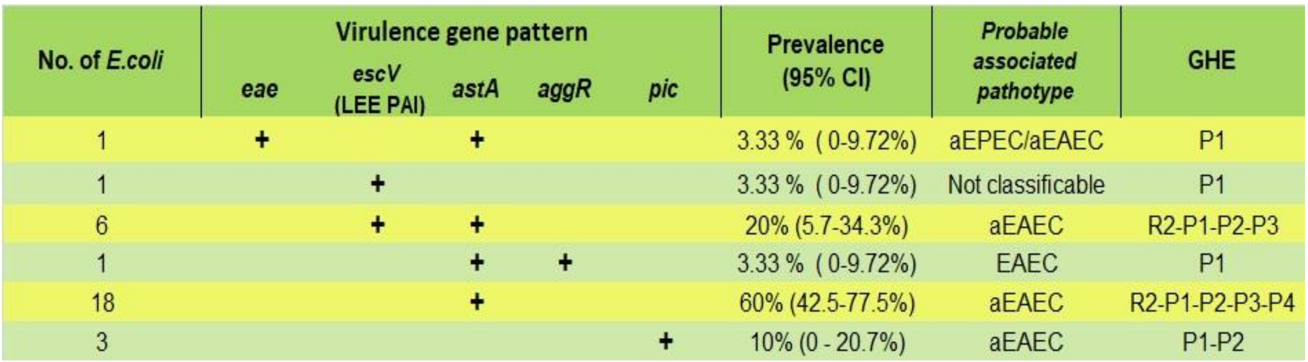
Table 7: MIC values of the Salmonella *strains.*

7.4 Prevalence and virulence factors of *E. coli*

Escherichia coli strains were isolated from 76 out of 78 wild boar diaphragmatic muscle samples, with a prevalence of 97.43% (95% CI= 93.2 – 100%).

None of the isolates exhibited phenotypic resistance to third-generation cephalosporins or carbapenems.

Negative results for all tested virulence genes were observed in 46 out of 76 *E. coli* isolates, corresponding to 60.52% (95% CI = 49.5 – 71%). The remaining 30 isolates (39.5%, 95% CI = 28.5 – 50%) tested positive for at least one virulence gene. The *eae* gene, associated with the EPEC pathotype, was detected in only 1 of 30 isolates (3.33%, 95% CI = 0 – 9.72%), while 7 of 30 strains (23.3%, 95% CI = 8.3 – 38.3%) carried the *escV* gene of the LEE PAI, associated with multiple pathotypes. The most frequently detected gene was *astA*, associated with EAEC, found in 26 of 30 *E. coli* isolates (86.7%, 95% CI = 74.7 – 98.7%). The *aggR* and *pic* genes, both linked to EAEC, were detected in 1 of 30 isolates (3.33%, 95% CI = 0 – 9.72%) and 3 of 30 isolates (10%, 95% CI = 0 – 20.7%), respectively. No *stx1* and *stx2* genes were detected, thus no STEC strains were identified. Similarly, no *E. coli* strains tested positive for the *bfpB, elt, estIa, estIb*, and *invE* genes, excluding the presence of ETEC, and EIEC pathotypes. Eight of the 30 *E. coli* isolates (26.6%, 95% CI = 10.8 – 42.4%) carried more than one virulence gene. Overall, the pathotypes most likely detected in wild boars were atypical EPEC *(eae*+ and *bfpB*-) and typical (*aggR*+) and atypical (*aggR*-) EAEC. A summary of the virulence gene patterns is provided in Table 8.



No. of *E.coli*	Virulence gene pattern					Prevalence (95% CI)	*Probable associated pathotype*	GHE
	eae	*escV* (LEE PAI)	*astA*	*aggR*	*pic*			
1	+		+			3.33 % (0-9.72%)	aEPEC/aEAEC	P1
1		+				3.33 % (0-9.72%)	Not classificable	P1
6		+	+			20% (5.7-34.3%)	aEAEC	R2-P1-P2-P3
1			+	+		3.33 % (0-9.72%)	EAEC	P1
18			+			60% (42.5-77.5%)	aEAEC	R2-P1-P2-P3-P4
3					+	10% (0 - 20.7%)	aEAEC	P1-P2

Table 8: Virulence gene patterns and their respective prevalence in the 30 E. coli *isolates, along with their probable associated pathotypes and the corresponding GHEs.*

8. Discussion

8.1 Microbial contamination of wild boar carcasses

The expanding wild boar population, primarily managed through hunting (Fulgione et al., 2022), has led to a significant increase in the availability of wild boar meat and products thereof (Fantechi et al., 2022). The microbiological quality of wild boar is generally considered comparable to that of domestic pigs, and this underpins the absence of specific standards for game meat in Regulation (EC) No 2073/2005 (Membrè et al., 2011). Nevertheless, the hygiene conditions during hunting and the subsequent handling of game differ significantly from those kept in slaughterhouses. Thus, the potential for microbial contamination in game meat is much higher, as the risk of secondary contamination during evisceration, as well as other manipulations in the field is considerably higher than in a controlled slaughterhouse environment. This situation introduces a range of variables that are more challenging to manage compared to conventional livestock production systems, including more limited veterinary inspections and insufficient guidance for hunters regarding the adoption of appropriate Good Hygiene Practices (GHP) (Borilova et al., 2016). In our study, the same process hygiene criteria set by Regulation (EC) No 2073/2005 for farmed animals were assessed to evaluate microbiological contamination of wild boar carcasses at the end of the skinning process in different GHEs, and to identify which hunting-related variables could influence the microbial load of carcasses. However, the differences in the processing of hunted game make it challenging to apply the hygiene criteria designed for livestock to wild boar carcasses, complicating direct comparisons. The ACC and EntC mean values detected in this study were 4.22 and 2.27 $\log_{10}$ CFU/cm^2, respectively. The Regulation (EC) No 2073/2005 defines the "m" and "M" limits for ACC and EntC criteria for farmed animal carcasses, establishing thresholds for classification as "satisfactory" (mean $\log_{10}$ CFU/cm^2 $\leq$ m), "acceptable" (m < mean $\log_{10}$ CFU/cm^2 < M), and "unsatisfactory" (mean $\log_{10}$ CFU/cm^2 > M). The Italian State-Regions Agreement No 41/2016 introduced modifications to these limits to accommodate non-destructive sampling methods, such as sponge sampling. Specifically, the values of "m" and "M" have been reduced by one-fifth of the values specified in Regulation (EC) No 2073/2005 (Table 9), as attested by the official veterinarians of the Local Health Authority (ASL) of Reggio-Emilia (Italy). Compared to the "m" and "M" limits established for farmed pig carcasses by the EU Regulation, the ACC and EntC mean values from this study were classified as "acceptable". However, it should be considered that farmed pig carcasses undergo scalding, dehairing, and singeing, thus retaining the skin, while wild boar carcasses are skinned (Bonardi et al., 2021). Since the skinning process might influence the microbial load of the carcass, it could be more reasonable to evaluate the microbial values of slaughtered wild boars with the process hygiene criteria of skinned animals (*i.e.*, bovines) (Stella et al., 2018). Nevertheless, when compared to the thresholds for bovine carcasses, the ACC and EntC mean values of the wild boars tested were also classified as “acceptable”.

		m [$\log_{10}$ CFU/cm^2]	M [$\log_{10}$ CFU/cm^2]
Carcasses of pigs	ACC	3.9	4.9
	EntC	1.9	2.9
Carcasses of cattle	ACC	3.4	4.9
	EntC	1.4	2.4

Table 9: The “m” and “M” values for ACC and EntC in pig and cattle carcasses (sampled by sponge technique) as defined by the Italian State-Regions Agreement No 41/2016.

The aerobic bacteria act as indicators of overall hygiene conditions, whereas the *Enterobacteriaceae* are more likely indicators of faecal contamination. The classification of “acceptable” rather than “satisfactory” underscores the challenges associated with adhering to GHPs during the hunting process, particularly during evisceration and skinning (Ramanzin et al., 2010). The contamination levels in wild boar carcasses may also be attributed to the potential transfer of microorganisms from the skin to the meat during the skinning step. In fact, wild boar skin tends to have higher microbial loads compared to that of ruminants, likely due to differences in their behaviour and ecology (Atanassova et al., 2008). Some Italian studies, evaluating the microbial contamination of wild boar carcasses via sponge sampling, have reported similar ACC and EntC values to ours (4.67 and 2.60 $\log_{10}$ CFU/cm^2, Orsoni et al., 2020; 4.61 and 3.00 $\log_{10}$ CFU/cm^2, Avagnina et al., 2012). Nevertheless, other studies have shown lower (3.21 and 1.32 $\log_{10}$ CFU/cm^2, Stella et al., 2018) or higher levels (5.88 and 5.39 $\log_{10}$ CFU/cm^2, Peruzy et al., 2022a). A European study reported slightly higher values of 5.2 and 3.6 $\log_{10}$ CFU/cm^2 for ACC and EntC respectively (Mirceta et al., 2017). In the present study, the contamination levels of both ACC and EntC showed statistically significant differences among the eight GHEs. R2 exhibited the highest ACC values, while M1 showed the lowest. For EntC, in P3 both the highest and lowest values were observed. This may suggest that while aerobic bacterial contamination primarily reflects hygiene conditions within the facilities, the presence of *Enterobacteriaceae* may be more influenced by the accuracy of the earlier steps in the hunting process. An Italian study on knives used during the slaughtering of wild boars and on chilling rooms in GHEs reported ACC and EntC levels of 3.03 $\log_{10}$ CFU/cm^2 and 2.24 $\log_{10}$ CFU/cm^2, respectively. These results underscore the importance of maintaining proper hygiene in instruments and facilities to reduce carcass contamination (Peruzy et al., 2022a). Nonetheless, microbial contamination of carcasses due to bacterial transfer from the hide or gastrointestinal tract can be significantly affected also by shooting accuracy and subsequent handling during processing (Ramanzin et al., 2010). Mirceta et al. (2017) identified significant differences in ACC and EntC levels among various GHEs, underscoring how variability in hunting practices and hunters' actions can impact meat microbiological quality. This highlights the need for adequate training of workers and strict adherence to GHP when handling and processing wild boar carcasses in GHEs. Differences in dressing techniques were associated with significantly varied microbial contamination levels, further increased by inadequate hand and knife hygiene (Mirceta et al., 2017). Conversely, Peruzy et al. (2022a) reported no significant differences in bacterial contamination levels between different GHEs, suggesting that factors beyond the hunting environment might also play a pivotal role in influencing microbial loads.

In this study, ACC and EntC values did not show statistically significant differences based on biometric data such as the sex and age of wild boars, consistent with findings from other Italian studies (Russo et al., 2016; Peruzy et al., 2022a). Conversely, Ranucci et al. (2021) and Orsoni et al. (2020) reported significant differences in ACC levels among age groups, with ACC increasing alongside animal weight, a parameter correlated with age. This suggests that older wild boars may carry higher microbial loads. Additionally, another study found that also EntC levels were influenced by both sex and age, with older males showing significantly higher contamination. This is likely due to the fact that male wild boars typically have higher live body weights (Stella et al., 2018). Indeed, heavier wild boars are more challenging to manage in the forest, requiring more time and effort to retrieve and transport to the collection point. For this reason, within a game meat supply chain, wild boars weighing less than 60 kg are often preferred (Ranucci et al., 2021).

The other variables considered in this study, particularly hunting methods, shooting accuracy and, the time intervals between different stages of the hunting process represent key factors of variability in hunting practices, highlighting the complexity of microbiological contamination in wild boar carcasses (Orsoni et al., 2020; Ranucci et al., 2021).

In our study, driven-hunting and "girata" were the methods employed by hunters for the wild boars sampled, as they are the most commonly used methods in Emilia-Romagna region. No statistically significant differences in ACC and EntC values were observed between the two hunting approaches. Both methods, despite their differences, involve the use of dogs and hunting groups. In particular, driven-hunting methods, can compromise carcass hygiene and warrant careful consideration within a game meat supply chain (Ranucci et al., 2021). Supporting this, Avagnina et al. (2012) observed significantly higher bacterial contamination in wild boars hunted using driven methods compared to individually hunted wild mammals, which employed spot-and-stalk techniques without the use of dogs that chase or attack the animals, forcing them toward hunters.

The hunting method is closely associated to shooting accuracy. It is reasonable to assume that driven-hunting may result in a higher incidence of inaccurate placed shots compared to stalking, where hunters target individual animals with greater precision (Ramanzin et al., 2010). As previously reported, an inaccurate shot to the abdominal region can result in non-fatal wounds, allowing the animal to continue running and thereby increasing the risk of contamination. Moreover, such shots can rupture the gastrointestinal tract, further contributing to carcass contamination. Avagnina et al. (2012) reported significantly higher ACC values, but not EntC values, in wild boars shot in the abdominal region compared to other areas, emphasizing the importance of precise shooting to minimize contamination risks. Contrary to this expectation, no statistically significant differences in ACC and EntC values based on shooting accuracy were found in this study, likely due to over 80% of the wild boars being killed with accurate shot placement. Similarly, other studies have found no significant impact of shooting accuracy on carcass contamination (Mirceta et al., 2017). However, lower microbial loads were observed when a single, effective shot ensured rapid death in wild boars (Peruzy et al., 2022a).

The evisceration is paramount in ensuring the microbiological quality of game meat. Practices such as performing evisceration in a lying position on the ground should be strictly avoided, with preference given to hanging evisceration, which has been associated with reduced contamination risks (Mirceta et al., 2017). However, a limitation of the present study is the lack of detailed information regarding the location and methods used for evisceration of the wild boars sampled.

The time period between killing and evisceration is another critical factor influencing game meat microbiological quality. It has been reported that a 3 h window is considered critical for minimizing bacterial spread from the gastrointestinal tract (Avagnina et al., 2012). In line with this, the present study found that wild boars eviscerated within 3 h post-killing exhibited significantly lower EntC values compared to those eviscerated after more than 3 h ($p < 0.05$). Nevertheless, ACC values did not vary significantly based on the killing-to-evisceration time interval. Some previous studies reported no significant differences in aerobic bacteria and *Enterobacteriaceae* contamination related to evisceration timing, likely due to shorter intervals of approximately 1 h (Orsoni et al., 2020; Peruzy et al., 2022a), although higher contamination levels were observed at 3 h compared to 1 h (Avagnina et al., 2012).

The primary source of wild boar carcass contamination is the hide, as the prolonged presence of bristles increases microbial loads on the carcasses (Avagnina et al., 2012). Accordingly, studies recommend prompt skinning to reduce contamination risks (Bonardi et al., 2021). In the present study, the time period between evisceration and skinning significantly influenced ACC and EntC values, with carcasses skinned within 10 h of evisceration showing lower microbial loads compared to those skinned after 10 h ($p < 0.05$). Ranucci et al. (2021) identified the duration of refrigeration time at the collection centre as the primary factor influencing both ACC and EntC values, when animals were stored unskinned after evisceration. This highlights the importance of minimizing storage time to maintain carcass hygiene. In this study, the longest recorded interval between evisceration and skinning was 60 h, observed in a single sample, which notably exhibited the highest ACC values. The total time between killing and skinning was also considered in this study. The samples of wild boar skinned within 3.5 hours of killing showed significantly lower ACC and EntC values compared to those skinned more than 3.5 hours after killing. An Italian study reported a significant increase in the *Enterobacteriaceae* load on carcasses between the evisceration and skinning period. This increase may be attributed to the bacterial species capable of proliferating at refrigeration temperatures, the temperature fluctuation in the chilling room, and the transportation conditions (Bonardi et al., 2021). Indeed, the rapid refrigeration of killed animals, along with the continuous and rigorous maintenance of the cold chain during storage, is essential to prevent spoilage and preserve its microbiological quality (Avagnina et al., 2012). In this study, while the specific times of refrigeration were not recorded, the temperatures of refrigeration rooms at the GHEs were monitored and consistently maintained below 7°C, in compliance with Regulation (EC) No 853/2004.

The significance of low temperatures in reducing bacterial contamination, including both ACC and EntC levels on carcasses, has been emphasized. Some studies observed higher bacterial counts in wild boars hunted at environmental temperatures exceeding 12°C–15°C (Peruzy et al., 2022a; Stella et al., 2018). This demonstrates that body temperature can decrease more rapidly when the environmental temperatures are below 10°C. Additionally, rainy conditions have been linked to increased faecal and ground contamination or the spread of pre-existing skin contamination (Ranucci et al., 2022). In this study, neither ambient temperature nor weather conditions during hunting were recorded. The hunting season in Italy typically occurs during autumn and winter, when cold temperatures prevail, however selective hunting for wild boar population control is carried out throughout the year.

8.2 *Salmonella* spp. detection in wild boars

Wild boars are recognized reservoirs of pathogenic microorganisms, including *Salmonella spp.,* a multi-host pathogen known for its long-term environmental persistence. This bacterium can survive under various environmental conditions, tolerating a wide range of temperatures (2°C to 54°C) and pH levels (3.7 to 9.4). Wild boars being omnivorous scavengers, known to forage on a wide variety of food sources, including carrion, insects, and reptiles, increases the likelihood of bacterial ingestion. A semi-quantitative risk assessment suggests that when *Salmonella* prevalence is low and game meat consumption is limited, the risk to public health remains minimal, though not entirely eliminated. Therefore, controlling *Salmonella* remains a high priority in ensuring the safety of wild boar meat (Chiari et al., 2013). In wild boars *Salmonella* is primarily located in the intestines, and the risk of carcass contamination arises during hunting, particularly due to intestinal rupture caused by shot placement or improper evisceration.

Additionally, bacteria can spread during handling and skinning procedures at game-processing facilities. Monitoring for *Salmonella* on farmed animal carcasses along with ACC and EntC, constitutes a process hygiene microbiological criterion. However, no reference limits are specified for game meat under Regulation (EC) No 2073/2005. For satisfactory results, *Salmonella* should ideally not be detected on carcasses, though the regulation allows minimal tolerances under specific limits. Referring to the thresholds set for bovine carcasses (given the similarity in slaughtering processes with wild boars), up to 2 positives per 50 carcasses tested (4.0%) is permissible, while for pig carcasses (due to the same species), the limit increases to 3 positives per 50 carcasses (6.0%). In the present study, the prevalence of *Salmonella* on wild boar carcasses was 3.48% (4 out of 115), aligning with the reported ranges but emphasizing the need for continuous monitoring and stringent control measures.

No pathological alterations were observed on the wild boar carcasses analyzed in this study, as noted also in other ones (Chiari et al., 2013; Razzuoli et al., 2021). Consequently, the isolation of *Salmonella* was not associated with macroscopic lesions indicative of salmonellosis, as *Salmonella* can frequently be present without causing visible signs of infection. This contrasts with findings in wild boars, where *Salmonella* Choleraesuis have been associated with pathological conditions (Longo et al., 2019).

The prevalence rates of *Salmonella* in wild boars reported across Italy vary significantly, ranging from negative findings (Orsoni et al., 2020; Peruzy et al., 2022a) to rates such as 2.5% (Ranucci et al., 2021), 3.6% (Rodas et al., 2014), 4.5% (Russo et al., 2016), 6.27% (Cilia et al., 2021), and up to 35.7% (Peruzy et al., 2022b). In Europe, *Salmonella* prevalence in wild boar samples also shows considerable regional variability, with lower rates observed in Spain (1.2%, Díaz-Sánchez et al., 2013) and Serbia (1.9%, Mirceta et al., 2017), compared to higher rates in Switzerland (12%, Wacheck et al., 2010) and Portugal (22%, Vieira-Pinto et al., 2011). In Central Europe, the prevalence of *Salmonella* on wild boar carcasses has been reported to range from less than 1% to approximately 7% (Paulsen et al., 2012). EFSA and ECDC (2024a) reported a prevalence of *Salmonella* positive wild boars of 8.0% in two Member States in 2023. This variability may be attributed to the sample matrix, as demonstrated in a study where *Salmonella* was detected in the caecal content and lymph nodes of wild boars, while negative results were observed on the carcasses (Stella et al., 2018). In another study, a *Salmonella* prevalence of 24.82% was found in the intestinal content of wild boars, with thirty different serovars isolated, belonging to three different *S. enterica* subspecies (Chiari et al., 2013).

Wild boars can harbour distinct microbial populations due to their lack of territorial boundaries and highly variable diets, leading to significant geographical variation in the prevalence of *Salmonella.* While some populations may be carriers of *Salmonella*, others have lower pathogen prevalence. Overall, the risk posed by *Salmonella* in large wild game is considered low due to its sporadic detection (Atanassova et al., 2008).

The four *Salmonella* isolates identified in this study included two *S*. Coeln, one *S.* Typhimurium, and one *S. diarizonae* O:50 (z), with *S. enterica* being the most prevalent subspecies. These serovars have been frequently isolated from wild boars in Italy (Zottola et al., 2013; Razzuoli et al., 2021; Bonardi et al., 2021; Peruzy et al., 2022b). However, Italy exhibits significant serovar variability, suggesting multiple potential sources of *Salmonella* exposure, including livestock farming, waste disposal, and contact with other wild species such as birds and amphibians (Chiari et al., 2013). Indeed, it has been demonstrated that *S.* Typhimurium isolates from wild boars share lineages with those isolated from bovines (Bolzoni et al., 2023). Moreover, an outbreak of salmonellosis in wild ruminants was attributed to their shared pastures with cattle, highlighting the critical role of interspecies interactions in *Salmonella* transmission (Paulsen et al., 2012). Despite these observations, Bonardi et al. (2019c) reported limited exchange of *Salmonella* serovars between farmed pigs and wild boars in the Emilia-Romagna region, showing that interspecies transmission may be context-dependent. *Salmonella* can be released into the environment by infected wild boars, resulting in surface water contamination and potentially causing direct or indirect contamination of crops. Additionally, it can be transmitted to farmed animals, raising concerns about its role in zoonotic transmission. Indeed, S*almonella* is the leading cause of outbreaks and cases linked to various types of food, thus involving processing by food business operators (FBOs). Surveillance strategies that start at the primary production level and extend across the entire food chain have proven particularly effective for *Salmonella*, facilitated by a unique collaboration between FBOs and competent authorities in control and monitoring efforts (EFSA and ECDC, 2024a). Similar approaches could also be adapted for the surveillance and control of hunted game.

S. Typhimurium is a frequently detected serovar in wildlife, including wild boars (Vieira-Pinto et al., 2011), as well as in birds and cold-blooded animals (Paulsen et al., 2012), and is also prevalent in farmed species. Its presence is particularly concerning, as it was the second most reported cause of human salmonellosis in the EU in 2023 (EFSA and ECDC, 2024a). Similarly, *S*. Coeln is often identified in wild boars and ranked fifth among serovars responsible for human salmonellosis in 2023, underscoring its potential public health impact (EFSA and ECDC, 2024a). *S. diarizonae* O:50 (z) is a serovar commonly isolated from beef, sheep, and snakes (Zottola et al., 2013), but also reported in wild boars (Bonardi et al., 2021). While this serovar is rarely implicated in human infections, its prevalence in wildlife warrants attention as a potential zoonotic agent. Additionally, an in vitro study demonstrated that, beyond *S.* Typhimurium and *S*. Coeln, which can penetrate intestinal epithelial cells and up-regulate the expression of genes for IL-8 and TNF-α, *S. diarizonae* has also been shown to trigger the up-regulation of the gene for INF-β, potentially provoking an inflammatory response (Razzuoli et al., 2017). This underlines the importance of *Salmonella* monitoring in wildlife populations. However, no cases of salmonellosis linked to the consumption of wild boar meat have been reported in Italy. This is likely due to the low prevalence of *Salmonella* in wild boars, the relatively limited consumption of wild game meat compared to total meat intake, as well as the challenges associated with conducting epidemiological investigations into foodborne diseases (Razzuoli et al., 2021).

Notably, the *S*. Coeln and *S*. Typhimurium isolates were recovered from wild boar carcasses processed at GHE P1, while S. *diarizonae* O:50 (z) was isolated at GHE P3. This means that *Salmonella* was detected in only two of the eight GHEs examined, with multiple isolates found in one establishment and none in the other six. These findings may reflect variations in *Salmonella* prevalence among wild boar populations in different ATCs of Emilia-Romagna region, as well as differences in evisceration and skinning practices at the GHEs. As an indicator of process hygiene, *Salmonella* positivity highlights the influence of hunting areas and processing methods on the contamination outcomes.

In this study, neither the sex nor the age of wild boars significantly influenced the presence of *Salmonella*. Similarly, other studies reported no significant association between these factors and pathogen prevalence (Stella et al., 2018; Cilia et al., 2021). Conversely, some studies have identified a significantly higher prevalence of *Salmonella* in younger animals (Chiari et al., 2013). Furthermore, Wacheck et al. (2010) and Rodas et al. (2014) reported higher pathogen prevalence in female wild boars compared to males. These findings may be attributed to behavioural differences: females and juveniles typically form social groups, which could facilitate pathogen transmission, while adult males are more solitary, potentially limiting their exposure.

The four wild boars in which *Salmonella* was detected were all hunted using the driven-hunting method, with three out of four being accurately shot and one lacking data on shooting accuracy. This suggests that, as observed for *Enterobacteriaceae*, *Salmonella* was still detected despite the hunting practices did not cause rupture of the intestinal tract. Notably, all *Salmonella* isolates were recovered from wild boars eviscerated > 3 h after killing, suggesting that, similar to *Enterobacteriaceae* contamination, the delay between killing and evisceration significantly influenced the bacterial load on the carcasses, including the presence of *Salmonella*. Conversely, neither the time between evisceration and skinning (which was always under 10 hours) nor variations in the killing-skinning time (both shorter and longer than 3.5 hours) had a significant impact on *Salmonella* prevalence. Similarly, previous studies have shown that the conditions under which wild boars were hunted and subsequently eviscerated did not have a significant impact on the prevalence of pathogenic bacteria on their carcasses (Peruzy et al., 2022a). However, another study reported that *Salmonella* contamination on wild boar carcasses increased threefold during storage at low temperature, in the period between evisceration and skinning (Bonardi et al., 2021). In contrast, EFSA (2011) suggested that bacterial recovery could be reduced at low temperatures due to bacterial stress caused by refrigeration.

Interestingly, in the present study, *Salmonella* was detected in samples with statistically significant higher levels of *Enterobacteriaceae* (EntC) ($p < 0.05$), and the sample showing the highest EntC load was found to be positive for *Salmonella*. A similar finding was reported also in a previous study (Mirceta et al., 2017). Notwithstanding, other investigations have shown no correlation between EntC values and *Salmonella* contamination (Ranucci et al., 2022; Altissimi et al., 2024).

The temperature is also a factor that can influence the detection of *Salmonella.* Indeed, increased prevalence of pathogenic bacteria has been observed at higher environmental temperatures (> 15°C) (Stella et al., 2018). Specific information on the temperature was not included in our study, although the wild boar hunting took place during the autumn-winter period.

As established by the Commission Implementing Decision (EU) No 2020/1729, the monitoring of AMR is mandatory for *Salmonella* spp. in major farmed animal populations and their derived meat products. However, such monitoring is not required for hunted game (EFSA and ECDC, 2024b). In this study, AMR was evaluated in the *Salmonella* isolates from wild boar carcasses. *S.* Coeln and *S.* Typhimurium demonstrated susceptibility to a panel of 15 antimicrobials from different classes. Similarly, *S. diarizonae* O:50 (z) exhibited high susceptibility, showing resistance only to sulfamethoxazole. This suggests that, since wild boars are not directly treated with antimicrobials, they are less likely to serve as reservoirs of antibiotic-resistant bacteria. AMR in wild animals is often linked to the environmental circulation of microorganisms deriving from farmed animals or human-related sources. Indeed, similarly, other studies conducted in northern Italy have reported low levels of resistance in *Salmonella* serovars, with resistance to sulfamethoxazole detected exclusively in *S. diarizonae* (Bonardi et al., 2021). In contrast, high levels of resistance in *Salmonella* isolates from wild boars have been reported in central Italy, particularly to sulfonamides, trimethoprim, colistin, streptomycin, gentamicin, tetracycline, and third generation cephalosporins (Zottola et al., 2013). The resistance to sulfonamides in *Salmonella* shows the highest frequency in both Italian and European wild boars, as well as resistance to tetracyclines and ampicillin. Indeed, high levels of AMR in *Salmonella* isolates from wild boars have also been reported in Germany (Methner et al., 2018) and Spain (Gil Molino et al., 2019). These findings support the hypothesis that antimicrobial agents used in agriculture, livestock production, and human medicine may contribute to the development of AMR in wildlife (Zottola et al., 2013; Razzuoli et al., 2021). Variations in the prevalence of AMR may result from differences in the distribution of specific *Salmonella* serovars and phage types across countries and regions, animal species, and wildlife. Certain *Salmonella* serovars or phage types within serovars may exhibit distinct and characteristic patterns of antimicrobial resistance to specific antimicrobials (EFSA and ECDC, 2021). According to EFSA, in 2022, *Salmonella* strains isolated from food-producing animals exhibited moderate to very high resistance to ampicillin, tetracyclines, and sulfonamides. The resistance to third-generation cephalosporins, such as cefotaxime and ceftazidime, was observed at low levels, while resistance to (fluoro)quinolones, including ciprofloxacin and nalidixic acid, ranged from low to moderate. In contrast, resistance to amikacin was rare, and colistin resistance was infrequently detected for pigs. Among antimicrobial resistant *Salmonella* isolates recovered from pigs, *S.* Typhimurium was one of the most common (EFSA and ECDC, 2024b). High levels of resistance to ampicillin, sulfonamides, and tetracyclines were also observed in *Salmonella* isolates from humans in 2022. However, in the same year, high rates of *Salmonella* isolates from both humans and farmed animals completely susceptible were also observed (EFSA and ECDC, 2024b).

The results from this study underscore the need for continuous and organized monitoring based on specific microbiological criteria for hunted game. The absence of clear microbiological standards at both the EU and national levels restricts competent authorities' ability to effectively enforce hygiene regulations. Moreover, this gap leaves hunters without adequate guidance for managing hygiene practices throughout the entire process, *i.e.* from "field to fork".

8.3 *Escherichia coli* in wild boars

E. coli can contaminate animal carcasses and meat if it penetrates the abdominal cavity, particularly when the animal is inaccurately shot. However, the risk of subsequent microbial contamination can be significantly minimized if the carcass is promptly and properly eviscerated. Indeed, *E. coli* is widely regarded as an indicator of GHPs (Barco et al., 2014).

According to Mirceta et al. (2017), the practice of removing the peritoneum and diaphragm is employed to reduce abdominal contamination arising from improperly placed shots and/or evisceration, highlighting the diaphragm as a source of bacterial contamination associated with improper hunting practices and inadequate hygiene procedures.

In the present study, *E. coli* was isolated from 97.43% (76/78) of the wild boar diaphragmatic muscle samples. Another Italian study reported a lower prevalence of 21.8% in fresh wild boar meat; however, the method employed for *E. coli* isolation does not include sample enrichment (Rega et al., 2021). Studies employing different sampling methodologies have shown higher prevalence rates, such as 100% in mesenteric lymph node samples (Bonardi et al., 2019a), and 87.5% in rectal swabs (Bertelloni et al., 2020). Across Europe, the prevalence of *E. coli* in wild boar populations shows variability. High prevalence rates have been reported in faecal samples from the Czech Republic (99%; Literak et al., 2010), Portugal (96%; Dias et al., 2015), and Serbia (95.28%; Velhner et al., 2018).

In our study, the high level of contamination may indicate unhygienic practices during carcass handling of the carcasses, likely caused by improperly performed evisceration. Given that shooting was accurate in most cases and that the time intervals between killing and evisceration (25 minutes to over 3 hours) and between killing and skinning (less than 30 minutes to over 3.5 hours) varied widely, these variables do not appear to have significantly influenced *E. coli* contamination.

In the EU, monitoring of AMR is mandatory for *E. coli* as an indicator commensal (Decision EU No 2020/1729) in food-producing animals and derived meat, with particular emphasis on ESβL-producing and carbapenemase-producing strains. The epidemiology of ESβL-producing microorganisms is complex, involving diverse factors such as geographic regions, healthcare settings, communities, hosts, and reservoirs like soil, water, livestock, wildlife, and pets. The role of wild animals, including wild boars, as carriers of ESβL-producing *E. coli* has been globally recognized. Moreover, the transferability of certain resistance plasmids, particularly those carrying ESβL genes, represents an additional concern (Mercato et al., 2022).

All *E. coli* isolates demonstrated phenotypic susceptibility to cephalosporins and carbapenems. In contrast, other Italian studies have identified ESβL-producing *E. coli* in wild boars, with prevalence rates of 15.96% (Formenti et al., 2021) and 23.3% (Mercato et al., 2022) in faeces, 0.9% in mesenteric lymph nodes (Bonardi et al., 2019a), and 6.5% in fresh meat (Rega et al., 2022). In other European countries, the prevalence in wild boar faeces was 10.4% in Portugal (Poeta et al., 2009), and 6% in the Czech Republic (Literak et al., 2010). This variability could primarily be attributed to variations in sampling methodologies; however, factors such as dietary differences among wild boars driven by food availability, higher levels of environmental AMR contamination, and proximity to farmed animals and human can also play a role. The prevalence of ESβL producing *E. coli* in farmed animals was reported at 33% in pigs and 38% in calves, while in pork meat and bovine meat prevalence values of 5.6% and 5% were found, respectively. Nevertheless, completely susceptible *E. coli* strains were also frequently identified both in pigs and in cattle (EFSA and ECDC, 2024b). Despite the findings of this study, monitoring of ESβL-producing *E. coli* in wild animals and derived game meat remains essential as part of the One Health approach, to trace the spread of AMR from primary sources such as farming, agriculture, and human activities to wildlife and the environment, and vice versa.

Another significant concern regarding *E. coli* in food-producing animals, including wild species and products thereof, is the occurrence of pathogenic strains. These include STEC, EPEC, EAEC, ETEC, and EIEC, which pose serious risks to food safety and public health. In the EU, STEC surveillance is mandatory in 24 Member States; however, data are not comparable across countries due to non-harmonized sampling programs. In Italy, STEC surveillance functions as a sentinel system, with a primary focus on the cases of haemolytic uremic syndrome (HUS) in the paediatric population. During 2023, the prevalence of STEC was reported to be 16.8% in cattle and 46.9% in pigs. In meat, the prevalence was 0.83% in fresh bovine meat, 20.2% in deer meat, and 10% in meat from other species, including wild boars. A single outbreak was linked to the consumption of bovine meat (EFSA and ECDC, 2024a). However, data related to hunting animals and game meat are scarce, despite various *E. coli* pathotypes having been detected in wild boars, with reports from Italy (Bertelloni et al., 2020; Peruzy et al., 2022b) and other European countries (Alonso et al., 2017; Dias et al., 2019).

In this study, the *E. coli* isolates were genotypically characterized for the presence of gene markers associated with five different pathotypes. Overall, 39.5% (30/76) of the *E. coli* isolates carried at least one virulence gene. Notably, inaccurate shooting was reported in only four of the 30 *E. coli* isolates harbouring virulence genes. However, no STEC, ETEC, or EIEC strains have been detected.

The prevalence of STEC in wild boar faeces varies widely across Europe, ranging from 0% to 28.3%. For instance, Spain reported a prevalence of 3.3% (Alonso et al., 2017), Portugal 4.8% (Dias et al., 2019), and Poland as high as 28.2% (Szczerba-Turek et al., 2019). Data on wild boar meat are more limited but reveal prevalence rates from 0% to nearly 43% (Altissimi et al., 2024). Bertelloni et al. (2020) reported a prevalence of 21.7% of STEC in wild boar rectal swabs in Italy, with the gene *stx2* being more frequently identified (24.6%) compared to the gene *stx1* (11.4%). In wild boar meat, STEC prevalence was lower (14.29%), although strains carrying both genes *stx1* and *stx2* have been detected (Peruzy et al., 2022b).

The negative results concerning STEC detection may be attributed to the isolation method we used, which primarily targeted commensal *E. coli* isolation followed by strain characterization. This focus could have limited the detection of specific pathogenic strains. EFSA and ECDC (2024a) recommend the ISO/TS 13136:2012 method for STEC detection, which includes a fundamental initial enrichment screening step. The importance of this method is underscored by Peruzy et al. (2022b), who reported a 42.86% detection rate for *stx1/stx2* genes in enrichment cultures, even though only 14.29% of STEC strains were subsequently isolated and characterized.

On the other hand, in accordance with the results of this study, ETEC and EIEC pathotypes are rarely isolated from asymptomatic wild animals (Bertelloni et al., 2020). In our study, one E. coli strain (3.33%) was positive for the *eae* gene, classifying it as atypical EPEC (aEPEC). Historically, typical EPEC (EPEC) have been defined as isolates possessing the attaching and effacing genotype (*eae*+) and the bundle-forming pilus gene (*bfp*+). *E. coli* strains that are negative for *bfp* gene but positive for *ea*e gene are classified as aEPEC (Hu et al., 2015). The role of aEPEC in human health remains uncertain (Hu et al., 2015), although some clinical cases, particularly in children, have been documented (Zhou et al., 2018). Similarly, another Italian study reported a prevalence of 3.4% for aEPEC in wild boar faeces, with the *eae* gene detected in 17.1% of samples and no positivity for *bfpB* (Bertelloni et al., 2020). Higher prevalence was observed in wild boar meat, where 32.14% of samples were positive for EPEC (Peruzy et al., 2022b). In Poland, EPEC were found in 30.9% of wild boar faeces (Szczerba-Turek et al., 2019).

The aEPEC strain identified in this study was also positive for the *astA* gene, classifying it additionally as atypical EAEC (aEAEC). The presence of the enteroaggregative heat-stable toxin gene (*astA*) in EPEC isolates has already been documented (EFSA, 2015). The occurrence of non-specific profiles, shared by multiple pathotypes, as well as hybrid strains, has become increasingly common and widespread (EFSA, 2015; Bertelloni et al., 2020). Regarding EAEC, most strains harbour a high molecular weight virulence plasmid (pAA) containing important virulence factors such as *astA* and *aggR*. However, these factors are not present in all isolates, as EAEC includes both virulent and non-virulent strains (Uber et al., 2006). Only typical EAEC strains carrying the *aggR* gene, which is essential for the expression of other virulence genes, are considered human pathogens (Sarantuya et al., 2004). Farmed animals are typically carriers of atypical EAEC (*aggR*-, *pic*+ and/or *astA*+) but seem to not play a role in their epidemiology (Uber et al., 2006; Zhou et al., 2018). In this study, one typical EAEC (*aggR*+, *astA*+) was identified (3.33%), along with 28 aEAEC strains exhibiting three distinct gene patterns: 19 strains (63.3%) were positive only for *astA* gene, 3 strains (10%) were positive only for *pic* gene, and 6 strains (20%) were positive for both *astA* and *escV* genes (LEE PAI+). The most frequently detected virulence gene was *astA* (86.7%), while the 23.3% of the isolates carried the LEE PAI (*escV*+). Bertelloni et al. (2020) reported a prevalence of 5.7% for EAEC in wild boar faeces. Specifically, the *astA* gene had a prevalence of 29.1%, while the *aggR* gene was detected in 3.4% of isolates. The prevalence of *escV* gene was found to be 4.6%.

EAEC is the second leading cause of travellers' diarrhoea after ETEC, and it is increasingly acknowledged as both an endemic and epidemic pathogen. It plays a critical role in causing persistent diarrhoea among children in developing countries. In the EU, the reported frequency of EAEC outbreaks has fluctuated, with cases ranging from six in 2012 to 24 in 2014. Foodborne outbreaks of EAEC are often linked to contamination by asymptomatic food handlers. Notably, there are no specific reporting requirements for the prevalence of EAEC in farmed animals or food within the EU (EFSA, 2015).

Interestingly, in this study, the aEAEC strains were isolated from wild boars processed in five out of eight GHEs. Conversely, aEPEC and EAEC strains were identified exclusively in samples from GHE P1. In this GHE, one wild boar tested positive for both aEAEC and *S.* Typhimurium. These finding emphasize the potential impact of factors such as geographic location, wild boar populations, hunting practices, and hygiene procedures at different GHEs on carcass contamination levels. These variables likely contribute to the observed differences in pathogen prevalence. Monitoring these pathogens, as well as antimicrobial resistance in hunted game, is crucial, even in the absence of high prevalence, to better understand their environmental spread and impact, through a One Health approach.

9. Conclusions

In conclusion, based on the results obtained in this study, it can be stated that despite the numerous variables associated with the hunting process, the overall values for ACC, EntC, and the prevalence of *Salmonella* may be considered “acceptable” under Regulation (EC) No 2073/2005. However, as previously emphasized, standardized microbiological criteria for hunted game at the European level should be set to monitor the entire hunting process. This should include comprehensive sampling plans, defined microbiological limits, and reference methods. Such measures would facilitate more targeted inspections by the Competent Authorities and could enhance hunters’ awareness through adequate training to reduce carcass contamination, whether for self-consumption or local marketing.

The game handling establishments (GHEs) play a pivotal role in the wild boar meat production chain. Proper utilization of designated workspaces is essential to prevent cross-contamination, as well as the implementation of GHPs. While shooting accuracy is essential for wild game welfare, causing immediate death and no pain, it also significantly reduces carcass contamination. However, in this study, shooting accuracy was not found to be a determining factor in the microbial load of wild boar carcasses. Moreover, the predominant hunting methods in Italy, such as driven hunting and “girata”, often culturally preferred, do not always allow for high accuracy in the killing process.

Timelines in the hunting process proved to be of critical importance. The interval between killing and evisceration should ideally be less than 3 hours, as longer durations were associated with increased contamination by *Enterobacteriaceae*, including pathogens like *Salmonella.* Similarly, the period between evisceration and skinning should not exceed 10 hours, as longer intervals significantly increased carcass contamination. Thus, completing the entire process in maximum 4 hours guarantees the best conditions to prevent microbial contamination of the carcasses. However, challenges related to long distances to collection centres or GHEs and impervious geographical areas make adhering to these timelines complex and logistically demanding.

Moreover, this study confirmed that wild boars, being free-ranging animals, can act as reservoirs of pathogens such as *Salmonella* and pathogenic *E. coli*. Although no resistance was detected to critically important antimicrobials (CIAs), resistance to sulfamethoxazole in *Salmonella diarizonae* was identified. These findings underline the importance of more frequent monitoring studies to safeguard both consumer and hunter health tracking the prevalence of foodborne pathogens and antimicrobial resistance (AMR) in wildlife.

For the above mentioned reasons, the present study highlights the urgent need for standardized microbiological criteria at EU level and integrated control measures in the hunting sector. Emphasizing a One Health approach, it calls for coordinated efforts to address the interconnected challenges of hygiene measures, pathogen transmission and AMR, bridging wildlife monitoring with broader public health and food safety goals.

Bibliography

Abrantes, A.C., and Vieira-Pinto, M. 15 years overview of European zoonotic surveys in wild boar and red deer: A systematic review. *One Health*, 16 ,100519, 2023, 10.1016/j.onehlt.2023.100519.

Agbaje, M., Begum, R.H., Oyekunle, M.A., Ojo, O.E., and Adenubi, O.T. Evolution of *Salmonella* nomenclature: a critical note. *Folia Microbiologica (Praha).* 56(6), 497-503, 2011, doi: 10.1007/s12223-011-0075-4.

Alhammadi, M. M., Godfrey, R. E., Ingram, J. O., Singh, G., Bathurst, C. L., Busby, S. J. W., and Browning, D. F. Novel organisation and regulation of the *pic* promoter from enteroaggregative and uropathogenic *Escherichia coli*. *Virulence*, *13*(1), 1393–1406, 2022, doi:10.1080/21505594.2022.2111754.

Alonso, C.A., Mora, A., Díaz, D., Blanco, M., González-Barrio, D., Ruiz-Fons, F., Simón, C., Blanco, J., and Torres, C. Occurrence and characterization of stx and/or eae-positive *Escherichia coli i*solated from wildlife, including a typical EPEC strain from a wild boar. *Veterinary Microbiology*, 207, 69-73, 2017, doi:10.1016/j.vetmic.2017.05.028.

Altissimi, C., Primavilla, S., Roila, R., Gavaudan, S., Morandi, B., Di Lullo, S., Coppini, M., Baldinelli, C., Cai, D., Branciari, R., Valiani, A., Paulsen, P., Ranucci, D. *Salmonella* in Wild Boar Meat: Prevalence and Risk Assessment in Central Italy (Umbria and Marche Region). *Foods*, 13(8), 1156, 2024, doi: 10.3390/foods13081156.

Amici, A., Serrani, F., Rossi, C.M., and Primi, R. Increase in crop damage caused by wild boar (*Sus scrofa* L.): the “refuge effect”. *Agronomy for sustainable development,* 32, 683–692, 2012, doi:10.1007/s13593-011-0057-6.

Amici, A., Cifuni, G.F., Contò, M., Esposito, L., and Failla, S. Hunting area affects chemical and physical characteristics and fatty acid composition of wild boar (*Sus scrofa*) meat. *Rendiconti Accademia Lincei ,* 26 (3), 527–534, 2015, doi:10.1007/s12210-015-0412-7.

Atanassova, V., Apelt, J., Reich, F., and Klein, G. Microbiological quality of freshly shot game in Germany. *Meat Science*, 78(4), 414-9, 2008, doi: 10.1016/j.meatsci.2007.07.004.

Avagnina, A., Nucera, D., Grassi, M.A., Ferroglio, E., Dalmasso, A., and Civera, T. The microbiological conditions of carcasses from large game animals in Italy. *Meat Science*, 9(3), 266-271, 2012, doi:10.1016/j.meatsci.2012.01.025.

Ballari, S.A., and Barrios-García, M.N. A review of wild boar *Sus scrofa* diet. *Mammal Review*, 44, 124-134, 2014, doi:10.1111/mam.12015.

Barco, L., Belluco,S., Roccato, A., and Ricci, A. *Escherichia coli* and *Enterobacteriaceae* counts on pig and ruminant carcasses along the slaughterline, factors influencing the counts and relationship between visual faecal contamination of carcasses and counts: a review. *EFSA Supporting Publication* ,11(8):EN-634, 111, 2014, doi:10.2903/sp.efsa.2014.EN-634.

Barrios-Garcia, M.N. and Ballari, S.A. Impact of wild boar (*Sus scrofa*) in its introduced and native range: a review. *Biological Invasions* 14, 2283–2300, 2012, doi:10.1007/s10530-012-0229-6.

Baskin, L., and Danell, K. Ecology of ungulates: A Handbook of Species in Eastern Europe and Northern and Central Asia. *Springer Science & Business Media.* Germany, 2003: 15-38.

Bassi, E., Gazzola, A., Bongi, P., Scandura, M., and Apollonio, M. Relative impact of human harvest and wolf predation on two ungulate species in Central Italy. *Ecological Research*, 35, 662–674, 2020, doi:10.1111/1440-1703.12130.

Beasley, J. C., Grazia, T. E., Johns, P. E., and Mayer, J. J. Habitats associated with vehicle collisions with wild pigs. *Wildlife Research*, 40(8), 654-660, 2014, doi:10.1071/WR13061

Bensaid, T., Bonnefoi-Kyriacou, B., Dupel-Pottier, C., Bellon, O., Lagier, E., and Chardon, H. *Streptococcus suis* meningitis following wild boar hunting. *Presse medicale (Paris, France: 1983)*, *32* (23), 1077-1078, 2003, PMID: 12910163.

Bertelloni, F., Cilia, G., Bogi, S., Ebani, V.V., Turini, L., Nuvoloni, R., Cerri, D., Fratini, F., and Turchi, B. Pathotypes and Antimicrobial Susceptibility of *Escherichia Coli* Isolated from Wild Boar (*Sus scrofa*) in Tuscany. *Animals,* , *10*, 744. 2020, doi:10.3390/ani10040744.

Bevins, S.N., Pedersen K., Lutman M.W., Gidlewski T., and Deliberto T.J. Consequences Associated with the Recent Range Expansion of Nonnative Feral Swine. *BioScience*, 64 (4), 291–299, 2014, doi:10.1093/biosci/biu015.

Bieber, C., and Ruf, T. Population dynamics in wild boar Sus scrofa: ecology, elasticity of growth rate and implications for the management of pulsed resource consumers. *Journal of Applied Ecology,* 42,1203–1213, 2005, doi:10.1111/j.1365-2664.2005.01094.x.

Bolzoni, L., Bonardi, S., Tansini, C., Scaltriti, E. Menozzi, I., Morganti, M., Conter, M., Pongolini, S. Different Roles of Wild Boars and Livestock in *Salmonella* Transmission to Humans in Italy. *EcoHealth,* 20, 122–132, 2023, doi:10.1007/s10393-023-01625-y.

Bonardi, S., Cabassi, C.S., Longhi, S., Pia, F., Corradi, M., Gilioli, S., and Scaltriti, E. Detection of Extended- Spectrum Beta-Lactamase producing *Escherichia coli* from mesenteric lymph nodes of wild boars (*Sus scrofa*). *Italian Journal of Food Safety*, 7(4), 7707, 2019a, doi:10.4081/ijfs.2018.7707.

Bonardi, S., and Pitino, R. Carbapenemase-producing bacteria in food-producing animals, wildlife and environment: A challenge for human health. *Italian Jourlan of Food Safety*, 8(2), 7956, 2019b doi: 10.4081/ijfs.2019.7956.

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*. 16(3), 420-428, 2019c, doi: 10.1007/s10393-019-01418-2.

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; *European Journal of Wildlife Research* , 67(5), 88, 2021, doi: 10.1007/s10344-021-01531-0.

Bongi, P., Tomaselli, M., Petraglia, A., Tintori, D., and Carbognani, M. Wild boar impact on forest regeneration in the northern Apennines (Italy). *Forest Ecology and Management*, 391, 230-238, 2017, doi:10.1016/j.foreco.2017.02.028.

Borilova, G., Hulankova, R., Svobodova, I., Jezek, F., Hutarova, Z., Vecerek, V., and Steinhauserova, I. The effect of storage conditions on the hygiene and sensory status of wild boar meat. *Meat Science*, 118, 71-77, 2016, doi:10.1016/j.meatsci.2016.03.024.

Brown, V.R., Bowen, R.A., and Bosco-Lauth, A.M. Zoonotic pathogens from feral swine that pose a significant threat to public health. *Transbound Emerging Disease,* 65, 649–659, 2018, doi:10.1111/tbed.12820.

Cahill, S., Llimona, F., Cabañeros, L., and Calomardo, F. 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, 221-233, 2012, doi:10.32800/abc.2012.35.0221.

Calosi, M., Gabbrielli, C., Lazzeri, Fattorini, N., Cesaretti, G., Burrini, L., Petrillo, O., and Ferretti, F. Seasonal and Ecological Determinants of Wild Boar Rooting on Priority Protected Grasslands. *Environmental Management* 74, 268–281, 2024, doi:10.1007/s00267-024-01952-y.

Cappa, F., Bani, L., and Meriggi, A. Factors affecting the crop damage by wild boar (*Sus scrofa*) and effects of population control in the Ticino and Lake Maggiore Park (North-western Italy). *Mammalian Biology,* 101, 451–463, 2021, doi:10.1007/s42991-021-00125-2.

Carey, M.P., Sanderson, B.L., Barnas, K.A. and Olden, J.D. Native invaders – challenges for science, management, policy, and society. *Frontiers in Ecology and the Environment*, 10, 373-381, 2012, doi:10.1890/110060.

Casamitjana, J. Italy to kill wild boars around Rome because their are disrupting the domestic pigs killing industries, Vegan FTA, 2022. Accessed on October 24: https://veganfta.com/2022/05/24/italy-to-kill-wild-boars-around-rome-because-they-are-disrupting-the-domestic-pig-killing-industry/.

Castanheira, M., Simner, P.J., and Bradford, P.A. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. *JAC Antimicrobial Resistance,* 3(3),dlab092, 2021, doi: 10.1093/jacamr/dlab092.

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

Castillo-Contreras, R., Marín, M., López-Olvera, J.R., Ayats, T., Aguilar, X.F., Lavín, S., Mentaberre, G., and Cerdà-Cuéllar, M. Zoonotic *Campylobacter* spp. and *Salmonella* spp. carried by wild boars in a metropolitan area: occurrence, antimicrobial susceptibility and public health relevance. *Science of The Total Environment*, 822, 2022, doi:10.1016/j.scitotenv.2022.153444.

Charrier, F., Rossi, S., Jori, F., Maestrini, O., Richomme, C., Casabianca, F., Ducrot, C., Jouve, J., Pavio, and N., Le Potier, M.F. Aujeszky's Disease and Hepatitis E Viruses Transmission between Domestic Pigs and Wild Boars in Corsica: Evaluating the Importance of Wild/Domestic Interactions and the Efficacy of Management Measures. *Frontiers in Veterinary Science*, 5:1, 2018, doi: 10.3389/fvets.2018.00001.

Chiari, M., Zanoni, M., Tagliabue, S. Lavazza, A., and Alborali, L. G.*Salmonella* serotypes in wild boars (*Sus scrofa*) hunted in northern Italy. *Acta Veterinaria Scandinavica* 55, 42, 2013, doi:10.1186/1751-0147-55-42.

Chinn, S. M., Schlichting,P.E., Smyser, T. J.,. Bowden, C. F., and Beasley, J. C. Factors influencing pregnancy, litter size, and reproductive parameters of invasive wild pigs. *Journal of Wildlife Management,* 86:e22304, 2022, doi:10.1002/jwmg.22304.

Cifuni, G.F., Amici, A., Contò, M., Viola, P., and Failla, S. Effects of the hunting method on meat quality from fallow deer and wild boar and preliminary studies for predicting lipid oxidation using visible reflectance spectra. *European Journal of Wildlife Research,* 60, 519–526,2014, doi:10.1007/s10344-014-0814-3.

Cilia, G.,Turchi, B., Fratini, F., Bilei, S., Bossù, T., De Marchis, M.L., Cerri, D., Pacini, M.I., and 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, 10*, 93, 2021, doi:10.3390/pathogens10020093.

Clavijo, A., Nikooienejad, A., Esfahani, M.S., Metz, R.P., Schwartz, S., Atashpaz-Gargari, E., Deliberto, T.J., Lutman, M.W., Pedersen, K., Bazan, L.R., Koster, L.G., Jenkins-Moore, M., Swenson, S.L., Zhang, M., Beckham, T., Johnson, C.D. and Bounpheng, M. Identification and Analysis of the First 2009 Pandemic H1N1 Influenza Virus from U.S. Feral Swine. *Zoonoses and Public Health*, 60, 327-335, 2013 doi:10.1111/zph.12006.

Clontz, L.M., Pepin, K.M., VerCauteren, K.C. and Beasley, J.C. Influence of biotic and abiotic factors on home range size and shape of invasive wild pigs (*Sus scrofa*). *Pest Management Science*, 78, 914-928, 2022, doi:10.1002/ps.6701.

CLSI (Clinical & Laboratory Standard Institute). M07 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, 12th Edition Clinical & Laboratory Standard Institute: Wayne, PA, USA, 2022. Accesse on October 2024: www.clsi.org.

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

Commission Implementing Regulation (EU) 2015/1375 of 10 August 2015 laying down specific rules on official controls for Trichinella in meat. *Official Journal of European Union L 212,* 11.8.2015, p. 7–34.

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. *Official Journal of European Union L* 131, 17.5.2019, p. 51–100.

Commission Implementing Decision (EU) 2020/1729 of 17 November 2020 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria and repealing Implementing Decision 2013/652/EU (notified under document C(2020) 7894). *Official Journal of European Union L 387,* 19.11.2020, p. 8–21.

Commission Implementing Regulation (EU) 2023/594 of 16 March 2023 laying down special disease control measures for African swine fever and repealing Implementing Regulation (EU) 2021/605. *Official Journal of European Union L* 79, 17.3.2023, p. 65–150.

Commission Notice on the guidelines on the prevention, control and eradication of African swine fever in the Union (ASF guidelines) C/2023/7855. *Official Journal of European Union, C/2023/1504, 18.12.2023, http://data.europa.eu/eli/C/2023/1504/oj.*

Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006*. Official Journal of European Union L* 119, 5.5.2023, p. 103–157.

Corradini, A., Marescotti, M.E., Demartini, E., and Gaviglio, A. Consumers' perceptions and attitudes toward hunted wild game meat in the modern world: A literature review. *Meat Scien*ce, 194, 2022, doi:10.1016/j.meatsci.2022.108955.

Cowled, B., and Garner, G. A review of geospatial and ecological factors affecting disease spread in wild pigs: Considerations for models of foot-and-mouth disease spread. *Preventive Veterinary Medicine*, 87 (3–4), 197-212, 2008, doi:10.1016/j.prevetmed.2008.03.012.

Darwich, L., Seminati, C., López-Olvera, J.R., Vidal, A., Aguirre, L., Cerdá, M., Garcias, B., Valldeperes, M., Castillo-Contreras, R., Migura-Garcia, L., Conejero, C., and Mentaberre, G. Detection of Beta-Lactam-Resistant *Escherichia coli* and Toxigenic *Clostridioides difficile* Strains in Wild Boars Foraging in an Anthropization Gradient. *Animals* 11(6),1585, 2021 doi:10.3390/ani11061585.

Decreto Legge n. 63/2024**.** "Disposizioni urgenti in materia di gestione della popolazione di cinghiali." Gazzetta Ufficiale della Repubblica Italiana, Anno 2024, n. 63. Data di pubblicazione: 15 maggio 2024.

Dias, D., Torres, R.T., Kronvall, G., Fonseca, C., Mendo, S., and Caetano, T. Assessment of antibiotic resistance of *Escherichia coli* isolates and screening of *Salmonella* spp. in wild ungulates from Portugal. *Research in Microbiology*, 166, 584-593, 2015, doi:10.1016/j.resmic.2015.03.006.

Dias, D., Caetano, T., Torres, R.T., Fonseca, C., Mendo, S. Shiga toxin-producing *Escherichia coli* in wild ungulates. *Science of the Total Environment, 651*, 203–209, 2019, doi:10.1016/j.scitotenv.2018.09.162.

Dias, D., Costa, S., Fonseca, C., Baraúna, R., Caetano, T,. and Mendo, S. Pathogenicity of Shiga toxin-producing *Escherichia coli* (STEC) from wildlife: Should we care? *Science of The Total Environment*, 812, ISSN 0048-9697, 2022, doi:10.1016/j.scitotenv.2021.152324.

Díaz-Sánchez, S., Sánchez, S., Herrera-León, S., Porrero, C., Blanco, J., Dahbi, G., Blanco, J.E., Mora, A., Mateo, R.,Hanning, I., Vidal, and D. 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. *Veterinary Microbiology*, 163 (3–4) 274-281, 2013, doi:10.1016/j.vetmic.2012.12.026.

Didero, N.M., Ernst, K.H., McKee, S.C., and Shwiff, S.A. A call and suggested criteria for standardizing economic estimates of wild pig damage. *Crop Protection*, 165, 2023, doi:10.1016/j.cropro.2022.106149.

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. *Official Journal of European Union L* 325, 12.12.2003, p. 31–40.

Dovrat, G., Perevolotsky, A., and Ne'eman, G. Wild boars as seed dispersal agents of exotic plants from agricultural lands to conservation areas. *Journal of Arid Environments*, 78, 49-54, 2012, doi:10.1016/j.jaridenv.2011.11.011.

EFSA (European Food Safety Authority), Ståhl, K., Boklund, A.E., Podgórski, T., Vergne, T., Abrahantes, J C., Cattaneo, E., Papanikolaou, A., Vergne, T., Abrahantes, J. C., Cattaneo, E., Papanikolaou, A., and Mur, L. Epidemiological analysis of African swine fever in the European Union during 2023. *EFSA Journal*, 22(5), e8809, 2024, doi:10.2903/j.efsa.2024.8809.

EFSA and ECDC(European Food Safety Authority and European Centre for Disease Prevention and Control). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017. *EFSA Journal,* 17(2):e05598, 2019, doi: 10.2903/j.efsa.2019.5598.

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control). The European Union One Health 2023 Zoonoses report. *EFSA Journal*, 22(12), e9106, 2024a, doi:10.2903/j.efsa.2024.9106

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2021–2022. *EFSA Journal*, 22, e8583, 2024b, doi:10.2903/j.efsa.2024.8583.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals. *EFSA Journal*, 9(8):2322, 95 pp. 2011, doi:10.2903/j.efsa.2011.2322.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). Scientific opinion on public health risks associated with Enteroaggregative *Escherichia coli* (EAEC) as a food-borne pathogen. *EFSA Journal*, 13(12):4330, 87 pp. 2015, doi:10.2903/j.efsa.2015.4330

EFSA BIOHAZ Panel, Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Jenkins C, Monteiro Pires S, Morabito S, Niskanen T, Scheutz F, da Silva Felício MT, Messens W and Bolton D. Scientific Opinion on the pathogenicity assessment of Shiga toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC. EFSA Journal, 18(1):5967, 105 pp. 2020, doi:10.2903/j.efsa.2020.5967.

EMA (European Medicine Agency), Categorisation of antibiotics in the European Union, 2019. Accessed on November 2024: https://www.ema.europa.eu/en/documents/report/categorisation-antibiotics-european-union-answer-request-european-commission-updating-scientific-advice-impact-public-health-and-animal-health-use-antibiotics-animals_en.pdf

ENETWILD-consortium, Pascual-Rico, R., Acevedo, P., Apollonio, M., Blanco-Aguiar, J.A.,Body, G., del Rio, L., Ferroglio, E., Gomez, A., Keuling, O., Plis, K., Podgórski, T., Preite, L., Ruiz-Rodriguez, C., Scandura, M., Sebastian, M., Soriguer, R., Smith, G.C., Vada, R., Zanet, S., Vicente, J., and Carpio, A. Wild boar ecology: a review of wild boar ecological anddemographic parameters by bioregion all over Europe. *EFSA supporting publications / European Food Safety Authority* Bd, 19, 3, 2022, doi:10.2903/sp.efsa.2022.en-7211.

Engeman, R.M., Massei, G., Sage, M., and Gentle, M.N. Monitoring wild pig populations: a review of methods. *Environmental Science and Pollution Research,* 20, 8077–8091, 2013, doi:10.1007/s11356-013-2002-5

EUCAST (European Committee on Antimicrobial Susceptibility Testing). Breakpoint Tables for Interpretations of MICs and Zone Diameters, Version 14.0. 2024. Accessed on November 2024: www.eucast.org/clinical_breakpoints.

FACE (European Federation of Associations for Hunting and Conservation). Hunting method and culture. *FACE Annual Report*, 2021. Accessed on October 2024: www.face.eu/about-face/.

Fantechi, T., Contini, C., Scozzafava, G., Casini, L. Consumer preferences for wild game meat: evidence from a hybrid choice model on wild boar meat in Italy. *Agriultural and Food Economics,* 10, 23, 2022, doi:10.1186/s40100-022-00231-w.

Fattorini, N., and Ferretti, F. Estimating wild boar density and rooting activity in a Mediterranean protected area. *Mammalian Biology* ,100, 241–251, 2020, doi:10.1007/s42991-020-00030-0.

Fernandez-de-Simon, J., Ferreres, J., and Gortázar, C. The number of hunters and wild boar group size drive wild boar control efficacy in driven hunts. *European Journal of Wildlife Research,* 69, 35, 2023, doi:10.1007/s10344-023-01661-7.

Fernandez-Llario, P., and Mateos-Quesada, P. Population structure of the wild boar (Sus scrofa) in two Mediterranean habitats in the western Iberian Peninsula. *Folia Zoologica*-Praha, 52.2, 143-148, 2003.

Fischer, A., Kerezi, V., Arroyo, B., Delibes-Mateos, M., Tadie, D., Lowassa, A., and Krange, O., Skogen, K. (De)legitimising hunting—discourses over the morality of hunting in Europe and eastern Africa. *Land Use Policy* 32,261–270, 2013, doi:10.1016/j.landusepol.2012.11.002.

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

Formenti, N., Calò, S., Parisio, G., Guarneri, F., Birbes, L., Pitozzi, A., Scali, F., Tonni, M., Guadagno, F., Giovannini, S., Salogni, C., Ianieri, A., Bellini, S., Pasquali, P., & Alborali, G. L. ESBL/AmpC-Producing *Escherichia coli* in Wild Boar: Epidemiology and Risk Factors. *Animals*, 11(7), 1855, 2021, doi:10.3390/ani11071855.

Frank, B., Monaco, A. and Bath, A.J. Beyond standard wildlife management: a pathway to encompass human dimension findings in wild boar management. *European Journal of Wildlife Research,* 61, 723–730, 2015, doi:10.1007/s10344-015-0948-y.

Frauendorf, M., Gethöffer, F., Siebert, U., and Keuling, O. The influence of environmental and physiological factors on the litter size of wild boar (*Sus scrofa*) in an agriculture dominated area in Germany. *Science of The Total Environment*, 541, 877-882, 2016, doi:/10.1016/j.scitotenv.2015.09.128.

Fulgione, D., and Buglione, M. The Boar War: Five Hot Factors Unleashing Boar Expansion and Related Emergency. *Land* , *11*, 887, 2022, doi:10.3390/land11060887.

Gamelon, M., Focardi, S., Baubet, E., Brandt, S., Franzetti, B., Ronchi, F., Venner, S., Sæther, B.E., and Gaillard, J.M. Reproductive allocation in pulsed-resource environments: a comparative study in two populations of wild boar. *Oecologia*, 183(4), 1065-1076, 2017, doi:10.1007/s00442-017- 3821-8.

Gauthier, A., Puente, J.L., and Finlay, B.B. Secretin of the enteropathogenic *Escherichia coli* type III secretion system requires components of the type III apparatus for assembly and localization. *Infection and Immununity*, 71(6), 3310-9, 2003, doi:10.1128/IAI.71.6.3310-3319.2003

Gaviglio, E., and Demartini, M.E. Marescotti. The creation of a local supply chain for large wild ungulates meat: Opportunities and limitations from an Italian alpine case study. *Quality - Access to Success,* 18, 215-222, 2017.

Geisser, H., and Reyer, H.-U. The influence of food and temperature on population density of wild boar *Sus scrofa* in the Thurgau (Switzerland). *Journal of Zoology*, 267, 89-96, 2005, doi:10.1017/S095283690500734X.

Gentsch, R.P., Kjellander, P. and Röken, B.O. Cortisol response of wild ungulates to trauma situations: hunting is not necessarily the worst stressor. *European Journal of Wildlife Research,* 64, 11, 2018, doi:10.1007/s10344-018-1171-4.

Gil Molino, M., Risco Pérez, D., Gonçalves Blanco, P., Fernandez Llario, P., Quesada Molina, A., García Sánchez, A., Cuesta Gerveno, J.M., Gómez Gordo, L., Martín Cano, F.E., Pérez Martínez, R., Varela Fernández, E., and Rey Pérez, J. Outbreaks of antimicrobial resistant *Salmonella* Choleraesuis in wild boars piglets from central-western Spain. *Transboundary and Emerging Diseases*, 66(1)225-233, 2019, doi: 10.1111/tbed.13003.

Giufrè, M., Mazzolini, E., Cerquetti, M., Brusaferro, S., CCM2015 One-Health ESBL-producing *Escherichia coli* Study Group. Extended-spectrum β-lactamase-producing *Escherichia coli* from extraintestinal infections in humans and from food-producing animals in Italy: a 'One Health' study. *International Journal of Antimicrobial Agents*. 58(5), 106433, 2021 doi: 10.1016/j.ijantimicag.2021.106433.

Giuggioli, G., Olivastri, A., Pennisi, L., Paludi, D., Ianieri, A., and Vergara, A. The hygiene-sanitary control in the wild game meats. *Italian Journal of Food Safety,* 6(4), 6875, 2018, doi: 10.4081/ijfs.2017.6875.

Gortázar, C., Acevedo, P., Ruiz-Fons, F., and Vicente, J. Disease risks and overabundance of game species. *European Journal of Wildlife Research*, *52*, 81-87, 2006, doi:10.1007/s10344-005-0022-2.

Gottstein, B., Pozio, E., and Nöckler, K. Epidemiology, diagnosis, treatment, and control of trichinellosis. *Clinical Microbiology Reviews*, 22, 127-145, 2009, doi: 10.1128/CMR.00026-08.

Greig, J., Rajić, A., Young, I., Mascarenhas, M., Waddell, L., and 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*, 62(4),69-84, 2015, doi: 10.1111/zph.12147.

Hearn, R., Watkins, C., and Balzaretti, R. The cultural and land use implications of the reappearance of the wild boar in North West Italy: A case study of the Val di Vara. *Journal of Rural Studies*, 36, 52-63, 2014, doi:10.1016/j.jrurstud.2014.06.004.

Hu, J., and Torres A.G. Enteropathogenic *Escherichia coli*: foe or innocent bystander? *Clinical Microbiology and Infection*, 21 (8), 729-734, 2015, doi:10.1016/j.cmi.2015.01.015.

ISO (International Organization for Standardization). Microbilogy of the food chain. Carcass sampling for microbiological analysism. ISO 17604:2015. Geneve, Switzerland, 2005.

ISO (International Organization for Standardization). Microbiology of food and animal feed. Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens. Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC). ISO/ TS 13136:2012. Geneve, Switzerland, 2012.

ISO (International Organization for Standardization). Microbiology of the food chain. General requirements and guidance for microbiological examinations. ISO 7218:2013. Geneve, Switzerland, 2013.

ISO (International Organization for Standardization). Microbiology of the food chain. Horizontal method for the detection, enumeration and serotyping of Salmonella – Part III: Guidelines for serotyping *Salmonella* spp. ISO 6579–3:2014. Geneve, Switzerland, 2014.

ISO (International Organization for Standardization). Microbiology of the food chain. Horizontal method for the detection, enumeration and serotyping of Salmonella – Part I: Detection of *Salmonella* spp. ISO 6579-1:2017. Geneve, Switzerland, 2017.

ISO (International Organization for Standardization). Microbiology of the food chain. Horizontal method for the detection and enumeration of *Enterobacteriaceae* – Part II: Colony count technique. ISO 21528–2:2017. Geneve, Switzerland, 2017.

ISO (International Organization for Standardization). Microbiology of the food chain. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 1: general rules for the preparation of the initial suspension and decimal dilutions. ISO 6887-1:2017. Geneve, Switzerland, 2017.

ISO (International Organization for Standardization). Microbiology of hte food chain. Horizontal method for the enumeration of microorganism. Part 1 : Colony count at 30°C by the pour plate technique. ISO ISO 4833-1:2022. Geneve, Switzerland, 2022.

ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale). Al 2021 stimato un milione e mezzo di cinghiali in Italia in sette anni, abbattimento aumentato del 45% danni all'agricoltura per 120 milioni. Comunicato stampa. January 13, 2023. Accessed on September 2024: www.isprambiente.gov.it/en/archive/news-and-other-events/ispra-news/files2023/area-stampa/comunicati-stampa/comunicatocinghiali-1.pdf

Italian State-Region Agreement No 41/2016. Intesa tra il Governo, le Regioni e le Provincie autonome di Trento e di Bolzabo sul documento concernente "Linee guida relative all'applicazione del Regolamento (CE) n. 2073/2005 e successive modifiche ed integrazioni sui criteri microbiologici applicabili agli alimenti". 41/CSR, 03/03/2016. Accessed on November 2024: http://archivio.statoregioni.it/DettaglioDocc9ac3.html?IDDoc=52685&IdProv=17032&tipodoc=2&CONF=CSR

Jakubas, D., Ryś, M., and Lazarus, M. Factors affecting wildlife-vehicle collision on the expressway in a suburban area in northern Poland. *North-Western Journal of Zoology*, 14(1), 107-116, 107-116, e171702(14), 2018.

Jay, M.T., Cooley, M., Carychao, D., Wiscomb, G.W., Sweitzer, R.A., Crawford-Miksza, L., Farrar, J.A., Lau, D.K., O'Connell, J., Millington, A. and Asmundson, R.V. *Escherichia coli* O157: H7 in feral swine near spinach fields and cattle, central California coast. *Emerging infectious diseases*, 13(12),1908- 1911, 2007, doi: 10.3201/eid1312.070763.

Jensen, P. Observations on the maternal behaviour of free-ranging domestic pigs. *Applied Animal Behaviour Science,* 16 (2), 131-142, 1986, doi:10.1016/0168-1591(86)90105-X.

Jones, B.A., Grace, D., Kock, R., Alonso, S., Rushton, J., Said, M.Y., McKeever, D., Mutua, F., Young, J., McDermott, J., and Pfeiffer, D.U. Zoonosis emergence linked to agricultural intensification and environmental change, *PNAS*, 110(21), 8399-8404, 2013, doi:10.1073/pnas.1208059110.

Kaper, J., Nataro, J. and Mobley, H. Pathogenic *Escherichia coli*. *Nature Review Microbiology,* 2, 123–140, 2004, doi:10.1038/nrmicro818.

Keuling, O., Stier, N., and Roth, M. Annual and seasonal space use of different age classes of female wild boar Sus scrofa L. *European Journal of Wildlife Research*, 54, 403-412, 2008 doi:10.1007/s10344-007-0157-4.

Keuling, O., Strauß, E., and Siebert, U. Regulating wild boar populations is "somebody else's problem"! - Human dimension in wild boar management. *Science of The Total Environment*, 554–555, 311-319, 2016, doi:10.1016/j.scitotenv.2016.02.159.

Keuling, O., Podgórski, T., Monaco, A., Melletti, M., Merta, D., Albrycht, M., Genov, P. V., Gethöffer, F., Vetter, S.G., Jori F., Scalera, R., and Gongora, J. Eurasian Wild Boar *Sus scrofa* (Linnaeus, 1758). In: Melletti M, Meijaard E, eds. *Ecology, Conservation and Management of Wild Pigs and Peccaries*. Cambridge University Press; 202-233, 2017.

Keuling, O.; Strauß, E.; Siebert, U. How Do Hunters Hunt Wild Boar? Survey on Wild Boar Hunting Methods in the Federal State of Lower Saxony. *Animals,*, *11*, 2658, 2021, doi:10.3390/ani11092658.

Kim HY. Statistical notes for clinical researchers: assessing normal distribution (2) using skewness and kurtosis. *Restorative Dentist and Endodontics*, 38(1), 52-4. 2013, doi: 10.5395/rde.2013.38.1.52.

Kock, R., Daniels-Haardt, I., Becker, K. Mellmann, A., Friedrich , A.W., Mevius, D., Schwarz, S., Jurke, A. Carbapenem-resistant Enterobacteriaceae *in wildlife, food-producing, and companion animals: a systematic review.* Clinical Microbiology and Infection*, 24(12). 1241 – 1250, 2018, doi:10.1016/j.cmi2018.04.004.*

Legge Regionale n. 8. Disposizioni per la protezione della fauna selvatica e per l'esercizio dell'attività venatoria, 15 febbraio, 1998. Accessed on January 2025: https://demetra.regione.emilia-romagna.it/al/articolo?urn=er:assemblealegislativa:legge:1994;8.

Lerner, H. and Berg, C. A comparison of three holistic approaches to health: One health, ecoHealth, and planetary health. *Frontiers in Veterinary Science*, *4*, 163, 2017 doi:10.3389/fvets.2017.00163.

Lestingi A. Use of Wild Boar (*Sus scrofa*) as a Sustainable Alternative in Pork Production. *Animals*, 13(14), 2258, 2023, doi:10.3390/ani13142258.

Lewis, J., Farnsworth, M., Burdett, C., Theobald, D., Gray, M., and Miller, R. Biotic and abiotic factors predicting the global distribution and population density of an invasive large mammal. *Scientific Reports,* 7, 44152, 2017, doi: 10.1038/srep44152

Li, A., Williams, N., Adams, P., Fenwick, S., and Hardy G. The spread of *Phytophthora cinnamomi* by feral Pigs. In: 5th IUFRO phytophthora diseases in forests and natural ecosystems, Auckland and Rotorua, 2010.

Li, X., Mowlaboccus, S., Jackson, B., Cai, C., Coombs, G.W. Antimicrobial resistance among clinically significant bacteria in wildlife: An overlooked one health concern. *International Journal of Antimicrobial Agents*, 64 (3), 107251, 2024, doi: 10.1016/j.ijantimicag.2024.107251.

Lindsey,R.L., Prasad,A., Feldgarden,M., GonzalezEscalona, N., Kapsak, C., Klimke,W. , Melton-Celsa, A., Smith, P., Souvorov, A., Truong, J., Scheutz, F. Identification and characterization of ten *Escherichia coli* strains encoding novel Shiga toxin 2 subtypes, Stx2n as well as Stx2j, Stx2m, and Stx2o, in the United States. *Microorganisms*, 11,2561, 2023 doi: 10.3390/microorganisms11102561.

Linee guida in materia di igiene delle carni di selvaggina selvatica. Bollettino ufficiale della regione Emilia-Romagna, DELIBERAZIONE DELLA GIUNTA REGIONALE 1 LUGLIO 2024, N. 1319. Accessed on November 2024: https://bur.regione.emilia-romagna.it/area-bollettini/n-225-del-09-07-2024-parte-seconda/linee-guida-in-materia-di-igiene-delle-carni-di-selvaggina-selvatica/print-inserzione?i=2b2c0bc33259471882d8e0e7b6f2e914

Linnell, J.D.C., Cretois, B., Nilsen, E.B., Rolandsen, C.M., Solberg, E.J., Veiberg, V., Kaczensky, P., Van Moorter, B., Panzacchi, M., Rauset, G.R., and Kaltenborn, B. The challenges and opportunities of coexisting with wild ungulates in the human-dominated landscapes of Europe's Anthropocene. *Biological Conservation*, 244, 2020, doi:10.1016/j.biocon.2020.108500.

Literak, M., Dolejska, T., Radimersky, J., Klimes, M., Friedman, F.M., Aarestrup, H., Hasman, and A. Cize. Antimicrobial-resistant faecal *Escherichia coli* in wild mammals in central Europe: multiresistant Escherichia coli producing extended-spectrum beta-lactamases in wild boars. *Jourlan of Applied Microbiology*, 108, 1702-1711, 2010, doi:10.1111/j.1365-2672.2009.04572.x.

Longo, A., Losasso, C., Vitulano, F., Mastrorilli, E., Turchetto, S., Petrin, S., Mantovani, C., Dalla Pozza, M.C., Ramon, E., Conedera, G., Citterio, C.V., Ricci, A., Barco, L., Lettini, A.A.

Insight into an outbreak of Salmonella Choleraesuis var. Kunzendorf in wild boars. *Veterinary Microbiology*, 238, 108423, 2019 doi: 10.1016/j.vetmic.2019.108423.

Macháčková, K., Zelený, J., Lang, D., and Vinš, Z. Wild Boar Meat as a Sustainable Substitute for Pork: A Mixed Methods Approach. *Sustainability*, 13(5), 2490, 2021 doi:10.3390/su13052490.

Marescotti, M.E., Caputo, V., Demartini, E., and Gaviglio, A. Discovering market segments for hunted wild game meat. *Meat Science*, 149,163-176, 2019, doi: 10.1016/j.meatsci.2018.11.019.

Marescotti, M. E., Caputo, V., Demartini, E., and Gaviglio, A. Consumer preferences for wild game cured meat label: do attitudes towards animal welfare matter?. *International Food and Agribusiness Management Review*, 23(4), 599-618, 2020, doi:10.22434/IFAMR2019.0203.

Marescotti, M.E., Demartini, E., Gibbert, M., Viganò, R., and Gaviglio, A. Disentangling Individual Phases in the Hunted vs. Farmed Meat Supply Chain: Exploring Hunters' Perceptions in Italy. *Foods,* 10*,* 174, 2021, doi:10.3390/foods10010174.

Markov, N., Economov, A., Hjeljord, O., Rolandsen, C.M., Bergqvist, G., Danilov, P., Dolinin, V., Kambalin, V., Kondratov, A., Krasnoshapka, N., Kunnasranta, M., Mamontov, V., Panchenko, D. and Senchik, A. The wild boar *Sus scrofa* in northern Eurasia: a review of range expansion history, current distribution, factors affecting the northern distributional limit, and management strategies. *Mammal Review*, 52, 519-537, 2022, doi:10.1111/mam.12301.

Marsico, G., Rasulo, A., Dimatteo, S., Tarricone, S., Pinto, F., and Ragni, M. Pig, F1 (wild boar x pig) and wild boar meat quality. *Italian Journal of Animal Science*, 6(sup1), 701-703, 2007, doi:10.4081/ijas.2007.1s.701.

Massei, G., Cowan, D. P., Coats, J., Gladwell, F., Lane, J. E. and Miller, L. A. Effect of the GnRH vaccine GonaConTM on the fertility, physiology and behavior of wild boar. *Wildlife Research,* 35, 540–547, 2008, doi: 10.1071/WR07132.

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. and Náhlik, A. Wild boar populations up, numbers of hunters down? A review of trends and implications for Europe. *Pest Management Science*, 71, 492-500, 2015, doi:10.1002/ps.3965.

Massei, G., Roy, S., and Bunting, R. "Too Many Hogs? A Review of Methods to Mitigate Impact by Wild Boar and Feral Hogs," *Human–Wildlife Interactions*, 5(1),10, 2011, doi:10.26077/aeda-p853.

Mayer, J.J. Wild Pig Population Biology. In book: *Wild Pigs: Biology, Damage, Control Techniques and Management.* Savannah River National Laboratory, Savannah River Nuclear Solutions, LLC, Savannah River Site, Aiken, South Carolina 29808, 2009.

Mayer, J.J., Edwards, T.B., Garabedian, J.E. and Kilgo, J.C. Sanitary Waste Landfill Effects on an Invasive Wild Pig Population. *Journal of Wildlife Management*, 85, 868-879, 2021, doi:10.1002/jwmg.22042.

Melis, C., Szafrańska, P. A., Jędrzejewska, B., and Bartoń, K. Biogeographical variation in the population density of wild boar (*Sus scrofa*) in western Eurasia. *Journal of Biogeography* 33, 803–811, 2006, doi:10.1111/j.1365-2699.2006.01434.x.

Melton-Celsa, A.R. Shiga Toxin (Stx) Classification, Structure, and Function. *Microbiol Spectrum,* 2(4), 2014, doi:10.1128/microbiolspec.ehec-0024-2013.

Membré, J.M., Laroche, M., and Magras, C. Assessment of levels of bacterial contamination of large wild game meat in Europe. *Food Microbiol*ogy, 28(5), 1072-9, 2011, doi:10.1016/j.fm.2011.02.015.

Mercato, A., Cortimiglia, C., Abualsha'ar, A., Piazza, A., Marchesini, F., Milani, G., Bonardi, S., Cocconcelli, P.S., and Migliavacca, R. Wild Boars as an Indicator of Environmental Spread of ESβL-Producing *Escherichia coli*. *Frontiers in Microbiology*, 13, 2022, doi:10.3389/fmicb.2022.838383.

Methner,U., Merbach, S., and Peters, M. *Salmonella enterica* subspecies *enterica* serovar Choleraesuis in a German wild boar population: occurrence and characterisation. *Acta Veterinaria Scandinavica*, 60, 65, 2018, doi:10.1186/s13028-018-0422-4.

Miller, R.S., Sweeney, S.J., Slootmaker, C., Grear, D.A., Di Salvo, P.A., Kiser, D., and Shwiff, S.A. Cross-species transmission potential between wild pigs, livestock, poultry, wildlife, and humans: implications for disease risk management in North America. *Scientific Reports,* 7, 7821, 2017, doi:10.1038/s41598-017-07336-z.

Mirceta, J., Petrovic, J., Malesevic, M., Blagojevic, B., and Antic, D. Assessment of microbial carcass contamination of hunted wild boars. *Europen Journal of Wildlife Research,* 63, 37, 2017. doi:10.1007/s10344-017-1096-3.

Monaco, A., Franzetti, B., Pedrotti, L., Toso, S. Linee guida per la gestione del cinghiale. Ministero Politiche Agricole e Forestali-I.N.F.S. 2003. Accessed on September 2024: www.isprambiente.gov.it/contentfiles/00006600/6683-linee-guida-gestione-cinghiale.pdf.

Monaco, A., Carnevali, L., and Toso, S. Linee guida per la gestione del Cinghiale (*Sus scrofa*) nelle aree protette II edizione, *Quaderni di Conservazione della Natura n 34,* ISPRA, Ministero dell'ambiente e della Tutela del Territorio e del Mare. 2023. Accessed on October 2024: www.isprambiente.gov.it/it/pubblicazioni/quaderni/conservazione-della-natura/linee-guida-per-la-gestione-del-cinghiale-sus-1.

Morán, L., Insausti, K., Barron, L.J.R., and Aldai, N. Wild Boar – Production, Meat Quality Traits and Derived Products. In: Lorenzo, J., Munekata, P., Barba, F., Toldrá, F. (eds) More than Beef, Pork and Chicken – The Production, Processing, and Quality Traits of Other Sources of Meat for Human Diet. Springer, Cham. 2019, doi:10.1007/978-3-030-05484-7_8.

Morelle, K., Podgórski, T., Prévot, C., Keuling, O., Lehaire, F., and Lejeune, P. A review of wild boar *Sus scrofa* movement ecology. *Mammal Review*, 45, 15-29, 2015, doi:10.1111/mam.12028.

Müller, D., Greune, L., Heusipp, G., Karch, H., Fruth, A., Tschäpe, H., and Schmidt, M.A.Identification of Unconventional Intestinal Pathogenic Escherichia coli Isolates Expressing Intermediate Virulence Factor Profiles by Using a Novel Single-Step Multiplex PCR. Applied *and Environmental Microbiology*, 73(10) 2007, doi:10.1128/AEM.02855-06.

Mysterud, A., and Ims, R.A. Functional responses in habitat use: availability influences relative use in trade-off situations. *Ecology,* 79, 1435–1441, 1998, doi:10.1890/0012-9658(1998)079[1435:FRIHUA]2.0.CO;2.

Nàhlik, A., Cahill, S., Cellina, S., Gál,J., Jánoska, F., Rosell, C., Rossi,S., and Massei, G. Wild boar Management in Europe:Knowledge and Practice. In:Melletti M, Meijaard E, eds. Ecology, Conservation and Management of Wild Pigs and Peccaries, Cambridge University Press, 339-353, 2017.

Needham, T., Bureš, D., Černý, J., and Hoffman, L.C. Overview of game meat utilisation challenges and opportunities: A European perspective. *Meat Science*, 204, 2023, doi:10.1016/j.meatsci.2023.109284.

Nores, C., Llaneza, L. and Álvarez, Á. Wild boar *Sus scrofa* mortality by hunting and wolf Canis lupus predation: an example in northern Spain. *Wildlife Biology*, 14,44-51, 2008, doi:10.2981/0909-6396(2008)14[44:WBSSMB]2.0.CO;2.

Nüesch-Inderbinen, M., Barmettler, K., Stevens, M.J.A., and Cernela, N. Shiga toxin-producing *Escherichia coli* isolated from hunted wild boar (*Sus scrofa*) in Switzerland. *Schweiz Arch Tierheilkd,* 166(3),131-140, 2024, doi: 10.17236/sat00419.

O'Brien, P., Vander Wal, E., Koen, E.L., Brown, C.D., Guy, J., van Beest, M.V., and Brook, K.R. Understanding habitat co-occurrence and the potential for competition between native mammals and invasive wild pigs (*Sus scrofa*) at the northern edge of their range. *Canadian Journal of Zoology*, 97(6),537-546, 2019, doi:10.1139/cjz-2018-0156.

Olivastri, A., Paoletti, B., Lauteri, C., Pennisi, L., Paludi, D., Festino, A.R., and Vergara, A. Parasitic cysts in wild boars hunted in Central Italy: The sanitary controls in the wild game meats chain. *Italian Journal of Food Safety*, 10(2),9383, 2021, doi:10.4081/ijfs.2021.9383.

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

Ortega, N., Fanelli, A., Serrano, A., Martínez-Carrasco, C., Escribano, F., Tizzani, P., and Candela, M.G. *Salmonella* seroprevalence in wild boar from Southeast Spain depends on host population density. *Research in Veterinary Science*,132, 400-403, 2020, doi:10.1016/j.rvsc.2020.07.026.

Paulsen, P., Smulders, F.J.M., and Hilbert, F. *Salmonella* in meat from hunted game: A Central European perspective. *Food Research International*, 45(2), 609-616, 2012, doi:10.1016/j.foodres.2011.06.055.

Perrat, A., Branchu, P., Decors, A., Turci, S., Bayon-Auboyer, M.H., Petit, G., Grosbois, V., Brugère, H., Auvray, F., and Oswald, E. Wild Boars as Reservoir of Highly Virulent Clone of Hybrid Shiga Toxigenic and Enterotoxigenic *Escherichia coli* Responsible for Edema Disease, France. *Emerging Infectious Disease*, 28(2), 382-393, 2022 doi:10.3201/eid2802.211491.

Peruzy, M.F., Capuano, F., Proroga, Y.T.R., Cristiano, D., Carullo, M.R.,and Murru N. Antimicrobial Susceptibility Testing for *Salmonella* Serovars Isolated from Food Samples: Five-Year Monitoring (2015–2019). *Antibiotics*, 9(7),365, 2020, doi:10.3390/antibiotics9070365.

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

Peruzy, M.F., Cristiano, D., Delibato, E., D'Alessio, N., Proroga, Y.T.R., Capozza, R.L., Rippa, A., and Murru, N. Presence of enteric bacterial pathogens in meat samples of wild boar hunted in Campania region, southern Italy. *Italian Journal of Food Safety*, 11(1), 9967, 2022b doi: 10.4081/ijfs.2022.9967.

Pittiglio, C., Khomenko, S., and Beltran-Alcrudo, D. Wild boar mapping using population-density statistics: From polygons to high resolution raster maps. *PLoS One*, 13(5), e0193295, 2018, doi:10.1371/journal.pone.0193295.

Podgórski, T., Lusseau, D., Scandura, M., Sönnichsen, L., and Jędrzejewska, B. Long-Lasting, Kin-Directed Female Interactions in a Spatially Structured Wild Boar Social Network. *PLoS ONE,* 9(6), e99875, 2014, doi:10.1371/journal.pone.0099875.

Poeta, P., Radhouani, H., Pinto, L., Martinho, A., Rego, V., Rodrigues, R., Gonçalves, A., Rodrigues, J., Estepa, V., Torres, C. and Igrejas, G. Wild boars as reservoirs of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* of different phylogenetic groups. *Journal of Basic Microbiology*, 49, 584-588, 2009, doi:10.1002/jobm.200900066.

Poirel, L., Madec, J., Lupo, A., Schink, A., Kieffer, N., Nordmann, P., and Schwarz, S. Antimicrobial Resistance in *Escherichia coli*. *Microbiology Spectrum*, 6(4), 10.1128/microbiolspec.arba-0026-2017, 2018 doi:10.1128/microbiolspec.arba-0026-2017.

Quaresma, M.A.G., Alves, S.P., Trigo-Rodrigues, I., Pereira-Silva, R., Santos, N., Lemos, J.P.C., Barreto, A.S., and Bessa, R.J.B. Nutritional evaluation of the lipid fraction of feral wild boar (*Sus scrofa scrofa*) meat. *Meat Science*, 89 (4),457-461, 2011, doi:10.1016/j.meatsci.2011.05.005.

Queenan, A.M., and Bush, K. Carbapenemases: the versatile beta-lactamases. *Clinical Microbiology Reviews*, 20(3), 440-58, 2007, doi: 10.1128/CMR.00001-07.

Quirós-Fernández, F., Marcos, J., Acevedo, P., and Gortazàr, C. Hunters serving the ecosystem: the contribution of recreational hunting to wild boar population control. *European Journal of Wildlife Research,* 63, 57, 2017, doi:10.1007/s10344-017-1107-4.

Ramanzin, M., Amici, A., Casoli, C., Esposito, L., Lupi, P., Marsico, G., Mattiello, S., Olivieri, O., Ponzetta, M.P., Russo, C., and Trabalza Marinucci, M. Meat from Wild Ungulates: Ensuring Quality and Hygiene of an Increasing Resource. *Italian Journal of Animal Science,* 9(3), 2010, doi:10.4081/ijas.2010.e61.

Ramey, A.M., and Ahlstrom, A.C. Antibiotic resistant bacteria in wildlife: perspectives on trends, acquisition and dissemination, data, gaps, and future directions. *Journal of Wildlife Disease,* 56 (1), 1–15, 2020, doi: doi:10.7589/2019-04-099.

Ramírez-Castillo, F.Y., Guerrero-Barrera, A.L., and Avelar-González, F.J. An overview of carbapenem-resistant organisms from food-producing animals, seafood, aquaculture, companion animals, and wildlife. *Frontiers in Veterinary Science*, 10, 2023, doi:10.3389/fvets.2023.1158588.

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,10*, 1548, 2021, doi:10.3390/foods10071548.

Razzuoli, E., Amadori, M., Lazzara, F., Bilato, D., Ferraris, M., Vito, G., and Ferrari, A. *Salmonella* serovar-specific interaction with jejunal epithelial cells. *Veterinary Microbiology*, 207, 219-225, 2017, doi:10.1016/j.vetmic.2017.07.002.

Razzuoli, E., Listorti, V., Martini, I., Migone, L., Decastelli, L., Mignone, W., Berio, E., Battistini, R., Ercolini, C., Serracca, L., Andreoli, T., Dellepiane, M., Adriano, D., Pitti, M., Meloni, D., and Modesto, P. Prevalence and Antimicrobial Resistances of *Salmonella* spp. Isolated from Wild Boars in Liguria Region, Italy. *Pathogens*. 10(5),568, 2021, doi: 10.3390/pathogens10050568.

Rega, M., Carmosino, I., Bonilauri, P., Frascolla, V., Vismarra, A., and Bacci, C. Prevalence of ESβL, AmpC and Colistin-Resistant *E. coli* in Meat: A Comparison between Pork and Wild Boar. *Microorganisms*. 9(2),214, 2021, doi:10.3390/microorganisms9020214.

Rega, M., Andriani, L., Cavallo, S., Bonilauri, P., Bonardi, S., Conter, M., Carmosino, I., and Bacci, C. Antimicrobial Resistant *E. coli* in Pork and Wild Boar Meat: A Risk to Consumers. *Foods 11*, 3662, 2022, doi: 10.3390/foods11223662.

Regulation 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, EC/178/2002. *Official Journal of European Union L* 31, 1. 2. 2002 , p.1 -24.

Regulation of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs EC/852/2004, *Official Journal of European Union L* 139, 30.4. 2004a, p. 1–54.

Regulation of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin EC/853/2004. *Official Journal of European Union L* 139, 30.04.2004b, p. 55 -205.

Regulation of the European Parliament and of the Council of 15 November 2005 on microbiological criteria for foodstuffs EC/2073/2005. *Official Journal of European Union L* 338, 22.12.2005, p. 1–26.

Ricci, S. Wild boars: 50 thousand farmers in the street throughout Italy in just one month. *Caccia Passione,* 2024. Accessed on October 2024: https://www.cacciapassione.com/en/wild-boars-50-thousand-farmers-in-the-streets-throughout-Italy-in-just-one-month/.

Risch, D.R., Ringma, J., and Price, M.R. The global impact of wild pigs (*Sus scrofa*) on terrestrial biodiversity. *Scientific Reports,* 11, 13256, 2021, doi:10.1038/s41598-021-92691-1.

Rodas, E.M., Bogdanova, T., Bossù, T., Pecchi, S., Tomassetti, F., De Santis, P., Tolli, R., Condoleo, R., Greco, S., Brozzi, A., Bilei, S., Micarelli, G., Martini, E., Palazzetti, M. Microbiological Assessment of Freshly-Shot Wild Boars Meat in Lazio Region, Viterbo Territory: a Preliminary Study. *Italian Journal of Food Safety*, 3(1), 1711, 2014, doi: 10.4081/ijfs.2014.1711.

Rossi, A., Santi, A., Barsi, F., Casadei, G., Di Donato, A., Fontana, M.C., Galletti, G., Garbarino, C.A., Lombardini, A., Musto, C., Prosperi, A., Pupillo, G., Rugna, G., and Tamba, M. Eleven Years of Health Monitoring in Wild Boars (*Sus scrofa*) in the Emilia-Romagna Region (Italy). *Animals*. 13(11):1832, 2023, doi:10.3390/ani13111832.

Ruiz-Fons, F. A Review of the Current Status of Relevant Zoonotic Pathogens in Wild Swine (*Sus scrofa*) Populations: Changes Modulating the Risk of Transmission to Humans. *Transbound and Emerging Disease*, 64, 68-88, 2017, doi:10.1111/tbed.12369.

Russo, L., Massei, G., and Genov, P. Daily home range and activity of wild boar in a Mediterranean area free from hunting. *Ethology Ecology & Evolution*, 9(3), 287-294, 1997, doi:10.1080/08927014.1997.9522888.

Russo, C., Balloni, S., Altomonte,I., Martini, M., Nuvoloni, R., Cecchi, F., Pedonese, F., Salari, F., Sant'ana Da Silva, A.M., Torracca, B. and Profumo A. Fatty Acid and Microbiological Profile of the Meat (Longissimus Dorsi Muscle) of Wild Boar (*Sus Scropha Scropha*) Hunted in Tuscany. *Italian Journal of Animal Science,* 16 (1), 1–8, 2016, doi:10.1080/1828051X.2016.1261006.

Saïd, S., Tolon, V., Brandt, S., Baubet, E. Sex effect on habitat selection in response to hunting disturbance: the study of wild boar. *European Journal of Wildlife Research,* 58, 107–115, 2012, doi:10.1007/s10344-011-0548-4.

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

Sanguinetti, J., and Kitzberger, T. Factors controlling seed predation by rodents and non-native *Sus scrofa* in *Araucaria araucana* forests: potential effects on seedling establishment. *Biologycal Invasions* 12, 689–706, 2010, doi:10.1007/s10530-009-9474-8.

Sarantuya, J., Nishi, J.,Wakimoto, N., Erdene, S., Nataro, J.P., Sheikh, J., Iwashita, M., Manago, K., Tokuda, K., Yoshinaga, M., Miyata, K., and Kawano, Y.Typical Enteroaggregative *Escherichia coli* Is the Most Prevalent Pathotype among *E. coli* Strains Causing Diarrhea in Mongolian Children. *Journal of Clinical Microbiology*, 42(1), 2004, doi:10.1128/jcm.42.1.133-139.2004.

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

Scillitani, L., Monaco, A., and Toso, S. Do intensive drive hunts affect wild boar (*Sus scrofa*) spatial behaviour in Italy? Some evidences and management implications. *Europena Journal of Wildlife Research,* 56, 307–318, 2010, doi:10.1007/s10344-009-0314-z.

Serraino, A., Marchetti, G., Sanguinetti, V., Rossi, M.C., Zanoni, R.G., Catozzi, L., Bandera, A., Dini, W., Mignone, W., Franzetti, F. and Gori, A. Monitoring of transmission of tuberculosis between wild boars and cattle: genotypical analysis of strains by molecular epidemiology techniques. *Journal of Clinical Microbiology*, *37*(9), 2766-2771, 1999, doi:10.1128/jcm.37.9.2766-2771.1999.

Seward, N. W., VerCauteren, K. C., Witmer, G. W., and Engeman, R. M. Feral swine impacts on agriculture and the environment. *Sheep & Goat Research Journal*, 12, 2004.

Siemann, E., Carrillo, J.A., Gabler, C.A., Zipp, R., and Rogers, W.E. Experimental test of the impacts of feral hogs on forest dynamics and processes in the southeastern US. *Forest Ecology and Management*, 258, 546–553, 2009, doi:10.1016/j.foreco.2009.03.056.

Simberloff, D. How common are invasion-induced ecosystem impacts?. *Biological Invasions* 13, 1255–1268, 2011, doi:10.1007/s10530-011-9956-3.

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

Stillfried, M., Fickel, J., Börner, K., Wittstatt, U., Heddergott, M., Ortmann, S., Kramer-Schadt, S., and Frantz, A.C. Do cities represent sources, sinks or isolated islands for urban wild boar population structure? *Journal of Applied Ecology*, 54, 272-281, 2017, doi:10.1111/1365-2664.12756.

Stokke, S., Arnemo, J.M., Brainerd, S.,Söderberg, A., Kraabøl, M., and Ytrehus, B. Defining animal welfare standards in hunting: body mass determines thresholds for incapacitation time and flight distance. *Scientific Reports,* 8, 13786, 2018, doi:10.1038/s41598-018-32102-0.

Swift, B.M.C., Bennett, M., Waller, K., Dodd, C., Murray, A., Gomes, R.L., Humphreys, B., Hobman, J.L., Jones, M.A., Whitlock, S.E., Mitchell, L.J., Lennon, R.J., and Arnold, K.E. Anthropogenic environmental drivers of antimicrobial resistance in wildlife. *Science of The Total Environment*, 649,12-20, 2019, doi:10.1016/j.scitotenv.2018.08.180.

Szczerba-Turek, A., Socha, P., Bancerz-Kisiel, A., Platt-Samoraj, A., Lipczynska-Ilczuk, K., Siemionek, J., Kończyk, K., Terech-Majewska, E., and Szweda, W. Pathogenic potential to humans of Shiga toxin-producing *Escherichia coli* isolated from wild boars in Poland. *International Journal of Food Microbiology*, 300, 8-13, 2019, doi:10.1016/j.ijfoodmicro.2019.04.004.

Tack, J. Wild Boar *(Sus scrofa)* populations in Europe A scientific review of population trends and implications for management. *European Landowners' Organization*, Brussels, 56 pp, 2018. Accessed on September 2024: https://wildbeimwild.com/wp-content/uploads/2023/08/12-Tack-J-Wild-Boar-Population-Trends-in-Europe-2018.pdf

Tajchman, K., Czyzowski P., Drozd, L., Karpinski M., and Wojtas J. Sustainable game management as one of the determinants of the welfare of hunting animals. *Journal of Animal Science, Biology and Bioeconomy*, 36(1), 19-30, 2018, doi:10.24326/jasbbx.2018.1.2.

Torres, R.T., Fernandes, J., Carvalho, J., Cunha, M.V. Caetano, T., Mendo, S., Serrano, E., and Fonseca, C. Wild boar as a reservoir of antimicrobial resi stance. *Science of The Total Environment*, 717, 2020, doi:10.1016/j.scitotenv.2019.135001.

Touzot, L., Schermer, E.,Venner, S., Delzon, S., Rousset, C., Baubet, E., Gaillard, J.M. and Gamelon, M. How does increasing mast seeding frequency affect population dynamics of seed consumers? Wild boar as a case study. *Ecological Applications,* 30(6), e02134, 2020, http://doi.org/10.1002/eap.2134.

Uber, A.P., Trabulsi, L.R., Irino, K., Beutin, L., Ghilardi, A.C.R., Gomes, T.A.T, Liberatore, A.M.A. de Castro, A.F.P., and Elias, W.P. Enteroaggregative *Escherichia coli* from humans and animals differ in major phenotypical traits and virulence genes. *FEMS Microbiology Letters*, 256 (2), 251–257, 2006, doi:10.1111/j.1574-6968.2006.00124.x.

Velhner, M., Todorović, D., Grego, E., Jovčić, B., Prunić, B., Stojanov, I., Kehrenberg, C. Fluoroquinolone-resistant and extended-spectrum beta-lactamase producing *Escherichia coli* isolates from free-living wild animals. *Veterinary Microbiology*, 223, 168-172, 2018, doi:10.1016/j.vetmic.2018.08.011.

Vetter, S.G., Ruf, T., Bieber, C., and Arnold, W. What Is a Mild Winter? Regional Differences in Within-Species Responses to Climate Change. *PLoS ONE,* 10(7), e0132178, 2015, doi:10.1371/journal.pone.0132178.

Vetter, S.G., Puskas, Z., Bieber, C., and Ruf, T.How climate change and wildlife management affect population structure in wild boars. *Science Reports,* 10, 7298, 2020, doi:10.1038/s41598-020-64216-9.

Vieira-Pinto, M., Morais, L., Caleja, C., Themudo, P., Aranha, J., Torres, C., Igrejas, G., Poeta, P., Martins, C. *Salmonella* spp. in wild boar (Sus scrofa): A public and animal health concern. In *Game Meat Hygiene in Focus*; Wageningen Academic Publishers: Wageningen, The Netherlands, 131–136, 2011, doi:10.3920/9789086867233_011.

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

von Essen, E. How Wild Boar Hunting Is Becoming a Battleground. *Leisure Sciences*, 42(5–6), 552–569, 2019, doi:10.1080/01490400.2018.1550456.

Wacheck, S., Fredriksson-Ahomaa, M., König, M., Stolle, A., and Stephan, R. Wild Boars as an important reservoir for foodborne pathogens. *Foodborne Pathogens and Disease, 7*, 307–312, 2010, doi:10.1089/fpd.2009.0367.

WHO (World Health Organization) Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR). Critically Important Antimicrobials for Human Medicine 6th Revision, 2018. Accessed on November 2024: https://iris.who.int/bitstream/handle/10665/312266/9789241515528-eng.pdf?sequence=1

WOAH (World Organization for Animal Health). Salmonellosis, Chapter 3.9.8., 2018. Accessed on October 2024: www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.09.08_SALMONELLOSIS.pdf .

Zhou, Y., Zhu, X., Hou, H., Lu, Y., Yu, J., Mao, L., Mao, L., and Sun, Z. Characteristics of diarrheagenic *Escherichia coli* among children under 5 years of age with acute diarrhea: a hospital based study. *BMC Infectious Disese,* 18, 63, 2018, doi:10.1186/s12879-017-2936-1.

Zottola, T.,Montagnaro, S., Magnapera, C., Sasso, S., De Martino, L., Bragagnolo, A., D'Amici, L., Condoleo, R.,Pisanelli, G., Iovane, G., and Pagnini, U. Prevalence and antimicrobial susceptibility of *Salmonella* in European wild boar (Sus scrofa); Latium Region – Italy. *Comparative Immunology, Microbiology and Infectious Diseases*, 36(2), 161-168, 2013, doi:10.1016/j.cimid.2012.11.004.

Zsolnai, A., Csókás, A., Szabó, L., Patkó, L., Csányi, S., Márton, M., Lakatos, A.E., Anton, I., Deutsch F., and Heltai, M.Genetic adaptation to urban living: molecular DNA analyses of wild boar populations in Budapest and surrounding area. *Mammlina Biology,* 102, 221–234, 2022, doi:10.1007/s42991-021-00212-4.

Zuberogoitia, I., del Real, J., Torres, J.J., Rodríguez, L., Alonso, M., and Zabala, J. Ungulate vehicle collisions in a peri-urban environment: consequences of transportation infrastructures planned assuming the absence of ungulates. *PLoS One*, 9(9):e107713, 2014, doi: 10.1371/journal.pone.0107713.

UNIONE EUROPEA
Fondo Sociale Europeo
Ministero dell'Università e della Ricerca
PON RICERCA E INNOVAZIONE 2014 - 2020
REACT EU
UNIVERSITÀ DI PARMA

La borsa di dottorato è stata cofinanziata con risorse del
Programma Operativo Nazionale Ricerca e Innovazione 2014-2020, risorse FSE REACT-EU
Azione IV.4 “Dottorati e contratti di ricerca su tematiche dell’innovazione”
e Azione IV.5 “Dottorati su tematiche Green”