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A PRELIMINARY STUDY FOR THE QUEST, RETRIEVAL, AND DISPOSAL OF WILD BOAR CADAVERS AT THE ONSET OF AN AFRICAN SWINE FEVER EPIDEMIC

Tesi di laurea in MONITORAGGIO E PIANIFICAZIONE DEL TERRITORIO

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SUMMARY

Abstract

ASF is a potentially panzootic viral pig disease, which has recently affected the Italian territory with multiple hotspots. According to the recent EU Regulation, immediate eradication measures must be taken as soon as it is detected. As no vaccine or drugs are available against ASF, preventive measures are crucial for disease management: in swine farms, these include depopulating, physically isolating vulnerable holdings, contact tracing of animals and related products, and enforcing biosecurity throughout the production chain. Outbreaks are managed by isolating the infected area, thus causing extremely harsh socioeconomic effects on both individual farms and downstream economies, exerted mainly by the restrictions applied on the commercialization of live swine and swine products.

A cornerstone of ASF prevention and management is the early detection and removal of viral reservoirs: in Europe, this is mainly represented by the wild boar, whose management is considered crucial for disease control: the measures to be implemented include the active quest and removal of cadavers, an important demographic decrement, and the predisposition of an infrastructural network for collecting, sampling and disposing of carcasses and cadavers.

Given the high resistance of ASFV, all these activities must be carried out observing stringent biosafety measures throughout a precise territorial compartmentalization, requiring high level skills, landscape expertise, coordination between different stakeholders, and the setup of an efficient operational hierarchy.

The present work describes the setup of these measures in preparation of an ASF outbreak in Pordenone EDR (ex-province), northeast Italy, and the main aspects to be considered for an efficient management of wild boar and wild boar cadavers in case of ASF introduction. An overview of ASF ecology and targeted legislation is given, in a view to draft a comprehensive framework of ASF management in wild boar at an operational scale.

1. Introduction

1.1. Wild boar

The Wild boar (*Sus scrofa* L., 1758) is an ungulate of the Suidae (Gray, 1821) family indigenous to Eurasia. Despite being the ancestor and a conspecific of the domestic pig, which is nowadays distributed worldwide in both its farmed and feral (i.e. farmed animals reverted to a wild state) kinds, the native form remains constrained to the Palearctic (Figure 1).

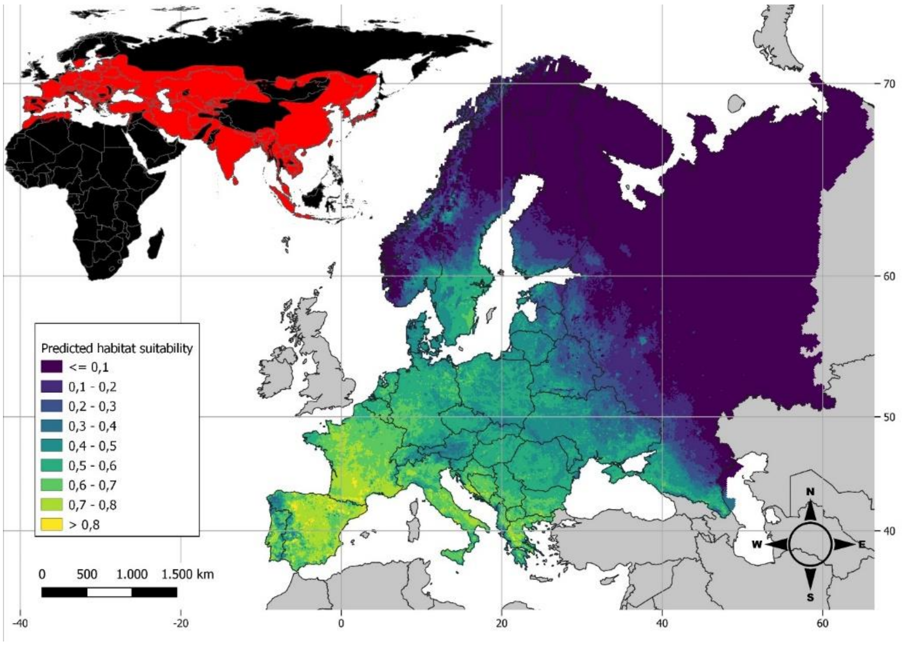
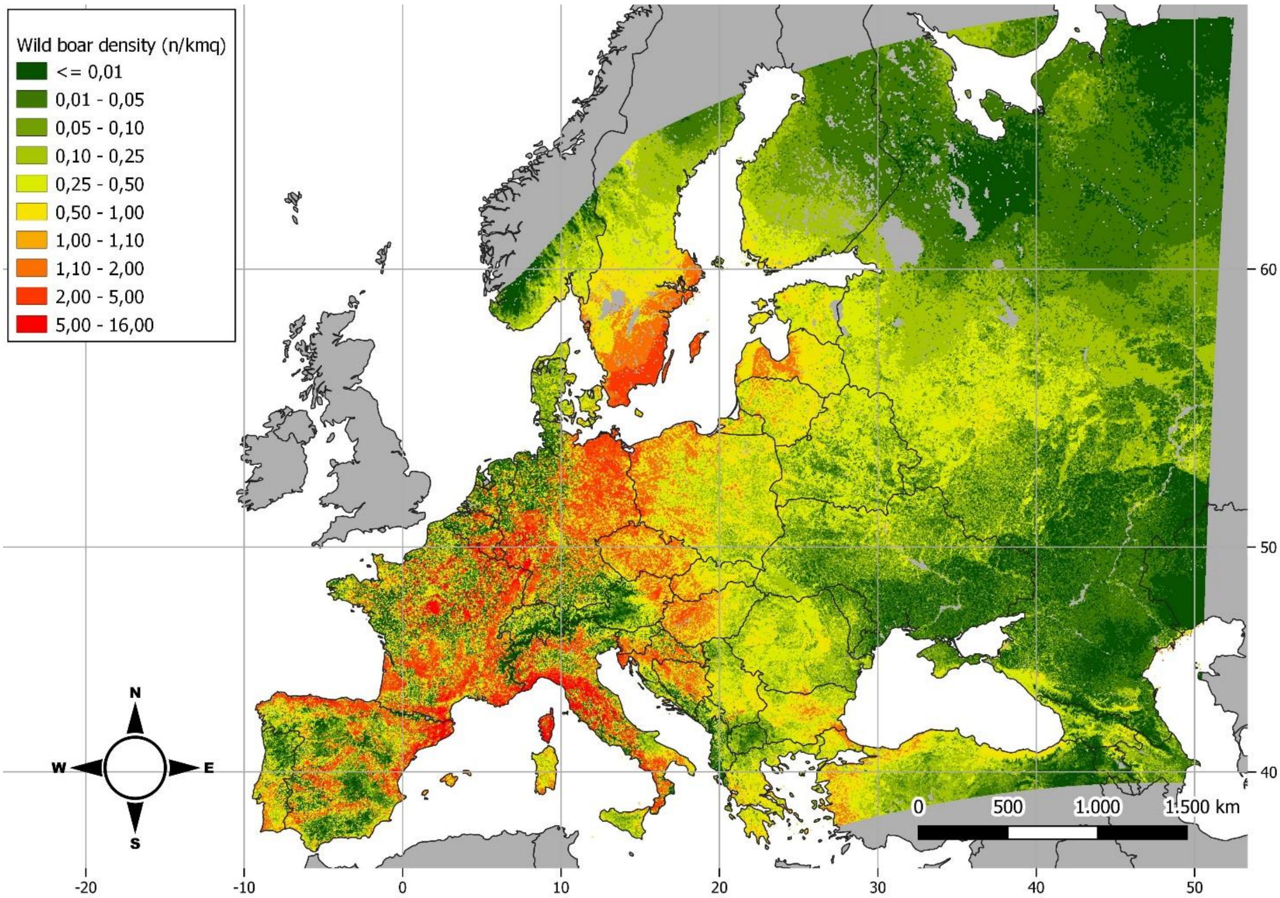


Figure 1: Distribution of wild boar in Afro-Eurasia (top left, IUCN data from Keuling & Leus, 2023) and habitat suitability model for wild boar in EU (data from ENETWILD-consortium, 2022.) EPSG: 3857

The Wild boar is a generally sedentary social species which aggregates in family groups, whose mean home range sizes is approximately 4.6±3.7 km^2 (ENETWILD-consortium, 2022). As the litter size is the largest amongst ungulates (3-7 to 11-15 cubs, Massei & Toso, 1993), local population densities show a strong seasonality, ranging from 2.7±2.7 individuals/km^2 in winter, to 28.4±25.7 individuals/km^2 in summer (ENETWILD-consortium, 2022). Generally, sexual maturity occurs within the 10^{th} month in males, and a couple of month later in females (Massei & Toso, 1993), with farrowing peaking during winter and early spring (Jori et al., 2021). However, as both fecundity and fertility in females are related to body mass rather than age, population productivity is constrained by environmental factors, such as water and food availability, and winter temperature (Jori et al., 2021; Pittiglio et al., 2018); thus, favourable conditions (e.g. warm winter, mast years) often coincide with earlier maturity (e.g. 23% fertile females as young as 9 - 12 months old) and bigger litters (Piol et al., 2022). Oestrus, which normally takes place between November and January, is also environmentally modulated, so that female reproductive season can extend all year round, allowing for the production of two breeds per year (Massei & Toso, 1993, but see also Piol et al., 2022), and yearly population growth possibly exceeding 200% (Keuling et al., 2013). Yearly natural mortality is often assumed to be approximately 10% (Guberti et al., 2022), raising to 15% if roadkill are included (Toïgo et al., 2008).

The wild boar population structure is generally reflected in the hunting bags, comprised by 50-60% of piglets (< 1 year old), 20-30% of subadults (one and two y.o.), and 10-20% of adults (≥ three y.o.).



Such a population structure is often itself a consequence of the hunting policies.

Figure 2: Wild boar population density as estimated in PITTIGLIO ET AL., 2018. EPSG: 3857

Wild boar is an opportunistic omnivore, whose diet include both plant and animal matter (PAOLUCCI & BON, 2022). Feeding occurs mainly through *rooting*, by which animals search for underground food sources such as plant roots, fungi or soil fauna.

During the past century, different factors have concurred to increase the size of both wild boar population and potential habitat throughout its distributional range, leading to an overall demographic increase in most EU countries (KEULING ET AL., 2013; MASSEI ET AL., 2015); notably:

1. Climate warming, which allowed wild boar to settle in previously unoccupied habitats (VETTER ET AL., 2015);
2. the development of industrial agriculture, that provided local populations with additional feeding resources (GUBERTI ET AL., 2022; JORI ET AL., 2021);
3. measures to increase hunting productivity, such as reintroductions, control of predators and supplementary winter feeding (MASSEI ET AL., 2015).

Given its general confidence towards anthropogenic habitats, this species is often regarded as problematic, especially for agriculture: negative impacts are exerted mainly through the *rooting* behaviour, with direct damages to the topsoil and the root apparatus of cultivated plants. As a consequence, populations are generally heavily managed through culling campaigns and targeted hunting; however, as these activities are often inefficient, local overabundance is frequently observed.

1.2. AFRICAN SWINE FEVER (ASF)

1.2.1. Aetiology and symptomatology

ASF is a viral disease caused by a double-strained DNA virus (ASFV) of the *Asfarviridae* family, genus *Asfivirus* (KING ET AL., 2011), affecting pig species of the *Suidae* family; these include all breeds of *Sus scrofa* (both domestic and wild) and various African species, such as warthogs (*Phacochoerus* spp.), bushpigs (*Potamochoerus* spp.) and the giant forest hog (*Hylochoerus meinertzhageni*) (:).

Although all *Suidae* taxa can be infected, the disease manifests clinically only in *Sus scrofa* (SÁNCHEZ-VIZCAÍNO ET AL., 2012): common symptoms include high fever, anorexia, vomiting, diarrhoea and respiratory complications, leading to haemorrhages and neurological disorders, and finally death (ARIAS & SANCHEZ-VIZCAINO, 2008).To date, twenty-four viral strains are acknowledged (QU ET AL., 2022), with genotype-specific pathogenicity and clinical characteristics (namely, mortality, morbidity, case fatality ratio), which varies greatly between strains.

ASFV Genotype II, which is currently circulating in the EU (see Distribution in Europe), is characterized by a very high case fatality (i.e. the proportion of infected individuals that die within a certain timeframe, THRUSFIELD ET AL., 2018), assessed at 94.5-100% in both domestic and wild suids (GERVASI & GUBERTI, 2021). Although estimates of prevalence (i.e., the proportion of infected individuals overall the population, THRUSFIELD ET AL., 2018) are hardly achievable in wild populations, wild boar demographic declines exceeding 80% are attributed to ASF (MORELLE ET AL., 2020). However, as the contagiosity of ASF is likely dose-dependent, a high viral transmission is bound to an exposure of high viral loads, as can be found in the bodily fluids (blood, especially) of infected animals; in situations where exposure to bodily fluids (e.g., high contact rate, cannibalism) is avoided, ASF transmission can be low (CHENAIS ET AL., 2018).

Figure 3: Some of the species targeted by ASFV: Sus scrofa *(A: domestic pig; B: wild boar);* Phacochoerus africanus (C); Potamochoerus porcus (D). All images are CC0.

Experimentally inoculated wild boars develop clinical signs after an incubation period of 3 – 4 days, while virus shedding starts after a latent period of 2 - 6 days, leading to the death of all tested specimens in seven to nine days post-inoculation (BLOME ET AL., 2012; GABRIEL ET AL., 2011).

ASFV is a very stable virus (MAZUR-PANASIUK ET AL., 2019): as its inactivation requires exposure to 60°C for at least 20 minutes, it can survive in fresh, frozen, putrefied and cured meat, as well as inside the lymph nodes and bone marrow of the few survivors, whose natural death and permanence in the field as infected cadavers is likely to start a new disease hotspot (BELLINI ET AL., 2016; PENRITH & VOSLOO, 2009). At environmental conditions, the infectivity of biological tissues (spleen, kidney, lung) is highly temperature-dependent, and spans from up to two years at -20°C, to 9-17 days at 23°C (MAZUR-PANASIUK & WOŹNIAKOWSKI, 2020); viral survival and infectivity in soil has been shown to be negatively impacted by acidic conditions (CARLSON ET AL., 2020).

1.2.2. Biological cycle

Depending on the presence of susceptible hosts, the characteristics of the pig production system, and the availability of an arthropod vector, ASF life cycle can be ascribed to four different, although non-exclusive, pathways (Figure 4).

1. Sylvatic cycle: prevalent in sub-Saharan Africa, where ASF is endemic in several countries (BELLINI ET AL., 2016). It is sustained by argasid ticks of the genus *Ornithodoros* and the common warthog (*Phacochoerus africanus*), which share burrowing sites. African suid hosts are mostly indifferent to the disease.
2. Tick-pig cycle: described in parts of sub-Saharan Africa and during the early 60s and 70s epidemics in Europe (BOINAS ET AL., 2011).
3. Domestic cycle: involved in the majority of oubreaks worldwide (PENRITH & VOSLOO, 2009); the virus in transmitted exclusively amongst domestic pigs, both via direct contact, and via contaminated porcine products, regardless of the arthropod host.
4. Wild boar-habitat cycle: characteristic of the current European outbreak, viral transmission is both direct amongst wild boars, and indirect via the environment, which is manly contaminated by infected cadavers.

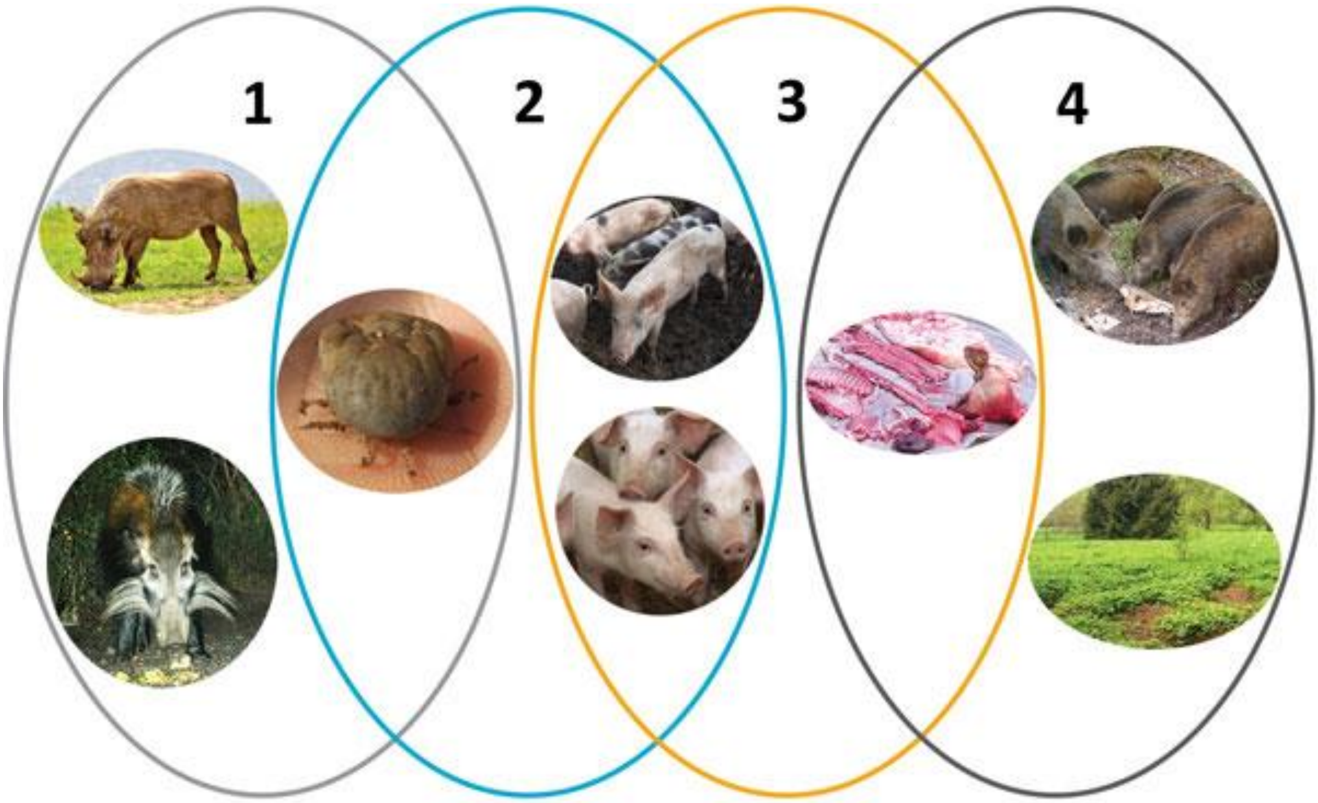


Figure 4: The biological cycles of African swine fever and main transmission agents. 1) Sylvatic cycle: the common warthog, bush pig, and soft ticks of Ornithodoros spp. The role of the bush pig in the sylvatic cycle remains unclear. 2) Tick–pig cycle: soft ticks and domestic pigs. 3) Domestic cycle: domestic pigs and pig-derived products (pork, blood, fat, lard, bones, bone marrow, hides). 4) Wild boar–habitat cycle: wild boar, infected meat and contaminated habitat (CHENAIS ET AL., 2018)

The aforementioned life cycles represent adaptations of ASFV to the local hosts' populations, at various phases of its evolution: the virus is suggested to have developed in *Ornithodoros* ticks around 1.5 million years ago (FORTH ET AL., 2020), remaining endemic amongst tick and local suids, mostly indifferent to the infection; the jump from African wildlife to disease-prone domestic pigs followed the increase of local breeds, leading to the definition of a *tick-pig* cycle first, and the establishment of a *domestic* cycle later; afterwards, the globalization of meat industry allowed ASFV to trespass previously restraining geographical barriers, to leave the African continent, and to further spread in Eurasian populations of domestic pig. The virus then gained the capability of surviving locally, often without major involvement of the domestic pig population (SAUTER-LOUIS ET AL., 2021), by exploiting the growing, free-ranging wild boar populations, and thus defining the *wild boar-habitat* cycle.

Although the *wild boar-habitat* cycle is independent of intermediate hosts, *Ornithodoros* ticks could still play an important role where present. In fact, they can act both as a vector, and a reservoir, being characterized by a long lifespan (up to 15 years), resistance to starvation and viral persistence (up to 5 years). However, their role in viral spread is considered negligible in most of Eurasia (EFSA, 2010).

The virus can spread either directly or indirectly: direct transmission occurs by close contact between healthy and sick animals, whereas indirect transmission takes place through infective environmental matrices (EFSA, 2014; PENRITH & VOSLOO, 2009), such as carcasses and offal, blood, faeces and urine, oral/nasal excretions/secretions, soil, grass and crops, raw meat, food/kitchen waste, fomites (e.g. clothes, tools, car tires), scavenging insects, hematophagous arthropods.

Contacts between living animals and infected carcasses is a particularly important source of indirect transmission, exerted mainly through specific behaviours such as sniffing, poking and cannibalism (Cukor, Linda, Václavek, Mahlerová, et al., 2020; Probst et al., 2017) (Figure 5).

*Figure 5: Wild boar (left) poking on the cadaver of a conspecific, likely feeding on diptera larvae. At the time the picture was taken, the cadaver underwent six days of environmental degradation. Courtesy of IZSVe (ongoing research project "*RC IZSVe 06/22 - Deathboars*")*

1.2.3. Epidemiology in WB

Typically, the spread of the ASFV amongst a naïve wild boar population follows four steps (Figure 6):

1. Incursion: is the introduction of the virus in a naïve population. It can occur either by natural spread from a neighbouring endemic, or by the importation of viral particles from non-adjacent territories. The former case is generally bounded to wild boar spatial behaviour (namely, home range size and dispersal distance), and particularly to the movement patterns of infected animals. The latter is mostly attributable to anthropogenic factors, such as transport and disposal of infected meat or meat products (Chenais et al., 2019); in this case, long distance "jumps" can be observed, which represent a major factor in ASF spatial dynamics (Pepin et al., 2020). Viral incursion is unrelated to population size and density.
2. Invasion: represents the initial successful spread of the virus. It is strictly dependent on intraspecific contact, and is thus theoretically bounded to a host density threshold (Nt), representing the minimum population density to sustain the spread. However, the resistance of ASFV to environmental degradation sets this threshold virtually to zero, and makes a reliable estimation practically unattainable (EFSA, 2018).
3. Epidemic: represents the progressive spread of the infection to the susceptible population. At an early stage, the epidemic curve usually follows an exponential growth, whose shape is

mostly determined by the basic reproductive number R_0 (i.e. the average number of secondary cases determined by one infected individual, during its entire infectious period, in a fully susceptible population, DIEKMANN ET AL., 1990), which for ASF Genotype two averages 1.67±0.22 (LOI ET AL., 2022; MARCON ET AL., 2020). Naturally, the speed of the epidemic spread ranges approximately from 1 km/month to 1 km/week (GUBERTI ET AL., 2022; LICOPPE ET AL., 2023; PODGÓRSKI & ŚMIETANKA, 2018).

4. Endemicity: is local persistence of the virus. It is supported by the host's critical community size (CCS), which represent the minimum population size with which a pathogen has 50% probability of fading out spontaneously (BARTLETT, 1960).

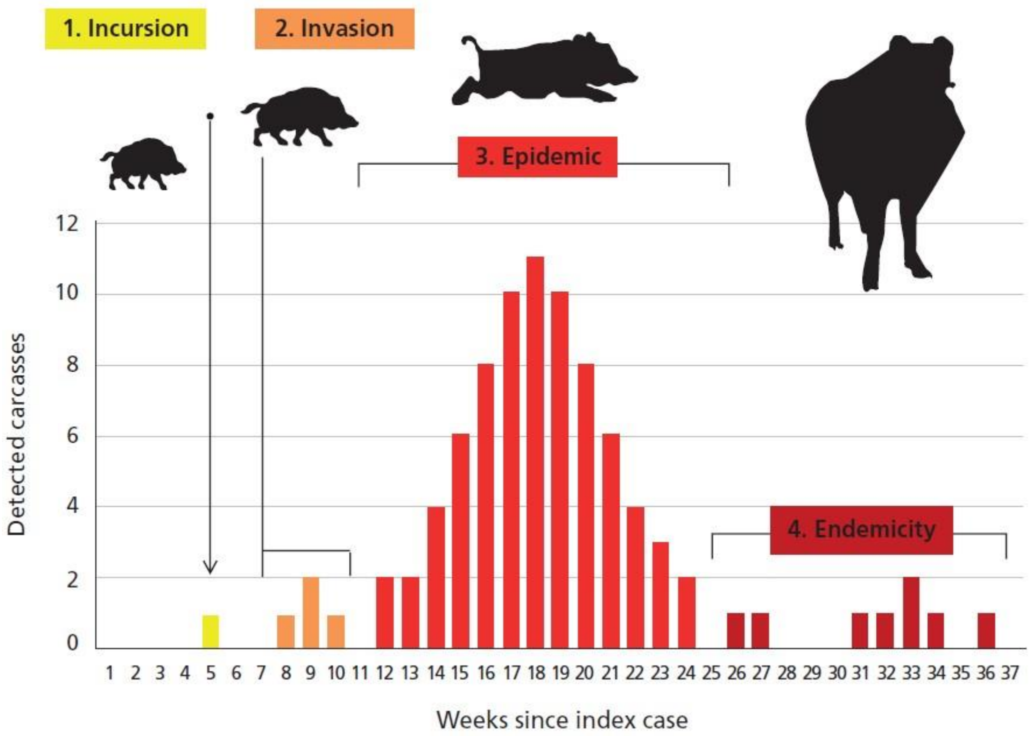


Figure 6: Hypothetical example of the four phases of the infection dynamic in a population of wild boar, measured through the number of carcasses detected weekly (GUBERTI ET AL., 2022)

Due to ASFV lethality, both Nt and CSS are often unrealized in the leftover wild boar population; however, the viral transmission can still be supported indirectly through the contaminated habitat. The long lasting viability of the virus largely outmatches the lifespan of infected animals: in fact, the role of cadaver-based transmission increases at decreasing population densities (PEPIN ET AL., 2020), making it possible to the virus to persist at very low prevalence (usually around 1%, GERVASI & GUBERTI, 2021), even in scanty host populations.

1.2.4. Distribution in Europe

To date, ASF has been reported almost exclusively in Afro-Eurasian countries, the only exception being the island of Hispaniola, where the virus has been reported in farmed animals; where both wild and domestic hosts are present, ASF affected both populations almost invariably. ASF affected countries based on WOAH case reports (https://wahis.woah.org/#/home, July 1st, 2005 – December 31st, 2023 data) is given in Figure 7.

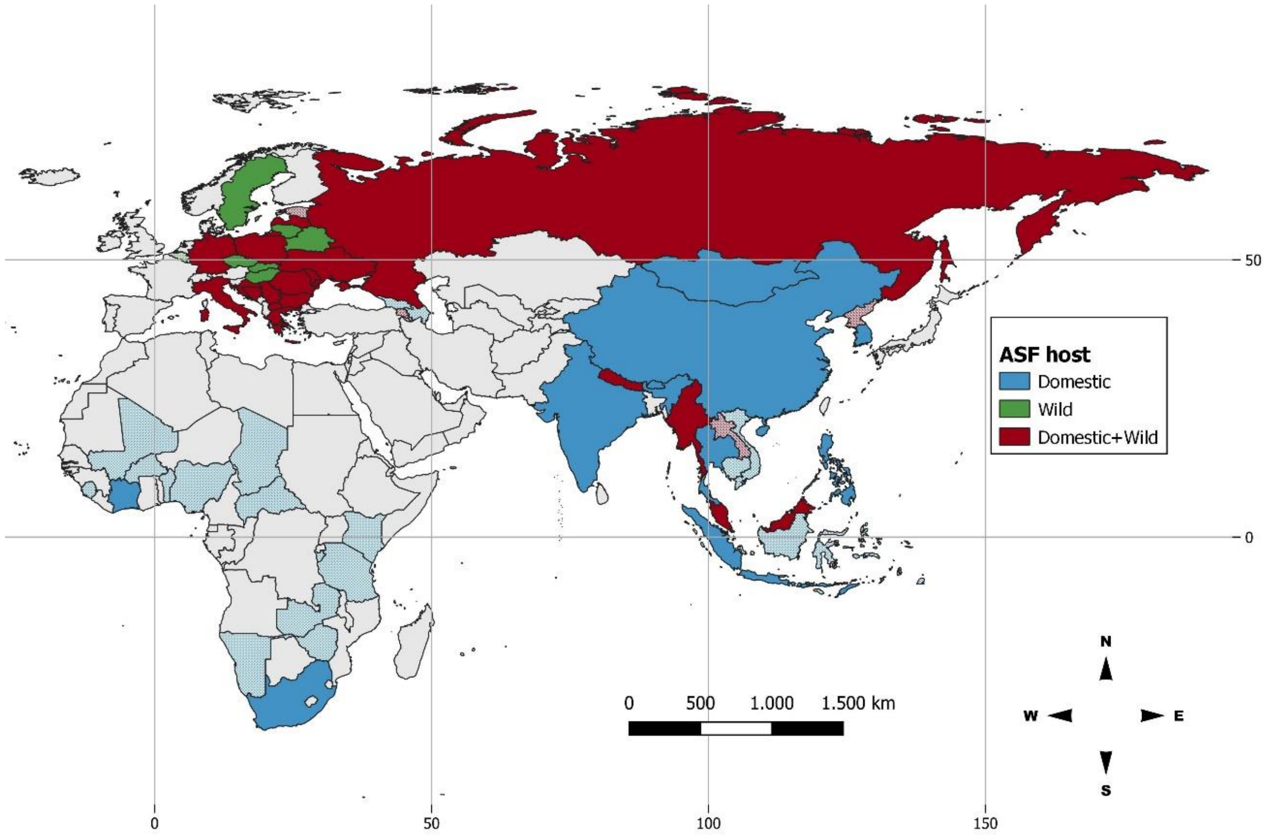


Figure 7 Afro-Eurasia and Indonesian countries affected by ASF in wildlife and domestic/farmed animals. Countries which had been affected before 2023, but were not during 2023, are shaded, while countries affected during 2023 (Jan 01st-Dec31st) are represented in bright tones. EPSG: 4326.

ASF is endemic in most countries of sub-Saharan Africa, where it was first reported in colonial Kenya in 1921 (EUSTACE MONTGOMERY, 1921), and where the highest genotypic richness is currently found (Figure 8).

In Europe, recurring Genotype I outbreaks have occurred during the second half of 1900; all of them have been successfully eradicated, except in the Italian island of Sardegna, where it remained endemic since 1978 (BELLINI ET AL., 2016; MUR ET AL., 2016) (see ASF occurrence).

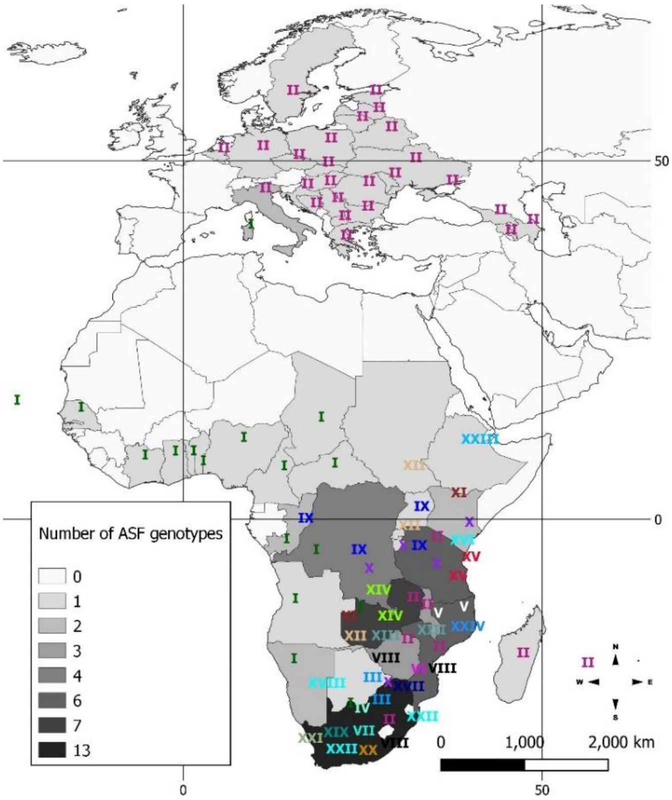


Figure 8: Distribution of ASF genotypes in Africa and Europe. Differently coloured roman numbers identify different genotypes (overlapping labels in genotypically rich countries have been removed). Data relative to Africa was obtained from Njau et al., 2021, while European data (December 2023) was downloaded from WOAH webGIS at https://wahis.woah.org/#/home. EPSG: 4326

A second ASFV strain (Genotype II) is affecting Eastern Europe since 2007 (Bellini et al., 2016; Blome et al., 2012; Guinat et al., 2014). Compared to ASFV Genotype I, Genotype II is more virulent and lethal, and appears to be highly wild boar density-dependent (Guberti et al., 2022). The European spread started from a spill-over event, likely originating from improper waste disposal by international ships in the Georgian docks of Poti (Beltran-Alcrudo et al., 2008). During the first stages of the epidemic, ASF infections were detected mostly in domestic pig farms with generally low biosecurity: arguably, the similarities with the predominantly "domestic" Genotype I outbreaks lead to overlook the role of wild boar in sustaining the epidemiological cycle of the virus (Sauter-Louis et al., 2021).

After occasional spill-overs to the wild boar population, ASF transmission pattern assumed the characteristics of a typical *wild boar-habitat* cycle (Chenais et al., 2018). During the subsequent years, the epidemic wave moved eastward affecting both farmed and wild hosts, through the Caucasus and the Russian Federation, with a pace ranging between 1 km/month to 1 km/week (Guberti et al., 2022). In 2014, ASF reached Lithuania and the EU borders, where it currently represents a constant threat to the livestock sector (Bellini et al., 2016).

To date, ASF has infected thirteen EU countries: Estonia, Lithuania, Latvia, Poland, Czech Republic, Bulgaria, Belgium, Romania, Hungary, Slovakia, Germany, Italy and, on early September 2023, Sweden. Among these, only two have achieved viral eradication: Czech Republic, which was declared ASF-free in 2019 after 21 months from the index case (OIE, 2019), and Belgium, which regain ASF-free status in November 2020 after 26 months of management (Licoppe et al., 2023; OIE, 2020). In both instances, ASF index case developed at more than 300 km from the nearest cases (Sauter-Louis et al., 2021), leading to ascribe the incursion to human transport. During December 2022, new outbreaks have been reported in Czech Republic, which likely followed the epidemic wave coming from Poland, while Belgium remains, to do date, ASF-free.

WOAH records for European ASF outbreaks in wild and domestic animals are represented in Figure 9 and Figure 10, respectively. Given that disease reports are the outcomes of voluntary surveillance activities, the distribution of cases must be interpreted with caution.

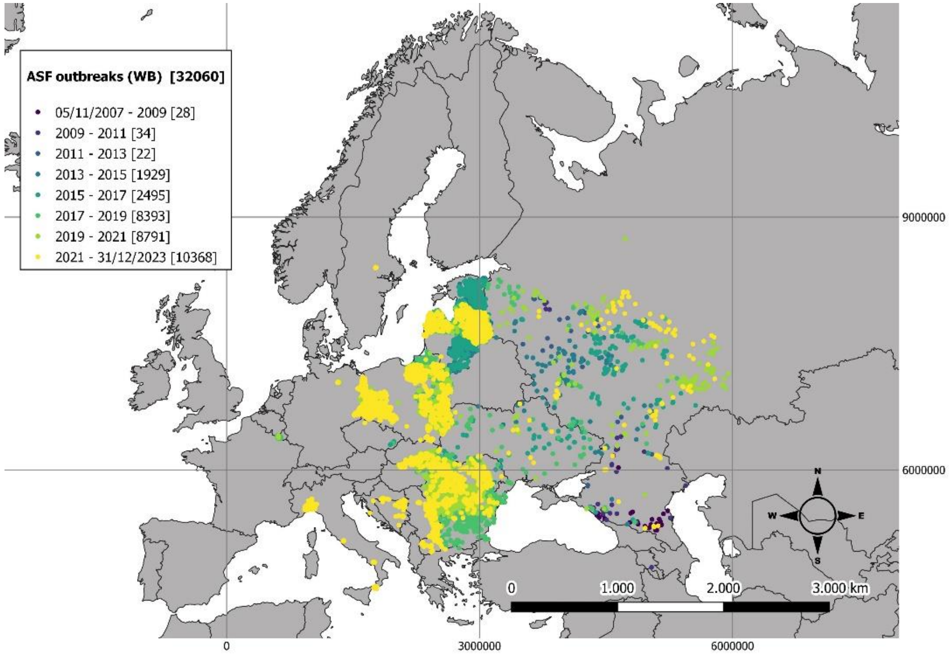


Figure 9: ASF outbreaks in wild boar (WB) in Europe (west to Ural mountains, 31/12/2023 data). Feature count is shown in square brackets in the map legend. Overlapping data. EPSG: 3857

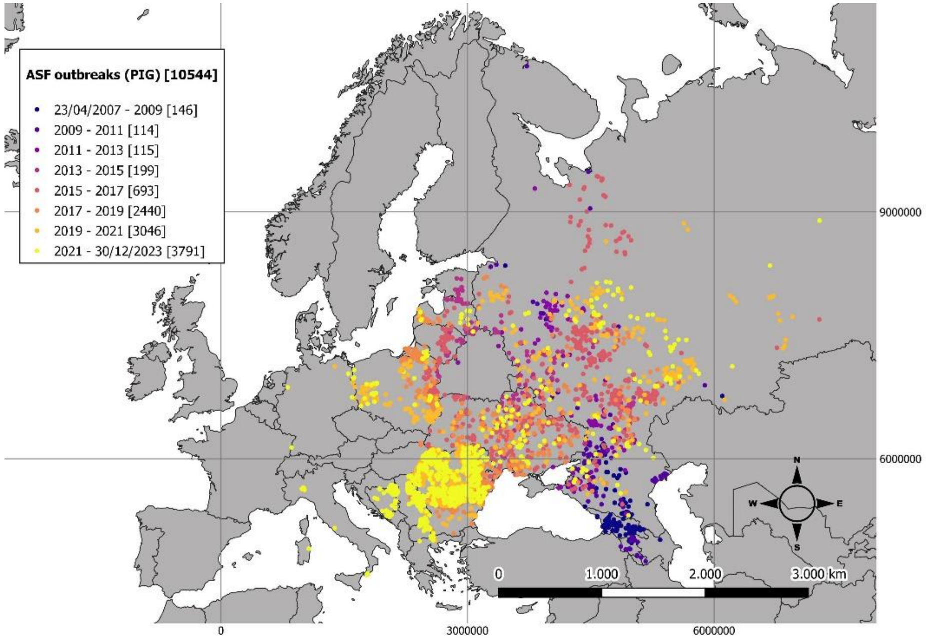


Figure 10: ASF outbreaks in domestic suids in Europe (west to Ural mountains, 31/12/2023 data). Feature count is shown in square brackets in the map legend. Overlapping data. EPSG: 3857

1.3. Strategies against ASF in the wild

1.3.1. EU legislation

All EU member States are members of the World Organization of Animal Health (WOAH), for which ASF is as "notifiable" disease; this includes "*transmissible diseases that have the potential for very serious and rapid spread, irrespective of national borders, that are of serious socio-economic or public health consequence and that are of major importance in the international trade of animals and animal products*" (OIE-WOAH, 2011 Art. 1.3.5.). WOAH member States can self-declare disease-free status after one year of absence of the virus, as proved through monitoring (OIE-WOAH, 2011).

In the EU, Regulation (EU) n. 429, 2016 ("Animal Health Law", AHL hereafter) provides the structure for prioritising and categorising diseases of Union concern. ASF is included amongst the five most important listed animal diseases, which include ASF, classical swine fever, foot and mouth disease, highly pathogenic avian influenza, and African horse sickness (AHL, art. 5). As a listed disease, ASF is subjected to categorization, provided through Implementing Regulation (EU) n. 1882, 2018; ASF is ascribed to three categories:

1. Category A: a disease that does not normally occur in the Union and for which immediate eradication measures must be taken as soon as it is detected (AHL, Article 9(1)(a));
2. Category D: a disease for which measures are needed to prevent it from spreading on account of its entry into the Union or movements between Member States (AHL, Article 9(1)(d))
3. Category E: a disease for which there is a need for surveillance within the Union (AHL, Article 9(1)(e))

As such, EU Countries must report ASF outbreaks to the EU Commission via a dedicated tool, the Animal Disease Information System (ADIS) (Regulation (EU) n. 429, 2016, Art. 22). Once ASF is detected, the area should be declared as infected, and subjected to zoning restrictions as per Delegated Regulation (EU) n. 687, 2020, Artt.63-64-65 (see Zoning). Implementing Regulation (EU) n. 594, 2023 also include specific measures for ASF control, according to the zoning status of the area.

Based on the EU experience, the success of ASF eradication strategy highly depends on prevention and early detection (Bellini et al., 2016), and must consist of an integrated approach based on zoning, population control, effective surveillance and removal and safe disposal of infected cadavers (EFSA, 2022); so far, none of this activity have single-handedly proven successful in stopping the viral spread (Licoppe et al., 2023). To carry on such an integrate strategy, an overall coordination amongst stakeholders is essential throughout the process (Licoppe et al., 2023).

To be effective, the management strategy must be timely and tailored to the epidemiological stage of the disease (Guberti et al., 2022). As the large number of infectious individuals makes ASF management a prohibitive task during the epidemic phase (Guberti et al., 2022), outbreak control should be reached ideally during the incursion and the invasion phases. However, as the former (i.e. the "patient zero") is virtually undetectable (Gervasi et al., 2020), the very first finding of an infected carcass is likely to mark the invasion phase, if not the onset of a latent epidemic, with a large number of infected and undetected carcasses already present in the field (Guberti et al., 2022).

Within 90 days from the ASF index case, the competent Authorities of affected member States should draft national surveillance and eradication programmes to be approved by EU Commission (REGULATION (EU) N. 429, 2016, Art. 31); this programmes should account for dedicated guidelines, as provided in SANTE/7113/2015, REV. 12, 2020, and should include a including a risk analysis of viral introduction.

1.3.2. Zoning

After the detection of the index case, and having assessed the spatial pattern of infections around the index case through on the active search of wild boar cadavers (see Surveillance), three "operational zones", as per LICOPPE ET AL., 2023 (Figure 11), should be defined:

1. Infected area: an area in which positive carcasses are found, and in which all ASF containment and eradication measures must be applied. This area must preferably be shaped according to the presence of artificial or natural barriers, to slow the viral spread. It includes the area of viral circulation, represented by a convex polygon around detected cases, plus a surrounding area which should be at least double in "size" (GUBERTI ET AL., 2022).
2. White area: a wild boar-free buffer zone, where the quasi-extinction (non-viability) of the host population should be looked forward
3. ASF free area: an external land strip in which usual hunting activities can be carried out, improving hunting efforts (e.g. double the previous year's hunting bag), and targeting adult and sub-adult females

Inside the infected area, a restriction on non-ASF related activities should be imposed to minimize the risk of spreading the virus through anthropogenic media, lifting the access ban only at the end of the epidemic phase (JORI ET AL., 2021). Culling and trapping activities must be carried out avoiding environmental exposure to infected biological ASFV matrices, especially blood, through the displacement of wounded animals or bloody carcasses. Every wild boar inside the infected area, either hunted or found dead, should be analysed for ASF, and thus be handled using stringent biosafety measures.

In the EU, the establishment of the infected area following an outbreak in wild animals is due for all AHL Category A diseases, (DELEGATED REGULATION (EU) N. 687, 2020, Artt.63-64-65); in addition, based on the epidemiological state of a territory, a second regionalization scheme ("restriction zoning", Figure 12) is also provided. Restriction zoning aims at regulating activities in and around the infected area, in particular the movement of animal and animal products, in a view to prevent the spread of the disease to naïve territories and commercial activities (IMPLEMENTING REGULATION (EU) N. 594, 2023; SANTE/7112/2015, REV. 3, 2019)

ASF affected territories are categorized into three Restriction Zones (RZs):

1. RZ I: ASF-free areas bordering with RZ II or RZ III
2. RZ II: areas in which ASF is found exclusively in wild boar
3. RZ III: areas in which ASF is found in domestic pigs, with or without cases in wild boar

Territories falling under these restriction zones (generally, at municipality level) are listed in Annex I of Implementing Regulation (EU) n. 594, 2023, which is regularly updated to account for new outbreak notifications and changes in the epidemiological situation of affected areas.

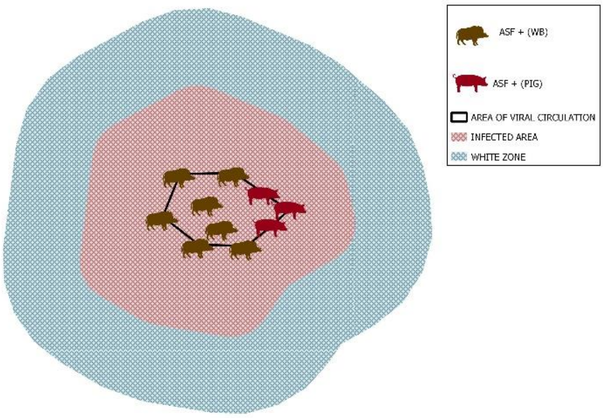


Figure 11: Operational zoning

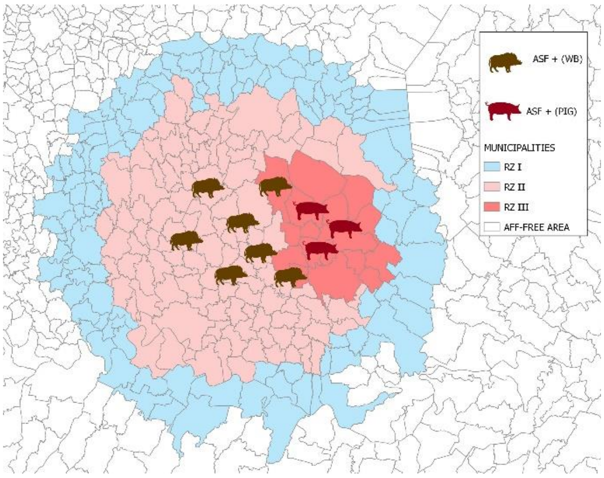


Figure 12: Restriction zoning

Although restriction zones and operational zones may overlap, they pursue different objectives: eventually, RZ II and RZ III will coincide with the Infected Area, and RZ I with the White area, thus making the institution of the Infected Area a provisional measure. However, the maintenance of an operational zoning system in parallel with EU zoning can facilitate the implementation of the ASF response strategy (LICOPPE ET AL., 2023). An updated, interactive map on ASF restriction areas in the EU can be found at https://santegis.maps.arcgis.com/apps/webappviewer/index.html?id=45cdd657542a437c84bfc9cf1846ae8c.

1.3.3. Fencing

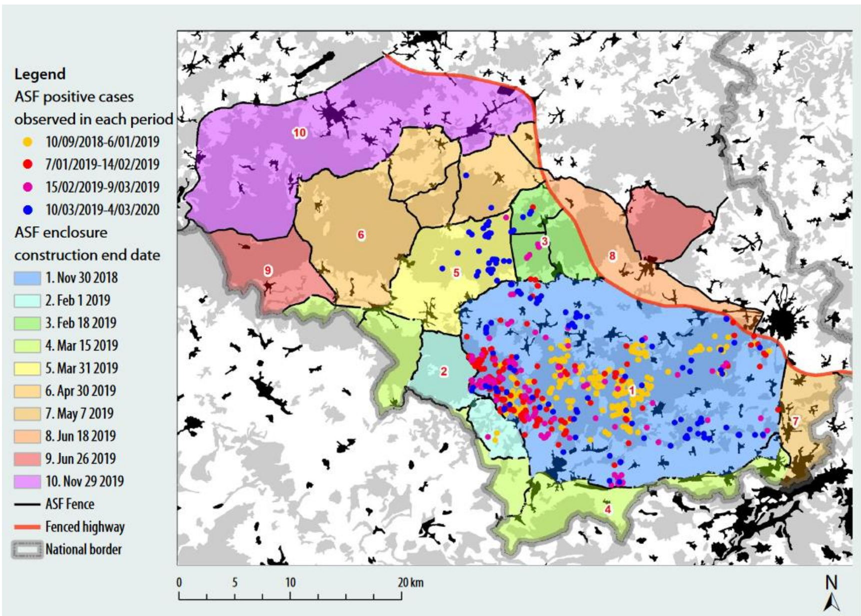


Figure 13: Fencing system around ASF cases in wild boar in Belgium (Jori et al., 2021)

In the context of ASF management in wildlife, fencing can be adopted to isolate infected areas, and to facilitate wild boar population management, by hindering animal movements. In particular, the creation of the "White zone" (see Zoning) requires an intense effort to prevent animal migration either in and out the ASF-free area (LICOPPE ET AL., 2023). "Ad hoc" or "focal" fencing can be implemented to increase the fragmentation of the habitat, in a view to slow down wildlife-mediated ASF spread (JORI ET AL., 2021).

To be effective, wild boar fences should provide an electrical shock, be buried at 50 centimetres below ground, and be regularly checked for breaks and impediments to the electric flow (JORI ET AL., 2021); as this is often too costly and too demanding to be implemented promptly, some compromise can be considered: for example, above-ground wire fences facilitated ASF management in Belgium, where approximately 300 km of 1.2 m high, unburied fences were laid down, resulting in approximately 270 m of fence per km^2 (Figure 13) (LICOPPE ET AL., 2023; ŠATRÁN, 2019). In fact, the Belgian solution was a compromise between efficiency and rapid installation, as compared to electric and buried fences, respectively. To further facilitate this operation, fencing could be carried out by enforcing existing barriers, such as highways, large rivers etc.

Despite being highly recommended for ASF management, the use of fencing for wildlife disease control is controversial, given to both scarce efficacy in containing indirect or human-mediated spread, and to various adverse ecologic and economic impacts (MYSTERUD & ROLANDSEN, 2019).

1.3.4. Wild boar management

After the introduction, the invasion stage on an ASF outbreak is mostly sustained by direct transmission; thus, the risk of developing an ASF epidemic in the wild could theoretically be minimized by maintaining populations in ASF-free areas below the host density threshold. Similarly, in areas characterized by an endemic persistence of the virus (which is mostly transmitted indirectly), this threshold would be represented by the critical community size (see Epidemiology). However, the resistance of ASFV to environmental degradation sets these thresholds virtually to zero, and makes a reliable estimation of both practically unattainable (EFSA, 2018). Furthermore, considering the high productivity of wild boar populations, such low numbers would probably be unreachable (GUBERTI ET AL., 2022; MASSEI ET AL., 2015; REICHOLD ET AL., 2022).

Conversely, an overall demographic decrease of wild boar in ASF free areas is considered desirable as a preventive measure (SANTE/7113/2015, REV. 12, 2020), aiming at slowing down an eventual ASF spread in the future. This includes culling, possibly targeted on sub-adult (i.e., one to two years old animals) and adult (≥ three y.o.) females (GUBERTI ET AL., 2022), avoiding supplementary feeding and limiting attractive baiting (e.g. SANTE/7113/2015, REV. 12, 2020).

As the demographic growth of wild boar populations can exceed 200% per year, inducing a decrease in the short term may require the removal of 65% of individuals from the local population (KEULING ET AL., 2013). However, this would likely determine compensatory migrations from nearby regions, and should therefore be implemented in a coordinated manner, possibly at a regional scale (JORI ET AL., 2021). At this conditions, achieving a substantial reduction is often unrealistic (GUBERTI ET AL., 2022; KEULING ET AL., 2016; MASSEI ET AL., 2015); however, this effort can be effective when applied in conjunction with supplementary measures, such as zoning, fencing and trapping (GUBERTI ET AL., 2022; JORI ET AL., 2021).

While a demographic decline of wild boar as a preventive measure against ASF is suggested overall, improving hunting in disease-ridden areas is controversial (APOLLONIO ET AL., 2017); the reason behind this lies in the operational aspects of killing, processing and transporting animals, which inherit a high risk of human-induced viral spread through infected bodily fluids; moreover, hunting chases are likely to induce dispersal of possibly infected animals towards ASF-free areas (PEPIN ET AL., 2020; SCILLITANI ET AL., 2010).

Therefore, the removal of animals for disease control should be strictly tailored on each ASF management area, as identified through the process of zoning (see Zoning). At first, hunting and feeding should be entirely banned inside the infected area, in a view to avoid viral spread; meanwhile, an intense hunting effort should aim at creating an area virtually free of all wild boars around the infected area (i.e., the "white zone"). Hunting practices should minimize animal movement; hence, driven hunting should be avoided in favour of standing hunts overall the surrounding areas (KEULING ET AL., 2008).

Once the virus has decimated most of the wild boars inside the infected area, an intensive hunting campaign should pursue the elimination of all of the few survivors. Given that hunting only slightly affects the high mortality induced by ASF (MORELLE ET AL., 2020), aids such as night visors and trapping devices are often suggested and implemented (LICOPPE ET AL., 2023).

1.3.5. Surveillance

In the context of animal health, *surveillance* is the systematic and ongoing production of data related to animal health and welfare, used to describe health hazard occurrence and to contribute to the planning, implementation, and evaluation of risk mitigation actions (HOINVILLE ET AL., 2013).

As regard to ASF, surveillance is performed by testing dead animals, both domestic and wild. Diagnosis for ASF is usually carried out by biomolecular (PCR) analysis on a spleen sample, but other tissues (kidney, lymph nodes, tonsils, blood, or bone marrow) and tests (antigenic test, RT-PCR, ELISA, immunoperoxidase) can be used, according to the state of the dead animal.

In wild boar, surveillance is carried out on animals which are either hunted (*active surveillance*) or found dead (*passive surveillance*), with the latter strategy largely outperforming the former in terms of efficacy (GERVASI ET AL., 2020).

To date, the vast majority of ASF outbreak in EU has been detected through passive surveillance (EFSA, 2022). However, wild boar density and viral prevalence may be too low to provide the required sample size (e.g. at the end of the epidemic phase): this can lead to overlook a latent endemic situation, where wild boar demographic recovery give rise to re-emerging ASF cases. Therefore, a combination of both active and passive surveillance is often required (GERVASI ET AL., 2020; NIELSEN ET AL., 2021).

In a particular case of passive surveillance, cadavers are actively looked for (*enhanced passive surveillance*, HOINVILLE ET AL., 2013). The scope of enhanced passive surveillance for ASF is twofold: it provides data for epidemiological surveillance, allowing to adjust management strategies accordingly (e.g. LICOPPE ET AL., 2023), while enforcing disease control through the removal of contaminating sources; to this point, a delay in detecting and removing infected cadavers impedes the early detection of the virus, thus favouring the development of ASF endemic contexts (GERVASI ET AL., 2020). Therefore, enhanced passive surveillance is considered a pillar for the eradication of ASF in wild boar (BOUÉ ET AL., 2017; GUBERTI ET AL., 2022; MORELLE ET AL., 2019; PROBST ET AL., 2017; ŠATRÁN, 2019).

To maximize surveillance outcomes, enhanced passive surveillance should target suitable habitats. More than 80% of wild boars die in forests, suggesting that sick animals perceive this environment as safe and comfortable (CUKOR, LINDA, VÁCLAVEK, ŠATRÁN, ET AL., 2020). Given the feverish symptoms induced by ASF, moribund and dead infected animals are often found around cool and moist environments, near water bodies (MORELLE ET AL., 2019), in young broad-lived forests or in meadows with significant vegetation (CUKOR, LINDA, VÁCLAVEK, ŠATRÁN, ET AL., 2020; PODGÓRSKI ET AL., 2020).

As for disease control, the quest for cadavers should aim to re-define the borders of the ASF management area, in a view to promptly concentrate management efforts where most needed; therefore, during the epidemic phase, enhanced passive surveillance should be focused inside and around the infected area.

These activities should be sustained during both disease-free and escalation periods (GUBERTI ET AL., 2022): given the effort they require, local authorities should well in advance identify dedicated personnel, develop all the protocols for carcass testing, collection and disposal, and be ready to promptly implement all of the strategies aimed at containing and extinguishing the virus.

Given the implications of manipulating, removing and destroying a possibly infected cadaver (see Carcass handling and disposal), a definition of "suspect case" must be clearly stated; given the ASF situation in Eurasia, however, it is largely considered that this definition should include "*any found carcass out of the context of hunting, including road killed animals and any diseased wild boar shot for sanitary reasons*" (GUBERTI ET AL., 2022; JORI ET AL., 2021). In practice, however, the definition of suspect case is largely dependent on local governments, which must provide the logistics for retrieving and disposing of each suspect case following adequate biosafety protocols (JORI ET AL., 2021).

The reliability of the enhanced passive surveillance system can be measured through the number of dead wild boar reported (GUBERTI ET AL., 2022): as natural mortality in wild boar (excluding roadkill) is approximately 10% of the population (KEULING ET AL., 2013; TOÏGO ET AL., 2008), the report of 10% of those is suggested as a threshold for an efficient passive surveillance (i.e. 1% of the whole estimated wild boar population, GUBERTI ET AL., 2022).

1.3.6. Handling and disposal of cadavers

Once sampling for ASFV had occurred, all cadavers and carcasses coming from the ASF management zone should be transported at a rendering plant using a dedicated truck (e.g., LICOPPE ET AL., 2023).

At the onset of an ASF outbreak, an intense effort should be spent looking for cadavers in the field, which should be tested for ASF in a view to define the infected area (SANTE/7113/2015, REV. 12, 2020). Afterwards, ASF management strongly relies on wild boar depopulation.

Both the retrieval of infected cadavers, and the act of killing a possibly infected animal, pose a high risk of spreading ASFV: to minimize this risk, all dead animals should be regarded as suspect ASF cases until the diagnostic results are available, and therefore treated with strict biosafety measures both during passive surveillance, and during hunting (GUBERTI ET AL., 2022).To this aim, all operators involved in manipulating cadavers should be trained, wear disposable clothing and undergo systematic disinfection at the end of the activities (LICOPPE ET AL., 2023). The use of private cars should be avoided, dedicating trucks to be used exclusively inside the infected area or, whether not possible, to be thoughtfully disinfected after each activity To avoid habitat contamination due to leakage of bodily fluid, ASF sampling should be carried out in a controlled environment, where the dead animals could be stored until diagnostic results are available. To this aim, intermediate collection point(s) between the killing ground/finding spot should be prepared, following biosafety protocols during the transport of dead animals, using plastic or metal tanks to transport the body.

In intermediate collection points, animals should be stored in batches, and released only when the entire batch is tested ASF negative. The equipment used for sampling and dressing should be disinfected after each use, and not leave the dressing facility. Disposal of offal must be regulated, and each hunting ground should be provided with one or more facility dedicated to animal dressing. Such facilities should be placed on permanently dry soil, and should be fenced against scavengers and unauthorized access. Water and a locked-up pit or container for offal and waste must be provided.

If transport of the dead animal is unfeasible, sampling can be provided on site, given that the area is thoughtfully disinfected, and the remains disposed of on site and made unavailable to scavengers. This can be accomplished either by incineration, local burning, single or mass burial (GUBERTI ET AL., 2022) or composting (CARRAU ET AL., 2023).

Given the high mortality of ASF, the means for disposing of a large number of cadavers must be prepared, especially in areas where wild boar population density is high. The logistics of disposal relies on the road network for transporting potentially infected material to the appropriate treatment plant, the availability of intermediate collection point(s), and the possibility of local disposal if rendering is considered unfeasible.

1.4. NATIONAL CONTEXT

1.4.1. Wild boar population

Wild boar had been largely absent from the Alpine territories between the 17th and the first half of the 20th centuries, persisting locally at a metapopulation state in central and southern Italy (APOLLONIO ET AL., 1988); the recolonization of the peninsula begun in the 1920s (Piemonte region: Torino, Cuneo and Imperia provinces) and the 1950s (Friuli-Venezia Giulia region: Udine province), proceeding from bordering States, and was favoured by both climate warming, and the rewilding of marginal habitats abandoned by humans (APOLLONIO ET AL., 1988).

Wild boar population size and distributional range have gradually increased during the second half of the 20th century, favoured by massive restocking of eastern European animals for hunting purposes (APOLLONIO ET AL., 1988; HAUFFE ET AL., 2007; PAOLUCCI & BON, 2022). At the national level, restocking of wild boars has been banned only in 2015, while supplementary feeding is allowed exclusively for controlled culling (LAW N. 221, 2015); nonetheless, although prohibited, supplementary feeding to facilitate hunting is still a common practice.

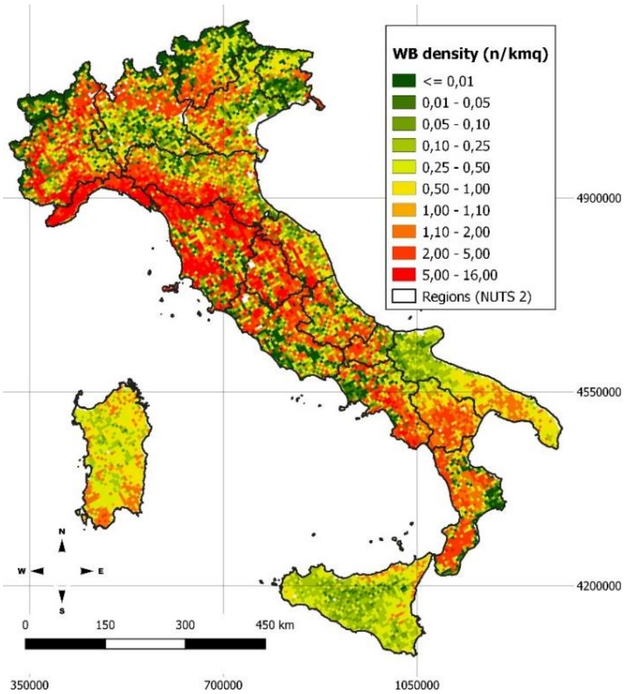


Figure 14: Wild boar population density in Italy (PITTIGLIO ET AL., 2018). EPSG: 32632

Currently, the Italian wild boar post-reproductive population is estimated at a plausible minimum of 1.5 million individuals (ISPRA, 2023, 2021 data); as its presence is reported in all Italian regions (Figure 14) (PAOLUCCI & BON, 2022), this species is the most widespread and abundant ungulate on the National territory. Locally, overabundance is often reported, representing a threat to both natural and agricultural ecosystems. As a consequence, its proliferation is contrasted by most administration. As a game species, the wild boar is hunted according to LAW N. 157, 1992, art. 18, and as a conflictive species is subjected to culling campaigns, which are regulated by means of specific quadrennial plans, as per LAW N. 157, 1992, art. 19. In the latter case, both administrative personnel and private hunters are involved in culling actions. During the period 2015-2021, overall wild boar harvest attributable to hunting and control activities were calculated at 86% (1.8 mln) and 14% (630000), respectively (ISPRA, 2023).

1.4.2. ASF occurrence

During the past century, Italy has been affected by multiple ASF Genotype I outbreaks, mostly linked to anthropogenic introduction. While viral eradication was achieved in all hotspots developed in the continental territory (DANZETTA ET AL., 2020), ASF Genotype I has been considered endemic in the island of Sardegna from 1978, up until very recently (October 25th, 2023). The persistence of the Sardinian hotspot was linked to traditional farming practices, frequently adopting free ranging of domestic pigs (MUR ET AL., 2016), and it has been regarded as the last remnant of ASF Genotype I in Europe.

In continental Italy, the first ASF National Surveillance and Prevention Plan (*Piano di sorveglianza e prevenzione in Italia*, NSPP hereafter) was adopted in 2020, as a consequence of the progressive expansion of the epidemic front in Eastern Europe (Figure 15), and in consideration of the risk represented by anthropogenic viral spread. Although most sectors were subjected to restrictions due to COVID-19 pandemic, the actions prescribed in the NSPP were deemed prerogative (https://resolveveneto.it/wp-content/uploads/2020/02/Nota-MS-su-Piano-sorveglianza-PSA_150420.pdf, in Italian), and lead to the detection of ASF index case through passive surveillance.

The ASF virus was first detected in Italy in a wild boar cadaver found on December 29th, 2021 in Ovada municipality, Piemonte region (northwest Italy, Figure 15). The sample tested positive for ASF on

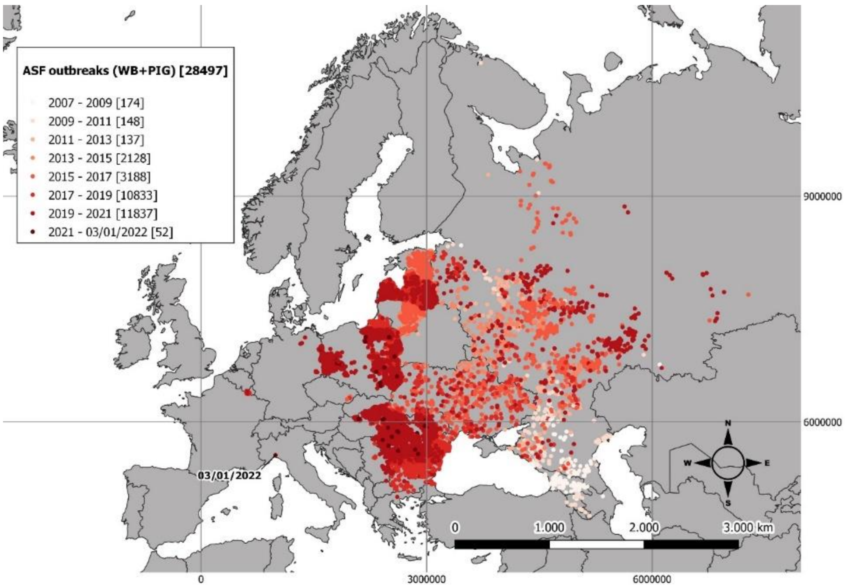
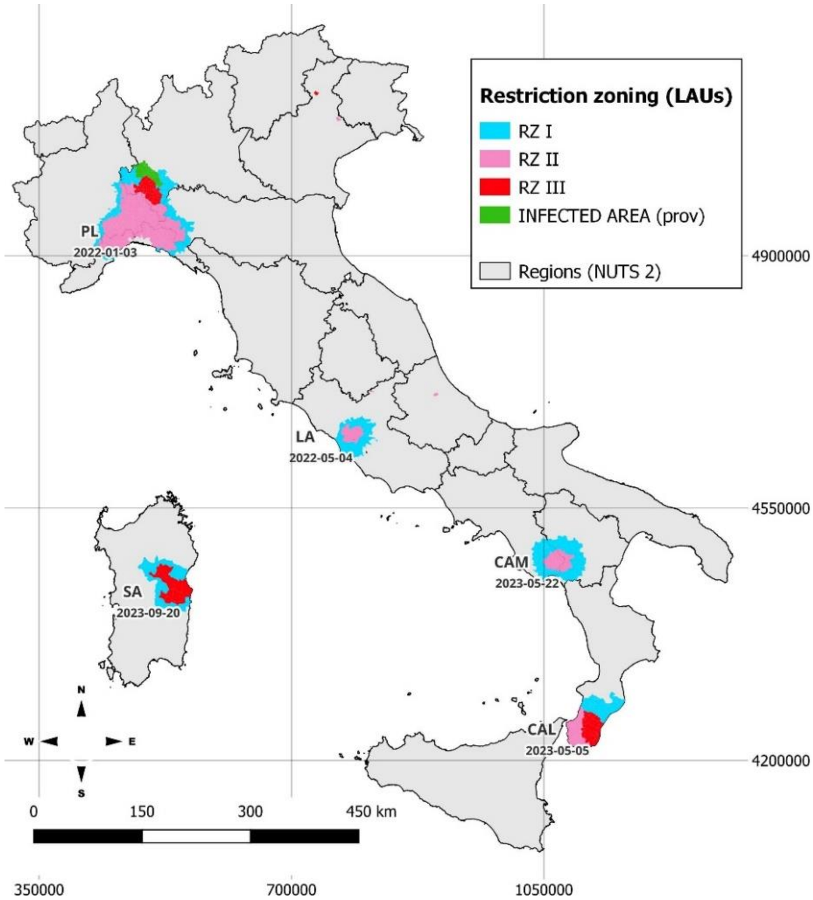


Figure 15: ASF cases (wild boar and domestic pig) in Europe at the times the Italian index case was found (January 3rd, 2022) (overlapping data). EPSG: 3857

January 5th, 2022, confirmed as ASFV Genotype II by the national reference lab two days later, and thus reported to EU Commission and WOAH (ISCARO ET AL., 2022), where the report was dated as January 3rd, 2022. The Italian index case was detected at approximately 800 km from the active epidemic front in Eastern Europe, thus suggesting the anthropogenic source of the introduction (Figure 15). Since then, three additional hotspots developed in central (Lazio region, 4th may, 2022) and Southern Italy (Calabria and Campania regions, April 27th and May 5th, respectively). Moreover, ASF Genotype II has been recently detected in a pig farm in Sardegna, and although the outbreak has been declared resolved in January 2024 (https://wahis.woah.org/%23/in-review/5491), the island could face further ASF developments (DEI GIUDICI ET AL., 2024).

Table 1: Summary of ASF Genotype II cases in Italy based on WOAH reports (Jan 23rd, 2024 data)

Hotspot	Region (NUTS 2)	ASF GEN II INDEX CASE	ASF GEN II LAST REPORT	N ASF CASES (22/01/2024)		
				WILD	DOMESTIC	TOT
PL	Piemonte	05/01/22	10/01/24	566	0	566
	Liguria	07/01/22	12/01/24	648	0	648
	Lombardia	19/06/23	16/01/24	34	19809	19843
	Emilia-Romagna	08/11/23	16/01/24	21	0	21
	TOT (PL)			1269	19809	21078
LA	Lazio	04/05/22	01/08/23	91	2	93
CAM	Campania	22/05/23	03/07/23	26	0	26
CAL	Calabria	27/04/23	14/11/23	17	413	430
SA	Sardegna	19/09/23	19/09/23	3		3
TOT				1406	20224	21630



A brief overlook of the first outbreaks up to January 2024 is summarized in Box 1; currently, four hotspots of ASF Genotype II are identified (Figure 16), tallying up to more than 1400 positive cases in wild boar only (Table 1, Figure 16).

Both the distance from the east-European epidemic front, and the spatial distribution of ASF hotspots on the national territory, indicate that a human-mediated incursion is highly plausible (EUROPEAN COMMISSION, 2022). This hypothesis is further supported by biomolecular data, which allowed to identify two different viral strains for the Piemonte-Liguria and Lazio outbreaks (https://www.izsplv.it/it/notizie/308-peste-suina-africana/1493-peste-suina-africana,-i-focolai-laziale-e-piemotese-ligure-hanno-origine-diversa.html, in Italian).

Figure 16: ASF hotspots and index cases in Italy (Jan 23rd, 2024 data), restriction zones and infected areas. PL = Piemonte-Liguria; LA = Lazio; CAL = Calabria; CAM = Campania; SA = Sardegna. EPSG: 32632

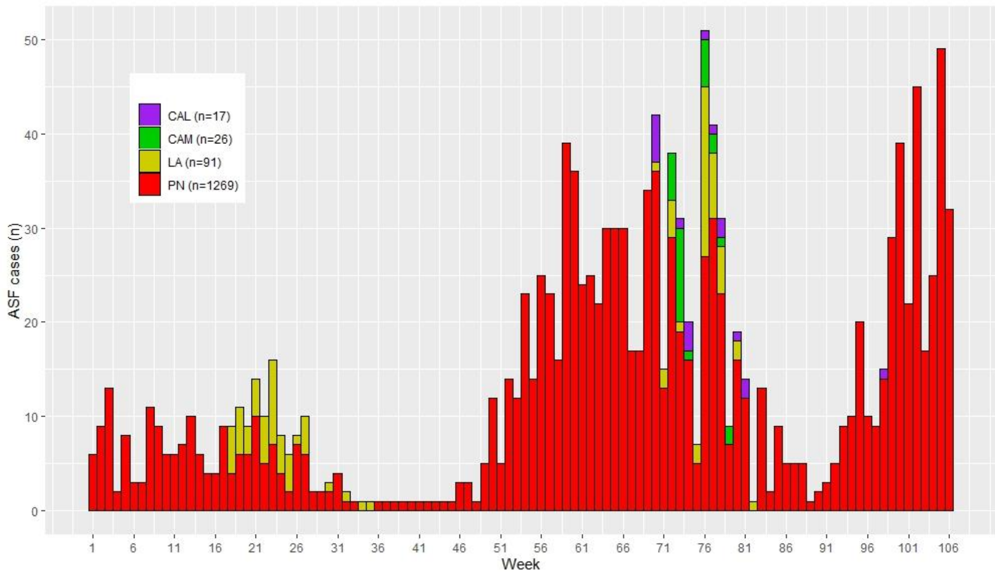


Figure 17: Weekly reports of ASF in wild boar from the ASF hotspots in continental Italy (Jan 22nd, 2024)

First reports of ASF in continental Italy

On January 5th, 2022, a wild boar carcass found during passive surveillance in Ovada (AL, Piedmont region) tested positive to ASF analysis, carried out by the local IZ. Two days later, the results were confirmed by CEREP as ASF Genotype II virus, and thus reported to EU Commission and WOAH. A Local Crisis Unit meeting was held, and the Central Crisis Unit was instituted. As ASF Expert Group identified the infected zone as comprising 63 municipalities, suspect case number two (Isola Del Cantone, GE, Liguria region) and three (Franconalto, AL, Piemonte region) were found.

On January 10th, 2022, cases two and three were confirmed ASF positive. Pending the amendment of 2021/594, Implementing Decision (EU) 2022/28 instituted the infected area as RZ II. The day after, three additional cases were found inside the RZ II (Voltaggio and Tagliolo-Monferrato, AL, Piemonte region).

On January 14th, 2022, Implementing Decision (EU) 2022/62 expanded RZ II to 115 municipalities, and two days later Implementing Regulation (EU) 2022/440 amended 2021/594.

On May 4th, 2022, CEREP confirms ASF in a dying wild boar found in Parco dell'Insugherata (Rome, Lazio region), approximately 450 km from the first outbreak. On June 9th, the virus entered a small free-range pig holding in the outskirt of Rome. No further ASF reports came from the Lazio hotspot since August, 2023.

On June 1st, 2022, as 136 ASF positive cases were already reported, fencing operations began in Ponzone-Voltri territories (GE, Liguria region).

On May, 5th, 2023, an infected wild boar was found in the hinterland of Reggio Calabria (RC, Calabria region), approximately 500 km from the nearest outbreak. The focus expanded in the Aspromonte area, involving also domestic pigs.

On May 22th, 2023, five wild boars were confirmed SAF positive in Cerreta Cognole forest (SA, Campania region), approximately 230 km from the nearest outbreak. EU RZs were instituted on September 19th by Implementing Regulation (EU) 2023/1799.

On June 19th, 2023, the PL hotspot northward expansion reached Lombardia region, where ASF was detected in wild boars. Between August and October, multiple farms in Lombardia were affected by the disease.

On August 27th, 2023, ASF was detected in a pig farm in Zinasco (PV, Lombardia); allegedly, the holding had been infected since early August, having experienced an important increase in mortality (apx. 400 deaths) that was kept from the Authorities; possibly infected animals would therefore have been sent towards multiple butcheries in Lombardia and other regions (Veneto and Emilia-Romagna), thus allowing the virus to spread with the meat trade. An investigation on these events is currently on the way.

On September 20th, 2023, ASF genotype II has been detected in a pig farm in Dorgali (NU, Sardegna region). As no further ASF cases have followed on the Island, the outbreak was declared resolved during January, 2024.

On November 8th, 2023, ASF was found in a wild boar in Ottone (PC, Emilia-Romagna region), following the eastward expansion of the PL hotspot.

Box 1: First ASF reports in continental Italy (January 2024 data)

1.4.3. Legislation and operational hierarchy

In Italy, REGULATION (EU) N. 429, 2016 (“*Animal Health Law*”, AHL hereafter) was implemented by EU DELEGATION LAW N. 53, 2021, Art. 14, prescribing the adoption of further acts to fit AHL in the national legislation; in large part, this is accomplished by, LEGISLATIVE DECREE N. 136, 2022.

With regards to ASF, the central competent Authority as per AHL is the Ministry of Health, through the National Centre for Animal Diseases (*Centro Nazionale di lotta ed emergenza contro le malattie animali*, CLEMA hereafter), while the local competent Authority as per AHL is the Local Health Unit (*Azienda Sanitaria Locale*, ASL hereafter) (LEGISLATIVE DECREE N. 136, 2022).

The strategies and operational hierarchy for intervening in case of epidemic emergencies and outbreaks of Category A diseases (which include ASF) are detailed in the National Contingency Plan (“*Piano Nazionale per le emergenze di tipo epidemico*" – ITAVETPLAN hereafter, ITALIAN MINISTRY OF HEALTH, 2014), which prescribes the institution of three crisis units: at the national level (CLEMA), the central crisis unit (*unità di crisi centrale*, UCC hereafter) coordinates the emergency on the basis of the scientific support given by specific expert groups (*gruppo operativo degli esperti*, GOE hereafter), by the Italian Institute for Environmental Protection and Research (*Istituto Superiore per la Protezione e la Ricerca Ambientale*, ISPRA hereafter) and by the Reference Lab for Pestivirus and Asfivirus diseases (*Centro di Referenza Nazionale per lo studio delle malattie da Pestivirus e da Asfivirus*, CEREP hereafter). After the detection of ASF index case, the CLEMA drafted a National Emergency and Eradication Plan (*Piano nazionale di sorveglianza ed eradicazione*, NEEP hereafter, EUROPEAN COMMISSION, 2022), according to LEGISLATIVE DECREE N. 136, 2022 and AHL, Art. 31. The NEEP includes the norms for ASF surveillance and eradication in infected Regions, as well as the measures to be undertaken in newly infected areas; these measures must be implemented in accordance with ITAVETPLAN and EU zoning strategy (see Zoning), and are further specified in the Operational Manual of Swine Flus (“*Manuale Operativo Pesti Suine”*, MOPS hereafter, ITALIAN MINISTRY OF HEALTH, 2022).

At lower levels of the operational hierarchy, the regional crisis unit (*unità di crisi regionale*, UCR hereafter) coordinate the corresponding ASLs at a regional (NUTS 2) scale, and the local crisis unit (*unità di crisi locale*, UCL hereafter) implements the strategic plans on the territory of each ASL (generally corresponding to a NUTS 3 territorial unit). Once the NEEP had been promulgated, each UCR had to articulate the actions prescribed therein in a specific Regional Plan of Urgent Interventions (*Piano Regionale di Interventi Urgenti*, PRIU hereafter), drafted in cooperation with ISPRA and CEREP. Each PRIU must be adopted by each UCL and enacted locally by each ASL through a specific ASF Territorial Operative Group (*Gruppo Operativo Territoriale*, GOT hereafter), which coordinate the UCL (ORDER N. 4, 2023). PRIUs are exempted from SEA and Natura 2000 EIA procedures (LAW N. 29, 2022).

Since 2022, a ASF Commissioner is instituted (LAW N. 29, 2022) to coordinate the strategy against ASF at national level, to approve the PRIUs in conjunction with the UCC, and to draft the eradication plans for ASF-infected regions; moreover, it coordinates the UCRs and the network of administrations responsible for implementing these actions. In the context of Italian jurisdiction, the Commissioner exerts derogative power through targeted *extra ordinem* acts (“Orders”).

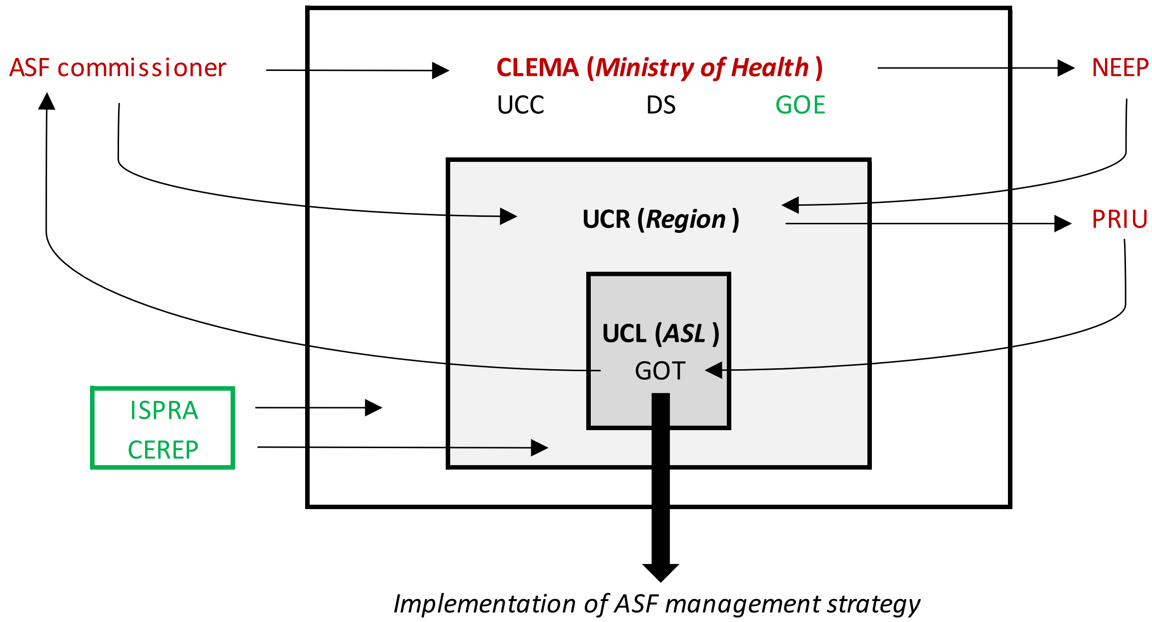


Figure 18: Schematization of ASF operational hierarchy in Italy. Sources of law are highlighted in red, while sources of scientific support are highlighted in green.

Following the NEEP, diagnosis for ASF must be carried on every wild boar found dead, and on every ASF suspect case. The definition of "suspect case" is not clearly stated in any piece of legislation, but it can de described in general terms as *"an animal showing ASF-like symptomatology, or a carcass found in the context of an overall enhanced mortality"* (e.g., NSPP, ORDER N. 5, 2023). However, what cases should be treated as "suspects" depends by the zoning status of the finding location: in Infected Areas, RZ II and RZ III, the aforementioned definition applies, while in buffer zones and RZ I, *"every suid cadaver showing symptoms ascribable to ASF"* must be regarded as suspect (ORDER N. 5, 2023). Finally, in ASF-free regions, DELEGATED REGULATION (EU) N. 689, 2019 definition of "suspect case" is adopted (ORDER N. 5, 2023); this includes animals or group of animals when:

a) *clinical, post-mortem or laboratory examinations conclude that clinical sign(s), post-mortem lesion(s) or histological findings are indicative of that disease;*
b) *result(s) from a diagnostic method are indicating the likely presence of the disease in a sample from an animal or from a group of animals; or*
c) *an epidemiological link with a confirmed case has been established.*

The test for ASF is carried out by RT-PCR on (preferably) spleen, kidney, lymph nodes or, in case of advanced decomposition, long bone tissue. Sampling from the dead animal is carried out by ASL, eventually supported by trained Personnel (Figure 19). If a finding is considered an ASF suspect case, ASL must immediately report it as such on SIMAN, while ASF test will be carried out at CEREP. For non-suspect cases, ASF test can be carried out by the local Zoophrophilactic Institute (*Istituto Zooprofilattico*, IZ hereafter): in case of a positive result, a second test must be provided, carried out either by the local IZ (infected regions) or by CEREP (ASF-naïve regions). CEREP must communicate the results of the test to the Ministry of Health as soon as they are available (MOPS) (Figure 20).

Figure 19: spleen identification for ASF sampling on domestic pig (above) and wild boar (below). Courtesy of IZSVe

Data regarding local outbreaks, collected by ASL, are channelled towards the EU Animal Disease Information System (ADIS, Regulation (EU) n. 429, 2016) through the National Animal Disease Information System ("*Sistema Informativo Malattie Animali Nazionale*", SIMAN), hosted on Veterinary Information System (VETINFO) portal. SIMAN notifications comply for both EU disease surveillance, and WOAH requirements for disease-free status declaration.

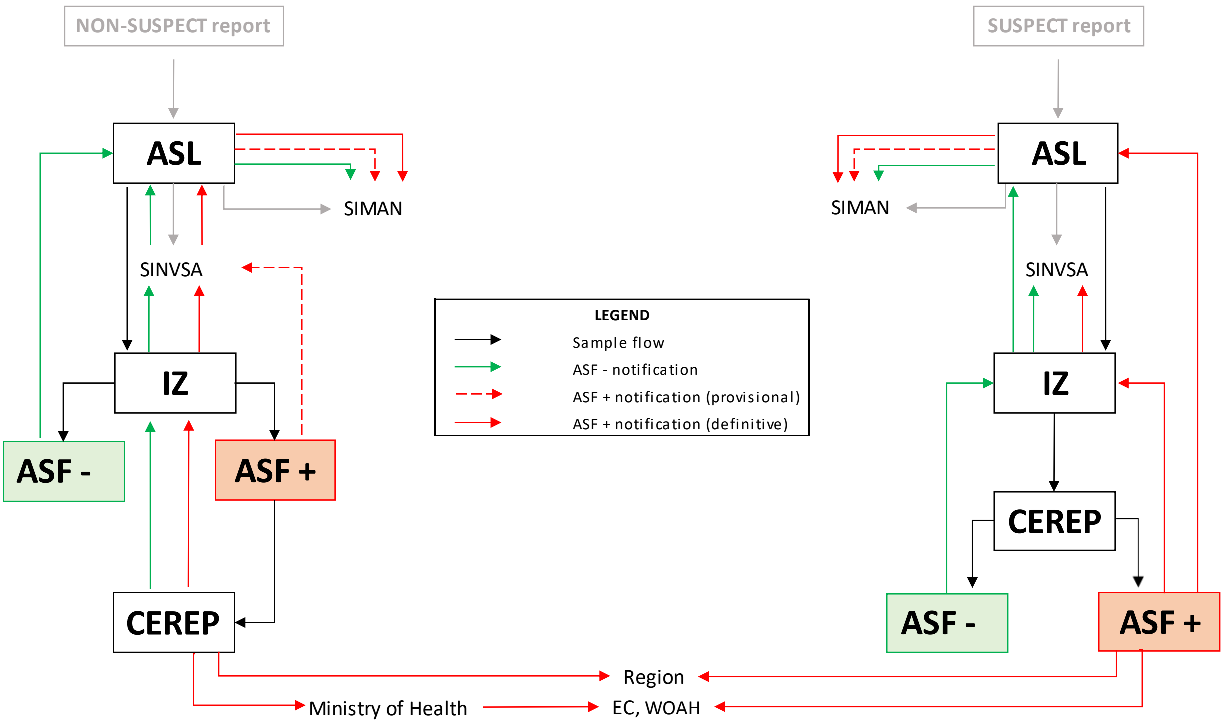


Figure 20: Notification flow for non-suspect (left) and suspect ASF cases (right)

The results of surveillance activities are published on VETINFO portal through the National Veterinary Information System (*Sistema Informativo Veterinario per la Sicurezza Alimentare*, SINVSA hereafter) Both SIMAN and SINVSA are accessible to restricted accounts only.

1.4.4. ASF management strategy

In case of an ASF outbreak, the infected area must be identified, delimited, and signalized. Pending the institution of EU restriction zones (IMPLEMENTING REGULATION (EU) N. 594, 2023), a preliminary infected area is defined by the area of active circulation (i.e., the minimum convex polygon around outbreaks, plus a 6 km buffer to account for wild boars movement pattern), plus a "high risk area" (MOPS), whose size should at least equal to that of the area of active circulation (GUBERTI ET AL., 2022). Around that, an area of improved passive surveillance ("surveillance area") should be identified (MOPS). Ecological barriers for fencing the RZs (see Fencing) must be identified and reinforced.

After EU zoning is instituted, ASF management activities must be undertaken accordingly; inside the infected area (RZ II and RZ III), specific activities must be carried on to improve disease surveillance, to thin the wild boar population, and to eradicate the virus, as detailed in Annex 3.

Once ASF virus has been eradicated, a proposal for scaling back the RZs can be advanced by the Ministry of Health to EU Commission, according to the outcomes of surveillance activities.

ASF surveillance is carried out by testing dead animals, which can be either hunted or culled (*active surveillance*), found opportunistically (*passive surveillance*) or actively searched for (*enhanced passive surveillance*) (see Surveillance). A summary of the main outcomes of these activities for Italian ASF hotspots (February 07th, 2024 data) is outlined in **Errore. L'origine riferimento non è stata trovata.**, while a comparison of the performances in terms of sample quality (i.e., proportion of samples yielding a reliable ASF diagnostic), and detection of ASF infected animals (i.e., ASF sample prevalence), is provided in Figure 21.

Table 2: Number of animals retrieved and tested for ASF through passive surveillance in Italian ASF hotspot (excluding Sardegna). CAM= Campania; CAL = Calabria; LA = Lazio; PL = Piemonte-Liguria

	CAM			CAL		LA			PL		
	AS	PS	EPS	AS	PS	AS	PS	EPS	AS	PS	EPS
ASF -	1041	422	6	1353	67	1561	648	9	10361	2799	47
ASF +	0	27	0	0	17	12	79	0	286	989	106
ND	0	0	0	0	0	0	0	7	10	57	11
TOT	***1041***	***449***	***6***	***1353***	***84***	***1573***	***727***	***16***	***10657***	***3845***	***164***

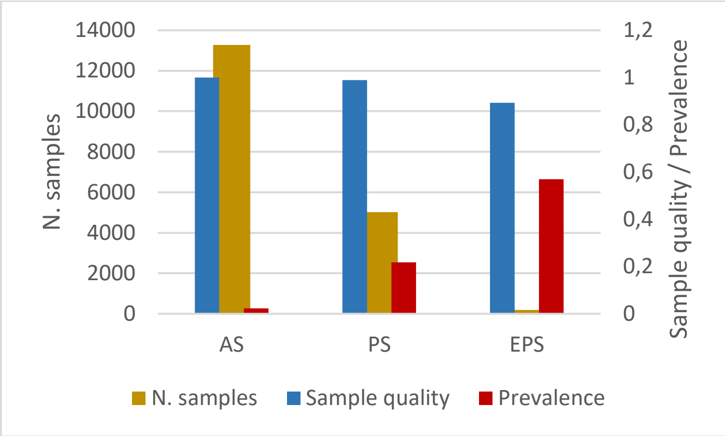


Figure 21: Aggregate performances of ASF surveillance strategies in Campania (CAM), Lazio (LA) and Piemonte-Liguria (PL) infected areas. Sample quality and sample prevalence are represented on the secondary axis.

The destiny of wild boar cadavers (either hunted or found dead) is also bounded to ASF zoning (Figure 22); namely:

- Inside the infected area or the RZ II, they constitute a Category 1 animal by-product (Regulation (EU) n. 1069, 2009). By way of derogation, ASF negative animals and animal parts derived from control activities can be commercialized outside the infected area and RZ II, only after risk-mitigating treatments as per Delegated Regulation (EU) n. 687, 2020, Annex VII are provided. Otherwise, wild boar cadavers are destined to destruction.
- Inside RZ I, they constitute a Category 3 animal by-product, as per Regulation (EU) n. 1069, 2009. By way of derogation. ASF negative animals and animal parts derived from hunting and control activities can be destined to self-consumption, only inside RZI; otherwise, commercialization outside RZI, by way of derogation, follows the same prescription as in RZII.

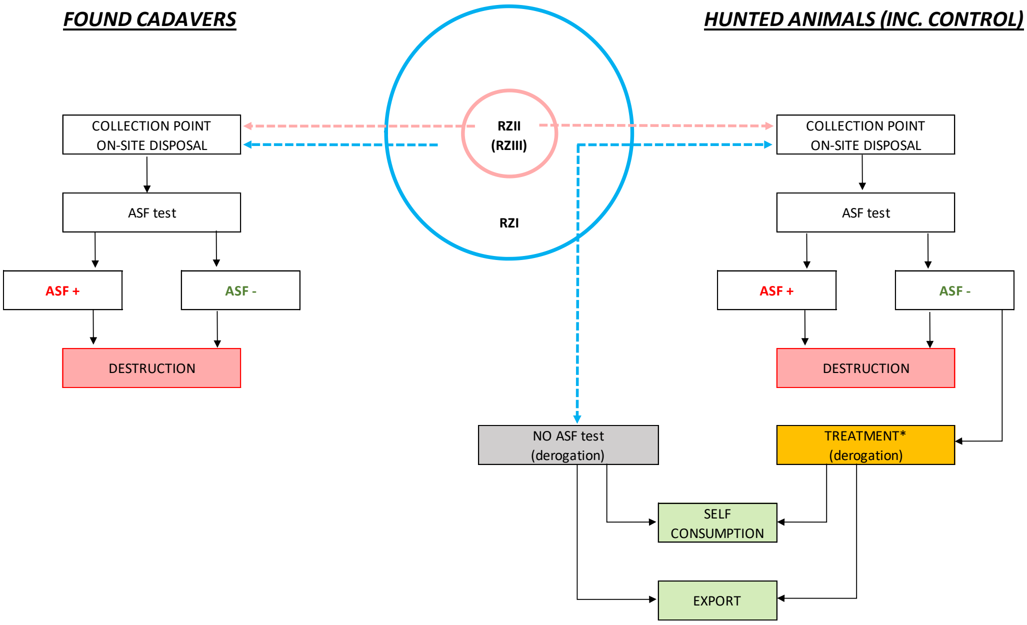


Figure 22: Destiny of WB cadavers found in the field (left) and carcasses derived by hunting and control activities (right) in Italy

As a measure to preventively slow down viral spread by direct contact amongst animals, a specific national plan for 2023 - 2028 (https://www.salute.gov.it/portale/documentazione/p6_2_2_1.jsp?lingua=italiano&id=3357, in Italian) encourage population thinning by setting the harvest quotas that Regions should reach, prescribing for each a severe increase of harvest by hunting (+ 116 ± 126%), and particularly by control (+ 468 ± 613%).

During peace time, passive surveillance should allow to detect suspect ASF cases. Nonetheless, as pointed out by JORI ET AL., 2021, the definition of "suspect case" is often lacking or unclear: to their point, in Italian legislation and guidelines, this concept is not well defined, being associated to diseased/found dead animals whose clinical-pathological picture is ascribable to ASF (i.e., by the intervening ASL operator), either in association with a context of "increased mortality", or not. As an example, ORDER N. 5, 2023 binds suspect cases to an increment of mortality inside the infected area (RZs II and III), but not in RZ I, while regional guidelines often associate this definition to spatio-temporally related" finding of more than two wild boar cadavers (e.g., https://salute.regione.emilia-romagna.it/normativa-e-documentazione/materiale-informativo/archivio/manuali/fauna_selvatica_2013.pdf, in Italian). This particular case not only is likely to add uncertainty to the operator's assignment, but also strikes as an epidemiological nonsense: in fact, inside the infected area, both an increased mortality amongst wild boar, and a higher frequency of ASF positive samples, are expected; conversely, in RZI, an increase in mortality should indeed be linked to a spread from the neighboring ASF infected area, given that natural mortality is expected to remain stable.

Sampling of dead animals (both carcasses, and cadavers) must be carried out in intermediate collection centres (ICCs hereafter) between the finding location, and the rendering plant. The transportation of the cadaver from the finding/culling location to the ICC must be carried out in strict biosafety, using solid trays and dedicated vehicles, and eventually a winch. Burial is allowed *in extrema ratio*, as per REGULATION (EU) N. 1069, 2009, Art. 19.

The characteristics of intermediate collection points are included in ORDER N. 2, 2023; in particular, they must include:

- Detergent and disinfectants.
- Access to clean water and electricity.
- A refrigerator or a freezer; alternatively, a sealed container if carcasses are disposed of before 48 hours.
- Sampling tools.
- Fencing to preclude access to animals and unauthorized persons.
- A cleansing area for tools and clothing.
- Disinfections barriers at entry point (e.g., pools of disinfectant).

The intermediate collection point can be emptied, only after ASF test results for all carcasses stocked therein is available. In case one carcass is ASF positive, all the stock must be considered infective. At that point, the use of the intermediate point is suspended, and the carcasses must be disposed of by ASL. All contaminated material, including the cadaver and the finding spot ("deathbed"), must be disinfected using agents listed in the MOPS; these include:

- Potassium peroxymonosulfate + malic acid + sulfamic acid + Sodium dodecylbenzenesulfonate + Sodium hexametaphosphate 1% (Virkon S)
- Sodium hydroxide 2%
- Sodium carbonate 40%
- 2-Phenylphenol 1% (Environ D)
- 2-Phenylphenol 5% (Lysol)

2. AIM OF THE STUDY

African Swine Fever (ASF) is a potentially panzootic viral pig disease, which has recently affected the territory of continental Italy with multiple hotspots. In Europe, the virus spreads mainly through transport of contaminated meat, then surviving locally in the wild boar population and in contaminated environments.

Introducing ASF translates into harsh economic and social impacts in affected regions, which must adopt severe countermeasures to eradicate the disease from both the domestic and the wild suid population, and to minimize the risk of viral spread. Accordingly, EU Regulation denote ASF as "*a disease for which there is a need for surveillance within the Union*", and "*a disease that does not normally occur in the Union and for which immediate eradication measures must be taken as soon as it is detected*".

In Italy, the competent Authority for the enactment of the ASF management strategy is the Local Health Unit (ASL), whose territory usually correspond to one EU NUTS 3 level. This is the case of the study area considered in the present work, which comprehends the ASF-free territory of Pordenone EDR (former Pordenone province).

In a nutshell, the study described herein aims at:

- building/increasing the ASF surveillance system.
- assessing the preparedness to a possible ASF introduction.
- supporting the ASF management capacities.

Epidemiological surveillance for ASF is based on the active search for wild boar cadavers (*enhanced passive surveillance*, EPS hereafter), an activity that should be undertaken in disease-free areas in order to detect the virus as early as possible. In this work, the definition of a set of transects for EPS is described, including a summary of the activities carried out during the last year, discussing the outcomes and highlighting possible weak spots of the strategy adopted so far.

In case an ASF outbreak is detected, a timely implementation of an integrated strategy for the quest, retrieval and disposal of possibly infected wild boar cadavers is crucial for disease management. This strategy pivots on sampling, transporting and disposing of these cadavers, relying on an infrastructural network that must be scaled to the number of dead animals it will have to cope with. Unfortunately, the likely outcomes of an eventual ASF outbreak are seldom predictable, leading to delaying decision making.

In the present study, a method for obtaining an indicative estimate of the expected wild boar mortality is presented, based on routinely collected census data, and on the outcomes of the successful ASF management experience carried out in Belgium. These bases allow to depict a likely scenarios of an eventual ASF epidemic, allowing to forward some hypotheses regarding the spatial distribution of the infrastructures needed for the management system.

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3. Study area

3.1. Environmental context

The study area (EPSG32633: 320872E, 5108678N) is the territory of the Regional Decentralization Institution (*Ente di Decentramento Regionale*, former Province, EDR hereafter) of Pordenone, a 2273 km² NUTS 3 (Regulation (EC) No 1059, 2003) territorial unit located in Friuli-Venezia Giulia region (FVG), in northeaster Italy. Geographically, two macro areas can be distinguished: a northern montane area, and a southern plain; in the context of the EU biogeographic regionalization (EEA, 2023), these two areas correspond to the "Alpine" and the "Continental" biogeographic region, respectively (Figure 23).

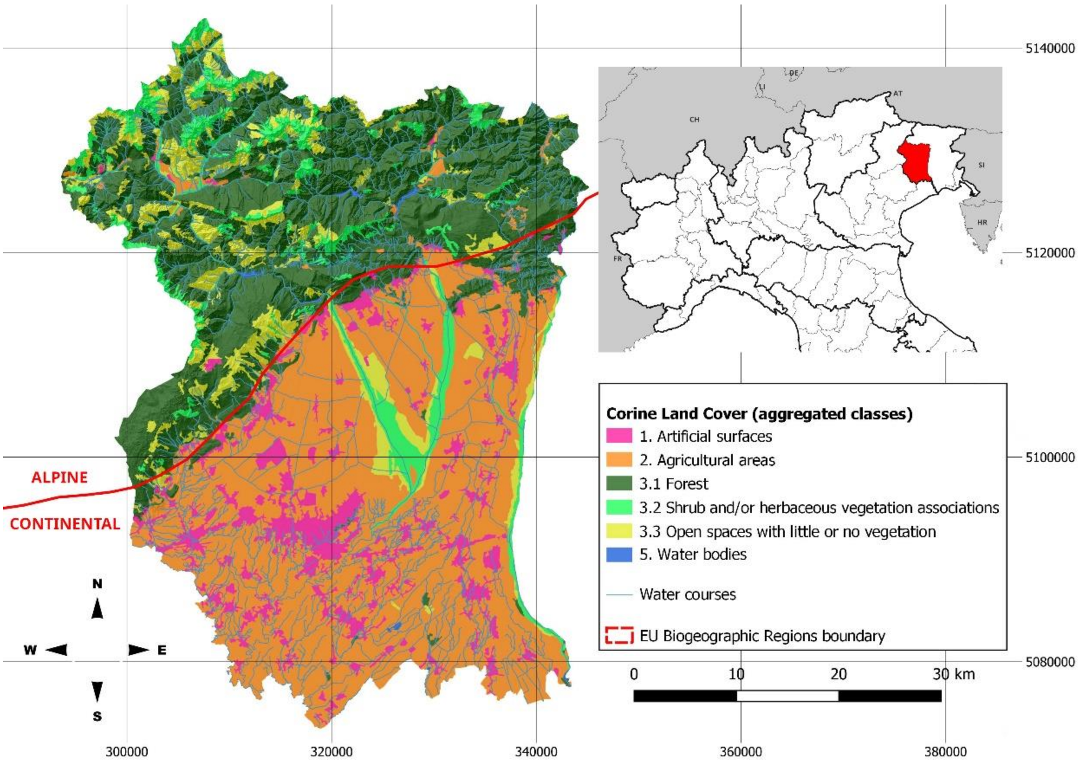

Figure 23: Pordenone EDR: Corine Land cover aggregated classification and water courses. Hillshade based on TINITALY DTM is used as a base layer. EPSG: 32633

The northern Alpine part spans over 992 km² (apx. 44% of the overall territory of the study area); it is characterized by a median altitude of 992 (IQR = 680) m a.s.l., and median terrain ruggedness index (TRI hereafter, Riley et al., 1999) of 16.82 (IQR=12.8). Land cover is mostly represented by forests and seminatural areas (96%), out of which 351 km² (37%) of broad-leaved forest and 313 km² (33%) of mixed forests. Approximately 25 km² are dedicated to agriculture, most of which (14 km²) interspersed with significant natural or semi-natural areas (CLC class 2.4.3).

The southern Continental portion of the study area spans over 1281 km² of mostly plain territory, with a median altitude of 56 (IQR=133.2) m a.s.l, and median TRI of 0.36 (IQR = 0.4). 72% of the land

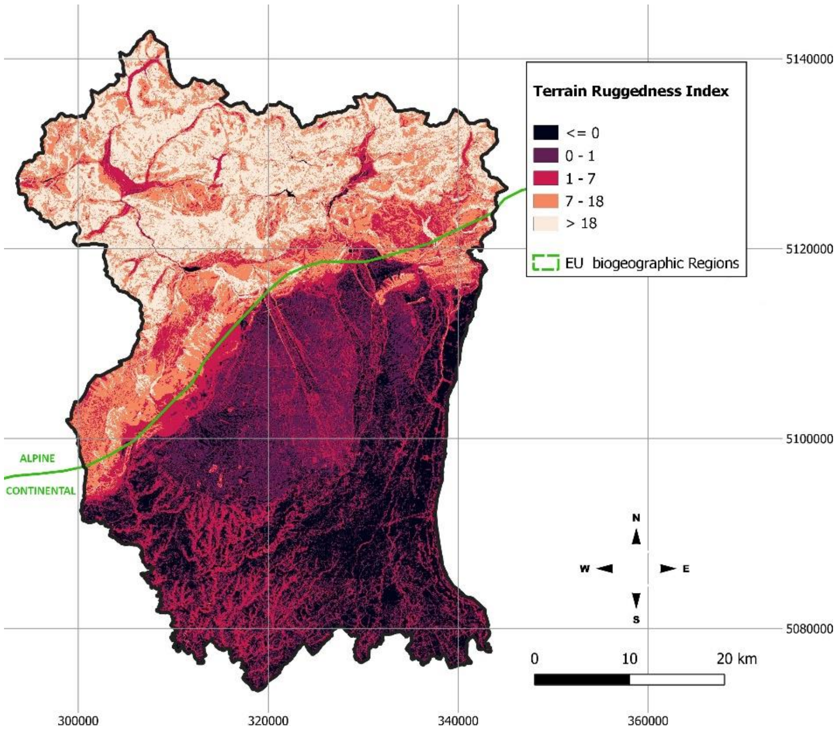

Figure 24: Terrain ruggedness index (TRI) and EU biogeographic regions in Pordenone EDR. EPSG: 32633

(916 km^2) is used for agricultural purposes, mainly non-irrigated crops (CLC class 2.1.1, 64%). Natural vegetation is found almost exclusively in marginal areas: forests are limited to the foothills, spanning over a 78 km^2 (6%) belt, while sparse shrub vegetation and non-vegetated natural areas occupy the riverbanks of rivers Cellina and Meduna, which converge halfway down the plains. 15% of the territory (187 km^2) is occupied by artificial surfaces, which consists mostly of discontinuous urban fabric (CLC class 1.1.2).

3.2. MANAGEMENT OF WILD BOAR

With regard to wildlife management, Pordenone EDR is subdivided in 56 hunting reserves (generally matching municipality boundaries, corresponding to Local Administrative Units as per REGULATION (EC) NO 1059, 2003), representing the territorial management units; these are grouped into four hunting districts (Figure 25, Figure 26). The local competent Authority for environmental and forestry policing is the Pordenone forestry inspectorate (*Ispettorato forestale di Pordenone*) of the Regional Forestry Corp, which is organized in six forest Stations, attending to a corresponding portion of the territory.

Concerning wild boar specifically, Pordenone EDR is subdivided in a "hunting area", and an "eradication area" (Figure 26), which are characterized by different management policies. In the hunting area, where wild boar is most abundant, wild boar management is directed towards the conservation of a manageable, healthy population, based on yearly census data. Hunting is implemented through the "selective" technique, which includes standing hunts (*posta*) and hunting with a scent hound (*girata*), and is carried out from May 15th to January 15th, eventually extended to April 1st (REGIONAL LAW N. 14, 1987). The hunting area includes the "Prealpi Carniche" and "Pedemontana pordenonese" hunting districts, occupying the mountains and foothills in the northern part of the study area.

In the eradication area, management aims at the eradication of residual, sporadic nuclei, whose size is also estimated yearly. Hunting is enacted using the "traditional" technique, consisting of driven hunts (*battuta*) which usually involve less than ten hunters, and is carried out from September 1st, to

December 31st. The eradication area consists of the "Alta Pianura pordenonese" and "Bassa Pianura pordenonese" hunting districts, located in the southern plains.

Population abundance, age structure and sex ratio are assessed through yearly censuses. Censuses are carried out by hunters during late winter through vantage point counts, which are performed simultaneously for all hunting reserves in each hunting district. The census data is then used to assign hunting quotas to each reserve, aiming at pursuing management goals. At the end of each season, this data is published on the regional website (https://www.regione.fvg.it/rafvg/cms/RAFVG/ambiente-territorio/tutela-ambiente-gestione-risorse-naturali/gestione-venatoria/FOGLIA9/).

In 2022-23, census data included 820 individuals (0.48±0.71 ind/km^2), corresponding approximately to 16% of the overall FVG census population (Figure 26).

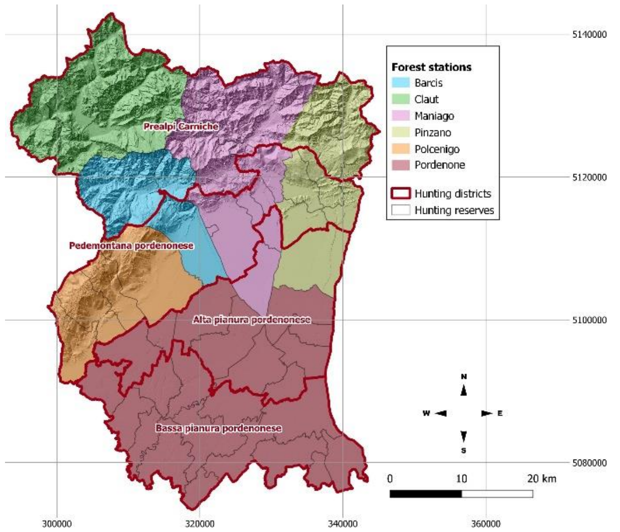

Figure 25: Hunting districts and forest stations. Hillshade based on TINITALY DTM is used as a base layer. EPSG: 32633

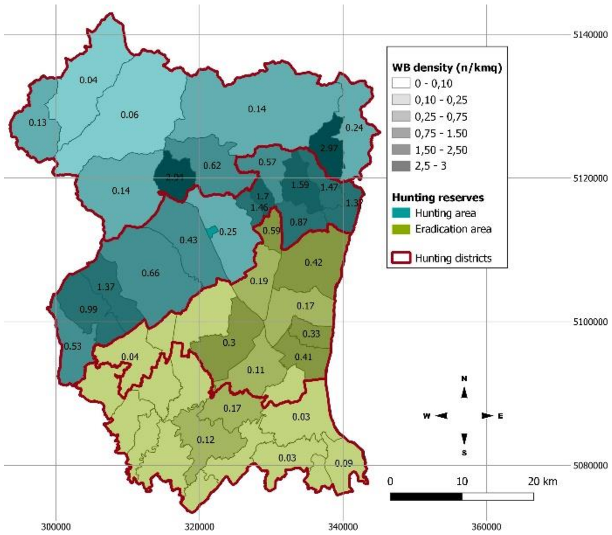

Figure 26: Hunting districts, hunting reserves and census data for 2022-23. Null values are not shown. EPSG: 32633

Wild boar hunting data for Pordenone EDR (2000-23) is represented in Figure 27. On average, 87±32% of the census population is assigned to harvest, out of which 51±14% is effectively harvested, leading to an average harvest offtake of 44±18%. Over the past twenty years, the wild boar population remained practically stable (β=-0.0002; R^2=0.02). According to census (2020-23 data), the population structure is based on a majority of juveniles, and a sex ratio slightly tilted towards males (J=0.59±0.02; M=0.18±0.02; F=0.23±0.03).

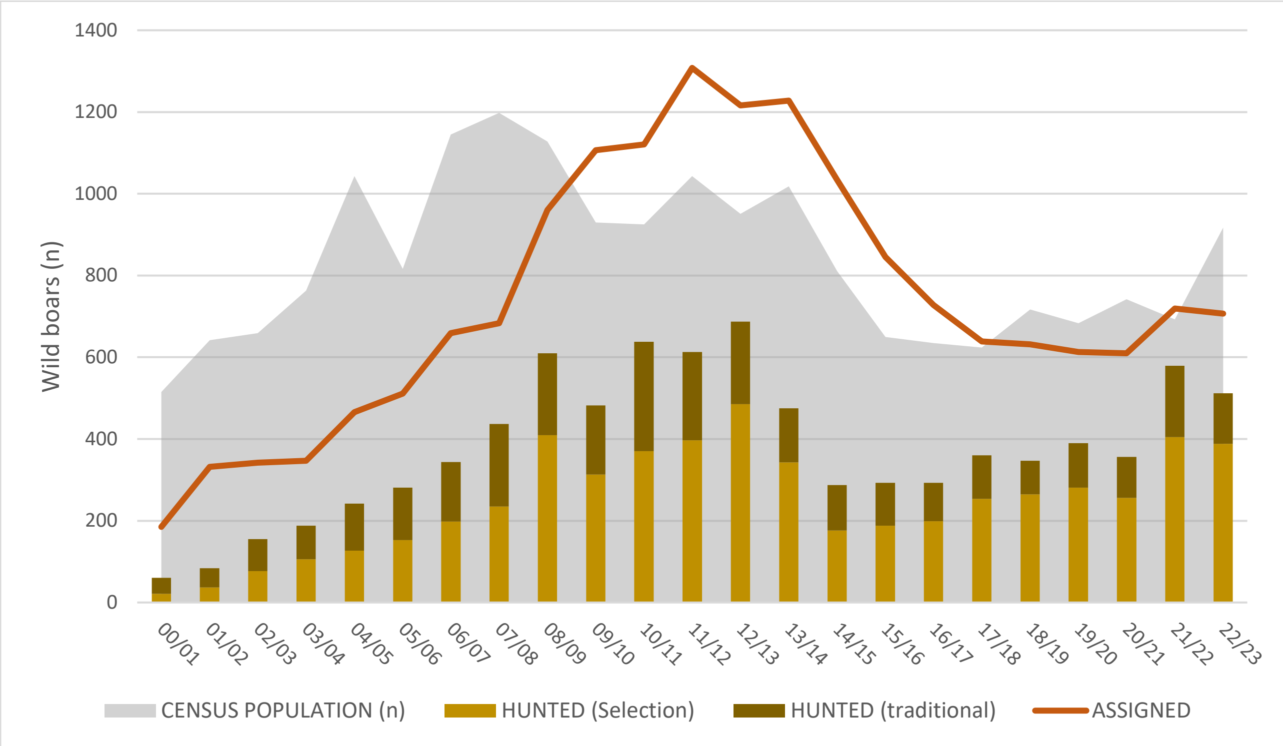

Figure 27: representation of Pordenone EDR overall hunting data (2000-2023).

Hunted animals are kept by hunters, either for auto consumption, for direct alienation or commercialization. In the latter case, the hunter must forward the carcass to a registered Game Handling Establishment (*centri di lavorazione selvaggina*), for veterinary control and further slaughtering phases, accordingly with REGULATION (EC) NO 853, 2004; beforehand, carcasses can eventually be gathered in collection centres (*centri di raccolta*), from one to five days accordingly to the equipment (REGIONAL DELIBERATION N. 943, 2021).

In addition to hunting, control measures against conflictive species (LAW N. 157, 1992; REGIONAL LAW N. 14, 2007) are applied, following specific quadrennial plans. Culling is carried out either by the Region Forestry Corp, or private trained operators ("*selecontrollori*" or "*bioregolatori*") all year round. In the case of carcasses arising from control measures, culled animals can either be destroyed, or destined to self-consumption by the shooter (up to three animals/person/year).

Animals found dead for any reason cannot enter the meat market, while commercialization is allowed for those that are culled (e.g. following a car crash)for which an ante-mortem examination, followed by a post-mortem exam at a CLS, is provided by trained personnel, as per REGULATION (EC) NO 853, 2004.

3.3. ASF PREPAREDNESS – CURRENT STATUS IN THE STUDY AREA

As no ASF case was detected in FVG region, nor in adjacent municipalities, FVG constitutes a minimum alert area (EUROPEAN COMMISSION, 2022; REGIONAL DELIBERATION N. 957, 2022). The ASF regional crisis unit (UCR) was instituted, and the Regional Plan of Urgent Interventions (*Piano Regionale di Interventi Urgenti*, PRIU hereafter) adopted, , on July 1st, 2022 (REGIONAL DELIBERATION N. 957, 2022).

In Pordenone EDR, the ASF local competent Authority is the Health Unit of Western Friuli (*Azienda Sanitaria del Friuli Occidentale*, ASFO hereafter).

Passive surveillance activities started in 2022. These include the geo-referencing, sampling and ASF testing of any wild boar which is either roadkill, found dead, or euthanized. Citizens can report wild boar cadavers either through the National Emergency Number (112), or directly to wildlife rescue agencies (https://www.regione.fvg.it/rafvg/cms/RAFVG/economia-imprese/agricoltura-foreste/foreste/FOGLIA100/). To that purpose, a regional toll-free number is available since 2019. To encourage citizen participation, a 10€ reward is offered for any case report (20€ if a sample is conferred), as suggested in SANTE/2017/10186, REV. 4, 2020.

Passive surveillance also includes the active search of carcasses at monthly cadence (see Enhanced passive surveillance), which should be carried out by the FVG Regional Forestry Corp.

FVG ASF strategy highly relies on wild boar management, prescribing a general thinning of the population: as an increase of hunting effort is considered unattainable, this purpose will be pursued by shifting hunting towards 0-12 month animals (60%) and adult females (65% of culled adults), as indicated in national guidelines (https://www.izsum.it/index.php?id_sezione=171152); this would induce a 3% increase of hunting harvest of adult females. Also, the intervention strategy includes the withdrawal from existing culling limitations: these include shifting the end of the selective hunting season from September 1st to December 31st, allowing to hunt more than 150% of the census population inside the hunting area, and allowing to harvest adult females with cubs inside the eradication area. Furthermore, night hunting using visors is allowed.

Furthermore, the PRIU plans to increase in the number of operators dedicated to population control, by incentivizing hunters to partake in this activity; this will be accomplished by allowing culled animals to enter the meat market, which was previously restricted to hunted animals, and by allowing hunters to keep more than the current maximum of two preys/hunter/year.

Conversely, national guidelines for 2023-28 (https://www.salute.gov.it/portale/documentazione/p6_2_2_1.jsp?lingua=italiano&id=3357), which were published after the latest FVG PRIU, prescribe for the region a more severe increase in wild boar harvest; in particular, yearly quota should increase of 59.2% for hunting, and 828.8% for control culling relative to the mean harvest of 2021-23 hunting seasons, reaching an overall expected harvest of 9100 wild boars per year (which is approximately 180% of the census population).

As the study area is currently ASF free, the definition of “suspect case” is only applied in presence of lesions or symptoms ascribable to ASF, and/or presence of two or more carcasses, and/or otherwise suspect-inducing conditions.

If the case is confirmed as non-suspect by ASL (or ASL designated) personnel, ASF sampling is carried on-site follows routinary biosecurity measures; the sampling material include:

- Dispensable gloves (nitrile/latex)
- Dispensable overshoes
- Airtight jar (primary sample container)
- UN3373 95KPa bags (secondary container)
- Biocide (sodium hypochlorite, Quaternary ammonium cation or similar)

- Waste plastic bag

The cadaver must then be reasonably hidden from people and scavenging animals until ASF test results are in; in case of a positive diagnosis, ASL operators must return on site, to dispose of the cadaver accordingly.

If the case is considered suspect, the carcass must be handled only with the supervision of ASL, which will provide sampling preferably at the collection point; the operators must then proceed as follows:

- Reach the target on an authorized vehicle; at the moment, one pick-up truck is available
- Wear the Personal protective equipment (PPE) before approaching the carcass
- Spread the carcass with disinfectant, as indicated in the MOPS
- Insert the carcass in a first bag (primary bag)
- Spread the bag with disinfectant
- Insert the primary bag containing the carcass in a secondary bag
- Load the bagged carcass on the authorized vehicle
- Remove the PPE and insert them in a bag, to be closed, disinfected and treated as 180202 EWC waste (Absolute Hazardous).
- Georeference the location
- Fill up the SINVSA sampling sheet, assigning a unique ID to the carcass
- Associate the carcass to the SINVSA sheet (i.e. writing the ID number on the bag)
- Bring the carcass to the rendering site/collection point
- Wash and disinfect the vehicle

As for the PRIU, the candidate endpoint of the cadavers retrieved in field activities from all FVG Region was identified in the rendering plant of Morsano al Tagliamento (SALGAIM ECOLOGIC S.p.A.); there, butchery waste is stabilized, and animal by-products (Regulation (EU) n. 1069, 2009) are sold as protein flours destined to incineration, and animal oils. As for a non-technical report published in 2014 (https://www.regione.fvg.it/rafvg/export/sites/default/RAFVG/ambiente-territorio/valutazione-ambientale-autorizzazioni-contributi/FOGLIA3/DITTE/allegati/PN-AIA-42R_sintesi.pdf), the plant treated 15000 tons of animal waste (corresponding, for example, to 300000 50kg cadavers per year, i.e. 822 cadavers per day), running at approximately 45% of its capacity. Given that the entire wild boar population in FVG is estimated at approximately 5400 animals, for the purposes of the present work the proposed plant was assumed to be compliant for the tasks required to it. Intermediate collection points have not been identified yet.

Sampling on the carcass is done by ASL at the rendering site or collection point; if the carcass can't be moved, sampling is conducted on site under ASL supervision, followed by burial or incineration on site; if tools have to be used for carcass removal (e.g. a winch), these have to be accurately disinfected afterwards.

4. MATERIALS AND METHODS

4.1. ENHANCED PASSIVE SURVEILLANCE

To maximize the likelihood of finding wild boar cadavers, the areas on which to identify the transects for enhanced passive surveillance were determined based the distribution of georeferenced salmonellosis (*Salmonella enterica* serovar *choleraesuis*, var. Kunzendorf) cases in wild boar (LONGO ET AL., 2019) (n=49, February 7th, 2012 - June 22nd, 2015 data). This choice was due to the symptomatology of this particular disease, which highly resembles that of ASF (notably, high fever), thus likely pushing infected animals to find relief in similar environments.

To highlight candidate areas, 3 km kernel density of Salmonellosis cases was calculated and plotted using QGIS (QGIS DEVELOPMENT TEAM, 2022). The transects were finally identified by operators of Pordenone Forestry Corp on their corresponding territory (forest stations), based on their prior knowledge of the territory and expected effort.

Operators were asked to identify candidate transects preferably alongside shallow water courses. This choice was based on recent literature, suggesting that these environments represent preferential deathbeds for sick animals (MORELLE ET AL., 2019).

Enhanced passive surveillance activities begun in April 2022, surveying each transect every two weeks; operators were asked to report and georeferenced all wild boar cadavers, to report any sign of presence of the species encountered during the activity, and note the time each survey started and ended, using a specific field note (Annex 2).

The outcomes of the surveys up to September 2023 are reported; these include the length of the tracks, the number of surveys carried out, the time required by each survey and the sampling effort (**SE**). The latter was calculated (for each transect and aggregated by forest station), as the product of the time required by the surveys and number of operators involved in the activities (Equation 5). Missing reports of sampling effort were replaced by the average value for the corresponding transect.

$$SE = time * N\ operators$$ *Equation 5*

The efficiency of the system (*sensu* PORTA, 2014: "*The effects or end results achieved in relation to the effort expended in terms of money, resources, and time*") was evaluated by dividing the number of cadavers found, by the sampling effort (Equation 6)

$$Efficiency = \frac{N\ found}{SE}$$ *Equation 6*

To evaluate the performances of the enhanced passive surveillance programme, its effectiveness (*sensu* PORTA, 2014: "*A measure of the extent to which an intervention or policy fulfills its objectives in practice"*) was calculated as the ratio between the number of wild boar cadavers found, and the 10%

of the census population (representing the quota of natural mortality expected to be present in the field, excluding roadkill, KEULING ET AL., 2013; TOÏGO ET AL., 2008) (Equation 7).

$$Effectiveness = \frac{N\ found}{10\%\ census}$$ *Equation 7*

4.2. EXPECTED WILD BOAR MORTALITY FOLLOWING ASF INTRODUCTION

To provide an estimate of ASF incidence, data from the former ASF infected area in Belgium was analysed. The choice of this particular area was due to the effectiveness of the management strategy in extinguishing the ASF outbreak, which is likely indicative of the detection of (virtually) all infected animals and cadavers.

Georeferenced ASF reports for Belgium were downloaded from WAHIS (https://wahis.woah.org/#/home); in order to identify the data subset most representative of epidemic stage of the outbreak, the cumulated dataset was visualized, setting a cut-off date at the reaching of a "plateau" at right-hand tail of the cumulated curve. Then, the infected area corresponding to the resulting subset (i.e. the convex hull containing all ASF cases) was determined, using the Minimum Binding Geometry tool in QGIS (QGIS DEVELOPMENT TEAM, 2022).

The number of wild boars present in the infected area, and therefore exposed to the infection, was derived from the 25 km^2 raster file produced by PITTIGLIO ET AL., 2018, who estimated wild boar population density on most of the European territory (Figure 2). Implicitly, an assumption of stability of the wild boar population from the publishing date (2018) to the cut-off date of the WOAH dataset, was made. The raster grid representing wild boar population density was resampled to a finer resolution (2.5 km^2) through bilinear interpolation using R package "terra" (HIJMANS R., 2023; R CORE TEAM, 2023). Then, the corresponding population size was calculated by multiplying the population density estimate, by the size of the infected area.

ASF cumulative incidence (**CI**) in wild boar over the epidemic period (**x**) for the chosen data subset was calculated as the number of ASF reports, divided by the estimated number of exposed animals (Equation 2).

$$CI_x = \frac{N\ ASF_x}{N_x}$$ *Equation 2*

This value was then extrapolated from the epidemic period (**t_x**) to a general timeframe **t_y** using Equation 3 (THRUSFIELD ET AL., 2018).

$$CI_y = 1 - (1 - CI_x)^{y/x}$$ *Equation 3*

Then, the time period **y** during which half of the population is expected to get infected was calculated, by solving Equation 2 for $CI_y = 0.5$ (Equation 4). Considering that, in case of an ASF outbreak, all diseased animals are actively looked for and eliminated, a 100% ASF case fatality rate was assumed: therefore, this index will be referred to as “*expected median lethal time*” ($expLT_{50}$).

$$expLT_{50} = \frac{x * \ln(1-0.5)}{\ln(1-CI_x)}$$

Equation 4

The distribution curve of CI as calculated by Equation 3, and the corresponding $expLT_{50}$, is plotted in Figure 28.

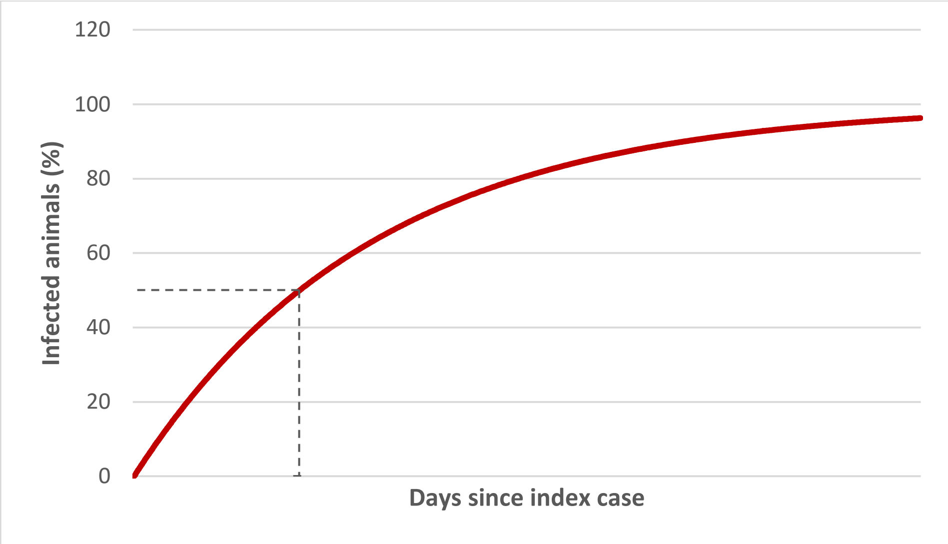

Figure 28: distribution curve of the expected CI as calculated using Equation 3; $expLT_{50}$ is highlighted.

To calculate the expected number of ASF positive cadavers to be disposed of in Pordenone EDR in case of an ASF outbreak, wild boar census data for 2022 were downloaded, filtered and collated from FVG region website (https://www.regione.fvg.it/rafvg/cms/RAFVG/ambiente-territorio/tutela-ambiente-gestione-risorse-naturali/gestione-venatoria/FOGLIA9/). The corresponding expected cumulate incidence at $expLT_{50}$ was calculated, for each hunting reserve, by multiplying the incidence estimated for the Belgian outbreak, by the number of wild boars present in the study area. A yearly 15% quota of the pre-existing wild boar population, representing animals that are expected to die by natural and vehicle mortality (Toïgo et al., 2008), was added to the result, thus obtaining the total expected mortality in wild boar, accounting for both ASF-and non ASF-related deaths. This number represents the number of dead animals that an efficient management strategy would need to test for ASF and destroy in Pordenone EDR. These calculations were performed using R (R Core Team, 2023), and the results were plotted using QGIS (QGIS Development Team, 2022).

In a view to provide a further contextualization of ASF management based on an estimate of the wild boar population, the size of the infected area in Gaume, and the cumulative incidence estimated therein at $expLT_{50}$, were compared to the corresponding outcomes of the Italian Piemonte-Liguria (PL) ASF outbreak.

4.3. SUPPORT FOR MANAGING CADAVERS IN THE FIELD

Currently, in Italy, if an area is declared infected by ASF, all wild boar cadavers found in the field have to be tested for ASF and destroyed; culled animal have to be tested as well, but only ASF positive animals have to be destroyed. To lower the risk of spreading ASFV through transportation of possibly infected cadavers, intermediate collection centres between the field and the rendering plant have to be set up. These sites must be accessible to ASF dedicated vehicle(s), and must be provided with electricity and water for conserving dead animals and disinfecting. Whether a safe transportation of the cadaver to the collection centre is not possible (e.g., in areas less served by the road network, "remote areas" hereafter), sampling of found cadavers can take place in the field, followed by in-situ burial or incineration of cadavers (either single or collective).

The areas eligible for hosting intermediate collection centres in Pordenone EDR were individuated based on the number of cadavers expected to be found as a consequence of ASF, natural mortality and vehicle collision (see Expected Wild Boar mortality following ASF introduction). To include only territories potentially occupied by wild boar from the computations, Corine Land Cover 2018 data (CLC hereafter, https://doi.org/10.2909/71c95a07-e296-44fc-b22b-415f42acfdf0) and the digital terrain model (DTM hereafter, TARQUINI ET AL., 2007) were analysed, retaining only habitats considered suitable for wild boar: namely, CLC class 2 (*Agricultural areas*), class 3.1 (*Forest*) and class 3.2.4 (*Transitional woodland/shrub*), only when placed at an altitude below 1800 meters above sea level.

A representation of the road network serving the study area was obtained using OpenStreetMap spatial data downloaded from GEOFABRICK (https://www.geofabrik.de/en/index.html), while data regarding forest roads was downloaded from FVG WebGIS (https://webgiscarnia.regione.fvg.it/it/map/viabilita_forestale/#); after removing overlapping data and roads unfit for vehicles, the two datasets were merged using QGIS (QGIS DEVELOPMENT TEAM, 2022). To identify remote areas, the Euclidian distance of any point in Pordenone EDR from roads was calculated at a resolution of 2.5 km^2, using R package "rgeos" (BIVAND R. & RUNDEL C., 2023; R CORE TEAM, 2023).

Finally, two raster images were obtained: one representing the expected number of wild boar cadavers, excluding the habitats considered unsuitable for wild boar, and another representing the inverse distance from the road network. After having standardized the two files by dividing the values of the two grids by the corresponding maximum, the product of the two raster was obtained. The candidate areas for intermediate collection points to be placed were identified by higher pixel values.

5. RESULTS

5.1. ENHANCED PASSIVE SURVEILLANCE

A set of seventeen transects were identified (Figure 29; Annex 1), tallying up to 61.254 (4.38 ± 2.6) km. Sixteen of them were included in the Pedemontana pordenonese hunting district, while only one (transect FNA) fell entirely the in Alta pianura pordenonese hunting district.

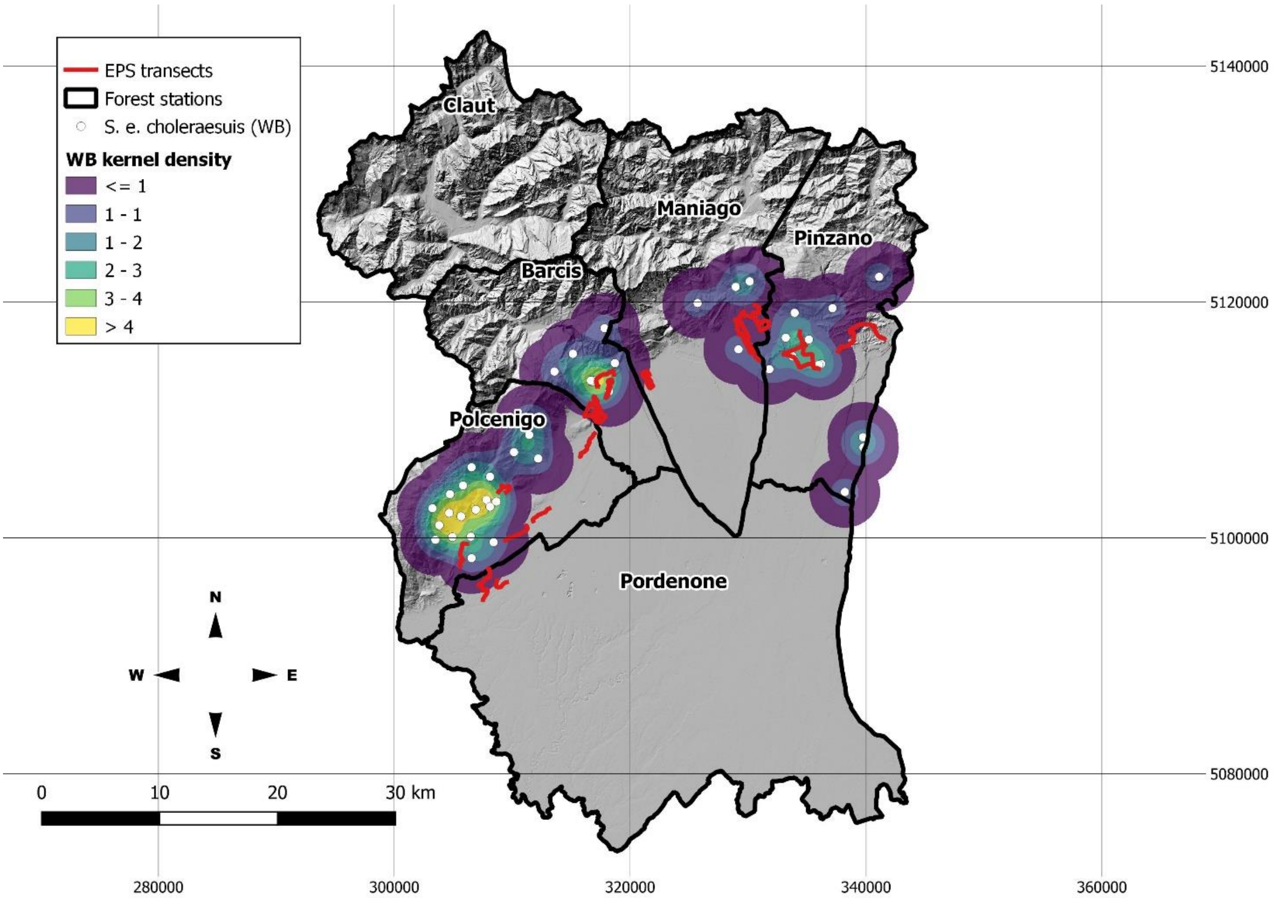

Figure 29: Deployment of transects for passive surveillance activities based on kernel density of S. choleraesuis *data from* LONGO ET AL.*, 2019 in Pordenone EDR. EPSG: 32633*

Enhanced passive surveillance (EPS) activities begun in April 2022 and were carried out twice a month by Pordenone Forestry Corp, inspecting transects falling in their corresponding forest station (FS hereafter). Only for transect FNA (Pordenone FS), the surveys were undertaken by Claut FS personnel.

Descriptive statistics regarding the sampling effort is included in Table 3. After seven surveys, transect BAL (2.98 km) of Barcis FS was replaced by MAS (7.20 km), as the growing crop became a visual impediment; transect MAS was later removed from the set in September 2023, since no sign of presence of the species was detected. Likewise, in Polcenigo FS, transect ART (2.48 km) was replaced by CAV (1.90 km) after 13 surveys, as operators found evidence of wild boar passage alongside the latter. Furthermore, since September 2023, transects MLI (2.43 km) and VAJ (2.41 km) of Maniago FS were replaced by MED (8.06 km), given to frequent reports of wild boar presence in the latter area.

Overall, the number of surveyed transects shifted from fourteen (61.2 km, April 2022 - September 2023) to 12 (57.3 km, September 2023 – December 2023). All transects were inspected twice a month, except for transects MAL and MAS (Barcis FS), for which only one survey was performed in July 2022. During this period, 83 operators were deployed in the search activities (1.38±0.67 operators per survey/transect), which took approximately five hours per month/FS.

Figure 30: Livenza (LIV) transect, in Polcenigo FS (September 23rd, 2022)

Figure 31: Artugna (ART) transect, in Polcenigo FS (September 23rd, 2022)

Missing reports of sampling effort affected 5% of surveys, while no wild boar cadaver was found during the timeframe considered (April 2022 – September 2023), indicating that both efficiency and effectiveness of the enhanced passive surveillance protocol were zero. Conversely, signs of presence of the species were reported for all but four transects, confirming its presence of the surveyed territory.

*Table 3: Sampling effort from April, 2022 to December, 2023. Dismissed transects and aggregated data including dismissed transects is enclosed in squared brackets. *hours are indicated in decimal form. BAL=Borgo Alzetta; GRI=Grizzo; MAL=Malnisio; MAS=Malnisio stradale; RCA=Rio Cavrezza; CAB=Cavasso-Arba; MLI=Maniago-Libero; MED=Meduno; VAJ=Vajont; BCA=Bonifica Casarotto; CCR=Col Cravest; SFA=Strada fantasma; ART=Artugna; CAV=Cavrezza; ART=Artugna; LIG=Ligont; LIV=Livenza; FNA=Fontanafredda-Nave.*

FOREST STATION	OPERATORS	TRANSECT ID	LENGTH	LENGTH (FS)	SURVEYS	TOT LENGTH	TOT LENGTH (FS)	EFFORT	SAMPLING EFFORT	SAMPLING EFFORT (FS)
	n		km	km	n	km	km	h (dec)*	h (dec)*	h (dec)*
BARCIS	9 (1.93 ± 0.79)	[BAL]	2.98	7.30 [17,48]	7	20.86	511.10	6.7 (0.16 ± 0.35)	12.4 (0.59± 1.28)	211.3 (10.06 ± 3.69)
		GRI	3.79		34	128.76		39.7 (0.95 ± 0.15)	77.6 (3.69 ± 1.33)	
		MAL	3.51		33	115.83		42,2 (1 ± 0.26)	83.6 (3.98 ± 1.93)	
		[MAS]	7.20		33	207.86		19.8 (0.47 ± 0.42)	37.8 (1.8 ± 1.7)	
CLAUT	7 (1.9 ± 0.29)	RCA	2.88	2.88	34	97.75	97.75	48 (1.14 ± 0.33)	90.9 (4.33 ± 1.23)	90.9 (4.33 ± 1.23)
MANIAGO	7 (1.85 ± 0.89)	CAR	8.70	16.76 [21.6]	34	295.73	594.39	34 (0.82 ± 0.09)	63.4 (3.02 ± 1.08)	125.6 (5.98 ± 1.93)
		[MLI]	2.43		34	82.62		13.3 (0.32 ± 0.18)	23.6 (1.12 ± 0.7)	
		MED	8.06		8	64.48		7.8 (0.19± 0.4)	15.7 (0.75 ± 1.58)	
		[VAJ]	2.41		34	81.97		12.9 (0.31 ± 0.17)	23 (1.09 ± 0.69)	
PINZANO	6 (1.95 ± 0.21)	BCA	8.32	17.23	34	282.71	585.92	45,9 (1.09 ± 0.79)	90.7 (4.32 ± 3.18)	306.7 (14.6 ± 9.23)
		CCR	1.37		34	46.68		63 (1.50 ± 0.78)	123 (5.86 ± 3.2)	
		SFA	7.55		34	256.53		47.5 (1.13 ± 0.81)	93 (4.43 ± 3.25)	
POLCENIGO	6 (1.52 ± 0.62)	[ART]	2.48	7.1 [9.58]	13	32.24	305.88	16.4 (0.39 ± 0.62)	24.5 (1.17 ± 1.92)	267.3 (12.73 ± 5.31)
		CAV	1.90		34	72.14		38,7 (0.92 ± 0.66)	52.8 (2.52 ± 2.16)	
		LIG	2.21		34	75.07		55.4 (1.32 ± 0.34)	80.3 (3.82 ± 2.79)	
		LIV	2.99		34	101.80		64 (1.52 ± 0.37)	109.7 (5.22 ± 1.85)	
PORDENONE	6 (2 ± 0.22)	FNA	6.00	6.00	34	204.10	204.10	50.2 (1.19 ± 0.43)	100.5 (4.79 ± 1.75)	100.5 (4.79 ± 1.75)
TOT (mean ± sd)	41 (1.83 ± 0.65)		61.254 (4.38 ± 2.6)	61.254 (10.21 ± 5.66)	474 (33.86 ± 0.36)	2071.92 (147.99 ± 87.78)	2071.92 (345.32 ± 190.73)	605.8 (35.6 ± 19.2)	1102.4 (64.8 ± 35.96)	1102.4 (183.7 ± 91.4)

5.2. EXPECTED WILD BOAR MORTALITY FOLLOWING ASF INTRODUCTION

The Belgian ASF outbreak lasted from September 09th, 2018, to March 04th, 2020 (543 days). Cumulated ASF reports (n=833) are represented in Figure 32: after visual inspection, only data collected up to June 21st, 2019 (286 days since the index case) was retained for downstream analysis. This subset (n=824) comprises 99% of outbreak reports, all of which included in Gaume region (cfr. LICOPPE ET AL., 2023).

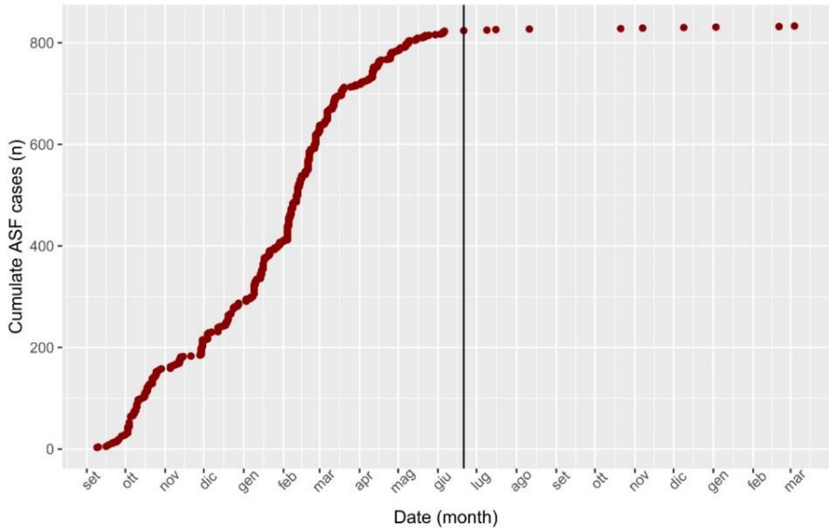

Figure 32: Cumulate data of ASF epidemic in Belgium (September 09th, 2018-March 04th, 2020, n=668); the cut-off point (21/06/2019, n=659) is indicated by a vertical line

On June 21st, 2019, ASF infected area in Gaume region extended 488,82 km^2; there, wild boar population density was estimated at 2.76 ± 1.4 ind/km^2, or 1348 ± 683 individuals overall (Figure 33). Over the 286-day period, ASF cumulative incidence was 0.61, and the expected median lethal time ($expLT_{50}$) as per Equation 3 was calculated at 211 days.

In Piemonte-Liguria (PL), where the index case occurred on January 3rd, 2022, $expLT_{50}$ would have fall on August 2th, 2022. At that time, PL infected area extended for 749.65 km^2, where wild boar population density averaged 2.91 ind/km^2, thus leading to a population size of 2181 individuals (Figure 34). 178 ASF positive cases were found in PL at $expLT_{50}$, so that ASF cumulative incidence results to be 0.0816; however, at $expLT_{50}$, cumulative incidence is supposed to be 0.5 (i.e., 1090 ASF reports): therefore, the outcomes of PL search efforts result to be approximately one sixth of those expected by a Belgian-like ASF management strategy.

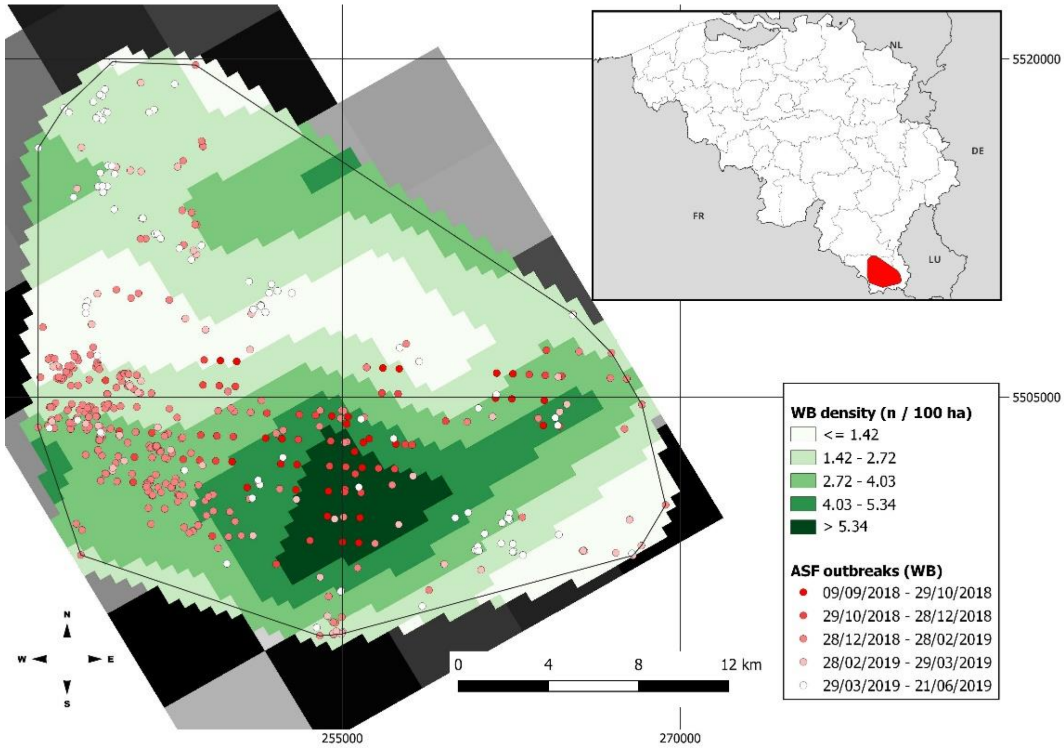

Figure 33: WB density and ASF cases in WB (n=833) in Gaume infected area (Belgium). Data collected after the cut-off date are represented as distinct features. Original density data from PITTIGLIO ET AL., 2018 is represented in the background (grayscale). EPSG: 3035

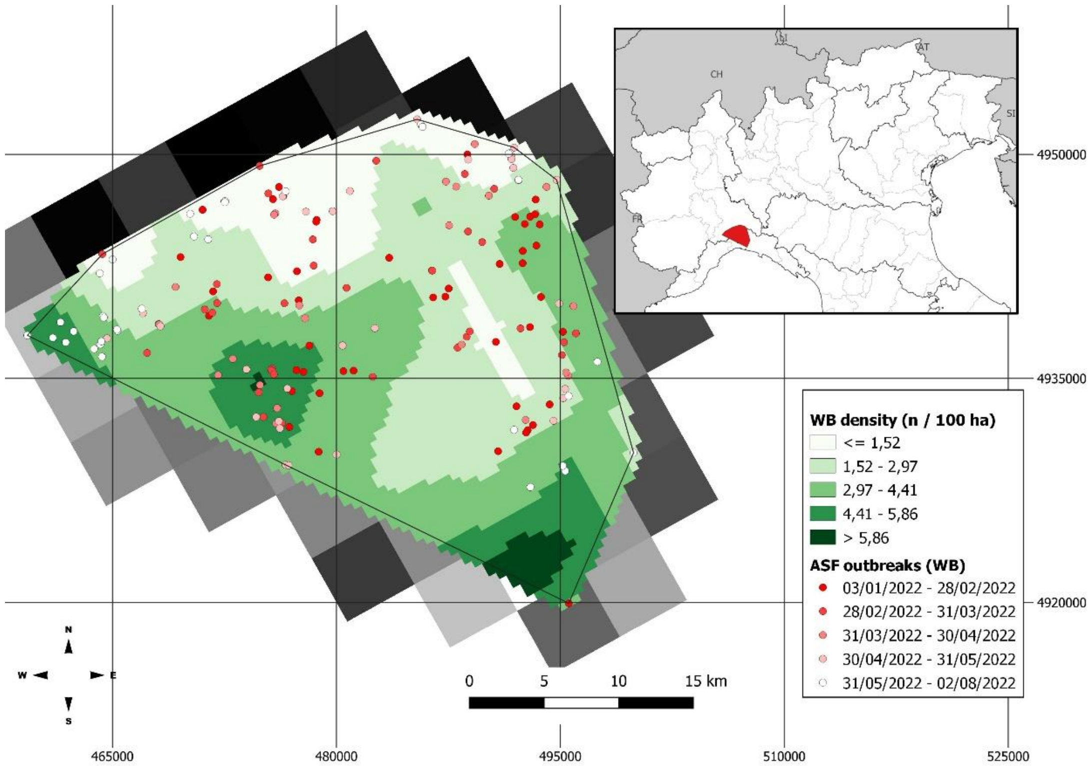

Figure 34: WB density and ASF cases in WB (n=178) in the ASF infected area (Piemonte-Liguria, Italy). Original density data from PITTIGLIO ET AL., 2018 is represented in the background (grayscale). EPSG: 3035

In 2022-23, Pordenone EDR wild boar census data included 820 individuals (0.48±0.71 ind/km^2). Assuming an expLT$_{50}$ of 211 days, as calculated on the basis of viral incidence found in the Belgian infected area (CI = 0.61 over 286-day period), ASF management system in Pordenone EDR should account for 411 infected wild boar cadavers over a 211-day period. Based on the indication of a 15% annual mortality due to natural deaths and vehicle collisions, an additional quota of 8.67% of the wild boar population should be included (n=71), thus tallying up to 482 wild boar cadavers for the whole study area at espLT$_{50}$. Given that 86% of the census population (n=706) occupies the northern hunting area included in Prealpi Carniche and Pedemontana pordenonese districts, in which wild boar population density reaches 0.93±0.85 ind/km^2, 414 wild boar cadavers should be expected in this area alone, averaging 18.8±13.5 cadavers per hunting reserve after 211 days from the index case (Figure 35).

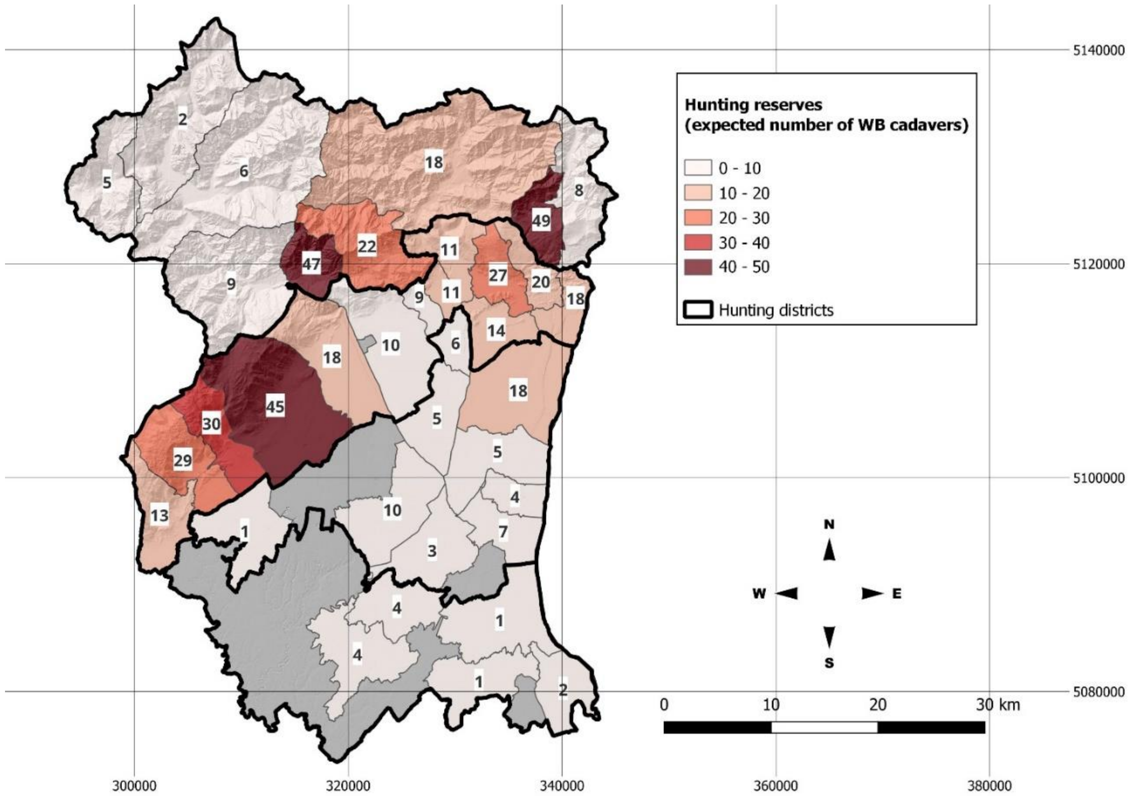

Figure 35: Expected number of cadavers at expLT$_{50}$ (211 days from the detection of the index case) in Pordenone EDR. Null values are not shown. Hillshade based on TINITALY DTM is used as a base layer. EPSG: 32633

5.3. Support for cadavers' management in the field

The habitat that was considered suitable for wild boar according to Corine Land Cover (CCL hereafter) and altitude is represented in Figure 36. Based on CLC classification and elevation data, 1797 km^2 (79%) of the Pordenone EDR territory resulted suitable for wild boar, out of which approximately 52% agricultural land, 43% forests and 5% transitional woodland and shrubs. 61 km^2 of the study area is located at more than 1800 m above sea level; however, the overlap between high elevation areas and CLC unsuitable habitat was high (89%), so that only a negligible proportion of excluded territory (0.4%) was due exclusively to altitude.

Remote areas identified through linear distance from the road network are represented Figure 37. Remote areas as identified by linear distance from the road network are found exclusively on the northern part of the study area.

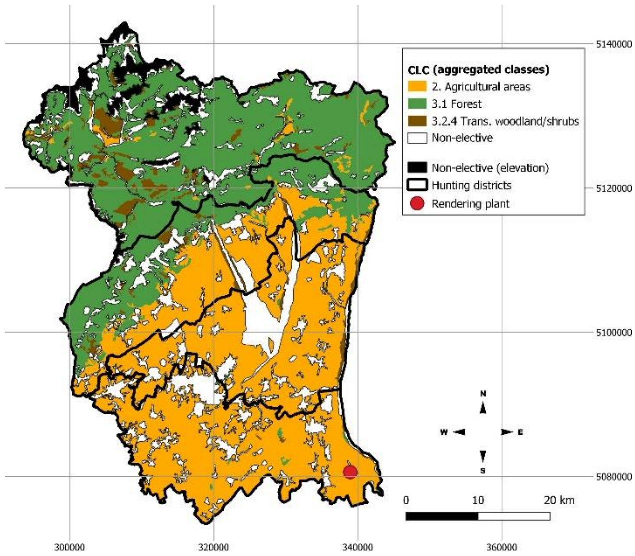

Figure 36: WB habitat eligibility based on Corine Land Cover classes. EPSG: 32633

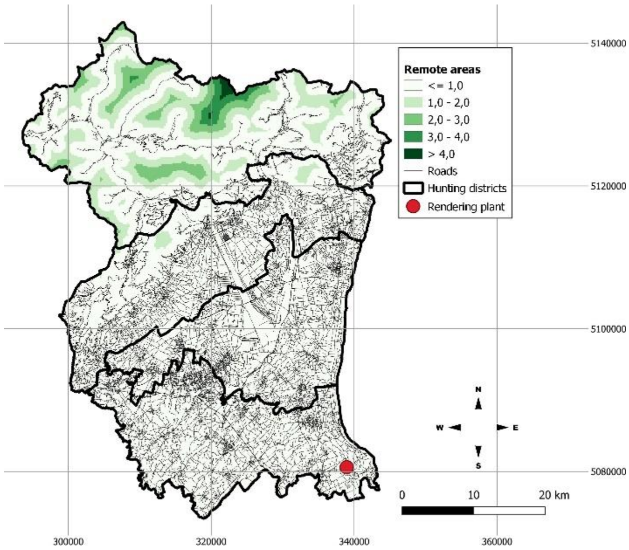

Figure 37: Remote areas, road network and rendering plant of Morasno al Tagliamento. EPSG: 32633

Site suitability for intermediate collection points based on habitat suitability, distance from the road network and expected wild boar cadavers is represented in Figure 38. Candidate sites for ICCs (suitability ≥ 0.75) were entirely determined on the territory of three hunting reserve (Aviano in "Pedemontana pordenonese" hunting district, Andreis and Clauzetto in "Prealpi carniche" hunting district)

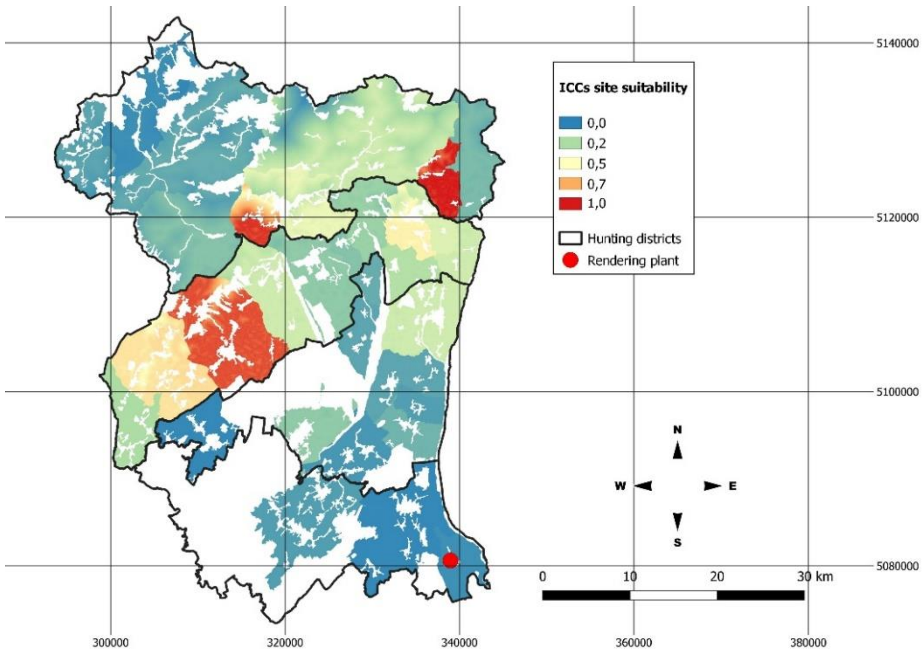

Figure 38: Site suitability for ICCs, based on expected number of WB cadavers, WB habitat suitability and distance from the road network. EPSG: 32633

6. DISCUSSION

6.1. ENHANCED PASSIVE SURVEILLANCE

Enhanced passive surveillance (EPS hereafter) is considered a pillar of ASF management in wild boar (BOUÉ ET AL., 2017; GUBERTI ET AL., 2022; MORELLE ET AL., 2019; PROBST ET AL., 2017; ŠATRÁN, 2019), providing data for both epidemiological surveillance by actively looking for samples, and disease control through the removal of viral reservoirs from the environment (LICOPPE ET AL., 2023, GERVASI ET AL., 2020).. The detection of 1% of the overall wild boar population is considered an indicative threshold to assess EPS reliability (GUBERTI ET AL., 2022), allowing to pinpoint an increase in mortality which should raise awareness in ASF-free areas. For this reason, this threshold is often used in the definition of “suspect ASF case”, as is the case in most Italian guidelines. However, if the size of the wild boar population is unknown, the concept of “increased mortality” could be misleading, and would benefit from a further refinement.

In Pordenone EDR, candidate sites for EPS were identified based on spatially clustered data (n = 49) relative to a *Salmonella* epidemic, comprising sick animals died and found opportunistically between February 7th, 2012, and June 22nd, 2015. Considering that the available data was not originally integrated with field measurement, and its spatial accuracy was likely too low to pinpoint potential deathbed sites, transects were ultimately individuated alongside suitable habitats by the field-expert operators of Pordenone Forestry Corp, and were thus expected to bear positive results. Unfortunately, this was not the case: in fact, no single wild boar cadaver was found during EPS activities, which encompassed considerable search efforts, exerted during a lengthy timeframe, thus leading to null efficacy and effectiveness of EPS activities. Such results were not expected in the study area, all the more reason considering that dead wild boars had been consistently found during the salmonellosis outbreak.

Although a mismatch between 2012-15 surveillance outcomes and the EPS activity reported herein could be partially linked to sample frequency (i.e., to the increased mortality caused by *Salmonella,* CONEDERA ET AL., 2014), and to different sampling conditions (i.e., to the longer span of surveillance activities, and to the broader sampling strategy), the absence of any finding in Pordenone EDR could be indicative of either an insufficient sampling effort for surveillance during peace times, or of the presence of bias/biases in the surveillance strategy.

Undoubtedly, protracted periods of null findings could demotivate both the operators and local administrations, whose workforce would seem to be negatively impacted by unfruitful search activities. As increasing sampling effort is often made impossible by limited resources, EPS could be improve by focusing search activities on potential deathbed sites. To this aim, spatial modelling could be considered: as an example, species distribution models including ordinary passive surveillance data could be implemented to highlight suitable areas. Data used for this purpose should be skimmed from accidental mortality such as roadkill or predation, and should be accompanied by field measurement in order to enhance spatial accuracy on a suitable scale. In fact, this activity has been considered for Pordenone EDR, and it will be undertaken in the foreseeable future using SINVSA notifications. Additionally, to quantify the reliability of human operators in detecting dead animals alongside EPS transects, scent hounds could be deployed as a golden standard.

However, it must be considered that in certain contexts (e.g., rugged territories, dense underwood), the search for wild boar cadavers could turn out all the way ineffective (DESVAUX ET AL., 2021): to this point, the wild boar population in Pordenone EDR is indeed located in the northern Alpine area, which can present morphological obstacles to EPS activities. Nonetheless, EPS has proven to be an important asset of ASF surveillance, and should be sustained during both disease-free and escalation periods (GUBERTI ET AL., 2022); it is therefore worth pursuing an optimization of search efforts, until the system is sensitive enough to detect suspicious shifts in mortality.

6.2. EXPECTED WILD BOAR MORTALITY FOLLOWING ASF INTRODUCTION

Knowledge regarding the size of a population affected by a disease is crucial for determining epidemiological parameters, such as disease prevalence and incidence: unfortunately, this data is often lacking, or missing altogether. In this study, in order to calculate cumulated incidence (CI hereafter) of ASF in Belgium, a large-scale wild boar population density estimate was used. However, this estimate was produced on the basis of spatially and temporally heterogeneous data, aiming at providing a tool for large-scale epidemiological analyses (PITTIGLIO ET AL., 2018). While an improvement in wild boar population assessment is undoubtedly desirable, the results presented herein should be interpreted with caution, aimed at providing a parsimonious scenario for disease management.

The characteristics of an epidemic event are strictly bounded to the underlying factors that determine the spread of the disease; notably, the demographic and behavioural dynamics of the affected population. If a population is naïve to a particular disease and can be considered closed (i.e., unaffected by recruitment, deaths, and migration), CI is representative of the average individual risk of developing that disease, during a certain timeframe. Considering the Belgian ASF outbreaks, the assumption of closure was closely met in terms of migrations, given that the emergence of the disease was followed by the implementation of severe restrictive measures. Conversely, both infected and non-infected animals were certainly removed during the timeframe considered for calculating CI, thus lowering the probability of viral transmission by direct contact: this has likely biased, by deficiency, the calculation of epidemiological CI. Moreover, the most relevant sources of infection (i.e, ASF positive cadavers) were actively removed from the field, thus further biasing CI calculation towards lower values. In fact, the CI value calculated in the present work from the beginning of the Belgian outbreak up to June 21st, 2019 (CI = 0.61), is highly similar to the “true” values reported for the same area, in overlapping periods, by LICOPPE ET AL., 2023 (CI = 0.65±0.04 up to March 19th, 2019; CI = 0.57±0.18 up to December 19th, 2019), whose calculations were affected by the same biases.

As for Pordenone EDR, the absolute abundance of the wild boar population was assessed through the technique of census by vantage point, which is susceptible to known biases (ENETWILD CONSORTIUM ET AL., 2018). Nonetheless, the local data used by PITTIGLIO ET AL., 2018 to estimate population density arose from the same technique: in fact, wild boar population census data obtained for 2021-22 in Pordenone EDR (0.40 ± 0.67 ind/km^2) was only slightly higher than the estimates available in PITTIGLIO ET AL., 2018 for the same area (0.35 ind/km^2). Given that the general trend of wild boar populations all over Europe has either been stable or increasing compared to the source data used by PITTIGLIO ET AL., 2018, the estimated effects of an ASF outbreak in Pordenone EDR in absolute terms (i.e., number of cadavers) is likely underestimated.

Considering all the above, integrating CI with a measure of the efforts to control ASF well adapts to the purpose of the present study: indeed, CI as calculated herein should not be considered in epidemiological terms, but rather as a parameter representing the proportion of ASF positive wild boar that are likely to arise from an efficient ASF management system, allowing to depicting a likely scenario and a baseline target for future strategies of disease management. As the both disease incidence, and efficacy of the depopulation, are susceptible to local variability, a common reference to evaluate the outcomes of different ASF management experiences, over different wild boar populations, is needed.

Given that both CI, and the number of detected animals, show variable growth rates at different stages of the outbreak, and especially near the beginning and the end of the emergency, it seems reasonable to set this reference at the median point, thus representing the number of days since the detection of the index case, after which half of the ASF positive wild boar population would have died, either as a consequence of the disease, or harvesting. Considering that ASF virus is highly resistant, remaining active in infected cadavers over a long period of time, the eradication of ASF before endemicity will only be accomplished by inducing both ASF lethality and case detectability to approach 100% (i.e., all infected animals die, are detected, and add up in assessing CI). As one of the very few cases in which ASF eradication was attained, such scenarios were likely closely met in the Gaume infected area (Belgium): therefore, in the present work, the reference timeframe spanning from the detection of ASF index case, to 50% expected CI, was calculated from this area. As no similar index was known to the authors, it is referred herein as "*expected median lethal time*" ($expLT_{50}$), based on the similarities with median lethal dose LD_{50}.

The comparison of CI calculated at $expLT_{50}$ in Piemonte-Liguria (PL, Italy) provides an example of the application of this indices as a tool for assessing the quality of ASF management strategies; in this case, the mismatch between the CI expected (0.5) and observed (0.08) suggest a high degree of underdetection of ASF positive animals.

6.3. Support for cadavers management in the field

Once the ASF index case has been detected, the size of the area subjected to restriction will depend on the distribution of positive animals, dead and alive, detected in the field The minimization of impacts exerted by ASF eradication strategy is therefore bound to a timely early detection on one hand (i.e., disease surveillance), and to prompt interventions on the other. To this second point, advancing territorial planning is essential: this include the definition of the network for transporting possibly ASF infected dead animals from the retrieval/culling site, to the rendering plant.

Although the transport outside Restriction Zones of dead animals derived from hunting and culling is clearly advised against by guidelines (e.g. MOPS, Guberti et al., 2022), it is allowed by national legislation. Accordingly, the sample for ASF testing from both cadavers retrieved from the field, and carcasses derived from harvesting, must be withdrawn inside the restriction zone in which they originated, and only then headed towards rendering or, eventually and exclusively for ASF negative carcasses derived from harvesting, meat treatment (Order n. 5, 2023).

However, FVG PRIU prescribes the identification of only one regional intermediate collection centre (ICC hereafter), which to date has not been identified yet: in a view to manage wild boar cadavers on smaller territorial units, thus lowering the risk of viral spread to larger areas, the individuation of further candidate sites for setting up ICCs is therefore advisable. To this point, it could be indicative to consider that the implementation of an efficient management strategy in Gaume (Belgium) allowed to contain the ASF spread inside a 488 km^2 infected area, which is approximately one quarter the size of Pordenone EDR: therefore, more than one ICC could be identified.

Considering that animal products destined to consumption must be kept physically separated from cadavers retrieved from the field, it follows that ICCs should include two distinct lines for sampling and transporting dead animals. As that the number of dead animals that the system is expected to produce is prone to changes, this network of infrastructures deployed to this end should allow for some adaptability; nonetheless, it should pursue a minimum target of an efficient management of expected ASF cases, of which an approximate indication was provided herein.

In the present work, considering that ICCs should be located where expected wild boar mortality is higher, and that their deployment would be optimized by avoiding manual transportation of carcasses for long distances, candidate sites for ICCs were identified based on census data, habitat suitability for wild boar, and linear distance from the road network, keeping in mind the following issues:

- Wild boar census data provided by vantage point is very likely affected by sampling bias: notably, inconsistent effort amongst hunting reserves due to different number of operators available (i.e., hunters and personnel of the Forestry Corp), and differential detectability of animals due to territorial heterogeneity. Considering that the current ASF management strategy pivots on wild boar depopulation, and that this activity relies upon the same operators that provide census data, the aforementioned biases will eventually be reflected on ASF management, unless additional resources are provided. Currently, a measure of these biases is not available for the study area, and should be the target of further studies.
- The identification of habitat suitability based solely on Corine Land Cover and elevation is certainly a rough methodological approach; nonetheless, given the ecological plasticity exhibited by this species, aggregated CLC classes are likely to give an indicative approximation of suitable territory, all the more reason when analysed in conjunction with census data.
- As a proxy of difficulty in transporting carcasses, linear distance from the road network can be misleading: this is especially true when rough territories are considered, where steep valleys and rock jumps can impede access to roads over small linear distances.

Three hunting reserves in Pordenone EDR resulted high suitable for ICCs (< 0.7): all three are located in the northern part of the study area, which present several characteristics that support this indication. Namely:

- it is densely populated by wild boar;
- it consist of mostly rough territory;
- it is far from the rendering plant.

Of course, a more precise definition of the ICC location(s) should take into account specific knowledge of local resources (e.g., availability of public space, the presence of abandoned buildings/laboratories, availability of electricity/water sources etc.) and available personnel. As an example, collection centres already deployed for temporarily field stocking of hunted animals could be considered a valuable option, whether they provide a suitable environment to apply the biosafety requirements of ICCs. However, a characterization of the number, size, type and distribution of these structures is not currently available. On-site burial is allowed *in extrema ratio* by national legislation. Transporting dead animals in rough areas can be arduous, implying a non-negligible risk of injury for the operators. Moreover, it inherits a considerable risk of bodily leakage, and consequent viral spread. Therefore, it seems appropriate to associate on-site burial with the distance from the road network. To this point, remote areas identified in the present work could provide an approximate indication for the location of burial sites, and could be integrated in the planning of ASF management strategy. Given that excavating pits for single burials would likely be unfeasible as a routine method, remote areas identified herein could represent approximate locations for mass burial trenches. In both cases, it must be considered that that burying corpses inherits a high risk of soil and water contamination, and should be pursued only after a fine characterization of the local environment: most importantly, local hydrology and pedology should be considered, in order to minimize the spread of both the bodily fluids leaking from the decomposing cadaver(s), and the disinfectants used to deactivate ASFV. To the latter point, citric acid has been suggested for soil disinfection due to ASFV low tolerance to acidic conditions (CARLSON ET AL., 2020), and could be considered as an alternative to traditional products.

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7. Conclusions

ASF is currently an existential threat to the European swine industry. Given the role of wild boar as a source of infection and viral reservoir, the activities of epidemic surveillance, population control and removal of cadavers represent key aspects of disease control. These tasks represent a conspicuous challenge, requiring solid bases for collaboration amongst stakeholders, information flow and coordination between operators in the various steps of the emergency. In order to maximize their efficiency and effectiveness, comprehensive territorial planning is necessary to identify the network of infrastructures on which they rely upon. The present work focussed on a small part of these requirements, while contributing in building the operative framework on which ASF preparedness is based.

The Italian experience with ASF underlines how viral introduction is often anthropogenic, and therefore unpredictable in time and space. The abundance of harsh territories will additionally hamper both early detection, and removal of cadavers from newly discovered infected areas; in this view, preparedness acquired during peace time is essential for eventually achieving ASF control and eradication. The enhanced passive surveillance (EPS hereafter) activities carried out in Pordenone EDR represent one of the few examples of active quest for wild boar cadavers implemented in ASF-free areas in Italy, and were realized thanks to the commitment of the Officers and field operators of the Pordenone forestry inspectorate. Despite having proven ineffective during the first 20 months of implementation, the EPS protocol will undergo further adjustments in order to improve its performances.

At the present state, the results presented herein stress the need for additional workforce for ASF management, particularly for carrying out EPS, and very likely for wild boar demographic control. As a rough comparison, the ASF outbreak in Belgium was controlled by deploying one operator each 20 hectares only to the quest for wild boar cadavers, while removal was carried out exclusively by Authorities (forest services and Civil Protection), in a view to avoid viral spread (Licoppe et al., 2023). In Italy, wildlife management pivots on leisure hunting. However, when facing epidemic emergencies such as ASF, the outreach of this activity will likely be insufficient: to this point, it is worth considering that inducing a decrease in the wild boar population is rarely achieved, although highly incentivised, even during peace times.

In a “One Health” perspective, the AHL encourages the participation of Veterinary Authorities in wildlife management; nonetheless, their activity relies on accurate and updated population assessments, which are rarely available for wild species, as underlined by the case of ASF in the wild boar population. This problem is often linked to a lack of personnel dedicated to wildlife monitoring: as an example, in the Italian context, this activity relies heavily on the voluntary efforts of leisure hunters, which are spatially skewed. In Pordenone EDR this factor is, currently, not accounted for, thus very likely leading to biased population assessments. To account for these biases, it seems therefore advisable to undertake targeted studies aimed at correlating routinary census data with a reliable estimate of population abundance.

Although describing the earliest steps required by ASF management in wild boar, many other are not addressed by the present work. Notably, the importance of adopting physical measures to retain the spread of the virus (i.e. fencing) seems self-evident, all the more reason considering the difficulties encountered in estimating and managing the wild boar population. These actions should follow careful mapping of ecological barriers and weak spots, thus defining the territorial units for downstream management actions. This would allow to anticipate the measures required by restriction zoning, leading to a timely intervention after the detection of the index case. In this view, further studies for identifying barriers and corridors in relation to wild boar movement are encouraged.

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