

RESEARCH NEWS

January 2022: Index case of new African Swine Fever incursion in mainland Italy

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African Swine Fever (ASF) is a haemorrhagic disease, which can cause high mortality in domestic pigs and wild boars; it does not affect humans but has a devastating socioeconomic impact. It can be transmitted directly through animal contact or indirectly via contaminated food and equipment. Humans can mechanically transport the ASF virus (ASFV) by human-mediated activities (the 'human factor') (EFSA AHAW Panel, 2014). The ongoing epidemiological wave in Europe originated in 2007 in Georgia and spread to other European countries in 2014; ASFV genotype II was responsible for these outbreaks, which affected both wild boar and domestic pigs (Blome et al., 2020). Since then, the disease has spread westward in Europe, with the epidemic reaching Germany (Sauter-Louis et al., 2020). At the same time, a long-distance jump transmitted the virus from infected countries to previously ASF-free countries, such as Belgium (Linden et al., 2019). It is suspected that the transmission via the human factor occurred in mainland Italy, which is also the latest European country to report the spread of ASF, where the first ASFV genotype II-positive wild boar was found in the Piedmont region (northwest region of the country) in January 2022 (ADIS, 2022). Subsequently, several other positive cases were reported in the wild boar population across the Liguria and Piedmont regions, a mountainous area between the

sea and the foothills of the Alps, as well as in the urban territory of Genoa.

1 | PASSIVE SURVEILLANCE PLAN IN FORCE IN ITALY

Owing to the threat from the ongoing European wave of ASFV outbreaks, the Italian government implemented a national surveillance plan for early detection of the infection in 2020 (Italian Ministry of Health, 2020). As per the surveillance plan, passive surveillance activities in the wild boar population and domestic pig farms should be performed; in addition, implementation of biosecurity measures should be verified, to avoid any risk factor of ASFV introduction (for instance, contact between pigs and wild boar population or swill feeding). The institutions involved are encouraged to implement management measures to control the density and range distribution of wild boar populations. The national plan places primary importance on awareness campaigns through training courses involving different stakeholders, such as veterinarians, breeders and hunters. Moreover, an assigned information flow was established to obtain an official database containing passive surveillance data (VETINFO).

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2 | ASF INDEX CASE DETECTION

On 29 December 2021, due to local passive surveillance activities, a dead wild boar was found in the Ovada municipality, the Province of Alessandria, in the Piedmont region; the animal was male, between 18 and 30 months old, with a weight of approximately 90 kg. The carcass was found in a rural environment and was freshly dead. Subsequently, in the next few days, other wild boars were found dead approximately 15–18 km from the location of the first sighting. In particular, a 2-year-old male wild boar was found moribund in the Isola del Cantone municipality, the Province of Genoa, in the Liguria region on 29 December 2021. Another young female wild boar was found dead on 3 January 2022, in the Fraconalto municipality, the Province of Alessandria, in the Piedmont region, and it was in the early stages of decay. Local veterinary authorities undertook sampling and transport of the tissue samples to the official laboratory-in-charge of the territory, which is located in Turin at the Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, and the relevant data were entered into the designated information system.

3 | LABORATORY TESTS

The local laboratory made the first diagnosis using the DNA extraction kit Blirt Extractme® Genomic DNA (BLIRT S.A., Poland), followed by commercial ID Gene™ African Swine Fever Duplex (ID.vet GENETICS, France) real-time PCR kit according to the manufacturer's instructions, on 5 January for the Ovada case and on 7 January 2022 for subsequent cases. After the samples tested positive for the presence of the viral genome, they were immediately sent to the Italian National Reference Laboratory for Swine Fevers (NRL) at the Istituto Zooprofilattico Sperimentale dell' Umbria e delle Marche, in Perugia. The NRL performed DNA extraction using the High Pure PCR Template Preparation Kit (Roche, Switzerland) as per the manufacturer's instructions, and the DNA samples were used for confirmatory tests and preliminary sequence analysis. The NRL performed real-time PCR assays as described in the World Organisation for Animal Health (OIE) Manual (OIE Manual for Diagnostic Tests & Vaccines for Terrestrial Animals, 2021), based on the protocols reported by King et al. (2003) and Fernández-Pinero et al. (2013), targeting conserved regions of the B646L gene. The NRL confirmed ASFV detection on 7 January 2022 for the Ovada sample and on 10 January 2022 for subsequent samples. Preliminary phylogenetic analysis of partial sequences of the B646L gene (VP72) and the B602L hypervariable region (CVR) was performed for the samples from Ovada via Sanger sequencing. Further, the NRL performed the haemadsorption assay using porcine peripheral blood mononuclear cell (PBMC)-derived macrophages, as described in the OIE Manual (OIE Manual for Diagnostic Tests & Vaccines for Terrestrial Animals, 2021). The virus was isolated from the Isola del Cantone and Fraconalto samples and subsequently tested. Strain virulence was determined at the BSL3 facility at NRL by intramuscularly administer-

ing two piglets with 1.5 ml of a tissue homogenate from the Ovada sample, in accordance with the current national legislation on the protection of animals used for scientific purposes (Italian Ministry of Health, 2014).

4 | CHAIN OF COMMAND ACTIVITIES

After the notification of the first ASF-positive case in mainland Italy, the operational expert group met on 7 January to support the competent authorities in assessing the epidemiological situation and its progression. On the same day, the local crisis unit involving the regional veterinary authorities, local official laboratories, other institutional bodies and stakeholder organizations also held a meeting. The central crisis unit met on 10 January, whereas additional cases suspected to be ASF were identified. As mandated by the EU legislation [Regulation (EU) 2016/429, Regulation (EU) 2020/687], an infected zone was established immediately via a ministerial arrangement (Italian Ministry of Health, 2022a). Currently, it includes an area of 2811 km² and covers 114 municipalities of the Liguria and Piedmont regions (Figure 1); additionally, a neighbouring zone with a 10 km radius was also identified. Over the next few days, Ministry of Health and NRL representatives inspected the infected area to obtain information for better management of the epidemiological situation.

5 | IMPLEMENTATION OF IMMEDIATE EMERGENCY MEASURES

Given the criticality of the situation, in adherence to EU legislation [Regulation (EU) 2016/429, Regulation (EU) 2020/687], the relevant authorities undertook emergency measures to prevent the spread of the disease by carrying out reinforced passive surveillance activities on pigs and wild boar. All farms in the infected area were subjected to checks regarding the implementation of surveillance and biosecurity measures, and the owners were asked to improve the implementation of biosecurity measures wherever relevant. Domestic pig management included backyard farm depopulation and slaughter management in the infected regions. In the infected area and neighbouring regions, hunting and other activities were prohibited, and active searching for wild boar carcasses as well as their removal was required. This activity was conducted by enrolling official veterinary authorities personnel, forest guards, hunters, civil defence personnel, and firefighting guards. These measures were specified in an official act issued by the Ministry of Health on 18 January 2022 (Italian Ministry of Health, 2022b) (Table 1). Further, proposals for fencing the infected area were formulated by May 2022, and considerations for disease management in the future were defined. In particular, the infected regions are required to draw up an eradication plan, whereas the neighbouring regions are required to reinforce a surveillance plan owing to the high risk of ASFV introduction. In the remainder of the country, the national surveillance plan continues to be in force.

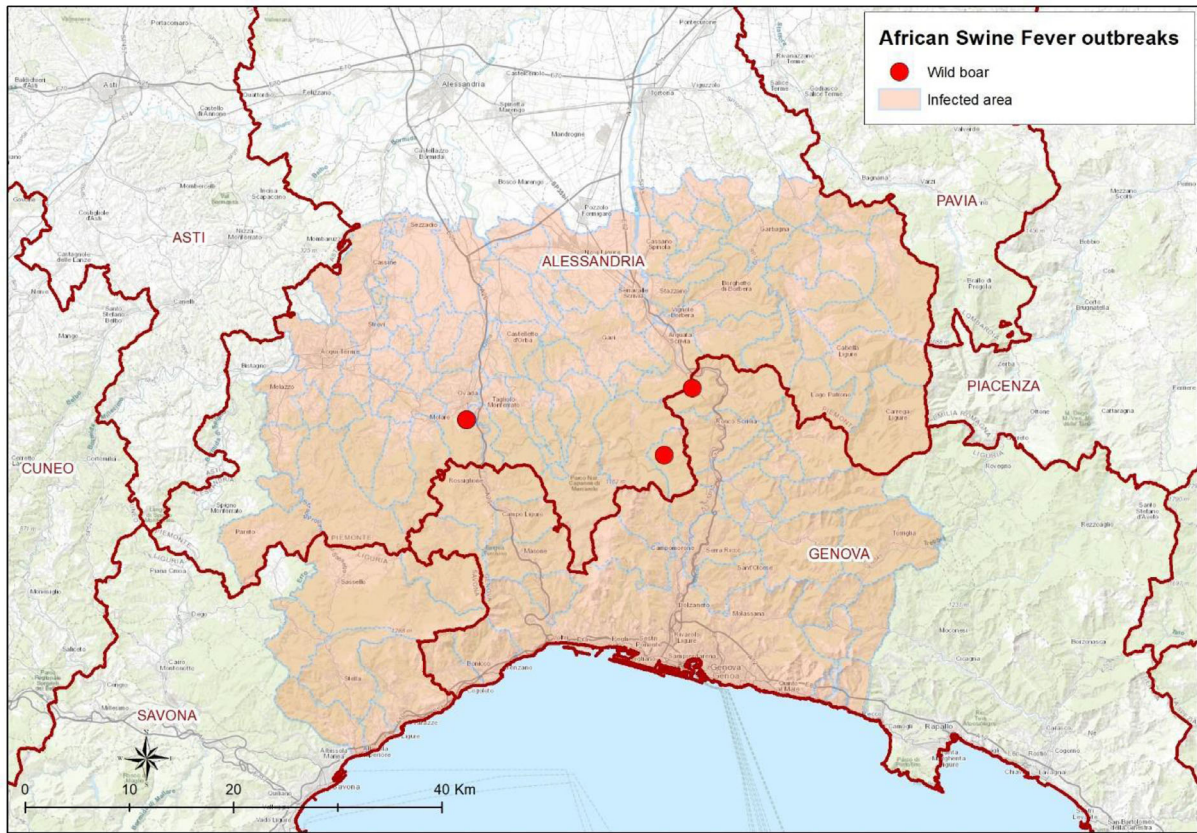


FIGURE 1 Mainland Italy ASF infected zone, Ministerial Arrangement, 11 January 2022

TABLE 1 Measures to control and prevent ASF spread in mainland Italy, Ministerial Arrangement, 18 January 2022

Infected area	Neighbouring zone (10 km)	Other regions
Hunting and other outdoor activities prohibition	Regulation of hunting and outdoor activities	-
Active wild boar carcasses searching starting from borders	-	-
Management and disposal of carcasses	-	-
Pig farm census	Pig farm census and immediate update of National Database	Pig farm census and immediate update of National Database
Depopulation of at-risk farms, included industrial farms and backyard farms, in the infected area and 6 months stop of reproduction and restocking	-	-
Stoppage of live pigs, pig meat products and ABPs movements	-	-
Virological test in pigs found dead	Virological test of pigs found dead	-
-	Enhancement of biosecurity measures at pig farms and checks starting from semi-wild farms	Enhancement of biosecurity measures at pig farms and checks starting from semi-wild farms
-	Enhancement of passive surveillance	-
-	Enhancement of checks on pig movements	-
-	Prohibition of movement of wild boars caught in protected areas and other wildlife establishments	Prohibition of movement of wild boars caught in protected areas and other wildlife establishments
-	-	Obligation of fences in semi-wild farms and individual identification of all breeders present there

6 | CONCLUSIONS

Based on current knowledge, passive surveillance is the most effective and efficient tool for the early detection of ASFV infection in wild boar (EFSA AHAW Panel, 2018). This report reveals that passive surveillance activities enabled the detection of the first ASFV case in wild boar in mainland Italy. The information acquired by veterinary authorities due to the implementation of the ASF surveillance plan since 2020 enabled the prompt application of reinforced surveillance measures. In mainland Italy, the initial spread of the virus in a susceptible wild boar population is ongoing (invasion phase), with a high number of unidentified cases. The involved ASFV strain shows high virulence capacity, as observed from the clinical phenotypes and mortality rates a few days after the administration of tissue homogenates from ASFV-infected wild boar to piglets, during *in vivo* experiments performed by the NRL (data not shown). Underestimation of cases could be due to several reasons, such as the large size of the infected zone and its geographical characteristics, including inaccessible areas where visibility of carcasses may be low due to the presence of dense forest, dense undergrowth, crevasses, ravines and snow. ASF has been prevalent in Italy since 1978 on the island of Sardinia (genotype I ASFV) and became endemic, involving domestic pigs, wild boar and illegal free-range pigs (Laddomada et al., 2019). However, in recent years, the Sardinian epidemiological situation has improved, with evidence of reduced viral circulation after the implementation of specific measures to reduce the prevalence of infection in endemic areas (Loi et al., 2020). In this context, the risk of importation of ASFV genotype I from the Sardinian-infected area into mainland Italy has decreased considerably; nevertheless, the risk of ASF introduction primarily due to the human factor has increased. The results of the initial molecular characterization studies showed that the viral strain isolated from mainland Italy was ASFV genotype II. High sequence homology (99.80%–99.90%) was observed with other representative genotype II strains characterized in the Caucasus regions, the Russian Federation, Southeast Asia and Central-Eastern Europe (data not shown). Phylogenetic data and the distance from the current endemic front indicate that ASFV introduction into mainland Italy was caused most likely by anthropogenic activities. The reported conditions in mainland Italy represent a novel scenario for controlling ASFV spread in the wild boar population, due to the mountainous and wooded involved territory. The application of new operational methods may be necessary for the Italian context.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data analysed in this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The National Legislation on the protection of animals used for scientific purposes was followed.

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