African swine fever in wild boar: Targeted surveillance, seasonal patterns and stakeholder perceptions

von Lisa Rogoll

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Aus dem Veterinärwissenschaftlichen Department
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ABBREVIATIONS

ASF	African swine fever
ASFV	African swine fever virus
ASP	Afrikanische Schweinepest
CSF	Classical swine fever
DIVA	Differentiating Infected from Vaccinated Animals
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority
ELISA	Enzyme-Linked Immunosorbent Assay
EMA	European Medicines Agency
EU	European Union
MSGIV	Ministerium für Soziales, Gesundheit, Integration und Verbraucherschutz des Landes Brandenburg
NRL	National Reference Laboratory
PCR	Polymerase Chain Reaction
PMI	Postmortem interval
SchwPestV	Schweinepestverordnung
TierGesG	Tiergesundheitsgesetz
TSN	Tierseuchennachrichtensystem
WAHIS	World Animal Health Information System
WOAH	World Organisation of Animal Health

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I. INTRODUCTION

African swine fever (ASF) is a viral disease that affects Eurasian wild boar (*Sus scrofa*) and domestic pigs (*Sus domesticus*) among other suid species. Originating from the African continent, the disease has spread widely throughout Europe and beyond in the last decade and has by now become a global challenge. Causing hemorrhagic fever, the disease can lead to severe symptoms and high lethality. Therefore, the disease is listed as notifiable to the World Organisation for Animal Health (WOAH) and as a Category A disease by the Animal Health Law of the European Union.

In addition to the original hosts of the *Suidae* family, soft ticks of the genus *Ornithodoros* serve as competent vectors of the disease. However, in absence of the tick vector, the disease is able to circulate in wild boar populations leading to sporadic spill overs to domestic pig holdings in most of the affected European countries. The lasting presence of ASF in wild boar causes a constant risk of transmission to domestic pigs and requires high biosecurity standards to prevent disease transmission. After ASF outbreaks, pigs from affected farms have to be culled. Furthermore, the lasting presence of ASF leads to trade restrictions causing high economic losses in the pig industries of affected countries. Thus, even though ASF has no zoonotic potential, its socio-economic impact is devastating.

In wild boar, the disease is transmitted directly from infected individuals or their contaminated carcasses to susceptible individuals. Indirect transmission through contaminated waste, vehicles or other fomites can also occur and might be facilitated by humans. To prevent ASF from spreading in the wild boar population, the rapid search, sampling and removal of carcasses is of utmost importance, since ASFV can persist in contaminated carcasses over long time periods. In addition, restriction zones are established and fences are constructed to hinder wild boar movement und the spread of ASF. Furthermore, to reduce the susceptible population and to monitor the disease spread, intensified hunting and sampling of wild boar is conducted in many countries.

Due to absence of any treatment or vaccine, control measures revolve around the mentioned strategies. However, they require huge amounts of resources, such as money or workforce, and have to be in place over long time periods. Thus, it is necessary to make targeted use of available resources. Additionally, effectiveness of surveillance and control is highly dependent on the compliance of the various stakeholders involved, such as hunters, farmers, veterinarians, laboratories and authorities. It is of utmost importance to consider involved stakeholders' perceptions and interests, since this may foster compliance and enhance efficient ASF surveillance and control.

To improve efficiency of surveillance and control strategies, targeted strategies making best use of available resources as well as improvement of stakeholder compliance are necessary. Thus, the present thesis aimed to provide a better understanding of surveillance strategies, seasonal patterns of disease occurrence as well as stakeholder perceptions in order to support the optimization of ASF surveillance and control in wild boar.

II. LITERATURE REVIEW

1 Characteristics of African swine fever virus

1.1 Taxonomy, morphology and genome

African swine fever virus (ASFV) is the causative agent of ASF. It belongs to the family of *Asfarviridae* and represents the family's only species in the sole genus *Asfivirus*. ASFV has a linear double-stranded DNA of 170-194 kbp and replicates in the cytoplasm primarily of cells of the mononuclear-phagocytic system, e.g. macrophages (ALONSO et al., 2018).

The extracellular enveloped virions have a size of 175-215 nm in diameter and consist of nucleoid and core shell, surrounded by two icosahedral protein capsids (inner and outer capsid), each enveloped by a lipid membrane and an outer envelope (Figure 1) (SALAS and ANDRÉS, 2013; ALONSO et al., 2018; ANDRÉS et al., 2020). The capsid is built from one major (p72) and four stabilizing minor proteins (H240R, M1249L, p17, p49) (WANG et al., 2019).

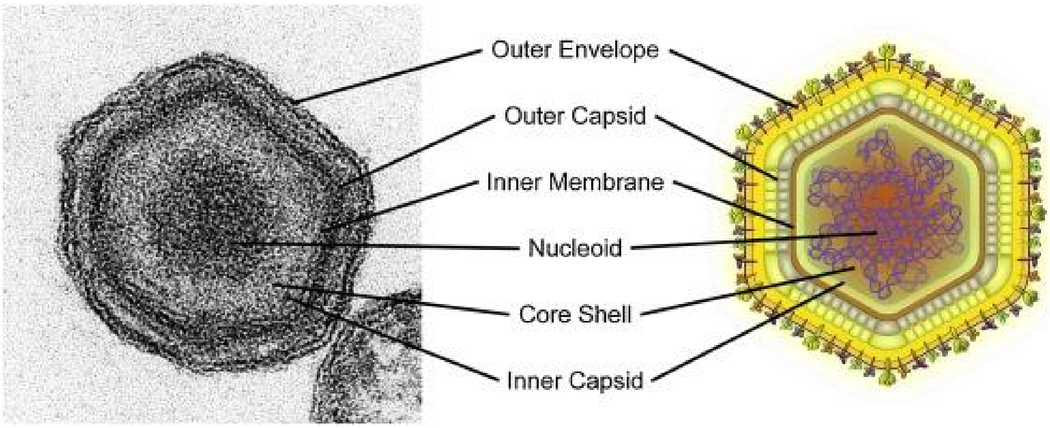

Figure 1. Virion structure of ASFV (BLOME et al., 2020). Left side: Electron microscope image of extracellular virion of ASFV. Right side: Schematic illustration of extracellular ASFV virions showing the virions components. The figure was taken from the publication by Blome et al. (2020) according to the CC-BY-NC-ND license (https://creativecommons.org/licenses/by-nc-nd/4.0).

1.2 Genetic variability

Variability in the gene B646L, which is encoding the capsid protein p72, is used for genotyping of ASFV (DIXON et al., 2013; BLOME et al., 2020). The combination with sequencing of the p54 and pB602L genes allows further genetic discrimination of subtypes (GALLARDO et al., 2009). So far, 24 genotypes of ASFV have been identified in Africa (BOSHOFF et al., 2007). Of these, only the genotypes I and II have been detected outside of Africa (BOSHOFF et al., 2007; ROWLANDS et al., 2008; SUN et al., 2021). Overall, the genome of ASFV showed high genetic stability and low mutation rates in the past (DIXON et al., 2020). Recently, however, five different linages of ASFV with at least ten different variants have been identified in Germany (FORTH et al., 2023). High-impact mutations were observed that had have never been identified before, leaving the potential impact of increased mutation rates of ASFV in the future unknown (FORTH et al., 2023).

1.3 Tenacity

ASFV appears to be stable for long periods in blood as well as in several organs and tissues. In liquid blood, the virus can survive up to several months or even years (DE KOCK et al., 1940; PLOWRIGHT and PARKER, 1967). In muscle, skin or subcutaneous fat the virus remains stable for several months (FISCHER et al., 2020a). Also in processed products such as Serrano or Iberian ham the virus can survive for more than 100 days (MEBUS et al., 1993). Thus, these tissues may represent long-term reservoirs for infectious virus, especially at low temperatures. Contrary, the stability of ASFV in urine or feces seems to be low, lasting only a few days (DAVIES et al., 2017; OLESEN et al., 2020).

However, the tenacity of the virus in tissues that may persist longer in the environment, such as bones, has been studied with variable results: Stability varied from less than one week (FISCHER et al., 2020a) up to several months (KOVALENKO et al., 1972). Furthermore, in a study in Lithuania by Zani et al. (2020) no infectious virus could be isolated from buried ASF-positive wild boar carcasses - only fragments of the viral genome were detected in the soil surrounding the carcasses (ZANI et al., 2020). This finding highlights that further research on the stability of the virus in materials that are considered relevant for indirect transmission of the virus such as soil, water, field crops or feed is of great interest. It has been shown that virus stability in soil ranging from a few days up to several weeks is dependent on the soil pH, its structure and the environmental temperature (STOIAN et al., 2019; CARLSON et al., 2020; MAZUR-PANASIUK and WOŹNIAKOWSKI, 2020). Furthermore, Niederwerder et al. (2019) showed that oral transmission of the virus through contaminated liquid and dry feed might be possible (NIEDERWERDER et al., 2019). It was shown by Sindryakova (2016) that ASFV may remain stable in compound feed up to 30 days and in water up to 60 days (SINDRYAKOVA et al., 2016). Furthermore, Fischer et al. (2021) demonstrated successful external ASFV contamination of commercial spiked spray-dried porcine plasma granules that are often added to the main feed of weaned pigs (FISCHER et al., 2021). However, when stored at room temperature, complete inactivation of ASFV was observed already after two weeks (FISCHER et al., 2021). In addition, Fischer et al. (2020b) did not detect infectious virus on field crops after two hours of drying, suggesting that risk of transmission through contaminated crops is low (FISCHER et al., 2020b).

To this end, the impact of feed, water and bedding on transmission of ASFV is still under discussion.

Inactivation of the virus can be achieved with lipid solvents and detergents as well as oxidizing agents (SÁNCHEZ-VIZCAÍNO et al., 2009).

1.4 Clinical signs and pathomorphological lesions

Depending on virulence of the respective ASFV strain and on host factors, the clinical signs and lethality rates of ASFV infection may vary (Figure 2). The host range of ASFV includes suids and soft ticks of the genus *Ornithodoros*. In the wild-living suid hosts in Africa, the warthogs (*Phacochoerus* spp.), the infection does not cause apparent disease (JORI et al., 2013).

High virulent strains of ASFV genotype II circulating outside Africa usually induce peracute and acute forms of ASF with high lethality rates in wild boar and domestic pigs (PIETSCHMANN et al., 2015; GUINAT et al., 2016; GALLARDO et al., 2018) (Figure 2). Infections with highly virulent strains may result in death within about five to ten days after infection, leading to lethality rates of up to 100% (BLOME et al., 2012; PIETSCHMANN et al., 2015; NURMOJA et al., 2017b; GALLARDO et al., 2018). Clinical signs of acute infection usually begin two to seven days post infection and usually include high fever (> 41°C) accompanied by a variety of other symptoms, such as lethargy, depression, ataxia, reddening of the skin, respiratory or gastrointestinal symptoms like anorexia, diarrhea or vomiting (GABRIEL et al., 2011; PIETSCHMANN et al., 2015; NURMOJA et al., 2017b). Neurological symptoms and abortion in pregnant sows due to severity of symptoms have also been observed (SCHLAFER and MEBUS, 1987; NURMOJA et al., 2017b). Infection with moderately virulent ASFV strains leads to similar clinical signs, however, lethality rates are lower and range from 30 to 70% (GALLARDO et al., 2018; BLOME et al., 2020). Typical pathomorphological lesions of acute ASFV infection include enlarged and hemorrhagic lymphnodes, pulmonary edema, renal petechia or hemorrhages, splenomegaly and gall bladder wall edema (GABRIEL et al., 2011; PIETSCHMANN et al., 2015; TAUSCHER et al., 2015; NURMOJA et al., 2017b). Both wild boar and domestic pigs show similar clinical signs and pathomorphological lesions (GABRIEL et al., 2011; PIETSCHMANN et al., 2015; TAUSCHER et al., 2015). However, attenuated phenotypes of certain virus strains, e.g. “Estonia 2014”, show higher virulence in wild boar and therefore result in more severe clinical signs (ZANI et al., 2018; SEHL et al., 2020).

Low virulence strains of ASFV show low lethality rates and the absence of the typical lesions mentioned above (BLOME et al., 2020). Chronical forms of the disease with mild and non-specific clinical signs can also be observed (SÁNCHEZ-VIZCAÍNO et al., 2015; GALLARDO et al., 2018) (Figure 2).

Surviving animals usually seroconvert and develop ASFV specific antibodies between 7 to 20 days post infection (MUR et al., 2016a; GALLARDO et al., 2018) (Figure 2). However, timing and level of antibody development may vary and is not predictive for disease outcome as antibodies are not fully able to neutralize the infection (ESCRIBANO et al., 2013; PIETSCHMANN et al., 2015; PIKALO et al., 2019; BLOME et al., 2020). There is still scientific debate as to whether surviving seropositive animals are potential carriers and shed ASFV (OĻŠEVSKIS et al., 2023). It has been reported that antibodies can be detected up to 7 months after primary infection without viremia or clinical signs (GALLARDO et al., 2018). The study by Petrov et al. (2018) showed that even though ASFV could be detected up to 91 days post infection in the blood of seroconverted animals, no transmission occurred from survivors to contact pigs (PETROV et al., 2018). Contrary, the study by Eblé et al. (2019) suggested that seropositive animals occasionally transmitted ASFV to other animals (EBLÉ et al., 2019). However, epidemiological analyses by Schulz et al. (2022) and Oļševskis et al. (2023) concluded that the number of exclusively seropositive animals found in the field is relatively small and therefore, they might not have a major impact on the disease dynamics (SCHULZ et al., 2022; OĻŠEVSKIS et al., 2023).

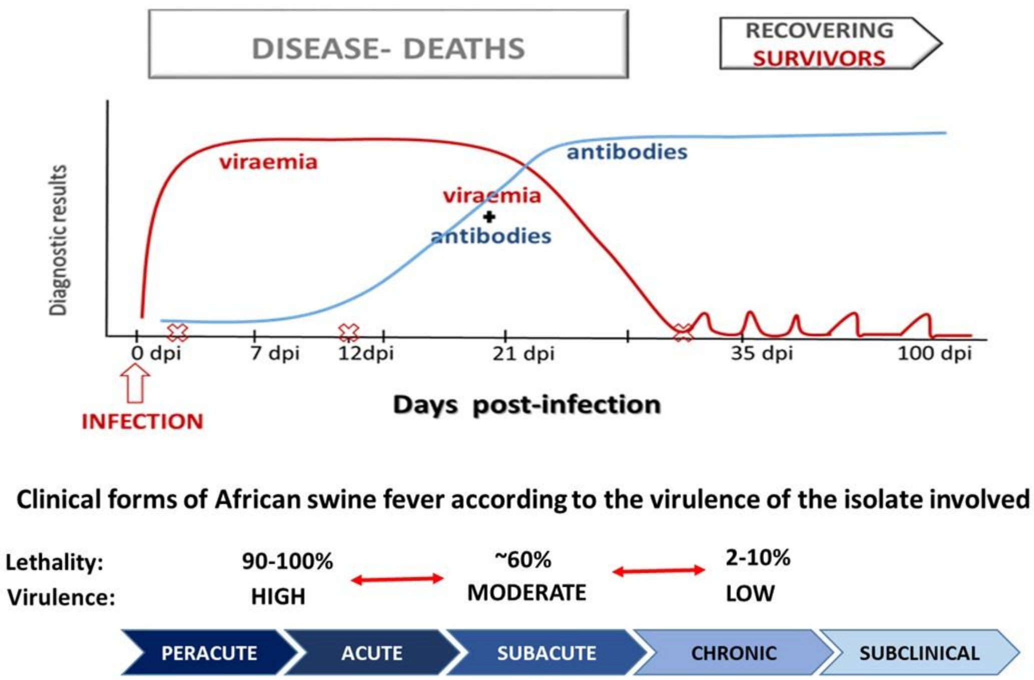

Figure 2. Overview of possible courses of disease after infection with ASFV. The figure is available at European Union Reference Laboratory for African Swine Fever (ASF), https://asf-referencelab.info/asf/en/procedures-diagnosis/diagnostic-procedures (accessed on 19 July 2023).

1.5 Laboratory diagnosis

Given its designation as an internationally notifiable disease by the WOAH, there are legal requirements and recommendations regulating laboratory diagnosis of ASF. The WOAH "Manual of Diagnostic Tests and Vaccines for Terrestrial Animals" (2023) and the European Union Reference Laboratory for ASF (https://asf-referencelab.info/asf/en/procedures-diagnosis/sops, accessed on 19 July 2023) offer comprehensive protocols and methods for laboratory diagnosis. To enable early warning, rapid intervention and surveillance of ASF, reliable and quick diagnosis is necessary (ARIAS et al., 2018).

There are two main categories of laboratory tests for ASF: virus detection and serology. The choice of specific tests is depending on prevailing disease situation and the diagnostic capabilities of the laboratory within the region or country.

Detection of ASFV using Polymerase chain reaction (PCR) protocols is the first choice for early detection of the disease. A large number of published protocols and fully validated test kits are available (BLOME et al., 2020). Several commercially available kits have been found suitable for ASFV detection in different studies (KING et al., 2003; SCHODER et al., 2020). In Germany, the National Reference Laboratory (NRL) for ASF provides a list of licensed kits in the German official collection of methods for notifiable diseases (https://www.fli.de/de/publikationen/amtliche-methodensammlung/, accessed on 19 July 2023). It has been shown that EDTA blood and spleen samples are best suited matrices for early detection using all types of PCR methods (PIKALO et al., 2021; ELNAGAR et al., 2021). As mentioned in the respective manuals, samples from tonsils, lymph nodes, bone marrow, lung, liver or kidney would also be suitable. For increased biosafety and easier sampling in the field, dry blood swaps were validated successfully as non-invasive sampling options to detect ASFV genome (PETROV et al., 2014; CARLSON et al., 2018; ELNAGAR et al., 2021). Virus isolation on porcine macrophages is used for confirmation and is necessary for further characterization of isolates (CARRASCOSA et al., 2011).

Antibody detection can be used to monitor the epidemiological disease situation in a region or country. It is usually performed with Enzyme-Linked Immunosorbent Assays (ELISA). As mentioned in the respective manuals, serum and plasma samples are best suitable for antibody detection. Several ELISA kits are available, however, a lack of quality in serum samples may affect test specificity negatively (GALLARDO et al., 2019).

2 Epidemiology of African swine fever

2.1 Hosts and transmission cycles

The host range of ASFV includes suids and soft ticks of the genus *Ornithodoros*. Furthermore, contaminated pig products and carcasses can contribute to disease transmission. Thus, four epidemiological cycles of ASF transmission are described: the sylvatic cycle, the domestic pig-tick cycle, the domestic pig cycle and the wild boar-habitat cycle (COSTARD et al., 2013; CHENAIS et al., 2018) (Figure 3). The four cycles are further described in the following chapters.

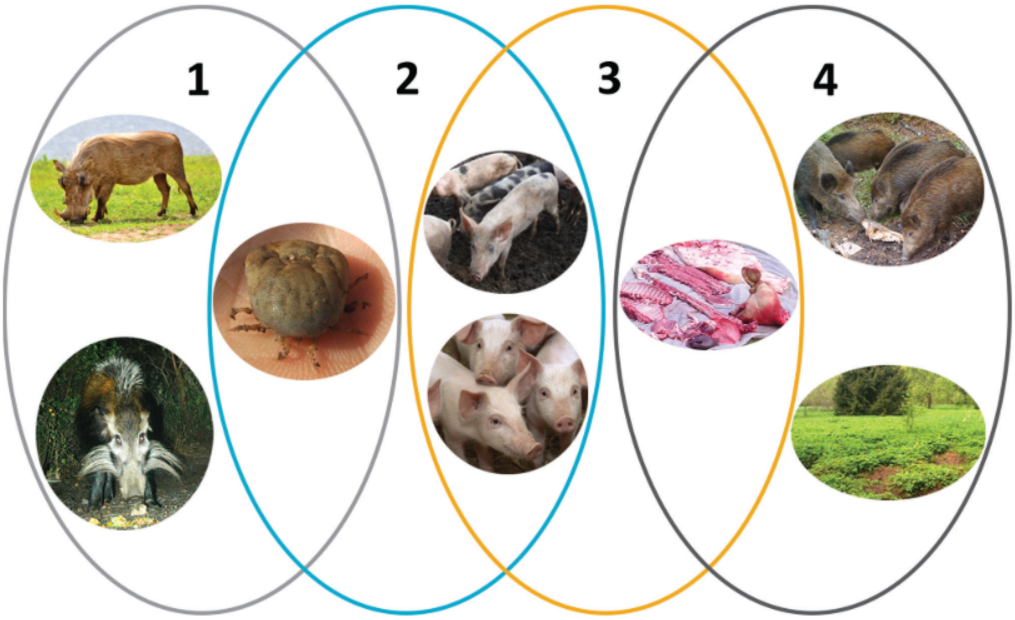

Figure 3. Hosts and transmission cycles of ASF (CHENAIS et al., 2018). Gray: Sylvatic cycle that involves warthogs (*Phacochoerus* spp.) or bushpigs (*Potamochoerus* spp.) as well as the soft tick vector of the genus *Ornithodoros*. Blue: Domestic pig-tick cycle that involves the soft tick vector and domestic pigs (*Sus domesticus*). Yellow: Domestic pig cycle that involves transmission among domestic pigs and contaminated products. Black: The wild-boar habitat cycle that involves contaminated carcass or pig products, wild boar (*Sus scrofa*) and their habitat. The figure was taken from the publication by Chenais et al. (2018) according to the CC-BY 4.0 license (https://creativecommons.org/licenses/by/4.0/).

2.2 Origin and spread of the disease in the 20th century

The first occurrence of ASF was described by Montgomery (1921). Referred to as “East African swine fever”, the disease was first diagnosed in 1910 on domestic pig farms in Kenya, at that time British East Africa (MONTGOMERY, 1921). A number of 15 outbreaks were recorded by 1915, in which 98.9% of all involved pigs died. It was already noted that wild living warthogs were present in the vicinity of all outbreak farms and that most farms were large and unfenced (MONTGOMERY, 1921). In the following years, the disease also occurred in Angola (1932) and South Africa (1940) (GAGO DA CÂMARA, 1932; DE KOCK et al., 1940). It was suspected that the virus persisted in warthogs, occasionally spilling over to domestic pigs. Thus, the first prevention and control measures in these countries aimed at separating domestic pigs from wild living warthogs (SCOTT, 1965). Nevertheless, the disease subsequently spread to Western Africa, when in 1959 the first outbreak was reported in Senegal (PENRITH and KIVARIA, 2022). Also Central African countries were subsequently infected, e.g. Cameroon (PENRITH and KIVARIA, 2022). Since then, ASF has become endemic in several sub-Saharan African countries.

Soft ticks of the genus *Ornithodoros* have been identified as a vector for ASF. This ancient transmission cycle of the disease is described as "sylvatic cycle" and considered as the origin of three other transmission cycles of ASF (COSTARD et al., 2013; CHENAIS et al., 2018) (Figure 3). Ticks can infect neonate warthogs in their burrows. In absence of clinical symptoms of the disease, warthogs develop considerable viremia and can, in turn, infect naïve ticks feeding on them (THOMSON, 1985). Mainly, ticks from the *Ornithodoros moubata* complex (East and Southern Africa) and *Ornithodoros erraticus* group (North and West Africa) are described to be responsible for disease maintenance on the African continent (JORI et al., 2013). In ticks, venereal and transovarial transmission have been observed (KLEIBOEKER and SCOLES, 2001). Besides warthogs, also bushpigs (*Potamochoerus* spp.) can be asymptomatically infected. However, since they exist in small numbers and prevalence of infection is much lower, their role in the epidemiology of ASF is thought to be minor, but remains unclear at last (JORI and BASTOS, 2009; JORI et al., 2013). Direct transmission from infected warthogs to other warthogs or to domestic pigs has not been described (JORI and BASTOS, 2009).

However, infected ticks being transported by warthogs to domestic pig farms appear to be a bridging link between the sylvatic cycle and the so-called "domestic pig-tick cycle" (JORI et al., 2013) (Figure 3). Ticks can serve as a reservoir for environmental persistence of ASFV in domestic pigs in addition to direct transmission between domestic pigs (WILKINSON, 1984). It has been described that soft ticks may colonize domestic pig farms and feed exclusively on domestic pigs (SANCHEZ-BOTIJA, 1963).

Additionally, the "domestic pig cycle", where ASF persists in the domestic pig population in absence of any natural reservoirs through direct transmission between infected and susceptible pigs as well as indirect transmission from contaminated fomites (e.g. clothing, vehicles, veterinary equipment) or feed (CHENAIS et al., 2019) has been described (Figure 3). Swill feeding of contaminated pork products and usage of blood products as protein source for domestic pigs can also function as source of infection (WEN et al., 2019). Through direct contact or improper disposal of waste, ASF can be transmitted from domestic pigs to free-living wild boar.

In 1957, the first jump of the virus (Genotype I) to the European continent, specifically to Portugal, happened when waste from airline flights, presumably from Angola, was fed to pigs near the Lisbon airport (BOINAS et al., 2011). The outbreak was quickly brought under control, but the virus was reintroduced into Portugal three years later in 1960. This time, the outbreak could not be contained. Subsequently, the disease also spread from the African continent to Spain (BOINAS et al., 2011; MUR et al., 2012a) and to several other European countries in the following decades, such as Belgium, the Netherlands, Italy, Malta, and France, where it affected domestic pig farms (BIRONT et al., 1987; TERPSTRA and WENSVOORT, 1986; SWANEY et al., 1987; WILKINSON et al., 1980). Sporadically, it also caused domestic pig outbreaks in the Americas, e.g. in Brazil, the Dominican Republic, Haiti and Cuba (REICHARD, 1978; MEBUS et al., 1978; ALEXANDER, 1992).

In the latter mentioned countries, the spread of ASF was most often brought under control within a few years. Contrary, ASFV subsequently became endemic on the Iberian Peninsula, where it affected domestic pigs and wild boar. Factors contributing to the persistence of the virus in Spain and Portugal were lack of biosecurity in domestic pig holdings, the presence of the soft tick vector *Ornithodoros erraticus* that served as reservoir and the presence of uncontrolled wild boar populations, that had been identified as natural hosts of the disease in certain affected areas (PÉREZ et al., 1998; BOINAS et al., 2011; MUR et al., 2012a). However, until 1981, only a few outbreaks in domestic pigs (approximately 6%) were attributed to direct disease transmission from wild boar (MUR et al., 2012b). In contrast, at that time, it was hypothesized, that the disease would not persist in wild boar once it had been eradicated from domestic pigs (PÉREZ et al., 1998).

After more than thirty years of efforts to control the disease, Portugal and Spain were declared free from ASF in 1993 and 1995, respectively (MUR et al., 2012a; BOINAS et al., 2011). The study by Mur et al. (2012a), in which wild boar from previously affected areas in Spain were examined for ASF in the period from 2006 to 2010, apparently proved the former hypothesis to be true: None of the examined wild boar was ASF positive, thus, it was concluded that the disease had not persisted in the wild boar population (MUR et al., 2012a).

2.3 Persistence of ASF in Sardinia

During the panzootic in the 20th century, ASFV (Genotype I) was also introduced into the Italian island Sardinia in 1978, probably through contaminated food waste that was fed to pigs (MANNELLI et al., 1998). Even though eradication plans were implemented from 1982 onwards, the disease became endemic on the island, causing outbreaks of ASF in domestic pigs and wild boar (MUR et al., 2016b; CAPPAI et al., 2018). Contrary to the epizootic situation on the Iberian

Peninsula, the tick vector was and is not present in Sardinia (MUR et al., 2016b). However, several other factors contributed to the persistence of ASF in Sardinia. There was a large number of small-sized non-professional domestic pig farms, implementing little to no biosecurity measures (MUR et al., 2016b; JURADO et al., 2018b). Additionally, due to lack of education programs for these farmers and delayed compensation payments, their compliance to report sick pigs was generally low (MUR et al., 2016b). Illegal breeding of pigs in free-ranging systems ("brado" pigs) was conducted in some regions of the island and illegal trade of animals or pig products frequently occurred, facilitating the spread and persistence of ASF (MUR et al., 2016b; JURADO et al., 2018b). Furthermore, population density of wild boar in combination with the mean altitude above sea level was identified as a risk factor for persistence (JURADO et al., 2018b). However, Cappai et al. (2018) found that the seroprevalence of ASF in wild boar in Sardinia was rather low (CAPPAI et al., 2018). It was assumed that wild boar did not play a key role in the disease persistence in Sardinia and no independent infection cycles in wild boar populations were established. More likely, disease persistence in the wild boar population was caused by repeated transmission events from domestic pigs to wild boar (MUR et al., 2016b; IGLESIAS et al., 2017; CAPPAI et al., 2018).

Several studies suggested that more likely the free-ranging "brado" pigs were the main reservoir of ASFV in Sardinia (JURADO et al., 2018b; CAPPAI et al., 2018; MUR et al., 2016b). Cadenas-Fernández et al. (2019) detected high interaction rates between free-ranging pigs and wild boar and Cappai et al. (2018) showed that high numbers of free-ranging pigs correlated with ASFV outbreaks on domestic pig farms, suggesting that free-ranging pigs might serve as a "bridge" to transmit ASFV between wild boar and domestic pigs (CAPPAI et al., 2018; CADENAS-FERNÁNDEZ et al., 2019). Therefore, an eradication plan aiming at depopulation of illegally bred, free-ranging pigs was put into place from 2017 onwards. Following the plan, between 2017 and 2020, more than 4000 of those pigs were culled and high disease prevalence (virus and antibody) were detected in culled "brado" pigs in the first year (LADDOMADA et al., 2019; FRANZONI et al., 2020). Prevalence as well as interactions between the suid populations decreased during implementation of the program until 2020, leading to a reduction of ASF outbreaks (LADDOMADA, 2020). The last outbreak in domestic pigs was detected in 2018 and the last case of ASF in wild boar occurred in 2019, suggesting that ASF is very closed to being eliminated in Sardinia (FRANZONI et al., 2020; LADDOMADA, 2020).

2.4 Introduction of ASF into the Caucasus region in the 21st century

Except for the endemic situation in Sardinia and one outbreak in Portugal in 1999 that could be contained quickly, the European continent remained free from ASF since 1995 (BOINAS et al., 2011). However, another transcontinental spread happened and ASFV was reported in Georgia in June 2007 at the Black Sea harbor of Poti (ROWLANDS et al., 2008) (Figure 4). Most likely, contaminated food waste from ships was the source of infection for free-ranging domestic pigs (ROWLANDS et al., 2008). This time, the causative ASFV strain belonged to the highly virulent genotype II, closely related to ASFV strains from the south eastern African countries, such as Mozambique, Madagascar and Zambia (ROWLANDS et al., 2008; KOLBASOV et al., 2018). The virus quickly spread throughout the country and by the summer of 2007 most districts of Georgia were affected, resulting in the death and culling of great numbers of pigs (ROWLANDS et al., 2008). Subsequently, the virus spread throughout the Caucasus region, affecting the neighboring countries Armenia later in 2007 and Azerbaijan in 2008 (EUROPEAN FOOD SAFETY AUTHORITY (EFSA) PANEL ON ANIMAL HEALTH AND WELFARE, 2010).

The first infection of a wild boar in the Russian Federation was confirmed close to the border with Georgia in November 2007 (EFSA PANEL ON ANIMAL HEALTH AND WELFARE, 2010). Several months later the first outbreak in domestic pigs was reported in summer 2008 (VERGNE et al., 2017). In the following years, ASF mainly circulated in the southern regions of the Russian Federation, affecting both domestic pigs and wild boar. The direct transmission among wild boar and between wild boar and free-ranging domestic pigs in backyard farms were considered to be the main drivers of the epizootic (GOGIN et al., 2013). High virulence of ASFV in wild boar of all ages was detected (BLOME et al., 2012). By 2011, the virus spread to the central and northern regions of the Russian Federation (VERGNE et al., 2017). It is believed that the spread of ASF throughout the country was facilitated by human activities, as illegal trade of pig products and swill feeding occurred (GOGIN et al., 2013). Numerous outbreaks in domestic pigs and cases in wild boar were reported, respectively. Studies by Kolbasov et al. (2018) and by Blokhin et al. (2020) suggested that contrary to the situation observed in the 20th century and in Sardinia involving genotype I, at this time ASF was indeed able to persist in wild boar populations (KOLBASOV et al., 2018; BLOKHIN et al., 2020).

The first ASF outbreak in the Ukraine was reported in 2012 close to the Black Sea coast. However, two years later, ASF occurrence in wild boar was reported in another area of the Ukraine close to the border with the Russian Federation.

In western Belarus, close to the Lithuanian border, an affected backyard holding was reported in 2013 (EFSA PANEL ON ANIMAL HEALTH AND WELFARE, 2014). Several months later, also a commercial pig holding close to the border with the Russian Federation reported an outbreak (EFSA PANEL ON ANIMAL HEALTH AND WELFARE, 2014). Belarus has officially reported only a few outbreaks. However, unofficial sources suggest that the country may have been affected even earlier and that ASF might sill circulate in the country's domestic pigs and wild boar population (EFSA PANEL ON ANIMAL HEALTH AND WELFARE, 2014).

2.5 The current situation in Europe

In January 2014, ASF reached the eastern area of the European Union (EU), when the first case of ASF in wild boar in Lithuania was reported (MAČIULSKIS et al., 2020) (Figure 4). Most likely, wild boar movements from the affected area in Belarus were the source of infection (PAUTIENIUS et al., 2018). Shortly thereafter, the first occurrence of ASF was also reported in wild boar in Poland (February 2014) and Latvia (June 2014) close to the countries' respective borders with Belarus (OĻŠEVSKIS et al., 2016; ŚMIETANKA et al., 2016). ASF was also reported in the northern regions of Latvia shortly after the first occurrence in the east. It is believed that this long-distance jump was human-mediated and caused by illegal disposal of waste in the forest (OĻŠEVSKIS et al., 2016; EFSA et al., 2018). These cases are believed to be epidemiologically linked to the first cases of ASF in wild boar in the south of Estonia in September 2014, that occurred close to the Latvian border (NURMOJA et al., 2017b). Almost simultaneously, ASF also emerged in the north-eastern area of Estonia close to the border with the Russian Federation (NURMOJA et al., 2017b).

Rapidly, ASF spread westwards throughout the wild boar populations of the three Baltic states in the following months and years (NURMOJA et al., 2020; MAČIULSKIS et al., 2020; OĻŠEVSKIS et al., 2020). Meanwhile, sporadic outbreaks in domestic pig holdings were reported. In Lithuania and Latvia mostly backyard-holdings were affected by ASF (MAČIULSKIS et al., 2020; OĻŠEVSKIS et al., 2020). In Estonia, ASF was notified in smallholder pig farms but also in bigger commercial farms (NURMOJA et al., 2020). In many cases, indirect transmission of the virus e.g. through contaminated fomites (vehicles, people, tools) combined with insufficient biosecurity, hygiene or swill feeding was believed to be the source of infection (NURMOJA et al., 2020; OĻŠEVSKIS et al., 2020). However, it was noted that ASF circulated in the wild boar populations close to the outbreaks in domestic pigs and in some cases, direct transmission from wild boar to domestic pigs was suspected (NURMOJA et al., 2020; OĻŠEVSKIS et al., 2020).

Compared to the situation in the Baltic states, the spread of ASF in Poland was initially much slower (WOŹNIAKOWSKI et al., 2016; ŚMIETANKA et al., 2016). Cases were limited to the regions close to the Belarusian border until 2016. It was suspected that repeated introductions from the infected, Belarusian wild boar population had occurred (ŚMIETANKA et al., 2016). During that time, ASF sporadically spread to domestic pig holdings. Mostly, backyard farms with low biosecurity measures allowing for direct transmission from wild boar to domestic pigs, were affected (WOŹNIAKOWSKI et al., 2016). A significant increase in the number of affected wild boar, as well as an increase in the number of affected areas, has occurred in 2017 and 2018 (PEJSAK et al., 2018). At the end of 2019, ASF was unexpectedly transmitted to wild boar in western Poland, presumably human-associated, and the number of cases in the area increased rapidly (MAZUR-PANASIUK et al., 2020). There were also outbreaks in larger domestic pig farms, leading to the culling of many thousand domestic pigs (MAZUR-PANASIUK et al., 2020).

Due to nearness of the epidemic front, surveillance efforts in Germany were intensified in the regions close to the border with Poland. In September 2020, ASF was detected in a wild boar found dead in the German federal state Brandenburg (SAUTER-LOUIS et al., 2021b). Similar to the situation in the afore mentioned countries, wild boar movements across the border were the suspected source of introduction (SAUTER-LOUIS et al., 2021b). One month later, also the federal state Saxony notified the first ASF case in wild boar in an area bordering with Poland (RICHTER et al., 2023). Sporadically, the disease was transmitted to domestic pig holdings in Germany as well.

In conclusion, a previously unseen epidemiologic scenario with different environmental and socioeconomical conditions and structures of pig industry occurred in the Baltic states, Poland and Germany. In these countries, mainly wild boar were affected by the epizootic and the numbers of cases in wild boar outnumbered outbreaks in domestic pigs by far (WOŹNIAKOWSKI et al., 2016; OĻŠEVSKIS et al., 2020; NURMOJA et al., 2020; MAČIULSKIS et al., 2020).

Large wild boar populations were present in these countries and served as a natural reservoir of ASF. Therefore, the new cycle was defined as "wild boar-habitat cycle" (CHENAIS et al., 2018) (Figure 3). In this cycle, ASF is transmitted directly between infected and susceptible wild boar and indirectly from contaminated carcasses (CHENAIS et al., 2018). It has been observed that wild boar sniff and poke on carcasses (PROBST et al., 2017) and that forms of cannibalism like consumption of muscles and organs can occur (CUKOR et al., 2020a; CUKOR et al., 2020b). Depending on environmental conditions, carcasses may remain infectious for several months especially at low temperatures contributing to ASF persistence in the habitat (FISCHER et al., 2020a). Through direct contact or improper disposal of waste, ASF can be transmitted between domestic pigs and wild boar, connecting the domestic pig and wild boar-habitat cycle.

The tick vector of the genus *Ornithodoros* does not play a role in this transmission cycle, since it is absent in most affected areas in Europe. Although the presence of *Ornithodoros* ticks has been described in some affected countries, involvement in the transmission is unlikely because wild boar do not use caves or burrows like warthogs (EFSA PANEL ON ANIMAL HEALTH AND

WELFARE, 2010; SAUTER-LOUIS et al., 2021a). There has been some discussion as to whether other vectors may be involved in the current disease scenario, but no evidence of this has been found. Herm et al. did not detect ASFV in various species of blood feeding arthropods that were collected in an Estonian area with high prevalence of ASF in wild boar in 2017 (HERM et al., 2021). However, experimental studies suggest that two hard tick species commonly distributed in Europe, *Ixodes ricinus* and *Dermacentor reticulatus,* or the stable fly *Stomoxys calcitrans* might play a role as potential mechanical rather than biological vectors (MELLOR et al., 1987; DE CARVALHO FERREIRA et al., 2014; OLESEN et al., 2018). However, there is no data verifying an epidemiologically relevant role of these potential vectors under field conditions.

Two main epidemiological scenarios of disease spread were observed in this new situation: On the one hand, the disease circulated in the wild boar populations and spread locally with slow average speed of 2 to 5 km per month (EFSA et al., 2017; MAČIULSKIS et al., 2020). On the other hand, long distance jumps of the virus have occurred, that were most likely human-mediated and caused e.g. by improper disposal of contaminated food.

The latter epidemiological scenario caused focal outbreaks of ASF in the Czech Republic in June 2017 and in Belgium in September 2018 more than 300 km away from the epidemic front (CHARVÁTOVÁ et al., 2019; LINDEN et al., 2019). In both countries, only wild boar were affected in relatively narrow areas and due to rapid intervention, the outbreaks could be contained (CHARVÁTOVÁ et al., 2019; WOAH, 2020). The Czech Republic declared freedom from the disease ten months after the first notification in 2018 (WOAH, 2019) and Belgium declared freedom from ASF in 2020 (WOAH, 2020). However, a new epidemiologic wave of ASF hit the Czech Republic in 2022, presumably caused by movement of infected wild boar from neighboring countries (STATE VETERINARY ADMINISTRATION OF THE CZECH REPUBLIC, 2023).

In the current epizootic, several other countries also reported ASF occurrence in wild boar or domestic pigs for the first time and have been affected ever since: Romania (2017), Hungary (2018), Bulgaria (2018), Slovakia (2019), Serbia (2019), Moldova (2020) and Greece (2020) (EFSA et al., 2021) (Figure 4). However, there is hardly any literature available on the epidemiological situation in these countries (SAUTER-LOUIS et al., 2021a). Most recently, ASF also emerged in North Macedonia (2022), Italy outside of Sardinia (2022), Croatia (2023), Bosnia and Herzegovina (2023), Kosovo (2023) and Sweden (2023) (World Animal Health Information System (WAHIS), https://wahis.woah.org/, accessed 11 September 2023) (Figure 4). It has to be noted that in several countries, particularly in southern Europe, e.g. Romania, Serbia or Moldova, outbreaks in domestic pigs dominate the ASF disease dynamics (LADOŞI et al., 2023) (Figure 5).

This is probably due to the local settings, which are dominated by backyard holdings with usually very low numbers of pigs and low biosecurity measures.

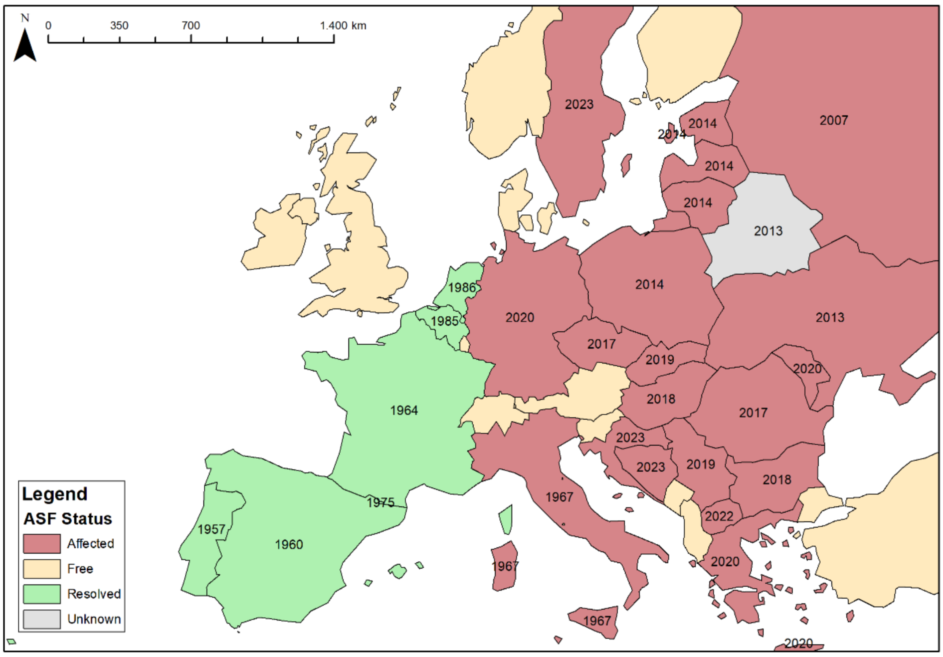

Figure 4. Overview of countries that have been or are still affected by ASF. The map displays the year of first occurrence as well as the current ASF status of the respective country. Administrative boundaries of countries were obtained from the Geoportal of the European Commission (Eurostat, available at https://ec.europa.eu/eurostat/web/gisco/geodata/reference-data/administrative-units-statistical-units/countries, accessed 31 July 2023). The data is based is on the geometry from EBM 2020 of EuroGeographics.

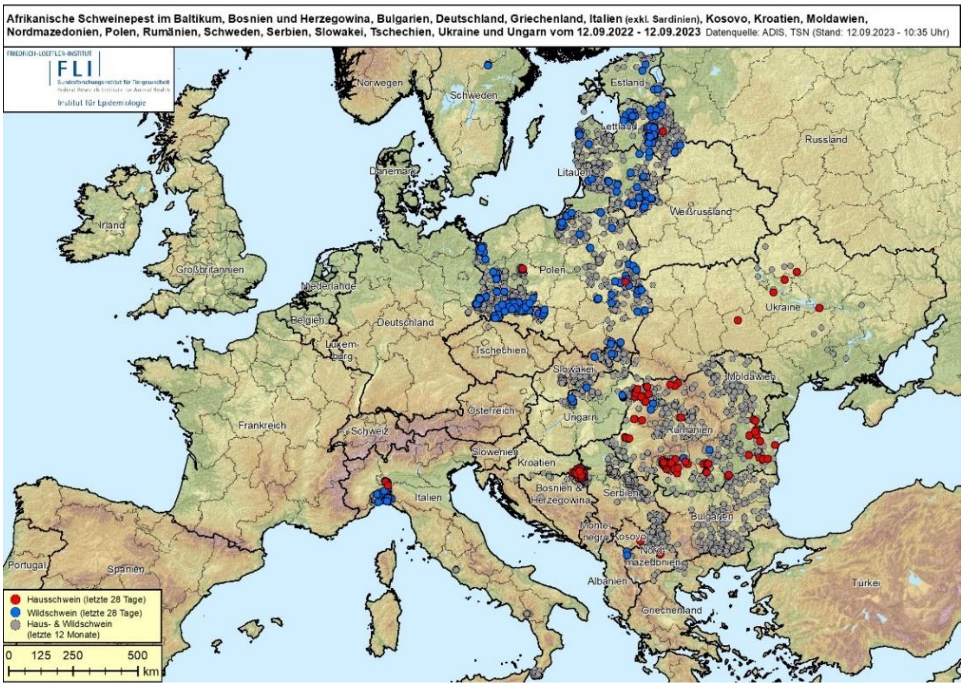

Figure 5. Current distribution of ASF cases in domestic pigs (red) and wild boar (blue) as of 12 September 2023. Older cases of the last 12 months (period from 12 September 2022 until 12 September 2023) are displayed in grey. The map is available at https://www.fli.de/de/aktuelles/tierseuchengeschehen/afrikanische-schweinepest/karten-zur-afrikanischen-schweinepest/ (accessed 12 September 2023).

2.6 Panzootic spread of ASF

ASF is not only widespread in European countries, but has become a panzootic global challenge. In August 2018, ASFV genotype II spread to China, which accounts for about half of the world's pig population (MIGHELL and WARD, 2021). Two years later, in 2021, also infections with ASFV genotype I with low virulence and lethality were detected in China (SUN et al., 2021). Subsequently, ASF spread to Vietnam, Mongolia, Cambodia, Laos, North Korea, Hong Kong, Myanmar, Philippines, Timor-Leste, and South Korea in 2019 (MIGHELL and WARD, 2021). Furthermore, ASF was also detected in India and Papua New Guinea in 2020, in Malaysia and Bhutan in 2021 and in Thailand and Nepal in 2022. Most recently, also Singapore was affected by ASF in 2023 (WAHIS, https://wahis.woah.org/, accessed 9 August 2023). In these countries, mainly domestic pig farms are affected by ASF. Long-distance pig movements, swill feeding and insufficient biosecurity appear to be common sources of disease transmission (MIGHELL and WARD, 2021; LI and TIAN, 2018). However, cases of ASF in wild boar were also reported in Asian countries and could contribute to disease transmission (MIGHELL and WARD, 2021; CADENAS-FERNÁNDEZ et al., 2022). It is suspected that ASF might circulate unnoticed in wild boar populations in Asia and that the epidemiologic role of wild boar in the current disease scenario is underestimated (CADENAS-FERNÁNDEZ et al., 2022; VERGNE et al., 2020).

Besides its emergence in Asia, ASF also reoccurred in the Americas. It was detected in the Dominican Republic and Haiti in 2021 after almost 40 years of absence (WAHIS, https://wahis.woah.org/, accessed 9 August 2023).

3 Surveillance and control of ASF in Europe

In the absence of therapeutic options to treat ASF or a vaccine to protect pigs from ASF infection, prevention and surveillance for early detection are essential strategies of ASF control. Furthermore, biosecurity and hygiene measures need to be applied. Prevention and control of ASF is regulated in the EU based on the Regulation (EU) 2016/429 (“Animal Health Law”) and its delegated and implementing acts Regulation (EU) 2018/1629, Regulation (EU) 2020/687 and Regulation (EU) 2023/594. These acts provide minimum requirements regarding disease control measures that member states have to implement. Listed as a Category A disease in the Animal Health Law, ASF requires immediate eradication measures to be applied when detected in a member state.

3.1 Surveillance strategies

In order to detect outbreaks and cases as soon as possible and to enable rapid and sufficient responses, effective surveillance strategies are needed. Both, active and passive surveillance are used in Europe.

According to the Animal Health Surveillance Terminology Final Report , passive (or reactive) surveillance is defined as an "observer-initiated provision of animal health related data (e.g. voluntary notification of suspect disease) or the use of existing data for surveillance” (HOINVILLE, 2013). In the context of ASF, passive surveillance includes notification, sampling and testing of domestic pigs or wild boar found dead or sick (DIXON et al., 2020; PALENCIA et al., 2023). It was suggested by a group of experts in the study by Jori et al. (2020) to distinguish between “routine” and “enhanced” passive surveillance (JORI et al., 2020). As long as an area is not affected by ASF and the perceived risk of ASF introduction is low, routine passive surveillance includes reporting wild boar found dead or shot sick. In case of ASF outbreaks or wild boar cases in an area, passive surveillance should be enhanced (JORI et al., 2020).

Active (or proactive) surveillance is defined as an “investigator-initiated collection of animal health related data using a defined protocol to perform actions that are scheduled in advance” according to the Animal Health Surveillance Terminology Final Report (HOINVILLE, 2013). Thereby, the investigator decides what information will be collected from which animals (HOINVILLE, 2013). Active surveillance of ASF includes targeted sampling of living domestic pigs or sampling of apparently healthy hunted wild boar (DIXON et al., 2020; PALENCIA et al., 2023).

Both passive and active surveillance are based on the cooperation with respective stakeholders such as farmers, hunters or rangers and their willingness to participate in surveillance. Even though the search for carcasses has been rated as less feasible activity by experts since it can be time-consuming and cost-intensive (PALENCIA et al., 2023; GUINAT et al., 2017), it has been demonstrated that passive surveillance is an effective tool for the early detection of ASF (ŚMIETANKA et al., 2016; SCHULZ et al., 2019b). Thus, it is of utmost importance to consider stakeholder perceptions and assess factors that might hinder engagement in surveillance.

3.2 Prevention and control strategies in domestic pigs

For domestic pig farms, an important strategy is to hinder potential interactions between domestic pigs and wildlife, e.g. by fencing outdoor areas or keeping pigs indoors (JURADO et al., 2018a). Thus, spill-overs in both directions can be prevented. Furthermore, illegal trade with pigs or pig products, illegal pig movements and improper waste disposal should be avoided (DIXON et al., 2020). Strict biosecurity measures should be applied e.g. cleaning and disinfection of facilities, equipment, clothing and vehicles (JURADO et al., 2018a). Furthermore, staff needs to be educated on these measures as well as of clinical signs of the disease (GAVIER-WIDÉN et al., 2015; JURADO et al., 2018a).

In the EU, in case of an outbreak, the legally required response includes depopulation of the affected farm, i.e. culling of all pigs. In addition, cleaning and disinfection of stables and potential fomites and implementation of movements restrictions are required according to the EU legislation. A protection zone (minimum 3 km) and a surveillance zone (minimum 10 km) are established around the outbreak farm, in which pig movements are restricted and neighboring farms are surveyed. Epidemiological investigations are necessary to identify the potential source of infection and to trace contacts (backwards and forwards) in order to detect or prevent potential secondary outbreaks.

3.3 Prevention and control strategies in wild boar

In almost all affected countries in Europe, wild boar are abundant and the population is constantly increasing (MASSEI et al., 2015). ASFV can persist in the wild boar population due to the high population density and the long survival of the virus in the environment especially at low temperatures (FISCHER et al., 2020a). Thus, contaminated wild boar carcasses pose a risk of ASFV transmission (CHENAIS et al., 2019). The complex interplay between the virus, wildlife hosts and environmental factors makes prevention and control of ASF in the wild boar population much more challenging than in domestic pigs. Intervention options to contain ASF in the wild boar population need to focus on preventive actions, wild boar population control and zoning according to the EU legislation (PALENCIA et al., 2023).

After confirmation of an ASF case in wild boar, restriction zones could be established around the index case (Figure 6). The infected zone could be fenced and hunting bans and entry bans for the general public should be established in order to hinder wild boar movements, following the example of the Czech Republic or Belgium (Figure 6). However, fencing is controversially discussed among stakeholders and experts due to its ecological impact (JORI et al., 2020; URNER et al., 2021a; STONČIŪTĖ et al., 2022). The effectiveness of fencing in controlling ASF is highly dependent on the local situation of the outbreak (JORI et al., 2020). It may be used successfully to control focal ASF outbreaks, like in the Czech Republic or in Belgium (CHARVÁTOVÁ et al., 2019). Also in front-like scenarios, like in Germany, it may contribute to controlling or at least slowing down the spread of ASF (RICHTER et al., 2023).

In a buffer zone surrounding the infected zone, population management should be applied carefully with minimal disturbance of wild life (Figure 6). In both zones, organized carcass searches and carcass removal contribute to reducing the risk of further ASF transmission from contaminated carcasses (CHENAIS et al., 2019). In the free zone around the buffer zone, depopulation measures should be implemented (Figure 6). Increased hunting contributes to preventively reducing the number of susceptible animals, thus reducing the risk of introduction and spread of ASF (GAVIER-WIDÉN et al., 2015; LANGE, 2015). Thereby, sanitary measures before, during and after hunting are of utmost importance. The usage of technical aids for hunting e.g. night vision or usage of wild boar traps can facilitate the depopulation (JORI et al., 2020). However, these strategies may be perceived controversially by stakeholders like hunters and need to be communicated carefully (JORI et al., 2020; STONČIŪTĖ et al., 2022; OELKE et al., 2022). Additionally, it is of utmost importance to raise awareness in the general public about the risk of ASF introduction and the pathways of ASF spread in so far non-affected regions (GAVIER-WIDÉN et al., 2015; SAUTER-LOUIS et al., 2021a).

However, described measures need to be adapted to the respective epidemiologic outbreak scenario. Whereas in the case of point introductions, measures can be applied locally and in a concentrated manner, in the case of frontal introductions measures often have to be applied over a large area or in several places at the same time. This requires more resources, such as financial means, technical tools and personnel, and is therefore much more challenging.

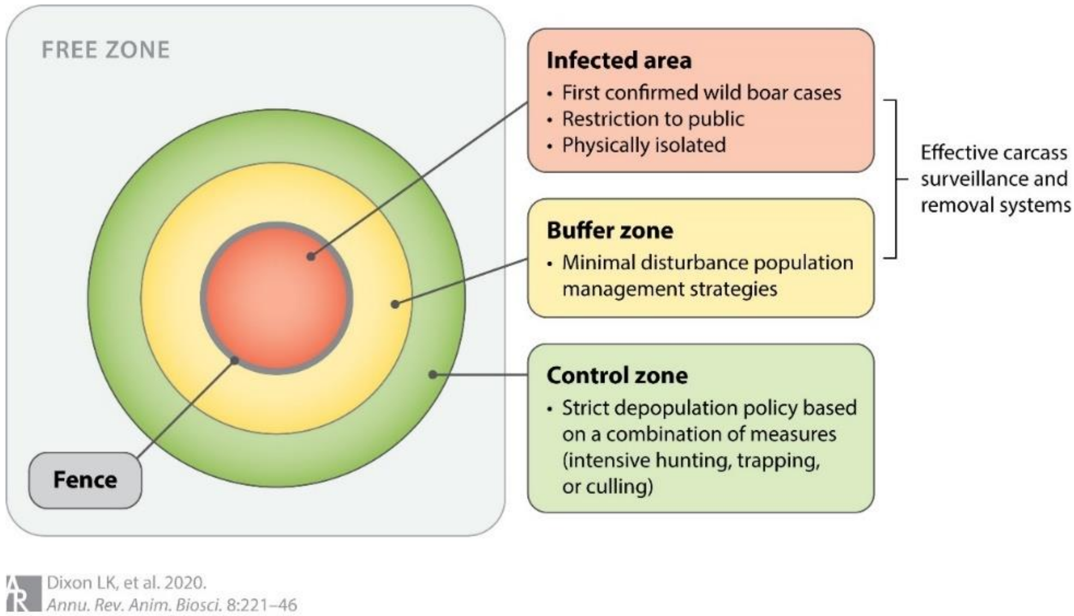

Figure 6. Restriction zones around an ASF case in the wild boar population (DIXON et al., 2020). In each area, different strategies are applied. The figure was taken from the publication by Dixon et al. (2020) according to the CC-BY 4.0 license (https://creativecommons.org/licenses/by/4.0/).

4 ASF in Germany: Spread, surveillance and control

In Germany, the diagnosis, surveillance and control of ASF is regulated by EU and by national legislation under the German Animal Health Act (Tiergesundheitsgesetz, TierGesG) and the Swine Fever Ordinance (Schweinepestverordnung, SchwPestV).

According to the legislation, diagnosis of ASF is performed initially in official state laboratories in the respective federal state according to the German official collection of methods for notifiable diseases (https://www.fli.de/de/publikationen/amtliche-methodensammlung/, accessed on 19 July 2023). Positive and uncertain results from state laboratories must be confirmed at the NRL. Only after verification by the NRL, positive cases are officially confirmed.

Since the first report of ASF in the EU in 2014, Germany started to increase surveillance activities and to prepare for eventual disease introduction (SAUTER-LOUIS et al., 2021b). These activities were even more intensified once the first case of ASF emerged in western Poland close to the German border in 2019 (MAZUR-PANASIUK et al., 2020). Additionally, a mobile fence was set up along parts of the border (SAUTER-LOUIS et al., 2021b). Thus, the first notification of ASF in wild boar in Germany in the federal state of Brandenburg in 2020 was not completely unexpected. However, ASF has since then spread in the wild boar populations in parts of the federal states of Brandenburg and Saxony resulting in great case numbers (Table 1). Wild boar movements from the infected area in Poland and within these German federal states are suspected to be the main driver of this epizootic. Several infection clusters have been detected in Germany, suggesting repeated introduction of ASF across the German-Polish border (SAUTER-LOUIS et al., 2022; FORTH et al., 2023). However, also human-associated spread might have occurred in Germany, e.g. in 2021 when ASF was notified in a district in Saxony that was more than 60 km distant from the initially affected region (RICHTER et al., 2023). Another “jump” of ASF was observed in a district of the federal state of Mecklenburg-Western Pomerania, when ASF was notified in wild boar approximately 140 km distant from the nearest wild boar case in 2021 (Figure 5). Only few cases were detected in the affected district in Mecklenburg-Western Pomerania in 2021 and 2022 (Table 1).

Several control measures were implemented building on the experiences of the Czech Republic and Belgium that were able to eliminate the disease successfully through fencing, depopulation and enhanced passive surveillance (CHARVÁTOVÁ et al., 2019; LINDEN et al., 2019). Likewise, in Germany restriction zones were established, intensified hunting as well as search for carcasses (using humans, dogs or drones) were implemented and fences were constructed to prevent further spread of ASF in the wild boar population (SAUTER-LOUIS et al., 2022). These strategies appear to be successful at least in slowing down the westwards spread of ASF (RICHTER et al., 2023). However, the German scenario differs from the ones in Belgium and the Czech Republic, since there is a constant infection pressure at the border with Poland leading to a constant risk of new introductions (SAUTER-LOUIS et al., 2022). Surveillance and control measures have to be implemented simultaneously in different locations for an unpredictable period of time. In such a scenario, the available resources must be used in the most targeted and efficient way possible.

The implementation of the described measures relies massively on the engagement of involved stakeholders e.g. local authorities, veterinarians, hunters or farmers. Thus, it is of utmost importance to keep up their willingness to engage in these measures. In order to motivate stakeholders, the affected federal states usually pay financial incentives to hunters and carcass searchers. However, the type of incentives and amount of payments varies across the different German federal states. For example, in Brandenburg financial rewards of up to 150€ are paid for notification of wild boar found dead and hunted wild boar (Ministerium für Soziales, Gesundheit, Integration und Verbraucherschutz des Landes Brandenburg (MSGIV), https://msgiv.brandenburg.de/msgiv/de/themen/verbraucherschutz/veterinaerwesen/tierseuchen/afrikanische-schweinepest/, accessed 31 July 2023).

ASF has sporadically spilled over to domestic pig farms in Germany as well (Table 1). So far, five outbreaks in domestic pigs occurred in the federal state Brandenburg and one outbreak occurred each in the federal states Mecklenburg-Western Pomerania, Lower Saxony and Baden-Wurttemberg (Table 1). Half of the outbreaks occurred on small-scale farms with 2 to 35 pigs. Three outbreaks occurred in medium-scale farms with 313 to 1,830 pigs, and one outbreak affected a large-scale farm with over 4,000 pigs (VAN DOOREN, 2023). In accordance with the EU and national legislation, all pigs on the affected farms were culled. Unfortunately, the respective sources for disease introduction could not be identified with certainty in epidemiological investigations for all the outbreaks. However, mostly, human-associated transmission or insufficient biosecurity were hypothesized.

Table 1. Confirmed cases of ASF in wild boar (WB) and outbreaks in domestic pigs (DP) per year in German federal states from the first notification of ASF on 10 September 2020 until 12 September, 2023**.** Data source: Tierseuchennachrichtensystem (TSN) (https://www.fli.de/en/services/information-systems-and-databases/tsn/, accessed 12 September 2023).

	2020		**2021**		**2022**		**2023***		**Total***	
	WB	**DP**	**WB**	**DP**	**WB**	**DP**	**WB**	**DP**	**WB**	**DP**
Brandenburg	386	0	1922	3	533	1	394	0	**3235**	**5**
Saxony	17	0	791	0	1027	0	430	0	**2265**	**0**
Mecklenburg-Western Pomerania	0	0	7	1	40	0	0	0	**47**	**1**
Lower Saxony	0	0	0	0	0	1	0	0	**0**	**1**
Baden-Wurttemberg	0	0	0	0	0	1	0	0	**0**	**1**
Total*	**403**	**0**	**2720**	**4**	**1600**	**3**	**824**	**1**	**5547**	**8**

* Numbers as of 12 September 2023.

5 Impact of ASF and its control in Europe

The panzootic spread of ASF and resulting control measures have a tremendous impact on economy, animal welfare, nature- and animal conservation as well as on the lives of all involved stakeholders.

Although in most European countries ASF is primarily a disease of the wild boar population, the pig industry has also been affected by the disease. Notification of ASF is usually followed by restrictions in pig movements and (inter-)national trade, since countries have the right to ban imports of pigs and pork products from ASF affected areas (NIEMI, 2020; WORLD TRADE ORGANIZATION, 2020). Thus, a decrease in the production and export of pork and pork products is observed in affected countries with industrialized domestic pig productions leading to great economic losses (HALASA et al., 2016a, 2016b; SÁNCHEZ-CORDÓN et al., 2018; NIEMI, 2020). In addition, high costs arise for the implementation of surveillance and control measures and eradication programs in both the domestic pig and wild boar sector (HALASA et al., 2016a, 2016b; MUR et al., 2016b; LADOŞI et al., 2023). As a consequence of implemented control measures, the structure of the pig industry may change, because the number of pig farms and the size of pig herds may decrease once a country has become affected by ASF (NIEMI, 2020; LADOŞI et al., 2023). In addition, it is likely that backyard holdings subsequently disappear (LADOŞI et al., 2023).

Aspects of animal welfare must not be neglected alongside the serious economic consequences of an ASF outbreak. Besides large numbers of deaths of domestic pigs and wild boar due to the severity of the disease, large numbers of animals have to be culled according to the legislation in order to stop the spread of ASF (SÁNCHEZ-CORDÓN et al., 2018; MIGHELL and WARD, 2021; LADOŞI et al., 2023). In addition to that, also certain preventive measures can have negative impacts on animal welfare, such as the requirement to keep domestic pigs indoors in affected areas, which is particularly challenging for organic-producing farms. These aspects of ASF prevention and control may also have a significant impact on affected people's livelihood. In the domestic pig sector, farmers, veterinarians and other involved stakeholders may experience severe mental distress due to the culling of large numbers of animals. It has been described that volunteers showed signs of post-traumatic stress disorder after being involved in the disease control of foot-and-mouth disease (HIBI et al., 2015).

In addition to the impact on animal welfare, ASF control measures in the wild boar sector, e.g. fencing can disrupt sensitive wildlife ecosystems and affect many wildlife species, compromising animal and nature conservation interests. As a result, this measure is controversial among stakeholders and experts (JORI et al., 2020; URNER et al., 2020; URNER et al., 2021b).

Especially in the current situation in Europe, where ASF is circulating in the wild boar population of many countries and therefore measures have to be implemented for a long time and in different areas at the same time, stakeholders may be stretched to their limits and as a result, acceptance and compliance for implementing measures may decrease. Therefore, it is of utmost importance to consider the interests and perceptions of stakeholders (JORI et al., 2020). Experts concluded that the communication with affected stakeholders is a key factor for efficient ASF prevention and control (GAVIER-WIDÉN et al., 2015; JORI et al., 2020). Communication channels and participation should be established prior to potential outbreaks and cases in order to be best prepared. Additionally, financial compensation for stakeholders appears to be a motivating factor that increases participation in measures such as carcass search and reporting (URNER et al., 2021a; STONČIŪTĖ et al., 2022)

III. Objectives

Objective 1: Identification of predilection sites for detecting ASF-positive wild boar carcasses *(Publication I and II)*

Carcasses of ASF-infected wild boar pose a risk of infection for their conspecifics (CHENAIS et al., 2019). Especially at low temperatures, carcasses may remain infectious over long time periods, increasing the risk for disease transmission (FISCHER et al., 2020a). However, the search for carcasses can be a challenging time-consuming activity, requiring skilled personal, technical aids and trained search dogs. Thus, to make best use of available resources, our studies aimed to identify predilection sites for detection of ASF-positive carcasses based on landscape characteristics and to assess whether real-time classification of field crops through satellite remote sensing can support targeted carcass search efforts.

Objective 2: Investigation of seasonal patterns of ASF occurrence in Europe *(Publication III and IV)*

Seasonal patterns of ASF occurrence have been observed both in domestic pigs (CHENAIS et al., 2019; NURMOJA et al., 2020) and wild boar (PAUTIENIUS et al., 2018; FRANT et al., 2020). However, study areas and analyzed periods varied and were most often limited to one country in these studies. Our studies aimed to gain a better understanding of the seasonal patterns of ASF in wild boar and domestic pigs in different European countries. Investigation of seasonal dynamics and the environmental, ecological and behavioral factors influencing them is necessary to adapt targeted surveillance and control efforts in high-risk periods.

Objective 3: Considering German hunters' perception of ASF surveillance and control in wild boar (*Publication V)*

Hunters appear to be crucial stakeholders when it comes to the implementation of surveillance and control measures, such as intensified hunting, carcass search and sampling of wild boar (JORI et al., 2020; URNER et al., 2021a). The efficiency of these measures is highly dependent on their participation and compliance. Our study aimed to elucidate how German hunters assess the effectiveness of these measures and which obstacles they experience when participating in ASF surveillance and control as well as to identify motivational factors to increase their participation.

IV. PUBLICATIONS

The publications included in this thesis are grouped according to their topic and presented as a part of the results section. The reference section of each manuscript is presented in the style of the respective journal and is not included at the end of this thesis. The numeration of figures and tables corresponds with the published form of each respective manuscript.

1 Publication I: Identification of predilection sites for wild boar carcass search based on spatial analysis of Latvian ASF surveillance data

Identification of predilection sites for wild boar carcass search based on spatial analysis of Latvian ASF surveillance data

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Identification of predilection sites for wild boar carcass search based on spatial analysis of Latvian ASF surveillance data

Lisa Rogoll[1✉], Katja Schulz[1], Christoph Staubach[1], Edvīns Oļševskis[2,3], Mārtiņš Seržants[2], Kristīne Lamberga[2], Franz Josef Conraths[1] & Carola Sauter-Louis[1]

Targeted search for wild boar carcasses is essential for successful control of African swine fever (ASF) in wild boar populations. To examine whether landscape conditions influence the probability of finding ASF-positive carcasses, this study analyzed Global Positioning System (GPS) coordinates of Latvian wild boar carcasses and hunted wild boar, extracted from the CSF/ASF wild boar surveillance database of the European Union, and random coordinates in Latvia. Geographic information system (GIS) software was used to determine the landscape type and landscape composition of carcass detection sites and to measure distances from the carcasses to nearest waterbodies, forest edges, roads and settlements. The results of the automated measurements were validated by manually analyzing a smaller sample. Wild boar carcasses were found predominantly in forested areas and closer to waterbodies and forest edges than random GPS coordinates in Latvia. Carcasses of ASF-infected wild boar were found more frequently in transitional zones between forest and woodland shrub, and at greater distances from roads and settlements compared to ASF-negative carcasses and random points. This leads to the hypothesis, that ASF-infected animals seek shelter in quiet areas further away from human disturbance. A detailed collection of information on the environment surrounding carcass detection sites is needed to characterize predilection sites more accurately.

African swine fever (ASF) is a viral disease that constitutes a threat for domestic pigs and wild boar worldwide. The disease is characterised by haemorrhagic fever, which leads to case/fatality ratios of up to 100%[1].

Since the introduction of ASF virus of genotype II into Georgia in 2007, the disease constantly spread over Europe and Asia, posing a constant threat to wild boar populations and domestic pigs[2,3]. In 2014, the first cases of ASF were detected in Latvia in wild boar in the eastern part of the country close to the border with Belarus[4]. Shortly after, a long-distance jump of the virus to the northern regions occurred, which was most likely human-mediated[5]. In the following years, ASF subsequently spread westwards throughout the country and reached the central part of Latvia in summer 2016[5]. By the end of 2019, the infection was present in wild boar in around 85% of the area of Latvia[6].

The lasting presence of ASF in wild boar populations increases the risk of introduction of the virus into domestic pig farms[7–10], which can lead to great suffering in affected pigs and massive socio-economic losses in the pig industry, caused in particular by trade and movement restrictions[11,12]. It is therefore of utmost importance to control the spread of the disease in wild boar populations.

In Latvia, long-lasting infection cycles in wild boar populations with endemic character established[13]. This infection cycle has been described as the "wild boar-habitat cycle" with direct transmission amongst infected and susceptible wild boar as well as indirect transmission through carcasses of infected wild boar present in the habitat[14]. Direct contact of wild boar with the carcasses such as sniffing and poking on carcasses[15] and forms of cannibalism like consumption of muscles and organs[16] have been observed. In addition, carcasses may remain infectious over long periods especially at low temperatures[17] causing local virus persistence in wild boar habitats. For these reasons, rapid search and disposal of wild boar carcasses is considered as one of the most important measures to control ASF in wild boar populations[18].

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Several studies have also shown that the probability of detecting ASF-positive wild boar is clearly higher among wild boar found dead compared to hunted animals[2,19,20]. This finding underlines the importance of passive surveillance through intensive carcass search and sampling.

Nevertheless, the search for wild boar carcasses is a time-consuming, cost-intensive and thus unpopular measure among hunters[21]. Although experts consider carcass search as an effective measure for ASF control, it is rated as less practical[22]. Accordingly, it seems reasonable to develop strategies for conducting carcass search in a more targeted and thus resource-efficient manner. One possible starting point for this is the hypothesis that ASF-infected wild boar prefer different habitats compared to their healthy conspecifics due to symptoms such as fever and depression.

Several studies already tested the hypothesis whether certain characteristics of the habitat increase the probability of finding an ASF-positive carcass and whether this can be used to identify possible predilection sites for carcass search—with variable results. It has been observed that ASF is more likely to occur in forest areas[23–25]. It has been demonstrated that ASF-positive carcasses are more likely to be found in younger forest stands up to 40 years of age in quiet places more distant from roads and forest edges[23], in areas of transition between woodland and shrub consisting of younger plants[26] and in cool and moist habitats further away from rivers[26]. Lim et al. reported that the numbers of ASF-infected carcasses were higher in regions with a low heat load index[27]. While it was observed in some studies that indicators of human activity such as the numbers of roads/settlements or human population density were positively associated with the notification of ASF in wild boar[24,27], others found an inverse influence of human activity on ASF case probability[23,25].

Based on these variable findings, our study aimed to make use of Latvian surveillance data to identify possible predilection sites for the search of ASF-positive carcasses in Latvia and thus to support the detection of wild boar carcasses.

Materials and methods

Data and study area

The data examined in this study originated from the EURL CSF/ASF wild boar surveillance database[28]. The following information was extracted from the database for each record: a unique identifier, the date of finding/shooting of each wild boar, the carcass type (found dead or shot dead), age (as estimated by the reporting person), sex and the results of virological and serological examination (ASF-positive or ASF-negative) as well as Global Positioning System (GPS) coordinates of the place, where the carcass had been detected. In case more than one animal was found/shot in a position with exactly the same coordinates on the same date, the coordinates were only considered once for the analysis. The final data set consisted of 11,577 records including 1444 ASF-positive and 606 ASF-negative wild boar found dead as well as a sample of 9527 ASF-negative wild boar hunted apparently healthy (randomly selected from all records of ASF-negative wild boar hunted apparently healthy in the database) in Latvia from June 2014 through to February 2021 (Fig. 1).

The study area covered the total area of Latvia with a size of 64,589 km^2[29] (Fig. 1). According to Corine Land Cover (CLC) 2018 map data (100 m/25 ha resolution)[30], the majority of the country is covered by agricultural area (39.6%), forests (37.6%) and transitional-woodland-shrub (16.0%). The forest area can furthermore be divided into broad-leaved (8.1%), coniferous (12.8%) and mixed forest (16.7%). The remaining area of Latvia is composed of waterbodies (2.0%), wetlands (2.5%), open spaces (0.1%), urban area (2.0%) and scrub and/or herbaceous vegetation association (0.1%). Based on hunting management units (HMU) of the State Forest Service in Latvia, the majority of agricultural area is composed of cereal fields (0.0% up to 48.7% per HMU), grassland (0.0% up to 43.1% per HMU) and rapeseed fields (0.0% up to 16.9% per HMU) (https://www.silava.

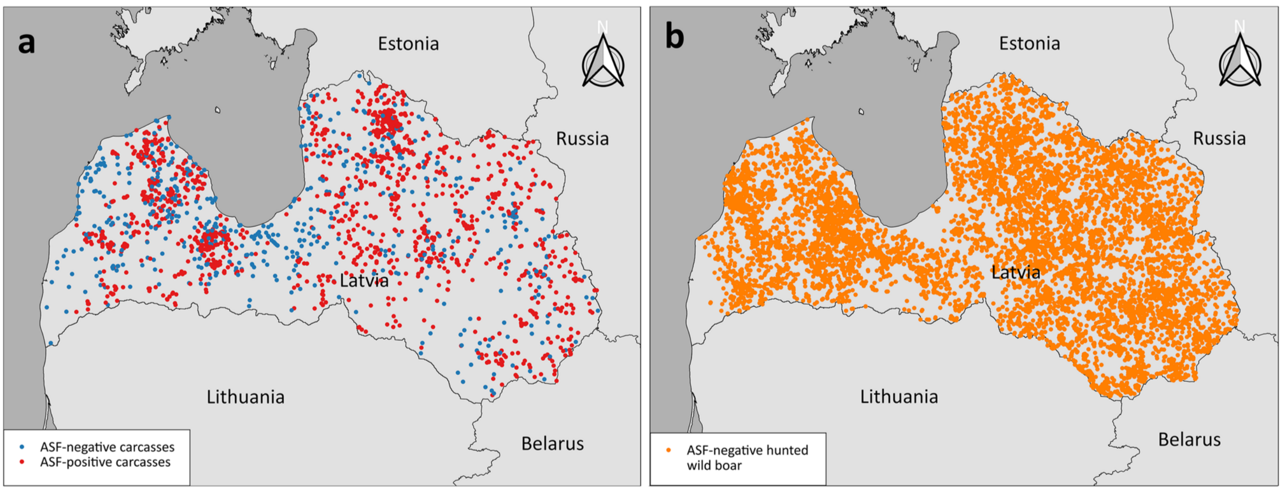

Figure 1. Overview of study area (Latvia) and GPS locations extracted from the EURL CSF/ASF wild boar surveillance database[28] for (**a**) ASF-negative (n = 606) and ASF-positive (n = 1444) carcasses found dead and (**b**) randomly chosen ASF-negative animals shot dead apparently healthy (n = 9527) from 2014 through to 2021. The map was created using QGIS Desktop 3.20.2[31].

lv/images/Petijumi/2022-LVM-Rekomendacijas-briezu-dzimtas-parnadzu-medibu-parvaldibas-pilnveidosanai/2023-LVM-Rekomendacijas-briezu-dzimtas-parnadzu-medibu-parvaldibas-pilnveidosanai-II-etaps.pdf, accessed 1 November 2023).

In 2015, no ASF-negative carcasses found dead were recorded with unique GPS-locations (Fig. 2). ASF-positive cases mainly originated from the years 2015 to 2017 (Fig. 2). The analysis of the monthly distribution of records (Supplementary Fig. S1) showed that animals were more frequently hunted in winter (n = 5702) than in summer months (n = 3825). In contrast, a higher proportion of carcasses were found in summer months (317 ASF-negative and 826 ASF-positive carcasses) than in winter months (289 ASF-negative and 618 ASF-positive carcasses).

The final data set contained information about the estimated age of the animals shot or found dead in 11,212 of 11,577 cases. The majority of animals (6107 out of 11,212; 55%) was approximately 1 to 2 years old at the time of death (Supplementary Fig. S2). The data set contained information about the sex of the animals for 10,609 of 11,577 cases, out of which 53% were male and 47% female (Supplementary Fig. S3).

In addition, a set of 10,000 random and independent GPS coordinates in Latvia was created as a control, whereby areas of water bodies were excluded.

Landscape type and landscape composition

The landscape type at the location, where a wild boar carcass had been detected or wild boar were hunted, and the landscape composition in a buffer zone with a radius of 3 km around the location to reflect potential moving distances and home ranges of wild boar[32,33] were analyzed using the geographic information system (GIS) software ArcGIS ArcMap 10.8.1[34]. The CLC map data was used within the projected coordinate system LKS92/Latvia TM (EPSG:3059). For both analyses, existing CLC categories were grouped together to the following landscape categories: (i) Forest (for analysis of landscape composition divided into broad-leaved, coniferous and mixed forest), (ii) agricultural area (including fields, arable land, crops and pastures), (iii) transitional woodland-shrub, (iv) waterbodies, (v) wetlands, (vi) open spaces with little or no vegetation (e.g. beaches, dunes, rocks), (vii) urban areas and (viii) scrub and/or herbaceous vegetation associations (including moors and heathland, natural grassland and sclerophyllous vegetation). For the analysis of the landscape type of detection sites and random points, a landscape category was allocated to each location using the ArcMap tool "Spatial Join". To analyze the landscape composition around the locations, the proportion of the area of different landscape categories in the buffer zones was calculated using the ArcMap tool "Intersect" and the field calculator.

Since CLC only provides landcover information for European countries, the landscape composition of buffers lying close to the border with Belarus and Russia could not be analyzed completely due to lack of data. This affected 115 records from the database and 87 random points. The respective records were excluded from the evaluation of the landscape composition.

Distance measurements

In a second step, the shortest distances of the locations, where wild boar carcasses had been detected or wild boar were hunted, to the next waterbody, road, settlement and forest edge were measured automatically using the GIS

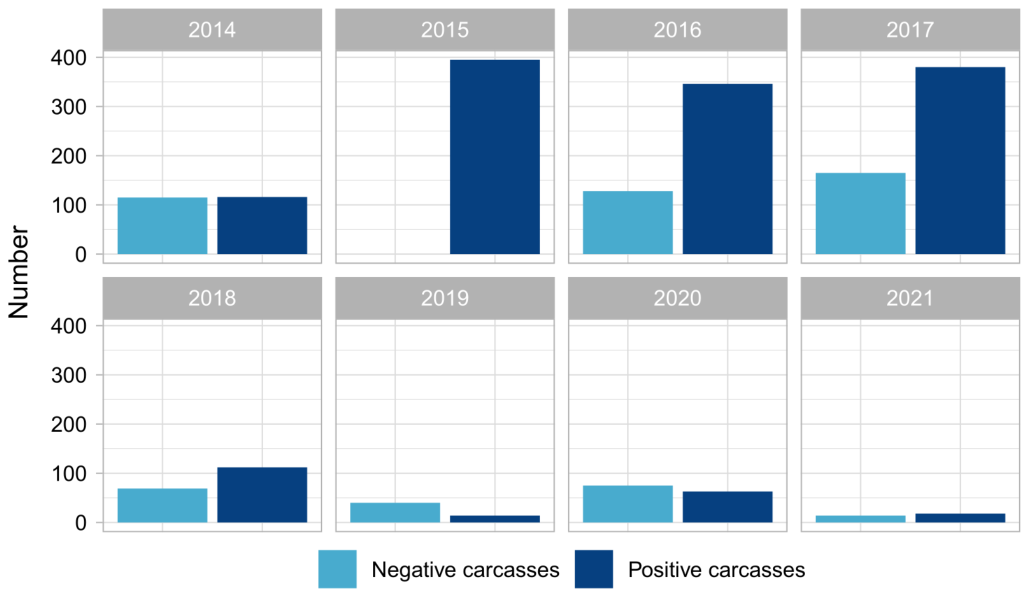

Figure 2. Numbers of records per year (from 2014 to 2021) extracted from the EURL CSF/ASF wild boar surveillance database[29] for ASF-negative carcasses (n = 606) and ASF-positive carcasses (n = 1444) found dead in Latvia used in the analysis.

software QGIS Desktop 3.20.2[31] and the plug-in "NNJOIN"[35]. For polygon layers, the shortest distance to the external boundary of the polygon was measured for both locations within and outside the polygon.

For the distance to the next waterbody, map data for rivers as well as inland and marine waterbodies from a Latvian topographic map (25 m resolution)[36] were used. To measure the distance to the next road, we used OpenStreetMap (0.4 m resolution)[37]. Only major roads suitable for cars were included in the measurements (feature classes "trunk", "primary", "secondary", "tertiary" as well as roads named with a "V"). Data for measuring the distance to the next settlement was obtained from the Copernicus European Settlement map (2 m resolution)[38]. The distance to the next forest edge was measured by using the Copernicus Forest Type map (10 m resolution)[39]. Thereby, the forest edge corresponded to the interface between forest and other habitat types.

Manual analysis

Manual analyses were performed to validate the results of the automated analyses of landscape types and distance measurements. Therefore, a smaller sample (n = 599) of ASF-positive carcasses (n = 249), ASF-negative carcasses (n = 175) and ASF-negative hunted wild boar (n = 175) was randomly selected from the original data set. For these locations, landscape type was examined and distances were measured manually within the record viewer of the EU CSF/ASF wild boar surveillance database using integrated orthophotos and a ruler tool. Beforehand, criteria for landscape analysis were set (Supplementary Table S1 and Supplementary Table S2).

Statistical analysis

Differences between ASF-positive carcasses, ASF-negative carcasses, ASF-negative hunted wild boar and random points were analyzed using non-parametric test methods, since the data were not normally distributed (tested with Kolmogorov–Smirnov-Test). Fisher's Exact and Kruskal–Wallis tests with subsequent pairwise Mann–Whitney-U-Test with Bonferroni correction were used for group comparisons. P-values of less than 0.05 were considered statistically significant.

Based on the date of detection or shooting of wild boar, respectively, two subgroups of the final data set were formed and tested for seasonal differences: (a) entries from summer months (April to September) and (b) entries from winter months (October to March). These analyses of the landscape type and landscape composition as well as distance measurements were performed with the whole data set and separately for the summer- and winter group.

In order to account for interactions between variables, multivariable logistic regression was performed to identify significant predictors, which increase the chance of finding ASF-positive carcasses. The outcome variable was the infection status of a carcass found dead (ASF-negative vs. ASF-positive). The tested predictors were the distances to certain landscape features (scaled to 100 m steps) and proportions of different landscape types (scaled to 10% steps).

To test for spatial autocorrelation, Moran's I, Geary's C and semi-variograms were calculated on the standardized deviance residuals of ordinary univariable generalized mixed models (GLM), as described by Cressie[40] and Diggle and Ribeiro[41]. To correct for spatial autocorrelation univariable generalized estimated equation (GEE) models were implemented[42]. The predictive quality of the univariable models was evaluated by calculating the area under the curve (AUC) of receiver operating characteristic (ROC) plots. Only predictors with p-values below 0.2 or AUC above 0.55 were retained in the multivariable GEE model. The final model was developed using backward elimination, whereby only predictors with p-values below 0.05 were included in the final model.

All statistical evaluations were conducted with the statistic software R[43] using Rstudio 4.0.3[44] as an interface. The packages tidyverse[45], dplyr[46] and lubridate[47] were used for data management and the package ggplot2[48] was used for visualizing of results. The package geoR was used to calculate semivariograms[49], the packages gee[50] and MASS[51] were used to implement GEE models, and the package epiDisplay[52] was used to create ROC plots. Supplementary Table S3 provides an overview of datasets and statistical methods used for each step of the analyses.

Results

Landscape type

The distribution of different landscape categories (Fig. 3 and Supplementary Table S4) of detection sites of ASF-positive carcasses differed statistically significantly from those of ASF-negative carcasses ($p < 0.001$). Although carcasses were often found in the forest, the proportion was higher for ASF-positive carcasses than for ASF-negative carcasses. By contrast, a higher proportion of ASF-negative carcasses was found on fields. The number of animals found in transitional woodland-shrub was higher for ASF-positive carcasses than for ASF-negative carcasses. In contrast to that, animals were mainly shot on fields and less frequently in forests and transition zones. In addition, the distribution of the landscape categories of random points differed statistically significantly from the results of ASF-positive carcasses found dead and ASF-negative hunted wild boar (both $p < 0.001$). Results of all pairwise comparisons are shown in Supplementary Table S5.

Only small proportions of records (animals and random points) were associated with other landscape types, such as urban areas, waterbodies, wetlands, open spaces with little or no vegetation and scrub and/or herbaceous vegetation associations (Supplementary Table S4). The proportion of ASF-positive carcasses found in urban areas (0.6%) was smaller compared to negative carcasses (3%) and random points (2%).

The analysis of the two seasonal subgroups showed that the distribution of detection or hunting sites, respectively, differed statistically significantly in the Fisher's Exact test between summer and winter months for ASF-positive carcasses ($p = 0.003$) and ASF-negative hunted wild boar ($p < 0.001$) (Supplementary Table S6).

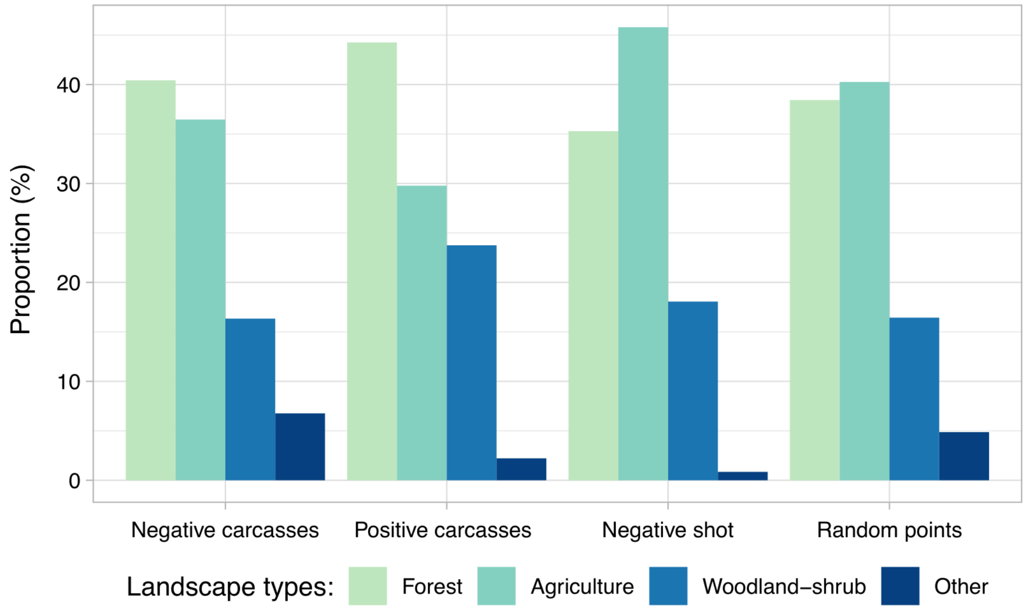

Figure 3. Proportions of landscape types (forest, agricultural area, transitional woodland-shrub and other locations) for ASF-negative carcasses (n = 606), ASF-positive carcasses (n = 1444), ASF-negative wild boar shot apparently healthy (n = 9527) between 2014 and 2021 and random points (n = 10,000) in Latvia. Other locations include waterbodies, wetlands, open spaces with little or no vegetation (e.g. beaches, dunes, rocks), urban areas and scrub and/or herbaceous vegetation associations (including moors and heathland, natural grassland and sclerophyllous vegetation).

Landscape composition

The main landscape components in a buffer zone of 3 km radius around the locations of ASF-positive (n = 1423) and -negative carcasses (n = 605), ASF-negative hunted animals (n = 9434) and random points (n = 9913) were forests, agricultural area and transitional woodland-shrub for all study groups (Fig. 4). The proportions of water,

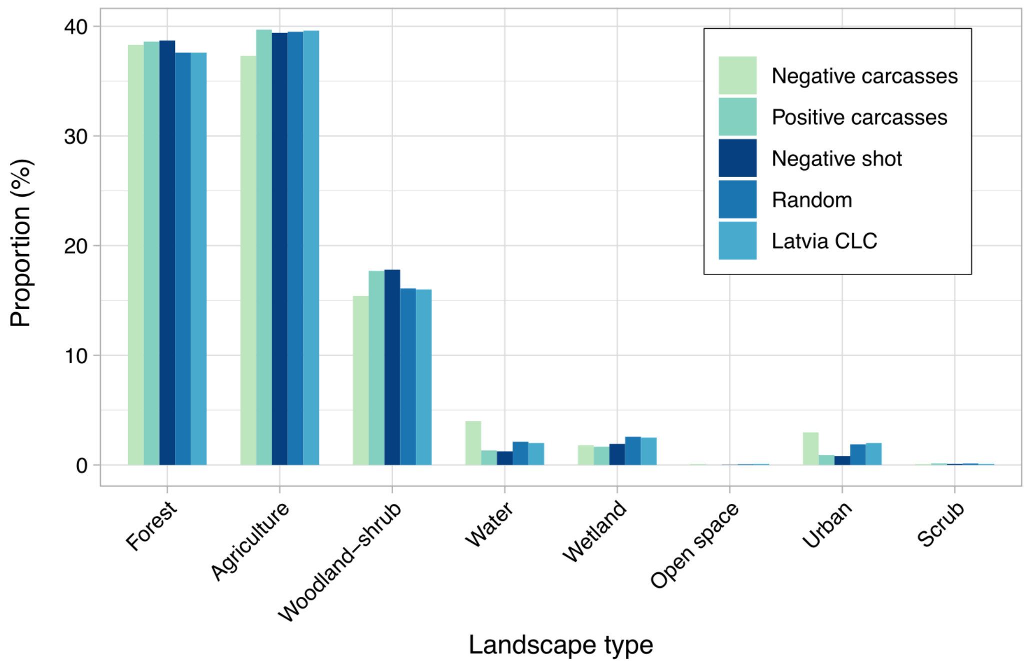

Figure 4. Mean proportions (in %) of different landscape types in a buffer zone with a radius of r = 3 km around the locations for ASF-positive carcasses (n = 1423), ASF-negative carcasses (n = 605), ASF-negative animals shot apparently healthy (n = 9434) from 2014 to 2021, random points (n = 9913) and Latvian landscape.

wetland, open spaces, urban areas as well as scrub and/or herbaceous vegetation were rather small in the buffer zones according to the CLC data.

The proportion of total forest area in the buffer zones was similar for ASF-positive carcasses, ASF-negative carcasses and hunted wild boar ($p = 1.000$ for all pairwise tests), whereas differences were observed in the proportions of different forest types: Around ASF-positive carcasses, the proportion of coniferous forest was significantly smaller ($p = 0.001$) and the proportion of broad-leaved ($p < 0.001$) and mixed forest ($p = 0.003$) significantly larger compared to ASF-negative carcasses. ASF-positive carcasses ($p = 0.02$) and ASF-negative hunted wild boar ($p < 0.001$) had a significantly larger median proportion of forest area in their buffer zones than the random points.

The proportion of agricultural area was significantly smaller for ASF-negative carcasses than for ASF-negative animals shot dead ($p = 0.07$). All remaining comparisons failed to result in statistically significant differences regarding the proportions of agricultural area ($p > 0.05$ for all pairwise tests regarding ASF-negative carcasses, ASF-positive carcasses, ASF-negative hunted animals and random points).

ASF-positive carcasses had a significantly greater proportion of transitional woodland shrub in their 3 km-buffer-zone than ASF-negative carcasses ($p < 0.001$), whereas the random points had a significantly smaller median proportion of transitional woodland-shrub than ASF-positive carcasses ($p < 0.001$) and ASF-negative hunted animals ($p < 0.001$).

In contrast, the proportion of urban area was significantly smaller for ASF-positive carcasses ($p < 0.001$) and hunted wild boar ($p < 0.001$) than for ASF-negative carcasses. The random points had a statistically significantly greater proportion of urban areas in their buffer zones than ASF-positive carcasses ($p < 0.001$) and hunted ASF-negative animals ($p < 0.001$), but a significantly smaller proportion of urban areas than ASF-negative carcasses ($p < 0.001$).

The analysis of seasonal differences in the landscape composition can be found in Supplementary Table S7.

Distance measurements

The results of the distance measurements of the locations of the carcasses, the hunted wild boar and the random points are displayed Table 1.

Animals of all three groups were found or shot, respectively, statistically significantly closer to forest edges than the random points ($p < 0.001$ for all pairwise tests). No significant differences were detected between ASF-negative carcasses, ASF-positive carcasses and ASF-negative hunted wild boar ($p > 0.05$ for all pairwise tests). Out of all animals, 70% were found or shot within a distance of 100 m to the nearest forest. Among the carcasses found dead, less than 5% were found in distances over 300 m to the next forest edge.

Wild boar were shot statistically significantly closer to waterbodies than the random points ($p < 0.001$). No statistically significant difference between ASF-positive and ASF-negative carcasses was detected regarding the distance to waterbodies ($p = 1.000$). The majority of analyzed locations (67%) was within a 200 m distance to the next waterbody, although the proportion was higher for ASF-positive carcasses (68%) and ASF-negative carcasses (70%) compared to the random points (65%). Only 6% of ASF-positive and 4% of ASF-negative carcasses were found beyond a distance of 500 m to the nearest waterbody.

Furthermore, ASF-positive carcasses and hunted animals were found statistically significantly further away from roads and settlements than ASF-negative carcasses ($p < 0.001$ for all pairwise tests). The greatest differences occurred in the distance to roads: 23% of ASF-positive and 46% of ASF-negative carcasses were found within a distance of 500 m to the next road. The random points were statistically significantly closer to roads than hunted wild boar ($p < 0.001$), but further away than ASF-negative carcasses ($p < 0.001$). Regarding the distance to settlements, the random points were statistically significantly closer than animals shot dead ($p = 0.002$), but further away than ASF-negative carcasses found dead ($p < 0.001$). The distance of ASF-positive carcasses and random points to settlements and roads was not statistically significantly different ($p > 0.05$).

In addition, the distances of ASF-positive carcasses and animals shot dead to waterbodies, roads, settlements and forest edges were not significantly different ($p > 0.05$ for all pairwise tests).

Regarding seasonal differences, ASF-negative carcasses were found significantly closer to roads ($p < 0.001$) in winter months (402 m) than in summer months (816 m) (Supplementary Table S8).

	ASF-negative carcasses	ASF-positive carcasses	ASF-negative hunted	Random points
	(n = 606)	(n = 1444)	(n = 9527)	(n = 10,000)
Waterbody	116	121	120	129
Road	611	1179	1224	1054
Settlement	356	453	497	462
Forest edge	53	49	51	75

Table 1. Median distances (in m) of ASF-negative carcasses, ASF-positive carcasses, ASF-negative wild boar shot dead and random points to the next waterbody, road, settlement and forest edge in Latvia from 2014 through to 2021.

Generalized estimation equation model

Ordinary univariable GLM models showed spatial correlation, which was tested by using Moran's I, Geary's C and semi-variograms calculated on the standardized deviance residuals (Supplementary Tables S9 and S10).

Univariable GEE models (Supplementary Table S11) examined potential factors that were associated with the infection status of the carcasses that were found dead (ASF-negative vs. ASF-positive). Based on the selection criteria of AUC and significance, the distance to waterbodies, the proportion of total forest area, agricultural area, scrub and mixed forests were excluded from the model. The proportion of wetlands was also excluded, since the AUC of the model was below 0.5. The final multivariable model (Table 2) with an AUC of 0.6575(Fig. 5) showed that the increasing distance to road had a positive effect on the chance of finding an ASF-positive carcass, whereas increasing distance to the forest edge, the proportion of open space and waterbodies and mixed forests had a negative effect. The semi-variogram of the GLM standardized deviance residuals used to correct the final GEE model, showed that the practical range[40] of the spatial correlation was 27.48 km (Supplementary Fig. S4).

Manual analysis

The evaluation of the smaller sample regarding the landscape type of carcass detection and hunting sites showed that most results were similar in both methods without statistically significant differences (Supplementary Table S12): ASF-positive carcasses were predominantly found in forests and transitional woodland-shrub areas, while the ASF-negative carcasses were found more frequently in agricultural areas and in other landscape types. Hunted animals were more often shot on agricultural areas than in forests.

There was a considerable difference between the manual evaluation and the automated approach regarding the ASF-positive carcasses found in forests (59% versus 48%) and in transitional woodland-shrub (20% versus 29%). However, the overall percentage of ASF-positive carcasses found in forests and transitional woodland-shrub combined was similar (79% versus 77%) with both methods.

Predictor	Estimate	P-value	OR (95% CI)
Intercept	0.4749	0.039	
Distance to road	0.0394	< 0.001	1.0400 (1.0330, 1.0480)
Distance to forest edge	− 0.0469	0.018	0.9542 (0.9180, 0.9919)
Proportion of open space	− 9.1972	0.030	0.0001 ($2.547 * 10^{-8}$, 0.4032)
Proportion of waterbodies	− 0.3238	< 0.001	0.7234 (0.6082, 0,8604)
Proportion of mixed forest	− 0.0862	0.018	0.9174 (0.8541, 0.9854)

Table 2. Results of the multivariable GEE model showing the estimates, p-values, odds ratios (OR) and approximate 95% confidence intervals (CI) of predictors based on naïve standard errors. The outcome variable was the infection status of the carcasses found dead (ASF-negative [n = 605] versus ASF-positive [n = 1423]) in Latvia from June 2014 through to February 2021.

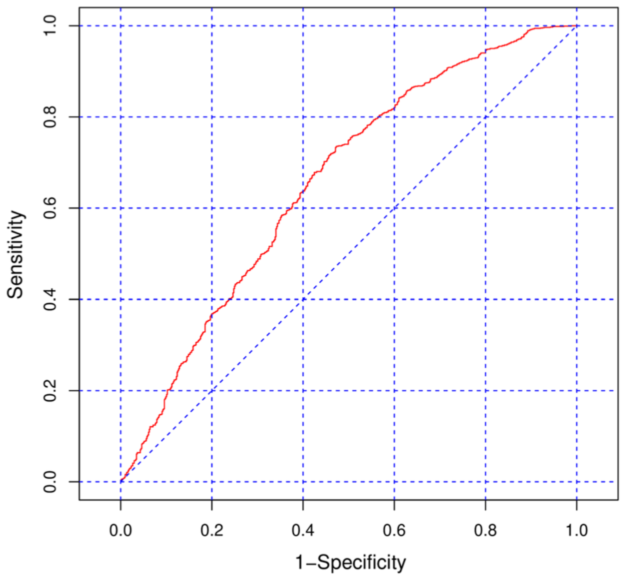

Figure 5. ROC plot of the final multivariable GEE model that included the predictors distance to forest edge, distance to roads, proportion of open space, proportion of waterbodies and proportion of broad-leaved forest. AUC = 0.6575.

Another difference was observed in the group of ASF-negative carcasses that were found in other landscape categories. This proportion was in the manual measurement twice as high (14%) than in the automated measurement (7%). In the manual measurement, 5% of the ASF-negative carcasses were found in urban areas, 3% in water bodies and 2% each on roads, in wetlands and on beaches. In the automated measurement, 4% of the ASF-negative carcasses were found in urban areas and 1% each in water bodies, wetlands and on beaches. The detection of carcasses found on roads was not possible in the automated evaluation, since the CLC map data does not contain information about infrastructure.

The comparison of manual and automated distance measurements (Table 3) using the Mann–Whitney-U test showed statistically significant differences in the distances to waterbodies ($p < 0.001$), settlements ($p = 0.003$) and forest edges ($p = 0.002$), but not in the distance to roads ($p = 0.797$). The locations were 145 m (median) closer to waterbodies (1st quartile Q1 = – 458 m, 3rd quartile Q3 = – 3 m), 5 m further away from roads (Q1 = – 1 m, Q3 = 11 m), 3 m closer to settlements (Q1 = – 190 m, Q3 = 34 m) and 3 m further away from forest edges (Q1 = – 6 m, Q3 = 26 m) in the automated measurements compared to the manual measurements.

Discussion

The continued spread of ASF constitutes a threat for wild boar and domestic pigs worldwide and sets a challenge for veterinary authorities, hunters and farmers. Among different measures to control the spread of ASF in wild boar, one essential strategy is the quick search for and removal of wild boar carcasses, which requires substantial financial and personal resources. We aimed to identify predilection sites for the search of wild boar carcasses to optimize the searches and save resources. To this end, a data set of 2050 GPS locations of detection sites of wild boar carcasses from Latvia, where ASF occurred since 2014, was analyzed to examine whether certain characteristics of the landscape influence the probability of finding a carcass of ASF-infected wild boar. All records of ASF-positive wild boar were considered in the evaluation, regardless of the testing method (PCR or serology). Three records out of 1444 had tested serologically positive, but virologically (i.e. PCR) negative for ASF. It is therefore unclear whether these three animals died of ASF. However, due to the small number, their influence on the overall results is regarded as extremely limited if not negligible.

Moreover, a dataset of 9527 GPS locations of hunting sites was analyzed. Overall, it was assumed that hunting locations represent wild boar habitats, as it seems reasonable that hunters predominantly hunt wild boar in places where wild boar are abundant. However, a bias in the data towards hunters' choice of where to hunt wild boar must be considered.

Since the landscape structure of Latvia is relatively homogenous and primarily consists of agricultural area and forests, a data set of 10,000 random GPS locations was created and analyzed in the same manner to put the possible results and correlations in relation to the general landscape structure present in Latvia.

When interpreting the study results, the possible accuracy of GPS data and available map data must be considered. It has been reported that GPS devices can reach precisions of under 10 m[53]. However, this precision can vary considerably and may be negatively influenced by lack of satellite availability and dense forest canopy cover[54]. Accordingly, a certain inaccuracy of GPS data submitted to the surveillance database must be tolerated, especially regarding locations in forests. However, this inaccuracy can eventually be neglected when put into relation to the resolution of the CLC map data. The minimum mapping unit of these data is 25 ha (500 × 500 m) and does therefore not capture smaller landscape features. Nevertheless, CLC provides good information on landscape composition with a coverage of 100% within Europe and has been used for similar purposes in several other studies[24,26,55,56].

Furthermore, inaccuracies have to be considered for the map data used for distance measurements: The median difference between automated and manual measurements of the distance to waterbodies suggested that there were discrepancies between map data and orthophotos. Random checks of GPS locations with large individual differences in this comparison revealed that small waterbodies and waterbodies covered by dense forest were easily overlooked or impossible to see in orthophotos. The median differences in the measurements of the distances to roads, settlements and forest edges were rather small, but the interquartile ranges suggested that outliers with large individual differences occurred. Random checks of GPS locations showed that this was at least in part due to incorrect classification of settlements by the European Settlement Map. Nevertheless, the

	ASF-negative carcasses		ASF-positive carcasses		ASF-negative hunted wild boar	
	(n = 175)		(n = 249)		(n = 175)	
	Measurement					
	Manual	Automated	Manual	Automated	Manual	Automated
Waterbody	290	89	347	94	354	110
Road	704	659	1320	1348	1360	1365
Settlement	410	370	573	535	550	430
Forest edge	40	53	41	60	40	46

Table 3. Comparison of the median distances (m) to the nearest waterbodies, roads, settlements and forest edges measured manually and in an automated way for a subset of ASF-negative, ASF-positive wild boar carcasses and ASF-negative hunted wild boar in Latvia from 2014 through to 2021.

median differences showed that individual divergences in both directions were eventually balanced, leading to the conclusion that the automated method is suitable for analysing large surveillance data sets.

Overall, the established GEE model (ASF-negative vs. ASF-positive carcasses) identified statistically significant factors influencing the probability of finding an ASF-positive carcass. According to the model, it was less likely to find an ASF-positive carcass with increasing distance to forest edges and increasing proportion of open spaces around the carcass. Combined with the fact that the proportion of carcasses found in forests was higher among ASF-infected wild boar than for non-infected, this might lead to the hypothesis that infected animals search for shelter in forest areas. This is in accordance with the results of other studies that observed associations between occurrence of ASF and forest coverage[24,25,55]. However, forest areas with nut-bearing trees and thickets generally represent a preferred natural habitat for wild boar, as they provide protection from predators and various food sources[57–59]. In our study, all carcasses were found close to forest edges, irrespective of their infection status. This was also observed by Cukor et al., who found that the vast majority of carcasses was found in forests and within a distance of up to 200 m to forest edges[23]. In both studies, that of Cukor et al. and in our own investigations, only few carcasses were found beyond distances of 500 m to the forest edge, indicating that forest edges are a potential key area for the detection of ASF-positive carcasses. Yet, these results may be biased by the fact that the search for carcasses is related to the accessibility of the terrain. Since peripheral areas of a forest may be easier to access, it could be more likely to find wild boar carcasses in these areas. This may also be a reason why increasing proportions of mixed forest area decreased the chance of finding a positive carcass in our model, since the density of trees and understorey vegetation are usually higher deep inside the forest and therefore limit detectability.

ASF-positive carcasses were found more frequently in areas of transitional woodland-shrub than negative carcasses, although the predictor was not statistically significant in the final model. They also had a greater proportion of this landscape type in their buffer zones compared to the random points. Similarly, Allepuz et al. identified an increased likelihood of finding positive carcasses in areas of transition between woodland and shrub[55]. These results may suggest that infected wild boar prefer to stay in border regions of forests to seek for shelter.

Beyond that, some studies concluded that ASF-infected animals might preferably stay close to water sources to cool down their body temperature if they have fever, which is a common clinical sign in ASF-infected pigs[23,24,26]. Yet, Allepuz et al. did not identify the distance to waterbodies as a statistically significant factor for the probability of finding ASF-positive carcasses[55]. Our model predicted a decreasing probability of finding an ASF-positive carcass with increasing proportion of waterbodies in the buffer zone around the location, which is most likely due to an artefact, since increasing proportions of waterbodies decrease the area that can be searched for carcasses due to the water coverage. Therefore, the probability to find a positive carcass or a carcass may generally decrease with increasing proportion of waterbodies. In our study, this was particularly obvious for areas close to the coast of the Baltic Sea, which contained large proportions of waterbodies.

Similar to the results of Cukor et al.[23], we found the majority of carcasses (70%) within a distance up to 200 m to the nearest waterbody, regardless of the infection status of the carcasses. Moreover, 64% of the random points were found within a distance of up to 200 m to the next waterbody, which implicates that Latvia is a water-rich country in general. It has a dense network of lakes, rivers, streams and ditches with a total surface area of approximately 2300 km^2[29], which represents 3.6% of the total area of the country. This implies that wild boar behavior and movement in Latvia might not be strongly influenced by the distance to water bodies in general, since it has been proven that wild boar adapt easily to the circumstances of their habitat and that the proximity to water bodies is more relevant for wild boar in dry regions than in water-rich areas[32,59].

In addition, climate conditions seem to influence the dependence of places, where wild boar chose their death bed, on water sources available nearby[23]. It has also been demonstrated that meteorological conditions such as temperature and precipitation, generally influence the spatial behavior of wild boar[32,58]. Some studies also observed a higher probability of ASF-occurrence in regions with lower mean temperatures[26,27]. Also, in our study, ASF-positive carcasses were found slightly closer to waterbodies in summer as compared to winter months. Yet, the results of the seasonal comparison of carcass finding sites in this study must be interpreted with care, since the analysis is based on the carcass detection dates, which might not necessarily be identical with season at the date of death. Considering the actual climate conditions at the time of death would require to assess the time between the death of a wild boar and its detection, the so-called post-mortem interval, based on the decomposition of the carcass. Such data was not available for the present study. Probst et al. showed that the decomposition process is highly variable and dependent on climatic and landscape conditions[60], which makes the estimation of post-mortem intervals difficult. Nevertheless, it is known that wild boar spatially adapt themselves to the seasonal variability of available food and shelter[59,61]. During the growing season in summer months, they move closer to fields and agricultural areas to feed on crops, while they dwell in winter especially in forests with broad-leaved and nut-bearing trees that provide food sources[59]. This effect was also visible in our results, since ASF-positive carcasses and hunted animals had a higher proportion of agricultural areas in their environment during summer months.

Besides, our results may also indicate that human activities have an impact on the probability of finding a carcass of an ASF-infected wild boar, based on the analysis of the distance to roads and settlements as well as the proportion of urban areas around finding sites. Negative carcasses were found closer to roads compared to ASF-positive carcasses, especially in winter months. Similarly, Cukor et al. observed that negative carcasses were found significantly closer to roads[23]. This might be due to the fact that road traffic accidents, apart from hunting, are a common cause of death of wild boar[62], especially in darker winter months. The ASF-negative wild boar found dead might therefore in many cases originate from road traffic accidents and may have been incorrectly classified in the EURL CSF/ASF wild boar surveillance database as a wild boar found dead. It has already been pointed out by Schulz et al. that road traffic accidents are most likely underreported in the database and only few cases have been reported during the whole study period from 2014 to 2021[63]. However, this finding may be biased by the fact that dense snow coverage in winter months might reduce the chances of detecting carcasses far from roads and paths. Nevertheless, the results may also indicate that infected animals are less mobile due to the severity of ASF symptoms and the inclination of infected wild boar to hide as much as possible from human disturbance.

The evaluations showed similarities between detection sites of ASF-positive carcasses and hunting locations of ASF-negative wild boar. Although the hunting sites differed from finding sites of carcasses, since the majority of hunted animals was shot on fields, the other results do not imply huge differences. This may be due to the fact that hunters are often the ones who find and report wild boar carcasses during their hunting activities. Moreover, active surveillance in Latvia included the sampling of hunted wild boar within a radius of 8 to 20 km around newly detected ASF cases[6], which have led to similarities in landscape composition in the buffer zones of wild boar in these areas.

Although the odds ratios of the predictors in our final logistic regression model were small, we can assume that the calculated p-values are truly significant, since we also corrected the model to account for the spatial correlation[64]. The predictive quality (AUC) of our model was moderate, which may be caused by the influence of many unknown factors, such as different surveillance efforts as well as the temporal course of the spatial spread of ASF during the study period.

In conclusion, we found that forest edges and clearings, as well as bushlands close to forests were predilection sites for the detection of wild boar carcasses in Latvia. Since wild boar can adapt to various habitats and their abundance is always influenced by local circumstances like availability of food resources and level of human interference[65], the results of this study may not be valid for other study areas with different habitat conditions. However, our results are in many aspects similar to those of other studies on the topic[24–26] and highlights the consistency of the influence of certain landscape characteristics across different study areas, time periods and methods. It seems therefore possible to use similar data from other regions to define predilection sites, on which the search for wild boar carcasses can focus in ASF-affected areas to save resources.

Data availability

The data that support the findings of this study are available from the responsible authority in Latvia upon reasonable request.

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Author contributions

L.R. designed the study, performed the GIS analysis and statistical evaluation and drafted the manuscript. K.S. carefully reviewed the manuscript. C.S. supported the design of the study, GIS analysis, statistical evaluation and reviewed the manuscript. E.O., M.S. and K.L. reviewed the manuscript, provided data and input towards the study design. F.J.C. supported the study with epidemiological considerations and carefully reviewed the manuscript. C.S.-L. designed and supervised the study, supported statistical analysis and carefully reviewed the manuscript.

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2 Publication II: Remote sensing provides a rapid epidemiological context for the control of African swine fever in Germany

Remote sensing provides a rapid epidemiological context for the control of African swine fever in Germany

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Remote Sensing Provides a Rapid Epidemiological Context for the Control of African Swine Fever in Germany

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Abstract: Transboundary disease control, as for African swine fever (ASF), requires rapid understanding of the locally relevant potential risk factors. Here, we show how satellite remote sensing can be applied to the field of animal disease control by providing an epidemiological context for the implementation of measures against the occurrence of ASF in Germany. We find that remotely sensed observations are of the greatest value at a lower jurisdictional level, particularly in support of wild boar carcass search efforts.

Keywords: African swine fever; epidemiology; remote sensing; geographical information systems; risk factor; risk assessment

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Introduction

African swine fever (ASF) is an internationally spreading, viral pig disease that severely damages agricultural pork production, economy and social welfare [1]. The ASF virus (ASFV) infects pigs, including wild boar, but is not harmful to humans. ASFV infection in pigs results in high virus concentrations in the blood and leads to generalised internal bleeding that usually kills affected pigs within several days [2]. In Europe, wild boars are considered important in the epidemiology of ASFV, from which it is assumed that the disease occasionally transfers into domestic pig holdings [3]. Considering wild boar biology and movement, the transmission of ASFV amongst wild boar could explain the dominating gradual spread of ASF observed in the current European epidemic. The complex behaviour of wild boar, uncertainties around the relevant ASF spread mechanisms and the current lack of a vaccine have made ASF difficult to control [4].

In the current epidemic, ASF has progressively spread across Europe and Asia from the original outbreak in Georgia in 2007. The ASF epidemic front in Europe has travelled from east to west and formed a satellite outbreak in West Poland in November 2019, approaching Germany. This threat to Germany elicited a sophisticated assortment of preventive disease control measures. Nevertheless, on 10 September 2020, near the Polish border, a wild boar carcass tested positive for ASF in Germany, marking the first ASF occurrence in this country [5].

Following the discovery of the disease, pre-planned responses were initiated. One of the key tasks was to determine the extent of the infected area as quickly as possible and based on this information, implement hazard and risk zones in the affected area.

In the past, mainly retrospective or static data, for example on land use, were applied for setting up these restriction zones (as mentioned in the European Commission working document on principles and criteria for geographically and temporally defining ASF regionalisation—working document SANTE/7112/2015) [6]. However, the currency of these approaches would be variable and largely ignore the important spatio-temporal fluidity of the wild boar habitats to implement effective ASF control measures.

When facing a disease incursion event, such as ASF, it is therefore critical to gain a timely and spatially explicit overview of the affected area. Systematic searches for wild boar carcasses and testing these for the presence of ASFV were, and continue to be, conducted throughout the risk area to guide the process of delimitating zones and to control the disease by removing infectious carcass material. Due to limited resources (e.g., personnel, finances), prioritization of the areas with high attractiveness for wild boars and consequentially increased permeability for ASF spread can increase the efficiency of targeted searches [7]. The attractiveness of the landscape for wild boar depends on various factors, some of which vary seasonally, e.g., maize fields, acorn/beech mast. Rather than using retrospective or static data, as has been used in the past, dynamic up-to-date information provides the opportunity to target the prioritization of such searches explicitly.

Besides sporadic outbreaks of ASF in domestic pigs, mainly wild boar have been affected by ASF in Germany [8,9]. Several carcasses of wild boar that tested positive for ASFV were found in harvested maize fields. Maize fields offer shelter and food for wild boar, and thus are an attractive habitat for this species. Once maize fields are harvested, the wild boar have to leave and find new areas to live in. Thus, for setting up restriction zones and control zones as regulated in the Commission Implementing Regulation (EU) 2023/594, it is of paramount importance to know where maize crops are located at the time and where wild boar could have moved from recently harvested fields. It is conceivable that wild boar will move into other still existing maize fields, or into forests that offer food and shelter, such as forests with oak and beech trees [10,11]. Remotely sensed observations can provide insights into important wild boar habitat factors, including food, water and shelter.

The use of satellite-based remote sensing data is one way to obtain up-to-date information quickly and easily. Conceptually, remote sensing has proven to be an effective tool for monitoring agricultural production. Due to a large variety of on-board sensors on an increasing number of civilian satellites, the spectral and temporal properties of the land surface resulting from human practices can be captured and monitored at different spatial and temporal scales [12]. Remote sensing is commonly applied in the field of agricultural crop production, including the monitoring of crop growth and detection of crop stress [13]. In addition, the application of remote sensing is well established in forest science for forest biomass monitoring [14] or forest tree species composition [15]. There are also approaches to use remote sensing data for early warning systems, e.g., remotely sensed sea water surface temperature as a predictor of the risk of Vibrio infections [16]. Whilst a considerable variety of remote sensing data is available and climate change instils an increasingly pressing need to interpret this information in a veterinary epidemiology context, many barriers still exist that prevent the wider use of such data for emerging disease management [17]. To our knowledge, this is particularly relevant for transferring satellite-based remote sensing technologies beyond research applications to the animal disease control sector in the field.

Here, we present how remotely sensed satellite observations can be applied for regional risk assessment in the context of ASF control in Germany and how remote sensing data are quickly prepared and provisioned to the competent authorities in the ASF outbreak area. A particular goal of this study was to better understand the relevance of satellite-based remote sensing for disease control efforts that followed the ASF incursion into eastern Germany on 10 September 2020.

1. Materials and Methods

1.1. Crop Classification

For the fastest possible provision of current landcover information, the development of fully automated and standardized methods for processing heterogeneous satellite data for large study areas was necessary. The large amount of data to be analysed requires cloud computing services, which provide the necessary data infrastructure and computing power.

Near real-time, remotely sensed information from the European Union's earth observation program 'Copernicus' (https://www.copernicus.eu/en, accessed on 15 November 2021) was acquired. Multitemporal Sentinel-1 Ground Range Detected (GRD) and Sentinel-2 Level 2A (L2A) scenes were used. They provide satellite-based high temporal, spectral and spatial resolution imagery to derive detailed and current land cover information on demand. These data were utilized for the categorization of primary crop types (refer to Table 2) and for assessing the current status of maize harvesting, as well as for identifying the presence and distribution of oak and beech trees. The primary aim of the classification was to rapidly and precisely predict those crucial landcover characteristics, all while upholding a high degree of spatial accuracy. This information was specifically directed towards regions in Germany where cases of African Swine Fever (ASF) in wild boars had been reported along the German–Polish border. Its purpose was to pinpoint suitable habitats for wild boars in those areas.

The crop type classification Is based on the very effective Random Forest classification algorithm [18]. Each crop type shows different spectral reflection characteristics due to its phenology. The standard characteristics, described by spectral indices, can be used for classification [19,20]. Here, a range of widely used indices were applied in a first model run, including spectral bands and possible band permutations based on the following equation:

$$\text{band permutations} = \frac{(x-y)}{(x+y)} \tag{1}$$

with x and y as different spectral bands. All indices tested are listed in Supplementary Table S1.

With all these predictors, a Random Forest classifier was trained with 10.000 training points to model the crop type classes (hyperparameters used: 450 trees and minimum leaf population of 4). The permutation-based model's variable importance, showing the variables with the highest distinctive power, revealed the most useful indices (Table 1). These indices were employed in constructing a conclusive classifier for crop type prediction. This approach resulted in a reduction in the data volume and processing time, leading to an acceleration of the entire workflow.

Table 1. Most useful indices.

Indices	
NDVI	Normalized Difference Vegetation Index [21]
NDYI	Normalized Difference Yellowness Index [22]
GNDVI	Green Normalized Difference Vegetation Index [23]
PVR	Photosynthetic Vigour Ratio [24]
MSAVI	Modified Soil Adjusted Vegetation Index [25]
MSR	Modified Simple Ratio [26]
REIP	Red-Edge Inflection Point [27]
VIS	Band 2, 3, 4
NIR and SWIR	Band 5, 6, 7, 8 and 12
Radar	VV max, VH max, VV/VH-Ratio max, VV Median, VH Median, VV/VH-Ratio Median

To evaluate the phenological changes in the index curve throughout the crop cycle, we generated standard curves for all indices in Table 1. The standard curves for the single crop types were derived from multitemporal Sentinel 2 and Sentinel 1 data from 2017 and 2018 by the use of IACS data for several regions in Germany (International Association of Classification Societies—https://iacs.org.uk/, accessed on 15 November 2021). The IACS crop-type classes were aggregated (Table 2). For each class, the corresponding IACS areas were used for the calculation of the statistics (mean, minimum, maximum, standard deviation) for all indices at every date. The mean plus and minus standard deviation values were calculated as well (meanadd, meansub). Through interpolation of the data points and smoothing (2^{nd} polynom), the standard curves "min", "max", "mean", "meanadd" and "meansub" were derived for each index in Table 1 (see NDVI in Figures 1 and 2). Upon examining all the standard curves and their intersections, it became evident that achieving a high classification accuracy would require a dense time series. Since cloudless images are infrequent in northern regions, the aggregation of various scenes became imperative.

Table 2. F-scores of the individual crop types for the year 2018 using satellite data from 12, 8 and 4 months, respectively (value range from 0 (bad) to 1 (perfect)).

Crop Type	12 Months	8 Months	4 Months
1: Grassland	0.83	0.76	0.71
2: Fallow land	0.46	0.28	0.22
3: Maize crop	0.87	0.39	0.21
4: Rye	0.74	0.29	0.20
5: Wheat	0.77	0.72	0.50
6: Potato	0.42	0.27	0.14
7: Sugar beet	0.71	0.32	0.24
8: Rapeseed	0.96	0.83	0.58
9: Barley	0.72	0.56	0.38
10: Oats	0.37	0.18	0.06
11: Woody plants	0.21	0.25	0.21
12: Other cereals	0.08	0.04	0.02
13: Root crops, rest	0.11	0.49	0.17

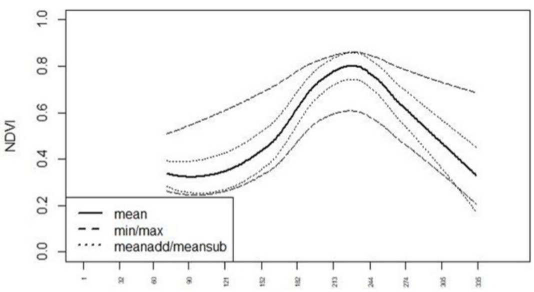


Figure 1. Normalized difference vegetation index (NDVI) standard curves for "corn", *x*-axis: Day of Year (DOY).

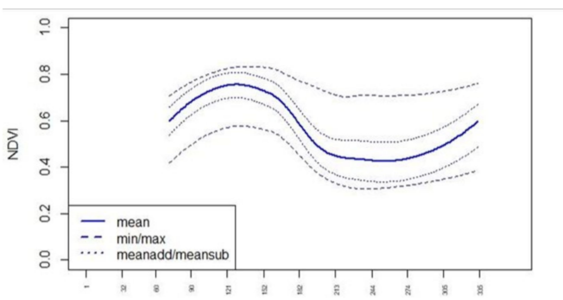
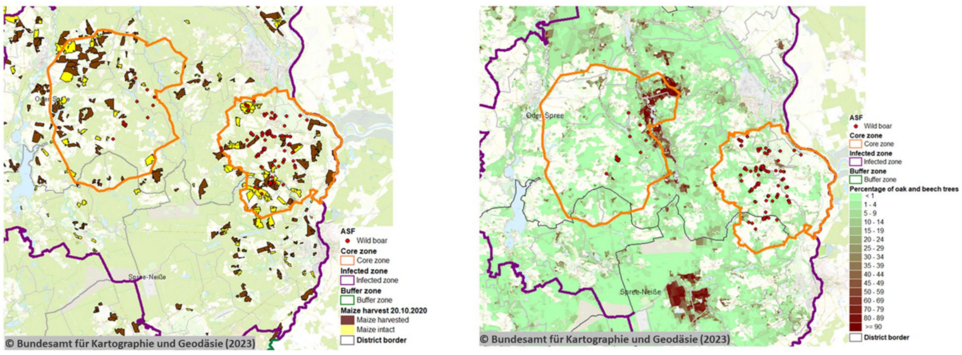


Figure 2. Normalized difference vegetation index (NDVI) standard curves for "canola". *X*-axis: Day of Year (DOY).

The classification was run for aggregated satellite data of two months each, starting on 1 October 2017. In total, a data set of 21 parameters (or 21 layers) was generated for two months each. That data set encompasses the seven spectral indices from Table 1 and all eight VIS, SWIR and NIR spectral bands and the six radar indices. The accuracy of the classification was assessed using the F-score (Table 2). The F-score corresponds to the harmonized mean of precision (rate of true positives vs. all positives) and recall (rate of all true positives vs. true positives and false negatives) and has a value range from 0 (bad) to 1 (perfect). IACS data were used as a reference.

1.2. Preparation and Provisioning of Remote Sensing Data to Competent Authorities

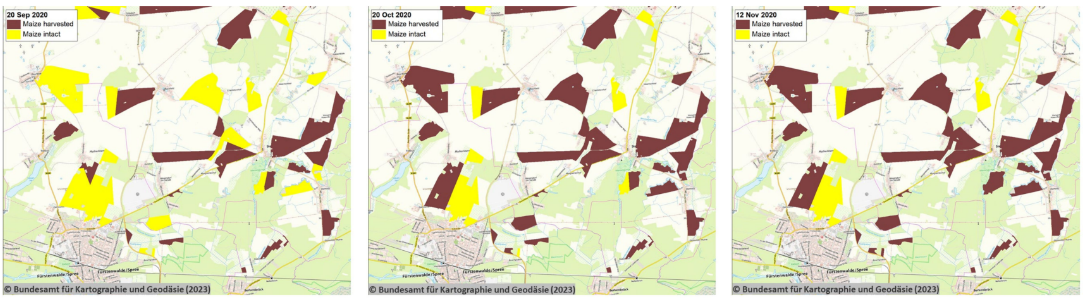
Satellite remote sensing data were prepared to show the location of maize crops, their harvesting status (Figure 3a (20 October 2020), FLI-Maps Links harvesting status: 20 September 2020; 20 October 2020; 12 November 2020) and forest-covered areas as well as their percentage of oak and beech trees on 20 October 2020 (Figure 3b, FLI-Maps Link: oak and beech trees). These data were prepared for the ASF outbreak area at the time with the current ASF control zones and reported wild boar ASF cases considered. The harvest of maize in the ASF outbreak zone was strictly regulated and coordinated to complement ASF control measures.



(**a**) (**b**)

Figure 3. Remote sensing of geospatial disease risk information using satellite-based imagery and geospatial interpretation for the context of the disease event at hand. (**a**) Maize crop harvest status and (**b**) the forest status on 20 October 2020. © Bundesamt für Kartographie und Geodäsie (2023), Datenquellen: https://sgx.geodatenzentrum.de/web_public/Datenquellen_TopPlus_Open (accessed on 7 July 2023).

Satellite remote sensing allowed spatial tracking of cropped maize areas over time to inform the implementation of ASF control measures (Figure 4).



(**a**) (**b**) (**c**)

Figure 4. Remote sensing of the ongoing maize crop harvest status on (**a**) 20 September, (**b**) 20 October, and (**c**) 12 November 2020. © Bundesamt für Kartographie und Geodäsie (2023), Datenquellen: https://sgx.geodatenzentrum.de/web_public/Datenquellen_TopPlus_Open (accessed on 7 July 2023).

The processed satellite information was then distributed through a pre-existing mapping tool known as 'FLI-Maps'. The tool FLI-Maps was developed by the Friedrich-Loeffler-Institut (FLI) to support Germany's surveillance obligation to record and control reportable animal diseases. In Germany, reportable animal disease events are recorded in a Geographical Information System (GIS) integrated disease reporting system called TSN ("Tierseuchennachrichtensystem" [28]). TSN utilises the FLI-Maps platform to geospatially summarise the status of reportable diseases and is readily accessible to relevant veterinary authorities in Germany.

1.3. Evaluating the Relevance of Remote Sensing Data for ASF Control

To evaluate the application of remotely sensed satellite observations in the early control phases following the month after ASF incursion in eastern Germany on 10 September 2020, an electronic questionnaire was administered to jurisdictional key personnel within the veterinary authorities engaged with ASF control in Germany. The questionnaire was circulated in the beginning of March 2021, thus capturing experiences from the first six months of ASF management in the entire affected area of Eastern Germany. The questionnaire is included with this article as Supplementary Materials (Supplementary File S1). It elicited the relevance of utilising remote sensing data by scoring different ASF management applications during the current outbreak management, as well as the jurisdictional working level of the respondent. The respondents were able to select semi-quantitative responses (rank 0, 1, 2, 3; according to the four response options in the questionnaire, see Supplementary Materials) regarding their experiences of applying satellite-based remote sensing data to ASF management, and to what extend this technology influenced their management of the disease. The relevance of remote sensing data for each queried ASF management-related application was calculated by summing the cumulative scores provided from all the respondents and presenting it as a proportion of the possible maximum score, stratified by jurisdictional level. The maximum score is given by the number of respondents multiplied with the highest possible rank 3.

2. Results

2.1. Accuracy of Crop Classification

When looking at the F-scores of the individual crop types (Table 2), it was noticeable that the quality of the classification of certain crop types also depended on the amount of satellite data used. Maize, rye, barley and sugar beet were identified very reliably if data from more than 8 months were available. Grassland, wheat and rapeseed, on the other hand, were correctly classified with less data. In the case of oats, fallow land and potatoes, only mediocre results were achieved at the end of the study period (30 September). The highly heterogeneous and mixed classes that grouped other crop types together hardly achieved any usable accuracies.

2.2. Evaluation of Relevance of Remote Sensing Data

At the lower jurisdictional level (district), the value of applying satellite remote sensing was assessed to be very relevant for the selection of areas targeted during the wild boar carcass searches for the choice of carcass search method and to guide the positioning of wild boar fencing (Figure 5). Overall, the value of remote sensing application in this context appeared to be of the greatest relevance in the field at lower jurisdictional levels, whereas representatives of higher-level jurisdictional authorities reported lower relevance of the technology for ASF control by comparison (Figure 5).

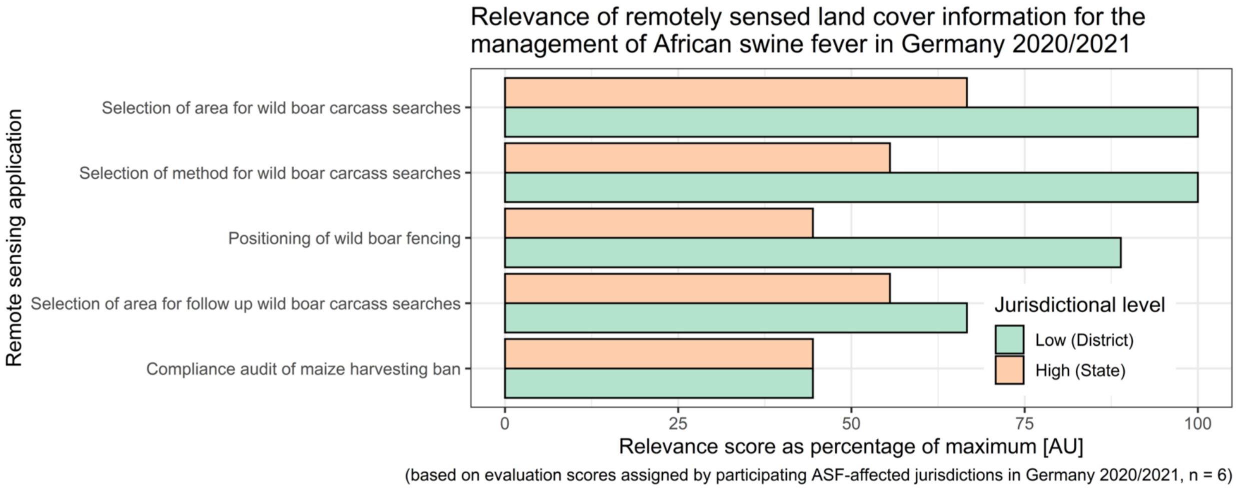


Figure 5. Relevance of remotely sensed land cover information for the management of African swine fever in Germany during 2020/2021. Relevance scores represent a percentage of the maximum score.

3. Discussion

Infectious disease epidemics result from direct interaction with environmental factors through space and time. It is therefore necessary to map the epidemiological context of environmental factors to have the best chance of comprehending relevant disease patterns suitable for intervention during disease incursion events. Whilst remotely sensed and disease-risk-related geospatial information is usually available to authorities, these types of data tend to be used extensively in retrospect only, rather than during an acute outbreak phase.

Applying and regularly updating (Figures 3 and 4) remotely sensed satellite data in several applications was found to be relevant for the implementation of ASF control measures in Germany, particularly at lower jurisdictional levels (Figure 5). Remote satellite sensing was applied to map the current extent of maize crops and the distribution of oak and beech forest in the ASF outbreak area. The prepared information was readily shared with the responsible authorities through the pre-existing FLI-Maps tool that had been integrated into the governmental veterinary TSN management software (TSN 3.3 R7a). In the field, remote sensing was applied to the selection of wild boar search areas and methods, including the planning and targeting of search missions by drones, helicopters, sniffer dogs and trapping teams. Remote sensing was also relevant for the positioning of wild boar fencing and monitoring compliance with the maize harvest ban regulations. As such, satellite-derived remote sensing data offered detailed information to implement risk-based targeting of previously described environmental ASF risk factors for efficient and sustainable disease control efforts [29].

In conclusion, we found that ensuring adequate synthesis and transfer of remotely sensed satellite observations provided a relevant and immediate epidemiological context for acute disease occurrence responses to ASF in the field. Rapid utilisation of GIS and remote sensing systems during the early phase following disease occurrence has the potential to greatly reduce long-term negative effects of such events by appropriately setting the course of disease management early on, likely yielding benefits in disease scenarios other than ASF. We therefore advance a concept to make sharable cartographic platforms and readily available, remotely sensed land cover information an integral part of epidemic preparedness strategies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/s23198202/s1, File S1: Questionnaire; Table S1: Indices tested.

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Conflicts of Interest: The authors declare no conflict of interests.

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3 Publication III: Seasonal Occurrence of African Swine Fever in Wild Boar and Domestic Pigs in EU Member States

Seasonal Occurrence of African Swine Fever in Wild Boar and Domestic Pigs in EU Member States

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Seasonal Occurrence of African Swine Fever in Wild Boar and Domestic Pigs in EU Member States

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Abstract: Since 2007, African swine fever (ASF) has spread widely within Europe and beyond. Most affected countries recorded outbreaks in domestic pigs and cases in wild boar. Outbreak data from 2014 to 2021 were used to investigate the seasonal pattern of ASF in domestic pigs and wild boar across affected member states of the European Union, since knowledge of seasonal patterns may provide the potential to adapt prevention, surveillance and control during times of increased risk. In domestic pigs, a yearly peak was observed in many European countries in summer (predominantly in July and August). In wild boar, the patterns showed more variability. In many countries, there was a seasonal peak of ASF occurrence in winter (predominantly in January and December), with an additional summer peak in the Baltic States (predominantly in July) and a further spring peak in Poland (predominantly in March). The observed seasonal effects may be related to the abundance and population dynamics of wild boar and to seasonality in pig farming. Moreover, ASF occurrence may also be influenced by human activities in both domestic pigs and wild boar.

Keywords: African swine fever; seasonality; surveillance; risk factor; epidemiology

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1. Introduction

African swine fever (ASF) is caused by African swine fever virus (ASFV), a large DNA virus from the family Asfarviridae [1]. It can cause haemorrhagic disease in suids and affects both Eurasian wild boar and domestic pigs, where it may lead to high case fatality [2–4]. From its original sylvatic cycle in sub-Saharan Africa, which involves warthogs and soft ticks of the genus *Ornithodoros* [5], ASF sporadically spilled over to domestic pigs and was occasionally transmitted to Europe in the 20th century, e.g., through the feeding of kitchen waste from ships or aircraft to domestic pigs. Most of these outbreaks were quickly brought under control, but long epidemic spells occurred after the introduction of ASF to Portugal and Spain [6,7]. Also, on the Italian island of Sardinia, ASF remained endemic since 1978 until very recently in a cycle including both domestic and feral pigs [6,8]. However, ASF was absent from the rest of Europe from 1995 until 2007, when ASFV, genotype II, was introduced into Georgia [6]. From there, ASF rapidly spread throughout several Eastern European countries, affecting Armenia and the Russian Federation later in 2007 and Azerbaijan in 2008 [6]. Ukraine and Belarus reported their first cases of ASF in 2012 and 2013, respectively [9]. During this "new" epidemic, Lithuania became affected as the first member state of the European Union (EU) in January 2014 [10], followed by Poland, Latvia and Estonia later that year [10–12]. Ever since, ASF has been spreading throughout Eastern, Central and Southern Europe, affecting, among others, the Czech Republic and Romania in 2017; Belgium, Bulgaria and Hungary in 2018 [13]; and Slovakia in 2019 [14]. Subsequently, the first outbreak of ASF in domestic pigs was reported in Greece, and the first cases in wild boar in Germany were confirmed in 2020 [15,16]. More recently, the Italian mainland was affected both in the wild boar and domestic pig sectors in 2022 [17], and Balkan Island countries were affected in 2023. Until recently, Belgium and the Czech Republic were the only two European countries in which ASF was eliminated in wild boar [18,19]. However, ASF was reintroduced into the Czech Republic in late 2022, presumably through wild boar that immigrated from Poland or Germany.

In many countries (e.g., the Baltic States, Bulgaria, Germany, Hungary and Slovakia), the so-called "wild boar habitat cycle" [20] is the main driver of ASF spread and persistence, leading to large case numbers in wild boar and sporadic outbreaks in domestic pigs. In Romania, a different situation was observed: large numbers of outbreaks in domestic pigs were reported, predominantly but not exclusively affecting farms with low levels of biosecurity [21].

Over the years, the seasonality of ASF occurrence has been analysed in a number of studies with variable results. Seasonal peaks in summer for outbreaks in domestic pigs were reported for different countries, e.g., the Baltic States, Poland and Romania [13,22,23]. Several studies detected seasonal patterns in the occurrence of ASF in wild boar with variable results regarding the respective periods and geographical areas [23–26].

Thus, the aim of this study was to identify seasonal patterns in EU member states that have reported ASF cases to the Animal Disease Information System (ADIS) for the period from 2014 to 2022. Seasonality was analysed in both domestic pigs and wild boar to elucidate the factors affecting these seasonal effects and to compare the results to those of previous studies.

2. Materials and Methods

2.1. *African Swine Fever Surveillance Data*

African swine fever surveillance data were used from the EU Animal Disease Information System (ADIS). This system contains information about each confirmed ASF outbreak in domestic pigs and about confirmed ASF cases in wild boar (regardless of the test method) in the European countries reporting to the system. Yet, it is possible that some countries sometimes report several wild boar cases in one record. Due to this potential inconsistency and lack of background information, our analysis was performed under the assumption that one record represents one wild boar case. For the current analysis, information about the country of origin, the date of confirmation and the subspecies (domestic pig or wild boar) was used. Only EU Member States were included in the analysis, as they are considered to have a consistent and reliable reporting system.

Table 1 shows the number of records and the first date of ASF occurrence per country for domestic pigs and for wild boar. The analysed period reaches from the first occurrence of ASF, genotype II, in wild boar in the EU in Lithuania on 24 January 2014, and in domestic pigs in Latvia on 26 June 2014, until 31 December 2022. Since the epidemiological situation in Sardinia differs from that in other European countries (ASFV of genotype I, endemic situation involving free-ranging domestic pigs and wild boar), data from Sardinia were excluded from further analysis.

Table 1. First date of ASF occurrence and total number (#) of records (as of 31 December 2022) used for the analysis per EU member state for domestic pigs and wild boar.

Country	Domestic Pigs		Wild Boar	
	First Date	# Records	First Date	# Records
Belgium	---	0	13 September 2018	648
Bulgaria	31 August 2018	72	23 October 2018	1453
Czech Republic	---	0	26 June 2017	231
Estonia	21 July 2015	28	8 September 2014	2956
Germany	15 July 2021	7	10 September 2020	4554
Greece	5 February 2020	1	---	0
Hungary	---	0	21 April 2018	8899

Table 1. *Cont.*

Country	Domestic Pigs		Wild Boar	
	First Date	# Records	First Date	# Records
Italy (excl. Sardinia)	9 June 2022	1	7 January 2022	268
Latvia	26 June 2014	75	26 June 2014	5367
Lithuania	24 July 2014	157	24 January 2014	4475
Poland	23 July 2014	502	17 February 2014	15,306
Romania	31 July 2017	5941	29 May 2018	3267
Slovakia	24 July 2019	44	8 August 2019	2634

2.2. Data Analyses

All analyses were conducted using the software R [27] with R Studio 4.0.3 [28] as an interface. For descriptive statistics and data management, the R package "tidyverse" [29] was used. Radar charts were produced for each individual country using the absolute and the relative frequency of notifications per month, using the package "fmsb" [30]. In the radar charts, the month of confirmation of ASF was used for the seasonal categorisation, irrespective of the year of the occurrence of ASF. For the detection of seasonal patterns of ASF occurrence, the confirmation dates of ASF for each wild boar record and each outbreak in domestic pigs were converted into a time series and dissected by using the function "decompose" within the R package "stats" [31]. Therefore, a time series is dissected into its components of an overall trend, seasonal effects and remaining random noise by using moving averages to remove the trend and by calculating seasonal figures via averaging over each time unit and over all periods [32]. The Friedman rank test was used to test for seasonality in the time series using the R package "seastests" [33,34]. In addition to global tests for all domestic pig outbreaks and wild boar cases included in the analysis, tests were performed for each country individually considering only the years from the first ASF occurrence until the last ASF occurrence. p-values below 0.05 were considered statistically significant and therefore considered as confirmation of seasonality in the tested time series.

3. Results

3.1. Domestic Pigs

In total, the dataset contained 6828 records of ASF outbreaks in domestic pigs. The radar charts (Figure 1) show an increased number of confirmed ASF outbreaks mainly in the summer months (June to September, with the largest numbers of outbreaks in July and August). This pattern was evident for all countries irrespective of the total number of outbreaks per country, except for Greece and Italy. Since only a single ASF outbreak occurred in these two countries (Greece, February 2020; Italy, June 2022) during the study period, seasonality could not be analysed in a meaningful way.

Decomposing the time series of all ASF outbreaks in all countries included in the analysis revealed a seasonal pattern with one yearly peak (Figure 2 and Appendix A, Figure A1). Figure 2 shows a summary of the seasonal pattern in the monthly course of the year for all analysed countries during the study period. Appendix A, Figure A1 shows the details of the components of the dissected time series. The seasonality in the time series was confirmed in the global Friedman test ($p < 0.001$) for all countries included in the analysis. The yearly peak according to the dissection of the time series occurred in summer, mainly in July and August, and it could be detected in Bulgaria, Estonia, Germany, Latvia, Lithuania, Poland, Romania and Slovakia (Figure 3). However, the Friedman rank test confirmed seasonality in Latvia ($p = 0.032$), Lithuania ($p = 0.026$), Poland ($p = 0.004$) and Romania ($p = 0.003$) only (Appendix A, Table A1).

Figure 1. Radar charts of domestic pig outbreaks. Radar charts show the seasonal distribution of number of ASF outbreaks per month in domestic pigs in different European Union countries, irrespective of the year (the scales are adjusted to the maximum number of outbreaks in each country).

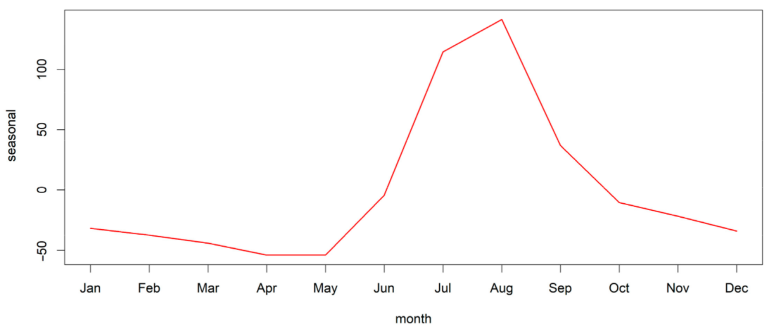

Figure 2. Seasonal pattern of domestic pig outbreaks in the European Union. The figure shows a summary of the seasonal pattern in the monthly course of the year of total ASF outbreaks in domestic pigs in the European Union (except for cases in Sardinia) throughout the time period from the first occurrence in 2014 until 31 December 2022. Further components of the time series are shown in Appendix A, Figure A1.

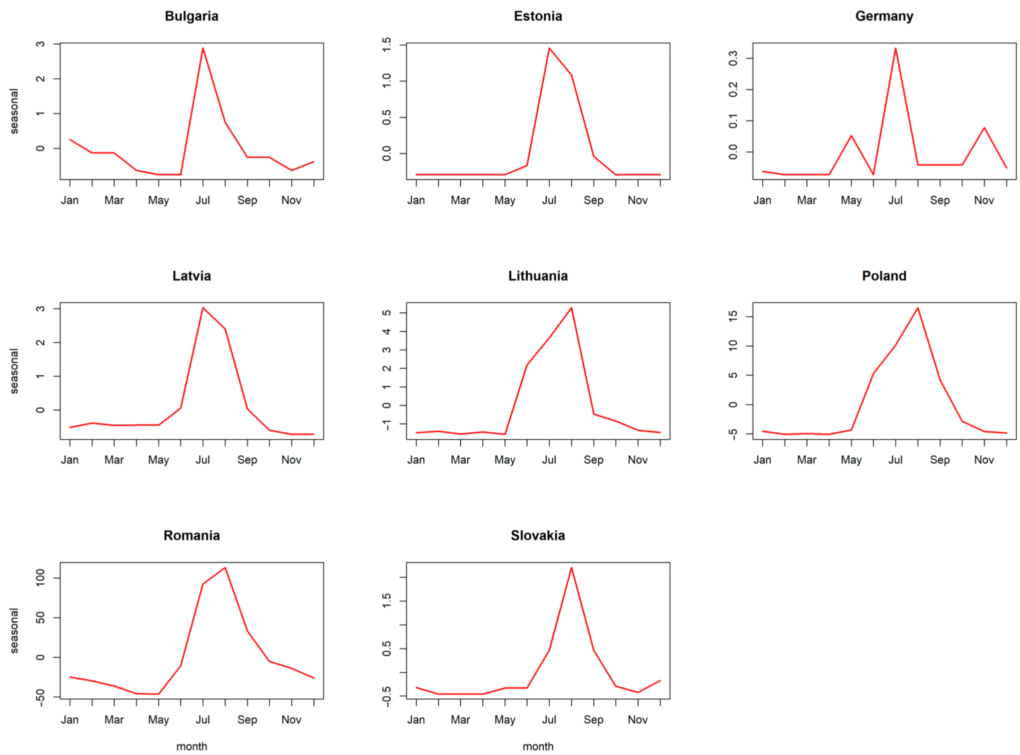

Figure 3. Seasonal pattern of domestic pig outbreaks per country. The figures show a summary of the seasonal pattern in the monthly course of the year of ASF outbreaks in domestic pigs for each country of the European Union, except for Greece and Italy, throughout the time period from the first occurrence in 2014 until 31 December 2022. Since Greece and Italy each had only one outbreak, the detection of a recurring seasonal pattern was not possible.

3.2. Wild Boar

The dataset contained a total number of 50,058 records of ASF cases in wild boar. The monthly distribution of the number of ASF cases in wild boar revealed a similar pattern for most of the analysed countries (Figure 4), but the distribution was not as uniform as for the outbreaks in domestic pig holdings. Decomposing the time series of all cases revealed a recurring yearly pattern with one bimodal peak in the winter months and a smaller peak in summer (Figure 5 and Appendix A, Figure A2). The seasonality in the time series was confirmed in the global Friedman rank test ($p < 0.001$) for all countries included in the analysis.

Large case numbers were observed in Bulgaria, Estonia, Germany, Latvia, Lithuania and Romania in the winter months, especially in December and January. Decomposing the data for each country individually (Figure 6) confirmed a seasonal peak in the winter months, mainly in January, for these countries. However, the Friedman rank test confirmed seasonality in Bulgaria ($p = 0.012$), Estonia ($p < 0.001$), Latvia ($p < 0.001$), Lithuania ($p = 0.018$) and Romania ($p = 0.003$) (Appendix A, Table A2).

Germany and the Baltic States (Estonia, Latvia and Lithuania) reported in addition large numbers of ASF cases during summer, mainly in July, leading to an extra summer peak. Only in the Czech Republic was a similar pattern seen. The largest numbers of cases were recorded in July and November, leading to peaks in summer (July) and autumn (November). Belgium recorded most cases in February leading to a winter peak. However, the seasonal pattern in both countries was not significant (Appendix A, Table A2).

By contrast, Hungary and Slovakia detected the largest number of cases in spring, especially in March and April, leading to a peak in this season. Poland reported the largest number of cases from December through to March leading to a winter peak (mainly in January) and a spring peak (mainly in March). The seasonal pattern was significant in

Hungary (p = 0.009) and Poland (p = 0.001) (Appendix A, Table A2). In Italy, most cases occurred in May and June, leading to a seasonal peak in May.

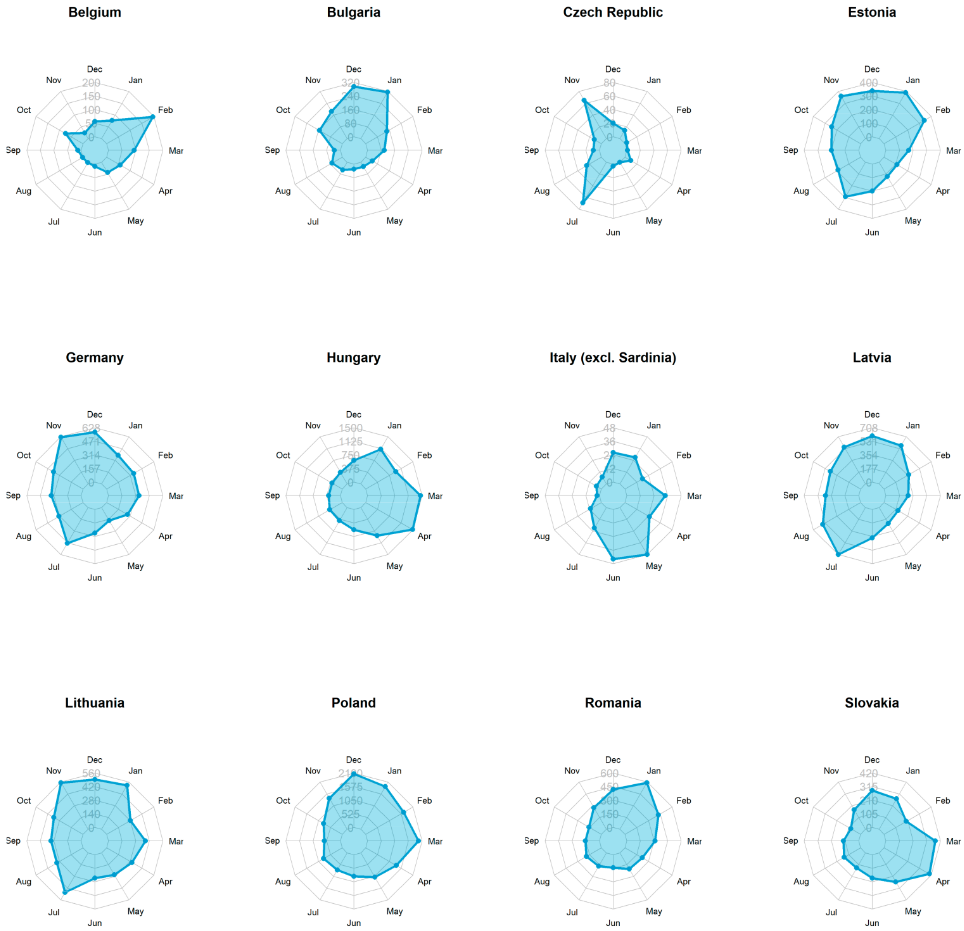

Figure 4. Radar charts of wild boar cases. Seasonal distribution of wild boar tested positively for ASF in different European Union countries across the months (except for cases in Sardinia), irrespective of the year of occurrence (the scales are adjusted to the maximum number of cases in each country).

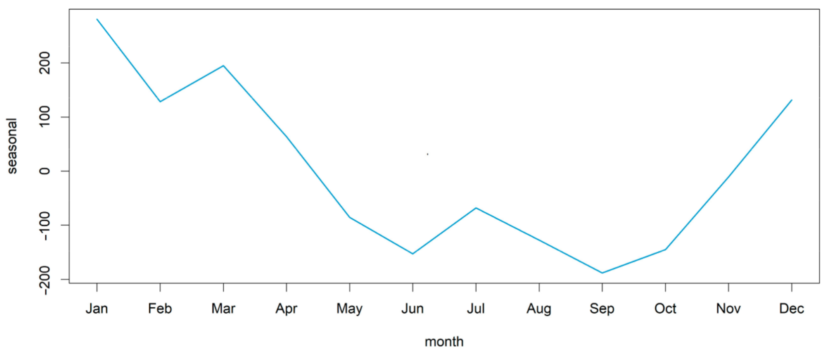

Figure 5. Seasonal pattern of wild boar cases in the European Union. The figure shows a summary of the seasonal pattern in the monthly course of the year of total ASF cases in wild boar in the European Union (except for cases in Sardinia) throughout the time period from the first occurrence in 2014 until 31 December 2022. Further components of the time series are shown in Appendix A, Figure A2.

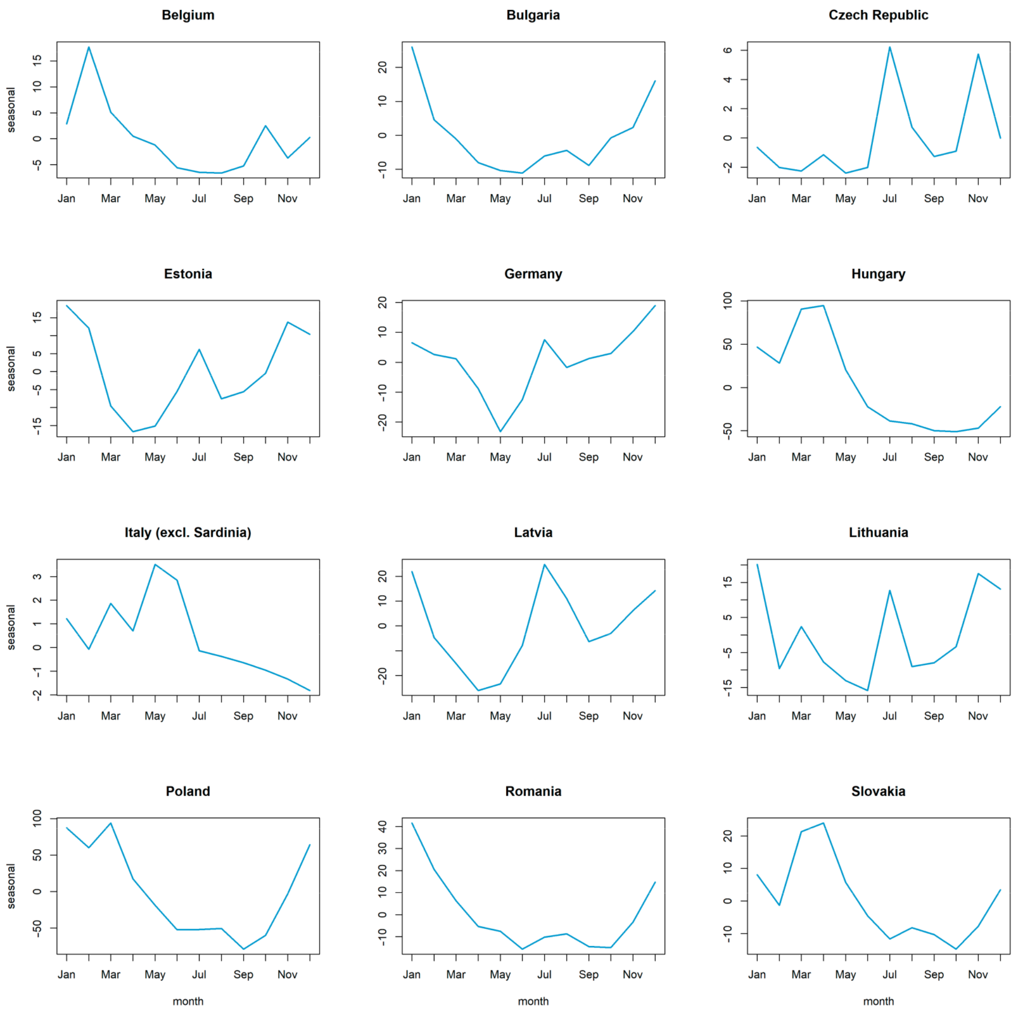

Figure 6. Seasonal pattern of wild boar cases per country. The figures show a summary of the seasonal pattern in the monthly course of the year of ASF cases in wild boar for each country of the European Union (except for cases in Sardinia) throughout the time period from the first occurrence in 2014 until 31 December 2022.

4. Discussion

The data used in our study originated from the ADIS database and contained official information about ASF cases in wild boar and outbreaks in domestic pigs in several European countries, particularly in member states of the European Union.

Although the databases hold information on confirmed cases or outbreaks, the test method used to identify and confirm ASF infection is not reported. The detection of ASFV or its genome in samples from domestic pigs or wild boar indicates acute infection at the time of sampling, thus providing relatively precise information on the period when the animal was infected. By contrast, the detection of antibodies only shows that the respective animal had been exposed to ASFV more than at least a few days before sampling. However, animals may remain seropositive for at least several weeks or even months [35,36], so it is not possible to determine the period of infection with ASFV precisely. The inclusion of serological test results for ASFV confirmation may therefore reduce the precision of the information on the true time of infection and may thus hamper seasonality analyses. However, the loss of precision is probably small in the entire dataset, since ASFV is usually detected and confirmed via PCR, i.e., ASFV genome detection. Moreover, it has been shown that the prevalence of ASF-specific antibodies in wild boar is below 2% in the Baltic States in the median [11,23,24,26]. These findings also indicate that the impact of ASFV-seropositive wild boar on the precision of seasonal analyses is very limited in our dataset.

Furthermore, only positive results were reported to ADIS (the case database). For such an analysis, the number of all sampled wild boar and the proportions of positive and negative results would be needed [37]. Moreover, the records in ADIS did not contain background information about the reason for sampling (i.e., passive or active surveillance). Seasonal differences in surveillance intensity may thus influence the overall seasonality patterns observed in our study, but their effect cannot be quantitatively assessed. Furthermore, information about cases and outbreaks were reported to ADIS after the confirmation of ASF, but no information about the putative date of death of the respective animals was included. Especially in wild boar, it cannot be excluded that animals may have died some time before their carcass was detected, which may bias the seasonal pattern. Probst et al. [38] described a method to assess the minimal post-mortem interval, which allows for the approximate time of death of wild boar to be estimated. However, this information was not available in the database. In addition, it is possible that some case records in wild boar each represent more than one ASF-positive wild boar. However, no background information was available, and we consider the likelihood of this issue causing a bias and influencing the seasonal occurrence to be low.

Overall, our analyses showed a relatively uniform seasonal peak of ASF occurrence in wild boar in winter months (mainly in December and January) in the Baltic States, Bulgaria, Germany, Poland and Romania, even though the seasonality in Germany was not significant in the Friedman rank test, presumably due to the shorter time period analysed. This observed seasonality may be an artefact related to an increased surveillance intensity in winter months: Winter is the main season when wild boar are usually hunted. This increases the number of wild boar that can be sampled in this season. Moreover, more hunters dwell in their hunting grounds during this period [39,40], which increases the chance of detecting carcasses of wild boar that succumbed to ASFV infection. In addition, the visibility of wild boar carcasses is higher in winter, since there is less vegetation. Moreover, carcasses decompose slowly because of low temperatures [41]. Altogether, these factors may increase the chance of detecting wild boar carcasses in winter, which could at least in part explain the observed winter peaks of ASF in wild boar.

However, in Lithuania, the ASF prevalence of wild boar found dead in winter was higher than that of animals found in summer between 2014 and 2017 [23] and in 2018 [24]. Also, in Poland, the chance of obtaining ASF-positive test results in wild boar found dead was higher in December and January [25]. This indicates that the detected seasonal pattern of ASF occurrence might not be exclusively caused by the increased sampling of wild boar at this time of the year. The winter peak could also be related to the reproductive behaviour of wild boar and to climate conditions. Since winter is the mating season of wild boar, increased contact rates between animals might lead to a higher risk of ASF transmission. Moreover, carcasses that can pose a risk of infection for living wild boar decompose more slowly in winter than in summer; thus, the capacity for disease transmission through contamination of the environment or interactions of wild boar with carcasses might be increased during this period [41–43]. By contrast, the higher temperatures in summer lead to the faster decomposition of carcasses, so they may vanish or be more frequently overlooked, and the low stability of ASFV at temperatures of 20 to 25 °C in different matrices has been shown [44–46]. Therefore, ASFV may be preserved for a longer time in winter than in summer months.

Nevertheless, our analyses indicated an additional, dominant summer peak in ASF cases in wild boar in the Baltic States and a less dominant summer peak in Germany, which was not detectable in other countries (except for in the Czech Republic, where the epidemic situation was different). Also, in Poland, we detected a bimodal pattern with peaks in winter (mainly in January) and spring (mainly in March).

Further epidemiological investigations of ASF spread in the Baltic States from 2014 to 2021 showed that the largest numbers of samples from passive surveillance were taken in July in Latvia and Lithuania [26], which might contribute to the increased numbers of positive wild boar in this period. Several studies examined the seasonality in wild boar in

Poland with variable results, which could be due to the study areas and periods analysed. Śmietanka et al. [47] observed the largest number of cases and the highest prevalence of ASF in summer and the lowest prevalence in spring and autumn in the period from 2014 to 2015. For the same period, another research group reported the largest number of analysed samples and ASF cases in "July and August but also during February and March" [12]. Similarly, other investigators detected seasonal patterns with peaks in ASF case probability in spring and summer for the period from 2014 to 2016 [48]. By contrast, Frant et al. [25] detected for the years 2017 and 2018 increased chances of obtaining positive results in passive surveillance in winter as compared to summer, while Lu et al. [49] found an increase in the trend for ASF cases in wild boar in October for the period from 2014 to 2017.

Large numbers of samples in summer with large numbers of ASF-positive results could be attributed to increased outdoor leisure activities during summer with increased chances of detecting wild boar carcasses [26]. Yet, the summer peak may also be related to the population dynamics of wild boar: Since spring is the farrowing season, the absolute number of young wild boar is increased during summer. Young pigs are more connected within the population; they have more contacts with wild boar outside their own social group than adults [50] and might have an increased interest in carcasses [51]. Furthermore, the study by Probst et al. [42] showed that most interactions of wild boar with carcasses of their conspecifics were observed in summer and early autumn. Wild boar showed special interest in the insects and maggots present in decomposing carcasses, which are present mostly in the warmer periods of the year [41]. Both factors might increase the risk of disease transmission among young wild boar in summer months.

The patterns of ASF seasonality in wild boar in Belgium and the Czech Republic cannot be compared to the ongoing epidemiological situation in other countries, since the epidemics in these countries were sparked by point infections [52]. The Czech Republic was only affected by ASF for 10 months from 26 June 2017 to 18 April 2018 in a small area of 89 km^2. During this time, an intensive carcass search and depopulation was conducted, especially in the beginning of the outbreak, as well as in March/April 2018 [18,53]. Similarly, Belgium was affected by ASF only in an area of 620 km^2 from 13 September 2018 until the last fresh wild boar carcass confirmed positive was found on 11 August 2019. During the outbreak, intensively organised carcass searches and intensified hunting were conducted in the infected zone [19]. Likewise, by the end of our study period, the mainland of Italy had only been affected by ASF in wild boar for one year. The first case was confirmed in January 2022, followed by increased surveillance activities and sampling efforts in the following months [17]. As more data become available, the seasonal patterns of the ongoing epidemics in the Czech Republic and Italy will have to be re-evaluated.

In contrast to wild boar ASF cases, the seasonal pattern for domestic pig outbreaks was relatively uniform in our study, with a summer peak (mainly in July) detected in the Baltic States, Bulgaria, Germany, Poland, Romania and Slovakia, irrespective of the absolute numbers of outbreaks in these countries. However, the seasonality in Bulgaria, Estonia, Germany and Slovakia was not significant in the Friedman rank test, presumably due to comparatively low case numbers in these countries. The summer peak in ASF outbreaks in domestic pigs has also been described in several other studies in EU countries, mainly the Baltic States, Poland and Romania [13,22,23,49,54]. Interestingly, similar summer peaks have also been reported from the Russian Federation [55–57] and Sardinia [58].

As for the Baltic States, several studies concluded that mostly domestic pig farms with low levels of biosecurity located in areas where ASF was also present in the wild boar population experienced large numbers of outbreaks in summer months [11,23,54]. In Lithuania and Latvia, mostly backyard pig holdings were affected, whereby shortcomings in biosecurity and the feeding of potentially contaminated fresh grass or crops (which are mainly available in summer) to pigs were considered the main factors for ASFV introduction [11,23]. In Estonia, the number of outbreaks in commercial pig farms exceeded the number of outbreaks in backyard farms, whereby ASFV was most likely introduced by contaminated fomites, such as clothing, vehicles, feed or bedding material [54].

Similar observations were made in domestic pig farms in Poland in the period from 2014 to 2021. The majority of outbreaks in domestic pigs occurred close to areas with previous ASF cases in wild boar [59]. In addition to spillover from the wild boar population, illegal trade, the burial of pigs from non-confirmed outbreaks and the introduction of ASFV by seasonal workers from other Eastern European countries were considered as other potential ways of virus introduction [59].

In Romania, the epidemic situation differs from that in other countries, since primarily outbreaks in domestic pig farms have been reported [21]. Nevertheless, mainly backyard holdings or farms with low levels of biosecurity were affected, and, therefore, transmission routes similar to those mentioned above are considered [13,21].

These observations led to the conclusion that the seasonal patterns of ASF occurrence in domestic pigs are closely linked to wild boar disease dynamics—at least in the Baltic States and Poland—with spillovers of the virus eventually occurring in both directions. Nevertheless, ASF has also occurred in domestic pig farms with high biosecurity settings and/or further away from the epidemic front in the wild boar populations and could not always be attributed to human-associated transmission [13]. This and the detected seasonal patterns with increased case numbers in wild boar and in domestic pigs in spring and summer led to the idea of the potential involvement of an arthropod vector in the transmission cycle of ASF in Europe. This was also concluded from epidemiological investigations by the EFSA, in which they found that most outbreak farms in Romania were located near water sources and that ASF spread increased in the time period following the rainy season, which would provide favourable conditions for insect resurgence [13]. However, no evidence has so far been presented demonstrating that an arthropod vector might currently be involved in ASFV transmission on the European continent [60–63].

It is known that soft ticks of the genus *Ornithodoros* are competent vectors of ASFV in Africa [64]. In Central Europe and the Baltic States, where soft ticks are almost absent, hard ticks have been checked for their potential role in the transmission of ASFV. In two very common hard tick species, *Ixodes ricinus* and *Dermacentor reticulatus*, no virus replication was observed, but still viral DNA could be detected in the ticks after several weeks, indicating that these ticks are very unlikely to be biological vectors but may play a role as potential mechanical vectors [61].

Also, the stable fly *Stomoxys calcitrans* was identified as a potential mechanical vector for ASFV [65]. Moreover, it has been proven that the ingestion of stable flies that previously fed on ASF-infected wild boar leads to the infection of domestic pigs [63]. However, there are no data from the field showing that this plays any epidemiologically relevant role. In a study by Herm et al., various species of blood-feeding arthropods were collected in an Estonian area with a high prevalence of ASF in wild boar in 2017 and tested for ASFV, all with negative results: no ASFV DNA was detected in *Ixodes ricinus* ticks, *Culicoides punctatus* and biting midges of the *C. obsoletus complex*; in *Aedes* spp., *Anopheles* spp. and *Culiseta annulata* mosquitoes; or in *Haematopota pluvialis* tabanids [62].

5. Conclusions

In conclusion, our study shows that seasonal patterns of ASF occurrence in domestic pigs and wild boar in EU countries exist. Knowledge of these patterns provides the potential to adapt control and prevention measures during certain times of high risk and could enable targeted surveillance for the detection of disease in previously unaffected areas and the detection of spread in affected areas. As for outbreaks in domestic pigs, the pattern was relatively uniform, with peaks in the summer months, mainly in July and August. On the contrary, the seasonal pattern for wild boar was not as uniform: most countries showed a peak in the winter months (mainly in December and January), and some showed additional peaks in spring (mainly in March) or summer (mainly in July). These findings suggest that there is a close link between disease dynamics in domestic pigs and wild boar populations, which is dependent on the survival of the virus in the environment, as well as seasonal changes in pig farming and wild boar population dynamics. However, human activities may strongly influence seasonal patterns of ASF occurrence.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

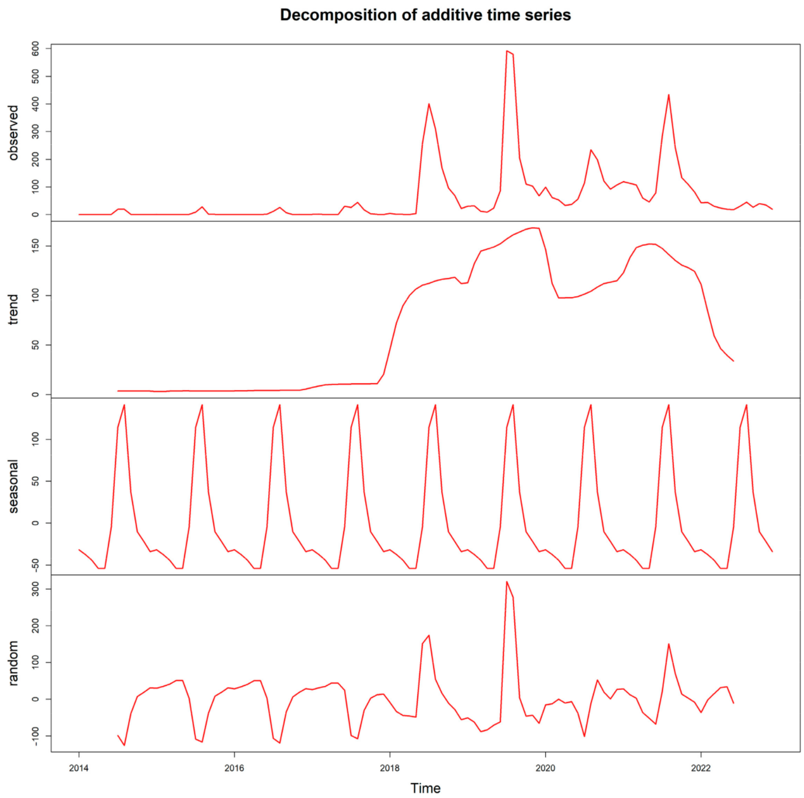

Figure A1. Decomposing the time series of ASF outbreaks in domestic pigs. The figure shows the results for outbreaks in domestic pigs for all countries of the European Union (except for outbreaks in Sardinia) from the first occurrence in 2014 until 31 December 2022 dissected into trends, seasonal patterns and remaining random noise.

Table A1. Results of the Friedman rank tests of time series of domestic pig outbreaks for each EU member state included in the analysis. The table shows test statistics, *p*-values and the tested periods. The period tested in each country begins in January of the year of the first ASF outbreak and ends in December of the last year of ASF outbreaks. Since Greece and Italy (excluding Sardinia) were only affected in one year of the study period, testing for seasonality with the Friedman rank test was not possible (n.a.). *p*-values below 0.05 are shown in bold.

Country	Test Statistic	*p*-Value	Year of First Occurrence	Year of Last Occurrence
Bulgaria	10.31	0.503	2018	2022
Estonia	5.51	0.904	2015	2021
Germany	7.77	0.734	2021	2022
Greece	n.a.	n.a.	2020	2020
Italy	n.a.	n.a.	2022	2022
Latvia	21.12	**0.032**	2014	2022
Lithuania	21.84	**0.026**	2014	2022
Poland	27.75	**0.004**	2014	2022
Romania	27.83	**0.003**	2017	2022
Slovakia	11.10	0.435	2019	2022

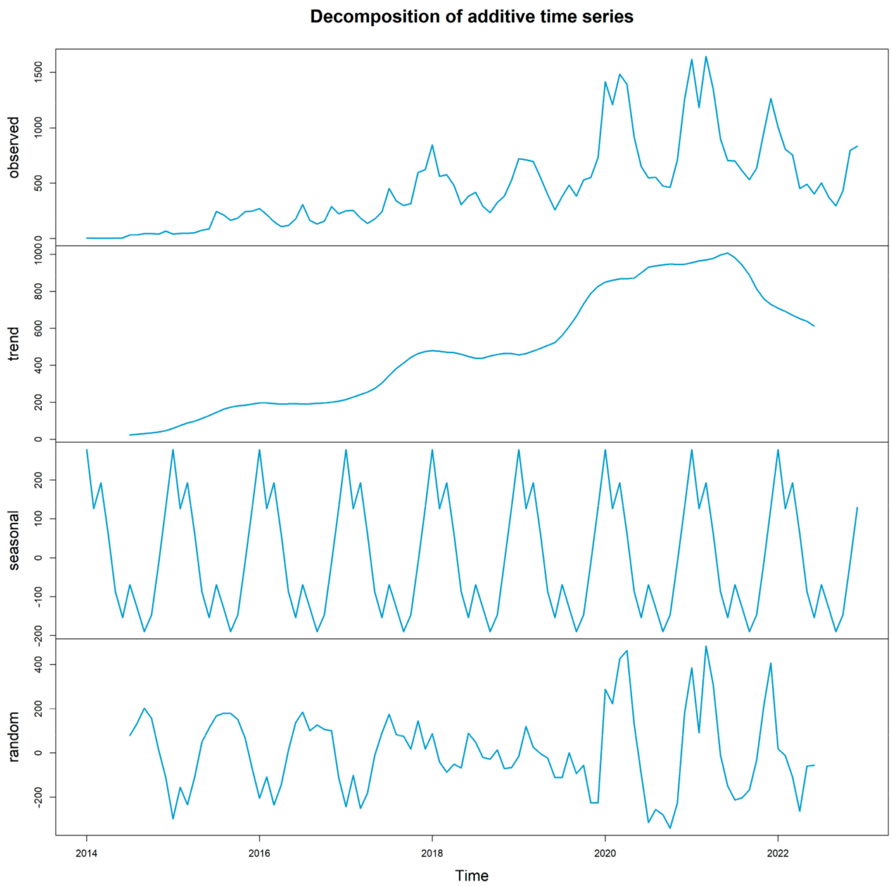

Figure A2. Decomposition of time series of ASF cases in wild boar. The figure shows the results for ASF cases in wild boar in all countries of the European Union (except for cases in Sardinia) from first occurrence in 2014 until 31 December 2022 dissectedinto trends, seasonal patterns and remaining random noise.

Table A2. Results of the Friedman rank tests of time series of wild boar cases for each EU member state included in the analysis. The table shows test statistics, *p*-values and the tested periods. The period tested in each country begins in January of the year of the first reported ASF case and ends in December of the year of the last reported ASF case. Since Italy (excluding Sardinia) was only affected in one year of the study period, testing for seasonality with the Friedman rank test was not possible (n.a.). *p*-values below 0.05 are shown in bold.

Country	Test Statistic	*p*-Value	Year of First Occurrence	Year of Last Occurrence
Belgium	10.92	0.450	2018	2019
Bulgaria	24.26	**0.012**	2018	2022
Czech Republic	2.55	0.995	2017	2022
Estonia	38.12	**<0.001**	2014	2022
Germany	17.92	0.083	2020	2022
Hungary	24.95	**0.009**	2018	2022
Italy	n.a.	n.a.	2022	2022
Latvia	46.81	**<0.001**	2014	2022
Lithuania	22.87	**0.018**	2014	2022
Poland	30.61	**0.001**	2014	2022
Romania	27.98	**0.003**	2018	2022
Slovakia	12.84	0.304	2019	2022

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4 Publication IV: Contrasting seasonality of African swine fever outbreaks and its drivers

Contrasting seasonality of African swine fever outbreaks and its drivers

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Contrasting seasonality of African swine fever outbreaks and its drivers

A B S T R A C T

The seasonality of African swine fever (ASF) outbreaks in domestic pigs differs between temperate and subtropical/tropical regions. We hypothesise that variations in the importance of wild boar-to-farm and farm-to-farm transmission routes shape these contrasting patterns, and we emphasise the implications for effective ASF control.

African swine fever (ASF) is a devastating viral disease that has affected many of the world's domestic pig and wild boar populations, rapidly spreading from sub-Saharan Africa to Europe, Asia, and the Americas (FAO, 2022). The co-circulation of the ASF virus (ASFV) in domestic pigs and wild boars exposes farms to a continued risk of ASFV introduction via both wild boar-to-farm and farm-to-farm transmission (Guinat et al., 2016). Yet, the effective control of ASFV in domestic pigs requires understanding the relative contributions of these two transmission routes to viral incursions on farms.

We present the contrasting seasonal patterns of ASF outbreaks on farms between different climatic zones. Based on this, we discuss how these patterns could result from variations in the dominant transmission route, highlight major uncertainties about the underlying epidemiological and ecological drivers, and suggest how further research could address these knowledge gaps.

The reported number of ASFV-infected herds show pronounced seasonality in Europe and temperate regions of Asia (Fig. 1A), with peaks in summer/autumn. It is unlikely that these summer/autumn peaks could be solely attributed to seasonal changes in pig production. Higher pig production would be expected to lead to more frequent movements of pigs, personnel, and vehicles—resulting in increased contacts with potentially contaminated pig value chain actors and environmental sources. However, pig production in Europe, where ASFV has been circulating for the longest time outside sub-Saharan Africa, and China did not increase during the summer and autumn (Fig. S1). In the Republic of Korea, pig production peaks in summer (Fig. S1), and farm-to-farm transmission has been suggested to have played a role in the country's first epidemic in 2019 (Yoo et al., 2021). However, since 2020, farm outbreaks in the country have remained too sporadic (with a median of 116 days between successively reported farm outbreaks) to conclude that farm-to-farm transmission alone drove the observed seasonal patterns.

These observations raise the possibility that ASFV transmission dynamics in wild boar populations has contributed to these summer/autumn peaks in ASF incidence on farms in temperate regions. In fact, wild boars engage in seasonal reproduction in temperate regions, mostly mating in winter and farrowing in spring, although due to mild winters, this seasonality in the reproduction cycle is less clear than it used to be (Pascual-Rico et al., 2022). The associated changes in social group size, structures, and behaviours could result in seasonal transmission among wild boars, thereby posing a seasonally varying risk of ASFV introduction to farms, as is the case for some other infectious diseases (Plowright et al., 2016). Furthermore, human and wild boar activities are likely to increase during crop harvesting seasons over the summer, further promoting spillover risk. This risk may be further increased by the higher abundance of hematophagous insects in summer, although their role in ASFV transmission outside Africa warrants further investigation (Guinat et al., 2016).

It must be noted that this alternative hypothesis does not seem to be supported by ASF incidence patterns observed in wild boars, considering that they show peaks mostly in winter (Fig. 1B). However, these observations could be strongly biased by seasonal variations in surveillance efforts due to wild boar hunting in temperate regions occurring mainly in winter (Schulz et al., 2022). Reflecting this, ASFV transmission dynamics in wild boars and its seasonal impact on viral incursions on farms are still poorly understood as most studies investigating the interface between wild boars and domestic pigs are based only on ASFV incidence in wild boars, without explicitly accounting for variations in surveillance efforts (Hayes et al., 2021). Furthermore, the occurrence of ASF outbreaks on farms can be influenced by diverse local factors, leading to variations within climatic zones and across years. For instance, in 2022, in Romania, ASF outbreaks among small-scale farms (i.e. fewer than 100 pigs) peaked in both summer and winter, while other European Union Member States continued to experience distinct peaks during the summer, regardless of farm size (European Food Safety Authority et al., 2023).

In subtropical/tropical Asian regions, reported farm cases show less pronounced seasonality (Fig. 1C). First, wild boar breeding in these regions is often protracted or occurs throughout the year (Indian River Lagoon Species Inventory), suggesting that wild boar population dynamics and, consequently, ASFV transmission dynamics in these populations and the risk they pose to farms may differ compared to

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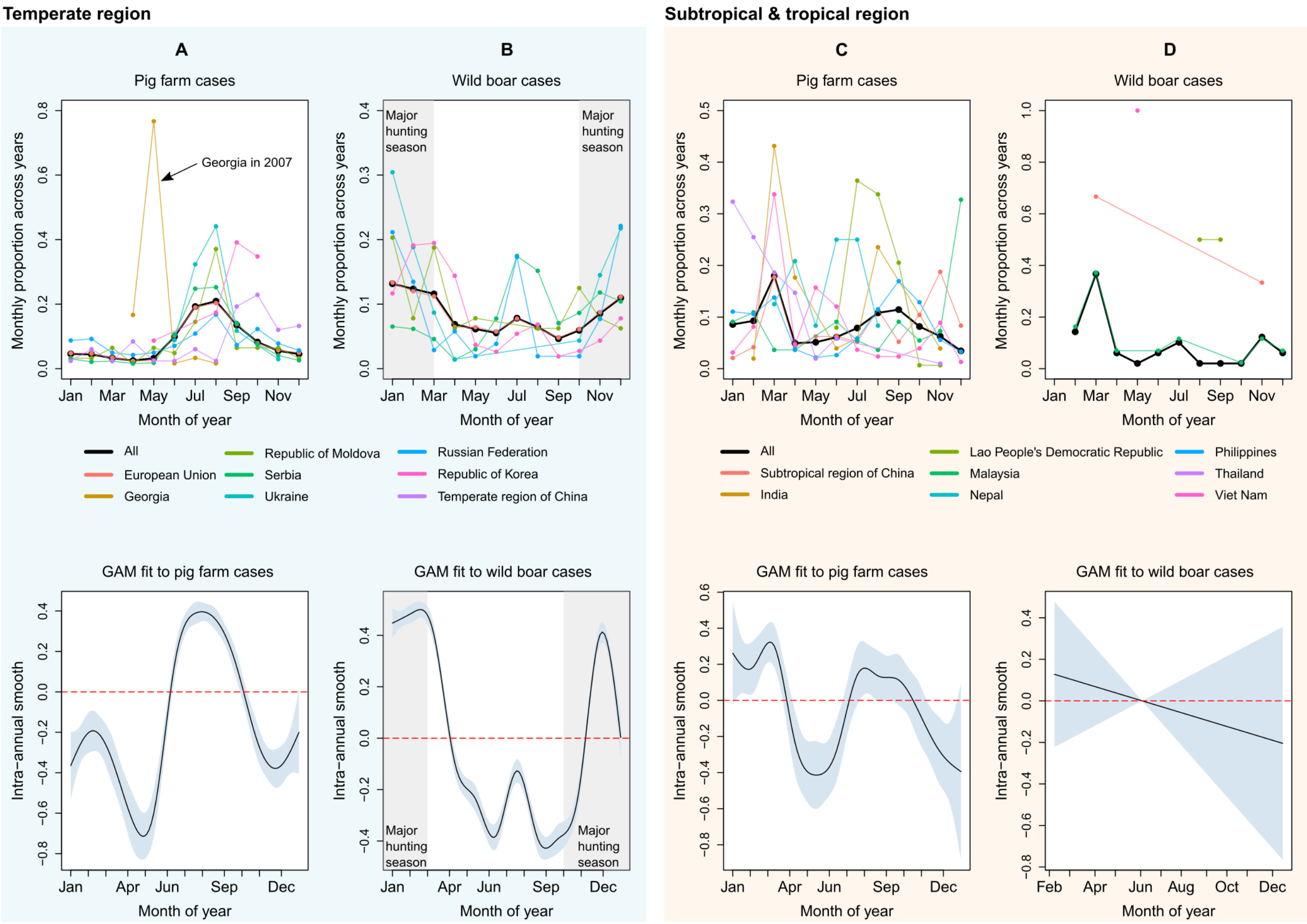

Fig. 1. The seasonal trend in ASF pig farm and wild boar cases by climatic zone. The plots on the first row show the proportion of ASF cases (y-axis) over month of year (x-axis), with different colours representing different countries or regions. Black points and lines represent the seasonal trend averaged over countries or regions with the same climatic zone. The plots on the second row show intra-annual smooth term estimates (y-axis) over month of year (x-axis) obtained by fitting a generalised additive model to incidence data by week of year. The analysis of the seasonal trend is based on FAO EMPRES Global Animal Disease Information System (EMPRES-i) data [downloaded September 24, 2022]. Only countries with more than 50 domestic pig farm cases before Sep 24, 2022, including those within the EU, are included to show the seasonal trend in countries where domestic pig farms are likely to have been exposed to a continuous risk of ASFV introduction. ASF cases in the countries of the EU are grouped, considering that ASF surveillance/control regulations are relatively more homogeneous within the EU compared with other countries. Conversely, ASF cases in China are separated by climatic zone, given that the country's land spans different climatic zones.

temperate climatic regions. In addition, if pig production varies seasonally, as observed for poultry production in some of those regions (Delabouglise et al., 2017), it could promote farm-to-farm viral transmission during high production periods. However, discerning the relative contribution of different transmission routes is expected to be more challenging in those regions considering the often limited availability of epidemiological and ecological data, especially of ASF surveillance data pertaining to wild boars (Fig. 1D) and farms (Vergne et al., 2020).

In conclusion, seasonal trends in farm outbreaks and their differences between climatic zones highlight the need to better understand the interface between wild boars and domestic pigs. To achieve this, it is crucial to assess ASFV transmission dynamics in wild boars and its potential associations with viral incursions on farms, taking into account wild boar ecology and variations in surveillance efforts. Furthermore, while these seasonal patterns differ between climatic zones, there are also variations within each zone and across years. These likely result from heterogeneous epidemiological contexts, emphasising the importance of characterising local pig value chains and understanding the way in which they may influence the observed seasonality in ASF outbreaks. Conducting such research would help quantify the relative contributions of wild boar-to-farm and farm-to-farm transmission routes to ASFV farm outbreaks, thus guiding optimal ASFV prevention and control strategies. These are challenging but important tasks.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.epidem.2023.100703.

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5 Publication V: African Swine Fever in Wild Boar: German Hunters' Perception of Surveillance and Control – A Questionnaire Study

African Swine Fever in Wild Boar: German Hunters' Perception of Surveillance and Control – A Questionnaire Study

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African Swine Fever in Wild Boar: German Hunters' Perception of Surveillance and Control—A Questionnaire Study

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Simple Summary: Effective control of African swine fever in wild boar relies on cooperation with hunters, who are involved in the local implementation of surveillance and control measures. This study focused on understanding German hunters' perceptions of different control measures and factors that influence compliance. Measures that hindered hunting were generally considered ineffective. Some measures were seen as controversial as they were seen as contrary to fair hunting practices. Effective communication and raising awareness are recommended to improve compliance with controversial measures. This study also highlighted the need to address hunters' concerns and provide adequate compensation to maintain their motivation to participate in ASF control efforts. Among others, financial incentives and reduced bureaucracy were identified as motivating factors.

Abstract: Since the first occurrence of African swine fever (ASF) in wild boar in Germany in 2020, the disease has primarily affected the wild boar population in the eastern part of the country close to the border with Poland. Local hunters play a crucial role in implementing surveillance and control. To evaluate their perceptions of existing control measures and analyze regional differences between hunters from ASF-affected and non-affected regions, a questionnaire study was conducted among the German hunting community. Hunters from non-affected areas held a more optimistic view regarding the effectiveness of control measures compared to hunters from affected areas. However, control measures that hinder hunting were generally perceived as ineffective. Measures that collided with hunters' understanding of fair hunting practices were regarded as controversial. Financial incentives and reducing bureaucracy were the most favored approaches to increase hunters' participation. Moreover, the possibility of eating or selling the meat of hunted wild boar and the provision of infrastructure for implementing ASF control were considered motivating. Thus, this study highlights the importance of compensating hunters and addressing their concerns to maintain their engagement in ASF control. To enhance compliance with controversial measures, thoughtful communication and raising awareness are essential.

Keywords: African swine fever; wild boar; hunters; participation; surveillance; control

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1. Introduction

African swine fever (ASF) is a severe hemorrhagic disease of different suid species, including domestic pigs and wild boar. The disease originates from the African continent and can cause a variation of symptoms, ranging from peracute death to subclinical infection [1]. The disease has been absent from the European continent since 1995 with the exception of the Italian island of Sardinia, where ASF was endemic from 1978 to recently [2]. The virus that currently circulates in Eastern, Southern, and Central Europe was introduced into Georgia in 2007 [3]. In the following years, ASF spread throughout the Caucasus Region and reached the Russian Federation, where it mainly affected domestic pigs [4]. Subsequently, the disease was introduced into the European Union, where it was first discovered in Lithuania in January 2014 and Poland one month later [5]. It then spread to Latvia in June 2014 [6] and Estonia in September 2014 [7]. The disease was subsequently introduced into several other countries including the Czech Republic, Romania, Belgium, Bulgaria, Greece, Hungary, Serbia, and Slovakia [8]. In November 2019, ASF unexpectedly emerged in wild boar in Western Poland [9]. Ten months later, on the 10th of September, the first case of ASF in wild boar in Germany was confirmed close to the Polish border [10]. Meanwhile, several other countries became affected by ASF, such as Bosnia and Herzegovina, Croatia, and the Italian mainland (outside of Sardinia).

The ongoing westward spread of the virus throughout the European Union is mainly driven by virus persistence in wild boar populations, described as the "wild boar habitat cycle" [11]. The virus can be transmitted from infected wild boar or contaminated carcasses to susceptible conspecifics [11]. In addition, humans represent a risk factor for the long-distance transmission of ASF and its introduction into domestic pig holdings [12]. The lasting presence of the ASF virus in wild boar populations poses a risk of infection for commercial domestic pig holdings and leads to trade restrictions that cause huge economic losses [13].

By July 2022, wild boar populations in three out of the sixteen federal states of Germany were affected by ASF: Saxony, Brandenburg, and Mecklenburg–Western Pomerania (as reported to the EU Animal Disease Notification System). The disease has apparently been eliminated in the affected wild boar population in Mecklenburg–Western Pomerania, and control seems to be successful in large parts of Brandenburg [14,15]. However, there are still cases emerging in Brandenburg and Saxony. So far, eight outbreaks in domestic pig farms occurred in the German federal states of Brandenburg, Mecklenburg–Western Pomerania, Saxony, Lower Saxony, and Baden–Wuerttemberg.

Mainly, passive surveillance is used for ASF detection in wild boar in Germany [10]. The importance of passive surveillance (i.e., searching, sampling, and removing wild boar carcasses) for the detection of ASF cases has been proven crucial by several studies showing that the probability of finding ASF-positive animals is much higher in animals found dead compared to hunted animals [16,17]. On the other hand, active surveillance (i.e., sampling apparently healthy wild boar through hunting) and increased hunting of wild boar to reduce the susceptible population and decrease wild boar reproduction rates is another important part of ASF control [18,19].

Regarding the implementation of the above-mentioned ASF control measures in wild boar, local hunters represent one of the most important stakeholders. Their willingness to participate in the implementation of measures such as intensified hunting, the search for wild boar carcasses, the sampling of dead wild boar, and increased biosecurity is of utmost importance for the success of surveillance and control measures. In addition, their knowledge of the local situation, in particular the wild boar population, is an important basis for the planning and implementation of measures. Thus, the necessity for research about hunters' perceptions of ASF control has been highlighted [20–23]. Different approaches have recently been used, e.g., performing participatory studies with the hunting communities of Latvia and Estonia [24,25] and conducting a questionnaire and a participatory study in Lithuania [26,27].

Building on these studies, we aimed to capture German hunters' perception of ASF surveillance and control in the German wild boar population by performing a web-based questionnaire study. To this end, we aimed to answer the following questions:

1. Which tasks do hunters fulfill in ASF surveillance and control and how do they assess the effectiveness of these tasks?
2. Which obstacles do hunters experience or expect when participating in ASF surveillance and control?
3. Which options do hunters consider as motivational to increase their participation in the intensified hunting of wild boar as well as intensified search for wild boar carcasses?

Based on the analysis of hunters' replies, starting points for optimizing surveillance and control of ASF in wild boar in Germany in cooperation with the national hunting community were identified.

2. Materials and Methods

2.1. Development and Content of the Questionnaire

The questionnaire was designed in the German language with the web-based survey tool "SoSci Survey" (https://www.soscisurvey.de/, accessed on 15 July 2022). The final draft of the questionnaire was pretested by four hunters in the first round and six hunters in the second round evaluating the clarity of questions and response options, the length of the questionnaire, and its technical implementation. After each round, improvements and corrections were made based on the comments of the testers.

The final version of the questionnaire (Supplementary Materials Document S1 and S2) included 26 questions that were estimated to take less than 15 min to answer. The questionnaire was divided into the four following parts:

1. Hunters' part in ASF control and surveillance measures (questions 1 to 4 and 8 to 11);
2. Hunters' perceptions regarding ASF control and surveillance (questions 5 to 7);
3. Motivational options for increased participation (questions 16 and 17);
4. General information about the participants (questions 12 to 15 and 18 to 26).

The questionnaire consisted of mandatory closed single-choice questions, closed and semi-closed multiple-choice questions, and three assessments based on five-point Likert scales. Voluntary open questions with the possibility of free-text input were included to allow participants to express the background of their decisions and to add suggestions to our proposed answer options.

At the end of the questionnaire, the participants also had the chance to add further comments on their perception of ASF control and surveillance. In addition, an option to submit contact data separately was implemented at the end of the questionnaire, if participants were interested in information about the results of the study or information about further participatory studies. Received contact data were saved and downloaded separately from study results, and no connection between the respective responses and the contact data of the participants was feasible.

2.2. Distribution of the Questionnaire

The anonymous and voluntary web-based data collection was conducted in a first period of 33 days from 29 April to 31 May 2022. The link to the questionnaire was distributed mainly via (a) private networks and social media accounts of authors and pretesters, (b) social media accounts and newsletter of the national German hunting association ("*Deutscher Jagdverband e.V. (DJV)*"), and (c) social media accounts and dashboards of German hunting magazines ("*Pirsch*", "*Wild und Hund*", "*Deutsche Jagdzeitung*").

Upon the request of hunting authorities, the data collection was extended by a further 40 days in a second period from 7 June to 20 July 2022, adding up to a total time of data collection of 77 days. In the second period, the link to the questionnaire was distributed via the contact networks of hunting authorities of the German federal states *("Oberste Jagdbehörden"*).

2.3. Ethics

The study received ethical clearance from the Ethics Committee of the University of Greifswald, University medicine, reference BB 044/22.

Participants were informed in writing of the background and the purpose of the study on the first page of the questionnaire. By taking part in the survey, participants agreed to an anonymous use of their answers for research and publication. No financial or other kind of compensation was rewarded for the participation. Since it was a web-based survey without any conditions of participation, everybody with access to the active questionnaire link could participate.

2.4. Questionnaire Analysis

For the analysis, only completed interviews with responses to all mandatory questions were considered. The data set was divided into three regional groups (Figure 1). The first group (a) ("affected") consisted of participants who stated to hunt mainly in areas (based on postal code) that were affected by ASF and considered as restriction zones as of 3 May 2022. The second group (b) ("vicinity") consisted of participants who hunted in at least one of the federal states neighboring the restriction zones with ASF outbreaks in wild boar. The third group (c) ("non-affected") consisted of participants who stated neither to hunt in affected federal states nor in any state adjacent to an affected federal state (Figure 1).

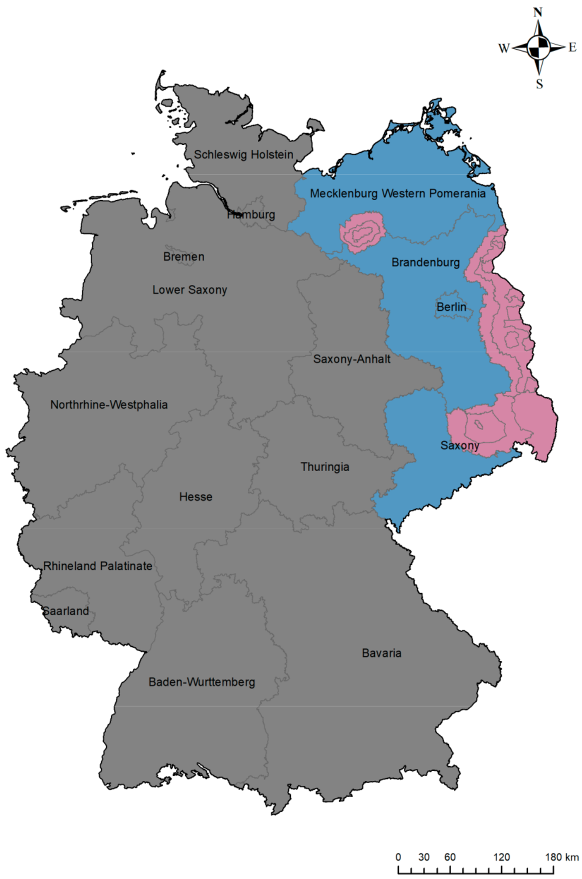

Figure 1. Overview of the study area. The map of Germany shows the ASF-restriction zones (red) as of 3 May 2022 based on the occurrence of ASF in wild boar ("affected"). The blue area represents the remaining areas of the federal states in the vicinity of ASF outbreaks ("vicinity"). The grey area represents federal states not affected by ASF in wild boar ("non-affected").

Statistical analyses were conducted using the statistic software R version 4.1.2 [28]. The packages "tidyverse" [29] and "dplyr" [30] were used for data management and descriptive statistics. Graphics were created with the package "gglplot2" [31].

For the analysis of single-choice and multiple-choice questions, the relative frequencies of the answers were calculated. The results were tested for statistical differences between the three groups using the chi-squared test. After applying the Bonferroni correction, p-values below 0.017 were considered statistically significant.

For the analysis of answers to Likert scale questions, the relative frequencies of each level of the scale (from 1 to 5) and the median level of all answers were calculated. The results were tested for statistical differences between each of the three groups using the pairwise Wilcoxon rank-sum test with the Bonferroni correction. P-values below 0.05 were considered statistically significant.

Free-text responses were manually analyzed by the authors using ATLAS.ti 22 [32]. Coding was used to categorize the contents of the free-text responses systematically in order to identify patterns and themes. The frequencies for each code were counted for hunters from areas affected by ASF, hunters in the vicinity of ASF, and hunters from non-affected areas, and relative frequencies were calculated for each code in relation to the total number of free-text responses for that question. We show the top three codes for each question. The complete list of code books, code explanations, and code frequencies for each free-text question can be found in the supplementary materials.

3. Results

3.1. Response and Dropout Rates

In total, 2707 filled-in questionnaires were received, out of which 1553 were complete (57%), i.e., they contained answers to all mandatory questions. Only these responses were used for further analysis. The majority of complete responses were received in the first period of data collection (n = 1019) (Figure 2). The distribution of the weblink to the questionnaire via the dashboards and social media accounts of hunting magazines led to the highest daily number of responses on 19 and 20 May 2022. No responses were received from 6 May to 9 May, on 14 July, 16 July, and from 19 July to 20 July 2022. From 1 June to 6 June 2022, the link to the questionnaire was offline; therefore, responses could not be received.

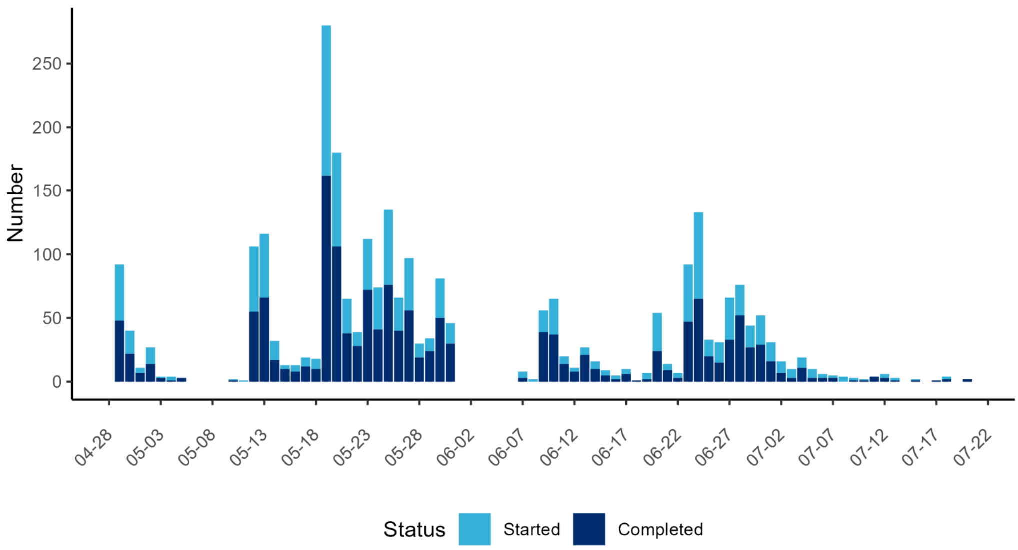

Figure 2. The number of started (light blue) and fully completed (dark blue) responses in a web-based questionnaire for German hunters for each day of the study period. The x-axis represents the date. The y-axis represents the number of responses received per day.

More than half of the participants who did not finish the questionnaire dropped out on the second page that contained the first set of questions on hunters' part in ASF control and surveillance measures (questions 1 and 2). An additional 17% and 10% left the questionnaire on page 3 (questions 3 and 4) and page 4 (questions 5 and 6), respectively. Only a few participants (<5%) terminated the questionnaire in other parts, except for page six, where another 8% left the questionnaire. Page six contained questions on implemented measures (questions 8 and 9).

3.2. General Information about Participants

The demographic distribution of the participants is shown in Table 1. The majority of the participants were male, over 40 years old, and had more than ten years of hunting experience. Regarding their hunting area, 83.9% of the participants selected only one federal state, 10.4% selected two federal states, and only 2.1% selected more than three federal states. Moreover, 1,391 hunters (89.6%) submitted the postal code of their main hunting area. Based on the postal codes, 414 participants (26.7%) hunted mainly in ASF restriction zones as of 3 May 2022, and were considered as "affected". A number of 457 participants (29.4%) hunted in Brandenburg, Mecklenburg–Western Pomerania, and Saxony outside the restriction zones and in the City of Berlin (a separate federal state, surrounded by Brandenburg) and were considered "in the vicinity of ASF". Another 682 participants (43.9%) hunted in federal states that have so far not been affected by ASF in wild boar and were, therefore, considered "non-affected".

Table 1. Demographic information of the participants (n = 1553). The table shows the relative proportion (in %) of gender, age, and years of hunting experience of the participants in a web-based questionnaire study for German hunters.

Gender (%)		Age (%)		Hunting Experience (%)	
Female	**12.1**	**Under 20 Years**	**1.5**	**Under 3 Years**	**5.7**
Male	87.4	20 to 40 years	27.9	3 to 10 years	25.7
Diverse	0.5	41 to 60 years	47.0	11 to 30 years	40.2
		Over 60 years	23.6	Over 30 years	28.4

The majority of participants were owners of walk-in certificates ("*Begehungsschein-inhaber*") for hunting districts (48.9%) or leased hunting districts (46.6%). Only 6.0% of the participants owned a hunting district themselves. While 2.9% of hunters provided no specific answer to the question, 9.9% stated to organize hunting in a different way and gave a free-text response (Supplementary Table S1). The majority of them (47.7% of 153 free-text answers) stated to be professional hunters. Another 13.7% of the free-text answers stated to be hunting officers ("*Jagdaufseher*") in their local districts, and 6.5% were regular or irregular guests in hunting districts. Hunters in affected areas and the vicinity went hunting for wild boar significantly more often than hunters in non-affected federal states ($p < 0.001$ and $p = 0.014$) and had a significantly greater estimated mean annual hunting bag of 20 and 22 wild boar hunted per year than hunters from non-affected areas with a mean annual hunting bag of 16 wild boar ($p < 0.001$) (Table 2).

Table 2. Proportion (in %) of hunters from ASF-affected regions (n = 414) in the vicinity of ASF (n = 457) and non-affected regions (n = 682) that hunted less than 1 time, 1 to 5 times, 6 to 10 times or more than 10 times per month as mentioned in a web-based questionnaire study for German hunters. Hunters from affected areas and in the vicinity of ASF hunted significantly more often than hunters from non-affected areas ($p < 0.001$ and $p = 0.014$). No statistically significant difference was detected between affected areas and vicinity areas ($p = 0.074$).

	Affected (%)	Vicinity (%)	Not Affected (%)
Less than 1 time	4.8	4.8	8.9
1 to 5 times	26.8	33.3	37.1
6 to 10 times	33.1	34.1	29.6
More than 10 times	35.3	27.8	24.3

Regarding the use of hunting tools, 38.5% of all hunters stated that they used silencers and night vision devices when hunting for wild boar. A total of 30.5% stated that they only used night vision devices. In contrast, 21.1% stated that they used neither silencers nor night vision devices.

More hunters in the affected federal states received financial compensation for hunted wild boar (a: 82.9%, b: 45.9%, c: 32.0%), sampling of wild boar (a: 51.0%, b: 35.9%, c: 25.8%), and notifications of detecting wild boar carcasses (a: 51.9%, b: 26.1%, c: 15.4%) than hunters in the vicinity of ASF and hunters in regions not affected by ASF. In total, 19.9% of the participants stated that they received no financial incentives and another 15.5% stated that they did not know if they were eligible to claim incentives.

A percentage of 12.5% of the participants stated that they also hunted in other European countries, and 18.0% reported that they had contact with domestic pig holdings on a regular basis.

3.3. The Role of Hunters in ASF Surveillance and Control

3.3.1. General Attitude of Hunters to the Success of ASF Control

Without any statistically significant differences between the groups (a vs. b: $p = 0.361$, b vs. c: $p = 0.950$, a vs. c: $p = 0.451$), 46.7% of the participants considered the elimination of ASF in wild boar in Germany possible. In contrast, 29.5% of the participants believed that the elimination of ASF in Germany is possible and 23.8% chose the answer option "I don't know".

In total, 1015 hunters used the free-text input to give reasons for their choice. A total of 657 hunters provided reasons for why they did not believe that ASF elimination is possible. The main reason was that they did not think it was possible to reduce the wild boar population to a level that allows ASF elimination, i.e., the population is too large and hunting is not possible in all areas (19.0% out of 657 free-text answers). They also pointed out that the routes of transmission of ASFV are too diverse and difficult to control, making it impossible to eliminate ASF (10.5%). In particular, they considered humans to be a key factor in the transmission of ASF through tourism, seasonal workers, immigration, or contamination of the environment with infectious food waste (16.6%). By contrast, 335 hunters provided reasons why they believed that the elimination of ASF is possible. They mainly stated that population reduction achieved through intensified hunting (28.7% out of 335 free-text answers) and the consistent enforcement and implementation of surveillance and control measures (21.8%) will lead to the elimination of ASF. Furthermore, success in other countries, such as Belgium or the Czech Republic, was seen as a good example that elimination might also be possible in Germany (17.6%). Out of 113 hunters who gave an explanation of why they were unsure whether the elimination would be possible or not, 9.7% thought that the elimination of ASF in Germany is hampered by continued infection pressure from Eastern European countries. However, 8.9% (out of 113 free-text answers) believed that slowing down the spread of ASF is possible.

Regarding the role of hunters in ASF control, without any statistically significant difference between the groups, 81.5% of the participants agreed with the statement that hunters play a crucial role in ASF control, 13.8% disagreed, and 4.8% chose "I don't know" (a vs. b: $p = 0.289$, b vs. c: $p = 0{,}330$, a vs. c: $p = 0.041$).

A total of 1194 hunters used the free-text option to explain the reasons behind their responses. Of these, 976 hunters explained why they play a key role in ASF control. The majority of them (51.3% out of 976 free-text answers) considered hunters as a key player because they have the authority, knowledge, and equipment to reduce and control the wild boar population, which is an important part of ASF surveillance and control. It was pointed out by 38.6% that hunters know the local conditions best and are familiar with the behavior of wild boar in their hunting area. In addition, 18.0% felt that only the hunting community had the necessary knowledge and skills to control ASF. By contrast, 187 hunters provided reasons why they did not consider the role of hunters in ASF control as crucial. They believed that increased hunting would not lead to the elimination of ASF (15.5% out of 187 free-text answers) or that the wild boar population in Germany is too large to be controlled (12.8%). However, some of them believed that hunters are important but not exclusively responsible for the success or failure of ASF control. They emphasized the importance of the interaction between different stakeholders (14.4%). In addition, some hunters who were unsure whether they play a crucial role in ASF control in Germany (31 answers) pointed out that many transmission routes of ASF (particularly humans or mechanical vectors) cannot be controlled or contained by hunters (19.4% out of 31 free-text answers) and that ASF surveillance and control cannot be carried out by hunters alone—a variety of other stakeholders also need to be involved (16.1%).

The complete analysis of free-text answers for both questions can be found in the Supplementary Materials (Supplementary Tables S2–S7).

3.3.2. Hunters' Knowledge of ASF

Hunters from affected areas assessed their knowledge of ASF as statistically significantly better compared to hunters in the vicinity ($p = 0.033$) and hunters in areas not affected by ASF ($p = 0.002$). In detail, 80.0% of the hunters from ASF-affected regions stated to have rather good or very good knowledge compared to 75.3% of hunters in the vicinity and 72.0% of hunters from non-affected areas. Less than 2.0% of the total participants assessed their knowledge as very or rather poor.

The sources for ASF knowledge that were used by more than half of the hunters were newspapers and hunting magazines (used by 71.0%), written information from the German hunting association (59.8%), personal conversations with other hunters (54.3%), and written information from the local veterinary office (51.6%) (Figure 3). The least used sources were social media (used by 22.7%), events like trade shows or lectures (22.7%), news or documentaries on television (22.6%), and websites of local veterinary authorities (22.3%). Other sources were used and explained further in a free-text input by 18.6% ($n = 289$) of the hunters (Supplementary Table S8). Of these, 24.6% stated that they acquired their knowledge about ASF in a professional context, e.g., in their professional work or during (university) studies. Another 18.4% reported that they gained their personal experience and knowledge through participation in ASF surveillance and control, and 11.8% stated that they had participated in exercises or workshops on ASF surveillance and control.

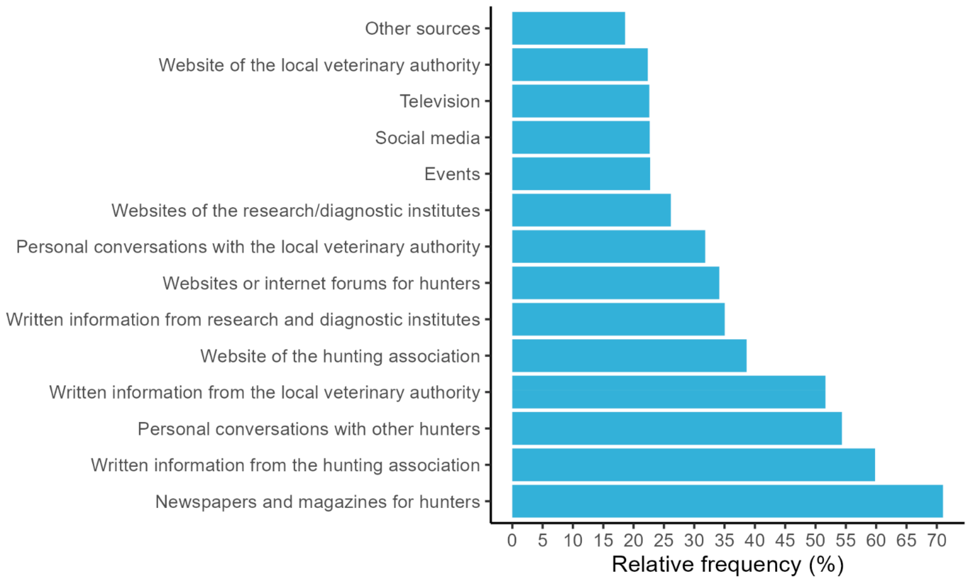

Figure 3. Relative frequency (in %) of hunters that used the respective source to obtain knowledge on ASF ($n = 1553$ responses to a web-based questionnaire for German hunters).

3.3.3. Surveillance and Control Measures Implemented by Hunters

As shown in Figure 4, measures were mainly implemented in terms of ASF surveillance rather than ASF control. The majority of the hunters took part in the increased hunting of wild boar (72.2%), sampling hunted wild boar that appeared sick and wild boar found dead (48.2%), and searching for carcasses as part of their usual hunting activities (47.3%) (Figure 4). The increased hunting of adult female wild boar and the removal of wild boar carcasses were carried out by 39.5% and 37.5% of the hunters as ASF surveillance measures and, to a lesser extent, ASF control measures (Figure 4).

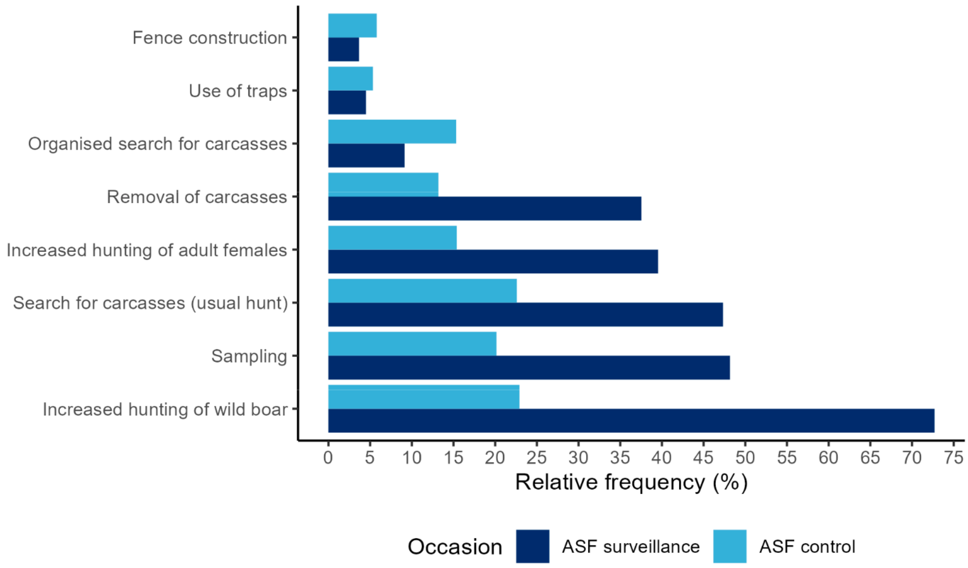

Figure 4. Relative frequency (in %) of hunters that implemented the respective measure in terms of ASF control or ASF surveillance (n = 1553 responses to a web-based questionnaire for German hunters).

Due to official ASF control measures, the organized search for carcasses with the help of human chains, search dogs, and drones (15.3%), the use of large boar traps (5.3%), and the construction of fences (5.8%) were used more frequently for ASF control than surveillance in the hunters' view (Figure 4). Furthermore, 53.0% of the participants stated that they were willing to use traps in case of an ASF outbreak in their area, while 47.0% stated they were not willing to use traps.

When asked if they had implemented measures other than those listed, 391 hunters provided free-text answers (Supplementary Table S9). Of these, 16.4% stated that they had not implemented any additional measures, and 12.5% repeated options that were already mentioned in the question. The main additional measures that hunters had implemented were attending seminars or lectures on ASF (17.4% out of 391 free-text answers), training and using their own dogs to search for carcasses (11.25%), and increased participation in driven hunts or in organizing them (8.6%).

3.3.4. Assessment of Effectiveness of Measures

The sampling of wild boar (hunted or found dead), increased hunting of wild boar, increased hunting of young animals, intensive carcass search and removal, and cleaning/disinfection were assessed as fairly effective (Figure 5 and Table 3). However, hunters from non-affected federal states assessed the increased hunting of young animals (p = 0.026) and intensive carcass search (p = 0.020) as significantly more effective compared to hunters from ASF-affected areas. Both hunters from non-affected regions and hunters in the vicinity of ASF occurrence assessed cleaning/disinfection as significantly more effective than hunters from ASF-affected areas ($p < 0.001$).

Moreover, hunters who were active in the vicinity of ASF and hunters from areas not affected by ASF considered the increased hunting of adult female wild boar as fairly effective, whereas it was assessed as moderately effective by hunters from ASF-affected regions (Table 3). Furthermore, all three groups rated the use of wild boar traps and the construction of fences as moderately effective. Hunters in regions not affected by ASF and hunters in the vicinity of ASF also assessed the temporary ban on driven hunts as moderately effective and, therefore, significantly more effective than hunters from ASF-affected regions ($p < 0.001$).

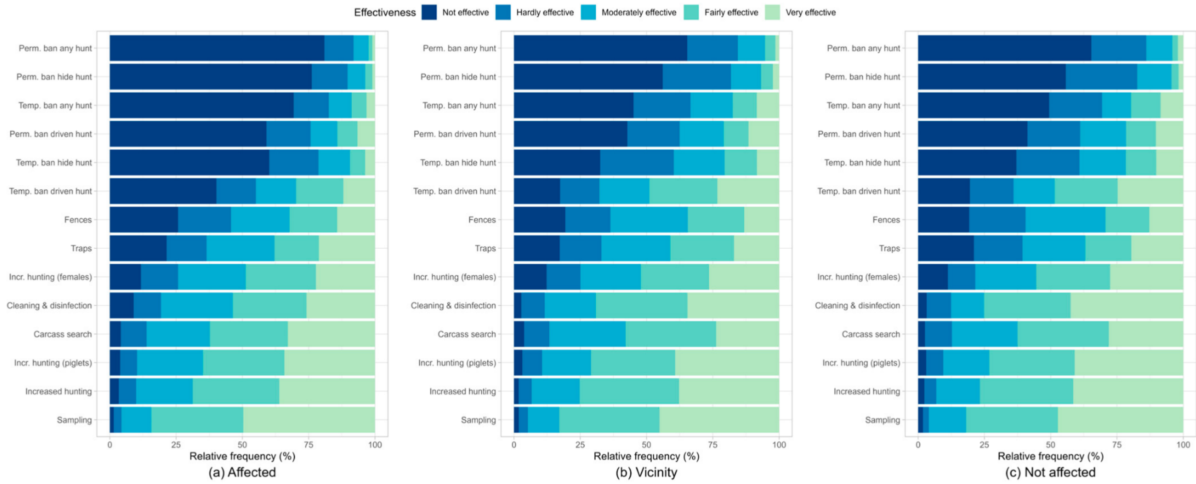

Figure 5. Relative frequency (in %) of hunters from ASF-affected areas (**a**), hunters from areas in the vicinity of ASF (**b**), and hunters from federal states not affected by ASF (**c**) who assessed the respective ASF control and surveillance measures in a web-based questionnaire for German hunters. The assessed measures were the sampling of wild boar hunted or found dead (sampling), increased hunting of wild boar (increased hunting), increased hunting of young animals (incr. Hunting (piglets)), an intensive search for and removal of wild boar carcasses (carcass search), cleaning and disinfection of hunting equipment/clothing and vehicles (cleaning and disinfection), increased hunting of adult females (incr. hunting (females)), use of wild boar traps (traps), construction of fences (fences), a temporary ban of driven hunts after an ASF outbreak (temp. ban driven hunt), a temporary ban of hide hunting after an ASF outbreak (temp. ban hide hunt), permanent ban of driven hunts after an ASF outbreak (perm. ban driven hunt), a temporary ban of any hunting activity after an ASF outbreak (temp. ban any hunt), a permanent ban of hide hunting after an ASF outbreak (perm. ban hide hunt), a permanent ban of any hunting activity after an ASF outbreak (perm. Ban any hunt).

Table 3. The median level of effectiveness of respective ASF control and surveillance measures as assessed by hunters from ASF-affected areas (n = 414), hunters in the vicinity of ASF (n = 457), and hunters from non-affected federal states (n = 682) in a web-based questionnaire for German hunters with the following levels: very effective (1), fairly effective (2), moderately effective (3), hardly effective (4), or not effective (5). Relative frequency (in %) of hunters who selected the alternative option ("I don't know") rather than assessing the effectiveness of respective measures. For the analysis of the median, the proportion of hunters who selected the alternative option ("I don't know") was excluded ("Excluded").

	Affected		**Vicinity**		**Not Affected**	
	Median	**Excluded (%)**	**Median**	**Excluded (%)**	**Median**	**Excluded (%)**
Sampling of wild boar (hunted or found dead)	2	1.2	2	3.9	2	2.6
Increased hunting of wild boar	2	0.2	2	2.0	2	1.8
Increased hunting of young animals	2	1.0	2	2.8	2	2.6
Intensive search for and removal of wild boar carcasses	2	0.7	2	3.3	2	3.4
Cleaning + disinfection of hunting equipment, clothing, and vehicles	2	3.6	2	5.7	2	4.4
Increased hunting of adult females	3	3.4	2	3.9	2	4.8
Use of wild boar traps	3	13.3	3	16.4	3	19.9
Construction of fences	3	3.4	3	8.5	3	9.5
Temporary ban on driven hunts after ASF outbreak	4	2.7	3	7.0	3	6.3
Temporary ban on hide hunting after ASF outbreak	5	2.4	4	8.5	4	9.4
Permanent ban on driven hunts after ASF outbreak	5	1.4	4	9.0	4	7.6
Temporary ban on any hunting activity after ASF outbreak	5	1.4	4	6.3	4	5.7
Permanent ban on hide hunting after ASF outbreak	5	1.7	5	7.2	5	7.9
Permanent ban on any hunting activity after ASF outbreak	5	1.2	5	5.9	5	5.3

As shown in Table 3, further kinds of temporary or permanent hunting bans were assessed as hardly effective or not effective at all by all groups. However, hunters from non-affected areas and the vicinity of ASF occurrence judged the effectiveness of a permanent and temporary ban on hide hunting, a permanent ban on driven hunts, and a permanent and temporary ban on any hunting activity as significantly higher compared to hunters from ASF-affected federal states (all $p < 0.001$).

Except for the assessment of cleaning/disinfection, where non-affected hunters assessed the effectiveness as significantly better ($p = 0.040$) compared to hunters in the vicinity, no statistically significant differences were detected between both groups (Supplementary Table S10).

The relative frequency of hunters who selected the option "I don't know" was generally higher for every measure in the groups of hunters from areas not affected by ASF and those from the vicinity of ASF occurrence compared to hunters in ASF-affected areas (Table 3). Regarding the effectivity of the use of wild boar traps, the proportion of hunters who selected the option "I don't know" was large compared to other control and surveillance measures (a: 13.3%, b: 16.4%, c: 19.9%).

In total, 388 respondents provided a free-text answer to the question if they considered measures other than those already listed to be effective. Of these, 9.5% negated and 14.7% repeated measures already mentioned in the question. In addition, a variety of different measures were mentioned by the participants (Supplementary Table S11), which mainly focused on options to promote and increase wild boar hunting and raise awareness of ASF among hunters and the general public. For example, 11.6% (out of 388 free-text answers) of the participants considered the increased use of technical aids for nighttime hunting (e.g., night vision, thermal imaging, artificial light) as helpful. The payment of financial compensation to hunters or the possibility of taking paid time off work to support ASF surveillance and control measures were mentioned by 7.5% as effective measures. Raising awareness and educating the general public about ASF and its surveillance and control was mentioned by 8.5% of respondents, and a ban on entering forests after an outbreak was supported by 7.5%.

3.4. Hunters' Perceptions regarding ASF Surveillance and Control

3.4.1. Consequences of ASF

The majority of participants (66.7% out of 1553 participants) selected three to five out of the ten proposed consequences of ASF surveillance and control, regardless of the location of their hunting area.

The top two consequences, which were chosen by approximately three-quarters of the participants, were the reduction in the wild boar population in their hunting areas and increased personal work and time load (Table 4). However, hunters from ASF-affected areas selected the reduction in the wild boar population significantly more often than hunters from unaffected areas ($p = 0.003$).

Approximately half of the participants expected or experienced local restrictions on their own hunting activity, regardless of the location of their hunting area (50.9%). However, hunters in the vicinity of ASF and hunters from non-affected areas significantly more often experienced or expected an increase in their own hunting activity (b: 56.7%, c: 58.2%) compared to ASF-affected hunters (40.6%, both $p < 0.001$). By contrast, hunters from ASF-affected areas significantly more often experienced a reduction in their own hunting activity (38.6%) compared to hunters in the vicinity (22.1%, $p < 0.001$) and hunters from non-affected regions (22.6%, $p < 0.001$).

Around one-fifth of the participating hunters expected or experienced conflicts with veterinary authorities (21.0%) and conflicts with other hunters (20.5%), regardless of the location of their hunting area. However, hunters from non-affected regions significantly more often expected or experienced conflicts with local farmers (32.1%) compared to hunters in the vicinity (23.0%, $p = 0.001$) and hunters from ASF-affected areas (17.6%,

$p < 0.001$). No other statistically significant differences between hunters in the vicinity and hunters from non-affected regions were found (Supplementary Table S12).

Table 4. Relative frequency (in %) of hunters who stated to expect or experience the respective consequences of ASF surveillance and control for hunters from ASF-affected areas ($n = 414$), hunters in the vicinity of ASF ($n = 457$), and hunters from regions not affected by ASF ($n = 682$) as mentioned in a web-based questionnaire for German hunters.

	Affected (%)	Vicinity (%)	Not Affected (%)
Reduction in the wild boar population in the hunting area	78.0	71.6	69.6
Increased personal work and time load	73.4	73.7	78.9
Increased personal costs	60.9	53.8	55.0
Local restrictions of own hunting activity	54.1	47.5	51.3
Increase in own hunting activity (single hunt)	40.6	56.7	58.2
Reduction in own hunting activity (single hunt)	38.6	22.1	22.6
Conflicts with other hunters	20.5	22.5	19.1
Conflicts with farmers	17.6	23.0	32.1
Conflicts with veterinary authorities	21.5	19.9	21.4
Other consequences	14.0	7.7	8.1
No consequences	1.7	3.3	4.3

Significantly more hunters from ASF-affected areas experienced further consequences (14.0%) compared to hunters in the vicinity (7.7%, $p = 0.004$) and hunters from unaffected regions (8.1%, $p = 0.002$).

A total of 148 respondents provided a free-text answer to explain further consequences (Supplementary Table S13). Of these, 18.9% reported conflicts with stakeholders other than those mentioned in the question, e.g., with forestry or animal rights activists, and in their private lives, e.g., with employers or family members. In addition, 14.9% argued that ASF surveillance and control measures, such as increased hunting or fencing, negatively affected other wildlife species, and 10.1% of respondents noted reduced or missing opportunities to sell wild boar meat and products or a price reduction for wild boar meat. Furthermore, 18.9% repeated one of the options already mentioned in the question.

3.4.2. Satisfaction with Cooperation and Appreciation by other Stakeholders

Based on the median level of satisfaction, hunters of all three groups were "rather satisfied" with the cooperation with the local hunting ring ("*Hegering*") (Tables 5–7). In addition, hunters from ASF-affected areas or the vicinity were "rather satisfied" with the cooperation with the local veterinary authorities (Table 5). By contrast, hunters from regions not affected by ASF were "rather satisfied" with the cooperation with the regional hunting association at the federal state level (state hunting association, "*Landesjagdverband*") (Table 6). The satisfaction with the cooperation with other listed stakeholders was assessed as "neutral" by all three groups (Tables 5–7). However, hunters from ASF-affected regions were significantly more satisfied with the cooperation with their local veterinary authorities and the local agriculture than hunters in the vicinity of ASF ($p = 0.003$ and $p = 0.11$) and hunters from areas not affected by ASF ($p < 0.001$) (Appendix A, Figure A1, and Supplementary Table S14). Hunters in the vicinity of ASF occurrence were also more satisfied with the cooperation with the local veterinary authority compared to hunters in non-affected areas ($p = 0.034$). Furthermore, hunters from affected regions and hunters in the vicinity were significantly more satisfied with the cooperation with external forces compared to hunters from non-affected areas ($p = 0.001$ and $p = 0.002$). By contrast, hunters from areas not affected by ASF were significantly more satisfied with the cooperation with the state hunting association and the national hunting association ("*Deutscher Jagdverband* *e.V. (DJV)*") than hunters from ASF-affected regions ($p < 0.001$) and hunters in the vicinity of ASF occurrence ($p < 0.001$). No statistically significant differences between the groups were detected regarding the satisfaction with the cooperation with the state laboratory (Supplementary Table S14).

Table 5. Assessment of the satisfaction with cooperation and appreciation by other stakeholders of hunters from ASF-affected regions ($n = 414$) as mentioned in a web-based questionnaire for German hunters. The table shows the median level of satisfaction of hunters' cooperation with respective stakeholders and the level of appreciation of hunters and their work by respective stakeholders. Levels 2 and 3 correspond to the answers "Rather satisfied" and "Neutral" regarding the level of satisfaction and "Rather yes" and "Neutral" regarding the question of whether they felt valued. To calculate the median, the share of hunters that selected the answer options "No cooperation" (no coop.) and "No specification" (no spec.) was excluded. The proportion (in %) of hunters that selected alternative answer options out of the total responses of hunters from affected areas ($n = 414$) is displayed.

	Satisfaction			Appreciation		
	Median	**No Coop. (%)**	**No Spec. (%)**	**Median**	**No Coop. (%)**	**No Spec. (%)**
Local veterinary authority	2	2.7	0.7	2	1.7	3.6
Hunting ring	2	6.5	2.4	2	5.3	5.1
State laboratory	3	11.6	8.7	3	9.9	11.8
External forces	3	21.0	6.8	3	18.6	10.1
Local agriculture	3	10.4	2.7	3	6.3	5.3
State hunting association	3	13.0	3.1	3	10.9	6.8
National hunting association	3	21.5	5.8	3	17.1	9.2

Hunters of all groups felt themselves and their work were valued by their local hunting ring (Tables 5–7), although the hunters from non-affected regions felt significantly more valued compared to hunters in the vicinity of ASF ($p = 0.040$) (Appendix A, Figure A2, and Supplementary Table S15). In addition, hunters from ASF-affected areas also felt significantly more valued by the local veterinary authority compared to hunters in the vicinity ($p = 0.004$) and hunters in non-affected regions ($p < 0.001$). They also felt more valued by the local agriculture compared to hunters from unaffected areas ($p = 0.015$). By contrast, hunters from non-affected areas felt significantly more valued by the state hunting association and the national hunting association compared to hunters from ASF-affected regions ($p < 0.001$) and hunters in the vicinity ($p < 0.001$). Regarding appreciation by the state laboratory and external forces, hunters remained neutral in the median without statistically significant differences between the groups (Tables 5–7, Supplementary Table S15).

For both questions described above, the proportion of hunters from non-affected regions who stated to have no cooperation with external forces was larger compared to hunters from ASF-affected areas (Table 5) and hunters in the vicinity (Table 6). In addition, a larger proportion of hunters from unaffected areas (Table 7) and hunters in the vicinity stated to have no cooperation with the state laboratory compared to hunters from ASF-affected regions. By contrast, a larger proportion of hunters from ASF-affected areas stated to have no cooperation with the state hunting association and the national hunting association compared to hunters in the vicinity and hunters from non-affected regions.

Table 6. Assessment of the satisfaction with cooperation and appreciation by other stakeholders of hunters from regions in the vicinity of ASF occurrence (*n* = 457) as mentioned in a web-based questionnaire for German hunters. The table shows the median level of satisfaction of hunters' cooperation with respective stakeholders and the level of appreciation of hunters and their work by respective stakeholders. Levels 2 and 3 correspond to the answers "Rather satisfied" and "Neutral" regarding the level of satisfaction and "Rather yes" and "Neutral" regarding the question of whether they felt valued. To calculate the median, the share of hunters that selected the answer options "No cooperation" (no coop.) and "No specification" (no spec.) was excluded. The proportion (in %) of hunters that selected alternative answer options out of the total responses of hunters from areas in the vicinity of ASF occurrence (*n* = 457) is displayed.

	Satisfaction			Appreciation		
	Median	**No Coop. (%)**	**No Spec. (%)**	**Median**	**No Coop. (%)**	**No Spec. (%)**
Local veterinary authority	2	5.0	4.2	3	5.0	8.3
Hunting ring	2	7.2	5.5	2	4.6	7.9
State laboratory	3	17.3	10.5	3	14.9	13.1
External forces	3	23.6	12.5	3	20.1	16.6
Local agriculture	3	10.9	7.4	3	7.9	8.3
State hunting association	3	10.5	4.4	3	7.9	7.4
National hunting association	3	15.8	7.9	3	12.7	9.2

Table 7. Assessment of the satisfaction with cooperation and appreciation by other stakeholders of hunters from regions not affected by ASF (*n* = 682) as mentioned in a web-based questionnaire for German hunters. The table shows the median level of satisfaction of hunters' cooperation with respective stakeholders and the level of appreciation of hunters and their work by respective stakeholders. The median levels 2 and 3 correspond to the answers "Rather satisfied" and "Neutral" regarding the level of satisfaction and "Rather yes" and "Neutral" regarding the question of whether they felt valued. To calculate the median, the share of hunters that selected the answer options "No cooperation" (no coop.) and "No specification" (no spec.) was excluded. The proportion (in %) of hunters that selected alternative answer options out of the total responses of hunters from non-affected areas (*n* = 682) is displayed.

	Satisfaction			Appreciation		
	Median	**No Coop. (%)**	**No Spec. (%)**	**Median**	**No Coop. (%)**	**No Spec. (%)**
Local veterinary authority	3	8.9	5.9	3	7.9	6.5
Hunting ring	2	5.9	5.0	2	5.0	5.7
State laboratory	3	22.1	10.6	3	19.2	10.6
External forces	3	26.7	15.4	3	24.6	14.4
Local agriculture	3	13.3	7.0	3	9.2	6.0
State hunting association	2	7.8	4.1	2	6.0	5.4
National hunting association	3	12.8	6.3	3	9.7	7.3

3.5. Motivational Options for Increased Participation of Hunters in ASF Surveillance and Control

Around half of the hunters (52.3% out of 1553 participants) selected three to five options, which might motivate them to increase their participation in intensified hunting in terms of ASF surveillance and control. By contrast, 4.1% of the hunters stated that none of the proposed options could motivate them. Reasons for this were given in the free text by 63 hunters (Supplementary Table S16). Of these, 22.2% stated that they already hunted as much as possible and did not have the time to increase their participation, and 19.0% considered the participation to be their duty as hunters and that no additional motivation was, therefore, needed. On the other hand, 20.6% of them did not think that an increase in the hunting of wild boar would be an effective way of controlling ASF and were, therefore, not interested in taking part.

The top three motivational options selected by more than half of hunters were the payment of appropriate financial incentives for hunted wild boar (a: 61.6%, b: 64.1%, c: 57.5%), the promotion of marketing and utilization of wild boar meat and products (a: 64.5%, b: 53.4%, c: 52.1%), and the reduction in the bureaucratic effort to receive financial incentives (a: 56.6%, b: 52.1%, c: 53.5%) (Figure 6). The proportion of hunters that selected appropriate incentives was significantly higher for hunters from ASF-affected regions compared to hunters in the vicinity ($p = 0.001$) and hunters from non-affected areas ($p < 0.001$). A noticeable proportion of hunters (a: 49.8%, b: 45.1%, c: 49.7%) also stated that an extension of the legal permission for using nighttime hunting devices would motivate them to hunt more often. In addition, the provision of additional hunting tools, such as night vision devices or silencers, was selected significantly more often by hunters from ASF-affected regions (44.7%) compared to hunters in the vicinity (36.6%, $p = 0.041$). No further statistically significant differences between the choices of the three groups were detected (Supplementary Table S17). However, a considerably larger proportion of hunters from non-affected regions considered an increase in the number of collection points for samples and support for shipping of samples (41.8%) and an increase in the number of storage sites for hunted wild boar (38.4%) as motivational compared to hunters from ASF-affected areas (36.5% and 34.8%) and those in the vicinity (37.4% and 33.9%).

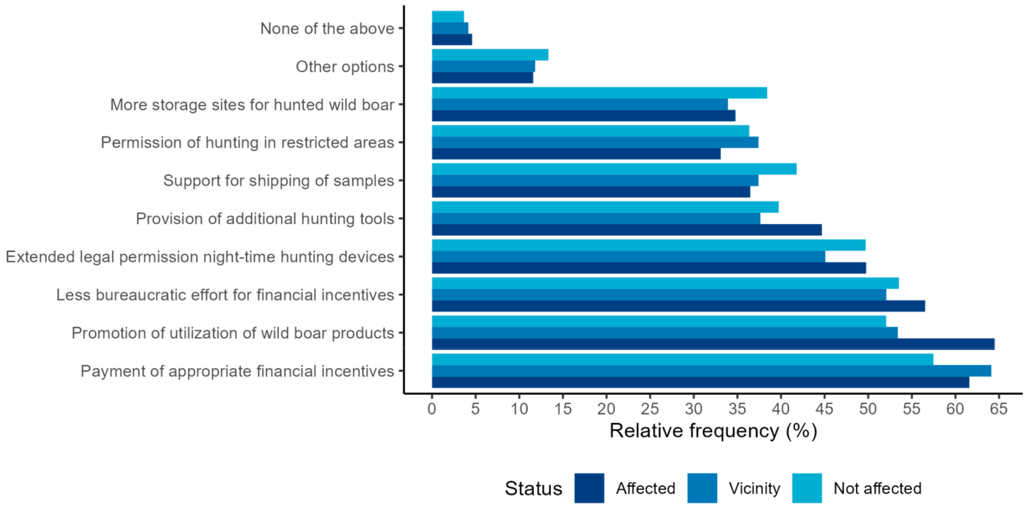

Figure 6. Relative frequency (in %) of hunters from ASF-affected areas ($n = 414$), hunters from areas in the vicinity of ASF ($n = 457$), and hunters from non-affected federal states ($n = 682$) who selected the respective options in a web-based questionnaire for German hunters as motivation to increase their participation in increased hunting of wild boar in terms of ASF surveillance and control.

Another 12.4% of the hunters stated that other options could motivate them to increase their participation. A total of 193 respondents proposed a number of different ideas, with a particular focus on options to facilitate wild boar hunting and compensate hunters for the increased workload and costs (Supplementary Table S18). The three most frequently mentioned options were the possibility of taking paid time off work to enable participation in ASF surveillance and control (8.8%), the provision of public facilities (e.g., confiscate bins) for free and the professional disposal of waste (8.3%), and changes in the hunting legislation to create more flexible hunting opportunities and changes in the system of how hunting districts are organized in Germany (7.8%).

Regarding motivational options for increased participation in carcass search, the majority of the hunters (64.0%) selected one to three of the proposed options. The total proportion of the hunters who stated that none of the listed options would motivate them to increase their participation in carcass searching and sampling (10.5%) was larger compared to motivational options for increased hunting, although the reasons were similar. A total of 163 participants explained the reasons for this in the free-text answers (Supplementary

Table S19). Of these, 28.8% stated that they already participated in the carcass search as much as possible and did not have the time to increase their participation even further, and 17.8% considered participation to be their duty as hunters and that they did not need additional motivation. A further 17.2% stated that their region was not affected by ASF and that there was currently no need for carcass searching and sampling. Hunters from ASF-affected regions (13.8%) were significantly more often selected that none of the listed options could motivate them compared to hunters in the vicinity of ASF (8.1%, $p = 0.010$).

The top three motivational options were the payment of appropriate financial incentives (a: 52.7%, b: 56.0%, c: 54.4%), the reduction in bureaucratic effort for receiving financial incentives (a: 39.9%%, b: 44.2%, c: 42.1%), and the increase in the number of collection and storage sites for wild boar carcasses (a: 30.2%, b: 40.9%, c: 43.1%) (Figure 7). The proportion of hunters who selected an increased number of storage sites for carcasses and the reduction in bureaucracy for the notification of wild boar (a: 42.7%, b: 39.6%, c: 29.0%) was significantly higher in the group of hunters in the vicinity ($p = 0.001$, $p < 0.001$) and hunters from non-affected regions ($p < 0.001$) compared to hunters from ASF-affected areas. Moreover, a significantly larger proportion of hunters from unaffected regions selected support for shipping of samples (c: 35.8%, a: 26.8%) as a motivational option compared to hunters from ASF-affected areas ($p = 0.003$). No statistically significant differences were detected between the choices of hunters in the vicinity and non-affected hunters (Supplementary Table S20).

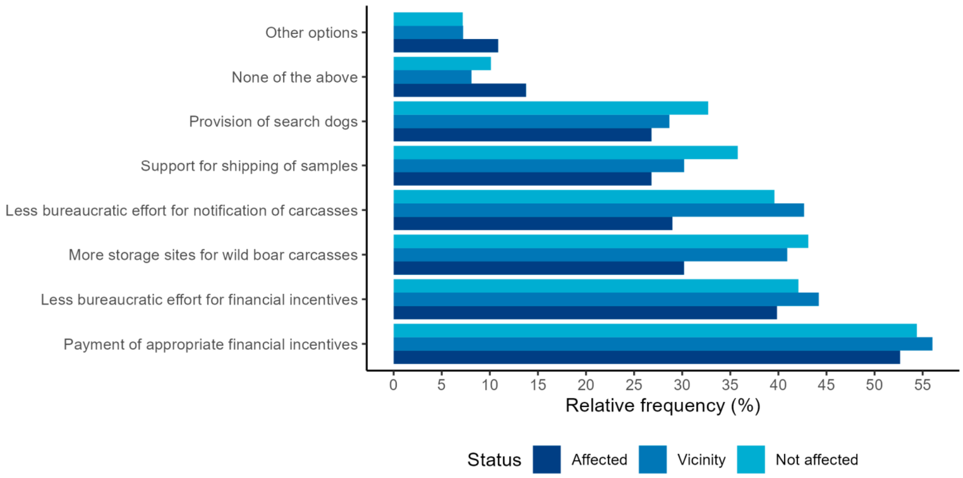

Figure 7. Relative frequency (in %) of hunters from ASF-affected areas ($n = 414$), hunters from areas in the vicinity of ASF ($n = 457$), and hunters from non-affected federal states ($n = 682$) who selected the respective options in a web-based questionnaire for German hunters as motivation to increase their participation in the search for and sampling of wild boar carcasses in terms of ASF surveillance and control.

A total of 127 participants suggested other options that could motivate them to increase their participation in the search for wild boar carcasses in the free text (Supplementary Table S21). The most frequently mentioned option (20.5%) was the payment of financial compensation for participation based on the time invested rather than the number of detected carcasses. Improving cooperation with the veterinary authorities, in particular, the flow of information and coordination of control measures, was also seen as a motivating factor by 12.6%. In addition, 10.2% considered increased opportunities or support to train their dog to search for carcasses and the possibility of taking paid time off work to participate in carcass searches as motivating.

3.6. Hunter's Additional Comments

A total of 294 participants left some additional comments before submitting the questionnaire (Supplementary Table S22). Of these, 7.5% stated that they had nothing further to say, and 10.2% made positive comments about the questionnaire, such as that they were happy to support the work or were grateful for the opportunity to express their opinions. In contrast, 3.7% were critical of the questionnaire. In addition, a large number of aspects mentioned in previous free-text responses were repeated. The most common (10.2%) was the criticism of fencing due to its negative impact on other wildlife species and the fragmentation of hunting areas. Furthermore, 7.1% expressed the wish to improve cooperation and communication with the authorities and 6.1% stressed the importance of raising awareness among the general public and stakeholders about ASF and its surveillance and control.

4. Discussion

ASF has been present in Germany since September 2020 and it mainly affects the wild boar population [10]. The circulation of ASF in wild boar populations poses a constant risk of spreading to pig farms, which can lead to major socio-economic losses and negative impacts on animal welfare [13]. Hunters are key players in implementing measures for ASF surveillance and control of wild boar, including carcass searches, the sampling of wild boar, and increased hunting to reduce the susceptible wild boar population.

A questionnaire study distributed among the German hunting community was conducted to elucidate which tasks hunters perform in ASF surveillance and control and how they perceive the effectiveness of these tasks, which obstacles they face when participating in ASF surveillance and control, and which options they consider to be motivating to increase their participation in certain activities.

As this was a public web-based survey, it cannot be ruled out that people participated who were not hunters. To address this issue, the survey mainly consisted of mandatory questions, and only fully completed questionnaires were analyzed. Answering the mandatory questions required a deep knowledge of hunting practices. This made it unlikely that a substantial number of people who were not hunters completed the questionnaire. Moreover, the survey link was made available through organizations associated with hunting, which made it less likely that members of the general public had access. Also, no outlying responses became apparent when managing and analyzing data. It can, therefore, be assumed that the vast majority of the participants were hunters engaged or interested in the topic of ASF prevention, surveillance, and control or otherwise stopped answering when confronted with the first part of the questionnaire. This view is also supported by the analysis of the dropout ratios per page. In addition, a larger number of participants dropped out when asked about which measures of ASF surveillance and control they had participated in, probably because the question was too complex, the instructions on how to answer it were unclear, or the participants had not been involved in such measures but were reluctant to admit this.

In total, 1553 participants completed the questionnaire, which represents a small group of approximately 403,000 hunters in Germany [33]. However, the repetition of codes throughout the free-text answers to a point where almost no new codes were identified in the data, suggesting that inductive thematic saturation may have already been reached [34,35].

The demographics of the participants in this study were similar to the results of a study of the hunting community by the German Hunting Association [33]. Nevertheless, a possible gender and age bias in the results of our study has to be taken into consideration.

Unsurprisingly, the level of participation in the questionnaire was particularly high in the ASF-affected federal states. However, responses from all federal states were received, showing that hunters outside affected areas are highly interested in ASF. Overall, few significant differences were found between the responses of hunters in the vicinity of ASF and non-affected hunters, suggesting that hunters' perceptions are not strongly influenced by the proximity of the epidemic front as long as their own hunting area is not affected.

In contrast to the present study, where less than a third of the hunters believed that ASF could be successfully eliminated in wild boar in Germany, the proportion (70%) was considerably higher in Lithuania [26]. This difference in the attitude of the hunters may be influenced by the epidemiological situation in the countries at the time of the questionnaire. This highlights the necessity to address hunters' perceptions and concerns toward ASF control to keep up their motivation to participate in surveillance and control measures in the long term.

Similar to their Lithuanian colleagues, German hunters mainly rated their knowledge of ASF as good or very good [26]. Therefore, affected hunters rated their knowledge of ASF significantly better than hunters in the vicinity of ASF and non-affected hunters, probably because of more experience and personal exposure to the disease. However, the self-evaluation of knowledge was not validated in the questionnaire. The listed sources of information indicate that hunters are generally interested in learning more about ASF, and media specifically created for hunters should be used primarily to provide hunters with relevant information and to increase awareness.

Overall, German hunters were rather satisfied with the cooperation with other stakeholders or remained neutral. Differences in hunters' satisfaction with hunting associations in different regions may be due to regional differences in the importance and structure of these associations, irrespective of the ASF status of the region. In the affected federal state of Saxony, the proportion of hunters who are members of these associations is considerably smaller than in other federal states [36]. The results indicate that it is only after an ASF outbreak that cooperation between the different actors at the local level is intensified, and this local cooperation mainly works satisfactorily in the event of an outbreak. However, conflicts with other hunters or other stakeholders were mentioned as a consequence of ASF control and surveillance, illustrating the big challenge of implementing ASF prevention, surveillance, and control measures [37]. Likewise, in a participatory study conducted by Jori et al., experts judged that ASF control has an impact on a large panel of stakeholders and concluded that "communication among and between stakeholders seems to be both difficult and essential". Yet, it is necessary to involve these stakeholders [38]. The desire for improved communication was expressed by participants in this study in their free-text answers and also by hunters from the Baltic states in previous studies [24,25]. This highlights the need for transparent and rapid communication and the need to prepare communication channels at an early stage in order to respond effectively to new outbreaks.

Overall, German hunters seem to assess the effectiveness of measures that support hunting to be more effective and rate hunting bans as the least effective measures of ASF control, which is in accordance with the results of other studies [23,26,27] but may also show some level of "vested interest".

The results may indicate that hunters in the vicinity of ASF and those from areas not affected by ASF are more optimistic about the success of ASF control than hunters from affected areas. They might have resigned due to the fact that the disease is still present after several months of ASF control, although great efforts were made and apparently led to an increase in personal workload and financial expenses. This burden was also reported by hunters from the Baltic states [23,27]. German hunters apparently also experienced an increase or reduction in their hunting activities, depending on the area. In affected regions, hunting activity was more likely to decrease due to hunting bans and a reduced wild boar population. By contrast, the consequence can also be an increase in hunting activity due to increased hunting as a surveillance measure in regions that have yet to be affected. Hunters have to face the moral challenge of a substantial reduction in the wild boar population in their hunting area and the need to deal with local restrictions on their hunting activity due to the establishment of restriction zones in affected areas or fencing. It was reported by Stončiūtė et al. that some hunters lost their joy and motivation for hunting [27].

Although the search for carcasses was rated as fairly effective by the hunting community, a substantial proportion of the participants (10%) did not find any motivation to increase their participation in this measure. This is understandable since the search for wild boar carcasses can be a time-consuming and sometimes tedious activity. It is also rated as less practical by some experts [19]. Likewise, Lithuanian hunters considered going into the forest specifically to search for wild boar carcasses to be less effective and were less willing to support this measure in comparison to searching for carcasses while they were already out in the forest [26]. However, several studies have shown that the search for carcasses is of utmost importance in the surveillance and control of ASF in wild boar since carcasses left in the forest pose a risk of infection to living wild boar and carcass sampling is useful for detecting new introductions of ASF [11,16,19].

Similar to hunters from the Baltic states [24–26], German hunters considered a payment of appropriate financial compensation and a reduction in bureaucracy as motivational options to increase participation in carcass search and wild boar hunting. The exact amount of money was not defined in the questionnaire since there are regionally different regulations in the German districts and federal states regarding eligibility and sums. For example, the compensation for hunted wild boar ranged from EUR 70 per animal in the non-affected federal state of Bavaria (Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, https://www.lgl.bayern.de/tiergesundheit/tierkrankheiten/virusinfektionen/asp/infos_jaeger.htm#aufwand, accessed on 28 August 2023) to EUR 150 per animal in the affected federal state of Brandenburg (Ministerium für Soziales, Gesundheit, Integration und Verbraucherschutz des Landes Brandenburg), https://msgiv.brandenburg.de/msgiv/de/themen/verbraucherschutz/veterinaerwesen/tierseuchen/afrikanische-schweinepest/, accessed on 28 August 2023). In our study, a significantly larger proportion of affected hunters stated to receive payments, which might have contributed to the significantly greater hunting bag and greater hunting frequency of these hunters. However, approximately one-fifth of the participants stated that they had not received any financial rewards. This could be due to the fact that there are no rewards in the participants' region or that not every hunter is eligible to receive the payments. Therefore, depending on the individual situation, the understanding of the "appropriate" amount may differ and could mean either increasing rewards or introducing rewards in general. In areas not affected by ASF, a predominant lack of available storage sites for carcasses and sample drop-off points might be an obstacle for carcass search and sampling. This stresses the need for good preparation and the establishment of infrastructure for the implementation of control measures at an early stage. In affected areas, the possibility of making use of and selling (ASF-negative) wild boar meat and products appeared to be of greater concern for hunters. Although increased hunting to reduce the susceptible wild boar population as a measure to hinder the spread of ASF was also assessed as a fairly effective measure, this strategy was perceived controversially by participants. Likewise, Oelke et al. reported different opinions on the topic that were gathered in interviews with German hunters [37]. Some hunters considered increased hunting as an unnecessary culling of wild animals to protect the domestic pig industry, opposing their ethical framework of fair hunting *("Waidgerechtigkeit")* [37,39]. In addition, restrictions in affected areas on selling and distributing wild boar meat as well as value loss of the meat are in contrast to the ethical standards and traditions of hunters to make use of the products of hunted animals. These issues may lead to reduced acceptance and compliance among hunters and should be addressed in order to keep up hunters' motivation and participation. However, it is important to note and communicate that the increased hunting of wild boar in the context of ASF control cannot be described as leisure hunting [40] but rather as a necessary component of ASF control.

Similar to German hunters, Lithuanian hunters considered the permission to use additional hunting tools as an effective measure to eliminate ASF [26]. As nighttime hunting can be challenging, the use of such aids may contribute to more effective hunting. However, some participants considered their use to be unethical as they stand against the traditional perception of fair hunting. This controversy was also reported by Oelke et al. [37].

Likewise, the construction of fences and the use of wild boar traps to reduce the wild boar population appear to be a controversial issue among German hunters. The construction of fences can interfere with individual property rights and has a strong impact on ecosystems of wildlife, eventually leading to lower acceptance of this measure [38]. Estonian and Latvian hunters also found fencing to be ineffective and a waste of time and money [24,25]. Even among other experts, there is disagreement on the effectiveness of fencing in controlling ASF [38], which seems to be highly dependent on the local situation of the outbreak. However, it was used successfully to control the focal ASF outbreak in the Czech Republic [41] and Belgium and has contributed to controlling or at least slowing down the westward spread of ASF in Germany [42].

Capturing and culling wild boar in traps was considered by experts as a feasible complementary measure to reduce the wild boar population in the event of an ASF outbreak, allowing for high biosecurity standards, but was opposed by hunters [38]. Similarly, the results of this study suggest that there is a disagreement among German hunters on this issue. Moreover, the assessment of the effectiveness of selective hunting of adult female wild boar was also heterogeneous. This measure was considered an unethical hunting practice that increases the risk of producing orphans [23,27].

Similar to hunters from Latvia and Lithuania [25,26], increased biosecurity, e.g., the cleaning and disinfection of hunting equipment, clothing, and vehicles was considered to be fairly effective by German hunters. It is vital that the hunting community is aware of the importance of increased biosecurity measures and is educated on how to implement them, as a notable proportion of German hunters reported that they travel to other European countries for hunting and have regular contact with domestic pig farms, which poses a risk for transmission of ASF through contaminated fomites.

5. Conclusions

Overall, the general perception of the effectiveness of specific ASF surveillance and control measures is consistent with the results of other studies in different countries. Consistently, measures that promote hunting were considered effective, whereas measures that hindered hunting were considered ineffective. The consequences for hunters were also perceived in a similar way, mainly in terms of an increase in workload and financial expenses. Therefore, financial compensation and a reduction in bureaucracy were consistently considered as motivational options. Intervention studies would be required to evaluate the real impact of these motivators. By showing regional differences between hunters affected by ASF, hunters in the vicinity of ASF, and non-affected hunters, our study highlights the necessity to consider hunters' perceptions and opinions on ASF surveillance and control to maintain their participation and motivation in the long term. The establishment of ways of communication between and among stakeholders is of utmost importance and must be in place early in preparation for potential outbreaks to ensure consistent education and flow of information about the ASF situation. It is of utmost importance to consider local hunters' perceptions and address their concerns at an early stage to increase their compliance when it comes to implementing restrictive control measures. This study highlights various challenges of bringing together different stakeholders, such as hunters, farmers, or authorities, in the context of ASF control and may thus indicate the need for inter-sectoral and complex approaches involving different stakeholders to identify adaptive solutions.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/ani13182813/s1, Document S1: Original questionnaire in the German language; Document S2: English translation of the questionnaire; Supplementary Table S1. Analysis of the free-text answers for different options of hunting organization; Supplementary Table S2. Analysis of the given free-text reasons why hunters believe that the elimination of ASF in wild boar in Germany is possible; Supplementary Table S3. Analysis of the given free-text reasons why hunters believe that the elimination of ASF in wild boar in Germany is not possible; Supplementary Table S4. Analysis of the given free-text reasons why hunters were unsure whether the elimination of ASF in wild boar in Germany is possible or not; Supplementary Table S5. Analysis of the given free-text reasons why hunters believe that they play a crucial role in ASF control in Germany; Supplementary Table S6. Analysis of the given free-text reasons why hunters believe that they do not play a crucial role in ASF control in Germany; Supplementary Table S7. Analysis of the given free-text reasons why hunters were unsure whether they play a crucial role in ASF control in Germany or not; Supplementary Table S8. Analysis of the free-text answers for further sources used to obtain knowledge about ASF; Supplementary Table S9. Analysis of the free-text answers for further measures that were implemented by hunters in terms of ASF surveillance or control; Supplementary Table S10. Pairwise Mann–Whitney U testing (with the Bonferroni correction) of the assessment of the effectiveness of different ASF-surveillance and control; Supplementary Table S11. Analysis of the free-text answers for additional effective measures to control ASF; Supplementary Table S12. Chi-squared testing of the selected consequences of ASF surveillance and control, which hunters from ASF-affected areas, hunters in the vicinity of ASF, and hunters from non-affected areas expect or experience; Supplementary Table S13. Analysis of the free-text answers for additional consequences of ASF surveillance and control; Supplementary Table S14. Pairwise Mann–Whitney U testing (with the Bonferroni correction) of the assessment of hunters' satisfaction with cooperation with other stakeholders involved in ASF surveillance and control; Supplementary Table S15. Pairwise Mann–Whitney U testing (with the Bonferroni correction) of the assessment of whether the hunters felt appreciated by other stakeholders involved in ASF surveillance and control; Supplementary Table S16. Analysis of the given free-text reasons why none of the provided options were considered to be motivational to increase participation in wild boar hunting; Supplementary Table S17. Chi-squared testing of the selected motivational options that would likely increase hunters' participation in the increased hunting of wild boar in terms of ASF surveillance and control; Supplementary Table S18. Analysis of the free-text answers for additional motivational options to increase participation in hunting wild boar; Supplementary Table S19. Analysis of the given free-text reasons why none of the provided options were considered to be motivational to increase participation in carcass search; Supplementary Table S20. Chi-squared testing of the selected motivational options that would likely increase hunters' participation in the search for wild boar carcasses in terms of ASF surveillance and control; Supplementary Table S21. Analysis of the free-text answers for additional motivational options to increase participation in carcass searches; Supplementary Table S22. Analysis of the additional free-text remarks or comments in the questionnaire.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the University of Greifswald, University medicine (BB 044/22, 04-06-2022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: The anonymized data used for the analysis can be obtained from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

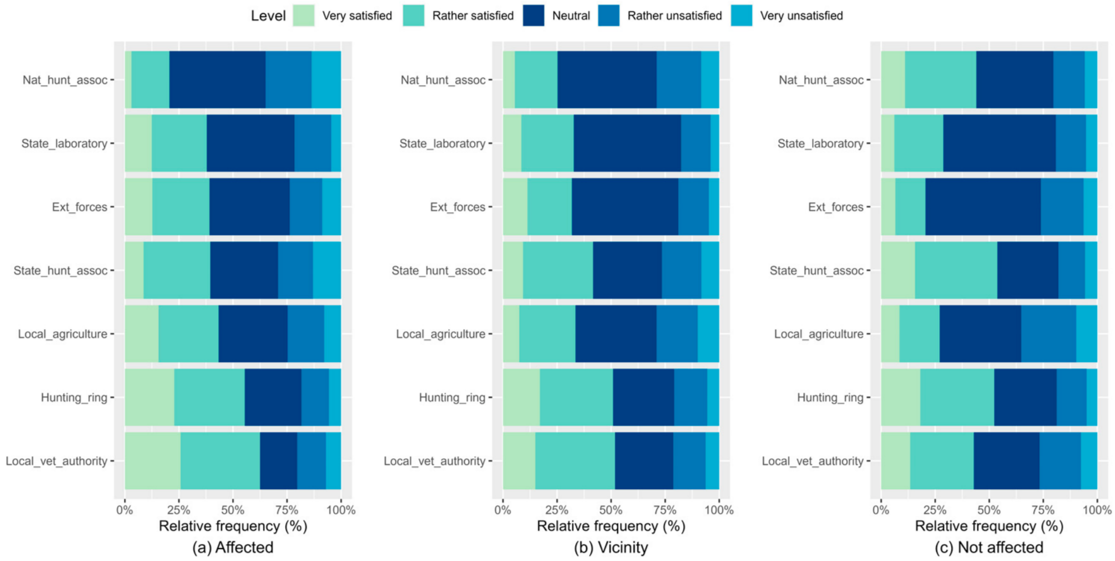

Figure A1. Relative frequency (in %) of hunters from ASF-affected federal states (**a**), hunters in the vicinity of ASF (**b**), and hunters from non-affected federal states (**c**) who selected the respective level of satisfaction with the cooperation with local veterinary authorities, the local hunting ring, the local agriculture, the state hunting association, external forces, the state laboratory, and the national hunting association in a web-based questionnaire for German hunters. The share of hunters who selected alternative options ("No cooperation" or "No specification") was excluded from the analysis, as displayed in Tables 5–7.

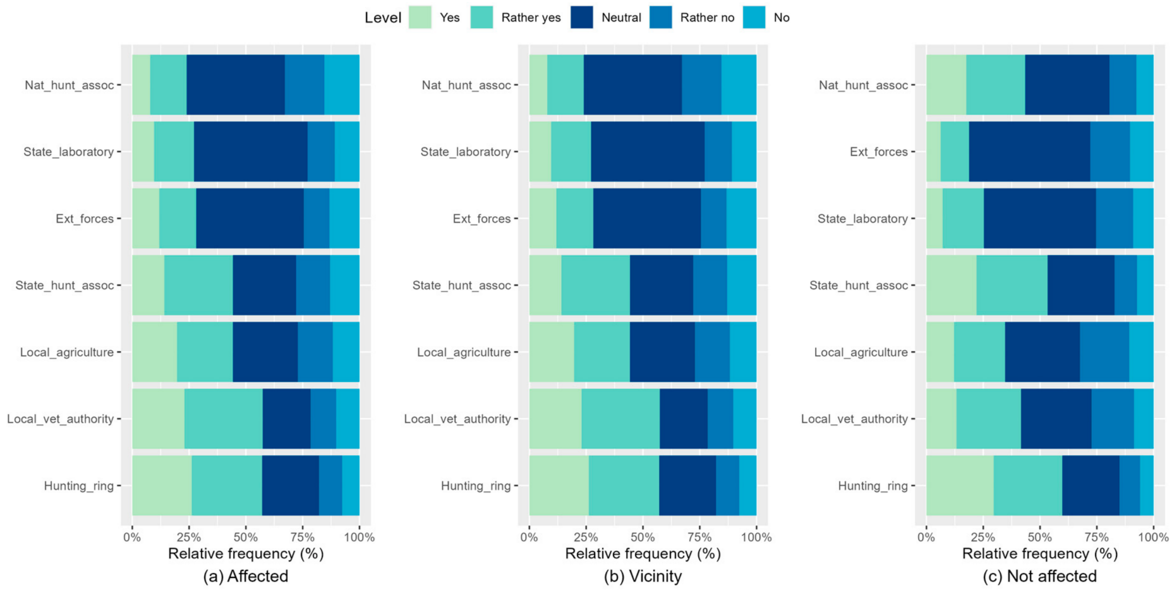

Figure A2. Relative frequency (in %) hunters from ASF-affected federal states (**a**), hunters in the vicinity of ASF (**b**), and hunters from non-affected federal states (**c**) who selected the respective level to rate whether they felt appreciated by the local hunting ring, the local veterinary authority, local agriculture, the state hunting association, external forces, the state laboratory, and the national hunting association in a web-based questionnaire for German hunters. The share of hunters who selected alternative options ("No cooperation" or "No specification") was excluded from the analysis, as displayed in Tables 5–7.

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V. DISCUSSION

The last decade has seen a panzootic, global spread of ASF. Many European countries have been affected for several years, including the Baltic states, Poland and Germany. In these countries, ASF is circulating and persisting in the wild boar population, posing an ongoing risk of disease transmission to domestic pig herds - with devastating consequences for animal welfare, the economy and stakeholders. With no effective treatment or vaccine available, control measures can solely focus on surveillance and prevention of disease spread.

In the Baltic states and also in Germany, passive surveillance, i.e. the search for and sampling of wild boar carcasses, has been intensified from 2014 onwards (PAUTIENIUS et al., 2018; SCHULZ et al., 2019b; SAUTER-LOUIS et al., 2021b). The rapid detection and removal of potentially contaminated wild boar carcasses reduces the risk of further transmission of the disease (CHENAIS et al., 2019). In addition to passive surveillance, often also active surveillance is carried out, i.e. samples are taken from apparently healthy hunted wild boars. The hunting of these animals also contributes to the targeted depopulation to prevent further spread of ASF (GAVIER-WIDÉN et al., 2015; LANGE, 2015).

However, the study by Schulz et al. (2019b) showed that implemented control measures in Latvia in the period from 2014 to 2017 did not result in decreasing ASF prevalence at first (SCHULZ et al., 2019b). Yet, more recently, a decrease in the number of PCR positive ASF cases was detected in Estonia, Latvia and Lithuania accompanied by an increase in serologically positive cases (SCHULZ et al., 2019a; OĻŠEVSKIS et al., 2020; SCHULZ et al., 2021a). These findings suggested a decreasing ASF incidence, leading to the assumption that the epizootics in these countries were in a decelerating phase.

Germany was able to benefit from the experience of other affected countries and to initiate appropriate measures at an early stage. Surveillance measures had already been intensified since the first occurrence of ASF in Lithuania in 2014 and preparations were made for potential outbreaks (SAUTER-LOUIS et al., 2021b). Following the example of Belgium and the Czech Republic, also fences were preventively constructed at parts of the German-Polish border after the introduction of ASF into Western Poland. Compared to the epizootic in Latvia, ASF spread more slowly in the German federal state of Saxony in the first 18 months after the first notification (RICHTER et al., 2023). The prevalence, average speed of spread and the size of the affected area after 18 months were also lower in Saxony compared to Latvia, suggesting that prevention and control strategies in Germany are at least successful in slowing down the spread of ASF

(RICHTER et al., 2023). Also in the federal states of Brandenburg, the restriction zones in the area first affected by ASF in 2020 were recently lifted (Press release, MSGIV, 21.07.2023). With the last confirmed case in wild boar in October 2021, the area is now considered free of ASF. Likewise, the restriction zone in the federal state of Mecklenburg-Western Pomerania was lifted recently (Press release, MINISTERIUM FÜR KLIMASCHUTZ, LANDWIRTSCHAFT, LÄNDLICHE RÄUME UND UMWELT, MECKLENBURG-VORPOMMERN, 15.09.2023). These examples highlight the importance of early preparation for potential outbreaks in high risk areas.

Nevertheless, repeated new introductions through movements of infected wild boar must be expected at any time. Additionally, humans can introduce the virus in unaffected areas or can facilitate the spread of ASF, e.g. through improper disposal of contaminated waste or insufficient biosecurity measures.

Hence, in Estonia ASF re-emerged after no pigs or wild boar tested positive for ASFV for 19 months (SCHULZ et al., 2020a). In the period from February 2019 to August 2020, only seropositive cases were reported in hunted wild boar and the last outbreak in domestic pigs had occurred in 2017 (NURMOJA et al., 2020; SCHULZ et al., 2021b). However, in August 2020 a wild boar found dead tested positive for ASFV, followed by several other case notifications and also an outbreak in domestic pigs in July 2021 (SCHULZ et al., 2021b), dashing the hope that ASF could be successfully eliminated in Estonia. Considering the implemented surveillance system and the decreasing wild boar population density it could not be ruled out that ASF had been present throughout that period at low prevalence below the detection limit (SCHULZ et al., 2021b). A new introduction appeared to be another possible reason for the re-emergence of ASF in Estonia. Additionally, the role of seropositive wild boar as potential virus carriers was discussed yet again (SCHULZ et al., 2021b). In the first place, there is still no consensus about the clear definition of a "carrier" in this context and evidence for a significant epidemiological role of carriers is lacking (STÅHL et al., 2019). Petrov et al. (2018) demonstrated before that pigs surviving an ASF infection were not able to transmit the virus to other pigs (NURMOJA et al., 2017a; PETROV et al., 2018). Additionally, epidemiological investigations in Latvia by Oļševskis et al. (2023) indicated that seropositive animals did not play a major role in the virus persistence in Latvia (OĻŠEVSKIS et al., 2023). To this end, the role of potential carriers in the current situation in Europe remains unclear.

Even more recently, ASF also re-emerged in the Czech Republic. The Czech Republic is one of two countries that were able to successfully eliminate ASF in the current epizootic scenario in Europe, declaring freedom of the disease in 2019 (CHARVÁTOVÁ et al., 2019). However, three years later, a wild boar carcass found close to the Polish border tested positive for ASFV in December 2022 (STATE VETERINARY ADMINISTRATION OF THE CZECH REPUBLIC, 2023) suggesting an introduction through the migration of infected Polish wild boar. Since then, several wild boar cases were confirmed.

Furthermore, many other countries in Europe have reported cases of ASF in wild boar for the first time in the last years, such as North Macedonia, Italy (outside of Sardinia), Croatia, Bosnia and Herzegovina or Sweden. These developments demonstrate how difficult and challenging the control and prevention of ASF in Europe is. Complete elimination of ASF in the affected countries seems unlikely in the current situation. Rather, it is likely that even more countries will become affected by ASF in the future (ANDRAUD et al., 2019; HALASA et al., 2019; JURADO et al., 2019; NEUMANN et al., 2021). To stop or at least to slow down the further spread of ASF and to protect domestic pig farms from transmission events, it is necessary to use available financial, human and technical resources in the most targeted way possible. Thereby, countries that have so far not been affected by ASF should learn from the experience of the affected countries in order to best prepare for potential outbreaks. Another important factor is to consider the opinions of affected stakeholders in order to increase their compliance in the implementation of surveillance and control activities. Therefore, this thesis aimed to provide a better understanding of surveillance strategies, seasonal patterns of disease occurrence, and stakeholder perceptions.

Several studies have highlighted the importance of passive surveillance for early detection of ASF outbreaks (ŚMIETANKA et al., 2016; NURMOJA et al., 2020; OĻŠEVSKIS et al., 2020; SCHULZ et al., 2021a). In these studies, prevalence of ASF was consistently significantly higher in wild boar found dead compared to the prevalence in wild boar hunted apparently healthy. Additionally, since living wild boar can become infected from contaminated carcasses (PROBST et al., 2017; CUKOR et al., 2020a; PROBST et al., 2020), quick detection and removal of carcasses is of utmost importance to hinder the spread of ASF.

Knowledge about environmental risk factors for ASF occurrence can contribute to developing an efficient and targeted passive surveillance system. For instance, determination of high-risk areas for the search for carcasses allows efficient use of resources (MORELLE et al., 2019).

Hence, several studies investigated environmental characteristics of wild boar deathbed choice in order to enable risk-based carcass search (MORELLE et al., 2019; CUKOR et al., 2020b; ALLEPUZ et al., 2022).The study by Morelle et al. (2019) indicated that due to high fever ASF-infected wild boar prefer cool and moist habitats, and therefore carcasses are likely to be found in such areas (MORELLE et al., 2019). Cukor et al. (2020b) reported that most carcasses in their study were found in forests, particularly in younger forest stands. Infected animals additionally preferred places more distant from roads and forest edges. It was concluded that sick animals search for calm and quiet places (CUKOR et al., 2020b). Allepuz et al. (2022) observed an increased likelihood of detecting positive carcasses in transitional areas between woodland and shrub, green urban areas and mixed forests (ALLEPUZ et al., 2022). Even though the studies were performed with different methods, in different areas and throughout different periods, some findings were consistent. Accordingly, also in the present study, carcasses in Latvia were found predominantly in forests (Publication I). Positive carcasses were more often found in transitional areas between woodland and shrub and in greater distances to roads or settlements compared to negative carcasses, emphasizing that infected wild boar might search for shelter in quiet places. Contrasting the hypothesis that infected wild boar prefer moist places due to high fever, no significant difference was observed in the distance to the next water source between positive and negative carcasses in Latvia (Publication I). However, Latvia is a water rich country and all carcasses were found closer to water sources compared to random locations (Publication I). This highlights that the habitat and deathbed preferences are of course dependent on the environmental conditions and available resources, indicating that high-risk areas for carcass search need to be determined and evaluated at the local level.

Especially in scenarios like currently observed in the Baltic states, where ASF seems to be persistent at low prevalence levels, targeted risk-based search could be a resource-efficient way to monitor wild boar populations for ASF occurrence over longer periods of time. It should be noted that ASF may be circulating at prevalence levels below the detection limit, particularly when wild boar densities have been greatly reduced (SCHULZ et al., 2021b). However, it can be expected that at some point the circulation would exceed the detection limit and the number of carcasses would increase again, making it unlikely that ASF could circulate undetected for long periods of time (SCHULZ et al., 2021b). It has been suggested that in addition to carcass searches also wild boar killed in road traffic accidents should be sampled regularly to increase detection probability (SCHULZ et al., 2020b).

Furthermore, passive surveillance strategies should be adapted seasonally. It is known that wild boar move closer to agricultural areas during the growing and harvesting season since they feed on grain or maize (MORELLE and LEJEUNE, 2015; PODGÓRSKI and ŚMIETANKA, 2018). Furthermore, it was observed in the study by Cukor et al. (2020b) that carcasses were found closer to water sources when temperatures were higher (CUKOR et al., 2020b). It might therefore seem useful to adjust the locations for carcass searches depending on the season. Satellite remote sensing of field crops can be used to support adjustment of locations for carcass search, especially on the lower jurisdictional level of disease control (Publication II). However, it is difficult to assess and analyze the direct impact of seasonality on the choice of deathbed in retrospective studies. The exact time of death and the postmortem interval (PMI) are often difficult to estimate once the carcass is found (PROBST et al., 2019; 2020). Therefore, the season in which a carcass is found is not necessarily the same as the season of death. The process of carcass decomposition can be highly variable depending on intrinsic factors such as body weight or size and extrinsic factors like temperature, humidity, precipitation and the existence of scavengers (PROBST et al., 2020). Thus, Probst et al. (2020) provided an adapted body scoring system and a checklist for wild boar carcasses found in the field in order to enable standardized PMI estimation (PROBST et al., 2020). Documentation of estimated PMI as well as environmental characteristics (e.g. landscape features, wetness, soil conditions) of carcass detection sites would enable future comprehensive analyses of wild boar deathbed choice.

Beyond the seasonal variation in the habitat choice of wild boar, also seasonal changes in the population dynamics of wild boar can be observed, eventually influencing ASF occurrence in general. In accordance with the present studies (Publication III and Publication IV), also in previous studies a peak of ASF occurrence in wild boar was detected in winter months, mainly December and January, in the Baltic states and Poland (PAUTIENIUS et al., 2018; FRANT et al., 2020; PAUTIENIUS et al., 2020). This finding might be related to the fact that winter is the mating season of wild boar with increased movement and contact rates, resulting in an increased ASF transmission risk. Furthermore, carcasses decompose much slower and ASFV is much more stable at lower temperatures (PROBST et al., 2019; FISCHER et al., 2020a), which might increase the transmission capacities even further. However, winter is the main hunting season and the chances of detecting positive wild boar and carcasses might be higher due to increased presence of hunters in hunting grounds during that time of the year (KEULING et al., 2010; QUIRÓS-FERNÁNDEZ et al., 2017). Therefore, the increase in the surveillance efforts might bias seasonal patterns of ASF occurrence (Publication III and IV). However, data to consider these surveillance efforts are hardly available at a larger scale for affected countries.

Since transmission of ASF to domestic pigs can occur through direct contact with wild boar, a correlation between peaks in the occurrence of ASF in wild boar and peaks in the occurrence in domestic pigs could be expected. Interestingly, in domestic pigs, seasonal peaks of ASF occurrence were detected consistently in the summer months, mainly in July, in the present studies (Publication III and IV) and in previous studies (PAUTIENIUS et al., 2018; CHENAIS et al., 2019; OĻŠEVSKIS et al., 2020; NURMOJA et al., 2020). The hypothesized interplay is not clearly apparent and it remains unclear which impact seasonal patterns of disease occurrence in wild boar might have on seasonal disease occurrence in domestic pigs (Publication IV).

On the one hand, seasonality in domestic pig outbreaks could be influenced by seasonal variations in the pig production. In some countries, e.g. Korea, pig productions peaks in summer (YOO et al., 2021), resulting in increased movements of pigs, workers and vehicles which can lead to increased risk of disease transmission between domestic pig farms. However, the described seasonality in pig farming is hardly observed in European countries (Publication IV). On the other hand, feeding of potentially contaminated fresh grass or crops could increase the risk for disease transmission in summer months. The latter was suspected to be a potential source of infection for domestic pig outbreaks in Latvia and Lithuania (OĻŠEVSKIS et al., 2016; PAUTIENIUS et al., 2018). Since summer is also the harvesting season, the presence of seasonal workers from abroad has also been considered as potential transmission route of ASF in Poland, but could not be confirmed (WOŹNIAKOWSKI et al., 2021).

To this end, seasonal patterns are not explored well enough to determine high-risk periods for ASF occurrence in wild boar and domestic pigs with certainty as they are influenced by the complex interplay of environmental, ecological, and anthropological factors. However, the discussed factors provide valuable insights to adapt prevention and surveillance strategies. For instance, domestic pig farmers should pay close attention to hygiene and biosecurity, especially during the high-risk periods. Feeding of fresh grass or crops from ASF-affected areas should be avoided. It is also very important to raise employees' awareness of the potential risks of disease transmission.

Beyond the knowledge about environmental risk-factors and seasonal variations, the efficiency of the ASF surveillance system is highly dependent of the willingness of stakeholders to participate in the system and to which extend they are involved in the surveillance (GERMAN et al., 2001; HOINVILLE et al., 2013). To adapt or improve a surveillance system, it is necessary to understand how stakeholders perceive the system and to identify potential ways to improve compliance (BRONNER et al., 2014; CALBA et al., 2015b; CALBA et al., 2015a). Thereby, also the value of non-monetary benefits should be considered and assessed, i.e. positive consequences for stakeholders resulting from the surveillance system (CALBA et al., 2015a).

The ASF prevention, surveillance and control system involves stakeholders from various branches, e.g. from the pig sector, animal health services, forest and wildlife exploitation, forestry services, civil services and many more (JORI et al., 2020). In the wild boar sector in the European countries, mainly hunters are responsible for detecting, reporting and sampling dead wild boar as well as depopulation of wild boar. Therefore, several studies were performed with hunters in the Baltic states to assess their perceptions and their willingness to participate in the current system, using questionnaires and participatory approaches (STONČIŪTĖ et al., 2021; 2022; URNER et al., 2020; 2021a; 2021b).

These studies revealed that the search for carcasses, i.e. passive surveillance, is considered as an undesirable activity among most stakeholders (STONČIŪTĖ et al., 2021; URNER et al., 2021a). Lithuanian hunters were less willing to participate in targeted carcass searches compared to looking for carcasses while they were already out in the forest for other reasons (STONČIŪTĖ et al., 2021). Accordingly, they rated this activity as a less effective measure to control ASF. Likewise, German hunters were unwilling to support passive surveillance already in the fight against Classical swine fever (CSF) (SCHULZ et al., 2016) and were less motivated to do so in the present study compared to participating in active surveillance (Publication IV). Financial compensation was mentioned as a considerable motivational option to increase participation in passive surveillance by hunters from the Baltic states and German hunters (STONČIŪTĖ et al., 2021; URNER et al., 2021a) (Publication V). Additionally, reduction of bureaucracy and a reduction of workload were considered motivational.

Hunters were more willing to support depopulation of wild boar through increased hunting and often assessed measures that promote hunting as more effective compared to measures that hinder or ban hunting (URNER et al., 2021a; STONČIŪTĖ et al., 2021) (Publication V). It should be noted, however, that hunting in this context refers more to depopulation for disease control than to normal leisure hunting (ESSEN and TICKLE, 2020). Thus, it has been shown that some hunters are ethically opposed to participating in active surveillance and depopulation (OELKE et al., 2022) (Publication V). In particular, the culling of female wild boar to prevent reproduction was considered unethical by hunters from the Baltic states because of the risk of producing orphans (STONČIŪTĖ et al., 2021; URNER et al., 2021a). The use of wild boar traps and the culling of entire groups of wild boar have also been discussed controversially in the present study (Publication V).

The efficacy of depopulation has also been discussed by experts. During the last years, the wild boar population in Europe has increased constantly with an estimated mean growth rate of approximately 20% per year (MASSEI et al., 2015). This makes even the management of the population extremely difficult, let alone the reduction of the population in the context of ASF control (QUIRÓS-FERNÁNDEZ et al., 2017). Modeling has shown that a large proportion of the wild boar population (around 80%) would need to be eliminated in order to stop the spread of ASF in an affected area (LANGE, 2015). In practice, this would not be feasible in many European countries due to the large population size. However, reducing the population density is considered to be useful in order to slow down the spread of ASF by preventing contact between and among groups of wild boar. The study by Yang et al. (2021) suggested that even low intensity population reduction can contribute to reducing the risk of ASF persistence (YANG et al., 2021).

In many countries, a drastic reduction of 85-95% of the wild boar population was observed in the years after ASF introduction (PALENCIA et al., 2023). However, it has been demonstrated that due to high lethality of ASF, the disease-induced mortality by far outweighs hunting-induced mortality (MORELLE et al., 2020). While the wild boar as a species is not threatened with extinction in Europe, the spread of ASF in Asia poses a significant threat to endemic and endangered wild pig species (EWERS et al., 2021; LUSKIN et al., 2021). Also in Haiti, it was already observed during the ASF outbreaks in the 20th century, that the creole pig, an important local Haitian pig breed, was eradicated through culling of the entire pig population (PENRITH and KIVARIA, 2022). Thus, Penrith and Kivaria (2022) referred to this as “an unwanted legacy and a warning against eradication of a single disease ahead of all other considerations” (PENRITH and KIVARIA, 2022).

In the domestic pig sector, great numbers of animals have already been culled globally (SÁNCHEZ-CORDÓN et al., 2018; MIGHELL and WARD, 2021; LADOȘI et al., 2023). Culling of pigs on affected pig farms is proven to be an effective ASF control measure to rapidly contain outbreaks in domestic pigs and thus it is mandatory according to EU legislation (GUINAT et al., 2017; JORI et al., 2020; PALENCIA et al., 2023). Nevertheless, it is ethically and morally challenging and can cause severe mental distress for involved stakeholders (HIBI et al., 2015). Thus, Hibi et al. (2015) suggested to provide mental health services for workers who deal with culling of animals due to infectious diseases (HIBI et al., 2015).

Beyond that, stakeholders have expressed their high hopes in the development of an efficient vaccine in order to stop the spread of ASF in the wild boar population in the present study (Publication V) and in studies from the Baltic states (URNER et al., 2020; URNER et al., 2021b). These hopes are probably raised from the positive experience with the oral vaccine against CSF, which supported the successful elimination of CSF from wild boar in Germany (KADEN et al., 2000; BLOME et al., 2017). However, the vaccine development for ASF is not comparable to this previous situation. There are still knowledge gaps regarding virus-host interactions, virulence genes and immune escape, the mechanisms of immune response and cross protection among different ASFV isolates, making the vaccine development particularly difficult (MUÑOZ-PÉREZ et al., 2021). There have been several approaches to ASFV vaccine development in the 20th and 21st century, out of which live attenuated vaccines appear to be the most promising advance towards sufficient protection (SANG et al., 2020; MUÑOZ-PÉREZ et al., 2021). For consideration for licensing in Europe, a vaccine candidate would have to meet high European Medicines Agency (EMA) standards of safety and protection including the implementation of the ‘Differentiating Infected from Vaccinated Animals (DIVA)’ strategy (SANG et al., 2020; MUÑOZ-PÉREZ et al., 2021). Thus, it is to be expected that it will be a number of years before a suitable vaccine for wild boar will be approved for use in Europe.

In view of this, it is likely that European countries will continue to struggle with the ASF epizootic in the wild boar population for a considerable time span, similar to the situation observed in Sardinia (MUR et al., 2016b). This setting will require an efficient toolbox of prevention, surveillance and control measures, making best use of available resources and adapting stakeholder perceptions and interests. Since there is a broad range of stakeholders involved, innovative holistic approaches will be needed in order to enhance communication among and between affected stakeholders and decision makers. Contrary to a top-down approach, involving stakeholders in decision-making and action planning can increase compliance (PALENCIA et al., 2023). Furthermore, transparent and sensitive stakeholder education is also important to increase the acceptance of certain measures and to further reduce the risk of ASF spread. Additionally, to advance vaccine development further research on viral-hosts interactions of ASFV will be necessary, demanding international collaboration.

VI. SUMMARY

African swine fever (ASF) is a viral disease that causes high lethality in domestic pigs and wild boar, resulting in significant economic and environmental impacts. The understanding of ASF transmission dynamics in wild boar populations and consideration of stakeholder perceptions are crucial for developing effective and targeted surveillance and control strategies, as there is no treatment or vaccine available.

ASF has emerged as a global threat in the last decade and many European countries, e.g. the Baltic states, Poland or Germany, have been suffering from the disease for several years now. In the current scenario, it is likely that ASF is going to persist even longer, particularly in wild boar populations of the affected countries. Thus, surveillance and control measures need to be implemented for long time spans and at several areas at the same time, which might push available resources and involved stakeholders to their limits.

To make best use of available resources and to enhance early detection strategies, one possible way would be to focus routine passive surveillance, i.e. carcass search and sampling, on high-risk areas, as identified and discussed in this thesis. However, strategies need to be adapted to the local conditions under consideration of stakeholder interests and experiences. Thereby, early preparation of infrastructure for carcass search, removal and sampling as well as communication are necessary to be best prepared for potential outbreaks.

Due to visible seasonal patterns of ASF occurrence in wild boar and domestic pigs, it appears necessary to adapt surveillance and control measures to these changes. Comprehensive research approaches are needed to investigate diverse seasonal patterns of disease transmission and their drivers in different regions.

Furthermore, the efficiency of surveillance and control measures is strongly dependent on stakeholders' acceptance and compliance. Thus, interdisciplinary collaboration between a broad range of stakeholders is required, which should start as early as possible in preparation for potential outbreaks. Awareness raising and education is also necessary to increase to enhance compliance and to reduce the risk of further ASF spread.

In conclusion, the integration of spatial analysis, temporal trends, and qualitative research methods strengthens the overall understanding of ASF dynamics and paves the way for more effective disease management strategies. The insights gained from this thesis provide guidance to resource

allocation and offer potential for facilitation of effective communication between different stakeholders in order to optimize ASF surveillance, prevention and control in wild boar.

VII. ZUSAMMENFASSUNG

Die Afrikanische Schweinepest (ASP) ist eine Viruserkrankung mit hoher Letalität bei Hausschweinen und Wildschweinen, die erhebliche wirtschaftliche und ökologische Auswirkungen hat. Aufgrund fehlender Therapiemaßnahmen oder einer Impfung, ist das Verständnis von Übertragungswegen in Wildschweinpopulationen und die Einbeziehung der Meinungen und Erfahrungen von beteiligten Akteuren entscheidend für die Entwicklung von effektiven und zielgerichteten Überwachungs- und Bekämpfungsstrategien.

Die ASP hat sich im letzten Jahrzehnt zu einer globalen Bedrohung entwickelt, und viele europäische Länder, wie die Baltischen Staaten, Polen oder Deutschland, sind mittlerweile mehrere Jahre von der Krankheit betroffen. Es ist wahrscheinlich, dass die ASP vor allem in den Wildschweinpopulationen der betroffenen Länder noch längere Zeit persistieren wird. Daher müssen Überwachungs- und Bekämpfungsmaßnahmen über lange Zeiträume und in mehreren Gebieten zur selben Zeit durchgeführt werden, was verfügbare Ressourcen und die beteiligten Akteure an ihre Grenzen bringen wird.

Um die verfügbaren Ressourcen optimal zu nutzen und die Früherkennung zu verbessern, besteht eine Möglichkeit darin, die routinemäßige passive Überwachung, d.h. Kadaversuche und -beprobung, auf Hochrisikogebiete zu fokussieren. Diese Strategien müssen jedoch an die lokalen Gegebenheiten angepasst werden, bestenfalls unter Berücksichtigung der Interessen und der Erfahrungen der beteiligten Akteure. Eine frühzeitige Vorbereitung der Infrastruktur für Kadaversuche und -beprobung sowie der Kommunikationskanäle ist für die bestmögliche Vorbereitung auf das Auftreten der Krankheit erforderlich.

Aufgrund erkennbarer saisonaler Muster des Auftretens der ASP bei Wild- und Hausschweinen erscheint es außerdem notwendig, die Überwachungs- und Bekämpfungsmaßnahmen an diese Gegebenheiten anzupassen. Weiterführende Studien sind erforderlich, um die unterschiedlichen saisonalen Muster der Krankheitsübertragung und deren Ursachen in verschiedenen Regionen zu untersuchen.

Darüber hinaus hängt die Wirksamkeit von Überwachungs- und Bekämpfungsmaßnahmen stark von der Akzeptanz und Compliance der beteiligten Akteure ab. Demnach ist eine interdisziplinäre Zusammenarbeit einer Bandbreite von Akteuren nötig, die so zur Vorbereitung auf mögliche Ausbrüche und Fälle so früh wie möglich beginnen sollte. Zusätzlich sind Aufklärung und Weiterbildung nötig, um die Einhaltung der Vorschriften zu verbessern und damit das Risiko einer weiteren Ausbreitung der ASP zu verringern.

Zusammenfassend lässt sich sagen, dass die Einbeziehung von räumlichen Analysen, zeitlichen Trends und qualitativen Forschungsmethoden das Gesamtverständnis der ASP-Dynamik verbessert und damit den Weg für effektivere Bekämpfungsstrategien ebnet. Die aus dieser Arbeit gewonnenen Erkenntnisse liefern Ansätze für eine zielgerichtete Zuteilung von Ressourcen und effektive Kommunikation zwischen Akteuren zur Optimierung der Überwachung, Prävention und Bekämpfung der ASP beim Wildschwein.

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