

LATVIJAS BIOZINĀTŅU UN TEHNOLOĢIJU UNIVĒRSITĀTE
LATVIA UNIVERSITY OF LIFE SCIENCES AND TECHNOLOGIES

Veterinārmedicīnas fakultāte
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**DEVELOPMENT OF A STRUCTURED OUTBREAK INVESTIGATION
AND EARLY DETECTION FRAMEWORK FOR AFRICAN SWINE
FEVER IN DOMESTIC PIG FARMS**

***ĀFRIKAS CŪKU MĒRA UZLIESMOJUMU STRUKTURĒTAS
IZMEKLĒŠANAS UN AGRĪNĀS ATKLĀŠANAS SISTĒMAS IZSTRĀDE
MĀJAS CŪKU NOVIETNĒS***

DOCTORAL THESIS based on a thematically coherent set of scientific publications
for the doctoral degree of Doctor of Science (Ph.D.) in Agriculture, Forestry and Veterinary
Sciences

PROMOCIJAS DARBA tematiski vienotu zinātnisko publikāciju kopa
zinātnes doktora grāda (Ph. D.) iegūšanai lauksaimniecības, meža un veterinārās
zinātnēs

Jelgava

2026

Research was carried out at/ *Pētījumi veikti:*

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“Call to Me, and I will answer you, and show you great and mighty things, which you do not know”

“Piesauc Mani, tad Es tevi uzklausīšu un tev atklāšu lielas un brīnišķas lietas, kas tev nebija zināmas”

Jer 33:3

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ABSTRACT

African swine fever (ASF) is one of the most important diseases affecting pigs due to its severe socioeconomic consequences, unpredictable cross-border spread, and the absence of effective therapeutic or vaccine-based prophylactic measures. The evolving epidemiological situation of ASF poses a continuous risk to pig farming within the European Union (EU) and in third countries. Since ASF first emerged in the EU in 2014, its spread has represented a major challenge for governmental institutions, industry experts, and various stakeholders.

ASF reached Latvia in 2014 through the eastern border via infected wild boar populations, thereby threatening domestic pig farms. Since then, ASF outbreaks have been detected in domestic pig holdings across Latvia. Although the first outbreaks occurred mainly in backyard farms, the first outbreak in a large commercial pig farm was recorded in January 2017, prompting immediate in-depth epidemiological investigations. Subsequent outbreaks in several commercial pig farms enabled more comprehensive epidemiological investigations under field conditions. This thesis summarizes three epidemiological studies conducted in four large commercial pig farms in Latvia, as well as a fourth study focusing on the opinions of farm workers, farmers, and other lay persons from different ASF-affected countries regarding possible routes of virus introduction.

Our studies were conducted to identify possible sources of virus introduction into the farms, determine the probable time of virus entry, and investigate the internal transmission pathways of the disease. Collectively, these investigations facilitated the development of a structured epidemiological approach for ASF outbreak investigations at the farm level. The biosecurity systems of the affected pig farms were assessed, leading to the development of a hypothesis-based approach for investigating potential pathways of virus introduction. Disease detection systems were analyzed in all study farms to evaluate whether ASF had been detected in a timely manner and to develop recommendations for improving surveillance aimed at earlier detection. By collecting and analyzing information on farming practices, as well as morbidity and mortality data from the study farms, the high-risk period (HRP) of ASF - defined as the time between virus introduction and disease detection - was established. Reconstruction of the HRP served as an important analytical tool linking virus introduction, disease detection, and outbreak control decisions. To assess the extent of infection within affected pig farms, a reliable sampling strategy was developed and applied in one of the studies. Several parameters, including mortality data and the movement of animals, personnel, and vehicles, were analyzed to better understand the mechanisms of virus spread within farms. A separate study investigated the opinions of lay persons regarding possible disease introduction routes into their farms, with particular emphasis on the epidemiological relevance and interpretation of such information.

Our findings demonstrated that ASF virus spread in large commercial pig farms initially occurs slowly. Enhanced passive surveillance was identified as a key tool for early disease detection and proved effective when systematically implemented. Recommendations were developed for the application of enhanced passive surveillance in pig farms to reduce the duration of the HRP. The hypothesis-based approach proved valuable for conducting structured epidemiological investigations of possible virus introduction pathways. The proposed sampling strategy, combining laboratory testing with pen-side diagnostic tests, enabled reliable assessment of ASF spread within different epidemiological units of affected farms. Furthermore, the opinions of lay persons regarding possible disease introduction routes highlighted the subjective nature of such information and emphasized the importance of experienced investigators in its appropriate interpretation during outbreak investigations. Overall, this thesis contributes to the development of a structured framework for ASF outbreak investigation and early disease detection, thereby supporting improved ASF control in domestic pig farms.

ABBREVIATIONS

Ab-LFD	Antibody lateral flow device
ADIS	Animal disease information system
Ag-LFD	Antigen lateral flow device
ASF	African swine fever
ASFV	African swine fever virus
CSF	Classical swine fever
Ct	Quantification cycle
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
EURL	European Union Reference Laboratory
FMD	Foot and Mouth disease
FVS	Food and Veterinary Service
HRP	High risk period
IPT	indirect immunoperoxidase technique
LFD	Lateral flow device
NRL	National Reference Laboratory
PCR	Polymerase chain reaction
qPCR	Quantitative Polymerase chain reaction
rtPCR	Real time Polymerase chain reaction
WAHIS	World Animal Health Information System
WOAH	World Organisation for Animal Health

ORIGINAL PUBLICATIONS

The thesis is based on four original publications (studies I - IV). Roman numerical is used within the text to refer to the publication.

- 1) Lamberga, K.; Seržants, M.; Oļševskis, E. African swine fever outbreak investigations in a large commercial pig farm in Latvia: A case report. Berl.Munch, Tierarztl. Wochenschr.2019, 132, 151–155
- 2) Lamberga, K., Oļševskis, E., Seržants, M., Bērziņš, A., Viltrop, A., & Depner, K. (2020). African swine fever in two large commercial pig farms in Latvia - Estimation of the high-risk period and virus spread within the farm. Veterinary Sciences, 7(3), 105. <https://doi.org/10.3390/vetsci7030105>
- 3) Lamberga, K., Depner, K., Zani, L., Oļševskis, E., Seržants, M., Ansonskā, S., Šteingolde, Ž., Bērziņš, A., Viltrop, A., Blome, S., & Globig, A. (2022). A practical guide for strategic and efficient sampling in African swine fever-affected pig farms. Transboundary and Emerging Diseases, 1–10. <https://doi.org/10.1111/tbed.14582>
- 4) Lamberga, K.; Ardelean, F.; Blome, S.; Busauskas, P.; Djuric, B.; Globig, A.; Guberti, V.; Miteva, A.; Oļševskis, E.; Seržants, M.; et al. African Swine Fever Outbreak Investigations - The Significance of Disease-Related Anecdotal Information Coming from Laypersons. Pathogens 2022, 11, 702. <https://doi.org/10.3390/pathogens11060702>

Contribution of the author

Publication/Study	Contribution	%
I	Designed and conducted the study, drafted the manuscript	70
II	Epidemiological investigation and data analysis, writing - original draft preparation	75
III	Conceptualization, methodology, farm investigations, formal analysis, data curation, and graphs, writing - original draft preparation	75
IV	Conceptualization, writing - original draft preparation, methodology, visualisation	65

1. INTRODUCTION

1.1. African swine fever: disease overview and epidemiology

African swine fever (ASF) is one of the most important animal infectious diseases of pigs due to its severe socioeconomic consequences, unpredictable international spread, and lack of effective treatment or vaccination options. ASF is a notifiable disease.

ASF was first described in Kenya in 1921 (Montgomery, 1921). The first introduction of ASF into Europe was to Portugal in 1957, where it was initially eradicated. However, it was introduced a second time to Portugal in 1960, from where it spread to Spain, becoming endemic in the Iberian Peninsula in the 1970s and 1980s and causing high economic losses. During this period, outbreaks also occurred sporadically in other European and American countries (e.g., the Netherlands, Belgium, France, northern Italy, Cuba, Brazil, the Dominican Republic, and Haiti) but were eradicated. In 1978, ASF was introduced to Sardinia. Since then, the animal disease has been endemic on the island (Mur et al., 2014) until recently, and the Sardinian authorities have succeeded in eradicating the disease (Cappai et al., 2022).

The source of infection in the primary outbreaks was mostly contaminated food waste originating from aircraft and ships involved in international traffic (Mur et al., 2012). Therefore, from an epidemiological perspective, it was concluded that humans are primarily responsible for both the intercontinental and regional spread of ASF through failure in biosecurity measures.

In 2007, ASF entered Georgia via the Black Sea port of Poti, presumably also through contaminated food waste, and spread rapidly in Georgia and in neighbouring Armenia and the Russian Federation. By the end of 2012, several regions in the European part of the Russian Federation were affected (Gogin et al., 2013; Organesyan et al., 2013). In the following years, outbreaks were reported from Ukraine and Belarus. In January 2014, ASF reached the eastern border of the EU, with Lithuania reporting the first ASF-positive wild boar. A month later, the first cases were reported from Poland, in June from Latvia, and in September 2014 from Estonia (EFSA, 2014; Pejsak and Stadejek, 2015). Due to the proximity of the first outbreaks in these countries to the Belarusian border, it can be assumed that ASF was introduced from Belarus to the Baltic States and Poland. However, officially no cases of ASF were reported from Belarus during this period.

Long before the current events in Europe and Asia, at the time when the virus was present in the Iberian Peninsula in addition to Africa, four countries in the Americas were also affected. Besides Cuba, which had the disease twice, in 1971 and 1980, these were the Dominican Republic (1978-1981), Haiti (1978-1983) and Brazil (1978 -1981). Very recently, in the summer of 2021, ASF has been detected for the second time after 40 years in the Dominican Republic.

It is undisputed that the virus was introduced in the Americas by boat or by plane. In other words, it was humans who introduced the virus, and that too in times when the world was not as interconnected as it is today. This transcontinental spread of the pathogen, presumably via ship and aircrafts, is not an exception with ASF, but the rule (EFSA, 2021; Sánchez-Vizcaíno et al., 2013). Sequence analyses of the most recent isolates from the Dominican Republic proved that it is the same virus strain that circulates in Europe and Asia.

It may be a long time since the outbreaks of the 1970s and 1980s in Europe and the Americas, and much has been learned about ASF since then, but it is good to remember that the virus has managed to cross oceans and enter Europe, Asia or the Americas several times with the help of humans. The recent 2021 entry into the Dominican Republic is yet another proof of this.

Historically, three epidemiological cycles were described: (i) the sylvatic cycle in Africa, where the virus is transmitted by soft ticks (*Ornithodoros spp.*) between African wild pigs (mainly warthogs) without causing disease in the vertebrate or arachnid host, (ii) the tick-pig cycle, in which the virus is transmitted between domestic pigs, with *Ornithodoros spp.* ticks

serving as vectors, and (iii) the domestic pig cycle, in which the virus is transmitted directly from pig to pig or via contact to contaminated materials, without the involvement of competent arthropod vectors. The vast majority of ASF outbreaks worldwide are attributed to the domestic pig cycle. However, when the disease arrived in the Baltic States and Poland in 2014 the epidemiological role of wild boar became evident, and a fourth infection cycle could be established: the wild boar-habitat cycle (Figure 1.1) (Chenais et al., 2018). These epidemiological characteristics determine the complexity of outbreak investigations.



Figure 1.1 The epidemiological cycles of ASF. 1 – sylvatic cycle, 2 – tick – pig cycle, 3 – domestic pig cycle, 4 – wild boar habitat cycle (Chenais et al., 2018)

It should be noted that the epidemiological situation observed in Europe after 2014 differs substantially from that experienced during the genotype I era in the Iberian Peninsula. The currently circulating genotype II viruses are characterized by very high lethality; consequently, control approaches and surveillance concepts that were developed during the earlier European outbreaks were not always directly applicable to the epidemiological conditions observed in the Baltic States and Poland in 2014. In particular, reliance on serological surveillance as an early detection tool proved to be of limited value under conditions where infected animals frequently died before developing detectable antibody responses.

From the point of view of animal disease control and epidemiology, the high tenacity of ASF virus to environmental influences is particularly noteworthy. Putrefaction and sunlight inactivate the ASF virus insufficiently and it remains infectious for several weeks, even under the conditions of the decomposition process. In carcasses and blood, the virus is capable of surviving for months, and in frozen meat, for years (Farez and Morley, 1997; EFSA, 2010). Thus, carcasses of infected animals and contaminated meat and meat products can be important sources of infection. Heat inactivation of ASF virus in meat and meat products occurs at 56 °C for 70 min or 60 °C for 20 min. During processing of meat and meat products, it is inactivated only when a core temperature of 69 °C is reached. The virus is stable in the wide pH range 3.0 to 13.4. Many basic disinfectants do not inactivate the virus, but acid-based ones do. Formic acid and also citric acid are very effective, the latter even on wood (Krug et al., 2012). Other effective disinfectants include sodium hydroxide (0.8% NaOH, 30 min), hypochlorite (2.3%,

30 min), formalin (0.3%, 30 min), orthophenylphenol (3%, 30 min), and iodine-containing disinfectants.

Considering hosts and vectors, both domestic pigs and wild boar are susceptible for the infection; other domestic species as well as humans are not susceptible. The African warthog is considered the natural reservoir of ASF virus. It does not usually show clinical signs of disease.

In some areas of Africa, as well as in the Iberian Peninsula, soft ticks of the genus *Ornithodoros* are competent vectors of the ASFV and play a major role in the spread of the disease (Oleaga-Perez et al., 1990). In *O. moubata* (occurring in sub-Saharan Africa), both transovarial and transstadial virus transmission occurs, it is fully competent vector. In *O. erraticus* (occurring in the Iberian Peninsula and northern and western Africa), transovarial transmission does not occur, but the virus persists in the tick for up to five years and is transmitted transstadially.

However, the presence of soft ticks is not essential for transmission or maintenance of the infection cycle. In Sardinia as well as in Central and Eastern Europe, there is no evidence so far that ticks are involved in the spread of ASF (EFSA, 2024). In Germany, wild boar sera were tested for the presence of antibodies against soft tick antigens and no evidence of contacts was found (Pietschmann et al., 2016), so it is not expected that soft ticks would be involved in an ASF cycle. Laboratory-based studies on the vector competence of other native ticks (e.g., *Ixodes Ricinus*) revealed no evidence of virus replication (de Carvalho Ferreira et al., 2014).

In the course of outbreak investigations in domestic pig herds, bloodsucking insects are often mentioned as potential mechanical vectors in the absence of clear evidence for the route of introduction. However, there are very few experimental and field studies to support that bloodsucking insects may play an epidemiologically relevant role in the spread of ASF. Mechanical transmission and prolonged detectability of replication-capable virus have been demonstrated for *Stomoxys calcitrans* (Mellor et al., 1987). A recent field study investigating the role of stable flies and biting midges as potential mechanical vectors of ASF virus concluded that potential role of incidental mechanical vector such as *Culicoides spp.* or *S. calcitrans* transmissions can be possible and further studies are important (Dhollander et al., 2025). Available scientific evidence suggests that stable flies and horse flies are exposed to ASF virus in affected areas in the EU with the capacity to transmit the virus to pigs (EFSA, 2024). Therefore, the relevance of bloodsucking insects to ASF epidemiology under field conditions remains unclear. Evidence is also lacking to observe relationship between ASF outbreak seasonality and the role of blood feeding insects.

In domestic pigs and wild boar, ASF typically manifests as an acute, highly lethal haemorrhagic disease characterised by rapid clinical progression and high case fatality rates. The course of the disease is the same in domestic and wild pigs. Both sexes and all age groups are equally affected. Susceptible animals become infected when they have direct contact with infected material or with diseased animals, usually by the oral route through consumption of contaminated feed, meat, etc. In areas where *Ornithodoros* ticks may be vectors, infection may occur via tick bites. Blood from viraemic animals is a critical factor from an epidemiological point of view, as one drop of blood can be sufficient for infection since the virus is an "arthropod borne" virus and therefore optimized for transmission by small amounts of blood.

After a relatively short incubation period of a few days, the animals develop a high fever (up to 42 °C), are fatigued and refuse to eat. Pregnant sows may abort due to the high fever. Haemorrhages in the skin and internal organs are often seen in an advanced stage of the disease (OIE, 2019). Death usually occurs within two weeks. Sometimes animals die peracutely without having shown pronounced signs of disease. Based on the clinical picture, ASF cannot be distinguished from severe courses of classical swine fever (CSF) and other severe general diseases in pigs; only laboratory diagnostic tests allow a reliable diagnosis. These non-specific early clinical signs contribute to delayed detection of ASF outbreaks under field conditions.

A special feature of ASF is the fact that infected animals, if they do not already die from the infection in the first two weeks, produce antibodies against the ASF virus, but these have no neutralizing effect and do not provide effective protection. The lack of protective antibodies is also a major reason why there are still no effective ASF vaccines, as the antibodies induced after vaccination are also not sufficiently protective. However, the detection of antibodies in an animal can be used very well epidemiologically. An antibody-positive finding indicates that the infection is at least two weeks old. If no antibodies are found, but only virus antigen or virus genome is detected using PCR, the infection is relatively recently introduced (< 12 days).

Often, ASF is described as a highly contagious diseases, similar as CSF or foot and mouth disease (FMD), in which the viruses are excreted in large quantities via various secretions and excretions. This does not apply to ASF in this way. It is a highly contagious disease only when blood contact is the primary route of transmission.

To summarise, epidemiological analysis of outbreak data indicates high lethality (case fatality) and low mortality in the initial phase of an outbreak and low contagiousness of the ASF virus. Lethality is the proportion of animals that died relative to the number of animals shown to be infected in period X; this is very high in the case of infection with genotype II of ASF virus. Over 90% of infected animals die within two weeks (Gabriel et al., 2011; Blome et al., 2012; Blome et al., 2013). Practically, this means that almost every animal that becomes infected also dies as a result of the infection. Experimental studies have also demonstrated the high lethality (Pietschmann et al., 2015; Gallardo et al., 2015). However, during the initial phase of African swine fever at the farm level - approximately two to three weeks after virus introduction - overall mortality may appear low despite high lethality, since only a limited number of animals within the epidemiological unit are affected. Mortality is defined as the proportion of animals that died in relation to the entire population in the period X.

The described epidemiological parameters underline the importance of field studies aimed at better understanding disease transmission dynamics within affected herds and the temporal development of infection at farm level. These epidemiological characteristics determine not only the clinical outcome of infection but also influence the design and implementation of outbreak control and surveillance strategies in affected regions.

These epidemiological patterns are closely linked to the biological characteristics of the ASF virus. ASF is caused by an enveloped double-stranded DNA virus of the *Asfarviridae* family. The virus possesses a complex multilayered structure and a genome encoding more than 150 genes, a substantial proportion of which remain functionally uncharacterized. The virus replicates predominantly in the cytoplasm of infected cells, particularly within perinuclear viral factories, and encodes many of the proteins required for its own transcription and replication. ASF virus shows a marked tropism for cells of the monocyte-macrophage lineage, which plays a central role in its pathogenesis. Infection is associated with dysregulation of the host immune response, including excessive production of proinflammatory cytokines and chemokines, contributing to severe clinical manifestations and high mortality rates. While some immune responses, such as early interferon production and T cell activation, are associated with protection, the mechanisms of protective immunity remain incompletely understood. The virus is characterized by exceptional environmental stability, allowing it to persist for extended periods in contaminated materials such as meat, blood, carcasses, and fomites. This resilience, together with multiple transmission pathways including direct contact, contaminated feed or equipment, and, in some regions, vector-borne transmission, complicates control efforts and contributes to the continued global spread of the disease (Mehinagic and Summerfield, 2026).

To date, 22 genotypes of the ASF virus have been described (Bastos et al., 2003). It should be noted that genotypes do not provide compelling information about virulence. The Sardinian, Iberian, and South American strains of ASF virus all belong to genotype I. In contrast, the virus strains currently circulating in Eastern Europe (Baltic States, Poland, Caucasus, Russian Federation, Ukraine) are classified as genotype II and consequently are not directly related to the Sardinian or Iberian isolates (Gallardo et al., 2009; Gallardo et al., 2014). The current

genotype II strains are identical in the routinely sequenced genomic regions and have minimal alterations when the whole genome is considered. However, in the search for discrimination possibilities, recently genome region that shows a pattern of sequence repeats that may make molecular epidemiological assessment possible were described (Gallardo et al., 2014; Goller et al., 2015; Forth et al., 2022).

The epidemiological situation observed in Latvia since 2014 provides a practical example of the described transmission dynamics and infection pressure in affected regions. Since its introduction in 2014, ASF has remained widely distributed in Latvia, with sustained circulation in wild boar populations and recurrent outbreaks in domestic pig herds (Figure 1.2). Currently, ASF cases have been detected in wild boar in almost the whole of Latvia. A total 11.211 wild boar cases and 102 outbreaks in domestic pig herds were recorded between 2014 and 2025, requiring the destruction of 81.590 domestic pigs (FVS website). These figures illustrate the persistent infection pressure from the wildlife reservoir and the ongoing risk of virus introduction into domestic pig holdings.

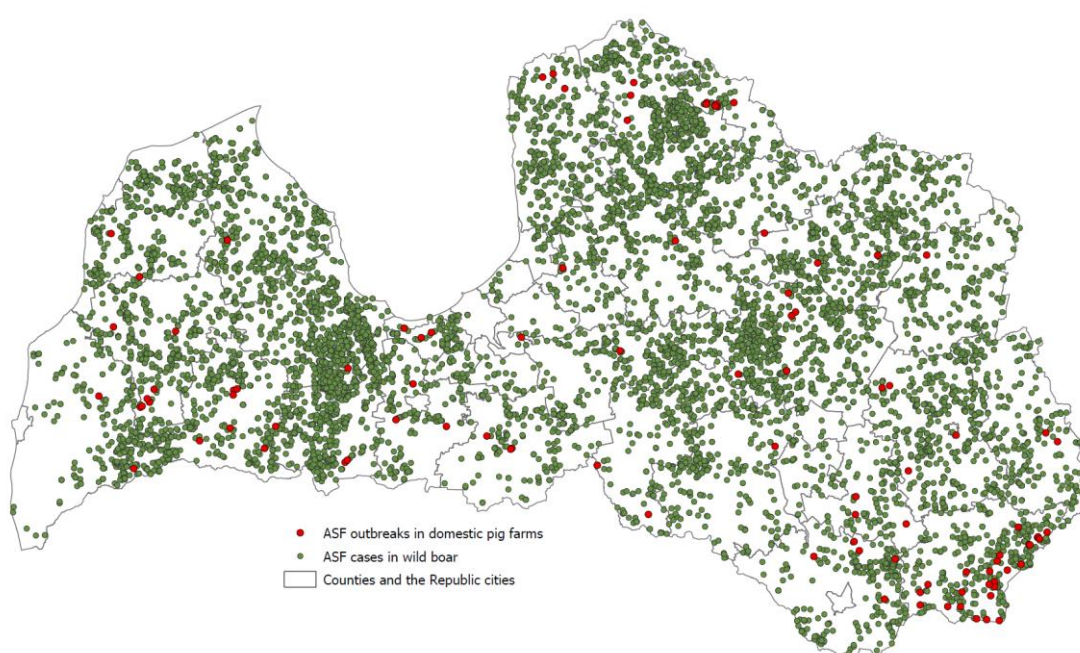


Figure 1.2 Distribution of ASF cases in wild boar and outbreaks in domestic pig farms in Latvia 2014-2025 (FVS, ASF surveillance data; visualisation using ESRI, ArcGIS Pro 3.1.0 version)

Within the framework of the ASF surveillance and control programme managed by FVS, both - wild boar found dead and hunted wild boar were tested for ASF. An epidemiological temporal and spatial analysis of the results of PCR tests and the results of the studies carried out have shown that the virus prevalence rates of ASF vary from year to year and also from region to region (Oļševskis et al., 2020.; BIOR, 2022). Although the spread of ASF resulted in a rapid reduction of the wild boar population, from about 74.000 individuals in 2013 to about 15.000 individuals in 2015, wild boar remain an ASF reservoir in Latvia and thus posing continuous and permanent threat for domestic pig sector – both for commercial farms and for backyard pigs (Figure 1.3).

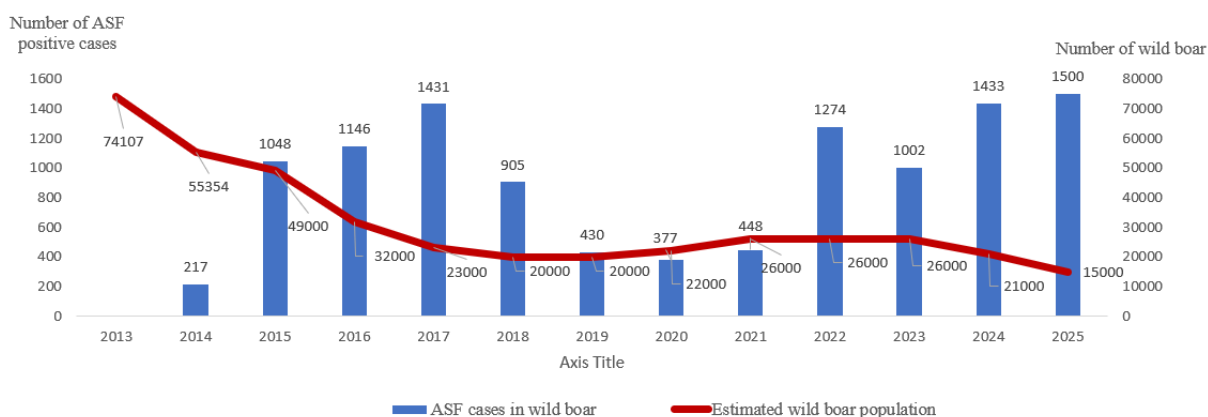


Figure 1.3 ASF cases in wild boar and estimated wild boar population per year (2013-2025) (Source: Food and Veterinary Service and State Forest Service)

Under such epidemiological conditions, characterised by continuous virus circulation in wild boar and recurrent spill-over into domestic pig herds, effective surveillance systems and clearly defined outbreak investigation and control measures become essential for limiting further spread of the disease.

1.2. ASF control, outbreak investigation and biosecurity

There are common basic category A disease control principles and requirements in the EU. ASF is a category A disease. Regulation (EU) 2020/687 lays down the basic rules on the disease control measures to apply in the affected pig farms in the event of an outbreak of ASF. EU legislation requires the competent authority to draw up and update contingency plans and provide detailed instruction manuals on implementing measures to be taken in case of ASF outbreak (EC, 2016). Contingency plans must ensure adequate allocation of resources and specify command structures at national, regional and local level. They must cover general support functions such as communication, logistics, human and material resources, as well as preparedness and control measures. The aim of the measures is to enable a rapid and effective response to outbreaks of category A diseases and to prevent the spread of the disease from affected animals and farms to unaffected animals. To provide effective disease control, it is essential to draw up, and keep up to date, detailed instruction manuals. Instruction manuals refer to and describe the operational instructions and procedures to be implemented in the event of an outbreak, to control and eradicate the disease. Instruction manuals contain documents that provide detailed technical guidance and procedures for specific animal diseases. They cover aspects such as disease procedure for notification, diagnostic criteria and capacities, epidemiological enquiries, establishment of the restricted zones and control measures to be applied in the restricted zones, vaccination plans, culling and disposal, cleaning and disinfection, and repopulation. From a scientific perspective, such preparedness planning is essential because ASF virus is environmentally resistant and capable of spreading through both direct and indirect transmission pathways, requiring coordinated and immediate response measures (EFSA, 2021; WOA, 2023).

Disease control and biosecurity measures should be applied immediately in the affected farms, particularly as regards movements of animals and products of animal origin. In order to prevent the spread of the infection from the affected pigs and holdings the following measures have to be implemented: isolation of all animals suspected of being infected, ceasing all movements of pigs from or to the establishment; keeping the manure, including litter and used bedding, and any product, material or substance likely to be contaminated until they are disposed of or processed or cleaned and disinfected. Additional biosecurity measures to avoid any risk of spreading the disease must be implemented. Furthermore, a restricted zone

(protection and surveillance) around the affected establishment needs to be established to prevent any further spread of the disease. Certain prohibitions of movements of animals and products within, from or through the protection and surveillance zones and the prohibitions of other activities that can pose a risk of spreading the disease are always established by the competent authorities. Control measures applicable within the surveillance zone include specific rules for animals, products obtained from those animals, and other substances and materials that may be contaminated. These measures are scientifically justified by the fact that ASF virus can persist for long periods in organic material such as manure, bedding and contaminated products, and can be transmitted indirectly via fomites, making strict movement control and biosecurity essential to interrupt transmission pathways (EFSA, 2021; WOA, 2022).

According to the EU legislation each country must have, implement and maintain a system that provides early detection of animal infectious diseases. EU Member States shall ensure that animal owners or keepers and other natural or legal persons immediately notify the competent authority where they suspect the presence of ASF or where the presence of a disease is detected. Veterinary services shall immediately conduct an investigation to confirm or rule out the presence of the suspected ASF (EC, 2019). Legislation requires the competent authority to carry out investigations on the occurrence of a disease at different stages: when the disease is suspected; when the disease is confirmed; and when it is necessary to rule out its spreading to epidemiologically linked establishments and locations. Those investigations include clinical examination and sampling for laboratory testing. Early detection and rapid notification are critical from an epidemiological perspective, as ASF outbreaks are often detected late due to non-specific early clinical signs, and delays in detection significantly increase the risk of further spread (EFSA, 2018).

For ASF outbreaks in pig farms, EU legislation requires also thorough outbreak investigation with the aim of identifying the likely origin of the virus and the high-risk period (HRP), that is, the estimated time that ASF has been on the farm before detection as well as to obtain information on the likely spread of the disease to other farms (EC, 2002; EC, 2016; EC 2019). EU legislation required (until 21 April 2021) the collection of random blood samples from pigs on the affected holding during the culling process for serological and virological testing. The minimum number of samples required should allow the detection of a seroprevalence of 10% in pigs in each sub-unit of the holding with a 95% confidence level (EC, 2003). Considering that the dominant ASF virus genotype II strains currently circulating are causing a case-fatality rate of >90%, seropositive healthy pigs are rarely found as majority of pigs do not survive the acute phase (Blome et al., 2020). Therefore, considering the circulation of virulent viruses, surveillance based on random serological sampling was no longer recommended as a method to estimate the spread of the virus within an affected farm (EFSA, 2021). The targeted surveillance approach was based on the assumption that infected animals would get sick and die (Busch et al., 2021; Blome et al., 2020). Nevertheless, antibody tests are important by providing additional information about the duration of virus presence on a farm and therefore are always employed.

A mandatory control measure is the killing of pigs that are infected or may be infected or may contribute to the spread of the disease. All animals of susceptible species kept in the affected establishment shall be killed as soon as possible on the spot, within the establishment, in such a way as to avoid any risk of spreading the disease agent during and after killing. Due to that reason all pigs from the outbreak farms independently of their size are killed and their carcasses safely disposed in the rendering plant. Although EU regulation allows to the competent authority to grant derogation from killing all the pigs in the epidemiological units in which the disease has not been confirmed if farm consists of more than one epidemiological unit. Such derogation can be granted only after carrying out a risk assessment, and after obtaining favorable results in laboratory examinations, and provided that the epidemiological enquiry has not revealed any epidemiological link between the epidemiological units in which

the disease has been confirmed (EC, 2019). Stamping-out is scientifically justified because ASF has no effective treatment or vaccine available, and infected animals represent a major source of virus shedding; therefore, rapid elimination of infected and potentially exposed animals is essential to remove infection sources and prevent further transmission (WOAH, 2023). Additionally, safe disposal of carcasses is critical, as ASF virus can remain infectious in carcasses for extended periods and contribute to environmental contamination and further spread (EFSA, 2018).

In Latvia all ASF suspected cases are notified to veterinary practitioner or to the veterinary service (FVS). Clinical, post-mortem inspection and sampling are carried out by veterinary practitioners or official veterinarians. Samples for laboratory investigation must be taken and sent to the NRL (MK, 2004). If ASF virus genome or antibodies are detected, the pig is considered infected and competent authority is in charge to confirm the outbreak. The laboratory costs for ASF investigations are covered by the State budget.

For the purpose of timely detection of outbreaks and due to high lethality (case fatality) and low mortality in the initial phase of an outbreaks and low contagiousness of the ASF virus it was decided to introduce an enhanced passive surveillance system in pig farms located in ASF risk areas. This was a consequence following the investigation of outbreaks in large farms, in particular the outbreak in a commercial farm in Latvia in January 2017. Afterwards the EU legislation was adapted accordingly, introducing the legal requirement to implement and maintain enhanced passive surveillance system. According to the current EU legislation the owner or keeper of the animals shall be obliged to ensure testing each week at least the first two dead pigs over the age of 60 days or, in the absence of such animals, on any dead pig after weaning, in each epidemiological unit (EC, 2023).

The FVS regularly informs the European Commission using ADIS (Animal Disease Information System) and the World Organization for Animal Health (WOAH) using WAHIS system (World Animal health Information System) about ASF cases in wild boar and outbreaks in domestic pig herds (EC, 2020; WOAH WAHIS). This is a way how information about a detected disease and the adopted measures is shared between the countries, allowing the competent authorities to react properly and to provide the necessary awareness of the disease risks and implement preventive measures.

To provide continuous evaluation and awareness of control measures the FVS organizes meetings for veterinary practitioners and official veterinarians, hunters' associations and pig farmers providing updated information about ASF epidemiological situation in wild boar population, and about the ASF outbreaks, the disease control measures and necessity to enforce biosecurity. Frequently media (press, radio and TV) are involved in awareness activities (PVD, 2021). Campaigns are supported by specially prepared videos and booklets containing different information depending on the message to be delivered and the target audience (FVS website 2).

Awareness of the disease is another important precondition to avoid or timely detect the infection. There are different target audiences for awareness campaigns, starting from the public in general, animal keepers, farmers, practicing veterinarians, official veterinarians, hunters etc.

Since there is no vaccine or treatment for ASF, the only way to prevent ASF virus introduction in the herd is the implementation of preventive measures. Biosecurity is the most effective preventive measure. It is a special system that needs to be developed, adopted, implemented and maintained respecting each individual establishment where pigs are kept. All holdings in which pigs are kept must ensure the implementation and compliance with the measures specified in Regulation of Cabinet of Ministers regarding a set of biosecurity measures for kept animals (MK, 2015). In addition, the operators of establishments of kept porcine animals located in ASF restricted zones shall ensure that reinforced biosecurity measures are implemented in pig farms, to receive the authorization for the movements of pigs within and outside those restricted zones (EC, 2023).

The main biosecurity requirements are as follows: there must be no direct or indirect contact between pigs and wild boar, appropriate hygienic measures such as a change of clothes

and footwear on entering and leaving the premises where pigs are kept, washing and disinfection of hands and disinfection of footwear at the entrance to the premises where porcine animals are kept, the absence of any contact with pigs for a period of at least 48 hours after any hunting of wild boar, a prohibition on unauthorized persons or means of transport entering the farms territory, including the premises, where pigs are kept, record-keeping of persons and means of transport accessing the pigs. Buildings where pigs are kept must be built in such a way that no other animals can enter the premises and buildings or have contact with pigs or their feed and bedding material, allow for the washing and disinfection of hands, have appropriate changing facilities for footwear and clothes at the entrance of the stables and stock-proof fencing of at least the premises where the pigs are kept and buildings where feed and bedding are kept.

The commercial farms have to elaborate biosecurity plan approved by the competent authority that include the establishment of the ‘clean’ and ‘dirty’ areas for personnel appropriate to the farm typology, such as changing rooms, shower, dining room; the setting up of the logistical arrangements for the entry of new animals into the farm; the procedures for the cleaning and disinfection of the facilities, transport, equipment and personnel hygiene, internal audit or self-evaluation for enforcing the biosecurity measures (EC, 2023; MK, 2015).

Despite the existence of established regulatory frameworks, surveillance systems, outbreak investigation requirements and biosecurity measures, ASF outbreaks in commercial pig farms continue to demonstrate challenges related to early detection, reconstruction of infection events and assessment of disease spread within affected holdings. These challenges underline the importance of strengthening epidemiological investigation approaches capable of providing reliable evidence to support outbreak management and control decisions at farm level. Therefore, there is a need to strengthen early detection approaches and to structure epidemiological investigations in affected pig farms to support evidence-based outbreak management.

2. HYPOTHESIS, OBJECTIVE AND TASKS

Hypotheses of the study:

- 1) A hypothesis-based epidemiological investigation approach, including structured information obtained from laypersons, can reliably identify the most probable pathways of African swine fever (ASF) virus introduction into pig herds.
- 2) Targeted sampling strategies based on the definition and categorization of epidemiological subunits, supported by the selection of clinically affected or dead animals and key epidemiological indicators, improve the effectiveness and timeliness of ASF outbreak investigations by enabling the spatial identification and classification of affected and unaffected units within pig farms, while rapid pen-side tests, such as lateral flow devices (LFDs), may serve as supportive tools alongside standard laboratory diagnostic methods (PCR, IPT, and ELISA).
- 3) Key epidemiological parameters can be systematically identified and applied to accurately reconstruct the high-risk period (HRP) of ASF outbreaks in affected pig farms.
- 4) ASF outbreaks in commercial pig farms may remain undetected for prolonged periods due to slow and heterogeneous within-farm spread and low initial farm-level mortality, highlighting the need for targeted surveillance for earlier detection.

The aim of this study was to investigate African swine fever (ASF) control strategies by improving and strengthening approaches for early outbreak detection and epidemiological investigations in affected pig farms through the evaluation of existing surveillance systems and outbreak investigation methodologies.

Tasks of the study:

- 1) To investigate probable pathways of ASF virus introduction into pig herds using a hypothesis-based epidemiological approach and evaluation of information obtained from laypersons (I, IV).
- 2) To evaluate and develop sampling strategies for assessing ASF virus spread within affected pig farms (II, III).
- 3) To assess the applicability of pen-side lateral flow device (LFD) tests as supportive diagnostic tools for ASF outbreak investigations and to compare their performance with standard laboratory diagnostic methods, including PCR, IPT, and ELISA (III).
- 4) To identify and analyze key epidemiological parameters required for the reconstruction of the high-risk period (HRP) of ASF outbreaks in affected pig farms (I, II).
- 5) To assess surveillance approaches applied in Latvia with regard to their effectiveness for the early detection of ASF outbreaks (I, II).

3. MATERIALS AND METHODS

3.1. Study design and study sites

Four interconnected epidemiological studies were carried out in commercial pig farms affected by African swine fever (ASF) in Latvia between 2017 and 2020. The studies focused on ASF virus introduction pathways, early outbreak detection, reconstruction of the high-risk period (HRP), virus spread within the farm, sampling strategies, rapid diagnostic methods, and interpretation of epidemiologically relevant field information.

Study I focused on ASF virus introduction pathways and early outbreak detection in a commercial, farrow-to-finish pig farm with approximately 5000 pigs located in the central part of Latvia.

Study II investigated estimation of the HRP and ASF virus spread within two large commercial farrow-to-finish farms. One farm, located in the south-eastern part of Latvia, housed approximately 6000 pigs, while the second farm, located in the south-western part of Latvia, housed approximately 16,000 pigs.

Study III focused on sampling strategies and their efficiency in ASF-affected farms. The study included three commercial farrow-to-finish farms with approximately 31, 1800, and 9000 pigs, respectively, located in the south-western part of Latvia.

Study IV investigated the significance of disease-related anecdotal information provided by laypersons during ASF outbreak investigations in several European countries, including Latvia.

At the time of outbreak confirmation, all study farms were located in areas under ASF-related restrictions due to virus circulation in wild boar populations (EC, 2014; Oļševskis et al., 2020; EFSA, 2021).

3.2. ASF outbreak confirmation and initiation of investigations

All field investigations were initiated immediately after ASF outbreaks had been officially confirmed by the Food and Veterinary Service of Latvia (FVS). According to national legislation, owners or keepers of pigs are obliged to report suspicion of ASF infection to a veterinary practitioner or to the FVS (MK, 2004).

Following notification, official veterinarians visited the suspected farms and collected blood (EDTA and serum samples in tubes) and tissue (spleen and lymph nodes) samples for laboratory testing in order to confirm or exclude ASF infection. If ASF virus genome or ASF-specific antibodies were detected, the pigs were considered infected, and the outbreak was officially confirmed by the FVS.

Further investigations were performed according to applicable European Union and national legislation regulating ASF outbreak investigations and disease control measures (EC, 2002; EC, 2016).

3.3. Epidemiological outbreak investigations and data collection

As soon as an ASF outbreak was confirmed, epidemiological investigations were initiated. The investigations were based on one or several on-site visits depending on the epidemiological situation in the affected farm.

To collect and cross-check epidemiologically relevant information, interviews were conducted with farm owners, veterinarians, zootechnicians, drivers, and other personnel involved in pig management and farm activities. In addition, farm biosecurity systems and management procedures were assessed.

Records related to animal movements, mortality, treatments, rendering of pigs, in-farm movements of pigs and laboratory test results were reviewed and analyzed.

Additional samples were collected from dead pigs or from live pigs prior to culling for epidemiological investigations and laboratory testing.

3.3.1. Hypothesis-based investigation of ASF virus introduction pathways

To facilitate and structure investigations of possible ASF virus introduction routes, a hypothesis-based epidemiological approach was developed and applied during Study I. Based on known ASF transmission pathways, three working hypotheses for possible virus introduction were formulated - the wild boar hypothesis; the meat/fomite hypothesis; the trade hypothesis.

Each hypothesis was investigated separately by evaluating epidemiological, laboratory, management, and biosecurity information to exclude unlikely transmission routes or identify the most plausible pathway of virus introduction. Assessment included evaluation of farm infrastructure, fences, vehicles, equipment hygiene procedures, internal workflow, movements of personnel, feed and food handling practices and movements of pigs and materials.

3.3.2. Reconstruction of the high-risk period (HRP)

Reconstruction of the high-risk period (HRP) was performed to estimate the probable period during which ASF virus had been circulating within the farm before outbreak detection. To estimate the HRP, timelines of epidemiologically important events were developed using morbidity and mortality data, post-mortem findings, laboratory test results, clinical observations, animal movement records, information on manure removal, records of in-farm pig movements and biosecurity-related observations.

Clinical findings together with genome and antibody detection results (ELISA and IPT) obtained from live and dead pigs originating from different pens and stables were collected, analyzed, and interpreted to reconstruct the probable spread of the virus within the farms. Particular attention was paid to possible epidemiologically relevant events involving staff hygiene, on-farm food consumption, transport activities, and internal farm management procedures.

3.3.3. Assessment of within-farm virus spread

Assessment of ASF virus spread within affected farms focused on identification of infected epidemiological units and evaluation of transmission patterns within the farm. The following clinical and pathological signs indicating possible ASF infection were evaluated and included: increased body temperature and fever, loss of appetite, abortions, diarrhea, vomiting with blood, hemorrhages in the skin and internal organs, enlarged or hemorrhagic lymph nodes, splenic and renal lesions.

Internal spread of infection between stables, pens, and production units was evaluated using clinical observations, mortality patterns, laboratory findings, and data on internal pig movements. The investigations also evaluated whether virus spread occurred uniformly throughout the farm or remained limited to specific epidemiological units.

3.3.4. Collection and interpretation of layperson information

Study IV evaluated epidemiologically relevant information provided by laypersons during ASF outbreak investigations. Personal views and statements from affected farmers, farm workers, veterinarians, and other individuals involved in farm activities were collected regarding possible ASF virus introduction routes and the probable timing of virus introduction into pig herds. The statements represented subjective opinions of the respondents and were categorized according to their epidemiological plausibility - plausible and requiring further investigation, implausible and rather implausible but not fully excludable.

The statements were interpreted together with epidemiological, laboratory, clinical, and biosecurity investigation findings.

3.4. Sampling strategy

3.4.1. Targeted sampling approach

Sampling for laboratory investigations was performed in each ASF outbreak farm included in the studies. Blood (EDTA and serum samples in tubes) and tissue samples (spleen and lymph nodes) were collected from pigs located in different stables and epidemiological units to assess ASF virus spread within the farm and reconstruct possible transmission pathways. Targeted purposive sampling was applied. Samples were primarily collected from clinically affected pigs, dead pigs and pigs with direct epidemiological contact with infected or suspected animals. Targeted sampling focused on selection of suspicious, sick, and dead pigs for laboratory testing.



Figure 3.1 Examples of clinically affected pigs identified during targeted outbreak investigations in ASF-affected farms: clinically sick sows showing haemorrhages in ears and loss of appetite (photo: K. Lamberg)



Figure 3.2 Examples of clinically affected pigs identified during targeted outbreak investigations in ASF-affected farms: clinically sick sows and abortion and enlarged spleen of ASF infected pig (photo: K. Lamberg)

3.4.2. Categorization of epidemiological units

A structured three-step sampling strategy was developed and applied during Study III, including identification of epidemiological subunits, categorization of subunits according to likelihood of infection and targeted selection of animals for testing.

For each epidemiological subunit, a category was assigned depending on the probability of ASF virus circulation based on clinical findings, mortality patterns, epidemiological links and previous laboratory findings.

Clinical inspection of pigs in all epidemiological subunits was performed before targeted selection of animals for sampling. All pigs showing clinical signs and pigs having direct contact with clinically affected animals were sampled.

3.4.3. Clinical scoring system

A clinical scoring system was developed and applied during Study III for evaluation of clinical findings and interpretation of rapid pen-side test results. Sampled pigs were classified according to severity of clinical signs into the following categories: clinically inconspicuous, mild clinical signs, severe clinical signs and dead pigs.

Laboratory and rapid diagnostic test results were analyzed and compared with the clinical scores.

3.5. Sample preparation and PCR analysis

All EDTA blood and tissue samples were stored, prepared, and processed according to the standard operating procedures of the NRL in a laminar flow cabinet.

Organ samples intended for nucleic acid detection were mechanically homogenized following dilution in phosphate-buffered saline (PBS) at a ratio of 1:10.

Viral DNA extraction was performed using the NucliSENS® easyMAG® automated nucleic acid isolation system (bioMérieux, France) according to the manufacturer's instructions. A heterologous internal control DNA (intype IC-DNA; QIAGEN, Germany) was used to assess extraction quality as previously described by Hoffmann et al. (2006).

Polymerase chain reaction (PCR) for ASFV genome detection was performed according to the protocol of the European Union Reference Laboratory for ASF (Fernández-Pinero et al., 2013).

Results were recorded as quantification cycle (Ct) values and interpreted as positive or negative according to the obtained Ct values.

3.6. Serological investigations (ELISA and IPT)

Serological investigations were performed to detect ASF virus specific antibodies. The commercially available Ingezim PPA Compac blocking ELISA kit (11.PPA.K.3, Ingenasa, Madrid, Spain) was used for testing serum samples according to the manufacturer's instructions. In addition, immunoperoxidase testing (IPT) was performed on serum samples or organ fluid samples according to the protocol of the European Union Reference Laboratory for ASF (European Union Reference Laboratory for ASF, 2018). IPT was used to detect low antibody titres that could not be identified by ELISA.

Combined interpretation of virological and serological test results was applied during epidemiological investigations. Detection of ASF virus genome by qPCR together with positive IPT and negative ELISA results in dead pigs was interpreted as indicating recent infection, likely within approximately 10 days prior to sampling.

3.7. Rapid pen-side lateral flow device (LFD) testing

Rapid pen-side lateral flow device (LFD) tests were evaluated under field outbreak conditions during Study III. Two types of LFD tests, including an antigen LFD for detection of ASFV antigen in whole blood samples and an antibody LFD for detection of ASFV-specific antibodies were used.

For antigen detection, the INgezim PPA CROM Ag LFD (11.ASF.K.42, Ingenasa, Madrid, Spain) was used. For antibody detection, the corresponding INgezim PPA CROM Ab LFD (11.PPA.K.41, Ingenasa, Madrid, Spain) was used. Both tests were performed according to the manufacturer's instructions. Results obtained under field conditions were compared with standard laboratory methods including qPCR, ELISA, and IPT.



Figure 3.3 Performing LFD testing in the field during ASF outbreak investigation (photo: M. Seržants)

3.8. Statistical analysis

Diagnostic sensitivity and specificity of antigen LFD tests were calculated separately for each farm included in Study III using qPCR results as the reference standard.

Sensitivity was calculated as true positive / (true positive + false negative) and specificity was calculated as true negative / (true negative + false positive).

Confidence intervals for sensitivity and specificity estimates were calculated using Epitools Epidemiological Calculators (Sergeant, 2018).

Spearman correlation analysis was performed using XLSTAT statistical software for Microsoft Excel (Addinsoft, 2019) to assess correlations between clinical scores and laboratory test results.

Combined interpretation of clinical, epidemiological, virological, and serological findings was applied throughout the investigations to reconstruct outbreak dynamics and support epidemiological decision-making.

4. RESULTS

4.1. Investigation of ASF virus introduction pathways and reconstruction of the high-risk period (HRP)

ASF was confirmed in the study farm in mid-January 2017. The animals were kept in seven separate stable buildings. The stable where the first clinically affected pigs were identified housed group-kept pregnant sows. Retrospective evaluation of post-mortem records revealed that the first dead sow could be traced back to 28 December 2016. Another four pigs became clinically ill on 4 January and died two days later. Additional dead sows were identified on 6, 9, 11, 12, and 13 January. In the meantime, several sows had been moved on 9 January to another stable for farrowing, and two of these animals were subsequently found dead on 12 and 13 January, suggesting possible virus introduction into the farrowing unit through movement of infected animals. The first dead boar in a separate breeding unit was identified on 7 January, followed by another on 14 January. The first laboratory testing for ASF virus was performed on pigs that died on 13 January. At the time of outbreak confirmation, 17 sows had already died. A worker responsible for insemination duties was identified as having daily contact with all three affected stables.

To support outbreak reconstruction and estimation of the HRP, a timeline integrating morbidity, mortality, post-mortem findings, pig movement records, and laboratory testing results was developed. Of the 75 sampled animals, ASF virus genome was detected in 23 pigs, while none of the tested animals were positive for ASF antibodies. The absence of antibodies indicated an acute course of infection. Based on the collected epidemiological and laboratory information, the estimated time of virus introduction into the farm was approximately six weeks before outbreak confirmation.

The observed virus spread within the farm was slow during the early phase of infection. Following outbreak confirmation, an additional 50 samples were collected from pigs in the affected sow unit, and only four animals tested positive for ASF virus genome detection.

Assessment of farm biosecurity showed that the farm infrastructure was generally well developed. The holding was fenced, vehicles entering the territory passed through sanitary control points, and dedicated farm clothing and footwear were available for staff. However, no sanitary filters were present at the entrances to pig stables. Epidemiological investigations and interviews revealed several weaknesses in implementation of biosecurity procedures. The layout of changing and resting rooms did not ensure effective separation between clean and dirty areas. Farm workers were allowed to bring food onto the premises and consume it within the production area. In addition, documentation regarding truck disinfection procedures during the winter period was incomplete.

A hypothesis-based epidemiological investigation approach was applied to evaluate possible ASF virus introduction pathways. Three possible introduction hypotheses were assessed: the wild boar hypothesis, the meat/fomite hypothesis, and the trade hypothesis.

The wild boar hypothesis was considered unlikely because the pigs were housed indoors, the farm was fenced, and no direct or indirect contact with wild boar could be identified. No bedding material or feed with possible wild boar contact had been used, and none of the farm personnel participated in hunting activities.

The meat/fomite hypothesis was considered the most likely route of virus introduction. Epidemiological investigations identified insufficient staff hygiene barriers and the possibility that contaminated meat products may have been brought onto the farm and subsequently introduced into pig areas. Furthermore, substantial staff turnover during the previous months raised concerns regarding awareness of ASF transmission risks and biosecurity compliance.

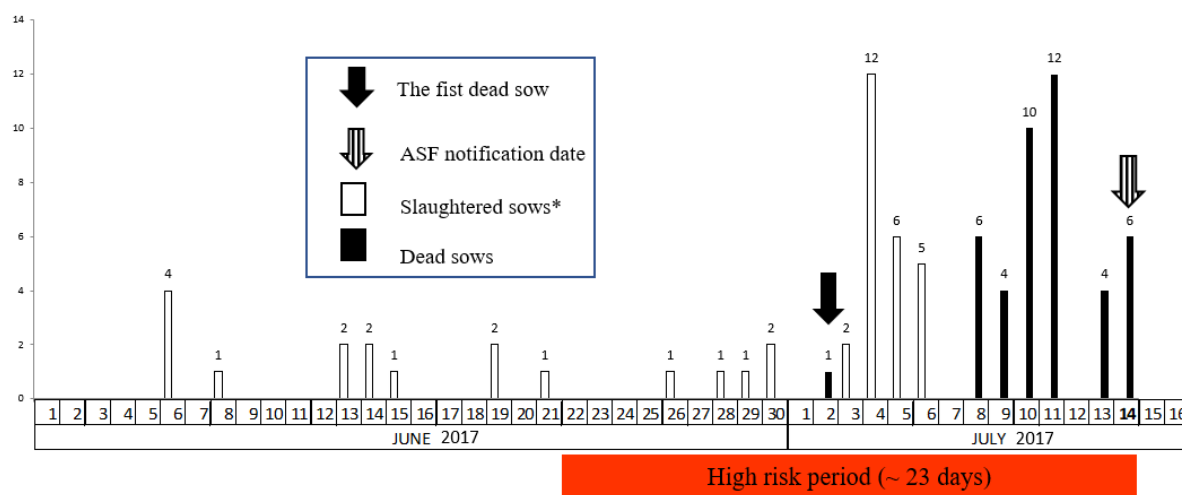
The trade hypothesis was excluded because no pigs had been introduced into the farm during the previous 12 months. However, piglets had been moved from the affected breeding

farm to a fattening farm belonging to the same owner during the estimated HRP. Subsequently, ASF was confirmed in the receiving farm and classified as a secondary outbreak.

During epidemiological investigations conducted in Latvia and several other ASF-affected European countries, information and opinions provided by farmers, farm workers, veterinarians, and other persons involved in farm activities were collected and evaluated in the context of possible ASF virus introduction routes. In total, 31 statements regarding possible virus introduction pathways were analyzed for their epidemiological relevance. Many statements were considered epidemiologically important and required further investigation, whereas others could be excluded immediately as implausible. A substantial proportion of the statements (n=13) were classified as rather implausible but not fully excludable. The investigations demonstrated that interpretation of such subjective information requires epidemiological expertise, outbreak investigation experience, and structured communication approaches.

To further investigate outbreak dynamics, additional epidemiological investigations were performed in two other ASF-affected commercial pig farms (Farms A and B), focusing on reconstruction of the high-risk period (HRP) and assessment of virus spread within affected holdings.

In Farm A, ASF was confirmed on 14 July 2017. The farm consisted of seven indoor stables. Severe clinical signs, including fever, abortions, and vomiting, were observed only in one stable housing group-kept pregnant sows. According to anamnesis data, the first dead sow had already been identified on 2 July following abortion. Between 2 July and outbreak confirmation, 43 sows died. An additional 25 sows were sent to slaughter due to health problems. The disease also spread to the farrowing stable after infected sows had been moved from the affected unit. Dead sows were subsequently identified in the farrowing unit on 7, 8, and 9 July. The reconstructed timeline of epidemiologically important events in Farm A and the estimated HRP are presented in Figure 4.1.



* Periodic slaughter of sows was performed within the farm in June, while sudden increase in numbers of slaughtered sows was observed from the 4th of July, but it neither can be proven or excluded that ASF was the cause for slaughtering.

Figure 4.1 Timeline of the disease event in Farm A, stable 2 and the estimated high-risk period

In Farm A, the sow that died on 2 July was considered the index case. Assuming an average survival period of approximately 10 days after infection, the estimated virus introduction date was around 22 June, resulting in an estimated HRP of approximately 23 days. More than two infection cycles were assumed to have occurred before outbreak detection. The probable spread of ASF virus between the stables in Farm A is illustrated in Figure 4.2.

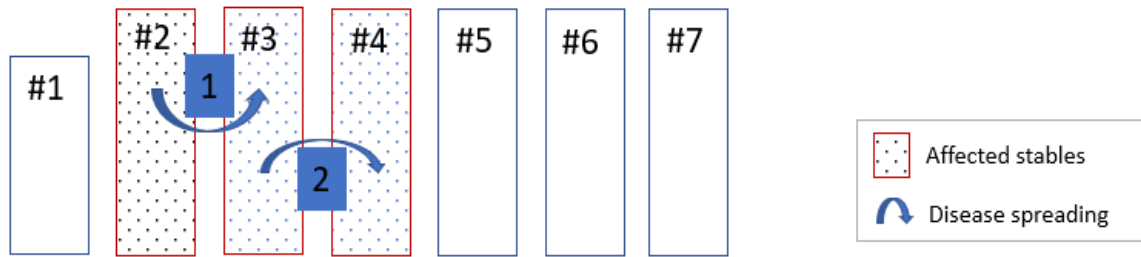


Figure 4.2 **Potential spread of ASF virus between the stables in a Farm A**

In Farm B, ASF was confirmed on 31 July 2018. The farm consisted of two geographically separate production locations. Enhanced passive surveillance was already implemented at the time of outbreak occurrence. Three dead weaners identified on 29 July were tested for ASF virus and found PCR positive. These pigs had previously been moved together with 277 other weaners from location B1 to location B2 on 23 July. After movement, mortality increased in the recipient group in location B2, whereas no increased mortality was observed in the remaining pigs in the source unit. A slight increase in mortality was observed in another stable of location B1 from mid-July onwards. Mortality data from stable 4 of location B1, which were used as part of the HRP reconstruction process, are presented in Figure 4.3.

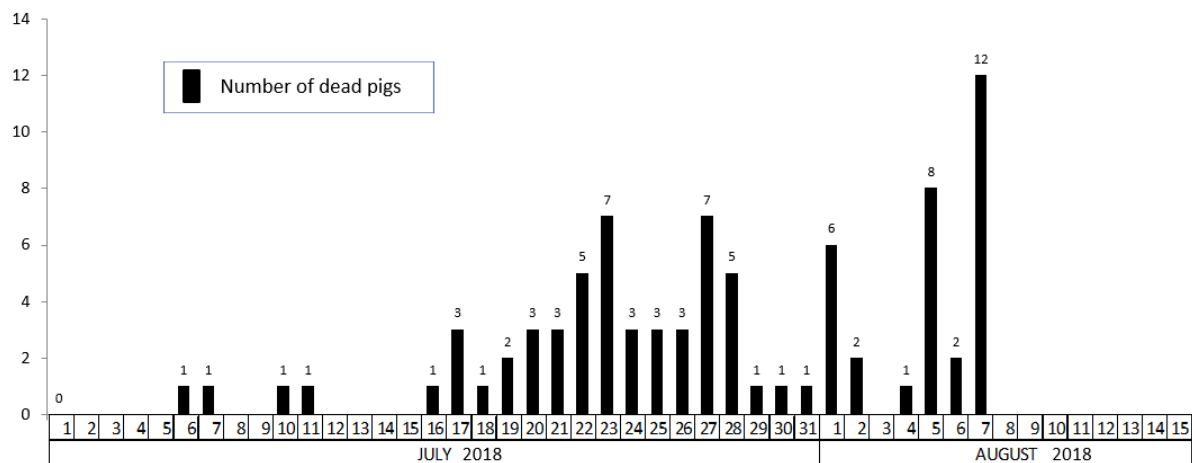


Figure 4.3 **Mortality data in stable 4 of Location B1**

The reconstructed timeline of epidemiologically important events in location B2 is presented in Figure 4.4.

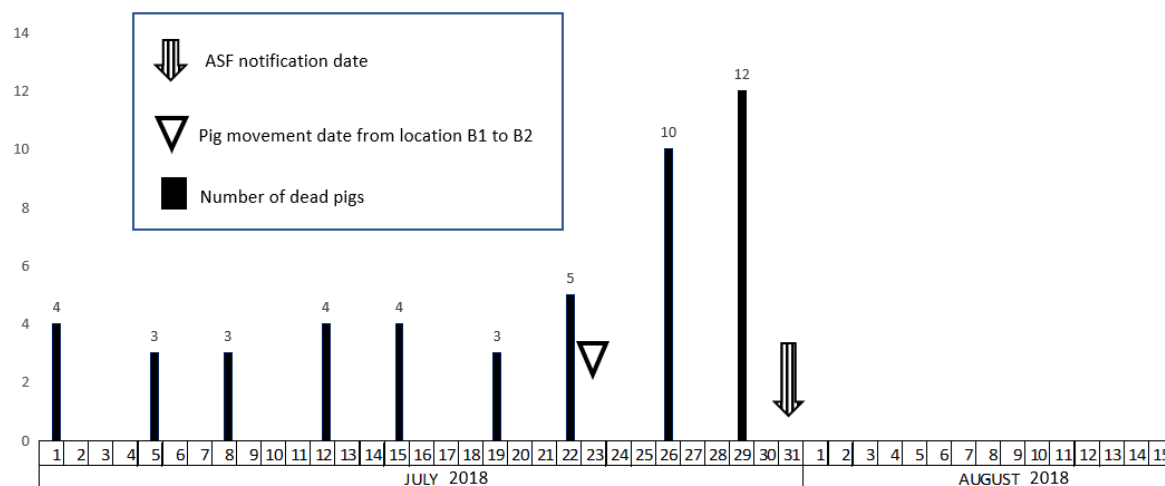


Figure 4.4 Timeline of the disease event in the stable where the first ASF positive pigs were detected (location B2)

In Farm B, the pigs found dead on 29 July were considered index cases. Based on combined virological and serological results, the estimated HRP was approximately two weeks. Because the pigs were PCR positive and antibody positive, infection was estimated to have occurred 7–10 days before death. Therefore, the estimated HRP was between 18 and 31 July. The probable spread of ASF virus between stables and locations in Farm B is illustrated in Figure 4.5.

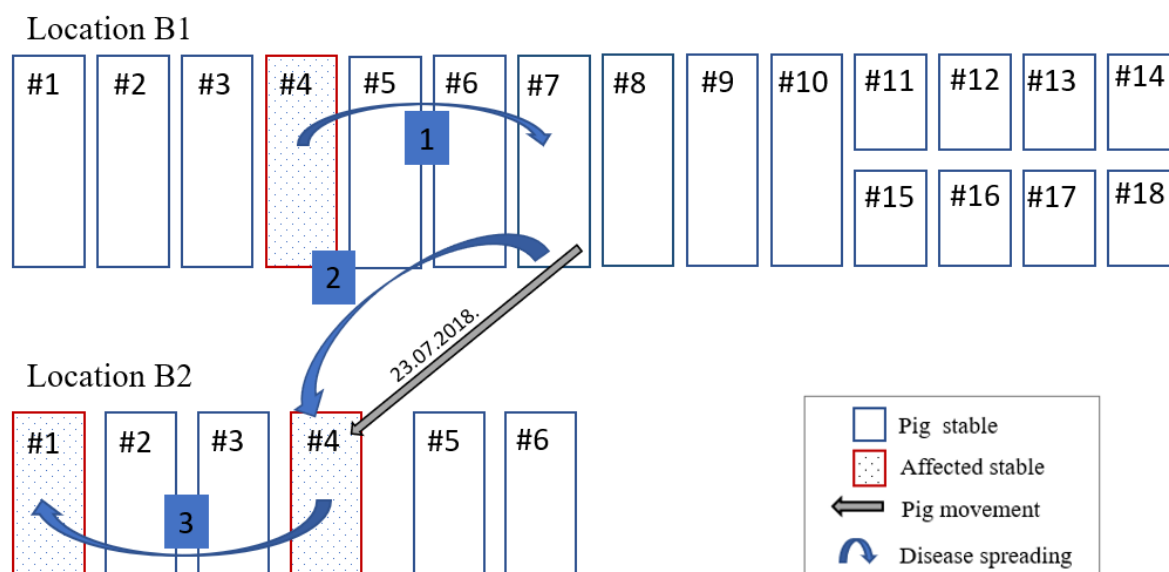


Figure 4.5 Potential spreading of ASF virus between the stables in a Farm B

Movement records and laboratory findings suggested that infection initially developed in stable 4 of location B1 and subsequently spread to location B2 through movement of infected weaners. Further spread between stables was considered most likely to have occurred through contaminated fomites.

4.2. Assessment of ASF virus spread within pig farms and sampling strategies

Assessment of ASF virus spread within affected pig farms demonstrated heterogeneous distribution of infection between epidemiological units. Although epidemiological links

between production units existed, several units remained free from detectable infection even during prolonged high-risk periods.

Following outbreak confirmation in Farms A and B, additional epidemiological investigations and targeted sampling were performed to evaluate the extent of ASF virus spread within the affected holdings. Samples were collected from pigs in different stables and epidemiological units before culling. The obtained laboratory results enabled identification of affected and non-affected units within the farms.

In Farm A, samples were collected from pigs in all seven stables before depopulation. Three out of seven stables were identified as epidemiologically affected. ASFV genome was detected mainly in stables housing sows, whereas most fattening units remained negative. The distribution of virological and serological results between the different stables of Farm A is presented in Table 4.1.

Table 4.1

RT PCR and serology results of pigs from Farm A sampled in different stables before culling

Stable Id	Number of pigs in the stable	Category of pigs	Number of samples taken in a stable	Laboratory testing results	
				RT PCR positive	Serology (ELISA, IPT) positive
1	213	Boars, sows	3	0	0
2	301	Sows *	4	4	2
3	696	Sows **	28	10	0
4	1761	Weaners	28	1	0
5	1157	Fatteners	29	0	0
6	1136	Fatteners	28	0	0
7	554	Fatteners	20	0	0
Total	5818	N/A	140	15	2

* Sows kept in group pens. ** Sows kept with piglets.

The investigations further demonstrated that spread of ASF virus within the farms was not uniform. In Farm A, epidemiological investigations suggested that infection initially developed in stable 2 and subsequently spread to the farrowing unit following movement of infected sows. Similarly, in Farm B, movement records and laboratory findings suggested that infection initially developed in stable 4 of location B1 and subsequently spread to location B2 through movement of infected weaners. Further spread between stables was considered most likely to have occurred through contaminated fomites. Despite existing epidemiological links between production units, several stables remained free from infection. The probable spread of ASF virus between production units in Farms A and B is illustrated in Figures 4.2 and 4.5, respectively.

To further evaluate within-farm transmission patterns and improve assessment of infection distribution, a targeted sampling strategy based on subdivision of farms into epidemiological subunits was applied in Study III. All study farms were divided into epidemiological subunits, which were subsequently categorized according to the probability of ASF infection. Clinical inspection of pigs in all subunits was performed prior to sampling. The categorization of epidemiological subunits and corresponding laboratory results are summarized in Table 4.2.

Table 4.2

Overview of testing results by the categories of farm sub-units

Category of farm subunit	Number of tested animals	Ag-LFD+	qPCR+ Ct<18	qPCR+ Ct>18	Ab-LFD+	ELISA+	IPT+
FARM A							
1	21	3	2	3	2	2	3
1	6	0	0	0	0	0	0
FARM B, unit B1							
1	4	3	3	0	0	0	0
2	11	1	1	4	0	0	1
3	5	1	1	0	0	0	1
FARM B, unit B2							
3	15	1	2	4	0	0	4
FARM C							
1	11	7	7	4	0	0	6
2	4	0	0	0	0	nd	nd
3	1	0	0	0	0	nd	nd
4	18	1	1	1	0	0	0

Application of the targeted sampling strategy enabled successful identification of infected epidemiological units within the affected farms. In Farm A, infected pigs were identified in one epidemiological unit, while another epidemiologically linked unit remained ASF negative. In Farm B, both farm units were affected. In Farm C, ASF-infected pigs were identified in three out of nine epidemiological subunits.

The investigations further demonstrated that purposive sampling of clinically affected and dead pigs enabled effective identification of epidemiologically affected units. Even in farms with prolonged HRP, several pens remained free from detectable ASF infection.

To evaluate rapid diagnostic testing under field outbreak conditions, 87 animals were additionally tested using antigen lateral flow devices (Ag-LFD) and compared with laboratory qPCR results. Of these animals, 17 were positive in Ag-LFD testing, whereas 34 were positive by qPCR. All qPCR-negative samples were also negative in Ag-LFD testing, resulting in a relative Ag-LFD specificity of 100%. Relative sensitivity compared with qPCR was 50% (CI 34.66%). Due to the low number of antibody-positive samples, diagnostic sensitivity and specificity of antibody LFD tests could not be calculated.

Comparison of qPCR Ct values and Ag-LFD-positive samples is presented in Figure 4.6. Ct values of Ag-LFD-positive samples ranged from Ct 14 to Ct 18, with a median Ct value of 17.

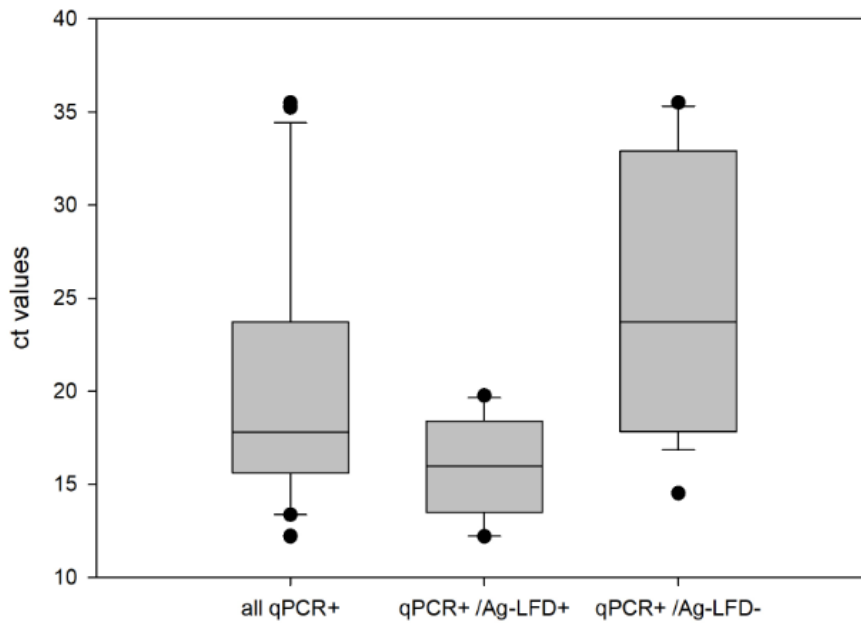


Figure 4.6 Comparison of Ct-values for all tested blood samples, samples that were qPCR and Ag-LFD positive and samples that were qPCR positive but Ag-LFD negative

Evaluation of clinical findings demonstrated that neither Ag-LFD nor qPCR produced positive results in clinically inconspicuous pigs. Positive laboratory and Ag-LFD results were detected only in pigs showing clinical signs. Distribution of ASF-positive pigs according to clinical score categories is presented in Figure 4.7.

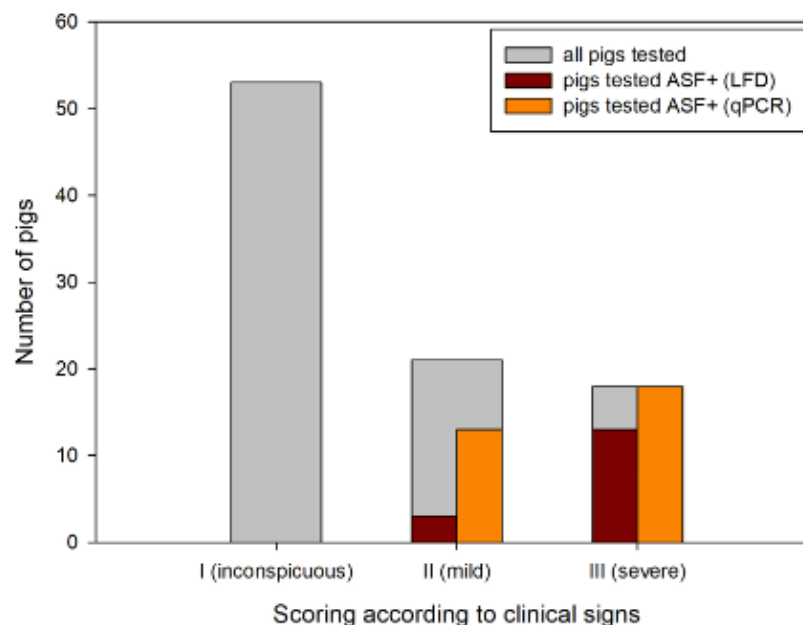


Figure 4.7 Pigs tested positive for ASF (qPCR, Ag-LFD) depending on clinical score

Correlation analysis demonstrated statistically significant associations between Ag-LFD results and clinical score, qPCR results and clinical score, as well as between antibody LFD and ELISA results. Results of the Spearman correlation analysis are presented in Table 4.3.

Table 4.3

Spearman correlation matrix for different ASF test results and ASF clinical score

Variables	Ag-LFD	Ab-LFD	qPCR	ELISA	IPT	Clinical score
Ag-LFD	1	0.008	0.246	0.008	0.271	0.531
Ab-LFD	x	1	0.065	1.000	0.296	0.227
qPCR	x	x	1	0.065	0.218	0.469
ELISA	x	x	x	1	0.296	0.227
IPT	x	x	x	x	1	0.201
Clinical score	x	x	x	x	x	1

Values in bold are different from 0 with a significance level $p < 0.05$

4.3. Early detection of ASF outbreaks in pig farms

The investigations demonstrated substantial differences in outbreak detection dynamics between the investigated farms and showed that ASF virus circulation in large commercial pig farms may remain undetected for prolonged periods during the early phase of infection. This was demonstrated in three different outbreak investigations included in the studies.

The January 2017 outbreak investigation demonstrated that ASF virus circulation within the affected farm remained undetected for several weeks before official confirmation. Epidemiological reconstruction indicated that the virus had been circulating for approximately six weeks prior to outbreak detection.

A similar retrospective analysis was performed in Farm A, where ASF was officially confirmed on 14 July 2017. Retrospective epidemiological investigations demonstrated that clinical signs and increased mortality had already been present before outbreak confirmation. According to anamnesis and mortality records, the first dead sow was identified on 2 July following abortion. During the following days, additional dead animals and abortions were recorded, and infected sows had already been moved between production units. The estimated HRP in Farm A was approximately 23 days.

The investigations demonstrated that mortality initially increased slowly and remained limited to a relatively small number of animals during the early phase of infection. This complicated timely outbreak recognition and allowed prolonged virus circulation within the farm before official confirmation.

In Farm B, ASF was confirmed on 31 July 2018. Three dead weaners identified on 29 July were tested for ASF virus and found PCR positive. These pigs had previously been moved together with 277 other weaners from location B1 to location B2 on 23 July. At the time of outbreak detection, widespread clinical signs and a marked increase in mortality had not yet been observed. ASF was identified through the implemented enhanced passive surveillance system, whereby dead pigs were tested weekly in the laboratory.

The reconstructed timeline of epidemiologically important events in location B2 is presented in Figure 4.3, while mortality data recorded in stable 4 of location B1 are presented in Figure 4.4.

The investigations demonstrated that the early stages of ASF outbreaks in large commercial pig farms may be characterized by slow virus spread and low mortality, resulting in delayed outbreak recognition.

Comparison of the investigated outbreaks demonstrated that systematic laboratory testing of dead pigs (enhanced passive surveillance) enabled earlier detection of ASF outbreaks before widespread clinical signs or substantial increases in mortality became evident.

4.4. Integrated epidemiological approach to ASF outbreak investigations

The investigations included assessment of possible ASF virus introduction pathways, reconstruction of the high-risk period (HRP), evaluation of within-farm virus spread, targeted sampling, laboratory investigations, and collection of epidemiologically relevant field information.

Hypothesis-based epidemiological investigations were applied to assess possible ASF virus introduction routes and evaluate their plausibility. Information and opinions provided by laypersons during outbreak investigations were collected and analyzed in the context of their epidemiological relevance.

Reconstruction of the HRP was performed using mortality records, clinical observations, laboratory results, animal movement data, and biosecurity information.

Assessment of within-farm virus spread, and application of targeted sampling strategies enabled identification of affected and non-affected epidemiological units within outbreak farms. Rapid pen-side diagnostic tests (LFDs) were evaluated under field outbreak conditions and compared with standard laboratory diagnostic methods.

Comparison of the investigated outbreaks showed differences in outbreak detection timing between farms and demonstrated prolonged undetected ASF virus circulation during the early phase of infection in some outbreaks.

5. DISCUSSION

5.1. Investigation of ASF virus introduction pathways and reconstruction of the high-risk period (HRP)

Our investigations demonstrated that human-mediated pathways and biosecurity failures were the most probable mechanisms of ASF virus introduction into affected pig farms. Furthermore, reconstruction of the high-risk period (HRP) proved essential for understanding outbreak dynamics and identifying secondary transmission risks.

The hypothesis of virus introduction via fomites and contaminated meat appeared to be the most likely route of infection, as infectious material could have entered the farm through staff or visitors. These findings emphasize the importance of maintaining awareness of disease transmission risks and implementation of biosecurity measures among different target groups involved in pig production. Human-related activities therefore require continuous attention within ASF prevention strategies. Our investigations confirmed that inadequate biosecurity remains one of the major risk factors for ASF introduction into pig farms and demonstrated that a structured, hypothesis-based epidemiological approach provides a useful methodological framework for outbreak investigations.

Good on-farm biosecurity is considered the most effective means of preventing ASF virus introduction into domestic pig holdings (Bellini et al., 2016). Following the first ASF cases detected in wild boar and domestic pigs in Latvia in 2014, national biosecurity requirements for pig holdings were gradually strengthened. However, field experience demonstrated that implementation of biosecurity measures was particularly challenging for many pig farmers, especially in smaller farms and backyard holdings, where biosecurity previously had not been systematically applied. In addition to financial investments in infrastructure, equipment, and farm fencing (“hardware”), effective biosecurity also requires implementation of appropriate management procedures (“software”), including hygiene measures, internal workflow organization, staff awareness, and compliance with daily routines. Our observations indicated that the “software” component represented the greatest long-term challenge, as maintaining awareness and changing habitual behavior requires continuous education, monitoring, and commitment.

When ASF outbreaks occur, epidemiological investigations must identify the most probable route of virus introduction into the holding. Such investigations require assessment of the entire farm system, including buildings, vehicles, equipment, fencing, animal movements, personnel movements, and management procedures linked to biosecurity implementation. In addition to biosecurity assessment, epidemiological investigations frequently rely on indirect epidemiological information, including observations and opinions provided by persons involved in farm activities.

Various pieces of information provided by laypersons during outbreak investigations were examined in greater detail. Farmers, farm workers, zootechnicians, and veterinary practitioners are commonly interviewed to obtain their opinions regarding possible virus introduction routes and the probable timing of introduction. Although these individuals may not possess detailed epidemiological knowledge of ASF, their observations and suspicions may provide useful contextual information. Numerous statements collected during outbreak investigations were therefore evaluated according to their potential epidemiological relevance and the need for further investigation.

However, interpretation of such subjective information is often difficult and requires careful evaluation in the context of clinical findings, biosecurity assessments, laboratory test results, and other epidemiological data. Some statements may appear highly speculative or initially seem to have little value, while others may represent important epidemiological clues. In some situations, anecdotal or misleading information may also be encountered (Trotta et al., 2022). Furthermore, farm representatives may be reluctant to discuss possible failures in

biosecurity implementation due to concerns regarding legal responsibility for the outbreak. These findings highlight the importance of trained investigators with expertise in ASF epidemiology, communication skills, and knowledge of legal requirements. Structured questionnaires and systematic interview approaches may improve the quality and consistency of information obtained during epidemiological investigations. Most statements collected during investigations were related to human activities, particularly in smaller farms, further emphasizing the central role of human-mediated pathways in ASF spread (Zani et al., 2019; Ardelean et al., 2021).

While identification of virus introduction pathways represents a critical component of outbreak investigations, understanding how long the virus has been circulating within the farm is equally important for evaluating the extent of exposure and the potential for secondary transmission. Therefore, reconstruction of the HRP becomes an essential analytical component of epidemiological investigations.

Our investigations demonstrated that ASF outbreaks in large commercial pig farms may remain undetected for prolonged periods, often involving more than one infection cycle before disease suspicion and laboratory confirmation. These findings highlighted the importance of distinguishing the incubation period from the HRP, as these concepts are frequently confused even among veterinary professionals. In the case of ASF, particularly in large commercial farms, several transmission cycles may occur before clinical suspicion arises.

The investigations showed that only a few animals died during the initial phase of the outbreaks and that mortality increased gradually over the following weeks. This observation suggests that ASF transmission within large pig farms initially occurs relatively slowly. At the beginning of infection, only a limited number of animals become infected, and time is required before sufficient environmental virus contamination develops to support wider transmission within the holding. Efficient transmission occurs when infected pigs begin to bleed, abort, or die, thereby exposing susceptible pigs to blood and other infectious excretions (Pietschmann et al., 2015). Experimental studies with genotype II ASF virus have demonstrated that one infection cycle, from infection to death, lasts approximately 10 days (Guinat et al., 2016; Pietschmann et al., 2015; Gabriel et al., 2011; Blome et al., 2013).

Field observations in Latvia and experimental studies further demonstrated that ASF contagiousness is lower than commonly assumed (Pietschmann et al., 2015). In 2014, 32 ASF outbreaks in Latvia were investigated. In 12 farms, all pigs were still alive when official veterinarians arrived, whereas in 20 farms one or more pigs had already died. Nevertheless, laboratory testing demonstrated that clinically healthy pigs housed in the same pen or barn had frequently not yet become infected with ASF virus (Oļševskis et al., 2016). These findings demonstrate that substantial delays may occur between infections of individual animals and that transmission to other pigs often occurs only after infected animals have died.

The methodology developed for HRP reconstruction, and the associated analytical approach are presented in Studies I and II. Timelines were established to reconstruct the probable course of infection within the farms. Various epidemiologically relevant data were collected and analyzed, including anamnestic information, morbidity and mortality records, post-mortem findings, laboratory results, internal pig movements, manure removal activities, and biosecurity-related observations. Particular attention was paid to staff-related risk factors, including hygiene practices, food consumption on farms, awareness of disease transmission risks, and transport vehicle movements. Relevant vulnerabilities identified during investigations were integrated into the analytical timelines.

The approach proved useful for systematic HRP estimation and provided important operational value for outbreak management. Reconstruction of the HRP enabled the competent authority to trace potential secondary outbreaks, monitor the health status of pigs moved to other farms or slaughterhouses during the HRP, and identify potentially contaminated meat products requiring recall. However, reconstruction of the HRP alone does not fully explain

outbreak dynamics. To support operational decision-making, the spatial and structural distribution of infection within affected farms must also be assessed.

5.2. Assessment of ASF virus spread within pig farms and sampling strategies

Our investigations demonstrated that ASF spread within large commercial pig farms was frequently heterogeneous, with some epidemiological units remaining unaffected even during prolonged high-risk periods. This finding highlighted the need for targeted sampling strategies to accurately assess within-farm virus distribution.

Assessment of virus spread within affected farms is essential not only for understanding outbreak dynamics but also for supporting implementation of appropriate disease control measures. Although epidemiological links between different farm units usually exist due to pig production practices and shared management procedures, not all epidemiological units necessarily become infected. Our investigations demonstrated that even during prolonged HRPs, some pens or farm sections remained unaffected. These findings indicate that ASF spread within large commercial farms is not uniform and follows identifiable transmission patterns.

A detailed understanding of within-farm infection distribution enables prioritization and coordination of disease control measures, including targeted culling strategies focused initially on the most affected units. Furthermore, detailed epidemiological assessment of infection distribution may support adaptation of disease control measures to specific outbreak situations, particularly in scenarios where regulatory frameworks allow derogations from total depopulation (EC, 2019).

The housing system and animal management practices may also influence within-farm transmission dynamics. When pigs are housed in larger groups, virus spread is generally faster than in systems where animals are housed individually or in smaller groups. Therefore, assessment of epidemiological links, internal animal movements, housing structures, and management practices becomes important for understanding transmission pathways within affected farms.

Once epidemiological units and potential transmission pathways have been identified, these analytical findings must be verified through an appropriate diagnostic sampling strategy. Such a strategy should be aligned both with reconstructed transmission patterns and with the epidemiological characteristics of ASF infection.

Prior to development of the targeted approach described in Study III, legislation required collection of random blood samples during culling for serological and virological testing. The minimum sample size was intended to detect a seroprevalence of 10% within each epidemiological subunit with a confidence level of 95% (EC, 2003). However, considering that highly virulent ASF virus strains were circulating in Europe, such random surveillance approaches became less appropriate for assessing active virus circulation (EFSA, 2021). Since virulent ASF infections result in death in the majority of infected pigs, surveillance strategies should primarily focus on virus detection in clinically sick and dead animals (Blome et al., 2020; Busch et al., 2021). Nevertheless, serological testing may still provide valuable epidemiological information regarding the duration of virus circulation within the farm.

Study III had two objectives: firstly, to develop a targeted and efficient sampling strategy for ASF-affected farms, and secondly, to evaluate the suitability of lateral flow devices (LFDs) under field conditions in comparison with standard laboratory methods, including PCR, ELISA, and IPT. Until then, LFDs had mainly been evaluated under experimental rather than field conditions in ASF-affected farms.

The underlying hypothesis was that ASF-infected animals would most likely be detected among clinically sick animals and that LFDs could support rapid identification of such animals under field conditions. Our results demonstrated that due to lower sensitivity compared with standard laboratory methods, LFDs cannot replace laboratory confirmation and are not suitable

for demonstrating disease freedom. However, they proved useful as complementary field tools during outbreak investigations in already confirmed ASF outbreaks, particularly when examining clinically suspect and dead pigs.

The principal objective of Study III, however, was to establish a reliable sampling strategy capable of providing an overview of virus distribution within affected farms. We developed a practical approach for subdividing farms into epidemiological units and categorizing them according to the likelihood of ASF virus circulation. Targeted sampling focused on clinically sick and dead animals, as well as pigs in close contact with infected animals. Our findings demonstrated that purposive sampling of clinically affected and dead pigs is highly effective for identifying infected epidemiological units and assessing active virus circulation within affected farms.

The proposed approach therefore provides a structured epidemiological tool for assessment of ASF spread within commercial pig farms and supports more evidence-based outbreak management. However, although targeted sampling improves outbreak investigations after disease confirmation, prevention of prolonged undetected virus circulation requires equally effective early detection systems. Therefore, surveillance strategies aimed at shortening the HRP remain a critical component of ASF control.

5.3. Early detection of ASF outbreaks in pig farms

The studies demonstrated that enhanced passive surveillance substantially improved early detection of ASF outbreaks and shortened the high-risk period (HRP) in affected pig farms. Study II, which analyzed two outbreak farms, one in 2017 and one in 2018, clearly illustrated the importance of enhanced surveillance approaches. On one farm, enhanced passive surveillance was not yet applied because the outbreak occurred before implementation of the system. As a result, ASF was detected with a delay. In contrast, on the second farm, the disease was detected considerably earlier due to the implementation of enhanced passive surveillance. Although mortality levels did not initially raise suspicion of ASF, the disease was detected because dead pigs were systematically tested for ASF.

Early detection is essential for minimizing the HRP and reducing opportunities for further virus transmission within and between farms. However, when Study I was conducted in 2017, there were no specific legal requirements for early ASF detection in pig farms. In addition, practical epidemiological knowledge regarding the behavior of ASF virus following its introduction into commercial pig farms was still limited. At that time, surveillance procedures for early ASF detection were mainly based on active surveillance and the ASF Diagnostic Manual in use during that period.

The findings from Study I contributed to a better understanding of ASF outbreak dynamics in large commercial pig farms, particularly by demonstrating that ASF virus circulation may remain undetected for a prolonged period due to low initial mortality. These observations highlighted the limitations of surveillance approaches primarily based on active clinical inspections and general mortality indicators. Following communication of these findings to the European Commission, enhanced passive surveillance approaches, particularly systematic testing of dead and clinically affected pigs, received greater emphasis in ASF surveillance strategies. Subsequently, surveillance approaches evolved both in Latvia and at the European Union level, with enhanced passive surveillance becoming an essential component of early ASF detection strategies (EC, 2014; EC, 2018). The present studies support the epidemiological value of this approach and demonstrate that systematic testing of dead pigs is a practical and epidemiologically justified method for improving early ASF detection and shortening the HRP.

5.4. Integrated epidemiological framework of ASF outbreak investigation and control

The findings of Studies I-IV demonstrate that effective ASF control at farm level requires an integrated epidemiological framework combining prevention, early detection, structured outbreak investigation, and evidence-based decision-making.

ASF remains a priority infectious disease in Latvia, where continuous circulation of the virus in wild boar populations maintains constant infection pressure on domestic pig farms. Temporal and spatial analyses have demonstrated variability in ASF prevalence among wild boar populations between years and regions (Oļševskis et al., 2020; BIOR, 2022). Wild boar therefore function as a long-term reservoir, creating a persistent epidemiological interface between wildlife and domestic pig production systems.

Within this epidemiological context, the present studies demonstrate that ASF control at farm level cannot rely on a single intervention but requires integration of multiple complementary epidemiological components.

First, the investigations of virus introduction pathways demonstrated that the most critical determinant of virus introduction is not structural farm design alone but the interaction between infrastructure (“hardware”) and human behavior (“software”). While physical barriers reduce direct contact with wild boar, human-mediated pathways, particularly those associated with food handling, staff movements, and procedural non-compliance, remain central to ASF introduction risk. This confirms that ASF epidemiology in domestic pigs is strongly influenced by anthropogenic factors, consistent with findings from other European studies (Zani et al., 2019; Ardelean et al., 2021). Furthermore, the structured hypothesis-based investigative approach applied in Study I provides a methodological framework that reduces subjectivity and increases transparency during outbreak investigations.

Second, reconstruction of the high-risk period (HRP) demonstrated the importance of reconstructive epidemiology in outbreak management. ASF outbreaks in large commercial pig farms may involve multiple infection cycles before disease detection, as supported by both field observations and experimental studies with genotype II ASF virus (Guinat et al., 2016; Pietschmann et al., 2015; Gabriel et al., 2011; Blome et al., 2013). The timeline-based analytical approach developed in the present studies integrates clinical, laboratory, management, and biosecurity data into a coherent epidemiological framework. This transforms HRP estimation from a descriptive exercise into a practical analytical tool with direct relevance for tracing secondary outbreaks and assessing onward transmission risks.

Third, assessment of within-farm spread demonstrated that infection dynamics are shaped by internal contact structures, pig movement patterns, and housing systems. Although epidemiological links between farm units frequently exist, infection is not necessarily distributed uniformly throughout the farm. This finding has important implications for risk-based disease control strategies and prioritization of control measures. It further demonstrates that ASF spread within intensive pig production systems follows identifiable transmission patterns that can be reconstructed and epidemiologically analyzed.

Fourth, development of targeted sampling strategies aligned diagnostic approaches with the biological and epidemiological characteristics of highly virulent ASF strains. Given the high case fatality rate (>90%) associated with genotype II ASFV (Guinat et al., 2016; Gabriel et al., 2011; Blome et al., 2013), purposive sampling of clinically sick and dead pigs is epidemiologically more appropriate than random serological and virological sampling for assessment of active virus circulation. The subdivision of farms into epidemiological units and categorization according to infection risk provides a structured decision-support tool for outbreak investigations. Although lateral flow devices (LFDs) cannot replace laboratory confirmation, their strategic application under field conditions may improve operational efficiency.

Finally, the transition from active surveillance towards enhanced passive surveillance represents an important paradigm shift in ASF control (EC, 2014; EC, 2018). The findings

generated in Studies I and II demonstrated that reliance solely on general mortality indicators is insufficient for early outbreak detection. Instead, systematic testing of dead pigs provides an epidemiologically justified and operationally feasible approach for improving early ASF detection and shortening the HRP.

Taken together, the studies presented in this thesis form an integrated epidemiological model for ASF outbreak investigation and control at farm level. The model consists of five interdependent components: structured assessment of virus introduction pathways, analytical reconstruction of the HRP, mapping of within-farm transmission patterns, risk-based targeted sampling strategies, and strengthened early detection through enhanced passive surveillance.

These components are closely interconnected. Accurate HRP reconstruction improves tracing activities, targeted sampling clarifies transmission patterns, and structured investigation of human-mediated introduction pathways strengthens preventive biosecurity measures. At the centre of this integrated framework lies the human factor - awareness, compliance, communication, and decision-making.

Therefore, effective ASF control requires not only regulatory enforcement and laboratory capacity but also sustained investment in behavioural biosecurity, structured epidemiological training, and systematic investigation methodologies. The methodological approaches developed in this thesis contribute to strengthening such capacity and provide a framework applicable beyond the Latvian context.

Overall, this work demonstrates that ASF outbreak management is not solely a matter of pathogen detection but a multidisciplinary epidemiological process integrating disease epidemiology, farm management systems, human behaviour, and regulatory structures into a coherent disease control strategy.

CONCLUSIONS

- 1) ASF in commercial pig farms may initially develop with slow and heterogeneous virus spread, allowing infection to remain undetected for extended periods. These findings demonstrate that early detection of outbreaks cannot rely solely on increased mortality or clinical suspicion and requires systematic surveillance approaches.
- 2) Reconstruction of the HRP represents a central element of epidemiological outbreak investigation, enabling identification of probable virus introduction events, assessment of transmission pathways, and improvement of tracing and outbreak management decisions.
- 3) Structured, hypothesis-based epidemiological investigations enable systematic assessment of possible ASF virus introduction pathways and support exclusion of unlikely transmission routes.
- 4) Assessment of within-farm transmission patterns demonstrated that ASF spread does not occur uniformly across production units. Identification of epidemiological units and application of targeted sampling strategies are essential for reliable assessment of infection distribution within affected farms. Evaluation of rapid pen-side diagnostic tests under field conditions further demonstrated their practical value as complementary tools alongside standard laboratory methods.
- 5) Enhanced passive surveillance based on systematic testing of dead pigs significantly improves early detection of ASF outbreaks and contributes to shortening the HRP, thereby reducing the probability of further disease spread.
- 6) Epidemiological investigations integrating structured data collection, professional interpretation of field information, and targeted diagnostic verification provide evidence for adapting disease control and management measures to the specific outbreak situation, including assessment of the possibility of applying derogations from total depopulation measures.
- 7) The findings of this study resulted in the development of a structured framework for ASF outbreak investigations that integrates early detection, high-risk period reconstruction, hypothesis-based epidemiological investigation, and targeted diagnostic approaches.

RECOMMENDATIONS

- 1) Enhanced passive surveillance based on systematic testing of dead pigs should be consistently applied and maintained in commercial pig farms, as it improves early detection of ASF outbreaks and contributes to reducing the duration of undetected infection.
- 2) A structured approach to reconstruction of the high-risk period (HRP), integrating epidemiological, clinical, laboratory, mortality, and movement data, should be applied during ASF outbreak investigations to support identification of virus introduction events, assessment of transmission pathways, and outbreak management decisions.
- 3) ASF outbreak investigations should follow an integrated, hypothesis-based epidemiological framework incorporating structured data collection, HRP reconstruction, identification of epidemiological units, targeted sampling strategies, and appropriate diagnostic methods to support evidence-based outbreak management and disease control.

ACKNOWLEDGEMENTS

Words cannot fully express my sincere gratitude to all those who inspired, supported, taught, and guided me throughout the journey of completing this doctoral thesis.

A very special collaboration with colleagues from the Friedrich-Loeffler-Institut (FLI), the Federal Research Institute for Animal Health (Germany), the Estonian University of Life Sciences, and the Institute of Food Safety, Animal Health and Environment “BIOR” developed not only into valuable scientific cooperation but also into lasting personal relationships and a shared passion for finding answers to the many epidemiological questions related to African swine fever. This collaboration enabled me to work with an exceptional team of supervisors and scientific advisors.

My supervisors, Prof. Dr. Aivars Bērziņš and Prof. Dr. Arvo Viltrop, together with my scientific consultant, Dr. Klaus Depner, have been a continuous source of support, advice, encouragement, and knowledge throughout my doctoral studies.

Throughout the study process, and particularly during the design of field investigations and periods of uncertainty, Klaus, your support, encouragement, and guidance were invaluable. Your scientific expertise, enthusiasm, and ability to see solutions where others saw obstacles have been a constant source of inspiration. I will always be deeply grateful for your support.

I sincerely thank the foundation “Vītolu fonds” for recognizing the potential of my doctoral research and supporting my studies through the LAB-AN scholarship.

I would also like to express my sincere appreciation for the recognition awarded by the Latvian Academy of Sciences, which acknowledged our research on African swine fever as one of the most significant achievements in applied science in 2025. Such recognition highlights the importance of applied research and serves as valuable encouragement for continued scientific work.

My deepest gratitude goes to all colleagues who co-authored the four publications included in this thesis. Coming from different countries and bringing diverse experiences with ASF, you made our joint work more comprehensive, insightful, and inspiring. The exchange of ideas, scientific discussions, and shared efforts to address the challenges of ASF control have been among the most rewarding aspects of this journey.

This thesis would never have been possible without the days, months, and years spent working alongside my colleagues at the Food and Veterinary Service since the beginning of ASF in Latvia. My special thanks go to Edvīns Oļševskis. You were the person who made me believe that pursuing doctoral studies was possible while balancing the responsibilities of daily work at the Food and Veterinary Service. I am also deeply grateful for involving me in numerous national and international research activities, which motivated and encouraged me to continue my scientific development.

I would also like to thank Mārtiņš Seržants for the countless discussions, questions, and hours spent working side by side during ASF outbreak investigations, data collection, and interpretation. Your ideas, practical solutions, critical thinking, and sense of humor have always been a source of support and inspiration.

All colleagues of the Veterinary Surveillance Department deserve my sincere gratitude for their support, cooperation, and encouragement throughout these years.

I am sincerely grateful to all official veterinarians and laboratory specialists who were involved in ASF outbreak investigations and field activities throughout the years. The collection of samples, diagnostic testing, data gathering, and countless hours spent in affected farms provided the foundation for this research. Without your commitment and professionalism, these studies would not have been possible.

I would like to express my sincere gratitude to Inga Bedrīte for her assistance with the technical formatting of this thesis and for her consistently supportive attitude throughout the process.

My gratitude also belongs to my parents, who always supported my decision to pursue further studies and provided invaluable practical assistance by taking care of my home and garden while I was occupied with research activities and business trips.

My husband Ivo and our sons, Pēteris and Kristaps - I cannot imagine a more supportive, loving, and wonderful family. Your patience, understanding, encouragement, and unconditional love have made this journey possible.

Finally, I thank God for His overwhelming love, wisdom, guidance, and blessings that have accompanied me throughout my life and this academic journey.

Thank you all.

ANOTĀCIJA

Āfrikas cūku mēris (ĀCM) ir viena no nozīmīgākajām cūku infekcijas slimībām, kas rada smagas sociālekonomiskās sekas, raksturojas ar neprognozējamu pārrobežu izplatību un kuras profilaksei joprojām nav pieejami efektīvi ārstniecības līdzekļi vai vakcīnas. Mainīgā ĀCM epidemioloģiskā situācija rada pastāvīgu risku cūkkopības nozarei gan Eiropas Savienībā (ES), gan trešajās valstīs. Kopš ĀCM pirmo reizi tika konstatēts ES 2014. gadā, tā izplatība ir radījusi būtiskus izaicinājumus valsts institūcijām, nozares ekspertiem un citām iesaistītajām pusēm.

ĀCM Latvijā tika konstatēts 2014. gadā, slimībai izplatoties mežacūku populācijā, tādējādi radot apdraudējumu mājas cūku novietnēm. Kopš tā laika ĀCM uzliesmojumi ir reģistrēti dažāda tipa cūku novietnēs visā Latvijā. Lai gan pirmie uzliesmojumi galvenokārt tika konstatēti piemājas saimniecībās, pirmais uzliesmojums lielā komerciālā cūku novietnē tika apstiprināts 2017. gada janvārī, kas kalpoja par pamatu padziļinātu epidemioloģisko izmeklēšanu uzsākšanai. Turpmākie uzliesmojumi vairākās komerciālās cūku novietnēs radīja iespēju veikt plašākas epidemioloģiskās izmeklēšanas reālos lauka apstākļos. Šajā promocijas darbā apkopoti trīs epidemioloģisko pētījumu rezultāti, kas veikti četrās lielās komerciālās cūku novietnēs Latvijā, kā arī ceturta pētījuma rezultāti, kurā analizēti fermu darbinieku, cūku audzētāju un citu nespeciālistu viedokļi par iespējamiem vīrusa iekļūšanas ceļiem saimniecībās dažādās ĀCM skartajās valstīs Eiropā.

Pētījumu mērķis bija identificēt iespējamās vīrusa iekļūšanas avotus saimniecībās, noteikt ticamāko vīrusa iekļūšanas laiku un izpētīt slimības izplatības ceļus novietņu ietvaros. Kopumā šo pētījumu rezultāti ļāva izstrādāt strukturētu epidemioloģisko pieeju ĀCM uzliesmojumu izmeklēšanai saimniecību līmenī. Skartajās cūku novietnēs tika izvērtētas biodrošības sistēmas, izstrādājot hipotēzēs balstītu pieeju iespējamo vīrusa iekļūšanas ceļu izmeklēšanai. Visās pētījumā iekļautajās novietnēs tika analizētas slimības atklāšanas sistēmas, lai novērtētu, vai ĀCM tika konstatēts savlaicīgi, un izstrādātu ieteikumus uzraudzības pilnveidošanai agrīnākai slimības atklāšanai. Apkopojot un analizējot informāciju par saimniekošanas praksi, kā arī saslimstības un mirstības datus, tika noteikts ĀCM augsta riska periods (HRP) – laika posms no vīrusa iekļūšanas novietnē līdz slimības atklāšanai. HRP rekonstrukcija kalpoja kā nozīmīgs analītisks instruments, kas ļāva sasaistīt vīrusa iekļūšanu, slimības atklāšanu un lēmumus par uzliesmojuma kontroli. Lai novērtētu infekcijas izplatību skartajās cūku novietnēs, vienā no pētījumiem tika izstrādāta un praktiski pielietota mērķtiecīga paraugu ņemšanas stratēģija. Lai labāk izprastu vīrusa izplatības mehānismus novietņu ietvaros, tika analizēti vairāki epidemioloģiskie rādītāji, tostarp mirstības dati, kā arī dzīvnieku, personāla un transportlīdzekļu pārvietošanās. Atsevišķā pētījumā tika analizēti nespeciālistu viedokļi par iespējamiem slimības iekļūšanas ceļiem saimniecībās, īpašu uzmanību pievēršot šādas informācijas epidemioloģiskajai nozīmei un interpretācijai.

Pētījumu rezultāti parādīja, ka ĀCM vīrusa izplatība lielās komerciālās cūku novietnēs sākotnēji norit lēni. Pastiprinātā pasīvā uzraudzība tika identificēta kā būtisks instruments agrīnai slimības atklāšanai, un tās efektivitāte tika apstiprināta, sistemātiski to ieviešot praksē. Tika izstrādāti ieteikumi pastiprinātās pasīvās uzraudzības īstenošanai cūku novietnēs, lai samazinātu HRP ilgumu. Hipotēzēs balstītā pieeja apliecināja savu lietderību strukturētu epidemioloģisko izmeklēšanu veidošanā, nosakot iespējamās vīrusa iekļūšanas ceļus. Piedāvātā paraugu ņemšanas stratēģija, apvienojot laboratoriskos izmeklējumus ar ātrajiem novietnē izmantojamiem diagnostiskajiem testiem, nodrošināja uzticamu ĀCM izplatības novērtēšanu skarto novietņu dažādās epidemioloģiskajās vienībās. Savukārt nespeciālistu viedokļu analīze par iespējamiem slimības iekļūšanas ceļiem apliecināja šādas informācijas subjektīvo raksturu un uzsvēra pieredzējušu epidemioloģisko izmeklētāju nozīmi tās korektā interpretācijā. Kopumā promocijas darbs sniedz ieguldījumu strukturēta ĀCM uzliesmojumu izmeklēšanas un agrīnas slimības atklāšanas ietvara izstrādē, tādējādi veicinot efektīvāku ĀCM kontroli mājas cūku novietnēs.

SAĪSINĀJUMI

b-LFD Antivielu laterālās plūsmas tests

ADIS Dzīvnieku slimību informācijas sistēma

Ag-LFD Antigēnu laterālās plūsmas tests

ĀCM Āfrikas cūku mēris

ĀCMV Āfrikas cūku mēra vīruss

KCM Klasiskais cūku mēris

Ct Kvantifikācijas cikls

DNS Dezoksiribonukleīnskābe

EFSA Eiropas Pārtikas nekaitīguma iestāde

ELISA Enzīmsaistītās imūnsorbcijas metode

ES Eiropas Savienība

EURL Eiropas Savienības references laboratorija

MNS (FMD) Mutes un nagu sērga

PVD Pārtikas un veterinārais dienests

HRP Augsta riska periods

IPT Netiešā imūnperoksidāzes metode

LFD Laterālās plūsmas tests

NRL Nacionālā references laboratorija

PĶR Polimerāzes ķēdes reakcija

Kvantitatīvā PĶR (qPCR) Kvantitatīvā polimerāzes ķēdes reakcija

Reāllaika PĶR (rtPCR) Reāllaika polimerāzes ķēdes reakcija

WAHIS Pasaules dzīvnieku veselības informācijas sistēma

WOAH Pasaules Dzīvnieku veselības organizācija

PUBLIKĀCIJAS

Promocijas darba pamatā ir četras oriģinālpublikācijas (I - IV pētījums). Turpmāk tekstā atsaucei uz konkrēto publikāciju izmantoti romiešu cipari.

1. Lambergā, K.; Seržants, M.; Oļševskis, E. African swine fever outbreak investigations in a large commercial pig farm in Latvia: A case report. Berl. Munch. Tierarztl. Wochenschr. 2019, 132, 151–155.
2. Lambergā, K., Oļševskis, E., Seržants, M., Bērziņš, A., Viltrop, A., & Depner, K. (2020). African swine fever in two large commercial pig farms in Latvia – Estimation of the high-risk period and virus spread within the farm. Veterinary Sciences, 7(3), 105. <https://doi.org/10.3390/vetsci7030105>
3. Lambergā, K., Depner, K., Zani, L., Oļševskis, E., Seržants, M., Ansonskā, S., Šteingolde, Ž., Bērziņš, A., Viltrop, A., Blome, S., & Globig, A. (2022). A practical guide for strategic and efficient sampling in African swine fever-affected pig farms. Transboundary and Emerging Diseases, 1–10. <https://doi.org/10.1111/tbed.14582>
4. Lambergā, K.; Ardelean, F.; Blome, S.; Busauskas, P.; Djuric, B.; Globig, A.; Guberti, V.; Miteva, A.; Oļševskis, E.; Seržants, M.; et al. African Swine Fever Outbreak Investigations – The Significance of Disease-Related Anecdotal Information Coming from Laypersons. Pathogens 2022, 11, 702. <https://doi.org/10.3390/pathogens11060702>

Autora ieguldījums

Publikācija/Pētījums	Ieguldījums	%
I	Izstrādāja pētījuma koncepciju, veica pētījumu un sagatavoja manuskripta projektu	70
II	Veica epidemioloģisko izmeklēšanu un datu analīzi, izstrādāja manuskripta sākotnējo redakciju	75
III	Izstrādāja pētījuma koncepciju un metodoloģiju, veica izmeklējumus saimniecībās, veica formālo datu analīzi, apkopoja datus un sagatavoja grafikus, sagatavoja manuskripta pirmo versiju	75
IV	Izstrādāja pētījuma koncepciju un metodoloģiju, sagatavoja manuskripta pirmo versiju un izstrādāja vizualizācijas	65

1. IEVADS

1.1. Āfrikas cūku mēris: slimības raksturojums un epidemioloģija

Āfrikas cūku mēris (ĀCM) ir viena no nozīmīgākajām cūku infekcijas slimībām, kas rada smagas sociālekonomiskās sekas, raksturojas ar neprognozējamu pārrobežu izplatību un kuras kontrolei joprojām nav pieejami efektīvi ārstēšanas vai vakcinācijas līdzekļi. ĀCM ir obligāti ziņojama dzīvnieku infekcijas slimība.

ĀCM pirmo reizi aprakstīja Kenijā 1921. gadā (Montgomery, 1921). Pirmoreiz Eiropā slimība tika konstatēta Portugālē 1957. gadā, kur sākotnēji to izdevās izskaust. Tomēr 1960. gadā ĀCM Portugālē tika ieviests atkārtoti, un no turienes izplatījās uz Spāniju, kļūstot endēmisks Ibērijas pussalā 20. gadsimta 70. un 80. gados un radot ievērojamus ekonomiskos zaudējumus. Šajā periodā uzliesmojumi sporādiski tika konstatēti arī citās Eiropas un Amerikas valstīs, tostarp Nīderlandē, Beļģijā, Francijā, Ziemeļtālrijā, Kubā, Brazīlijā, Dominikānas Republikā un Haiti, tomēr šajās valstīs slimību izdevās izskaust. 1978. gadā ĀCM tika konstatēts Sardīnijā, kur tas saglabājās endēmisks vairāk nekā četras desmitgades (Mur et al., 2014), līdz Sardīnijas kompetentās iestādes nesen sekmīgi panāca slimības izskaušanu (Cappai et al., 2022).

Primāro uzliesmojumu infekcijas avots visbiežāk bija piesārņoti pārtikas atkritumi no starptautiskajā satiksmē iesaistītiem gaisa kuģiem un kuģiem (Mur et al., 2012). Tādēļ epidemioloģiskajā skatījumā secināts, ka cilvēka darbība un biodrošības prasību neievērošana ir galvenie faktori, kas veicina gan starpkontinentālu, gan reģionālu ĀCM izplatību.

2007. gadā ĀCM nonāca Gruzijā caur Poti ostu Melnās jūras piekrastē, visticamāk, arī ar piesārņotu pārtikas atkritumu starpniecību, un strauji izplatījās Gruzijā, Armēnijā un Krievijas Federācijā. Līdz 2012. gada beigām slimība bija izplatījusies vairākos Krievijas Federācijas Eiropas daļas reģionos (Gogin et al., 2013; Organesyan et al., 2013). Turpmākajos gados par uzliesmojumiem tika ziņots Ukrainā un Baltkrievijā. 2014. gada janvārī ĀCM sasniegta Eiropas Savienības austrumu robežu, kad Lietuvā tika konstatēta pirmā ar ĀCM inficētā mežacūka. Mēnesi vēlāk pirmie gadījumi tika reģistrēti Polijā, jūnijā Latvijā, bet 2014. gada septembrī – Igaunijā (EFSA, 2014; Pejsak and Stadejek, 2015). Ņemot vērā pirmo uzliesmojumu ģeogrāfisko tuvumu Baltkrievijas robežai, tiek pieņemts, ka ĀCM Baltijas valstīs un Polijā nonāca no Baltkrievijas, lai gan šajā periodā par ĀCM gadījumiem Baltkrievijā oficiāli netika ziņots.

Jau pirms pašreizējās ĀCM izplatības Eiropā un Āzijā, laikā, kad slimība bija sastopama Āfrikā un Ibērijas pussalā, uzliesmojumi tika reģistrēti arī četrās Amerikas valstīs. Papildus Kubai, kur slimība tika konstatēta divas reizes – 1971. un 1980. gadā, ĀCM tika reģistrēts Dominikānas Republikā (1978 - 1981), Haiti (1978 - 1983) un Brazīlijā (1978 - 1981). Pēc vairāk nekā 40 gadu pārtraukuma 2021. gada vasarā ĀCM atkārtoti tika konstatēts Dominikānas Republikā.

Nav šaubu, ka ĀCM vīruss Amerikas kontinentā nonāca ar kuģu vai gaisa transporta starpniecību. Citiem vārdiem sakot, tieši cilvēka darbība nodrošināja vīrusa pārvešanu starp kontinentiem. Šāda patogēna pārvešana ar kuģiem un lidmašīnām nav izņēmums, bet gan raksturīga ĀCM epidemioloģijas iezīme (EFSA, 2021; Sánchez-Vizcaíno et al., 2013). Dominikānas Republikā 2021. gadā konstatēto vīrusa izolātu sekvenēšanas rezultāti apliecināja, ka tas pieder tam pašam vīrusa genotipam, kas cirkulē Eiropā un Āzijā.

Lai gan kopš 20. gadsimta 70. un 80. gadu uzliesmojumiem Eiropā un Amerikā ir pagājis ilgs laiks un zināšanas par ĀCM ir būtiski paplašinājušās, ir svarīgi atcerēties, ka vīruss vairākkārt spējis pārvarēt okeānus un nonākt Eiropā, Āzijā un Amerikā cilvēka darbības rezultātā. Arī atkārtotā slimības konstatēšana Dominikānas Republikā 2021. gadā kalpo kā vēl viens apliecinājums šim faktam.

Vēsturiski ir aprakstīti trīs galvenie ĀCM epidemioloģiskie cikli: (i) silvātiskais cikls Āfrikā, kurā vīruss cirkulē starp mīkstajām ērcēm (*Ornithodoros spp.*) un Āfrikas savvaļas cūku

sugām, galvenokārt kārpčūkām, neizraisot saslimšanu ne mugurkaulnieku, ne posmkāju saimniekorganismos; (ii) ērcu - cūku cikls, kurā *Ornithodoros spp.* ērces darbojas kā vektori un nodrošina vīrusa pārnesi starp mājas cūkām; un (iii) mājas cūku cikls, kurā vīruss izplatās tiešā kontaktā starp cūkām vai netieši ar kontaminētu materiālu starpniecību bez kompetentu posmkāju vektoru iesaistes. Lielākā daļa ĀCM uzliesmojumu pasaulē ir saistīti tieši ar mājas cūku ciklu.

Tomēr pēc slimības ievazāšanas Baltijas valstīs un Polijā 2014. gadā kļuva acīmredzama mežacūku nozīme slimības epidemioloģijā, ļaujot izdalīt ceturto epidemioloģisko ciklu – mežacūku un apkārtējās vides ciklu (1.1. attēls) (Chenais et al., 2018). Šīs epidemioloģiskās īpatnības nosaka ĀCM uzliesmojumu izmeklēšanas sarežģītību un nepieciešamību pēc daudzpusīgas epidemioloģiskās pieejas.



1.1 attēls. ĀCM epidemioloģiskie cikli. 1 - silvātiskais cikls, 2 – ērcu - cūku cikls, 3 - mājas cūku cikls, 4 – mežacūku - vides cikls (Chenais et al., 2018)

Jāatzīmē, ka epidemioloģiskā situācija Eiropā pēc 2014. gada būtiski atšķiras no situācijas, kas tika novērota genotipa I dominances laikā Ibērijas pussalā. Pašlaik cirkulējošajiem II genotipa vīrusiem raksturīga ļoti augsta letalitāte, tādēļ slimības kontroles pieejas un uzraudzības koncepcijas, kas tika izstrādātas agrāko Eiropas uzliesmojumu laikā, ne vienmēr bija tieši piemērojamas epidemioloģiskajiem apstākļiem, kas 2014. gadā tika novēroti Baltijas valstīs un Polijā. Īpaši jāuzsver, ka seroloģiskā uzraudzība kā agrīnas slimības atklāšanas instruments izrādījās ierobežoti efektīva apstākļos, kad inficētie dzīvnieki bieži gāja bojā vēl pirms nosakāmu antivielu veidošanās.

No dzīvnieku infekcijas slimību kontroles un epidemioloģijas viedokļa īpaši nozīmīga ir ĀCM vīrusa augstā noturība apkārtējā vidē. Trūdēšanas procesi un saules gaisma vīrusu inaktivē nepietiekami, un tas saglabā inficēt spēju vairākas nedēļas pat sadalīšanās procesā esošos audos. Līķos un asinīs vīruss spēj saglabāties vairākus mēnešus, bet sasaldētā gaļā - pat vairākus gadus (Farez and Morley, 1997; EFSA, 2010). Tādēļ inficētu dzīvnieku līķi, kā arī kontaminēta gaļa un gaļas produkti var kalpot par nozīmīgiem infekcijas avotiem.

ĀCM vīrusa termiskā inaktivācija gaļā un gaļas produktos tiek panākta, tos karsējot 56 °C temperatūrā 70 minūtes vai 60 °C temperatūrā 20 minūtes. Gaļas produktu pārstrādes laikā

vīruss tiek inaktivēts tikai tad, ja produkta iekšējā temperatūra sasniedz vismaz 69 °C. Vīruss ir stabils plašā pH diapazonā no 3,0 līdz 13,4. Daudzi tradicionāli lietotie dezinfekcijas līdzekļi nav efektīvi pret ĀCM vīrusu, savukārt skābju saturoši līdzekļi nodrošina efektīvu inaktivāciju. Īpaši efektīva ir skudrskābe un citronskābe, turklāt citronskābe ir efektīva arī uz koka virsmām (Krug et al., 2012). Efektīvi dezinfekcijas līdzekļi ir arī nātrijs hidroksīds (0,8 % NaOH, 30 min), hipohlorīts (2,3 %, 30 min), formalīns (0,3 %, 30 min), ortofenilfenols (3 %, 30 min) un jodu saturoši dezinfekcijas līdzekļi.

Runājot par saimniekorganismiem un vektoriem, uzņēmīgas pret ĀCM infekciju ir gan mājas cūkas, gan mežacūkas, savukārt citas mājdzīvnieku sugas un cilvēki pret šo infekciju nav uzņēmīgi. Āfrikas kārpčūka tiek uzskatīta par dabisko ĀCM vīrusa rezervuāru, un tai parasti neattīstās slimības klīniskās pazīmes.

Dažos Āfrikas reģionos, kā arī Ibērijas pussalā, *Ornithodoros* ģints mīkstās ērces ir kompetenti ĀCM vīrusa vektori un tām ir būtiska nozīme slimības izplatībā (Oleaga-Perez et al., 1990). Sugai *Ornithodoros moubata*, kas sastopama Subsahāras Āfrikā, raksturīga gan transovariāla, gan transstadiāla vīrusa pārņemšana, un tā tiek uzskatīta par pilnībā kompetentu vektoru. Savukārt sugai *Ornithodoros erraticus*, kas sastopama Ibērijas pussalā un Ziemeļāfrikā, transovariāla pārņemšana nenotiek, tomēr vīruss ērcē var saglabāties līdz pat pieciem gadiem un tiek pārņemts transstadiāli.

Tomēr mīksto ērcu klātbūtne nav obligāts priekšnoteikums infekcijas pārnei vai epidemioloģiskā cikla uzturēšanai. Sardīnijā, kā arī Centrālajā un Austrumeiropā līdz šim nav iegūti pierādījumi par ērcu iesaisti ĀCM izplatībā (EFSA, 2024). Vācijā mežacūku asins serumos tika noteiktas antivielas pret mīksto ērcu antigēniem, taču netika konstatētas pazīmes, kas liecinātu par kontaktu ar šīm ērcēm (Pietschmann et al., 2016). Tādēļ nav pamata uzskatīt, ka mīkstās ērces būtu iesaistītas ĀCM epidemioloģiskajā ciklā Eiropā. Arī laboratoriskie pētījumi par citu vietējo ērcu sugu, piemēram, *Ixodes ricinus*, vektorkompetenci neuzrādīja vīrusa replikāciju (de Carvalho Ferreira et al., 2014).

Izmeklējot ĀCM uzliesmojumus mājas cūku novietnēs, asinssūcēji kukaiņi bieži tiek minēti kā iespējami mehāniskie vektori gadījumos, kad nav iespējams skaidri noteikt infekcijas iekļūšanas ceļu. Tomēr eksperimentālo un lauka pētījumu skaits, kas apstiprinātu šādu kukaiņu epidemioloģiski nozīmīgu lomu ĀCM izplatībā, ir ļoti ierobežots. Mehāniska vīrusa pārņemšana un ilgstoša replikācijspējīga vīrusa saglabāšanās ir pierādīta sugai *Stomoxys calcitrans* (Mellor et al., 1987). Nesenā lauka pētījumā, kurā tika vērtēta mušu un knišļu iespējamā loma ĀCM vīrusa mehāniskā pārnēsē, secināts, ka tādu nejaušu mehānisko vektoru kā *Culicoides* spp. un *S. calcitrans* iesaiste vīrusa pārnēsē ir iespējama, tomēr nepieciešami turpmāki pētījumi (Dhollander et al., 2025). Pieejamie zinātniskie dati liecina, ka mušas un dunduri ĀCM skartajās teritorijās Eiropas Savienībā var nonākt kontaktā ar vīrusu un potenciāli pārņemt to cūkām (EFSA, 2024). Tādēļ asinssūcēju kukaiņu nozīme ĀCM epidemioloģijā lauka apstākļos joprojām nav pilnībā noskaidrota. Tāpat nav pietiekamu pierādījumu, kas ļautu saistīt ĀCM uzliesmojumu sezonālo raksturu ar asinssūcēju kukaiņu aktivitāti.

Mājas cūkām un mežacūkām ĀCM parasti izpaužas kā akūta, ļoti letāla hemorāģiska slimība, kurai raksturīga strauja klīnisko pazīmju attīstība un augsta letalitāte. Slimības norise mājas cūkām un mežacūkām ir līdzīga. Infekcijai vienlīdz uzņēmīgi ir abu dzimumu un visu vecuma grupu dzīvnieki. Uzņēmīgie dzīvnieki inficējas, nonākot tiešā kontaktā ar infekciozu materiālu vai slimiem dzīvniekiem, visbiežāk perorālā ceļā, uzņemot kontaminētu barību, gaļu vai citus produktus. Teritorijās, kur *Ornithodoros* ģints ērces darbojas kā vektori, infekcija var tikt pārņemta arī ar ērcu kodumiem.

No epidemioloģiskā viedokļa īpaši nozīmīgas ir viremiska dzīvnieka asinis, jo infekcijas ierosināšanai var pietikt pat ar vienu asins pilienu. Tas saistīts ar faktu, ka ĀCM vīruss evolūcijas gaitā ir pielāgojies pārnesei ar posmkāju vektoru starpniecību, tādēļ efektīvi izplatās arī ar ļoti nelielu asiņu daudzumu.

Pēc salīdzinoši īsa inkubācijas perioda, kas ilgst dažas dienas, dzīvniekiem attīstās augsta ķermeņa temperatūra (līdz 42 °C), novērojama nomāktība un ēstgribas zudums.

Grūsnām sivēnmātēm augstā drudža dēļ var notikt aborts. Slimības vēlīnākajās stadijās bieži novēro asinsizplūdumus ādā un iekšējos orgānos (OIE, 2019). Nāve parasti iestājas divu nedēļu laikā. Atsevišķos gadījumos dzīvnieki iet bojā, neizrādot izteiktas klīniskās pazīmes. Pamatojoties vienīgi uz klīnisko ainu, ĀCM nav iespējams atšķirt no smagām klasiskā cūku mēra (KCM) formām vai citām smagām sistēmiskām cūku slimībām. Drošu diagnozi iespējams noteikt tikai ar laboratoriskajiem izmeklējumiem. Šīs nespecifiskās agrīnās klīniskās pazīmes ir viens no iemesliem novēlotai ĀCM uzliesmojumu atklāšanai lauka apstākļos.

Viena no ĀCM īpatnībām ir tā, ka inficētie dzīvnieki, ja tie pirmajās divās nedēļās neiet bojā infekcijas dēļ, izstrādā antivielas pret ĀCM vīrusu. Tomēr šīm antivielām nav neitralizējošas iedarbības, un tās nenodrošina efektīvu aizsardzību pret infekciju. Aizsargājošu antivielu trūkums ir viens no galvenajiem iemesliem, kādēļ joprojām nav pieejamas efektīvas vakcīnas pret ĀCM, jo arī vakcinācijas rezultātā inducētās antivielas nenodrošina pietiekamu aizsardzību. Vienlaikus antivielu noteikšanai ir būtiska epidemioloģiska nozīme. Antivielu klātbūtne norāda, ka infekcija dzīvniekā bijusi vismaz divas nedēļas. Savukārt, ja antivielas netiek konstatētas, bet tiek noteikts tikai vīrusa antigēns vai vīrusa genoms ar polimerāzes ķēdes reakcijas (PĶR) metodi, tas liecina par salīdzinoši nesenu infekcijas iekļūšanu (mazāk nekā 12 dienas).

ĀCM nereti tiek raksturots kā ļoti lipīga slimība, līdzīgi kā klasiskais cūku mēris vai mutes un nagu sērga (MNS), kuru ierosinātāji lielā daudzumā izdalās ar dažādiem organisma sekrētiem un ekskretiem. Tomēr šāds raksturojums pilnībā neatbilst ĀCM epidemioloģijai. ĀCM var uzskatīt par ļoti lipīgu slimību tikai tajos gadījumos, kad galvenais transmisijas ceļš ir kontakts ar asinīm.

Kopumā uzliesmojumu epidemioloģiskā analīze liecina, ka ĀCM raksturīga augsta letalitāte, zema mirstība uzliesmojuma sākumposmā un salīdzinoši zems lipīgums. Letalitāte ir to dzīvnieku īpatsvars, kuri noteiktā laika periodā gājuši bojā no kopējā inficēto dzīvnieku skaita. ĀCM vīrusa II genotipa gadījumā letalitāte ir ļoti augsta – vairāk nekā 90 % inficēto dzīvnieku iet bojā divu nedēļu laikā (Gabriel et al., 2011; Blome et al., 2012; Blome et al., 2013). Praktiski tas nozīmē, ka gandrīz katrs inficētais dzīvnieks iet bojā infekcijas rezultātā. Augstu letalitāti apstiprina arī eksperimentālie pētījumi (Pietschmann et al., 2015; Gallardo et al., 2015).

Tomēr ĀCM uzliesmojuma sākumposmā novietnes līmenī – aptuveni divas līdz trīs nedēļas pēc vīrusa iekļūšanas – kopējā mirstība var būt zema, neskatoties uz augsto letalitāti, jo skarts ir tikai ierobežots dzīvnieku skaits attiecīgajā epidemioloģiskajā vienībā. Mirstība ir to dzīvnieku īpatsvars, kuri noteiktā laika periodā gājuši bojā attiecībā pret visu populāciju.

Minētie epidemioloģiskie rādītāji uzsver lauka pētījumu nozīmi, lai labāk izprastu slimības izplatības dinamiku skartajos ganāmpulkos un infekcijas attīstību laika gaitā novietnes līmenī. Šīs epidemioloģiskās īpatnības nosaka ne tikai infekcijas klīnisko iznākumu, bet arī ietekmē uzliesmojumu kontroles un uzraudzības stratēģiju izstrādi un īstenošanu skartajos reģionos.

Šie epidemioloģiskie raksturlielumi ir cieši saistīti ar ĀCM vīrusa bioloģiskajām īpašībām. ĀCM izraisa apvalkots divpavedienu DNS vīruss, kas pieder *Asfarviridae* dzimtai. Vīrusam ir sarežģīta daudzslāņaina struktūra un genoms, kas kodē vairāk nekā 150 gēnus, no kuriem ievērojamas daļas funkcijas joprojām nav pilnībā noskaidrotas. Vīruss galvenokārt replicējas inficēto šūnu citoplazmā, īpaši perinukleārajās vīrusu replikācijas struktūrās, un kodē daudzus proteīnus, kas nepieciešami tā transkripcijai un replikācijai.

ĀCM vīrusam raksturīgs izteikts tropisms pret monocītu - makrofāgu līnijas šūnām, kurām ir centrāla nozīme slimības patoģenēzē. Infekcija izraisa būtiskus imūnās atbildes traucējumus, kas veicina smagu klīnisko pazīmju attīstību un augstu mirstību. Lai gan noteiktas imūnās reakcijas, piemēram, agrīna interferonu producēšana un T limfocītu aktivācija, tiek saistītas ar aizsardzību pret infekciju, aizsargājošās imunitātes mehānismi joprojām nav pilnībā izprasti.

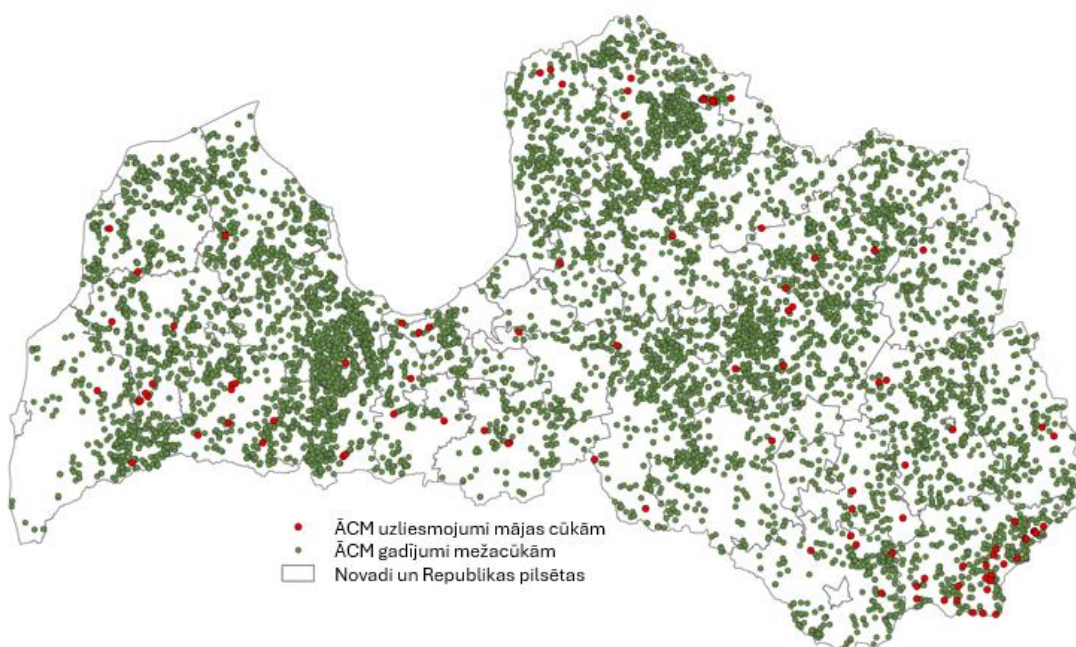
Vīrusam raksturīga liela noturība apkārtējā vidē, kas ļauj tam ilgstoši saglabāties kontaminētā gaļā, asinīs, liķos un citos infekciju pārnesošos objektos. Šī noturība kopā ar daudzveidīgajiem transmisijas ceļiem - tiešu kontaktu, kontaminētu barību vai aprīkojumu, kā arī atsevišķos reģionos - vektoru izraisītu pārnesei, būtiski apgrūtina slimības kontroli un veicina tās turpmāku izplatību pasaulē (Mehinagic and Summerfield, 2026).

Līdz šim ir aprakstīti 22 ĀCM vīrusa genotipi (Bastos et al., 2003). Jāuzsver, ka genotips pats par sevi nesniedz pietiekamu informāciju par vīrusa virulenci. Sardīnijā, Ibērijas pussalā un Dienvidamerikā cirkulējušie ĀCM vīrusa celmi pieder I genotipam. Savukārt vīrusa celmi, kas pašlaik cirkulē Austrumeiropā (Baltijas valstīs, Polijā, Kaukāza reģionā, Krievijas Federācijā un Ukrainā), pieder II genotipam un tādēļ nav tieši saistīti ar Sardīnijā vai Ibērijas pussalā sastopamajiem izolātiem (Gallardo et al., 2009; Gallardo et al., 2014).

Pašlaik cirkulējošie II genotipa vīrusi rutīnas sekvenēšanā izmantotajos genoma reģionos ir ģenētiski identiski, un arī pilna genoma līmenī starp tiem konstatējamās tikai minimālas atšķirības. Tomēr, meklējot iespējas detalizētākai celmu diferenciācijai, pēdējos gados ir aprakstīti genoma reģioni ar atkārtotu nukleotīdu secību variācijām, kas potenciāli var tikt izmantoti molekulāri epidemioloģiskos pētījumos (Gallardo et al., 2014; Goller et al., 2015; Forth et al., 2022).

Latvijā kopš 2014. gada novērotā epidemioloģiskā situācija ir praktisks piemērs iepriekš aprakstītajai infekcijas izplatības dinamikai un pastāvīgajam infekcijas apdraudējumam skartajos reģionos. Kopš ĀCM ievazāšanas Latvijā 2014. gadā slimība ir saglabājusies plaši izplatīta, turpinot cirkulēt mežacūku populācijā un periodiski izraisot uzliesmojumus mājas cūku novietnēs (1.2. attēls).

Pašlaik ĀCM gadījumi mežacūkām ir konstatēti gandrīz visā Latvijas teritorijā. Laikposmā no 2014. līdz 2025. gadam reģistrēti 11211 ĀCM gadījumi mežacūkām un 102 uzliesmojumi mājas cūku novietnēs, kuru apkarošanas pasākumu ietvaros tika iznīcinātas 81590 mājas cūkas (PVD dati). Šie rādītāji raksturo infekcijas apdraudējumu, ko rada mežacūkas, un pastāvīgo risku vīrusa iekļūšanai mājas cūku novietnēs.

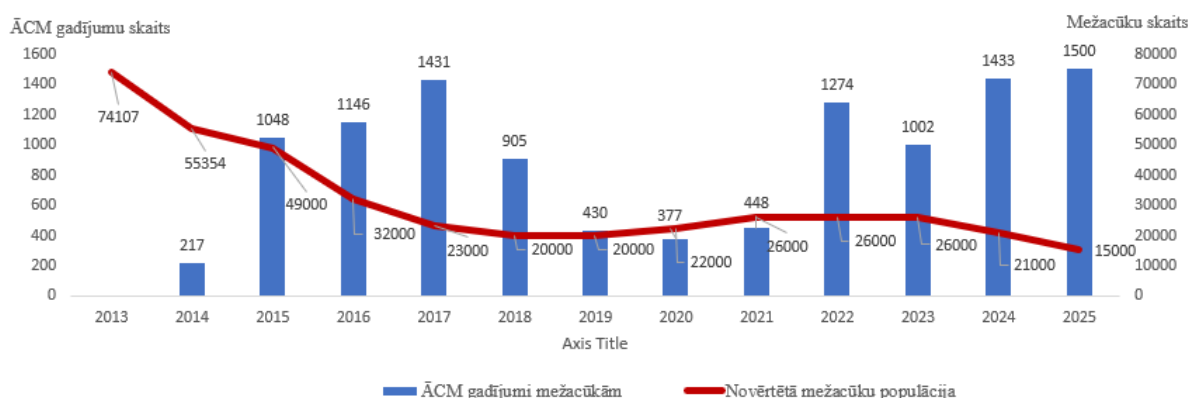


1.2 attēls. Āfrikas cūku mēra gadījumu izplatība mežacūku populācijā un uzliesmojumi mājas cūku novietnēs Latvijā 2014. - 2025. gadā (PVD ĀCM uzraudzības dati; vizualizācija sagatavota, izmantojot ESRI ArcGIS Pro 3.1.0 programmatūru)

Pārtikas un veterinārā dienesta īstenotās ĀCM uzraudzības un apkarošanas programmas ietvaros ĀCM izmeklējumi tika veikti gan atrastām beigtām, gan nomedītām mežacūkām.

Veiktā PQR izmeklējumu rezultātu analīze, kā arī citu pētījumu rezultāti liecina, ka ĀCM vīrusa izplatība mežacūku populācijā būtiski atšķiras gan starp gadiem, gan dažādiem Latvijas reģioniem (Oļševskis et al., 2020; BIOR, 2022).

Lai gan ĀCM izplatības rezultātā mežacūku populācija strauji samazinājās no aptuveni 74000 dzīvnieku 2013. gadā līdz aptuveni 15000 dzīvnieku 2015. gadā, mežacūkas joprojām saglabājas kā nozīmīgs ĀCM vīrusa rezervuārs Latvijā, tādējādi radot pastāvīgu un ilgstošu apdraudējumu mājas cūku sektoram - gan komerciālajām cūku novietnēm, gan piemājas saimniecībām (1.3. attēls).



1.3 attēls. ĀCM gadījumi mežacūkām un novērtētais mežacūku populācijas lielums pa gadiem (2013 - 2025) (Avots: Pārtikas un veterinārais dienests un Valsts meža dienests)

Šādos epidemioloģiskajos apstākļos, kam raksturīga nepārtraukta vīrusa cirkulācija mežacūku populācijā un periodiska vīrusa iekļūšana mājas cūku novietnēs, īpaši būtiska nozīme ir efektīvām uzraudzības sistēmām, kā arī skaidri definētiem uzliesmojumu izmeklēšanas un kontroles pasākumiem, lai ierobežotu turpmāku slimības izplatību.

1.2. Āfrikas cūku mēra kontrole, uzliesmojumu izmeklēšana un biodrošība

Eiropas Savienībā ir noteikti vienoti A kategorijas dzīvnieku slimību kontroles principi un prasības. Āfrikas cūku mēris ir A kategorijas slimība. Regula (ES) 2020/687 nosaka pamatnoteikumus slimības apkarošanas pasākumiem, kas piemērojami skartajās cūku novietnēs ĀCM uzliesmojuma gadījumā. Eiropas Savienības tiesību akti paredz, ka kompetentajai iestādei ir jāizstrādā un regulāri jāatjaunina ārkārtas rīcības plāni, kā arī jānodrošina detalizētas instrukcijas par pasākumiem, kas īstenojami ĀCM uzliesmojuma gadījumā (EC, 2016).

Ārkārtas rīcības plāniem jānodrošina atbilstošs resursu sadalījums un skaidri jānosaka vadības struktūra valsts, reģionālajā un vietējā līmenī. Tajos jāietver vispārējās atbalsta funkcijas, piemēram, komunikācija, loģistika, cilvēkresursu un materiālo resursu pārvaldība, kā arī gatavības un slimības kontroles pasākumi. Šo pasākumu mērķis ir nodrošināt ātru un efektīvu reaģēšanu uz A kategorijas slimību uzliesmojumiem un novērst slimības izplatīšanos no skartajiem dzīvniekiem un novietnēm uz neskartiem dzīvniekiem un novietnēm.

Efektīvai slimības kontrolei būtiska nozīme ir detalizētu instrukciju izstrādei un regulārai aktualizēšanai. Instrukcijās tiek noteiktas un aprakstītas darbības procedūras, kas īstenojamas uzliesmojuma gadījumā, lai kontrolētu un izskaustu slimību. Tās ietver detalizētus tehniskos norādījumus par konkrētu dzīvnieku infekcijas slimību apkarošanu, tostarp slimības paziņošanas kārtību, diagnostikas kritērijiem un iespējām, epidemioloģisko izmeklēšanu, ierobežojumu zonu noteikšanu un tajās piemērojamajiem kontroles pasākumiem, vakcinācijas

plāniem, dzīvnieku nogalināšanu un liķu iznīcināšanu, tīrīšanu un dezinfekciju, kā arī novietņu atjaunošanu pēc uzliesmojuma. No zinātniskā viedokļa šāda sagatavotības plānošana ir īpaši svarīga, jo ĀCM vīruss ir noturīgs apkārtējā vidē un spēj izplatīties gan tiešā, gan netiešā ceļā, tādēļ slimības ierobežošanai nepieciešama koordinēta un nekavējoša rīcība (EFSA, 2021; WOA, 2023).

Slimības kontroles un biodrošības pasākumi skartajās novietnēs jāievieš nekavējoties, īpašu uzmanību pievēršot dzīvnieku un dzīvnieku izcelsmes produktu pārvietošanas ierobežošanai. Lai novērstu infekcijas izplatīšanos no skartajiem dzīvniekiem un novietnēm, nepieciešams izolēt visus dzīvniekus, par kuriem ir aizdomas par inficēšanos, pārtraukt jebkādu cūku pārvietošanu uz novietni vai no tās, kā arī nodrošināt, ka kūstmēsli, pakaiši un citi materiāli vai vielas, kas varētu būt kontaminēti, tiek uzglabāti līdz to iznīcināšanai, apstrādei vai tīrīšanai un dezinfekcijai. Papildus jāsteno biodrošības pasākumi, lai novērstu jebkādu turpmāku slimības izplatīšanās risku.

Ap skarto novietni jānosaka ierobežojumu zona, kas ietver aizsardzības un uzraudzības zonu, lai novērstu turpmāku slimības izplatīšanos. Kompetentā iestāde nosaka ierobežojumus dzīvnieku un produktu pārvietošanai aizsardzības un uzraudzības zonās, no tām vai caur tām, kā arī aizliedz citas darbības, kas varētu radīt slimības izplatīšanās risku. Uzraudzības zonā piemērojamie kontroles pasākumi ietver īpašas prasības attiecībā uz dzīvniekiem, no tiem iegūtajiem produktiem, kā arī citām vielām un materiāliem, kas var būt kontaminēti.

Šo pasākumu zinātniskais pamatojums balstās uz ĀCM vīrusa spēju ilgstoši saglabāties organiskajos materiālos, piemēram, kūstmēšos, pakaišos un kontaminētos produktos, kā arī izplatīties netiešā ceļā ar kontaminētu priekšmetu un aprīkojuma starpniecību. Tādēļ stingra pārvietošanas kontrole un biodrošības prasību ievērošana ir būtiski priekšnosacījumi infekcijas transmisijas ķēžu pārtraušanai un slimības tālākas izplatības novēršanai (EFSA, 2021; WOA, 2022).

Saskaņā ar Eiropas Savienības tiesību aktiem katrai dalībvalstij ir jāizveido, jāievieš un jāuztur sistēma, kas nodrošina dzīvnieku infekcijas slimību agrīnu atklāšanu. Eiropas Savienības dalībvalstīm jānodrošina, ka dzīvnieku īpašnieki, turētāji un citas fiziskas vai juridiskas personas nekavējoties informē kompetento iestādi par aizdomām par ĀCM vai slimības konstatēšanu. Savukārt veterinārajam dienestam nekavējoties jāuzsāk izmeklēšana, lai apstiprinātu vai izslēgtu aizdomas par ĀCM klātbūtni (EC, 2019).

Tiesību akti paredz, ka kompetentajai iestādei jāveic izmeklēšana vairākos slimības uzraudzības posmos: ja ir radušās aizdomas par slimību, pēc slimības apstiprināšanas, kā arī gadījumos, kad nepieciešams izslēgt slimības izplatīšanos uz epidemioloģiski saistītām novietnēm vai citām vietām. Šādas izmeklēšanas ietver klīnisko izmeklēšanu un paraugu ņemšanu laboratoriskajiem izmeklējumiem. No epidemioloģiskā viedokļa agrīnai slimības atklāšanai un savlaicīgai ziņošanai ir izšķiroša nozīme, jo ĀCM uzliesmojumi bieži tiek atklāti novēloti nespecifisko sākotnējo klīnisko pazīmju dēļ, bet jebkura kavēšanās būtiski palielina slimības tālākas izplatības risku (EFSA, 2018).

ĀCM uzliesmojumu gadījumā mājas cūku novietnēs Eiropas Savienības tiesību akti paredz arī padziļinātas epidemioloģiskās izmeklēšanas veikšanu, lai noteiktu iespējamo vīrusa izcelsmi, rekonstruētu augsta riska periodu (HRP), tas ir, novērtētu laika posmu, kurā vīruss atradies novietnē pirms tā atklāšanas, kā arī iegūtu informāciju par iespējamo slimības izplatīšanos uz citām novietnēm (EC, 2002; EC, 2016; EC, 2019).

Līdz 2021. gada 21. aprīlim Eiropas Savienības tiesību akti paredzēja nejauši atlasītu asins paraugu ņemšanu no cūkām skartajā novietnē dzīvnieku likvidēšanas laikā, lai veiktu seroloģiskos un virusoloģiskos izmeklējumus. Minimālajam paraugu skaitam bija jānodrošina iespēja konstatēt 10 % seroprevalenci katrā novietnes apakšvienībā ar 95 % ticamības līmeni (EC, 2003).

Nemot vērā, ka pašlaik cirkulējošie ĀCM vīrusa II genotipa celmi izraisa vairāk nekā 90 % letalitāti, seropozitīvas un klīniski veselas cūkas tiek konstatētas reti, jo lielākā daļa inficēto dzīvnieku neizdzīvo slimības akūto fāzi (Blome et al., 2020). Tādēļ virulentu ĀCM

vīrusa celmu cirkulācijas apstākļos uz nejašu seroloģisko paraugu ņemšanu balstīta uzraudzība vairs netika rekomendēta kā metode vīrusa izplatības novērtēšanai skartajā novietnē (EFSA, 2021). Mērķtiecīgās uzraudzības pieejas pamatā bija pieņēmums, ka inficētie dzīvnieki saslims un ies bojā (Busch et al., 2021; Blome et al., 2020). Vienlaikus antivielu noteikšanai joprojām ir būtiska nozīme, jo tā sniedz papildu informāciju par vīrusa klātbūtnes ilgumu novietnē, tādēļ seroloģiskie izmeklējumi joprojām tiek izmantoti epidemioloģiskās izmeklēšanas ietvaros.

Viens no obligātajiem ĀCM apkarošanas pasākumiem ir tādu cūku nogalināšana, kuras ir inficētas, iespējami inficētas vai var veicināt slimības izplatīšanos. Visas uzņēmīgo sugu dzīvnieku grupas skartajā novietnē pēc iespējas ātrāk jānogalina uz vietas tādā veidā, lai nogalināšanas procesa laikā un pēc tā tiktu novērsts jebkāds slimības ierosinātāja izplatīšanās risks. Tādēļ ĀCM uzliesmojuma gadījumā tiek likvidētas visas cūkas skartajā novietnē neatkarīgi no tās lieluma, un dzīvnieku līķi tiek droši iznīcināti dzīvnieku izcelsmes blakusproduktu pārstrādes uzņēmumos.

Tomēr Eiropas Savienības tiesību akti paredz iespēju kompetentajai iestādei noteiktos gadījumos atkāpties no prasības likvidēt visas cūkas novietnē, ja tā sastāv no vairākām epidemioloģiskajām vienībām. Šāda atkāpe var tikt piemērota tikai pēc riska novērtējuma veikšanas, labvēlīgu laboratorisko izmeklējumu rezultātu saņemšanas un ar nosacījumu, ka epidemioloģiskajā izmeklēšanā nav konstatēta saikne starp epidemioloģiskajām vienībām, kurās slimība apstiprināta, un vienībām, kurās tā nav konstatēta (EC, 2019).

Pilnīga dzīvnieku likvidēšana ir zinātniski pamatota pieeja, jo pret ĀCM nav pieejama efektīva ārstēšana vai vakcīna, bet inficētie dzīvnieki ir nozīmīgs vīrusa izdalīšanas avots. Tādēļ ātra inficēto un potenciāli inficēto dzīvnieku likvidēšana ir būtiska, lai novērstu infekcijas avotus un pārtrauktu slimības tālāku izplatīšanos (WOAH, 2023). Tāpat būtiska nozīme ir drošai dzīvnieku līķu iznīcināšanai, jo ĀCM vīruss tajos spēj saglabāt infekciozitāti ilgstoši un var veicināt apkārtējās vides kontamināciju un slimības tālāku izplatību (EFSA, 2018).

Latvijā par visiem aizdomīgajiem ĀCM gadījumiem tiek ziņots praktizējošam veterinārārstam vai Pārtikas un veterinārajam dienestam (PVD). Klīnisko izmeklēšanu, pēcnāves apskati un paraugu ņemšanu veic praktizējoši veterinārārsti vai oficiālie veterinārārsti. Paraugi laboratoriskajiem izmeklējumiem jānosūta uz Nacionālo references laboratoriju (NRL) (MK, 2004). Ja laboratoriskajos izmeklējumos tiek konstatēts ĀCM vīrusa genoms vai antivielas pret ĀCM vīrusu, dzīvnieks tiek uzskatīts par inficētu, un kompetentā iestāde pieņem lēmumu par uzliesmojuma apstiprināšanu. Ar ĀCM izmeklēšanu saistītās laboratorisko analīžu izmaksas sedz valsts budžets.

Lai nodrošinātu savlaicīgu ĀCM uzliesmojumu atklāšanu, ņemot vērā vīrusa augsto letalitāti, zemo mirstību uzliesmojuma sākumposmā un salīdzinoši zemo lipīgumu, tika pieņemts lēmums ieviest pastiprinātas pasīvās uzraudzības sistēmu cūku novietnēs, kas atrodas ĀCM riska teritorijās. Šādas pieejas ieviešanu būtiski ietekmēja lielo komerciālo cūku novietņu uzliesmojumu izmeklēšanas rezultāti, īpaši 2017. gada janvārī konstatētais uzliesmojums komerciālā cūku novietnē Latvijā. Vēlāk attiecīgi tika pilnveidoti arī Eiropas Savienības tiesību akti, nosakot juridisku pienākumu ieviest un uzturēt pastiprinātas pasīvās uzraudzības sistēmu. Saskaņā ar pašlaik spēkā esošajām Eiropas Savienības prasībām dzīvnieku īpašniekam vai turētājam katrā epidemioloģiskajā vienībā katru nedēļu jānodrošina laboratoriska izmeklēšana vismaz pirmajām divām nobeigušajām cūkām, kas vecākas par 60 dienām, vai, ja šādu dzīvnieku nav, jebkurai cūkai, kas nobeigusies pēc atšķiršanas (EC, 2023).

PVD regulāri sniedz informāciju Eiropas Komisijai, izmantojot Dzīvnieku slimību informācijas sistēmu (ADIS), un Pasaules Dzīvnieku veselības organizācijai (WOAH), izmantojot Pasaules dzīvnieku veselības informācijas sistēmu (WAHIS), par ĀCM gadījumiem mežacūkām un uzliesmojumiem mājas cūku novietnēs (EC, 2020; WOAH WAHIS). Šāda informācijas apmaiņa nodrošina savlaicīgu informācijas apriti starp valstīm par konstatētajiem slimības gadījumiem un īstenotajiem kontroles pasākumiem, ļaujot kompetentajām iestādēm atbilstoši reaģēt, novērtēt riskus un īstenot nepieciešamos preventīvos pasākumus.

Lai nodrošinātu pastāvīgu slimības kontroles pasākumu izvērtēšanu un veicinātu informētību par ĀCM, PVD regulāri organizē sanāksmes praktizējošiem veterinārārstiem, oficiālajiem veterinārārstiem, mednieku organizācijām un cūku audzētājiem, sniedzot aktuālo informāciju par epidemioloģisko situāciju mežacūku populācijā, ĀCM uzliesmojumiem, slimības apkarošanas pasākumiem un biodrošības prasību ievērošanas nozīmi. Sabiedrības informēšanas pasākumos bieži tiek iesaistīti arī masu saziņas līdzekļi – prese, radio un televīzija (PVD, 2021). Informatīvās kampaņas tiek papildinātas ar speciāli sagatavotiem videomateriāliem un informatīviem bukletiem, kuru saturs tiek pielāgots attiecīgajai mērķauditorijai un komunikācijas mērķiem (PVD tīmekļvietne).

Informētība par slimību ir viens no būtiskākajiem priekšnosacījumiem infekcijas novēršanai vai savlaicīgai atklāšanai. Informatīvajās kampaņās tiek uzrunātas dažādas mērķgrupas, tostarp sabiedrība kopumā, dzīvnieku turētāji, lauksaimnieki, praktizējošie veterinārārsti, oficiālie veterinārārsti, mednieki un citas iesaistītās personas.

Tā kā pret ĀCM nav pieejama ne efektīva vakcīna, ne ārstēšanas iespējas, vienīgais veids, kā novērst vīrusa iekļūšanu novietnē, ir preventīvo pasākumu ieviešana. Biodrošība ir efektīvākais profilakses līdzeklis pret ĀCM. Tā ir pasākumu sistēma, kas jāizstrādā, jāievieš un pastāvīgi jāuztur, ņemot vērā katras konkrētās novietnes īpatnības. Visām novietnēm, kurās tiek turētas cūkas, jānodrošina Ministru kabineta noteikumos noteikto biodrošības prasību ievērošana (MK, 2015).

Papildus tam cūku novietņu operatoriem, kuru novietnes atrodas ĀCM ierobežojumu zonās, jānodrošina pastiprinātu biodrošības pasākumu ieviešana, lai saņemtu atļauju cūku pārvietošanai ierobežojumu zonu ietvaros vai ārpus tām (EC, 2023).

Galvenās biodrošības prasības paredz, ka jānovērš jebkāda tieša vai netieša saskare starp mājas cūkām un mežacūkām. Novietnēs jāievēro atbilstoši higiēnas pasākumi, tostarp apģērba un apavu maiņa, ieejot telpās, kur tiek turētas cūkas, un izejot no tām, roku mazgāšana un dezinfekcija, kā arī apavu dezinfekcija pie ieejas cūku turēšanas telpās. Personām, kuras piedalījušās mežacūku medībās, vismaz 48 stundas pēc medībām aizliegts nonākt kontaktā ar cūkām. Tāpat jānodrošina, ka novietnes teritorijā, tostarp cūku turēšanas telpās, neiekļūst nepiederošas personas vai transportlīdzekļi, kā arī jāveic personu un transportlīdzekļu uzskaitē, kas iekļūst teritorijā, kur tiek turētas cūkas.

Ēkām, kurās tiek turētas cūkas, jābūt izbūvētām tā, lai tajās nevarētu iekļūt citi dzīvnieki un nonākt saskarē ar cūkām, to barību vai pakaišiem. Tām jānodrošina iespēja veikt roku mazgāšanu un dezinfekciju, jābūt piemērotām vietām apavu un apģērba maiņai pie ieejas dzīvnieku turēšanas telpās, kā arī jābūt ierīkotam drošam nožogojumam vismaz ap teritoriju, kur tiek turētas cūkas, un vietām, kur tiek uzglabāta barība un pakaiši.

Komerציālajām cūku novietnēm ir jāizstrādā kompetentās iestādes apstiprināts biodrošības plāns. Tajā jāparedz „tīro” un „netīro” zonu nodalīšana personālam atbilstoši novietnes tipam, piemēram, ģērbtuves, dušas un ēdināšanas telpas, kā arī jānosaka kārtība jaunu dzīvnieku ievietošanai novietnē. Biodrošības plānā jāiekļauj procedūras telpu, transportlīdzekļu un aprīkojuma tīrīšanai un dezinfekcijai, personīgās higiēnas prasības, kā arī iekšējā audita vai pašnovērtējuma sistēma biodrošības prasību ievērošanas kontrolei (EC, 2023; MK, 2015).

Neraugoties uz izstrādātajiem normatīvajiem regulējumiem, uzraudzības sistēmām, uzliesmojumu izmeklēšanas prasībām un biodrošības pasākumiem, ĀCM uzliesmojumi komerciālajās cūku novietnēs joprojām rada būtiskus izaicinājumus saistībā ar slimības agrīnu atklāšanu, infekcijas notikumu rekonstrukciju un slimības izplatības novērtēšanu skartajās novietnēs. Šie izaicinājumi uzsver nepieciešamību pilnveidot epidemioloģiskās izmeklēšanas pieejas, kas spētu nodrošināt ticamus pierādījumus uzliesmojumu pārvaldības un kontroles lēmumu pieņemšanai novietnes līmenī. Tādēļ pastāv nepieciešamība pilnveidot agrīnas slimības atklāšanas pieejas un strukturēt epidemioloģisko izmeklēšanu skartajās cūku novietnēs, lai veicinātu uz pierādījumiem balstītu uzliesmojumu kontroli.

2. HIPOTĒZE, MĒRĶIS UN UZDEVUMI

Pētījuma hipotēzes

- 1) Hipotēzēs balstīta epidemioloģiskā izmeklēšanas pieeja, kas ietver strukturētu no nespeciālistiem iegūtas informācijas izvērtēšanu, ļauj ticami identificēt ticamākos Āfrikas cūku mēra (ĀCM) vīrusa iekļūšanas ceļus cūku ganāmpulkos.
- 2) Mērķtiecīgas paraugu ņemšanas stratēģijas, kuru pamatā ir epidemioloģisko apakšvienību definēšana un kategorizēšana, klīniski slimu vai nobeigušos dzīvnieku atlase un būtiskāko epidemioloģisko rādītāju izvērtēšana, uzlabo ĀCM uzliesmojumu izmeklēšanas efektivitāti un savlaicīgumu, nodrošinot skarto un neskarto vienību telpisku identificēšanu un klasificēšanu cūku novietnēs, savukārt ātrie novietnē izmantojamie testi, piemēram, laterālās plūsmas testi (LFD), var kalpot kā papildu rīks līdztekus standarta laboratoriskajām diagnostikas metodēm (PQR, IPT un ELISA).
- 3) Galvenos epidemioloģiskos parametrus iespējams sistemātiski identificēt un izmantot precīzai ĀCM uzliesmojumu augsta riska perioda (HRP) rekonstrukcijai skartajās cūku novietnēs.
- 4) ĀCM uzliesmojumi komerciālajās cūku novietnēs var ilgstoši palikt neatklāti lēnās un nevienmērīgās infekcijas izplatības novietnes ietvaros, kā arī zemās mirstības dēļ uzliesmojuma sākumposmā, kas uzsver nepieciešamību pēc mērķtiecīgas uzraudzības agrīnākai slimības atklāšanai.

Pētījuma mērķis

Pētījuma mērķis bija pilnveidot Āfrikas cūku mēra (ĀCM) kontroles stratēģijas, uzlabojot un stiprinot agrīnas uzliesmojumu atklāšanas un epidemioloģiskās izmeklēšanas pieejas skartajās cūku novietnēs, izvērtējot esošās uzraudzības sistēmas un uzliesmojumu izmeklēšanas metodoloģijas.

Pētījuma uzdevumi

- 1) Izmeklēt iespējamos ĀCM vīrusa iekļūšanas ceļus cūku ganāmpulkos, izmantojot hipotēzēs balstītu epidemioloģisko pieeju un izvērtējot no nespeciālistiem iegūto informāciju (I, IV).
- 2) Izvērtēt un pilnveidot paraugu ņemšanas stratēģijas ĀCM vīrusa izplatības novērtēšanai skartajās cūku novietnēs (II, III).
- 3) Novērtēt novietnē izmantojamo laterālās plūsmas testu (LFD) pielietojamību kā papildu diagnostikas rīku ĀCM uzliesmojumu izmeklēšanā un salīdzināt to veikspēju ar standarta laboratoriskajām diagnostikas metodēm, tostarp PQR, IPT un ELISA (III).
- 4) Identificēt un analizēt galvenos epidemioloģiskos parametrus, kas nepieciešami ĀCM uzliesmojumu augsta riska perioda (HRP) rekonstrukcijai skartajās cūku novietnēs (I, II).
- 5) Izvērtēt Latvijā izmantotās uzraudzības pieejas attiecībā uz to efektivitāti ĀCM uzliesmojumu agrīnā atklāšanā (I, II).

3. MATERIĀLI UN METODES

3.1. Pētījuma dizains un pētījuma vietas

Laikposmā no 2017. līdz 2020. gadam Latvijā tika veikti četri savstarpēji saistīti epidemioloģiskie pētījumi komerciālās cūku novietnēs, kurās bija konstatēts Āfrikas cūku mēris (ĀCM). Pētījumi bija vērsti uz ĀCM vīrusa iekļūšanas ceļu noteikšanu, agrīnu uzliesmojumu atklāšanu, augsta riska perioda (HRP) rekonstrukciju, vīrusa izplatību novietnes ietvaros, paraugu ņemšanas stratēģijām, ātrajām diagnostikas metodēm un epidemioloģiski nozīmīgas lauka apstākļos iegūtas informācijas interpretāciju.

I pētījumā tika analizēti ĀCM vīrusa iekļūšanas ceļi un agrīna slimības atklāšana komerciālā pilna cikla cūku novietnē Latvijas centrālajā daļā, kurā tika turētas aptuveni 5000 cūkas.

II pētījumā tika vērtēta augsta riska perioda (HRP) noteikšana un ĀCM vīrusa izplatība divās lielās komerciālās pilna cikla cūku novietnēs. Vienā no novietnēm, kas atradās Latvijas dienvidaustrumu daļā, tika turētas aptuveni 6000 cūkas, savukārt otrā novietnē Latvijas dienvidrietumu daļā – aptuveni 16 000 cūkas.

III pētījums bija veltīts paraugu ņemšanas stratēģijām un to efektivitātei ĀCM skartās cūku novietnēs. Pētījumā tika iekļautas trīs komerciālas pilna cikla cūku novietnes Latvijas dienvidrietumu daļā ar attiecīgi aptuveni 31, 1800 un 9000 cūkām.

IV pētījumā tika analizēta nespeciālistu sniegtās ar slimību saistītās informācijas nozīme ĀCM uzliesmojumu izmeklēšanā vairākās Eiropas valstīs, tostarp Latvijā.

Uzliesmojumu apstiprināšanas laikā visas pētījumā iekļautās novietnes atradās teritorijās, kurās bija noteikti ar ĀCM saistīti ierobežojumi saistībā ar vīrusa cirkulāciju mēžacūku populācijā (EC, 2014; Oļševskis et al., 2020; EFSA, 2021).

3.2. ĀCM uzliesmojuma apstiprināšana un izmeklēšanas uzsākšana

Visas lauka izmeklēšanas tika uzsāktas nekavējoties pēc tam, kad ĀCM uzliesmojumu bija oficiāli apstiprinājis Pārtikas un veterinārais dienests (PVD). Saskaņā ar Latvijas normatīvajiem aktiem cūku īpašniekiem vai turētājiem ir pienākums ziņot par aizdomām par ĀCM infekciju praktizējošam veterinārārstam vai PVD (MK, 2004).

Pēc paziņojuma saņemšanas oficiālie veterinārārsti apmeklēja attiecīgo novietni un laboratoriskajiem izmeklējumiem noņēma asins paraugus (EDTA asinis un asins serumu) un audu paraugus (liesu un limfmezglus), lai apstiprinātu vai izslēgtu ĀCM infekciju.

Ja laboratoriskajos izmeklējumos tika konstatēts ĀCM vīrusa genoms vai ĀCM specifiskas antivielas, dzīvnieks tika uzskatīts par inficētu un PVD oficiāli apstiprināja uzliesmojumu.

Turpmākās izmeklēšanas tika veiktas saskaņā ar spēkā esošajiem Eiropas Savienības un Latvijas normatīvajiem aktiem, kas reglamentē ĀCM uzliesmojumu izmeklēšanu un slimības apkarošanas pasākumus (EC, 2002; EC, 2016).

3.3. Epidemioloģiskā uzliesmojumu izmeklēšana un datu vākšana

Pēc ĀCM uzliesmojuma apstiprināšanas nekavējoties tika uzsākta epidemioloģiskā izmeklēšana. Atkarībā no epidemioloģiskās situācijas skartajā novietnē izmeklēšana ietvēra vienu vai vairākus apmeklējumus uz vietas.

Lai iegūtu un pārbaudītu epidemioloģiski nozīmīgu informāciju, tika veiktas intervijas ar novietņu īpašniekiem, veterinārārstiem, zootehniķiem, transportlīdzekļu vadītājiem un citiem darbiniekiem, kuri bija iesaistīti cūku aprūpē vai novietnes darbības nodrošināšanā. Papildus tika izvērtētas novietņu biodrošības sistēmas un saimniekošanas procedūras.

Tika pārskatīti un analizēti dati par dzīvnieku pārvietošanu, mirstību, ārstēšanu, kritušo dzīvnieku izvešanu, cūku pārvietošanu novietnes ietvaros, kā arī laboratorisko izmeklējumu rezultāti.

Epidemioloģiskās izmeklēšanas un laboratorisko izmeklējumu vajadzībām papildus tika ņemti paraugi no nobeigušām cūkām vai no dzīvām cūkām pirms to likvidēšanas.

3.3.1. Hipotēzēs balstīta ĀCM vīrusa iekļūšanas ceļu izmeklēšana

Lai atvieglotu un strukturētu iespējamo ĀCM vīrusa iekļūšanas ceļu izmeklēšanu, I pētījuma ietvaros tika izstrādāta un praktiski pielietota hipotēzēs balstīta epidemioloģiskās izmeklēšanas pieeja. Balstoties uz zināmajiem ĀCM transmisijas ceļiem, tika formulētas trīs darba hipotēzes par iespējamajiem vīrusa iekļūšanas ceļiem: mežacūku hipotēze, gaļas/kontaminētu priekšmetu hipotēze un tirdzniecības hipotēze.

Katra hipotēze tika izvērtēta atsevišķi, analizējot epidemioloģisko, laboratorisko, saimniekošanas un biodrošības informāciju, lai izslēgtu maz ticamos transmisijas ceļus vai identificētu ticamāko vīrusa iekļūšanas ceļu. Izvērtēšana ietvēra novietnes infrastruktūras, nožogojumu, transportlīdzekļu, aprikojuma higiēnas procedūru, iekšējo darba plūsmu, personāla pārvietošanās, barības un pārtikas aprites prakses, kā arī dzīvnieku un dažādu materiālu pārvietošanas analīzi.

3.3.2. Augsta riska perioda (HRP) rekonstrukcija

Augsta riska perioda (HRP) rekonstrukcija tika veikta, lai noteiktu iespējamo laika posmu, kurā ĀCM vīruss bija cirkulējis novietnē pirms uzliesmojuma atklāšanas. HRP noteikšanai tika izstrādātas epidemioloģiski nozīmīgu notikumu laika līnijas, izmantojot saslimstības un mirstības datus, pēcnāves izmeklēšanas rezultātus, laboratorisko izmeklējumu rezultātus, klīniskos novērojumus, dzīvnieku pārvietošanas uzskaites datus, informāciju par kūstmēslu izvešanu, datus par cūku pārvietošanu novietnes ietvaros un ar biodrošību saistītos novērojumus.

Lai rekonstruētu iespējamo vīrusa izplatību novietnēs, tika apkopoti, analizēti un interpretēti klīniskie novērojumi, kā arī vīrusa genoma un antivielu noteikšanas rezultāti (ELISA un IPT), kas iegūti no dzīvām un nobeigušām cūkām dažādos aizgaldos un kūtīs. Īpaša uzmanība tika pievērsta epidemioloģiski nozīmīgiem notikumiem, kas saistīti ar personāla higiēnu, pārtikas lietošanu novietnē, transporta aktivitātēm un novietnes iekšējām pārvaldības procedūrām.

3.3.3. Vīrusa izplatības novērtēšana novietnes ietvaros

ĀCM vīrusa izplatības novērtēšana skartajās novietnēs bija vērsta uz inficēto epidemioloģisko vienību identificēšanu un infekcijas izplatības rakstura izvērtēšanu novietnes ietvaros. Tika izvērtētas un analizē iekļautas šādas klīniskās un patoloģiski anatomiskās pazīmes, kas var liecināt par iespējamu ĀCM infekciju: paaugstināta ķermeņa temperatūra un drudzis, ēstgribas zudums, aborti, caureja, vemšana ar asinīm, asinsizplūdumi ādā un iekšējos orgānos, palielināti vai hemorāģiski limfmezgli, kā arī liesas un nieru bojājumi.

Infekcijas izplatība starp kūtim, aizgaldiņiem un ražošanas vienībām tika vērtēta, izmantojot klīnisko novērojumu datus, mirstības rādītājus, laboratorisko izmeklējumu rezultātus un informāciju par cūku pārvietošanu novietnes ietvaros. Izmeklēšanas gaitā tika izvērtēts arī tas, vai vīrusa izplatība notikusi vienmērīgi visā novietnē vai arī infekcija bijusi ierobežota atsevišķās epidemioloģiskajās vienībās.

3.3.4. Nespeciālistu sniegtās informācijas iegūšana un interpretācija

IV pētījumā tika analizēta nespeciālistu sniegtās epidemioloģiski nozīmīgās informācijas loma ĀCM uzliesmojumu izmeklēšanā. No skarto novietņu īpašniekiem, darbiniekiem, veterinārārstiem un citām novietnes darbībā iesaistītām personām tika iegūta informācija par iespējamiem ĀCM vīrusa iekļūšanas ceļiem un iespējamo vīrusa iekļūšanas laiku cūku ganāmpulkā.

Respondentu izteiktie viedokļi un pieņēmumi tika uzskatīti par subjektīvu informāciju un klasificēti atbilstoši to epidemioloģiskajai ticamībai: ticami un tālāk izmekļējami, maz ticami, kā arī maz ticami, bet pilnībā neizslēdzami.

Iegūtā informācija tika interpretēta kopā ar epidemioloģiskās, laboratoriskās, klīniskās un biodrošības izmeklēšanas rezultātiem.

3.4. Paraugu ņemšanas stratēģija

3.4.1. Mērķtiecīga paraugu ņemšanas pieeja

Praugu ņemšana laboratoriskajiem izmeklējumiem tika veikta visās pētījumos iekļautajās ĀCM skartajās cūku novietnēs. Lai novērtētu ĀCM vīrusa izplatību novietnes ietvaros un rekonstruētu iespējamās transmisijas ceļus, no cūkām dažādās kūtīs un epidemioloģiskajās vienībās tika ņemti asins paraugi (EDTA asinis un asins serums) un audu paraugi (liesa un limfmezgli). Pētījumos tika izmantota mērķtiecīga izlases veida paraugu ņemšana. Paraugi galvenokārt tika ņemti no klīniski slimiem dzīvniekiem, nobeigušām cūkām, kā arī no dzīvniekiem, kuriem bija tiešs epidemioloģiskais kontakts ar inficētiem vai iespējami inficētiem dzīvniekiem. Mērķtiecīga paraugu ņemšana bija vērsta uz aizdomīgu, slimu un nobeigušos dzīvnieku atlasīšanu laboratoriskajiem izmeklējumiem.



3.1 attēls. Klīniski slimu cūku piemēri, kas identificēti mērķtiecīgas uzliesmojuma izmeklēšanas laikā ĀCM skartās novietnēs: klīniski slimas sivēnmātes ar asinsizplūdumiem ausīs un ēstgribas zudumu (foto: K. Lamberga)



3.2 attēls. **Klīniski slimu cūku piemēri, kas identificēti mērķtiecīgas uzliesmojuma izmeklēšanas laikā ĀCM skartās novietnēs: klīniski slimas sivēnmātes, abortēti augļi un ĀCM inficētas cūkas palielināta liesa** (foto: K. Lamberga)

3.4.2. Epidemioloģisko vienību kategorizācija

III pētījuma ietvaros tika izstrādāta un pielietota strukturēta trīs posmu paraugu ņemšanas stratēģija, kas ietvēra epidemioloģisko apakšvienību identificēšanu, apakšvienību kategorizēšanu atbilstoši infekcijas iespējamībai un mērķtiecīgu dzīvnieku atlasīšanu laboratoriskajiem izmeklējumiem.

Katrai epidemioloģiskajai apakšvienībai tika piešķirta kategorija atbilstoši iespējamībai, ka tajā cirkulē ĀCM vīruss. Kategorizācija tika veikta, ņemot vērā klīniskos novērojumus, mirstības rādītājus, epidemioloģiskās saiknes un iepriekš iegūtos laboratorisko izmeklējumu rezultātus.

Pirms mērķtiecīgas dzīvnieku atlasīšanas paraugu ņemšanai visās epidemioloģiskajās apakšvienībās tika veikta cūku klīniskā apskate. Paraugi tika ņemti no visām cūkām ar klīniskām slimības pazīmēm, kā arī no dzīvniekiem, kuriem bija tiešs kontakts ar klīniski slimām cūkām.

3.4.3. Klīniskās vērtēšanas sistēma

III pētījuma ietvaros tika izstrādāta un izmantota klīniskās vērtēšanas sistēma klīnisko pazīmju izvērtēšanai un ātro novietnē izmantojamo diagnostisko testu rezultātu interpretācijai. Paraugu ņemšanai atlasītās cūkas tika klasificētas atbilstoši klīnisko pazīmju smaguma pakāpei šādās kategorijās: bez klīniskām pazīmēm, vieglas klīniskās pazīmes, smagas klīniskās pazīmes un nobeigušās cūkas.

Laboratorisko izmeklējumu un ātro diagnostisko testu rezultāti tika analizēti un salīdzināti ar piešķirtajiem klīniskajiem vērtējumiem.

3.5. Paraugu sagatavošana un PĶR analīze

Visi EDTA asiņu un audu paraugi tika uzglabāti, sagatavoti un apstrādāti saskaņā ar Nacionālās references laboratorijas standarta operāciju procedūrām, izmantojot laminārās plūsmas drošības kabinetu.

Orgānu paraugi, kas paredzēti nukleīnskābju noteikšanai, tika mehāniski homogenizēti pēc atšķaidīšanas fosfātu buferšķīdumā (PBS) attiecībā 1:10.

Vīrusa DNS ekstrakcija tika veikta, izmantojot automatizēto nukleīnskābju izolēšanas sistēmu NucliSENS® easyMAG® (bioMérieux, Francija) saskaņā ar ražotāja norādījumiem. Ekstrakcijas kvalitātes kontrolei tika izmantota heteroloģā iekšējās kontroles DNS (intype IC-DNA; QIAGEN, Vācija), kā aprakstīts Hoffmann et al. (2006).

Polimerāzes ķēdes reakcija (PĶR) ĀCM vīrusa genoma noteikšanai tika veikta saskaņā ar Eiropas Savienības references laboratorijas ĀCM diagnostikas protokolu (Fernández-Pinero et al., 2013).

Rezultāti tika reģistrēti kā kvantifikācijas cikla (Ct) vērtības un interpretēti kā pozitīvi vai negatīvi atbilstoši iegūtajām Ct vērtībām.

3.6. Seroloģiskie izmeklējumi (ELISA un IPT)

Seroloģiskie izmeklējumi tika veikti, lai noteiktu ĀCM vīrusam specifiskas antivielas. Asins seruma paraugu izmeklēšanai saskaņā ar ražotāja norādījumiem tika izmantots komerciāli pieejamais bloķējošās ELISA tests Ingezim PPA Compac (11.PPA.K.3, Ingenasa, Madride, Spānija).

Papildus seruma vai orgānu šķidruma paraugiem tika veikta netiešā imūnperoksidāzes metode (IPT) saskaņā ar Eiropas Savienības references laboratorijas ĀCM diagnostikas protokolu (European Union Reference Laboratory for ASF, 2018). IPT tika izmantota zemu antivielu titru noteikšanai, kurus ar ELISA metodi nebija iespējams identificēt.

Epidemioloģiskās izmeklēšanas laikā tika veikta virusoloģisko un seroloģisko izmeklējumu rezultātu kombinēta interpretācija. ĀCM vīrusa genoma noteikšana ar kvantitatīvo polimerāzes ķēdes reakciju (qPĶR) kopā ar pozitīvu IPT un negatīvu ELISA rezultātu nobeigušām cūkām tika interpretēta kā nesenās infekcijas pazīme, kas, visticamāk, bija notikusi aptuveni 10 dienas pirms paraugu ņemšanas.

3.7. Ātrie laterālās plūsmas testi (LFD)

III pētījuma ietvaros tika novērtēta ātro laterālās plūsmas testu (LFD) pielietojamība uzliesmojumu apstākļos lauka pētījumos. Tika izmantoti divu veidu LFD testi: antigēna LFD ĀCM vīrusa antigēna noteikšanai asins paraugos un antivielu LFD ĀCM vīrusam specifisku antivielu noteikšanai.

Antigēna noteikšanai tika izmantots INgezim PPA CROM Ag LFD tests (11.ASF.K.42, Ingenasa, Madride, Spānija), savukārt antivielu noteikšanai tika izmantots atbilstošais INgezim PPA CROM Ab LFD tests (11.PPA.K.41, Ingenasa, Madride, Spānija). Abi testi tika veikti saskaņā ar ražotāja norādījumiem.

Lauka apstākļos iegūtie rezultāti tika salīdzināti ar standarta laboratorisko diagnostikas metožu rezultātiem, tostarp kvantitatīvās polimerāzes ķēdes reakcijas (qPĶR), ELISA un IPT rezultātiem.



3.3 attēls. Ātrā laterālās plūsmas testa (LFD) izmantošana lauka apstākļos ĀCM uzliesmojuma izmeklēšanas laikā (foto: M. Seržants)

3.8. Statistiskā analīze

Antigēna LFD testu diagnostiskā jutība un specifiskums tika aprēķināti katrai III pētījumā iekļautajai novietnei atsevišķi, izmantojot qPQR rezultātus kā references metodi. Jutība tika aprēķināta pēc formulas: **jutība = patiesi pozitīvi / (patiesi pozitīvi + viltus negatīvi)**. Savukārt specifiskums tika aprēķināts pēc formulas: **specifiskums = patiesi negatīvi / (patiesi negatīvi + viltus pozitīvi)**.

Jutības un specifiskuma novērtējumu ticamības intervāli tika aprēķināti, izmantojot Epitools epidemioloģiskos kalkulatorus (Sergeant, 2018).

Lai novērtētu saistību starp klīniskajiem vērtējumiem un laboratorisko izmeklējumu rezultātiem, tika veikta Spīrmēna korelācijas analīze, izmantojot statistiskās analīzes programmatūru XLSTAT Microsoft Excel videi (Addinsoft, 2019).

Visā izmeklēšanas procesā tika izmantota klīnisko, epidemioloģisko, virusoloģisko un seroloģisko rezultātu kombinēta interpretācija, lai rekonstruētu uzliesmojumu attīstības gaitu un pamatotu epidemioloģisko lēmumu pieņemšanu.

4. REZULTĀTI

4.1. ĀCM vīrusa iekļūšanas ceļu izmeklēšana un augsta riska perioda (HRP) rekonstrukcija

ĀCM pētījumā iekļautajā novietnē tika apstiprināts 2017. gada janvāra vidū. Dzīvnieki tika turēti septiņās atsevišķās kūtīs. Kūtī, kurā tika identificētas pirmās klīniski slimās cūkas, atradās grupās turētas grūsnas sivēnmātes. Retrospektīvi izvērtējot pēcnāves izmeklēšanas datus, tika konstatēts, ka pirmā nobeigusies sivēnmāte bija reģistrēta jau 2016. gada 28. decembrī. Vēl četras cūkas saslima 4. janvārī un nobeidzās divas dienas vēlāk. Turpmākas nobeigušās sivēnmātes tika konstatētas 6., 9., 11., 12. un 13. janvārī.

Tikmēr vairākas sivēnmātes 9. janvārī tika pārvietotas uz citu kūti atnešanās vajadzībām, un divas no šīm cūkām tika atrastas nobeigušās 12. un 13. janvārī, kas liecināja par iespējamu vīrusa ievazāšanu atnešanās sektorā, pārvietojot inficētus dzīvniekus. Pirmais nobeigušais vaislinieks atsevišķā vaislas dzīvnieku vienībā tika konstatēts 7. janvārī, bet nākamais – 14. janvārī. Pirmie laboratoriskie izmeklējumi ĀCM vīrusa noteikšanai tika veikti cūkām, kas nobeidzās 13. janvārī. Uzliesmojuma apstiprināšanas brīdī jau bija nobeigušās 17 sivēnmātes. Tika konstatēts, ka darbinieks, kura pienākumos ietilpa mākslīgā apsēklošana, ikdienā strādāja visās trīs skartajās kūtīs.

Lai atbalstītu uzliesmojuma rekonstrukciju un augsta riska perioda (HRP) novērtēšanu, tika izveidota laika līnija, kurā apvienoti dati par saslimstību, mirstību, pēcnāves izmeklēšanas rezultātiem, cūku pārvietošanas uzskaiti un laboratorisko izmeklējumu rezultātiem. No 75 izmeklētajiem dzīvniekiem ĀCM vīrusa genoms tika konstatēts 23 cūkām, savukārt nevienam no izmeklētajiem dzīvniekiem netika konstatētas antivielas pret ĀCM vīrusu. Antivielu neesamība liecināja par akūtu infekcijas norisi. Pamatojoties uz iegūto epidemioloģisko un laboratorisko informāciju, tika secināts, ka vīruss novietnē bija iekļuvis aptuveni sešas nedēļas pirms uzliesmojuma apstiprināšanas.

Novērotā vīrusa izplatība novietnē infekcijas sākumposmā bija lēna. Pēc uzliesmojuma apstiprināšanas no cūkām skartajā sivēnmāšu vienībā tika noņemti vēl 50 paraugi, un tikai četrām cūkām tika konstatēts ĀCM vīrusa genoms.

Novietnes biodrošības izvērtējums liecināja, ka infrastruktūra kopumā bija labi attīstīta. Novietnes teritorija bija iežogota, visi transportlīdzekļi, kas iebruca teritorijā, šķērsoja sanitārās kontroles punktus, un darbiniekiem bija pieejams speciāls darba apģērbs un apavi. Tomēr pie ieejām cūku turēšanas telpās nebija ierīkotas sanitārās caurlaides. Epidemioloģiskā izmeklēšana un intervijas atklāja vairākas nepilnības biodrošības prasību īstenošanā. Ģērbtuvju un atpūtas telpu izvietojums nenodrošināja efektīvu tīro un netīro zonu nodalīšanu. Darbiniekiem bija atļauts ienest pārtiku novietnes teritorijā un lietot to ražošanas zonā. Turklāt dokumentācija par transportlīdzekļu dezinfekciju ziemas periodā bija nepilnīga.

Lai izvērtētu iespējamās ĀCM vīrusa iekļūšanas ceļus, tika izmantota hipotēzēs balstīta epidemioloģiskās izmeklēšanas pieeja. Tika izvērtētas trīs iespējamās vīrusa iekļūšanas hipotēzes: mežacūku hipotēze, gaļas/kontaminētu priekšmetu hipotēze un tirdzniecības hipotēze.

Mežacūku hipotēze tika uzskatīta par maz ticamu, jo cūkas tika turētas slēgtās telpās, novietnes teritorija bija iežogota un netika konstatēta ne tieša, ne netieša saskare ar mežacūkām. Novietnē netika izmantoti pakaiši vai barība, kas varētu būt bijuši kontaktā ar mežacūkām, un neviens no novietnes darbiniekiem nepiedalījās medībās.

Par ticamāko vīrusa iekļūšanas ceļu tika atzīta gaļas/kontaminētu priekšmetu hipotēze. Epidemioloģiskajā izmeklēšanā tika konstatētas nepietiekamas personāla higiēnas barjeras, kā arī iespēja, ka novietnē tika ienesti kontaminēti gaļas produkti, kas vēlāk nonāca cūku turēšanas zonās. Papildus tam iepriekšējo mēnešu laikā novietnē bija vērojama ievērojama darbinieku mainība, kas radīja bažas par informētību attiecībā uz ĀCM ievazāšanas riskiem un biodrošības prasību ievērošanu.

Tirdzniecības hipotēze tika izslēgta, jo iepriekšējo 12 mēnešu laikā novietnē netika ieviesta neviena cūka. Tomēr aprēķinātā HRP laikā sivēni no skartās vaislas novietnes tika pārvietoti uz tam pašam īpašniekam piederošu cūku nobarošanas novietni. Vēlāk šajā novietnē tika apstiprināts ĀCM uzliesmojums, kas tika klasificēts kā sekundārs uzliesmojums.

Epidemioloģisko izmeklēšanu laikā Latvijā un vairākās citās ĀCM skartajās Eiropas valstīs tika apkopota un izvērtēta lauksaimnieku, novietņu darbinieku, veterinārārstu un citu novietnes darbībā iesaistīto personu sniegtā informācija un viedokļi par iespējamajiem ĀCM vīrusa iekļūšanas ceļiem. Kopumā epidemioloģiskās nozīmības ziņā tika analizēts 31 apgalvojums par iespējamajiem vīrusa iekļūšanas ceļiem.

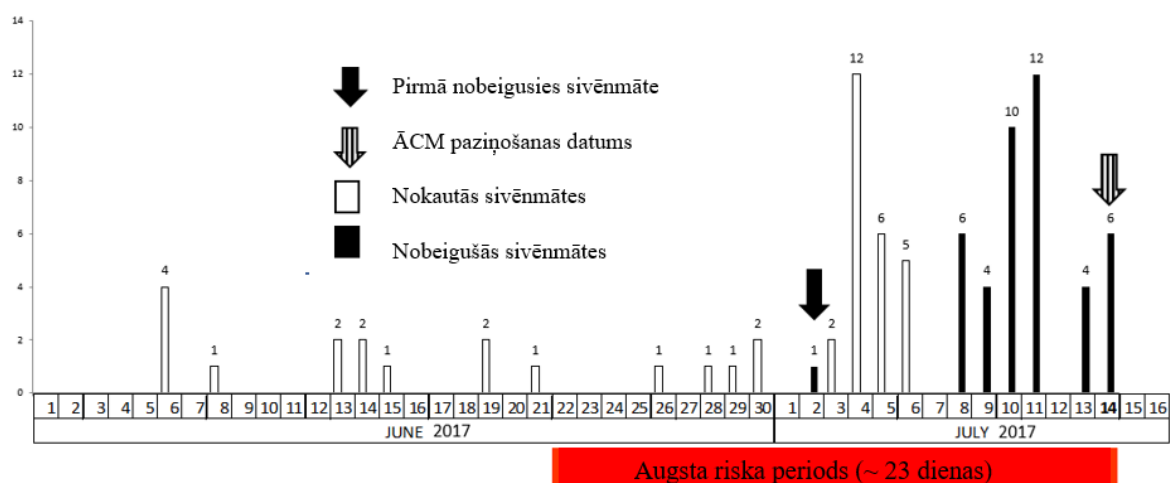
Daudzi no šiem apgalvojumiem tika atzīti par epidemioloģiski nozīmīgiem un prasīja turpmāku izmeklēšanu, savukārt citi tika nekavējoties izslēgti kā maz ticami. Būtiska daļa apgalvojumu (n = 13) tika klasificēti kā maz ticami, bet pilnībā neizslēdzami. Pētījuma rezultāti parādīja, ka šādas subjektīvas informācijas interpretācijai nepieciešamas epidemioloģiskās zināšanas, pieredze uzliesmojumu izmeklēšanā un strukturēta komunikācijas pieeja.

Lai padziļināti izpētītu uzliesmojumu attīstības gaitu, divās citās ĀCM skartās komerciālās cūku novietnēs (A un B novietne) tika veiktas papildu epidemioloģiskās izmeklēšanas, koncentrējoties uz augsta riska perioda (HRP) rekonstrukciju un vīrusa izplatības novērtēšanu skartajās novietnēs.

A novietnē ĀCM tika apstiprināts 2017. gada 14. jūlijā. Novietni veidoja septiņas slēgta tipa kūtis. Smagas klīniskās pazīmes, tostarp drudzis, aborti un vemšana, tika novērotas tikai vienā kūtī, kurā grupās tika turētas grūsnas sivēnmātes. Saskaņā ar anamnēzes datiem pirmā nobeigusies sivēnmāte pēc aborta tika konstatēta jau 2. jūlijā. Laikposmā no 2. jūlija līdz uzliesmojuma apstiprināšanai nobeidzās 43 sivēnmātes. Vēl 25 sivēnmātes veselības problēmu dēļ tika nosūtītas uz kautuvi.

Slimība izplatījās arī uz atnešanās kūti pēc tam, kad no skartās vienības uz to tika pārvietotas inficētas sivēnmātes. Atnešanās vienībā nobeigušās sivēnmātes tika konstatētas 7., 8. un 9. jūlijā.

A novietnē rekonstruētā epidemioloģiski nozīmīgo notikumu laika līnija un novērtētais HRP ir attēloti 4.1. attēlā.



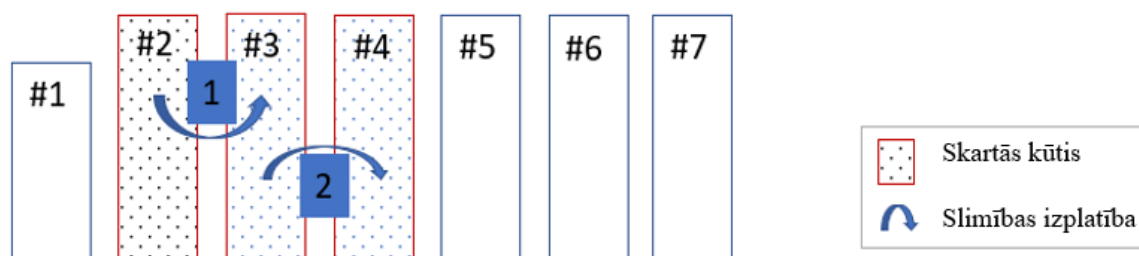
*Jūnijā periodiski tika kautas sivēnmātes, un pastiprināta kaušana novērota no 4.jūlija, bet nav pierādāma saikne ar ĀCM

4.1 attēls. Epidemioloģiski nozīmīgo notikumu laika līnija saimniecībā A (kūtī Nr. 2) un novērtētais augsta riska periods (HRP)

A novietnē par indeksgadījumu tika uzskatīta sivēnmāte, kas nobeidzās 2. jūlijā. Pieņemot, ka vidējais izdzīvošanas periods pēc inficēšanās ir aptuveni 10 dienas, tika

aprēķināts, ka vīruss novietnē, visticamāk, iekļuva ap 22. jūniju. Tādējādi novērtētais augsta riska periods (HRP) bija aptuveni 23 dienas.

Tika pieņemts, ka līdz uzliesmojuma atklāšanai bija notikuši vairāk nekā divi infekcijas cikli. ĀCM vīrusa iespējamā izplatība starp kūtīm A novietnē ir attēlota 4.2. attēlā.



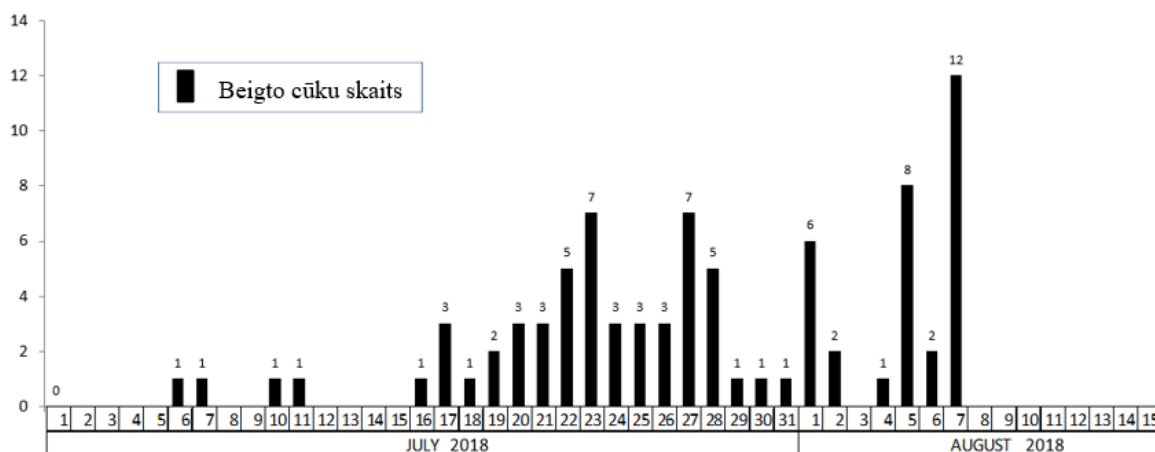
4.2 attēls. Iespējamā ĀCM vīrusa izplatība starp kūtīm novietnē A

B novietnē ĀCM tika apstiprināts 2018. gada 31. jūlijā. Novietne sastāvēja no divām ģeogrāfiski atdalītām ražošanas vietām (lokācijām). Uzliesmojuma konstatēšanas laikā novietnē jau bija ieviesta pastiprinātā pasīvā uzraudzība.

Trīs nobeigušies atšķirti sivēni, kas tika konstatēti 29. jūlijā, tika izmeklēti uz ĀCM, un PQR rezultāti bija pozitīvi. Šie dzīvnieki iepriekš, 23. jūlijā, kopā ar vēl 277 atšķirtiem sivēniem bija pārvietoti no ražošanas vietas B1 uz ražošanas vietu B2.

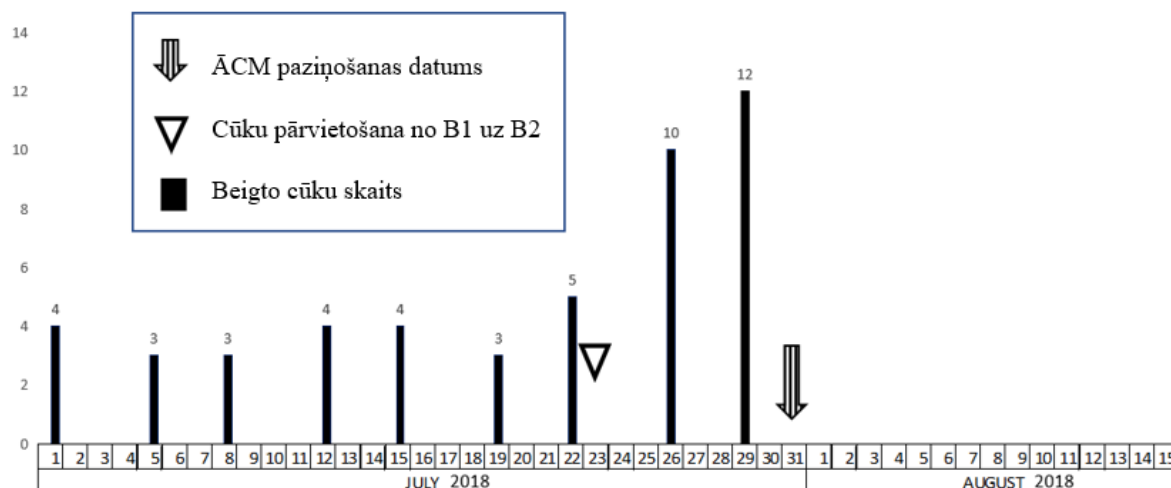
Pēc pārvietošanas ražošanas vietā B2 tika novērota paaugstināta mirstība, savukārt pārējām cūkām izcelsmes vienībā paaugstināta mirstība netika konstatēta. Vienlaikus neliels mirstības pieaugums tika novērots citā kūtī ražošanas vietā B1 jau kopš jūlija vidus.

Mirstības dati no B1 ražošanas vietas 4. kūts, kas tika izmantoti augsta riska perioda (HRP) rekonstrukcijā, ir attēloti 4.3. attēlā.



4.3 attēls. Mirstības dinamika kūtī Nr. 4, ražošanas vietā B1

B2 ražošanas vietā rekonstruētā epidemioloģiski nozīmīgo notikumu laika līnija ir attēlota 4.4. attēlā.



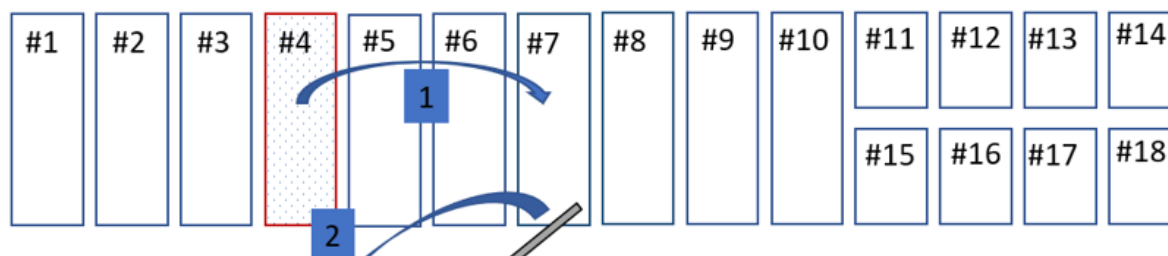
4.4 attēls. Epidemioloģiski nozīmīgo notikumu laika līnija novietnē, kurā konstatētas pirmās ĀCM pozitīvās cūkas (ražošanas vieta B2)

B novietnē par indeksgadījumiem tika uzskatīti sivēni, kas tika atrasti nobeigušies 29. jūlijā. Pamatojoties uz virusoloģisko un seroloģisko izmeklējumu rezultātu kombinētu interpretāciju, tika novērtēts, ka augsta riska periods (HRP) bija aptuveni divas nedēļas.

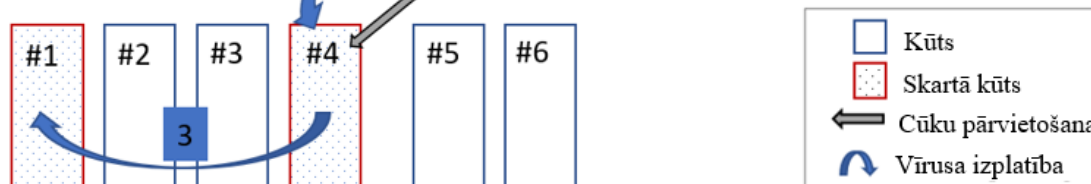
Tā kā cūkām tika konstatēti pozitīvi gan PQR, gan antivielu noteikšanas rezultāti, tika pieņemts, ka inficēšanās notikusi 7 - 10 dienas pirms dzīvnieku nobeigšanās. Līdz ar to novērtētais HRP bija laikposmā no 18. līdz 31. jūlijam.

ĀCM vīrusa iespējamā izplatība starp kūtīm un ražošanas vietām B novietnē ir attēlota 4.5. attēlā.

Ražošanas vienība B1



Ražošanas vienība B2



4.5 attēls. Iespējamā ĀCM vīrusa izplatība novietnē B

Dzīvnieku pārvietošanas uzskaites dati un laboratorisko izmeklējumu rezultāti liecināja, ka infekcija sākotnēji attīstījās B1 ražošanas vietas 4. kūtī un vēlāk izplatījās uz B2 ražošanas vietu, pārvietojot inficētus atšķirtos sivēnus. Savukārt turpmākā infekcijas izplatība starp kūtīm visdrīzāk notika ar kontaminētu priekšmetu starpniecību.

4.2. ĀCM vīrusa izplatības novērtēšana cūku novietnēs un paraugu ņemšanas stratēģijas

ĀCM vīrusa izplatības novērtēšana skartajās cūku novietnēs parādīja neviendabīgu infekcijas izplatību starp epidemioloģiskajām vienībām. Lai gan starp dažādām ražošanas vienībām pastāvēja epidemioloģiskas saiknes, vairākās vienībās infekcija netika konstatēta pat ilgstoša augsta riska perioda laikā.

Pēc uzliesmojumu apstiprināšanas A un B novietnēs tika veiktas papildu epidemioloģiskās izmeklēšanas un mērķtiecīga paraugu ņemšana, lai novērtētu ĀCM vīrusa izplatības apmēru skartajās novietnēs. Pirms dzīvnieku likvidēšanas paraugi tika ņemti no cūkām dažādās kūtīs un epidemioloģiskajās vienībās. Iegūtie laboratorisko izmeklējumu rezultāti ļāva identificēt skartās un neskartās vienības novietņu ietvaros.

A novietnē pirms depopulācijas paraugi tika ņemti no cūkām visās septiņās kūtīs. Par epidemioloģiski skartām tika atzītas trīs no septiņām kūtīm. ĀCM vīrusa genoms galvenokārt tika konstatēts kūtīs, kurās tika turētas sivēnmātes, savukārt lielākajā daļā nobarošanas vienību laboratorisko izmeklējumu rezultāti bija negatīvi. Virusoloģisko un seroloģisko izmeklējumu rezultātu sadalījums starp dažādām A novietnes kūtīm ir attēlots 4.1. tabulā.

4.1. tabula

PĶR un seroloģisko izmeklējumu rezultāti A novietnes cūkām, no kurām paraugi ņemti dažādās kūtīs pirms dzīvnieku likvidēšanas

Kūts numurs	Cūku skaits kūtī	Cūku kategorija	Kūti ņemto paraugu skaits	Laboratorisko testu rezultāti	
				PĶR pozitīvi	Seroloģiski (ELISA, IPT) pozitīvi
1	213	Kuīli, sivēnmātes	3	0	0
2	301	Sivēnmātes *	4	4	2
3	696	Sivēnmātes **	28	10	0
4	1761	Atšķirtie sivēni	28	1	0
5	1157	Nobarojamās	29	0	0
6	1136	Nobarojamās	28	0	0
7	554	Nobarojamās	20	0	0
Kopā	5818	N/A	140	15	2

* Sivēnmātes grupu aizgaldos ** Sivēnmātes ar sivēniem

Pētījuma rezultāti apliecināja, ka ĀCM vīrusa izplatība novietnēs nenotika vienmērīgi. A novietnē epidemioloģiskā izmeklēšana liecināja, ka infekcija sākotnēji attīstījās 2. kūtī un vēlāk izplatījās uz atnešanās vienību pēc inficētu sivēnmāšu pārvietošanas. Līdzīgi arī B novietnē dzīvnieku pārvietošanas uzskaites dati un laboratorisko izmeklējumu rezultāti liecināja, ka infekcija sākotnēji attīstījās B1 ražošanas vietas 4. kūtī un vēlāk izplatījās uz B2 ražošanas vietu, pārvietojot inficētus atšķirtos sivēnus. Savukārt turpmākā infekcijas izplatība starp kūtīm visdrīzāk notika ar kontaminētu priekšmetu starpniecību.

Neraugoties uz epidemioloģisko saikņu pastāvēšanu starp ražošanas vienībām, vairākas kūtis saglabājās brīvas no infekcijas. ĀCM vīrusa iespējamā izplatība starp ražošanas vienībām A un B novietnēs ir attēlota attiecīgi 4.2. un 4.5. attēlā.

Lai detalizētāk izvērtētu infekcijas izplatības raksturu novietnes ietvaros un uzlabotu infekcijas izplatības novērtēšanu, III pētījumā tika izmantota mērķtiecīga paraugu ņemšanas stratēģija, kuras pamatā bija novietņu sadalīšana epidemioloģiskajās apakšvienībās. Visas pētījumā iekļautās novietnes tika sadalītas epidemioloģiskajās apakšvienībās, kuras pēc tam tika klasificētas atbilstoši ĀCM infekcijas iespējamībai.

Pirms paraugu ņemšanas visās epidemioloģiskajās apakšvienībās tika veikta cūku klīniskā apskate. Epidemioloģisko apakšvienību kategorizācija un tai atbilstošie laboratorisko izmeklējumu rezultāti ir apkopoti 4.2. tabulā.

4.2. tabula

Laboratorisko izmeklējumu rezultātu pārskats atbilstoši novietņu epidemioloģisko apakšvienību kategorijām

Fermas apakšvienības kategorija	Testēto dzīvnieku skaits	Ag-LFD+	PQR+ Ct<18	PQR+ Ct>18	Ab-LFD+	ELISA+	IPT+
A NOVIETNE							
1	21	3	2	3	2	2	3
1	6	0	0	0	0	0	0
B NOVIETNE, B1 RAŽOŠANAS VIENĪBA							
1	4	3	3	0	0	0	0
2	11	1	1	4	0	0	1
3	5	1	1	0	0	0	1
B NOVIETNE, B2 RAŽOŠANAS VIENĪBA							
3	15	1	2	4	0	0	4
C NOVIETNE							
1	11	7	7	4	0	0	6
2	4	0	0	0	0	nd	nd
3	1	0	0	0	0	nd	nd
4	18	1	1	1	0	0	0

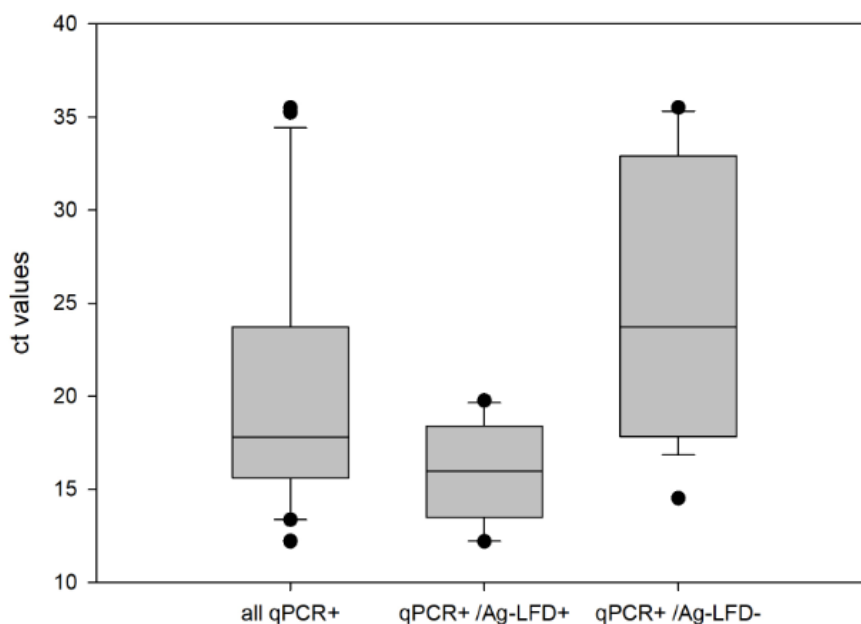
Mērķtiecīgās paraugu ņemšanas stratēģijas izmantošana ļāva sekmīgi identificēt inficētās epidemioloģiskās vienības skartajās novietnēs. A novietnē inficētas cūkas tika konstatētas vienā epidemioloģiskajā vienībā, savukārt cita epidemioloģiski saistīta vienība saglabājās brīva no ĀCM infekcijas. B novietnē infekcija tika konstatēta abās novietnes vienībās. C novietnē ar ĀCM inficētas cūkas tika identificētas trijās no deviņām epidemioloģiskajām apakšvienībām.

Pētījuma rezultāti arī parādīja, ka mērķtiecīga klīniski slimu un nobeigušos dzīvnieku atlase paraugu ņemšanai ļāva efektīvi identificēt epidemioloģiski skartās vienības. Pat novietnēs ar ilgstošu augsta riska periodu vairākos aizgaldos netika konstatētas ĀCM infekcijas pazīmes.

Lai novērtētu ātro diagnostisko testu pielietojamību uzliesmojumu apstākļos lauka pētījumos, papildus tika izmeklēti 87 dzīvnieki, izmantojot antigēna laterālās plūsmas testus (Ag-LFD), un iegūtie rezultāti tika salīdzināti ar qPQR rezultātiem. No šiem dzīvniekiem 17 bija pozitīvi Ag-LFD testā, savukārt 34 dzīvniekiem ĀCM vīrusa genoms tika konstatēts ar qPQR. Visi qPQR negatīvie paraugi bija negatīvi arī Ag-LFD testā, kā rezultātā Ag-LFD relatīvais specifiskums bija 100 %. Relatīvā jutība, salīdzinot ar qPQR, bija 50 % (ticamības intervāls 34,66 %).

Tā kā antivielu pozitīvo paraugu skaits bija neliels, antivielu LFD testu diagnostisko jutību un specifiskumu nebija iespējams aprēķināt.

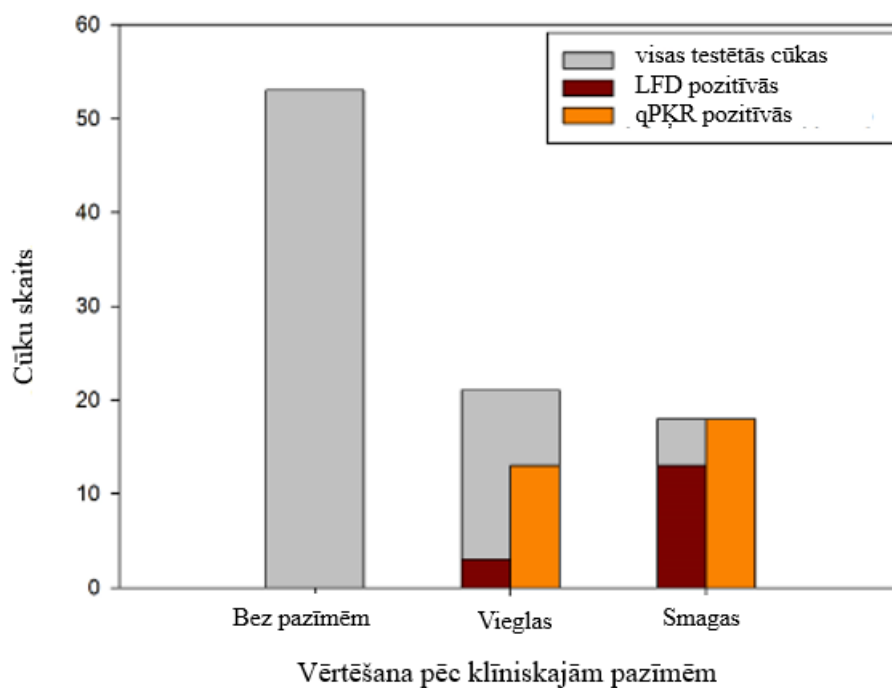
qPQR Ct vērtību un Ag-LFD pozitīvo rezultātu salīdzinājums ir attēlots 4.6. attēlā. Ag-LFD pozitīvajiem paraugiem Ct vērtības bija robežās no 14 līdz 18, un mediānā Ct vērtība bija 17.



4.6 attēls. Ct vērtību salīdzinājums visiem izmeklētajiem asins paraugiem, paraugiem ar pozitīviem qPĶR un Ag-LFD rezultātiem, kā arī paraugiem ar pozitīvu qPĶR rezultātu un negatīvu Ag-LFD rezultātu

Klīnisko pazīmju izvērtējums parādīja, ka ne Ag-LFD, ne qPĶR testos netika iegūti pozitīvi rezultāti cūkām bez klīniskām slimības pazīmēm. Pozitīvi laboratorisko izmeklējumu un Ag-LFD rezultāti tika konstatēti tikai cūkām ar klīniskām slimības pazīmēm.

ĀCM pozitīvo cūku sadalījums atbilstoši klīniskā vērtējuma kategorijām ir attēlots 4.7. attēlā.



4.7 attēls. Ar ĀCM inficēto cūku (qPĶR, Ag-LFD) sadalījums atbilstoši klīniskā vērtējuma kategorijām

Korelācijas analīze parādīja statistiski nozīmīgu saistību starp Ag-LFD rezultātiem un klīnisko vērtējumu, starp qPQR rezultātiem un klīnisko vērtējumu, kā arī starp antivielu LFD un ELISA rezultātiem.

Spīrmēna korelācijas analīzes rezultāti ir apkopoti 4.3. tabulā.

4.3. tabula

Spīrmēna korelācijas matrica dažādu ĀCM diagnostisko testu rezultātiem un ĀCM klīniskajam vērtējumam

Mainīgie lielumi	Ag-LFD	Ab-LFD	qPQR	ELISA	IPT	Klīniskais vērtējums
Ag-LFD	1	0.008	0.246	0.008	0.271	0.531
Ab-LFD	x	1	0.065	1.000	0.296	0.227
qPQR	x	x	1	0.065	0.218	0.469
ELISA	x	x	x	1	0.296	0.227
IPT	x	x	x	x	1	0.201
Klīniskais vērtējums	x	x	x	x	x	1

Treknrakstā attēlotās vērtības ir statistiski nozīmīgi atšķirīgas no 0 pie nozīmības līmeņa $p < 0,05$

4.3. ĀCM uzliesmojumu agrīna atklāšana cūku novietnēs

Pētījuma rezultāti parādīja būtiskas atšķirības uzliesmojumu atklāšanas dinamikā starp izmeklētajām novietnēm un apliecināja, ka ĀCM vīrusa cirkulācija lielās komerciālās cūku novietnēs infekcijas sākumposmā var ilgstoši palikt neatklāta. To apstiprināja trīs pētījumos iekļauto uzliesmojumu izmeklēšanas rezultāti.

2017. gada janvāra uzliesmojuma izmeklēšana parādīja, ka ĀCM vīruss skartajā novietnē bija cirkulējis vairākas nedēļas pirms tā oficiālas apstiprināšanas. Epidemioloģiskā rekonstrukcija liecināja, ka vīruss novietnē bija cirkulējis aptuveni sešas nedēļas pirms uzliesmojuma atklāšanas.

Līdzīga retrospektīva analīze tika veikta A novietnē, kur ĀCM tika oficiāli apstiprināts 2017. gada 14. jūlijā. Retrospektīvā epidemioloģiskā izmeklēšana parādīja, ka klīniskās pazīmes un paaugstināta mirstība bija novērojama jau pirms uzliesmojuma apstiprināšanas. Saskaņā ar anamnēzes un mirstības datiem pirmā nobeigusies sivēnmāte pēc aborta tika konstatēta 2. jūlijā. Turpmākajās dienās tika reģistrēti papildu nobeigušies dzīvnieki un aborti, un inficētās sivēnmātes jau bija pārvietotas starp ražošanas vienībām. Novērtētais augsta riska periods (HRP) A novietnē bija aptuveni 23 dienas.

Izmeklēšana parādīja, ka infekcijas sākumposmā mirstība pieauga lēni un skāra salīdzinoši nelielu dzīvnieku skaitu. Tas apgrūtināja savlaicīgu uzliesmojuma atklāšanu un ļāva vīrusam ilgstoši cirkulēt novietnē pirms tā oficiālas apstiprināšanas.

B novietnē ĀCM tika apstiprināts 2018. gada 31. jūlijā. Trīs nobeigušies atšķirtie sivēni, kas tika konstatēti 29. jūlijā, tika izmeklēti uz ĀCM, un PQR rezultāti bija pozitīvi. Šie dzīvnieki iepriekš, 23. jūlijā, kopā ar vēl 277 atšķirtiem sivēniem bija pārvietoti no B1 ražošanas vietas uz B2 ražošanas vietu. Uzliesmojuma atklāšanas brīdī novietnē vēl netika novērotas plaši izplatītas klīniskās pazīmes vai izteikts mirstības pieaugums. ĀCM tika identificēts, pateicoties ieviestajai pastiprinātās pasīvās uzraudzības sistēmai, kuras ietvaros katru nedēļu laboratoriski tika izmeklētas nobeigušās cūkas.

B2 ražošanas vietā rekonstruētā epidemioloģiski nozīmīgo notikumu laika līnija ir attēlota 4.3. attēlā, savukārt B1 ražošanas vietas 4. kūtī reģistrētie mirstības dati ir attēloti 4.4. attēlā.

Pētījuma rezultāti parādīja, ka ĀCM uzliesmojumu agrīnajās stadijās lielās komerciālās cūku novietnēs raksturīga lēna vīrusa izplatība un zema mirstība, kas var izraisīt novēlotu uzliesmojuma atklāšanu.

Izmeklēto uzliesmojumu salīdzinājums parādīja, ka sistemātiska nobeigušos cūku laboratoriskā izmeklēšana pastiprinātās pasīvās uzraudzības ietvaros ļauj atklāt ĀCM uzliesmojumus agrīnāk, vēl pirms parādās plaši izplatītas klīniskās pazīmes vai būtiski palielinās mirstība.

4.4. Integrēta epidemioloģiskā pieeja ĀCM uzliesmojumu izmeklēšanā

Izmeklēšanas ietvērā iespējamo ĀCM vīrusa iekļūšanas ceļu izvērtēšanu, augsta riska perioda (HRP) rekonstrukciju, vīrusa izplatības novērtēšanu novietnes ietvaros, mērķtiecīgu paraugu ņemšanu, laboratoriskos izmeklējumus, kā arī epidemioloģiski nozīmīgas lauka informācijas apkopošanu.

Lai izvērtētu iespējamās ĀCM vīrusa iekļūšanas ceļus un noteiktu to ticamību, tika izmantota hipotēzēs balstīta epidemioloģiskās izmeklēšanas pieeja. Uzliesmojumu izmeklēšanas laikā tika apkopota un analizēta arī lauksaimnieku, novietņu darbinieku un citu personu sniegtā informācija un viedokļi, izvērtējot to epidemioloģisko nozīmību.

Augsta riska perioda (HRP) rekonstrukcija tika veikta, izmantojot mirstības datus, klīniskos novērojumus, laboratorisko izmeklējumu rezultātus, dzīvnieku pārvietošanas informāciju un datus par biodrošības pasākumu ievērošanu.

Vīrusa izplatības novērtēšana novietnes ietvaros un mērķtiecīgu paraugu ņemšanas stratēģiju izmantošana ļāva identificēt skartās un neskartās epidemioloģiskās vienības uzliesmojuma skartajās novietnēs. Ātrie diagnostiskie testi uz vietas novietnē (LFD) tika izvērtēti uzliesmojumu apstākļos un salīdzināti ar standarta laboratoriskajām diagnostikas metodēm.

Izmeklēto uzliesmojumu salīdzinājums parādīja atšķirības uzliesmojumu atklāšanas laikā starp novietnēm un apliecināja, ka atsevišķos uzliesmojumos ĀCM vīruss infekcijas sākumposmā cirkulēja neatklāts.

5. DISKUSIJA

5.1. ĀCM vīrusa iekļūšanas ceļu izmeklēšana un augsta riska perioda (HRP) rekonstrukcija

Mūsu pētījumi parādīja, ka ar cilvēka darbību saistīti faktori un biodrošības prasību neievērošana bija visdrīzākie ĀCM vīrusa iekļūšanas mehānismi skartajās cūku novietnēs. Turklāt augsta riska perioda (HRP) rekonstrukcija izrādījās būtiska uzliesmojuma attīstības izpratnei un sekundārās infekcijas izplatīšanās risku identificēšanai.

Hipotēze par vīrusa iekļūšanu ar kontaminētu priekšmetu vai kontaminētu gaļas produktu starpniecību tika atzīta par ticamāko infekcijas ievazāšanas ceļu, jo infekciozs materiāls novietnē varēja nonākt ar darbinieku vai apmeklētāju starpniecību. Šie rezultāti uzsver nepieciešamību uzturēt augstu informētības līmeni par slimības izplatīšanās riskiem un biodrošības pasākumu nozīmi dažādās cūkkopībā iesaistītajās mērķgrupās. Tādēļ ar cilvēka darbību saistītajiem riska faktoriem jāpievērš pastāvīga uzmanība ĀCM profilakses stratēģijās. Mūsu pētījumi apstiprināja, ka nepietiekama biodrošība joprojām ir viens no galvenajiem ĀCM ievazāšanas riska faktoriem cūku novietnēs, kā arī parādīja, ka strukturēta, hipotēzēs balstīta epidemioloģiskā pieeja nodrošina lietderīgu metodoloģisko ietvaru uzliesmojumu izmeklēšanai.

Par visefektīvāko līdzekli ĀCM vīrusa iekļūšanas novēršanai cūku novietnēs tiek uzskatīta laba biodrošības prakse (Bellini et al., 2016). Pēc pirmo ĀCM gadījumu konstatēšanas mežacūku populācijā un mājas cūkām Latvijā 2014. gadā biodrošības prasības cūku novietnēs pakāpeniski tika pastiprinātas. Tomēr praktiskā pieredze liecināja, ka biodrošības pasākumu ieviešana daudziem cūku audzētājiem bija būtisks izaicinājums, īpaši mazajās saimniecībās un piemājas novietnēs, kur biodrošības pasākumi iepriekš netika sistemātiski īstenoti. Papildus finanšu ieguldījumiem infrastruktūrā, aprīkojumā un novietņu norobežošanā ("hardware"), efektīva biodrošība prasa arī atbilstošu pārvaldības procedūru ("software") ieviešanu, tostarp higiēnas pasākumu ievērošanu, iekšējo darba procesu organizēšanu, personāla informētību un ikdienas procedūru ievērošanu. Mūsu novērojumi liecināja, ka tieši "software" komponente rada lielākos ilgtermiņa izaicinājumus, jo informētības uzturēšana un ierastu paradumu maiņa prasa nepārtrauktu izglītošanu, uzraudzību un iesaisti.

ĀCM uzliesmojuma gadījumā epidemioloģiskās izmeklēšanas galvenais uzdevums ir noteikt visticamāko vīrusa iekļūšanas ceļu novietnē. Šādas izmeklēšanas prasa visaptverošu novietnes sistēmas izvērtēšanu, tostarp ēku, transportlīdzekļu, aprīkojuma, nožogojuma, dzīvnieku pārvietošanas, personāla kustības un biodrošības procedūru analīzi. Papildus biodrošības izvērtējumam epidemioloģiskajās izmeklēšanās bieži tiek izmantota arī netieša epidemioloģiskā informācija, tostarp novietnes darbībā iesaistīto personu novērojumi un viedokļi.

Atsevišķi tika detalizētāk analizēta uzliesmojumu izmeklēšanas laikā no nespeciālistiem iegūtā informācija. Lauksaimnieki, novietņu darbinieki, zootehniķi un veterinārārsti bieži tiek intervēti, lai noskaidrotu viņu viedokli par iespējamiem vīrusa iekļūšanas ceļiem un iespējamo infekcijas ievazāšanas laiku. Lai gan šīm personām ne vienmēr ir padziļinātas zināšanas epidemioloģijā vai par ĀCM, viņu novērojumi un pieņēmumi var sniegt vērtīgu kontekstuālu informāciju. Tādēļ uzliesmojumu izmeklēšanas laikā iegūtie apgalvojumi tika izvērtēti atbilstoši to iespējamajai epidemioloģiskajai nozīmībai un nepieciešamībai veikt turpmāku izmeklēšanu.

Tomēr šādas subjektīvas informācijas interpretācija bieži ir sarežģīta un prasa rūpīgu izvērtēšanu kontekstā ar klīniskajiem novērojumiem, biodrošības novērtējumu, laboratorisko izmeklējumu rezultātiem un citiem epidemioloģiskajiem datiem. Daži apgalvojumi var šķist izteikti spekulatīvi vai sākotnēji mazvērtīgi, savukārt citi var sniegt nozīmīgas epidemioloģiskas norādes. Atsevišķos gadījumos var tikt iegūta arī anekdotiska vai maldinoša informācija (Trotta et al., 2022). Turklāt novietņu pārstāvji var nevēlēties apspriest iespējamās

nepilnības biodrošības prasību ievērošanā, baidoties no juridiskas atbildības par uzliesmojuma izcelsmi. Šie novērojumi uzsver apmācītu izmeklētāju nozīmi, kuriem ir zināšanas par ĀCM epidemioloģiju, komunikācijas prasmes un izpratne par normatīvo aktu prasībām. Strukturētu aptaujas anketu un sistemātisku intervēšanas pieeju izmantošana var uzlabot epidemioloģiskajās izmeklēšanās iegūtās informācijas kvalitāti un salīdzināmību. Lielākā daļa izmeklēšanas laikā iegūto apgalvojumu bija saistīti ar cilvēku darbībām, īpaši mazajās saimniecībās, vēlreiz apliecinot cilvēka faktora centrālo lomu ĀCM izplatībā (Zani et al., 2019; Ardelean et al., 2021).

Lai gan vīrusa iekļūšanas ceļa noteikšana ir būtiska uzliesmojuma izmeklēšanas sastāvdaļa, ne mazāk svarīgi ir noskaidrot, cik ilgi vīruss ir cirkulējis novietnē. Šī informācija ir nepieciešama, lai novērtētu iespējamās izplatības apmēru un sekundārās infekcijas izplatīšanās risku. Tādēļ augsta riska perioda (HRP) rekonstrukcija ir būtiska epidemioloģiskās izmeklēšanas analītiskā sastāvdaļa.

Mūsu pētījumi parādīja, ka ĀCM uzliesmojumi lielās komerciālās cūku novietnēs var ilgstoši palikt neatklāti un ka līdz slimības aizdomu rašanās brīdim un laboratoriskai apstiprināšanai bieži ir noticis vairāk nekā viens infekcijas cikls. Šie rezultāti uzsver nepieciešamību skaidri nošķirt inkubācijas periodu no augsta riska perioda (HRP), jo praksē šie jēdzieni nereti tiek sajaukti pat veterinārmedicīnas speciālistu vidū. ĀCM gadījumā, īpaši lielās komerciālās novietnēs, pirms klīnisku aizdomu rašanās var noritēt vairāki secīgi infekcijas cikli. Pētījumi parādīja, ka uzliesmojumu sākumposmā nobeidzās tikai daži dzīvnieki, bet mirstība pakāpeniski pieauga turpmāko nedēļu laikā. Šis novērojums liecina, ka ĀCM transmisija lielās cūku novietnēs sākotnēji notiek salīdzinoši lēni. Infekcijas sākumposmā inficējas tikai neliels dzīvnieku skaits, un ir nepieciešams laiks, līdz vidē uzkrājas pietiekams vīrusa daudzums, lai nodrošinātu plašāku infekcijas izplatīšanos novietnē. Efektīva transmisija notiek brīdī, kad inficētās cūkas sāk asiņot, abortēt vai nobeidzas, tādējādi pakļaujot uzņēmīgās cūkas saskarei ar asinīm un citiem infekcioziem izdalījumiem (Pietschmann et al., 2015). Eksperimentālie pētījumi ar II genotipa ĀCM vīrusu ir parādījuši, ka viens infekcijas cikls - no inficēšanās līdz dzīvnieka nobeigšanās brīdim - ilgst aptuveni 10 dienas (Guinat et al., 2016; Pietschmann et al., 2015; Gabriel et al., 2011; Blome et al., 2013).

Lauka novērojumi Latvijā un eksperimentālie pētījumi papildus parādīja, ka ĀCM lipīgums ir zemāks, nekā bieži tiek pieņemts (Pietschmann et al., 2015). Latvijā 2014. gadā tika izmeklēti 32 ĀCM uzliesmojumi. Divpadsmit novietnēs visas cūkas vēl bija dzīvas brīdī, kad ieradās oficiālie veterinārārsti, savukārt 20 novietnēs viena vai vairākas cūkas jau bija nobeigušās. Tomēr laboratoriskie izmeklējumi parādīja, ka klīniski veselās cūkas, kas atradās tajā pašā aizgaldā vai kūtī, bieži vēl nebija inficējušās ar ĀCM vīrusu (Oļševskis et al., 2016). Šie rezultāti liecina, ka starp atsevišķu dzīvnieku inficēšanos var pastāvēt ievērojamas laika nobīdes un ka infekcijas pārvešana citām cūkām bieži notiek tikai pēc inficēto dzīvnieku nobeigšanās.

HRP rekonstrukcijai izstrādātā metodoloģija un ar to saistītā analītiskā pieeja ir aprakstīta I un II pētījumā. Lai rekonstruētu iespējamo infekcijas attīstības gaitu novietnēs, tika izveidotas epidemioloģisko notikumu laika līnijas. Tika apkopoti un analizēti dažādi epidemioloģiski nozīmīgi dati, tostarp anamnēzes informācija, saslimstības un mirstības dati, pēcnāves izmeklēšanas rezultāti, laboratorisko izmeklējumu dati, cūku pārvietošanas uzskaites novietnes ietvaros, kūtsmēsļu izvešanas dati un ar biodrošību saistīti novērojumi. Īpaša uzmanība tika pievērsta ar personālu saistītajiem riska faktoriem, tostarp higiēnas paradumiem, pārtikas lietošanai novietnē, informētībai par slimības izplatīšanās riskiem un transportlīdzekļu kustībai. Izmeklēšanās identificētie riska faktori tika integrēti analītiskajās laika līnijās.

Izstrādātā pieeja izrādījās noderīga sistemātiskai HRP novērtēšanai un sniedza būtisku praktisku ieguldījumu uzliesmojumu pārvaldībā. HRP rekonstrukcija ļāva kompetentajai iestādei izsekot iespējamās sekundārās uzliesmojumus, uzraudzīt to cūku veselības stāvokli, kuras HRP laikā tika pārvietotas uz citām novietnēm vai kautuvēm, kā arī identificēt potenciāli kontaminētus gaļas produktus, kurus bija nepieciešams atsaukt no aprites. Tomēr HRP

rekonstrukcija viena pati nesniedz pilnīgu izpratni par uzliesmojuma attīstības dinamiku. Lai atbalstītu operatīvu lēmumu pieņemšanu, nepieciešams novērtēt arī infekcijas izplatību skartajā novietnē.

5.2. ĀCM vīrusa izplatības novērtēšana cūku novietnēs un paraugu ņemšanas stratēģijas

Mūsu pētījumi parādīja, ka ĀCM izplatība lielās komerciālās cūku novietnēs bieži ir neviendabīga un ka atsevišķas epidemioloģiskās vienības var saglabāties neskartas pat ilgstoša augsta riska perioda (HRP) laikā. Šis novērojums uzsvēra nepieciešamību izmantot mērķtiecīgas paraugu ņemšanas stratēģijas, lai precīzi novērtētu vīrusa izplatību novietnes ietvaros.

Vīrusa izplatības novērtēšana skartajās novietnēs ir būtiska ne tikai uzliesmojuma attīstības izpratnei, bet arī atbilstošu slimības kontroles pasākumu plānošanai un īstenošanai. Lai gan starp dažādām novietnes vienībām parasti pastāv epidemioloģiskas saiknes, ko nosaka cūku audzēšanas tehnoloģija un kopīgas pārvaldības procedūras, ne visas epidemioloģiskās vienības obligāti kļūst inficētas. Mūsu pētījumi parādīja, ka pat ilgstoša HRP laikā atsevišķi aizgaldi vai novietnes sektori var saglabāties neskarti. Šie rezultāti liecina, ka ĀCM izplatība lielās komerciālās novietnēs nenotiek vienmērīgi un seko noteiktiem transmisijas modeļiem.

Detalizēta izpratne par infekcijas izplatību novietnes ietvaros ļauj prioritizēt un koordinēt slimības kontroles pasākumus, tostarp mērķtiecīgi plānot dzīvnieku likvidēšanu, sākot ar visvairāk skartajām vienībām. Turklāt detalizēts infekcijas izplatības epidemioloģiskais novērtējums var palīdzēt pielāgot slimības kontroles pasākumus konkrētajai uzliesmojuma situācijai, īpaši gadījumos, kad normatīvais regulējums pieļauj atkāpes no pilnīgas novietnes depopulācijas (EC, 2019).

Arī turēšanas sistēma un dzīvnieku pārvaldības prakse var ietekmēt infekcijas izplatības dinamiku novietnē. Ja cūkas tiek turētas lielākās grupās, vīrusa izplatība parasti notiek straujāk nekā sistēmās, kur dzīvnieki tiek turēti individuāli vai nelielās grupās. Tādēļ epidemioloģisko saikņu, dzīvnieku pārvietošanas, novietnes struktūras un pārvaldības procedūru izvērtēšana ir būtiska, lai izprastu transmisijas ceļus skartajās novietnēs.

Pēc epidemioloģisko vienību un iespējamo transmisijas ceļu identificēšanas šie secinājumi jāapstiprina ar atbilstošu diagnostisko paraugu ņemšanas stratēģiju. Šādai stratēģijai jābūt saskaņotai gan ar rekonstruētajiem transmisijas modeļiem, gan ar ĀCM infekcijas epidemioloģiskajām īpatnībām.

Pirms III pētījumā aprakstītās mērķtiecīgās pieejas izstrādes normatīvie akti paredzēja nejauši izvēlētu asins paraugu ņemšanu dzīvnieku likvidēšanas laikā virusoloģiskajiem un seroloģiskajiem izmeklējumiem. Minimālais paraugu skaits bija noteikts tā, lai katrā epidemioloģiskajā apakšvienībā ar 95 % ticamības līmeni būtu iespējams konstatēt 10 % seroprevalenci (EC, 2003). Tomēr, ņemot vērā, ka Eiropā cirkulēja augsti virulenti ĀCM vīrusa celmi, šāda nejauša uzraudzības pieeja kļuva mazāk piemērota aktīvas vīrusa cirkulācijas novērtēšanai (EFSA, 2021). Tā kā virulenta ĀCM infekcija vairumam inficēto cūku izraisa nobeigšanos, uzraudzības stratēģijām galvenokārt jābūt vērstām uz vīrusa noteikšanu klīniski slimām un nobeigušām cūkām (Blome et al., 2020; Busch et al., 2021). Vienlaikus seroloģiskie izmeklējumi joprojām var sniegt vērtīgu epidemioloģisko informāciju par vīrusa cirkulācijas ilgumu novietnē.

III pētījumam bija divi mērķi. Pirmais bija izstrādāt mērķtiecīgu un efektīvu paraugu ņemšanas stratēģiju ĀCM skartajām novietnēm, bet otrais – novērtēt laterālās plūsmas testu (LFD) piemērotību lauka apstākļos, salīdzinot tos ar standarta laboratoriskajām metodēm, tostarp PQR, ELISA un IPT. Līdz tam LFD testi galvenokārt bija novērtēti eksperimentālos apstākļos, nevis reālu ĀCM uzliesmojumu laikā novietnēs.

Pētījuma pamatā bija pieņēmums, ka ar ĀCM inficēti dzīvnieki visdrīzāk tiks identificēti starp klīniski slimajiem dzīvniekiem un ka LFD testi varētu palīdzēt tos ātri identificēt lauka apstākļos. Mūsu rezultāti parādīja, ka zemākas jutības dēļ salīdzinājumā ar

standarta laboratoriskajām metodēm LFD testi nevar aizstāt laboratorisko apstiprināšanu un nav piemēroti slimības neesamības pierādīšanai. Tomēr tie izrādījās noderīgi kā papildinošs lauka diagnostikas instruments jau apstiprinātu ĀCM uzliesmojumu izmeklēšanā, īpaši klīniski aizdomīgu un nobeigušos cūku izmeklēšanā.

Tomēr III pētījuma galvenais mērķis bija izstrādāt uzticamu paraugu ņemšanas stratēģiju, kas ļautu novērtēt vīrusa izplatību skartajās novietnēs. Mēs izstrādājām praktisku pieeju novietņu sadalīšanai epidemioloģiskajās vienībās un to klasificēšanai atbilstoši ĀCM vīrusa cirkulācijas iespējamībai. Mērķtiecīga paraugu ņemšana tika vērsta uz klīniski slimajiem un nobeigušajiem dzīvniekiem, kā arī cūkām, kas atradās ciešā kontaktā ar inficētiem dzīvniekiem. Mūsu rezultāti parādīja, ka mērķtiecīga klīniski slimu un nobeigušos cūku izmeklēšana ir ļoti efektīva, lai identificētu inficētās epidemioloģiskās vienības un novērtētu aktīvu vīrusa cirkulāciju skartajās novietnēs.

Tādējādi piedāvātā pieeja nodrošina strukturētu epidemioloģisko instrumentu ĀCM izplatības novērtēšanai komerciālās cūku novietnēs un veicina uz pierādījumiem balstītu uzliesmojumu pārvaldību. Tomēr, lai gan mērķtiecīga paraugu ņemšana uzlabo uzliesmojumu izmeklēšanu pēc slimības apstiprināšanas, ilgstošas neatklātas vīrusa cirkulācijas novēršanai nepieciešamas tikpat efektīvas agrīnās atklāšanas sistēmas. Tādēļ uzraudzības stratēģijas, kuru mērķis ir saīsināt HRP, joprojām ir būtiska ĀCM kontroles sastāvdaļa.

5.3. ĀCM uzliesmojumu agrīna atklāšana cūku novietnēs

Pētījumi parādīja, ka pastiprinātā pasīvā uzraudzība būtiski uzlabo ĀCM uzliesmojumu agrīnu atklāšanu un saīsina augsta riska periodu (HRP) skartajās cūku novietnēs. II pētījums, kurā tika analizētas divas uzliesmojuma skartas novietnes - viena 2017. gadā un otra 2018. gadā, skaidri parādīja pastiprinātas uzraudzības pieejas nozīmi. Vienā no novietnēm pastiprinātā pasīvā uzraudzība vēl netika piemērota, jo uzliesmojums notika pirms šīs sistēmas ieviešanas. Rezultātā ĀCM tika atklāts ar novēlošanos. Savukārt otrajā novietnē slimība tika konstatēta ievērojami agrāk, pateicoties ieviestajai pastiprinātajai pasīvajai uzraudzībai. Lai gan mirstības līmenis sākotnēji neradīja aizdomas par ĀCM, slimība tika atklāta, jo nobeigušās cūkas tika sistemātiski izmeklētas uz ĀCM.

Agrīna slimības atklāšana ir būtiska, lai samazinātu HRP un ierobežotu turpmākas vīrusa izplatīšanās iespējas gan novietnes ietvaros, gan starp dažādām novietnēm. Tomēr laikā, kad 2017. gadā tika veikts I pētījums, nepastāvēja specifiskas normatīvo aktu prasības attiecībā uz ĀCM agrīnu atklāšanu cūku novietnēs. Turklāt praktiskās epidemioloģiskās zināšanas par ĀCM vīrusa uzvedību pēc tā iekļūšanas komerciālās cūku novietnēs vēl bija ierobežotas. Tajā laikā agrīnas ĀCM atklāšanas uzraudzības procedūras galvenokārt balstījās uz aktīvo uzraudzību un attiecīgajā periodā spēkā esošo ĀCM diagnostikas rokasgrāmatu.

I pētījuma rezultāti veicināja labāku izpratni par ĀCM uzliesmojumu attīstības dinamiku lielās komerciālās cūku novietnēs, īpaši parādot, ka zemas sākotnējās mirstības dēļ ĀCM vīrusa cirkulācija var ilgstoši palikt neatklāta. Šie novērojumi atklāja ierobežojumus uzraudzības pieejām, kas galvenokārt balstās uz klīniskās apskates rezultātiem un vispārējiem mirstības rādītājiem. Pēc šo secinājumu paziņošanas Eiropas Komisijai lielāka uzmanība ĀCM uzraudzības stratēģijās tika pievērsta pastiprinātajai pasīvajai uzraudzībai, īpaši sistemātiskai nobeigušos un klīniski slimu cūku izmeklēšanai. Turpmāk gan Latvijā, gan Eiropas Savienības līmenī uzraudzības pieejas tika pilnveidotas, un pastiprinātā pasīvā uzraudzība kļuva par būtisku ĀCM agrīnas atklāšanas stratēģiju sastāvdaļu (EC, 2014; EC, 2018).

Šajā promocijas darbā iegūtie rezultāti apstiprina šīs pieejas epidemioloģisko nozīmi un parāda, ka sistemātiska nobeigušos cūku laboratoriskā izmeklēšana ir praktiska un epidemioloģiski pamatota metode ĀCM agrīnas atklāšanas uzlabošanai un HRP saīsināšanai.

5.4. Integrēts epidemioloģiskais ietvars ĀCM uzliesmojumu izmeklēšanai un kontrolei

Pētījuma rezultāti parādīja, ka efektīvai ĀCM kontrolei novietnes līmenī ir nepieciešams integrēts epidemioloģiskais ietvars, kas apvieno profilaksi, agrīnu slimības atklāšanu, strukturētu uzliesmojumu izmeklēšanu un uz pierādījumiem balstītu lēmumu pieņemšanu.

ĀCM joprojām ir viena no prioritārajām infekcijas slimībām Latvijā, kur nepārtraukta vīrusa cirkulācija mežacūku populācijā uztur pastāvīgu infekcijas risku mājas cūku novietnēm. Laika un telpas analīzes ir parādījušas būtiskas atšķirības ĀCM izplatībā mežacūku populācijā pa gadiem un reģionos (Oļševskis et al., 2020; BIOR, 2022). Tādējādi mežacūkas darbojas kā ilgtermiņa infekcijas rezervuārs, veidojot pastāvīgu epidemioloģisko saikni starp savvaļas dzīvnieku populāciju un mājas cūku audzēšanas sistēmām.

Šādos epidemioloģiskajos apstākļos šajā darbā iegūtie rezultāti parāda, ka ĀCM kontrole novietnes līmenī nevar balstīties uz vienu atsevišķu pasākumu, bet tai nepieciešama vairāku savstarpēji papildinošu epidemioloģisko komponentu integrācija.

Pirmkārt, vīrusa iekļūšanas ceļu izmeklēšana parādīja, ka izšķirošais faktors vīrusa ievazāšanā nav tikai novietnes infrastruktūra, bet gan mijiedarbība starp tehniskajiem biodrošības risinājumiem un cilvēku rīcību. Lai gan fiziskās barjeras samazina tieša kontakta iespējamību ar mežacūkām, ar cilvēka darbību saistītie transmisijas ceļi, īpaši tie, kas saistīti ar pārtikas apriti, personāla pārvietošanos un biodrošības procedūru neievērošanu, joprojām ir galvenie ĀCM ievazāšanas riska faktori. Tas apstiprina, ka ĀCM epidemioloģiju mājas cūku populācijā būtiski ietekmē antropogēnie faktori, kas atbilst arī citu Eiropas pētījumu secinājumiem (Zani et al., 2019; Ardelean et al., 2021). Turklāt I pētījumā izmantotā strukturētā, hipotēzēs balstītā izmeklēšanas pieeja nodrošina metodoloģisku ietvaru, kas samazina subjektivitāti un palielina uzliesmojumu izmeklēšanas caurskatāmību.

Otrkārt, augsta riska perioda (HRP) rekonstrukcija parādīja rekonstruktīvās epidemioloģijas nozīmi uzliesmojumu kontrolē. ĀCM uzliesmojumi lielās komerciālās cūku novietnēs var ietvert vairākus infekcijas ciklus pirms slimības atklāšanas, ko apstiprina gan lauka novērojumi, gan eksperimentālie pētījumi ar II genotipa ĀCM vīrusu (Guinat et al., 2016; Pietschmann et al., 2015; Gabriel et al., 2011; Blome et al., 2013). Šajā darbā izstrādātā uz laika līnijām balstītā analītiskā pieeja integrē klīniskos, laboratoriskos, pārvaldības un biodrošības datus vienotā epidemioloģiskā sistēmā. Tādējādi HRP novērtēšana kļūst ne tikai par aprakstošu darbību, bet arī par praktisku analītisku instrumentu sekundāro uzliesmojumu izsekošanai un turpmākās infekcijas izplatīšanās risku izvērtēšanai.

Treškārt, vīrusa izplatības novērtēšana novietnes ietvaros parādīja, ka infekcijas dinamiku būtiski ietekmē iekšējie kontakti, dzīvnieku pārvietošanas plūsmas un turēšanas sistēmas. Lai gan starp dažādām novietnes vienībām bieži pastāv epidemioloģiska saikne, infekcija neizplatās vienmērīgi visā novietnē. Šim novērojumam ir būtiska nozīme uz risku balstītu slimības kontroles stratēģiju plānošanā un kontroles pasākumu prioritizēšanā. Tas arī apliecina, ka ĀCM izplatība intensīvās cūkkopības sistēmās seko identificējamiem transmisijas modeļiem, kurus iespējams rekonstruēt un epidemioloģiski analizēt.

Ceturtkārt, mērķtiecīga paraugu ņemšanas stratēģijas izstrāde ļāva pielāgot diagnostikas pieeju augsti virulentu ĀCM vīrusa celmu epidemioloģiskajām īpatnībām. Ņemot vērā II genotipa ĀCM vīrusa izraisīto augsto letalitāti (>90 %) (Guinat et al., 2016; Gabriel et al., 2011; Blome et al., 2013), klīniski slimu un nobeigušos cūku mērķtiecīga izmeklēšana ir epidemioloģiski piemērotāka nekā nejauša seroloģiskā vai virusoloģiskā paraugu ņemšana aktīvas vīrusa cirkulācijas novērtēšanai. Novietņu sadalīšana epidemioloģiskajās vienībās un to klasificēšana atbilstoši infekcijas riskam nodrošina strukturētu lēmumu pieņemšanas atbalsta instrumentu uzliesmojumu kontrolē. Lai gan laterālās plūsmas testi (LFD) nevar aizstāt laboratorisko apstiprināšanu, to stratēģiska izmantošana lauka apstākļos var uzlabot izmeklēšanas operatīvo efektivitāti.

Visbeidzot, pāreja no aktīvās uzraudzības uz pastiprināto pasīvo uzraudzību ir nozīmīga paradigmas maiņa ĀCM kontroles jomā (EC, 2014; EC, 2018). I un II pētījuma rezultāti parādīja, ka paļaušanās tikai uz vispārējiem mirstības rādītājiem nav pietiekama agrīnai uzliesmojumu atklāšanai. Savukārt sistemātiska nobeigušos cūku izmeklēšana ir epidemioloģiski pamatota un praktiski īstenojama pieeja, kas uzlabo ĀCM agrīnu atklāšanu un saīsina HRP.

Kopumā šajā promocijas darbā ietvertie pētījumi veido integrētu epidemioloģisko modeli ĀCM uzliesmojumu izmeklēšanai un kontrolei novietnes līmenī. Modelis sastāv no pieciem savstarpēji saistītiem komponentiem: strukturētas vīrusa iekļūšanas ceļu izvērtēšanas, analītiskas HRP rekonstrukcijas, infekcijas izplatības noteikšanas novietnes ietvaros, uz risku balstītas mērķtiecīgas paraugu ņemšanas stratēģijas un agrīnas atklāšanas stiprināšanas, izmantojot pastiprināto pasīvo uzraudzību.

Šie komponenti ir cieši savstarpēji saistīti. Precīza HRP rekonstrukcija uzlabo izsekošanas pasākumu efektivitāti, mērķtiecīga paraugu ņemšana palīdz precizēt infekcijas izplatību, savukārt strukturēta ar cilvēka darbību saistīto vīrusa iekļūšanas ceļu izmeklēšana stiprina preventīvos biodrošības pasākumus. Šī integrētā ietvara centrā atrodas cilvēka faktors - informētība, prasību ievērošana, komunikācija un lēmumu pieņemšana.

Tādēļ efektīva ĀCM kontrole prasa ne tikai normatīvo prasību ievērošanu un laboratorisko kapacitāti, bet arī ilgtermiņa ieguldījumus uzvedības biodrošībā, strukturētā epidemioloģiskajā apmācībā un sistemātiskās izmeklēšanas metodēs. Šajā promocijas darbā izstrādātās metodoloģiskās pieejas veicina šādas kapacitātes stiprināšanu un nodrošina ietvaru, kas ir piemērojams arī ārpus Latvijas.

Kopumā šis darbs parāda, ka ĀCM uzliesmojumu pārvaldība nav tikai patogēna noteikšana, bet gan daudzdisciplinārs epidemioloģisks process, kas vienotā slimības kontroles stratēģijā apvieno slimības epidemioloģiju, novietņu pārvaldības sistēmas, cilvēku uzvedību un normatīvo regulējumu.

SECINĀJUMI

- 1) ĀCM komerciālās cūku novietnēs sākotnēji var izplatīties lēni un neviendabīgi, ļaujot infekcijai ilgstoši palikt neatklātai. Šie rezultāti parāda, ka uzliesmojumu agrīna atklāšana nevar balstīties tikai uz mirstības pieauguma rādītājiem vai klīniskām aizdomām, un prasa sistemātisku uzraudzības pieeju.
- 2) Augsta riska perioda (HRP) rekonstrukcija ir būtiska epidemioloģiskās izmeklēšanas sastāvdaļa, kas ļauj identificēt iespējamus vīrusa iekļūšanas notikumus, izvērtēt izplatīšanās ceļus un pilnveidot izsekošanas un uzliesmojumu kontroles pasākumus.
- 3) Strukturēta, hipotēzēs balstīta epidemioloģiskā izmeklēšana nodrošina sistemātisku pieeju iespējamo ĀCM vīrusa iekļūšanas ceļu izvērtēšanai un ļauj izslēgt maz ticamus vīrusa iekļūšanas ceļus.
- 4) Vīrusa izplatības novērtēšana novietnes ietvaros parādīja, ka ĀCM neizplatās vienmērīgi starp ražošanas vienībām. Epidemioloģisko vienību identificēšana un mērķtiecīgu paraugu ņemšanas stratēģijas izmantošana ir būtiska, lai ticami novērtētu infekcijas izplatību skartajās novietnēs. Ātro diagnostisko testu izmantošanas izvērtējums lauka apstākļos apliecināja to praktisko nozīmi kā papildinošu instrumentu epidemioloģiskajā izmeklēšanā līdztekus standarta laboratoriskajām metodēm.
- 5) Pastiprinātā pasīvā uzraudzība, kas balstīta uz sistemātisku nobeigušos cūku laboratorisko izmeklēšanu, būtiski uzlabo ĀCM uzliesmojumu agrīnu atklāšanu un veicina HRP saīsināšanu, tādējādi samazinot turpmākas slimības izplatīšanās risku.
- 6) Epidemioloģiskā izmeklēšana, kas apvieno strukturētu datu apkopošanu, profesionālu lauka informācijas interpretāciju un mērķtiecīgu diagnostisko apstiprināšanu, nodrošina pierādījumus slimības kontroles un pārvaldības pasākumu pielāgošanai konkrētajai uzliesmojuma situācijai, tostarp izvērtējot iespēju piemērot atkāpes no pilnīgas depopulācijas.
- 7) Pētījuma rezultāti ļāva izstrādāt strukturētu ĀCM uzliesmojumu izmeklēšanas ietvaru, kas apvieno agrīnu slimības atklāšanu, HRP rekonstrukciju, hipotēzēs balstītu epidemioloģisko izmeklēšanu un mērķtiecīgu diagnostiku.

REKOMENDĀCIJAS

- 1) Pastiprinātā pasīvā uzraudzība, kas balstīta uz sistemātisku nobeigušos cūku laboratorisko izmeklēšanu, būtu konsekventi jāpiemēro un jāuztur komerciālajās cūku novietnēs, jo tā uzlabo ĀCM uzliesmojumu agrīnu atklāšanu un veicina neatklātas infekcijas perioda saīsināšanu.
- 2) ĀCM uzliesmojumu izmeklēšanā būtu jāizmanto strukturēta augsta riska perioda (HRP) rekonstrukcijas pieeja, integrējot epidemioloģiskos, klīniskos, laboratoriskos, mirstības un dzīvnieku pārvietošanas datus, lai atbalstītu vīrusa iekļūšanas notikumu identificēšanu, transmisijas ceļu izvērtēšanu un lēmumu pieņemšanu uzliesmojumu pārvaldībā.
- 3) ĀCM uzliesmojumu epidemioloģiskajai izmeklēšanai būtu jābalstās uz integrētu, hipotēzēs balstītu epidemioloģiskās izmeklēšanas ietvaru, kas ietver strukturētu datu apkopošanu, HRP rekonstrukciju, epidemioloģisko vienību identificēšanu, mērķtiecīgas paraugu ņemšanas stratēģijas un atbilstošas diagnostikas metodes, lai nodrošinātu uz pierādījumiem balstītu uzliesmojumu pārvaldību un slimības kontroles pasākumu īstenošanu.

PATEICĪBA

Vārdi nespēj pilnībā izteikt manu patieso pateicību visiem tiem cilvēkiem, kuri mani iedvesmoja, atbalstīja, mācīja un vadīja šajā promocijas darba tapšanas ceļā.

Īpaša sadarbība ar kolēģiem no Frīdriha Leflera institūta (Friedrich-Loeffler-Institut, FLI), Vācijas Federālā dzīvnieku veselības pētniecības institūta, Igaunijas Dzīvības zinātņu universitātes un Pārtikas drošības, dzīvnieku veselības un vides zinātniskā institūta "BIOR" izvērtās ne tikai vērtīgā zinātniskā sadarbībā, bet arī ilgstošās personīgās attiecībās un kopīgā aizrautībā meklēt atbildes uz neskaitāmiem epidemioloģiskajiem jautājumiem, kas saistīti ar Āfrikas cūku mēri. Šī sadarbība man deva iespēju strādāt kopā ar izcilu darba vadītāju un zinātnisko konsultantu komandu.

Mani darba vadītāji prof. Dr. Aivars Bērziņš un prof. Dr. Arvo Viltrops, kā arī zinātniskais konsultants Dr. Klaus Depners visā doktorantūras laikā ir bijuši nepārtraukts atbalsta, padomu, iedrošinājuma un zināšanu avots.

Studiju procesā, īpaši izstrādājot lauka pētījumus un brīžos, kad piedzīvota nedrošība vai apjukums, Klaus, Tavs atbalsts, iedrošinājums un vadība bija nenovērtējami. Tava zinātniskā kompetence, entuziasms un spēja saskatīt risinājumus tur, kur citi redzēja šķēršļus, man vienmēr ir bijis iedvesmas avots. Es vienmēr būšu dziļi pateicīga par Tavu atbalstu.

Sirsnīgi pateicos fondam "Vītolu fonds" par mana promocijas darba ieceres novērtēšanu un studiju atbalstīšanu, piešķirot LAB-AN stipendiju.

Vēlos izteikt arī patiesu pateicību Latvijas Zinātņu akadēmijai par mūsu pētījuma par Āfrikas cūku mēri atzinību, iekļaujot to starp nozīmīgākajiem sasniegumiem lietišķajā zinātnē 2025. gadā. Šāds novērtējums apliecina lietišķo pētījumu nozīmīgumu un kalpo kā vērtīgs iedrošinājums turpmākajam zinātniskajam darbam.

Mana visdziļākā pateicība pieder visiem promocijas darbā iekļauto publikāciju līdzautoriem. Jūs pārstāvat dažādas valstis un esat devuši unikālu pieredzi Āfrikas cūku mēra jomā, padarot mūsu kopīgo darbu daudzpusīgāku, vērtīgāku un iedvesmojošāku. Ideju apmaiņa, zinātniskās diskusijas un kopīgie centieni risināt Āfrikas cūku mēra kontroles izaicinājumus ir bijuši viens no nozīmīgākajiem ieguvumiem šajā ceļā.

Šis promocijas darbs nebūtu tapis bez dienām, mēnešiem un gadiem, kas pavadīti, strādājot kopā ar kolēģiem Pārtikas un veterinārajā dienestā. Īpaša pateicība Edvīnam Oļševskim - Tu man liki noticēt, ka doktorantūras studijas ir iespējams apvienot ar ikdienas darbu Pārtikas un veterinārajā dienestā. Esmu arī dziļi pateicīga par iesaistīšanu daudzās nacionālā un starptautiskā līmeņa pētniecības aktivitātēs, kas mani motivēja turpināt zinātnisko izaugsmi.

Vēlos pateikties arī Mārtiņam Seržantam par neskaitāmajām diskusijām, jautājumiem un stundām, kas pavadītas, strādājot plecu pie pleca Āfrikas cūku mēra uzliesmojumu izmeklēšanā, datu vākšanā un interpretācijā. Tavas idejas, praktiskie risinājumi, kritiskā domāšana un humora izjūta vienmēr ir bijuši atbalsta un iedvesmas avots.

Visi Veterinārās uzraudzības departamenta kolēģi ir pelnījuši manu sirsnīgu pateicību par atbalstu, sadarbību un iedrošinājumu šo gadu garumā.

Esmu patiesi pateicīga visiem veterinārajiem inspektoriem un laboratorijas speciālistiem, kuri gadu gaitā bija iesaistīti Āfrikas cūku mēra uzliesmojumu izmeklēšanā un lauka darbā. Paraugu ņemšana, laboratoriskā diagnostika, datu apkopošana un neskaitāmās stundas, kas pavadītas skartajās novietnēs, veidoja pamatu šim pētījumam. Bez jūsu profesionalitātes, atbildības sajūtas un ieguldījuma šie pētījumi nebūtu bijuši iespējami.

Vēlos izteikt sirsnīgu pateicību Ingai Bedrītei par palīdzību promocijas darba tehniskajā noformēšanā un par vienmēr atbalstošo attieksmi visa procesa laikā.

Pateicība pieder arī maniem vecākiem, kuri vienmēr ir atbalstījuši manu lēmumu turpināt studijas un snieguši nenovērtējamu praktisku palīdzību, rūpējoties par manu māju un dārzu laikā, kad biju aizņemta ar pētniecisko darbu vai komandējumiem.

Mans vīrs Ivo un mūsu dēli Pēteris un Kristaps - es nespēju iedomāties atbalstošāku, mīlošāku un brīnišķīgāku ģimeni. Jūsu pacietība, sapratne, iedrošinājums un beznosacījumu mīlestība ir padarījusi šo ceļu iespējamu.

Visbeidzot, pateicos Dievam par Viņa neizmērojamo mīlestību, gudrību, vadību un svētībām, kas mani pavadījušas visas dzīves un šī akadēmiskā ceļa laikā.

REFERENCES

- Addinsoft. (2019). Xlstat statistical and data analysis solution, Addinsoft.
- Ardelean, F.; Globig, A.; Năvălici, A.I.G.; Blome, S.; Dietze, K.; Depner, K.; Zani, L. The course of African swine fever in Romanian backyard holdings—A case report. *Veter. Med. Sci.* 2021, 7, 2273–2279. [CrossRef]
- Bastos, A. D., Penrith, M. L., Cruciare, C., Edrich, Hutchings, J. L., G., Roger, F., Couacy-Hymann, E., and Thomson, R. (2003): Genotyping field strains of African swine fever virus by partial p72 gene characterization. *Arch. Virol.* 148, 693–706.
- Bellini S, Rutili D, Guberti V (2016): Preventive measures aimed at minimizing the risk of African swine fever virus spread in pig farming systems. *Acta Vet Scand* 58: 1–10. DOI 10.1186/s13028-016-0264-x.
- Blome, S., Franzke, K., & Beer, M. (2020). African swine fever—A review of current knowledge. *Virus Research*, 287, 198099. <https://doi.org/10.1016/j.virusres.2020.198099>.
- Blome, S., Gabriel, C., Beer, M. (2013): Pathogenesis of African swine fever in domestic pigs and European wild boar. *Virus Research*, 173, 122–130.
- Blome, S., Gabriel, C., Dietze, K., Breithaupt, A. and Beer, M. (2012): High virulence of African swine fever virus Caucasus isolate in European wild boar of all ages. *Emerging Infectious Diseases*, 18, 708.
- Blome, S., Goller, K.V., Petrov, A., Dräger, C., Pietschmann, J., Beer, M. (2015): Alternative sampling strategies for passive classical and African swine fever surveillance in wild boar--extension towards African swine fever virus antibody detection. *Vet Microbiol.* 2014 Dec 5; 174(3-4):607-8. doi: 10.1016/j.vetmic.2014.09.018. Epub 2014 Oct 5. PubMed PMID: 25448452.
- Busch, F., Haumont, C., Penrith, M.-L., Laddomada, A., Dietze, K., Globig, A., Guberti, V., Zani, L., & Depner, K. (2021). Evidence-based African swine fever policies: Do we address virus and host adequately? *Frontiers in Veterinary Science*, 8, 637487. <https://doi.org/10.3389/fvets.2021.637487>.
- Cappai, S., Baldi, I., Desini, P., Pintore, A., Denurra, D., Cherchi, M., Rolesu, S., Mandas, D., Franzoni, G.; Fiori, M.S.; et al. Changes in Estimating the Wild Boar Carcasses Sampling Effort: Applying the EFSA ASF Exit Strategy by Means of the WBC-Counter Tool. *Viruses* 2022, 14, 1424. <https://doi.org/10.3390/v14071424>
- de Carvalho Ferreira, H.C., Tudela Zúquete, S., Wijnveld, M., Weesendorp, E., Jongejan, F., Stegeman, A., Loeffen, W.L. (2014): No evidence of African swine fever virus replication in hard ticks. *Ticks Tick Borne Dis.* 2014 Sep;5(5):582-9. doi: 10.1016/j.ttbdis.2013.12.012. Epub 2014 Jun 26. PubMed PMID: 24980962.
- Dhollander S., Balmoş O.M., Cattaneo E., Cortiñas J.A., Anette Ella Boklund A.E., Szczotka-Bochniarz A., Mihalca A.D., Mur L., Frant M., Gal-Cisoñ A., Kwasnik M, Rozek W., Malakauskas A., Masiulis M., Turcinaviciene J., Chesnoiu T., Jazdzewski K., Rola J., Barbuceanu F., Miguel Ángel Miranda¹³ and Stegeman J.A.: Investigating the role of stable flies (*Stomoxys calcitrans*) and biting midges of the genus *Culicoides* as potential mechanical vectors of African swine fever virus in Lithuania, Poland and Romania. *Parasites & Vectors* (2025) 18:312 <https://doi.org/10.1186/s13071-025-06816-w>

(EC, 2002) Council Directive 2002/60/EC of 27 June 2002 Laying Down Specific Provisions for the Control of African Swine Fever and Amending Directive 92/ 119/EEC as Regards Teschen Disease and African Swine Fever. Available online: <https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32002L0060> (accessed on 25 April 2023).

EC (2003). Commission Decision 2003/422/EC of 26 May 2003 Approving an African Swine Fever Diagnostic Manual. Available online: <https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32003D0422> (accessed on 25 April 2023).

(EC 2014) Commission Implementing Decision 2014/709/EU of 9 October 2014 Concerning Animal Health Control Measures Relating to African Swine Fever in Certain Member States and Repealing Implementing Decision 2014/178/EU. Available online: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32014D0709> (accessed on 25 April 2023).

EC (2016). “Regulation (EU) 2016/429 of the European parliament and of the council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health (‘Animal Health Law’)” Council and Parliament of the European Union. Available online: <https://eur-lex.europa.eu/eli/reg/2016/429/2021-04-21> (accessed on 25 April 2023).

EC (2018). “Working document Strategic approach to the management of African Swine Fever for the EU”. SANTE/7113/2015. Available online: https://food.ec.europa.eu/animals/animal-diseases/diseases-and-control-measures/african-swine-fever_en (accessed on 25 April 2023).

(EC, 2019) Commission Delegated Regulation (EU) 2020/687 of 17 December 2019 supplementing Regulation (EU) 2016/429 of the European Parliament and the Council, as regards rules for the prevention and control of certain listed diseases, Available online: https://eur-lex.europa.eu/eli/reg_del/2020/687/oj (accessed on 25 April, 2023).

(EC, 2020) Commission implementing regulation (EU) 2020/2002 of 7 December 2020 laying down rules for the application of Regulation (EU) 2016/429 of the European Parliament and of the Council with regard to Union notification and Union reporting of listed diseases, to formats and procedures for submission and reporting of Union surveillance programmes and of eradication programmes and for application for recognition of disease-free status, and to the computerised information system (accessed on 25 April, 2023).

(EC, 2023) Commission Implementing Regulation (EU) 2023/594 of 16 March 2023 laying down special disease control measures for African swine fever and repealing Implementing Regulation (EU) 2021/605, Available online: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32023R0594> (accessed on 25 April, 2023).

EFSA (2010): Scientific Opinion on African swine fever, EFSA J., 8, 1556

EFSA (2014): Scientific Opinion on African swine fever. EFSA J., 12, 3628

EFSA (2020): Epidemiological analyses of African swine fever in the European Union (November 2018 to October 2019), European Food Safety Authority, 18(1), e05996. <https://doi.org/10.2903/j.efsa.2020.5996>

EFSA (2021): Scientific Opinion on the assessment of the control measures of the category A diseases of Animal Health Law: African Swine Fever. EFSA Journal, 19(1), e06402. <https://doi.org/10.2903/j.efsa.2021.6402>

EFSA Panel on Animal Health and Welfare (AHAW). (2018). African swine fever in wild boar. EFSA Journal, 16(7), e05344. <https://doi.org/10.2903/j.efsa.2018.5344>

EFSA (2024) Boklund, A. E., Stahl, K., Miranda Chueca, M. A., Podgórski, T., Vergne, T., Cortinas Abrahantes, J., Cattaneo, E., Dhollander, S., Papanikolaou, A., Tampach, S., & Mur, L. (2024). Risk and protective factors for ASF in domestic pigs and wild boar in the EU, and mitigation measures for managing the disease in wild boar. *EFSA Journal*, 22(12), e9095. <https://doi.org/10.2903/j.efsa.2024.9095>

EFSA Panel on Animal Health and Welfare (AHAW). (2021). Assessment of the ability of different matrices to transmit African swine fever virus (ASFV). *EFSA Journal*, 19(4), e06360. <https://doi.org/10.2903/j.efsa.2021.6360>

European Union Reference Laboratory for ASF. (2018). “Standard operating procedure for the detection of antibodies against African swine fever by indirect immunoperoxidase technique”.

Farez, S. und Morley, R.S. (1997) – Potential animal health hazards of pork and pork products. *Rev. sci. tech. Off. Int. Epiz.*, 16 (1), 65-78

Fernández-Pinero, J., Gallardo, C., Elizalde, M., Robles, A., Gómez, C., Bishop, R., Heath, L., Couacy-Hymann, E., Fasina, F. O., Pelayo, V., Soler, A., & Arias, M. (2013). Molecular diagnosis of African Swine Fever by a new real-time PCR using universal probe library. *Transboundary and Emerging Diseases*, 60(1), 48–58. <https://doi.org/10.1111/j.1865-1682.2012.01317.x>

FVS Website 1: <https://www.pvd.gov.lv/lv/afrikas-cuku-mera-uzliesmojumi-latvija> (accessed on 25 April 2023).

FVS Website 2: <https://www.pvd.gov.lv/lv/afrikas-cuku-meris-0> (accessed on 27 April 2023)

Gabriel, C., Blome, S., Malogolovkin, A., Parilov, S., Kolbasov, D., Teifke, J.P. and Beer M. (2011): Characterization of African Swine Fever Virus Caucasus Isolate in European Wild Boar. *Emerging Infectious Diseases*, 17, 2342-2345.

Gallardo, C., Fernández-Pinero, J., Pelayo, V., Gazeaev, I., Markowska-Daniel, I., Pridotkas, G., Nieto, R., Fernández-Pacheco, P., Bokhan, S., Nevolko, O., Drozhzhe, Z., Pérez, C., Soler, A., Kolvasov, D., Arias, M. (2014): Genetic variation among African swine fever genotype II viruses, eastern and central Europe. *Emerg Infect Dis.* 2014 Sep; 20(9):1544-7. doi: 10.3201/eid2009.140554

Gallardo, C., Mwaengo, D. M., Macharia, J. M., Arias, M., Taracha, Soler, E. A., A., Okoth, E., Martin, E., Kasiti, J. and Bishop, R. P. (2009): Enhanced discrimination of African swine fever virus isolates through nucleotide sequencing of the p54, p72, and pB602L (CVR) genes. *Virus Genes* 38, 85–95.

Gallardo, C., Soler, A., Nieto, R., Cano, C., Pelayo, V., Sánchez, M.A., Pridotkas, G., Fernandez-Pinero J., Briones, V. and Arias, M. (2015): Experimental infection of domestic pigs with African swine fever virus Lithuania 2014 genotype II field isolate. *Transboundary and Emerging Diseases*, 2015 Mar 22. doi: 10.1111/tbed.12346

Gogin, A., Gerasimov, V., Malogolovkin, A. and Kolbasov, D. (2013): African swine fever in the North Caucasus region and the Russian Federation in years 2007–2012. *Virus Research*, 173, 204–211.

Goller, K.V., Malogolovkin, A.S., Katorkin, S., Kolbasov, D., Titov, I., Höper, D., Beer, M., Keil, G.M., Portugal, R., Blome, S. (2015): Tandem repeat insertion in African swine fever virus, Russia, 2012. *Emerg Infect Dis.* 2015 Apr;21(4):731-2. doi: 10.3201/eid2104.141792.

Guinat, C.; Gubbins, S.; Vergne, T.; Gonzales, J.L.; Dixon, L.; Pfeiffer, D.U. Experimental pig-to-pig transmission dynamics for African swine fever, Georgia 2007/1 strain. *Epidemiol. Infect.* 2016, 144, 25–34.

Hoffmann, B., Depner, K., Schirrmeier, H., & Beer, M. (2006). A universal heterologous internal control system for duplex real-time RT-PCR assays used in a detection system for pestiviruses. *Journal of Virological Methods*, 136(1–2), 200–209. <https://doi.org/10.1016/j.jviromet.2006.05.020>

Krug, P.W., Larson, C.R., Eslami, A.C., Rodriguez, L.L. (2012) - Disinfection of foot-and-mouth disease and African swine fever viruses with citric acid and sodium hypochlorite on birch wood carriers. *Veterinary Microbiology* 156, 96–101; doi:10.1016/j.vetmic.2011.10.032

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

Mehinagic, K., Summerfield, A. (2026). African swine fever virus. *Trends in Microbiology*. <https://doi.org/10.1016/j.tim.2026.02.006>

Mellor, P.S., Kitching, R.P., Wilkinson, P.J. (1987): Mechanical transmission of capripox virus and African swine fever virus by *Stomoxys calcitrans*. *Res Vet Sci.* 1987 Jul; 43(1): 109-12.

(MK, 2004) Ministru kabineta noteikumi Nr.83 Rīgā 2004.gada 17. februārī (prot. Nr.7 17.§) Āfrikas cūku mēra likvidēšanas un draudu novēršanas kārtība, Available online: <https://likumi.lv/ta/id/84573-afrikas-cuku-mera-likvidesanas-un-draudu-noversanas-kartiba>, (Accessed on 25 April, 2023).

(MK, 2015) Ministru kabineta noteikumi Nr.291 Rīgā 2015.gada 9. jūnijā (prot. Nr.28 14.§)

MONTGOMERY, R. E., 1921. On a form of swine fever occurring in British East Africa (Kenya Colony). *Journal of Comparative Pathology*, 34, 159–191 & 243-262.

Mouchantat, S., Globig, A., Böhle, W., Petrov, A., Strebelow, H.G., Mettenleiter, T.C., Depner K. (2014b): Novel rope-based sampling of classical swine fever shedding in a group of wild boar showing low contagiousity upon experimental infection with a classical swine fever field strain of genotype 2.3. *Vet Microbiol.* 2014 Jun 4;170(3-4):425-9.

Mouchantat, S., Haas B., Böhle, W., Globig, A., Lange, E., Mettenleiter, T.C., Depner, K. (2014a): Proof of principle: non-invasive sampling for early detection of foot-and-mouth disease virus infection in wild boar using a rope-in-a-bait sampling technique. *Vet Microbiol.* 2014 Aug 6; 172 (1-2):329-33.

Mur, L., Atzeni, M., Martinez-Lopez, B., Feliziani, F., Rolesu, S. and Sanchez-Vizcaino, J. M. (2014): Thirty-Five-Year Presence of African Swine Fever in Sardinia: History, Evolution and Risk Factors for Disease Maintenance

Mur, L., Martinez-Lopez, B., Sanchez-Vizcaino, J.M. (2012): Risk of African swine fever introduction into the European Union through transport-associated routes: returning trucks and waste from international ships and planes. *BMC Vet Res*, 8, 149.

Noteikumi par biodrošības pasākumu kopumu dzīvnieku turēšanas vietām. Available online: <https://likumi.lv/ta/id/274930-noteikumi-par-biodrosibas-pasakumu-kopumu-dzivnieku-turesanas-vietam> (Accessed on 25 April, 2023).

Oganesyana, A.S., Petrovaa, O.N., Korennoya, F.I., Bardinaa, N.S., Goginb, A.E., Dudnikova, S.A. (2013): African swine fever in the Russian Federation: Spatio-temporal analysis and epidemiological overview. *Virus Research* 173 (2013) 204– 211

Oleaga-Perez, A., Perez-Sanchez, R. and Encinas-Grandes, A. (1990): Distribution and biology of *Ornithodoros erraticus* in parts of Spain affected by African swine fever. *Vet. Rec.* 126, 32–37.

Oļševskis, E., Guberti, V., Seržants, M., Westergaard, J., Gallardo, C., Rodze, I., Depner, K. (2016): African swine fever virus introduction into the EU in 2014: Experience of Latvia. *RVSC*, doi: 10.1016/j.rvsc.2016.01.006

Oļševskis, E., Schulz, K., Staubach, C., Seržants, M., Lambergā, K., Pūle, D., Ozoliņš, J., Conraths, F. J., & Sauter-Louis, C. (2020). African swine fever in Latvian wild boar—A step closer to elimination. *Transboundary and Emerging Diseases*, 67(6), 2615–2629. <https://doi.org/10.1111/tbed.13611>

Pejsak, Z. and Stadejek, T. (2015): Epidemiology of African swine fever in Poland. Available at: <https://www.pig333.com/print/9390>

Petrov, A., Schotte, U., Pietschmann J., Dräger, C., Beer, M., Anheyer-Behmenburg, H., Goller, K.V., Blome, S. (2015): Alternative sampling strategies for passive classical and African swine fever surveillance in wild boar. *Vet Microbiol.* 2014 Oct 10; 173(3-4):360-5. doi: 10.1016/j.vetmic.2014.07.030. Epub 2014 Aug 15. PubMed PMID: 25179470.

Pietschmann, J., Guinat, C., Beer, M., Pronin. V., Tauscher, K., Petrov, A., Keil, G. and Blome, S. (2015): Course and transmission characteristics of oral low dose infection of domestic pigs and European wild boar with a Caucasian African swine fever virus isolate. *Arch Virol*, DOI 10.1007/s00705-015-2430-2

Pietschmann, J., Mur L., Blome, S., Beer, M., Pérez-Sánchez, R., Oleaga, A., Sánchez-Vizcaíno, J.M. (2016): African swine fever virus transmission cycles in Central Europe: Evaluation of wild boar-soft tick contacts through detection of antibodies against *Ornithodoros erraticus* saliva antigen. *BMC Vet Res.* 2016 Jan 4; 12(1):1. doi: 10.1186/s12917-015-0629-9. PubMed PMID: 26728767; PubMed Central PMCID: PMC4698789.

PVD, 2021; <https://www.pvd.gov.lv/lv/publiskais-parskats>

OIE Technical Disease Card: African swine fever, https://www.woah.org/en/document/african_swine_fever/ 15/06/2019, assessed on 25 April 2023

Sánchez-Vizcaíno, J. M., Mur, L., & Martínez-López, B. (2013). African swine fever (ASF): Five years around Europe. *Veterinary Microbiology*, 165(1–2), 45–50. <https://doi.org/10.1016/j.vetmic.2012.11.030>

Sergeant, E. S. G. (2018). Epitools epidemiological calculators. Ausvet. <http://epitools.ausvet.com.au>

Trotta, A.; Marinaro, M.; Cavalli, A.; Cordisco, M.; Piperis, A.; Buonavoglia, C.; Corrente, M. African Swine Fever—How to Unravel Fake News in Veterinary Medicine. *Animals* 2022, 12, 656. [CrossRef]

WOAH WAHIS. <https://wahis.woah.org/#/home> (Accessed on 27 April 2023)

World Organisation for Animal Health & Food and Agriculture Organization of the United Nations. (2022). African swine fever in wild boar: Ecology and biosecurity (2nd ed.). <https://www.woah.org/app/uploads/2022/07/asf-in-wild-boar-ecology-and-biosecurity-2nd-ed.pdf>

World Organisation for Animal Health. (2023). African swine fever (infection with African swine fever virus). In Terrestrial Animal Health Code. <https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/>

Wozniakowski, G., Kozak, E., Kowalczyk, A., Łyjak, M., Pomorska-Mol, M., Niemczuk, K., Pejsak, Z. (2015): Current status of African swine fever virus in a population of wild boar in eastern Poland (2014-2015). Arch. Virol. DOI 10.1007/s00705-015-2650-5

Zani, L.; Dietze, K.; Dimova, Z.; Forth, J.H.; Denev, D.; Depner, K.; Alexandrov, T. African Swine Fever in a Bulgarian Backyard Farm—A Case Report. Veter. Sci. 2019, 6, 94. [CrossRef] [PubMed]

**PUBLICATIONS / PROMOCIJAS DARBA PAMATĀ ESOŠĀS
PUBLIKĀCIJAS**

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Open Access

Berl Münch Tierärztl Wochenschr
DOI 10.2376/0005-9366-18031

© 2018 Schlütersche
Verlagsgesellschaft mbH & Co. KG
ISSN 0005-9366

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Eingegangen: 12.03.2018
Angenommen: 18.06.2018

Online first: 22.10.2018
[http://vetline.de/open-access/
158/3216/](http://vetline.de/open-access/158/3216/)

Summary

Zusammenfassung

U.S. Copyright Clearance Center
Code Statement:
0005-9366/2018/18031 \$ 15.00/0

Case report/Fallbericht

Food and Veterinary Service, Riga, Latvia¹
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African swine fever outbreak investigations in a large commercial pig farm in Latvia: a case report

Untersuchungen eines Ausbruchs der Afrikanischen Schweinepest in einem großen kommerziellen Schweinebestand in Lettland: ein Fallbericht

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In Mid-January 2017 a large commercial pig breeding farm in Latvia with almost 5000 animals was affected by African swine fever (ASF). Epidemiological investigations using a hypothesis based approach were conducted to trace the source of virus and to reconstruct the course of the disease until notification.

Although the farm was located in an area where the disease is circulating in the wild boar population, it was found highly unlikely that wild boar might have played a direct role in this outbreak. Management inconsistencies related to human behavior proved to be the main vulnerabilities for virus introduction. Farm management did not fulfill biosecurity requirements in all aspects to prevent virus introduction. Therefore, the human factor could not be ruled out and has been considered to be the most serious risk factor. Most probably the ASF virus entered the farm beginning of December 2016, about six weeks before disease confirmation.

It was concluded that early detection of ASF by passive surveillance is most crucial and has to be improved. In risk areas dead pigs should be compulsory tested for ASF even if farm mortality is below the normal threshold.

Keywords: Outbreak investigation, Biosecurity, African swine fever

Mitte Januar 2017 kam es zu einem Ausbruch der Afrikanischen Schweinepest (ASP) in einer großen kommerziellen Schweinezuchtanlage in Lettland. Es waren fast 5000 Tiere betroffen. Um die Quelle des Virus nachzuverfolgen und den Verlauf der Krankheit vor der Anzeige zu rekonstruieren, wurden epidemiologische Nachforschungen durchgeführt.

Obwohl sich der Betrieb in einem Gebiet, in dem die ASP innerhalb der Wildschweinpopulation zirkuliert, befand, wurde es als sehr unwahrscheinlich angesehen, dass das Wildschwein eine direkte Rolle in diesem Ausbruchsgeschehen gespielt hat. Es konnte nachgewiesen werden, dass Unregelmäßigkeiten im Management, die mit menschlichem Verhalten zusammenhingen, die größten Schwachstellen hinsichtlich des Viruseintrags darstellten. Das Betriebsmanagement erfüllte nicht in allen Bereichen die Biosicherheitsanforderungen, um einen Viruseintrag zu verhindern. Dementsprechend konnte der Faktor Mensch nicht ausgeschlossen werden und wurde als wichtigster Risikofaktor angesehen. Sehr wahrscheinlich kam es zu Beginn Dezember 2016, sechs Wochen vor Krankheitsbestätigung, zum ASP-Viruseintrag.

Schlussfolgernd ist die Früherkennung von ASP durch passive Überwachung wesentlich und muss verbessert werden. In Risikogebieten sollten tote Schweine verpflichtend auf ASP getestet werden, selbst wenn die Mortalität des Betriebes unterhalb des normalen Grenzwertes liegt.

Schlüsselwörter: Ausbruchsuntersuchung, Biosicherheit, Afrikanische Schweinepest

Background

The epidemic currently affecting parts of Eastern Europe started in Georgia in 2007 and has reached the eastern borders of the European Union at the beginning of 2014 (European Food And Safety Authority 2015, European Food Safety Authority et al. 2017, Sanchez-Vizcaino et al. 2013,). First cases of infected wild boar were reported by Lithuania in January 2014, followed by Poland, Latvia and Estonia. The African swine fever virus (ASFV) entered Latvia from the South East of the country and continued to spread westwards and northwards. By May 2018 Latvia notified over 3000 cases in wild boar and 53 outbreaks in domestic pigs (Table 1, Figure 1). The course of ASF in wild boar proved to be very complex and difficult to control (European Food Safety Authority et al. 2017) and about 87% of Latvia had to be placed under trade and sanitary restrictions (European Commission 2014)). Apart from the Baltic States and Poland where the disease became endemic in wild boar populations further cases and outbreaks were reported from Romania, Moldova, Czech Republic and Hungary during 2017 and 2018. In contrast the outbreaks in domestic pigs could be controlled timely and without major problems. Apart from three outbreaks which affected large commercial farms, all other outbreaks occurred in small farms or back yard holdings.

In case of an outbreak a timely epidemiological investigation has to be conducted to identify the possible origin of the ASFV and to estimate the high risk period, which is the length of time during which ASFV was on the farm before notification (Anonymus 2002). All direct

TABLE 1: Latvian notifications of positive African swine fever cases in domestic pigs and in wild boar from 2014 to May 2018

Year	Domestic pigs	Wild boar	Total
2014	32	148	180
2015	10	752	762
2016	3	865	868
2017	8	947	955
2018	0	323	323
Total	53	3035	3088

or indirect contacts with the outbreak holding during the high risk period have to be regarded as suspect and followed up accordingly.

Case presentation and chronology of events

The case report presented is an observational study in a large pig breeding farm with almost 5000 animals (sows, piglets and boars) in Krimuldas County where ASF was confirmed mid-January 2017. After weaning, piglets were delivered to a fattening farm, which was located 50 km away and belonged to the same company. The last delivery of piglets took place one day before outbreak confirmation. Three weeks later ASF was confirmed also in the fattening farm.

The animals were kept in seven separate stable buildings with a common yard. At the entrances of each stable there were no sanitary filters. Based on the information received

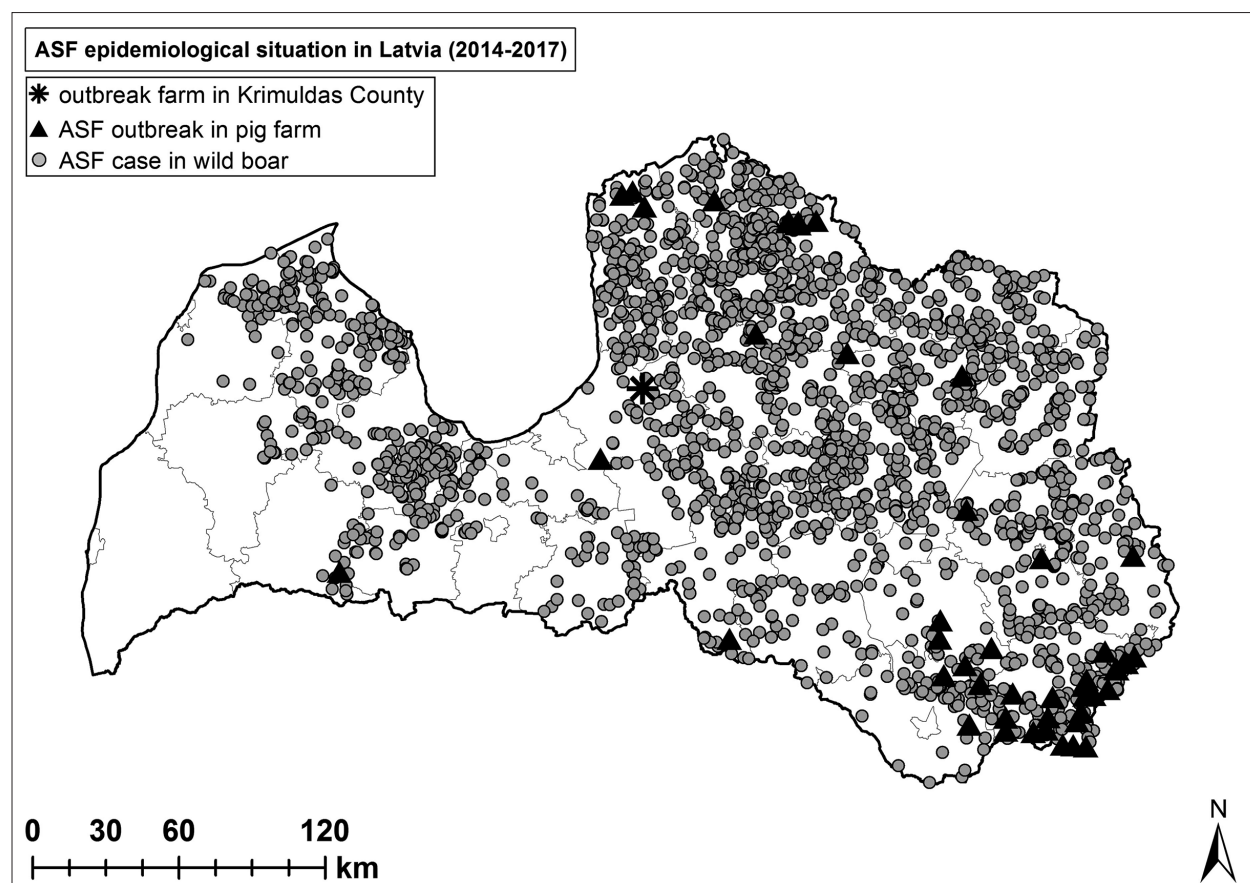


FIGURE 1: African swine fever (ASF) epidemiological situation in Latvia (2014–2017). Data extracted from the Animal Disease Notification System – ADNS.

from the farm manager apparently the first health problems started in stable No. 5 where pregnant sows were kept in groups. According to the farm records (necropsy documents) the first sow with lesions died on 28th of December, 2016. Four pigs showed loss of appetite and vomiting on the 2nd of January and were found dead on 4th of January. Another dead sow was found on 6th of January, followed by one on 9th, one on 11th, 4 on 12th and 6 sows on 13th of January. On 9th of January several sows were moved from stable No. 5 to stable No. 6A for farrowing. Two of those pigs were found dead on 12th and 13th of January. The movement of sows from stable No. 5 to stable 6A was considered as ASFV introduction to stable No. 6A. Breeding boars were kept in a separate stable (No. 2). In this stable, the first boar was found dead on the 7th January and a second one on the 14th January. However, no sampling was carried out for laboratory investigations. On the 14th January another boar was found dead and tested positive for ASF.

Only pigs which died on 13th and 14th of January were sampled and tested for ASF.

All affected stables (5, 6A and 2) were closely linked by the farm staff (inseminator) and the movement of pregnant pigs between different stables.

Methodology

We aimed to investigate how the virus might have entered the breeding farm and how the disease evolved within the farm. The study included several farm visits comprising interviews with the farm manager, veterinarian and farm workers. The interviews were informal, with simultaneous note-taking. Additional information was obtained from the local veterinary service and the rendering company servicing the farm. Information on biosecurity was extracted from the interview data and from observations made during the farm visits. Basically two different aspects were addressed: the biosecurity of the farm setup (buildings, fences, equipment, vehicles, etc.) and the biosecurity linked to management procedures (hygiene protocols, internal work flow, movements of people and vehicles, etc.).

To structure the investigations concerning the origin of the ASFV a hypothesis based approach was chosen. Each hypothesis was then addressed separately to prove or reject it. Three working hypotheses were formulated (Table 1):

- wild boar hypothesis – direct or indirect contacts with infected wild boar
- meat hypothesis – contact with contaminated meat, meat products or any other fomites
- trade hypothesis – introduction of infected pigs

The hypotheses were based on possible virus transmission routes, e.g. ingestion of infected meat or meat products, contact between sick and healthy animals and fomites (European Food and Safety Authority 2015). The option concerning soft tick involvement was not considered since *Ornithodoros* ticks were not reported to play a role in Latvia (Salmane 2012).

Discussion and Conclusion

Possible length of time during which ASF may have existed on the farm

A time-line of the potential course of the disease was created based on laboratory results, anamnestic data,

and records on morbidity, mortality and post mortem findings. In December (19th and 28th December) pathological changes which were indicative for ASFV (enlarged spleen and hemorrhages) were noted in two dead sows of one stable. However, no samples were taken for laboratory confirmation. During the first two weeks of January an increased morbidity and mortality of sows was noticed, particularly in one stable where 17 sows died. ASF was suspected and first samples were taken from five sows which died on 13th January. All five samples were positive for ASF viral DNA. In addition, 50 live pigs from the same stable were sampled and only four of them were positive for ASF viral DNA. In total, 75 animals were sampled and tested for the presence of ASF viral DNA and antibodies. No ASF antibodies were detected, while ASF viral DNA was found in 23 animals. The absence of seropositive animals supports the assumption that no chronic or sub clinical forms of ASF occurred. All infected animals must have died, before detectable antibodies were present in the blood.

Taking into consideration the incubation period of ASF of few days up to one week (Blome et al. 2013), most probably the ASFV entered the farm at the beginning of December 2016 about six weeks before disease confirmation. However, regardless of the time point of virus introduction (early December or later) the virus spread within the farm was very slow; only relative few animals were affected until beginning of January. This slow spread can be explained by the low contagiousness of the virus and the housing of sows within the stables.

It indicates that virus transmission from one animal to the next is rather a slow process. Infected blood plays a major role in virus transmission. Efficient transmission takes place when diseased animals start bleeding or when they die and susceptible animals start licking and nibbling on their carcasses (Pietschmann et al. 2015).

Our findings do not support a massive virus introduction. If a high amount of virus would have been introduced (e.g. via contaminated feed or several infected animals), a rapid and fulminating disease development would have been expected. More probable is the introduction of small amount of virus infecting initially only one or few animals. The mortality during the beginning phase of the disease did not exceed the normal mortality threshold of the farm, which might have been the main reason for not suspecting ASF much earlier. If the dead sows from December, which showed pathological changes, had been tested for ASF, it is most likely the disease would have been diagnosed much earlier and infection of the fattening farm, which took piglets from the breeding unit, could have been avoided.

Biosecurity and risk pathways

The initial impression of the farm setup was that it seemed to be suitable for pig farming in an area, where ASF infected wild boar is in the neighborhood. However, interviews and in-depth assessment revealed that the daily management of the farm did not fulfill biosecurity requirements in all aspects to prevent virus introduction (Table 1). The weakest points were the sanitary filters (locker rooms) for the staff and the entry points of the farm. The locker and resting rooms were not aligned to provide clean separation between staff reception areas and the animal accommodation. The farm employees could bring and consume their own food inside the farm. Taking into consideration that it was the cold period of

the year (winter time) when ASF was circulating on the farm, failure in disinfection efficiency cannot be excluded and also documental evidence of proper disinfection of vehicles was missing.

It was considered that a significant contribution to poor biosecurity may have been the large turnover of staff (almost 50%) in the 10 weeks prior to confirmation of disease, which had poor biosecurity training and awareness of the risks of ASF.

In a previous ASF study in Latvia in 2014 it was reported that biosecurity shortcomings were the common finding and the most serious factor responsible for virus introduction in pig holdings, either via the link to infected wild boar or via swill feeding (Oļševskis et al. 2016). Our findings confirmed that inadequate farm biosecurity is still a major issue. Good farm biosecurity is considered to be the most important tool for preventing ASFV introduction on a holding (Bellini et al. 2016).

The possible origin of ASFV on the holding (Table 2)

Wild boar hypothesis

Although the study farm was located in a restricted area due to ASF in wild boar, no direct or indirect contacts with wild boar were identified. The farm is fenced in by a newly build fence which effectively prevents the access of wild boar. No wild boar or traces of wild boar have been seen in the nearby vicinity. The closest ASF positive wild boar was found about 4.4 km away from the study farm 4 months before outbreak confirmation. Furthermore, no indirect contacts to the wild boar environment were identified. No feed or bedding originating from areas where infected wild boar might have had access entered the farm. No farm employees or other persons visiting the farm participated in hunting or were otherwise involved in activities linked to wild boar. Based on these findings it is highly unlikely that wild boar might have played a direct or indirect role in this outbreak.

Fomites and meat hypothesis

The role of fomites or of contaminated meat or meat products as a potential source of ASFV could be addressed only indirectly by interpreting the biosecurity findings (Table 1). Due to the insufficient biosecurity, infectious material could have entered the farm anytime and ASFV introduction by people could not be ruled out.

Trade hypothesis

The study farm did not receive any live pigs during previous 12 months. Therefore, virus introduction via infected pigs could be excluded (trade hypothesis). However, the opposite was the case. Weaning piglets were regularly delivered to a fattening unit belonging to the same company where ASF was later confirmed. The fattening unit was located around 50 km from the breeding unit. Apart from movements to the fattening farm, one gilt was sold to a small holding and one boar to another small holding in October 2016. However, none of these holdings became infected. Considering

TABLE 2: Hypotheses of introduction routes of African swine fever virus into the pig farm: summary of the epidemiological investigations

Working hypothesis of ASF virus introduction	Risk factors and biosecurity	Description	Probability of introduction pathway
1. Link to infected wild boar	Setup of the farm	Farm has a newly built fence which effectively prevents the access of wild boar. No evidence of wild boar in the vicinity of the farm.	Excluded
	Indirect contact to wild boar environment	No feed or bedding originating from areas/ establishments, where infected wild boar might have had access, entered the farm. Wood used for heating of separate buildings of the farm was kept for 2 years in fenced area. The farm provided pest control, although infestation of mice was observed within few stables.	Excluded
	Role of personnel (anthropogenic factor)	No connection of personnel to hunting activities or other activities linked to wild boar.	Low
	Role of vehicles entering the farm (feed trucks, pig trucks, farms tractor when moving pig carcasses to containers located outside the fenced territory)	The farm has two separate gates, equipped with disinfection installations. One gate used for trucks and other for tractors. Taking into consideration cold weather conditions during December and January, failure in disinfection efficiency cannot be excluded. Evidence of proper disinfection of vehicles was missing.	Moderate
2. Link to contaminated meat or meat products or fomites	Setup of the farm	No proper sanitary filter for farm workers and visitors, no strict clean separation between reception and animal areas. This allows bringing potentially contaminated pig meat products for self-consumptions within the farm and if some leftovers are given to pigs, the infection could be introduced.	High
	Role of personnel (anthropogenic factor)	May bring in food from home, no strict internal rules to eat in designated place. No clear evidence that each person was properly instructed about the biosecurity and risks related to ASF introduction. Huge turnover of employees (almost 50%) during the last two months, no proper risk awareness of newly employed stuff. Lack of awareness of employers may result in failure of the biosecurity measures.	High
3. Trade	Introduction of infected animals	No live pigs have been introduced during previous year prior to ASF notification	Excluded

incubation period and possible time when ASFV was on the farm, the above mentioned movements took place before the breeding farm was infected.

Conclusions and lessons to be learned

The hypothesis based working approach proved to be a suitable tool to conduct epidemiological investigation, particularly to exclude some potential ways of virus introduction. However, certain risk factors remained unclear, particularly some aspects related to human activities on the farm.

Nevertheless, the human factor proved to be the main risk for ASFV introduction on pig farms. Therefore, farm biosecurity has to be addressed more rigorously, particularly all aspects related to human activities. Information campaigns with all stake holders (farmers, veterinarians and staff) are a vital issue.

Early detection of disease is essential to keep the high-risk period as short as possible; passive surveillance must be enhanced in ASF restricted and risk areas. In breeding farms all dead gilts, sows and boars must be compulsory tested for ASF, even if farm mortality is below the normal threshold. In fattening farms at least the first two deaths in each production unit each week, should be tested for ASF virus.

Acknowledgements

The authors wish to thank Dr. Klaus Depner for a critical review of the manuscript and to Dr. Fred Landeg for reviewing the English. The authors also want to thank Dr. Katja Schulz and Nicole Reimer for helping to draft the manuscript and Figure 1.

Conflict of interest

The authors declare that they have no competing interests.

Funding

This article is based upon work from COST Action (ASF-STOP CA15116), supported by COST (European Cooperation in Science and Technology).

Ethical Approval

Not applicable.

Authors contribution

KL, MS, and EO designed and conducted the study, KL and EO drafted the manuscript.

References

- Anonymus (2002):** Council Directive 2002/60/EC of the 27th of June 2002 laying down specific provisions for the control of African swine fever and amending Directive 92/119/EEC as regards Teschen disease and African swine fever. Official Journal of the European Union 192: 27.
- Bellini S, Rutili D, Guberti V (2016):** Preventive measures aimed at minimizing the risk of African swine fever virus spread in pig farming systems. *Acta Vet Scand* 58: 1–10. DOI 10.1186/s13028-016-0264-x.
- Blome S, Gabriel C, Beer M (2013):** Pathogenesis of African swine fever in domestic pigs and European wild boar. *Virus Res* 173: 122–130.
- European Commission (2014):** Commission implementing decision of 9 October 2014 concerning animal health control measures relating to African swine fever in certain Member States and repealing Implementing Decision 2014/178/EU. Official Journal of the European Union.
- European Food And Safety Authority (2015):** Scientific opinion on African swine fever. *EFSA J* 13: 4163.
- European Food Safety Authority, Cortinas Abrahantes J, Gogin A, Richardson J, Gervelmeyer A (2017):** Scientific report on epidemiological analyses on African swine fever in the Baltic countries and Poland. *EFSA J* 15: 4732.
- Oļševskis E, Guberti V, Serzants M, Westergaard J, Gallardo C, Rodze I, Depner K (2016):** African swine fever virus introduction into the EU in 2014: Experience of Latvia. *Res Vet Sci* 105: 28–30.
- Pietschmann J, Guinat C, Beer M, Pronin V, Tauscher K, Petrov A, Keil G, Blome S (2015):** Course and transmission characteristics of oral low-dose infection of domestic pigs and European wild boar with a Caucasian African swine fever virus isolate. *Arch Virol* 160: 1657–1667.
- Salmane I (2012):** Ticks (Acari, Ixodida: Ixodidae & Amblyommiidae) of Latvia. *Latvijas Entomologs* 51: 153–154.
- Sanchez-Vizcaino JM, Mur L, Martinez-Lopez B (2013):** African swine fever (ASF): Five years around Europe. *Vet Microbiol* 165: 45–50.

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Case Report

African Swine Fever in Two Large Commercial Pig Farms in LATVIA—Estimation of the High Risk Period and Virus Spread within the Farm

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Received: 6 July 2020; Accepted: 3 August 2020; Published: 7 August 2020

Abstract: African swine fever (ASF) was first detected in Latvia in wild boar at the Eastern border in June 2014. Since then ASF has continued to spread in wild boar populations covering almost whole territory of the country. Sporadic outbreaks occurred at the same time in domestic pig holdings located in wild boar infected areas. Here we present the results of the epidemiological investigation in two large commercial farms. Several parameters were analyzed to determine the high risk period (HRP) and to investigate the ASF virus spread within the farm. Clinical data, mortality rates and laboratory results proved to be good indicators for estimating the HRP. The measures for early disease detection, particularly the enhanced passive surveillance that is targeting dead and sick pigs, were analyzed and discussed. Enhanced passive surveillance proved to be a key element to detect ASF at an early stage. The study also showed that ASF virus might spread slowly within a large farm depending mainly on direct contacts between pigs and the level of internal biosecurity. Findings suggest improvements in outbreak prevention, control measures and may contribute to a better understanding of ASF spreading patterns within large pig herds. Culling of all pigs in large commercial farms could be reconsidered under certain conditions.

Keywords: African swine fever; commercial farms; early detection; high risk period; disease control

1. Introduction

The first set of cases of African swine fever (ASF) in Latvia were detected in the wild boar population at the eastern border with Belarus in June 2014 [1–3]. The disease continued to spread over most of the territory of Latvia within the following years [4–6]. Similar to other ASF affected countries, the infection cycle is maintained by wild boar and this remains a permanent threat for the domestic pig industry [1,7,8]. In parallel, to the epidemic in wild boar, sporadic ASF outbreaks occurred mostly in backyard farms located in areas where wild boar were affected [1]. Due to their low level of biosecurity, backyard farms are more vulnerable to ASF virus introduction, while large

commercial pig farms are better protected although biosecurity may vary from farm to farm [9]. The first outbreak in a large commercial farm occurred in January, 2017 followed by a secondary outbreak three weeks later in another farm owned by the same company [10].

In total 37,744 domestic pigs died or had to be culled so far due to ASF. An overview of the ASF epidemic in Latvia since 2014 is shown in Table 1.

Table 1. African swine fever (ASF) outbreaks and cases in Latvia during 2014–2020.

Year	Outbreaks in Domestic Pigs			Cases ** in Wild Boar	Territory Affected by ASF in Wild Boar (%) ***
	Commercial *	Backyard *	No of Culled Pigs		
2014	13	19	552	247	31.83
2015	4	6	213	1048	51.03
2016	3	0	308	1146	78.08
2017	5	3	19,914	1431	87.47
2018	5	5	16,705	905	95.01
2019	1	0	52	430	96.09
2020 (1st June)	0	0	0	133	96.39
Total	31	33	37,744	5340	N/A

* pig farms are categorized according to the Strategic approach to the management of African Swine Fever for the EU, SANTE/7113/2015—Rev 12. ** Case is defined according to the European Union (EU) legislation [11]. *** Estimate based on the size of restriction zones by the end of a particular year. Restrictions zones are defined according to the EU legislation [12,13].

In case of an ASF outbreak, a timely epidemiological investigation has to be conducted to identify the possible source and to estimate the high risk period (HRP), which is the likely length of time that ASF has been present on the farm. It is very important to detect infected farms as early as possible after ASF virus introduction to avoid further spread and to minimize the losses to the pig sector and reduce governmental costs associated with outbreak eradication [14]. Animal experiments as well as field observations have shown that a low to moderate transmissibility of ASF virus between pigs is leading to a slow spread of the disease within a farm [10,15–19]. Therefore, the inadvertent introduction of the virus may remain unnoticed by the farmer or the veterinarian for extended time. Under unfavorable circumstances, the HRP may be several weeks or even months during which the virus can spread within a farm or to other farms.

The main strategic aim of ASF surveillance in domestic pigs is to keep the HRP as short as possible by early detection of infected holdings. To facilitate the early detection of outbreaks, new measures were introduced in Latvia in 2017 requiring regular sampling and testing of sick and dead pigs for ASF virus. Each week, at least the first two deaths, including post weaning pigs or pigs older than two months in each production unit, had to be sampled and tested [12,20]. This enhanced passive surveillance approach was based on the assumption that due to the high case fatality (>90%) of ASF almost all infected pigs will become sick and die [15,21,22]. Therefore, any sick or dead animal would be a good candidate for ASF testing.

In this report, we present the results of epidemiological investigations conducted in two large pig farms, affected by ASF in Latvia. One outbreak (Farm A) occurred in 2017 before the enhanced passive surveillance was introduced, while the second outbreak (Farm B) occurred in 2018, when enhanced surveillance was already in place. We focused on the early detection strategy, the HRP and the virus spread within the farms. Our case report contributes to a better understanding of ASF spread patterns within large pig herds and suggests how outbreak prevention and control measures can be improved.

2. Case Description

2.1. Farm A

Farm A is located in south-east of Latvia, a farrow to finish farm with almost 6000 pigs, including nearly 600 breeding sows notified ASF suspicion to the veterinary authority on 14th July 2017 and the outbreak was confirmed by the veterinary authority at the same day. At that time, an active

spread of the infection was ongoing in the local wild boar population [23] (Figure 1). The closest ASF positive wild boar was found less than 3 km from the farm. Pigs were kept indoors in seven separate stables according to their production category (sows, boars, gilts and finishers). Sows were kept in three stables depending on their reproduction phase—sows for artificial insemination, pregnant sows kept in groups and farrowing sows. The farm had its own slaughterhouse located within the farm's territory. The meat from the slaughterhouse was delivered to meat processing and retail establishments in Latvia.

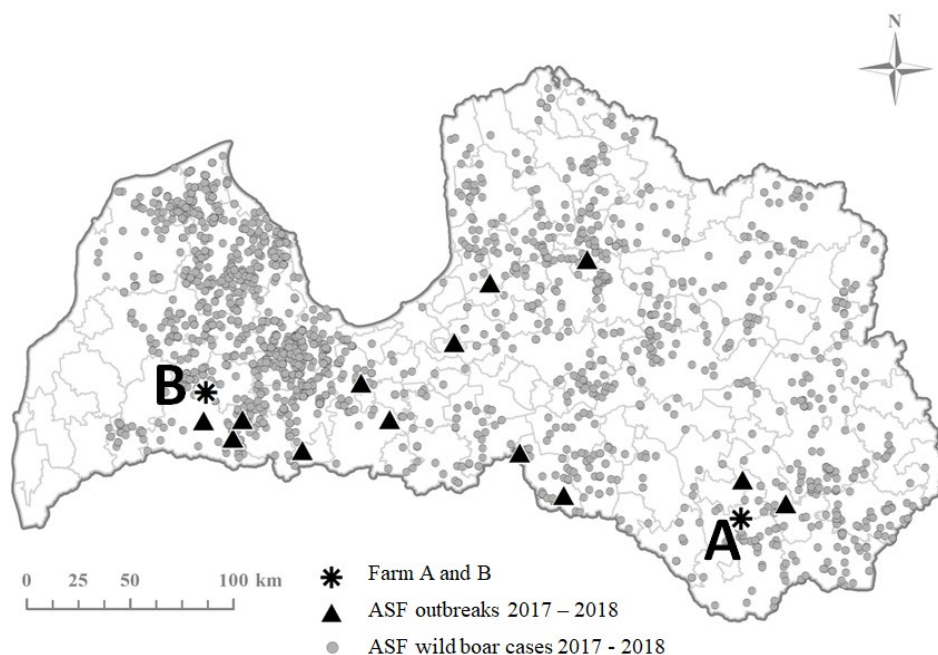


Figure 1. ASF in Latvia in 2017–2018 and the location of the two outbreak farms described in this study.

2.2. Farm B

Farm B, a large farrow to finish farm with around 16,000 pigs, including nearly 1600 breeding sows, was reported ASF positive to the veterinary authority by the laboratory on 31st of July 2018. The farm is located in south-western part of Latvia, also in a restriction area due to ASF in wild boar (Figure 1). Pigs were kept indoors in two separate locations (B1 and B2) three km apart. In B1 breeding pigs, including boars, pregnant and farrowing sows with piglets and weaned pigs were kept in 18 stables. Fattening pigs and gilts were kept in six stables in location B2. There were regular movements of different pig consignments between the two locations on a daily basis. The fatteners were sold to slaughterhouses in Latvia and Lithuania.

2.3. Epidemiological Investigations

The epidemiological investigations were conducted according to the European Union legislation [11,24] and were performed by the veterinary authority. The investigations included onsite inspections, interviews with relevant personnel from the farm's administration, veterinarians and workers directly involved in the daily care of the animals. The documentation regarding animals, personnel, visitors and vehicles entering and leaving the farms were checked and the farm biosecurity status was evaluated. Hypothetical virus introduction routes were identified and checked as previously described [10].

For the estimation of HRP and the reconstruction of the spreading pathways within the farm, the information obtained from the farm records and interviews with farm staff, clinical and post-mortem findings and laboratory results were evaluated. If clinical data were interpreted, an infection cycle from infection until death of ten days was assumed [17,18,22]. For laboratory findings showing

positive RT-PCR and immunoperoxidase test (IPT) results after a period of up to 10 days, it was assumed that might have passed from infection until death of the sampled pigs (C. Gallardo, personal communication (European Union reference laboratory (INIA-CISA), Valdeolmos, Madrid, Spain).

In addition, samples for laboratory testing were taken before culling from pigs of different groups and different stables to obtain a better picture of virus spread within the farms (Tables 2 and 3). The samples were tested for the presence of viral genome (RT-PCR test) and ASF antibodies (Ab-ELISA and IPT) at the Institute of Food Safety, Animal Health and Environment “BIOR” (National Reference Laboratory, NRL) according to the manufactures instructions of the test kit.

Table 2. RT PCR and serology results of pigs of Farm A sampled in different stables before culling.

Stable Id	Number of Pigs in the Stable	Category of Pigs	Number of Samples Taken in a Stable	Laboratory Testing Results	
				RT PCR Positive	Serology (ELISA, IPT) Positive
1	213	Boars, sows	3	0	0
2	301	Sows *	4	4	2
3	696	Sows **	28	10	0
4	1761	Weaners	28	1	0
5	1157	Fatteners	29	0	0
6	1136	Fatteners	28	0	0
7	554	Fatteners	20	0	0
Total	5818	N/A	140	15	2

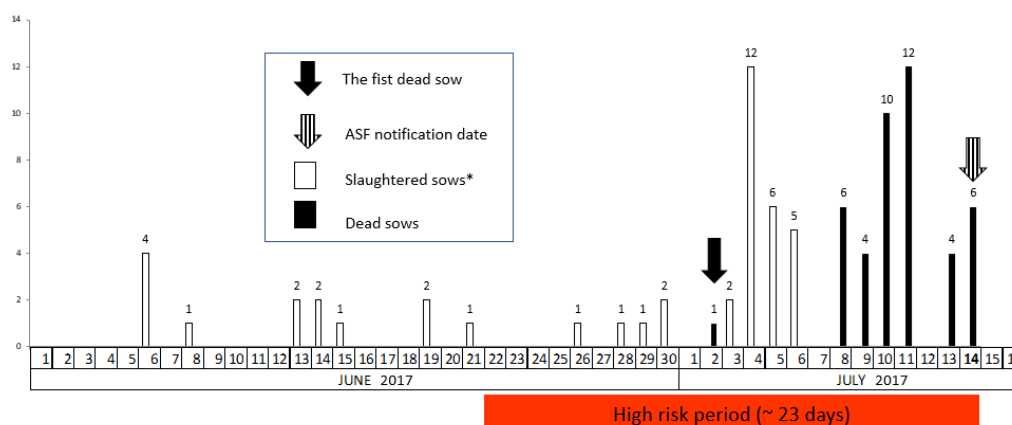
* Sows kept in group pens. ** Sows kept with piglets.

Table 3. RT PCR and serology results of pigs sampled before culling in different stables of Farm B at location B2.

Stable Id	Number of Pigs in the Stable	Category of Pigs	Number of Samples Taken in a Stable	Laboratory Testing Results	
				RT PCR Positive	Serology (IPT) Positive
1	685	Gilts, Fatteners	13	1	0
2	877	Gilts, Fatteners	10	0	0
3	946	Gilts, Fatteners	12	0	0
4	997	Gilts, Fatteners	18	7	5
5	848	Gilts, Fatteners	4	0	0
6	1118	Fatteners	7	0	0
Total	5471	N/A	64	8	5

2.4. Chronology of Events in Farm A

Timeline of the disease event in Farm A is summarized in Figure 2. ASF suspicion was notified by the farmer based on severe clinical signs and increased mortality in stable 2 on the 14th of July. The observed clinical signs were abortions, vomiting, lethargy, high fever and loss of appetite. The disease was officially confirmed in the evening of the same day. Four dead pigs sampled on the 14th July were PCR positive and two of them were also antibody positive (ELISA and IPT). The clinical events started most probably in stable 2 where pregnant sows were kept in groups. On 2nd of July one sow, which previously aborted, died. However, the animal was not sampled and tested for ASF and no suspicion was reported. According to the farm workers, the remaining sows of the same pen were moved to other pens within the same stable. In the following days, several other sows aborted and mortality increased, reaching the peak of 12 dead sows on the 11th of July. A total of 43 sows died between the 2nd and 14th of July, while 25 sows were slaughtered due to severe health problems. Five more sows died in the farrowing stable 3, all coming from stable 2. One of them died on 7th of July and the other four on the 8th and 9th of July, respectively.



* Periodic slaughter of sows was performed within the farm in June, while sudden increase in numbers of slaughtered sows was observed from the 4th of July, but it neither can be proven or excluded that ASF was the cause for slaughtering.

Figure 2. Timeline of the disease event in Farm A, stable 2 and the estimated high risk period.

Additional samples were taken from pigs in all other stables the day after outbreak confirmation to investigate further the disease situation in each separate unit and to reconstruct the potential spread scenario within the farm. Purposive samples were taken from pigs with any disease signs and randomly to detect 10% prevalence with 95% confidence in each sampling unit (stable) [10,17,25–27]. The laboratory results are shown in Table 2.

2.5. Chronology of Events in Farm B

The ASF suspicion in Farm B was notified to the veterinary authority by the NRL on the 31st of July. Three pigs older than four months died on the 29th of July and tested PCR positive for ASF during routine testing in the frame of enhanced passive surveillance. The pigs were from location B2, stable 4. The timeline of the disease event in that stable is presented in Figure 3. Alongside RT-PCR positive results, the three pigs mentioned above were also positive for ASF antibodies (IPT test). According to the information provided by the owner and the farm veterinarian, no clinical signs indicating ASF were noticed prior to the deaths of the pigs. The ASF positive pigs belonged to a group of 280 weaners, which were moved on the 23rd of July from location B1 stable 7 to location B2 stable 4. A few days after the movement an increased mortality was observed in stable 4 in the group of the moved animals (Figure 3B). The increase in mortality was associated with very hot weather conditions and previous experience with edema disease (*E.coli* enterotoxaemia). However, mortality in location B1 stable 7 did not increase notably (2 to 3 weaned pigs per week). In the farm's records a slight increase of mortality could be seen in stable 4 of location B1 starting mid-July (Figure 3A).

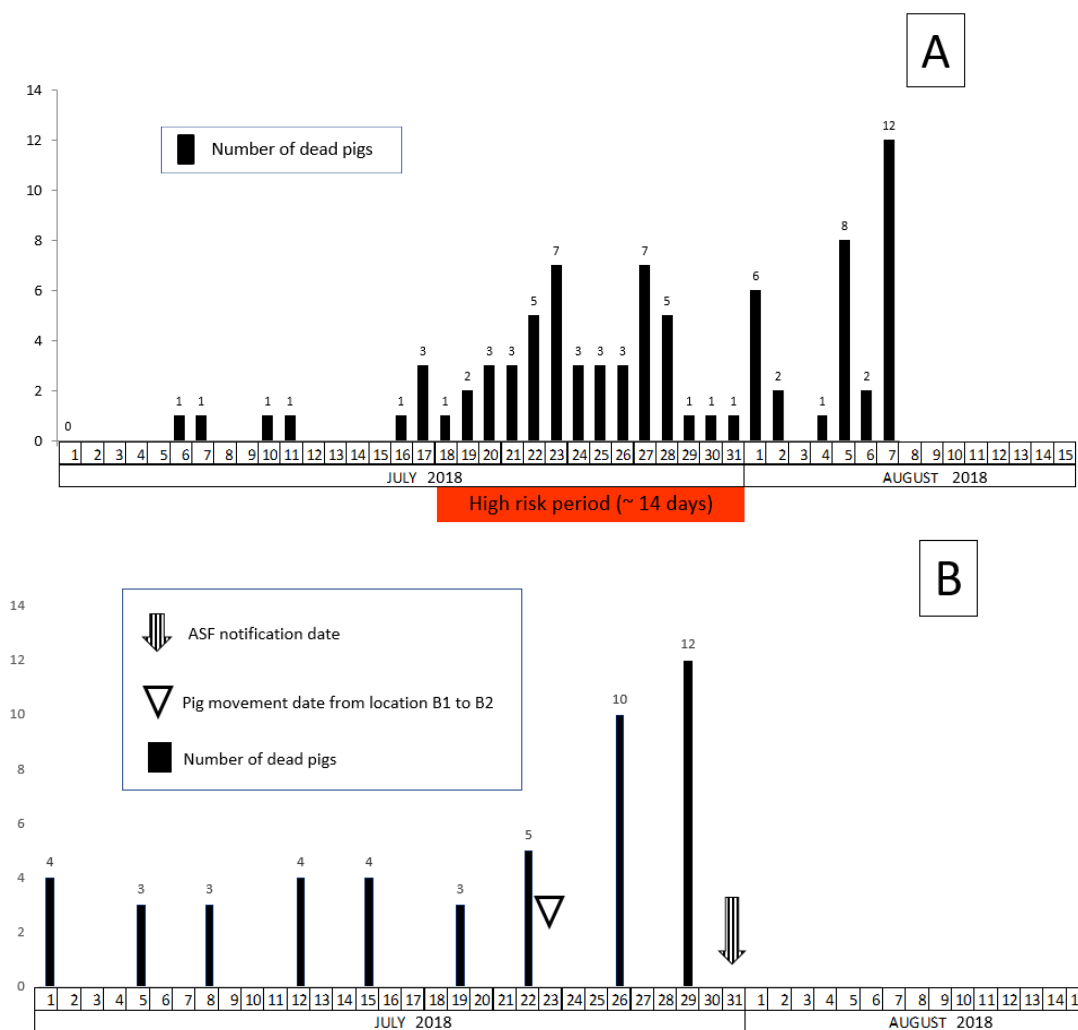


Figure 3. Timeline of the disease event in location B2 stable 4 (**A**) and B1 stable 4 (**B**) and the estimated high risk period for farm B.

After disease notification, on the 1st of August samples were taken from pigs of both locations to identify possible virus spread within the farm. Considering the large number of stables and animals, the sampling was targeted to dead pigs and pigs with clinical signs.

In location B1, seven dead pigs from stable 4 were sampled and six tested RT-PCR positive. Four of these pigs tested negative for antibodies to ASF by IPT. In addition, 40 live pigs from different stables were sampled and all of them tested negative for antibodies (ELISA) and virus genome.

In location B2, samples were taken from 13 dead and 51 sick pigs suspected of being infected. These pigs were selected since they were less active and reluctant to move. One dead pig in stable 1 and two dead and five suspect live pigs from stable 4 were found RT-PCR positive. Two RT-PCR positive dead pigs from the stable 4 were positive by IPT. All the suspect live pigs were tested for virus genome and antibodies (ELISA). The five PCR positive suspected pigs were tested for antibodies with both—ELISA test and IPT and three tested positive in IPT. Details of sample size and laboratory results are shown in Table 3.

2.6. Estimation of the High Risk Period in Farm A

The first pig, which aborted and died on 2nd July was presumed to be the first animal which got infected (index case) in farm A. Thus, ASF virus could have entered the farm about 10 days earlier, around 22nd of June, considering the maximum survival time of 10 days after the infection with an ASF genotype II virus strain [22]. The suspicion of the outbreak was notified on 14th of July, which

makes a total of 23 days of HRP for the farm A. In addition, two dead pigs sampled on 14th July were seropositive (ELISA and IPT). This finding supports the estimate of HRP being longer than 10 days. A further indicator for prolonged HRP was the increased mortality during the last three weeks before notification supporting the hypothesis that more than two infectious cycles should have passed.

2.7. Estimation of the High Risk Period in Farm B

Due to the enhanced passive surveillance, conducted on Farm B we assumed that the RT-PCR positive animals which died on the 29th of July were also the first infected animals (index cases) on the farm. In addition to the RT-PCR positive results, these pigs were tested positive also by IPT method. Therefore, we assume that they got infected 7 to 10 days earlier, around the 18th of July. The estimated HRP would be around two weeks.

2.8. Potential Spreading Scenario within Farm A

The potential spreading Scenario within Farm A is shown in Figure 4. Most probably, ASF infection started in stable 2 with a sow which aborted and died on the 2nd of July (index case). This sow came from the insemination stable 1 around 10–12 days before. However, the clinical status of the animals in the insemination stable as well as the laboratory results were not indicating an ongoing infection there. Pigs were moved from stable 1 to stable 2 using an outdoor path. It can be hypothesized that the index sow picked up infection from potentially contaminated environment during the outdoor walk. The path which connected two stables was badly constructed, with no solid floor and with partly damaged fencing, which may allow the inadvertent escape of pigs into the farm's territory. If the farm's territory was contaminated with ASF virus it was possible for pigs to get infected. After the index sow died, the sows from that pen were moved to other places within the same stable and they were the next ones who aborted and died.

Based on the disease timeline, we assume that the five ASF positive sows in the farrowing stable 3 became infected while still being in stable 2. Most probably, these animals were moved to the farrowing stable during the incubation period.

One weaned pig was found PCR positive in stable 4. The weaners in stable 4 were brought from stable 3. Therefore, it can be assumed that infection in stable 4 was introduced through weaned piglets. ASF virus spread between the stables in Farm A could occur due to movements of infected animals. However, pigs in other stables, where no animal movements took place during the HRP, remained unaffected.

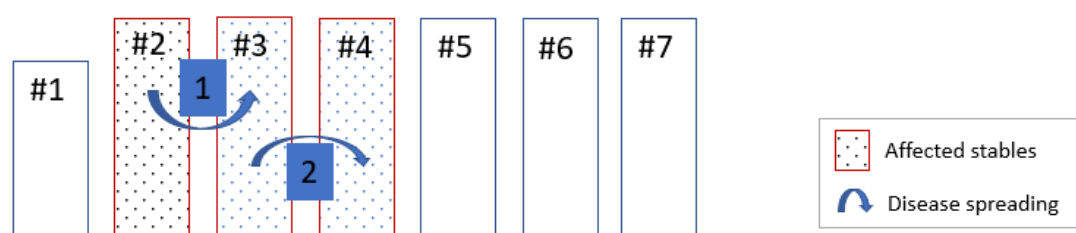


Figure 4. Potential spread of ASF virus between the stables in a Farm A.

2.9. Potential Spreading Scenario in Farm B

The potential spreading scenario in Farm B is shown in Figure 5. The three pigs (index cases) in stable 4 of location B2 tested RT-PCR positive, were moved from location B1, stable 7 six days before they died. Since the pigs were also seropositive, it can be excluded that infection happened during the transport.

The positive RT-PCR results from pigs sampled after disease notification proved that ASF was also present in stable 4 of location B1 and in stable 1 of location B2. Most probably infection started

in stable 4, location B1 and then probably jumped over via stable 7 to location B2. This can be justified by the movement records between both locations. However, it remains unclear how the infection spread between stables in location B1. Most probably, it might have happened via movement of farm workers or via shared stable equipment. All stables were connected through corridors where workers and equipment, including wheel borrows, could move without additional biosecurity measures. Furthermore, it remained unclear how the virus entered stable 1 in location B2. Based on the farm's records, movement of pigs between stables did not take place. Most probably shared equipment or farm workers could have played a role in virus transmission.

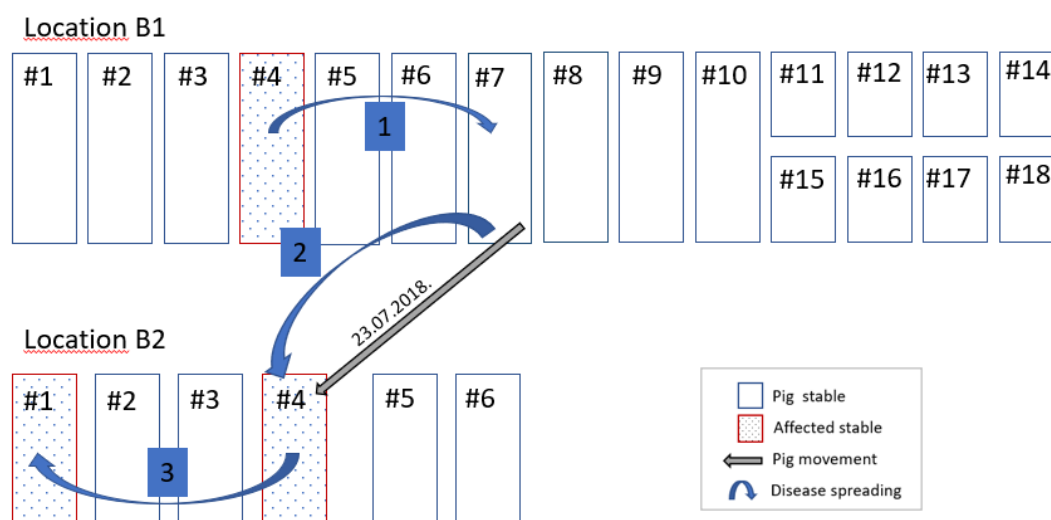


Figure 5. Potential spreading of ASF virus between the stables in a Farm B.

3. Discussion

3.1. Early Detection of the Disease and HRP

Early detection would mean the detection of the very first ASF infected pigs on the farm at the shortest period of time after the infection was established. Ideally, these should be the first pigs which show clinical signs and die due to ASF. This might happen about one week after infection. However, seldom reliable data and information are found indicating the start of an infection (index case) on a farm. Due to the rather low infectivity of the circulating ASF virus, only few animals may become infected and die during the first weeks [19]. Therefore, the general mortality rate may not exceed normal levels and the initially low ASF induced mortality can easily be overlooked, particularly in larger farms. It could be shown that ASF virus might circulate in a herd for nearly a month before it causes a marked increase in mortality [15,17,18]. Under such conditions, enhanced surveillance targeting sick and dead animals, independently from the mortality rate, would be the only practical option for early ASF detection. In contrast, sampling of clinically healthy pigs would fail to detect ASF on a farm during the initial stage.

In Farm A, ASF was suspected and reported due to increased morbidity and mortality of pregnant sows at a late stage. No enhanced passive surveillance system for early detection was in place targeting dead or suspect animals. An HRP of longer than 3 weeks was estimated. In Farm B, the enhanced passive surveillance was implemented and ASF could be detected before mortality noticeably raised. The HRP was around two weeks.

A reasonably accurate estimate of the HRP may allow the veterinary authorities to trace efficiently potential secondary outbreaks or virus spreading via animal movement, contaminated products, vehicles, people and other means. However, under field conditions estimating the HRP is rather difficult and often unsatisfactory due to insufficient or questionable data. The history based on clinical signs and morbidity records might not always be reliable or relevant laboratory examinations might be missing. However, mortality records and laboratory test results proved to be

useful indicators for estimating the HRP. Experimental studies with genotype 2 ASF virus showed that in average an infection cycle, the time from infection until death of an animal, is around 10 days [15,16,21,22]. Usually antibodies detectable in an ELISA test are found in animals, which do not die within the first 10 days of infection [22]. The timeline showing the increased mortality on Farm A is a strong indicator that more than two infectious cycles of about 10 days each might have occurred, meaning that more than 20 days must have past, since virus introduction. However, in a worst-case scenario the HRP could have been even longer. Seropositivity detected by ELISA test is a very clear indicator that the virus must have been on the farm for more than ten days. If the concept of weekly testing of dead pigs would have been in place on Farm A, the outbreak could have been detected much earlier, most probably when the first sow was found dead. On Farm B none of the pigs tested positive by ELISA but the first PCR positive animals were also positive in IPT. Such results indicate a relative recent infection. Seroconversion can be seen by IPT around 7 to 10 days after infection [28], (C. Gallardo, personal communication (European Union reference laboratory (INIA-CISA), Valdeolmos, Madrid. Spain).

The sooner the early detection surveillance scheme for detecting potentially infected holdings is implemented, the earlier farms are found in which only one or few virus positive animals are present. This leads to the dilemma regarding the acceptance of the depopulation measures, which have to follow without any delay. Due to this dilemma, alternative culling and surveillance schemes for large farms where only few infected animals are present could be developed.

3.2. Virus Spreading within the Farms

Reconstructing the potential spreading pathway of the virus within the farm may help to identify how and where the virus might have entered and to which degree the farm was affected. Additional blood or organ samples for laboratory examinations taken from suspect animals, from all production units during culling or shortly before, can prove the presence or absence of the virus in different farm units. However, under field conditions, sampling of pigs before culling or during culling is difficult because of time pressure and other logistical arrangements. In Farm A the ASF infection most probably started in the stable 2 and spread to the stables 3 and 4, while four other stables were not affected. For Farm B reconstruction of the virus pathway was more difficult compared to Farm A, mainly due to the complex epidemiological situation ongoing in parallel in the two separate locations. The clinical events started most probably in stable 4, location B1 and then jumped over via stable 7 to location B2. Direct contacts with infected live pigs or carcasses posed the highest risk of spreading the infection [9,29]. As in the case of Farm A, we could see how infection spread from one stable to the next following the movements of infected pigs. Non-compliance with farm biosecurity measures left space for virus introduction and spread within the farm (biosecurity [30]).

Experimental findings as well as field studies have proven a low transmissibility of ASFV, which may delay the virus spread inside a pen and between pens [15,16,18]. The calculated period from infection until onset of infectiousness was around 10 days within a pen and around 13 days between pigs in different pens in a study by Guinat et al. [15]. Our findings support these experimental studies, confirming the relatively slow spread of the infection and are based on the analysis of ASF spread within both farms, considering the results of clinical and laboratory investigations, as well as on the estimated length of the HRP. Although relatively long HRP was detected, particularly for Farm A, there were still negative animals within the affected stables and there were stables not affected at all on the date of investigation. However, the speed of virus spread is very much influenced by the local biosecurity measures, the local farm infrastructure and the farm management. Therefore, daily maintenance of high biosecurity measures must have highest priority [9,31]. Farms located in infected areas due to ASF virus circulation in wild boar populations are under particular risk.

4. Conclusions

ASF rather spreads slowly within pig herds, particularly in the initial stage of the outbreak. Several weeks can pass with ASF infection within the farm until mortality rises above the average level.

Enhanced passive surveillance by testing dead or sick animals is an effective method for the early detection of ASF in large pig farms. In addition, it may lead to a considerable reduction of the HRP.

Mortality records and laboratory results proved to be useful indicators for estimating the HRP.

With early detection and a short HRP it can be assumed that large parts of the farm are not yet affected by ASF. Under the precondition of slow virus spread, non-affected farm units could be excluded from culling measures. Good farm management and strict internal biosecurity measures as well as an intelligent surveillance scheme should be in place to reach this goal.

Veterinary authorities may take into consideration slow spread of the virus within a farm when planning and performing stamping out procedures thus gaining time for epidemiological investigations, sampling for laboratory investigations, as well as time to prepare properly for culling and synchronize it with carcass destruction.

Author Contributions: L.K., E.O. and M.S.: epidemiological investigation and data analysis; K.D. and A.V.: methodology, L.K.: writing—original draft preparation; K.D.: review and editing; M.S. and E.O.: visualization, A.V. and A.B. review and supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This work received no external funding.

Acknowledgments: The authors wish to thank to Inese Raižģe from the Joint Stock Company “Latvian State Forests” for the Figure 1, to the regional veterinary inspectors who carried out sampling of pigs in both affected farms and to Ieva Rodze, Svetlana Cvetkova and Žanete Šteingolde for providing good quality laboratory data which were used in the study. The authors also wish to thank Fred Landeg for reviewing the English.

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

References

1. Oļševskis, E.; Guberti, V.; Seržants, M.; Westergaard, J.; Gallardo, C.; Rodze, I.; Depner, K. African swine fever virus introduction into the EU in 2014: Experience of Latvia. *Res. Vet. Sci.* **2016**, *105*, 28–30.
2. EFSA (European Food Safety Authority); Depner, K.; Gortazar, C.; Guberti, V.; Masiulis, M.; More, S.; Olsevskis, E.; Thulke, H.-H.; Viltrop, A.; Wozniakowski, G.; et al. Scientific report on the epidemiological analyses of African swine fever in the Baltic States and Poland. *EFSA J.* **2017**, *15*, e5068, doi:10.2903/j.efsa.2017.5068.
3. EFSA (European Food Safety Authority); Cortinas Brahanes, J.; Gogin, A.; Richardson, J.; Gervelmeyer, A. Scientific report on epidemiological analyses on African swine fever in the Baltic countries and Poland. *EFSA J.* **2017**, *15*, e4732, doi:10.2903/j.efsa.2017.4732.
4. Schulz, K.; Olsevskis, E.; Staubach, C.; Lamberg, K.; Serzants, M.; Cvetkova, S.; Conraths, F.J.; Sauter-Louis, C. Epidemiological evaluation of Latvian control measures for African swine fever in wild boar on the basis of surveillance data. *Sci. Rep.* **2019**, *9*, 4189, doi:10.1038/s41598-019-40962-3.
5. EFSA (European Food Safety Authority); Boklund, A.; Cay, B.; Depner, K.; Foldi, Z.; Guberti, V.; Masiulis, M.; Miteva, A.; More, S.; Olsevskis, E.-H.; et al. Scientific report on the epidemiological analyses of African swine fever in the European Union (November 2017 until November 2018). *EFSA J.* **2018**, *16*, e5494, doi:10.2903/j.efsa.2018.5494.
6. EFSA (European Food Safety Authority); Miteva, A.; Papanikolaou, A.; Gogin, A.; Boklund, A.; Bøtner, A.; Linden, A.; Viltrop, A.; Schmidt, C.G.; Ivanciu, C.; et al. Scientific report on the epidemiological analyses of African swine fever in the European Union (November 2018 to October 2019). *EFSA J.* **2020**, *18*, e5996, doi:10.2903/j.efsa.2020.5996.
7. Pautienius, A.; Grigas, J.; Pileviciene, S.; Zagrabkaite, R.; Buitkuviene, J.; Pridotkas, G.; Stankevicius, R.; Streimikyte, Z.; Salomskas, A.; Zienius, D.; et al. Prevalence and Spatiotemporal Distribution of African Swine Fever in Lithuania, 2014–2017. *Virol. J.* **2018**, *15*, 177.

8. Pejsak, Z.; Niemczuk, K.; Frant, M.; Mazur, M.; Pomorska-Mol, M.; Zietek-Barszcz, A.; Bocian, L.; Lyjak, M.; Borowska, D.; Wozniakowski, G. Four Years of African Swine Fever in Poland. New Insights into Epidemiology and Prognosis of Future Disease Spread. *Pol. J. Vet. Sci.* **2018**, *21*, 835–841.
9. Bellini, S.; Rutili, D.; Guberti, V. Preventive measures aimed at minimizing the risk of African swine fever virus spread in pig farming systems. *Acta Vet. Scand.* **2016**, *58*, 82.
10. Lamberga, K.; Seržants, M.; Oļševskis, E. African swine fever outbreak investigations in a large commercial pig farm in Latvia: A case report. *Berl.Munch, Tierarztl. Wochenschr.* **2019**, *132*, 151–155.
11. European Commission, 2002. Council Directive 2002/60/EC of 27 June 2002 Laying Down Specific Provisions for the Control of African Swine Fever and Amending Directive 92/119/EEC as Regards Teschen Disease and African Swine Fever. Available online: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02002L0060-20080903> ((accessed on 18 June 2020).
12. Commission Implementing Decision 2014/709/EU of 9 October 2014 Concerning Animal Health Control Measures Relating to African Swine Fever in Certain Member States and Repealing Implementing Decision 2014/178/EU. Available online: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32014D0709> (accessed on 26 June 2020).
13. Working Document SANTE/7112/2015/Rev. 3, Principles and Criteria for Geographically defining ASF Regionalization. Available online: https://ec.europa.eu/food/sites/food/files/animals/docs/ad_control-measures_asf_wrk-doc-sante-2015-7112.pdf (accessed on 2 July 2020).
14. Arias, M.; Jurado, C.; Gallardo, C.; Fernandez-Pinero, J.; Sanchez-Vizcaino, J.M. Gaps in African swine fever: Analysis and priorities. *Transbound. Emerg. Dis.* **2017**, *1* (Suppl 65), 235–247, doi:10.1111/tbed.12695.
15. Guinat, C.; Gubbins, S.; Vergne, T.; Gonzales, J.L.; Dixon, L.; Pfeiffer, D.U. Experimental pig-to-pig transmission dynamics for African swine fever, Georgia 2007/1 strain. *Epidemiol. Infect.* **2016**, *144*, 25–34.
16. Pietschmann, J.; Guinat, C.; Beer, M.; Pronin, V.; Tauscher, K.; Petrov, A.; Keil, G.; Blome, S. Course and transmission characteristics of oral low-dose infection of domestic pigs and European wild boar with a Caucasian African swine fever virus isolate. *Arch. Virol.* **2015**, *160*, 1657–1667.
17. Nurmoja, I.; Motus, K.; Kristian, M.; Niine, T.; Schulz, K.; Depner, K.; Viltrop, A. Epidemiological analysis of the 2015–2017 African swine fever outbreaks in Estonia. *Prev. Vet. Med.* **2018**, S0167-5877(18)30361-1, doi:10.1016/j.prevetmed.2018.10.001.
18. Zani, L.; Dietze, K.; Dimova, Z.; Forth, J.H.; Denev, D.; Depner, K.; Alexandrov, T. African Swine Fever in a Bulgarian Backyard Farm—A Case Report. *Vet. Sci.* **2019**, *6*, 94, doi:10.3390/vetsci6040094.
19. Chenais, E.; Depner, K.; Guberti, V.; Dietze, K.; Viltrop, A.; Ståhl, K. Epidemiological considerations on African swine fever in Europe 2014–2018. *Porc. Health Manag.* **2019**, *5*, 6, doi:10.1186/s40813-018-0109-2.
20. European Commission (EC). Strategic Approach to the Management of African Swine Fever for the EU, SANTE/7113/2015—Rev12. Available online: https://ec.europa.eu/food/sites/food/files/animals/docs/ad_control-measures_asf_wrk-doc-sante-2015-7113.pdf (accessed on 6 November 2020).
21. Gabriel, C.; Blome, S.; Malogolovkin, A.; Parilov, S.; Kolbasov, D.; Teifke, J.P.; Beer, M. Characterization of African swine fever virus Caucasus isolate in European wild boars. *Emerg. Infect. Dis.* **2011**, *17*, 2342–2345, doi:10.3201/eid1712.110430.
22. Blome, S.; Gabriel, C.; Beer, M. Pathogenesis of African swine fever in domestic pigs and European wild boar. *Virus Res.* **2013**, *173*, 122–130, doi:10.1016/j.virusres.2012.10.026.
23. Oļševskis, E.; Schulz, K.; Staubach, C.; Seržants, M.; Lamberga, K.; Pūle, D.; Ozoliņš, J.; Conraths, F.; Sauter-Louis, C. African swine fever in Latvian wild boar—A step closer to elimination. Running head: Epidemiology of African swine fever in Latvia. *Transbound. Emerg. Dis.* **2020**, doi:10.1111/tbed.13611.
24. Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on Transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law'); Council of the European Union: Brussels, Belgium, 2016.
25. Gallardo, C.; Soler, A.; Nieto, R.; Cano, C.; Pelayo, V.; Sanchez, M.A.; Pridotkas, G.; Fernandez-Pinero, J.; Briones, V.; Arias, M. Experimental Infection of Domestic Pigs with African Swine Fever Virus Lithuania 2014 Genotype II Field Isolate. *Transbound. Emerg. Dis.* **2015**, *64*, 300–304, doi:10.1111/tbed.12346.
26. Olesen, A.S.; Lohse, L.; Boklund, A.; Halasa, T.; Gallardo, C.; Pejsak, Z.; Belsham, G.J.; Rasmussen, T.B.; Bøtner, A. Transmission of African swine fever virus from infected pigs by direct contact and aerosol routes. *Vet. Microbiol.* **2017**, *211*, 92–102, doi:10.1016/j.vetmic.2017.10.004.

27. European Commission, 2003. Commission Decision 2003/422/EC of 26 May 2003 Approving an African Swine Fever Diagnostic Manual. Available online: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32003D0422> (accessed on 18 June 2020).
28. Gallardo, C.; Fernández-Pinero, J.; Arias, M. African swine fever (ASF) diagnosis, an essential tool in the epidemiological investigation. *Virus Res.* **2019**, *271*, 197676.
29. Guiant, C.; Gogin, A.; Blome, S.; Keil, G.; Pollin, R.; Pfeiffer, D.U.; Dixon, L. Transmission routes of African swine fever virus to domestic pigs: Current knowledge and future research directions. *Vet. Rec.* **2016**, *178*, 262–267 doi:10.1136/vr.103593.
30. Kristīne Lamberga. Veterinary Surveillance Department, Food and Veterinary Service, Riga, Latvia. Personal communication with Food and Veterinary Service, 2020.
31. Penrith, M.-L.; Vosloo, W. Review of African swine fever: Transmission, spread and control. *J. South. Afr. Vet. Assoc.* **2009**, *80*, 58–62.



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A practical guide for strategic and efficient sampling in African swine fever-affected pig farms

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Funding information

FLI ASF

Abstract

In the case of African swine fever (ASF) outbreaks in pig farms, EU legislation requires a thorough epidemiological investigation to determine, among other tasks, the extent of infection in the affected farm. The main aim of this study was to implement a reliable sampling strategy to quickly obtain an overview of the extent of ASF virus spread in an affected pig farm. We developed and tested a three-step approach: (i) identification of sub-units within the affected farm, (ii) categorization of sub-units, and (iii) targeted selection of animals for testing. We used commercially available lateral flow devices (LFDs) to detect ASF antigen and antibodies under field conditions and compared them with routinely performed laboratory tests (qPCR, ELISA, IPT). The study was conducted in three commercial farms in Latvia that were affected by ASF in July 2020. One of the affected farms was relatively small with only 31 pigs, whereas the other two were large with 1800 and 9800 animals, respectively. The approach proved to be helpful and practical for efficient and reliably assess the ASF situation on the farm and to identify sub-units within a farm where infected animals are present and sub-units which might (still) be free of infection. This important epidemiological information helps to better estimate the high-risk period and to track the potential spread of infection outside the farm. It allows also to prioritize culling and, if appropriate, to pursue a partial culling strategy taking into account the absence of clinical signs, implemented biosecurity measures, quarantine and negative test results, among others. This might be of interest for large commercial farms where the infection was identified very early and has not yet spread widely. Due to its limited sensitivity, the antigen LFD test is useful for testing animals showing signs of disease.

KEYWORDS

African swine fever, domestic pigs, lateral flow device, outbreak investigation, sampling strategy

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1 | INTRODUCTION

African swine fever (ASF) is a deadly viral animal disease that significantly affects domestic and wild suids (*Sus scrofa*) (Plowright et al., 1994; Chenais et al., 2019). The current epizootic is caused by ASF virus (ASFV) of genotype II and started in 2007 in Georgia, from where it spread to neighbouring Caucasian countries and the Russian Federation, reaching the European Union in 2014, including Latvia (EFSA, 2020; OIE, 2021; Viltrop et al., 2021). In 2018, China, the world's largest pig producer, reported its first ASF outbreaks, and by the end of 2019 China had lost more than half of its pig population (Berthe, 2020). During the summer of 2021, ASF arrived in the Dominican Republic and Haiti thus threatening the pig sectors in North and Latin America (Gonzales et al., 2021). The huge losses are caused not only by the infection itself, which kills the affected animals, but also by the trade restrictions and the culling of all animals on an affected farm.

For ASF outbreaks in pig farms, EU legislation requires a thorough outbreak investigation with the aim of identifying the likely origin of the virus and the high-risk period (HRP), that is, the estimated time that ASF has been on the farm before detection as well as to obtain information on the likely spread of the disease to other farms (EC, 2002, 2016). To do this, it would be important to estimate the extent of an outbreak on a farm, that is, how far the virus has already spread within the farm, by identifying the affected pig units. For outbreak investigations after disease confirmation, EU legislation required (until 21 April 2021) the collection of random blood samples from pigs on the affected holding during the culling process for serological and virological testing. The minimum number of samples required should allow the detection of a seroprevalence of 10% in pigs in each sub-unit of the holding with a 95% confidence level (EC, 2003). Analyses of domestic pig outbreaks in the current epizootic as well as experimental studies have shown that the contagiousness of ASF is rather low, leading to a slow spread within pig herds (Chenais et al., 2019). This in turn usually leads to low mortality on farms in the early phase, usually within 2 to 3 weeks after virus introduction, though after this period most of the animals in the same pen will be infected. To date, all ASF viruses detected in domestic pigs and the majority of wild boar in Latvia were virulent strains despite the finding of an attenuated strain in a hunted wild boar in 2017 (Gallardo, Soler, et al., 2019; Gallardo et al., 2021). Taking into account that the dominant ASFV genotype II strains currently circulating are causing a case-fatality rate of >90%, seropositive healthy pigs are rarely found as majority of pigs do not survive the acute phase (Blome et al., 2020). Therefore, considering the circulation of virulent viruses, surveillance based on random serological sampling is no longer recommended for early detection of ASF or as a method to estimate the spread of the virus within an affected farm (EFSA, 2021). The targeted surveillance approach is based on the assumption that virulent viruses are still the ones infecting domestic pigs and therefore the majority of infected animals would get sick and die (Busch et al., 2021; Blome et al., 2020). Therefore, targeted sampling focuses on the selection of suspicious, sick, and dead animals to be tested for the presence of the virus (risk-based surveillance). Nevertheless, antibody tests are important findings that provide

additional information about the spread of the disease in a farm (EC, 2018).

Under field conditions, rapid detection of ASF-infected animals can be increased with the help of lateral flow devices (LFDs) for ASF virus antigen (Ag-LFD) and antibody (Ab-LFD). Such pen-side or point-of-care tests have been developed in recent years and are commercially available (Cappai et al., 2017; Gallardo, Fernandez-Pinero, et al., 2019). While the sensitivity and specificity of the Ab-LFD is over 90% compared to antibody ELISA tests, the sensitivity of the Ag-LFD has been shown to be below standard tests routinely used in diagnostic laboratories, for example, qPCR (Carlson et al., 2017; Pikalo et al., 2020, 2021; Sastre et al., 2016).

The main aim of our study was to implement a reliable sampling strategy that would enable us to quickly obtain an overview of the extent of virus spread on an ASF-affected pig farm. A fast and reliable overview would allow decision-makers to quickly take further appropriate action in controlling the outbreak. The hypothesis we are pursuing is that ASF-infected animals are most likely to be found in the group of sick animals, and LFDs may help in identifying such animals. Our study had two objectives: (i) to develop a targeted and efficient sampling strategy for ASF-affected farms and (ii) test the suitability of LFDs under field conditions compared to standard laboratory tests (PCR, ELISA, and IPT). So far, LFDs have mainly been tested under experimental conditions and not as an additional tool on ASF-affected farms.

In large commercial pig farms in endemic areas, consideration is being given to exempting animals not affected by the disease from culling, where legislation and biosecurity allow (Costard et al., 2022). On farms where the disease has not yet spread widely, unaffected sub-units could be exempted from culling following an appropriate legal, biosecurity, and surveillance concept (Costard et al., 2022). In order to consider this partial culling approach, it is necessary to efficiently and carefully monitor the extent of virus spread on the farm after the disease has been notified.

2 | MATERIALS AND METHODS

2.1 | Outbreak information and farm characteristics

The study was conducted in three commercial farms in Latvia that were affected by ASF in July 2020. It was a purely random choice of farms that resulted from the epidemiological situation at the time. The farms were of different sizes and there was no epidemiological link between them. All three farms were located in regions with active ASF virus circulation in the wild boar population (Oļševskis et al., 2020). The following brief farm descriptions reflect the ASF situation on the farms when ASF was confirmed and before we started our study. The information was provided by the farm owners or the farm veterinarians.

Farm A was a small commercial pig farm with 31 pigs in two separate stables (A1 and A2) only a few metres apart. Stable A1 had 25 pigs in six pens: one gilt in a pen and 24 finishers (30–80 kg weight) in five pens. In stable A2, two sows and four piglets were kept. ASF was suspected

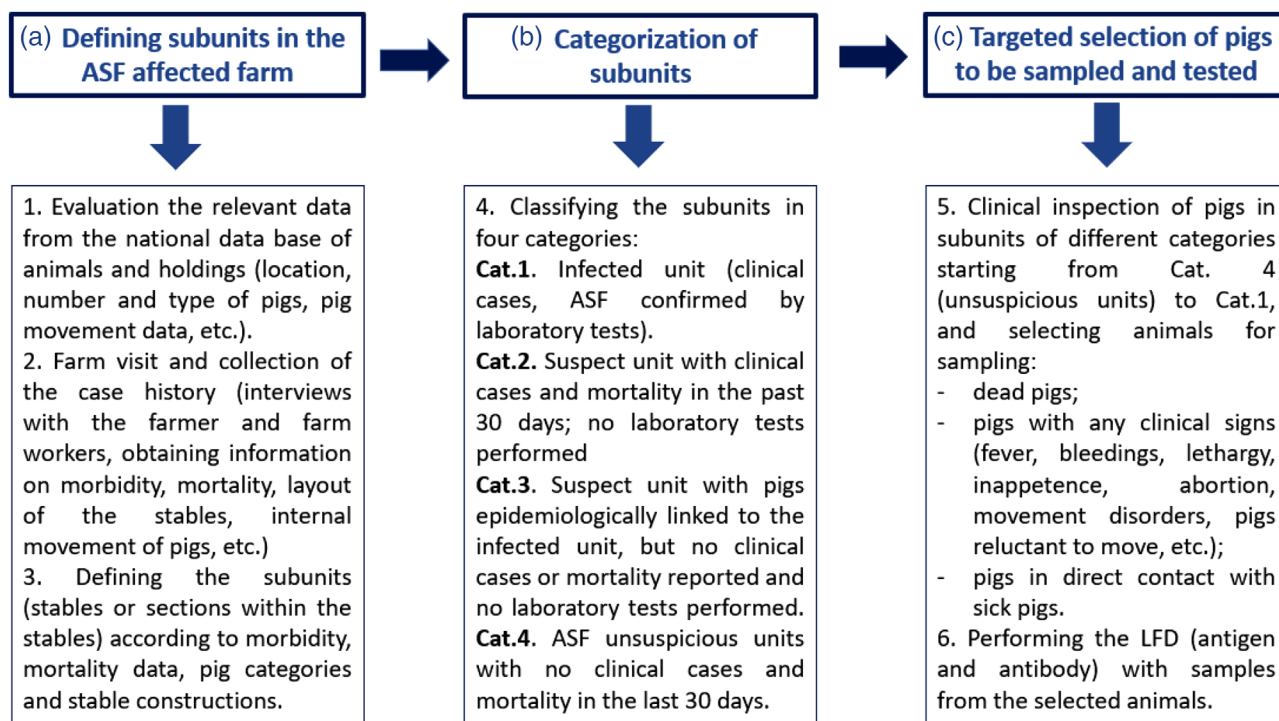


FIGURE 1 Sampling strategy of pigs in African swine fever (ASF)-affected farms for the purpose of outbreak investigations

and confirmed after four pigs had died in one pen of stable A1. All other pigs were clinically unsuspicious at the time the disease was notified.

On *farm B*, almost 1800 pigs were kept in two farm units (B1 and B2). Unit B1 consisted of three separate stables and unit B2 of one stable. The distance between the farm units was about 3 km. Breeding animals, piglets, and part of the fattening animals were kept in B1, the remaining finishers in B2. The units were epidemiologically linked through animal movements, shared farm equipment and vehicles, and farm workers who worked on both sites. ASF was confirmed after three pregnant sows from a pen in B1 had aborted and died.

Farm C, a farrow to finish farm, had almost 9800 pigs when ASF was confirmed. The pigs were kept in eight separate stables which were divided into sections or rooms according to pig categories: breeding animals, gilts, weaners, and finishers. Injured or sick pigs were isolated in a separate section.

The outbreak was confirmed in a breeding sow found dead. According to the farmer, several sows aborted, and there was increased mortality and morbidity in one of the stables where pregnant sows were kept.

2.2 | Sampling strategy for assessing the virus spread on the farms

A three-step sampling procedure was developed and applied as described in Figure 1.

Our first step was to identify sub-units for sampling, then categorize these units according to their likelihood of having ASF-infected pigs, and finally select potentially infected pigs within the sampling units for

testing. All pigs appearing sick during the investigation were sampled. Animals that had contact to sick appearing animals were also sampled (see below).

Blood was collected from the jugular vein or orbital sinus in serum and EDTA tubes. Tissue samples (spleen and lymph nodes) were taken from deceased pigs and packed in plastic bags.

2.2.1 Farm A

Stables A1 and A2 of farm A were considered as two sub-units and were assigned to category 1 (Figure 2). Apart from five severely ill pigs found in A1, all other pigs were clinically inconspicuous. Due to the small number of pigs ($n = 27$) and the likelihood that all could be infected, all animals were sampled, starting in A1 ($n = 21$) and followed by A2 ($n = 6$).

2.2.2 Farm B

Figure 3 shows the sub-units and the number of samples taken at farm B. The assigned categories for the sub-units and the sampled animals are shown in Table 1. In unit B2, 15 out of 447 pigs were sampled, including nine inconspicuous pigs which had direct contact with suspect animals and six pigs with mild clinical signs. In the different sub-units of B1, a total of 20 pigs were sampled. Ten of these showed no clinical signs but had direct contact with suspect animals and six had mild signs. One sub-unit of B1 was assigned to category 1 since several sows aborted and were severely sick. Four sick sows were sampled.



Stable identification	No. of sub-units per stable	Assigned category for sub-unit	No. of pigs sampled
Farm unit B1			
B1.1	1	3	5
B1.2	1	1	4
B1.3	2	2	11
Farm unit B2	1	3	15
Total			35

Stable 5 had two separate sections as shown in Figure 1; one for injured and sick pigs (C5a, hospital) and another for gilts and fattening pigs.

2.3 | Scoring of clinical signs

The sampled animals were classified into three clinical categories: (i) *inconspicuous*: animals with no particular signs, apart from being less

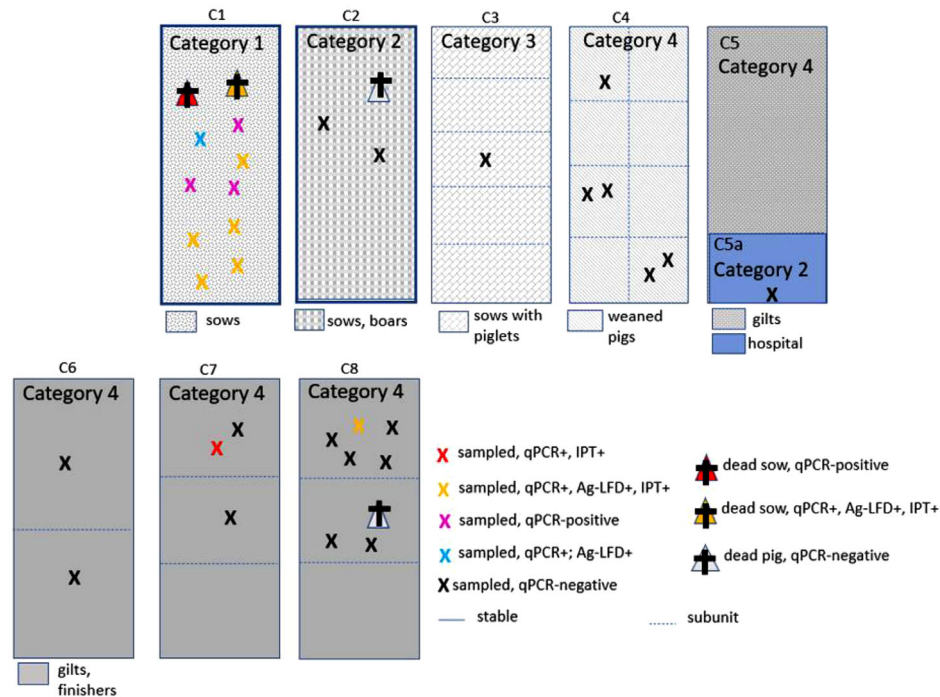


FIGURE 4 Schematic view of farm C with sampling units (C1, C2, C3, C4, C5, C5a, C6, C7, and C8) and results of testing

TABLE 2 Assigned categories for sampling units and sampled animals (farm C)

Stable identification	No. of sub-units per stable	Assigned category for the sub-unit	No. of pigs sampled
C 1	1	1	11
C 2	1	2	3
C 3	5	3	1
C 4	8	4	5
C 5	1	4	0
C 5a	1	2	1
C 6	2	4	2
C 7	3	4	3
C 8	3	4 ^a	8
Total			34

^aOne dead pig was found during clinical inspection, thus after categorization of sub-units according to Figure 1.

active; (ii) *mild*: animals with mild clinical signs, for example, with some visible skin lesions, but standing up when prompted; (iii) *severe*: moribund animals with high fever, with haemorrhagic lesions, not standing up when prompted. The dead animals are not included in any of three clinical categories.

2.4 | Testing

On the farms for antigen detection in whole blood samples, the Ingezim PPA CROM Ag LFD (11.ASF.K.42, Ingenasa, Madrid, Spain)

was used. For ASF-specific antibodies, we used the corresponding Ingezim PPA CROM Ab LFD (11.PPA.K.41, Ingenasa, Madrid, Spain). Both tests were performed according to the manufacturer's instructions.

The samples were also tested at the National Reference Laboratory. For genome detection, quantitative PCR (qPCR) was performed with all EDTA and tissue samples after extraction of the viral DNA using the Nuclisens® Easymag® assay (bioMérieux, France) according to the producer's manual. The qPCR was performed according to the protocol of the European Union Reference Laboratory for ASF (Fernández-Pinero et al., 2013). A heterologous control DNA—intype IC-DNA (QIAGEN, Germany) was used to assess the quality of extracted DNA as described earlier (Hoffmann et al., 2006). Results were recorded as quantification cycle (Ct) values and further interpreted as positive or negative according to the obtained value.

For antibody detection, the commercially available Ingezim PPA Compac blocking ELISA kit (11.PPA.K.3, Ingenasa, Madrid, Spain) was used. In farm A, all sampled animals were tested for ASFV-specific antibodies, while in farms B and C only the qPCR-positive animals were tested. The test and the interpretation of the results were carried out according to the manufacturer's instructions.

Antibody tests were also carried out on all qPCR-positive samples using IPT. The IPT was performed according to the protocol of the European Union Reference Laboratory for ASF (European Union Reference Laboratory for ASF, 2018). The reason for the additional use of the IPT was to detect low antibody titres that were not detected with the ELISA.

All results were plotted in simple sketches of the layout of the farms (Figures 2–4).

TABLE 3 Overview of testing results by categories of farm sub-units

Category of farm sub-unit	No. of tested animals	Ag-LFD+	qPCR+ Ct<18	qPCR+ Ct>18	Ab-LFD+	ELISA+	IPT+
Farm A							
1	21	3	2	3	2	2	3
1	6	0	0	0	0	0	0
Farm B, unit B1							
1	4	3	3	0	0	0	0
2	11	1	1	4	0	0	1
3	5	1	1	0	0	0	1
Farm B, unit B2							
3	15	1	2	4	0	0	4
Farm C							
1	11	7	7	4	0	0	6
2	4	0	0	0	0	nd	nd
3	1	0	0	0	0	nd	nd
4	18	1	1	1	0	0	0

2.5 | Statistical analysis

Sensitivity and specificity were calculated for qPCR and antigen LFD separately for each farm. Sensitivity was calculated as true positive/(true positive + false negative). Specificity was calculated as true negative/(true negative + false positive). Confidence intervals for sensitivity and specificity estimates were calculated using EpiTools Epidemiological Calculators (Sergeant, 2018).

Spearman correlation analysis was performed using XLSTAT (Addinsoft, 2019) to assess the correlation between the results of different tests and the clinical score.

3 | RESULTS

3.1 | Test results

Table 3 gives an overview of the results by different categories, and Figures 2–4 show the recorded results for all three farms.

3.1.1 Farm A

A total of five pigs from A1 were found qPCR positive (Figure 2, Table 3). In A2, no qPCR positive pigs were detected despite the epidemiological link. Three of the qPCR positive pigs also reacted positively with the Ag-LFD.

Three pigs were seropositive with the IPT, two of them were also positive with the Ab-LFD and ELISA. One of these pigs was qPCR positive but negative with the Ag-LFD (Table 3).

3.1.2 Farm B

In farm B, 15 pigs were found qPCR positive. Six of these pigs with Ct values below 15 also reacted positively with Ag-LFD (Figure 3). There were no pigs that tested positive with ELISA or Ab-LFD, while six animals tested IPT positive (Table 3).

According to the results, all stables and both farm units were affected.

3.1.3 Farm C

A total of 13 pigs tested qPCR positive. Eight of these pigs also tested positive with Ag-LFD (Figure 4). There were no pigs that tested positive with ELISA or Ab-LFD, while six animals tested IPT positive (Table 3).

According to the results, ASF-infected pigs were located in C1 (category 1), C7, and C8 (both category 4).

3.2 | Sensitivity and specificity of LFD compared to qPCR

Out of 87 animals tested with Ag-LFD, 17 reacted positive. By qPCR, 34 of 87 pigs tested positive. All animals showed mild to severe clinical signs. The median Ct value was 17. The Ct values of the samples that were positive for Ag-LFD ranged from Ct 14 to 18 (Figure 5).

The relative sensitivity of Ag-LFD compared to PCR was 50% (CI 34.66%).

None of the qPCR-positive samples with a Ct value of >20 was detected as positive by the Ag-LFD (Figure 5). Samples that gave

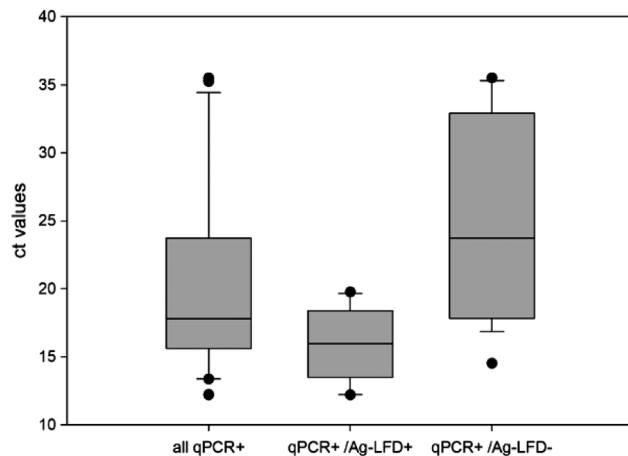


FIGURE 5 Comparison of Ct-values for all tested blood samples, samples that were qPCR and Ag-LFD positive and samples that were qPCR positive but Ag-LFD negative. The boundaries of the boxes indicate the 25th and 75th percentiles, and the line within the box marks the median

negative results in the qPCR were also negative in the Ag-LFD. The relative specificity was thus 100%.

With a single exception (sampling unit C7), all sampling units that were positive by qPCR ($n = 9$) were also positive by Ag-LFD ($n = 8$).

Two animals tested seropositive with the Ab-LFD. The results were confirmed in the laboratory with the ELISA test. Thirteen samples which reacted positive in the IPT were not detected with the Ab-LFD and the ELISA. At the same time, all antibody positive animals were also qPCR positive. Since the number of samples showing ASFV-specific antibodies was low ($n = 2$), relative specificity and sensitivity could not be calculated for the Ab-LFD.

Whisker boundaries indicate minimum and maximum values.

3.3 | Ag-LFD / Ab-LFD and laboratory test results versus clinical signs

Comparison of the Ag-LFD results with the clinical score of the sampled pigs showed that most animals with advanced or severe clinical signs were tested positive. However, three pigs with mild clinical signs were also Ag-LFD positive. Neither Ag-LFD nor qPCR gave positive results in inconspicuous pigs (Tables S1 and S2, Figure 6). Positive antibody results were only found in pigs with clinical signs (Table S1). The results of correlation analysis for the different ASF tests and the ASF clinical scores showed significant correlations with Ab-LFD and ELISA, Ag-LFD and clinical score, and qPCR and clinical score (Table 4).

4 | DISCUSSION

The measures to be taken following confirmation of ASF in domestic pig holdings are quite explicit according EU legislation. After confirmation of the disease outbreak, investigations must be carried out, obtain-

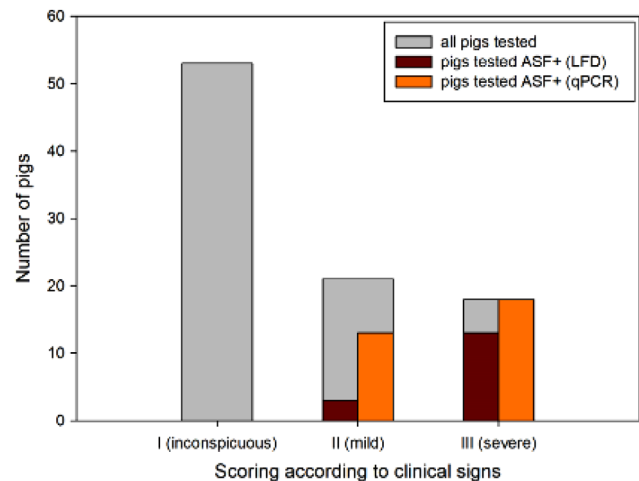


FIGURE 6 Number of pigs tested positive for African swine fever (ASF) (qPCR, Ag-LFD) depending on clinical score of sampled pigs

ing information on the movement of animals, persons, products, vehicles, and so on that may have spread the pathogen during the period in question prior to notification. Some ASF farms are detected at a very early stage of infection when only few animals are infected, while other farms are found ASF positive after weeks of virus circulation within the farm with many pens and sub-units affected (Lamberga et al., 2020). To assess the virus spread within the holding and to other holdings, it is important to have an overview of virus circulation within the farm. Assessing the spread of the virus within a farm gives also reliable information about the high-risk period, that is, the estimated time that ASF has been on the farm before detection. For that purpose, sampling and testing of animals from different sub-units of the affected farm are necessary. This is a challenging task, especially under the time pressure of mandatory control measures.

Targeted sampling of sick or dead animals instead of random sampling is nowadays recommended to detect ASF-infected animals (Busch et al. 2021; EFSA, 2021). We used a simple and straightforward approach to divide farms into sub-units and clearly categorize them for targeted sampling in order to identify the units where ASF virus is likely circulating and units that are likely not to be infected. However, on small farms, where a categorization is difficult due to closer contact of the pigs among each other, testing all individual pig is possible, as we did on farm A.

The clinical score helped to better understand and interpret the LFD test results. In particular, we could correlate the test results to the clinical categories (inconspicuous, mild and severe). None of the inconspicuous pigs ($n = 53$) that were tested because they had contact to suspicious pigs, reacted positively neither in Ag-LFD nor qPCR, whereas severely diseased animals had a significantly high chance of being detected by Ag-LFD. The progression of clinical disease correlates with the viral load in the blood. Samples from mildly diseased pigs with qPCR Ct values above 20 were negative when tested with Ag-LFD. These field results are in line with experimental studies (Pikalo et al., 2021). A recent study found a high positivity rate for Ag-LFD during the acute phase of disease between days 4 and 7 post-infection

TABLE 4 Spearman correlation matrix for different African swine fever (ASF) test results and ASF clinical score

Variables	Ag-LFD	Ab-LFD	qPCR	ELISA	IPT	Clinical score
Ag-LFD	1	0.008	0.246	0.008	0.271	0.531
Ab-LFD	x	1	0.065	1.000	0.296	0.227
qPCR	x	x	1	0.065	0.218	0.469
ELISA	x	x	x	1	0.296	0.227
IPT	x	x	x	x	1	0.201
Clinical score	x	x	x	x	x	1

Note: Values in bold are different from 0 with a significance level $p < .05$.

(dpi) when testing EDTA-blood as sample matrix or up to 10 days when serum is used as the sample matrix (Deutschmann et al., 2021; Pikalo et al., 2021). Therefore, it should be kept in mind that the sensitivity of Ag-LFD limits its use to acutely sick animals 4–10 dpi. The same authors also found that at 10 dpi antibodies can be detected by IPT, ELISA, or Ab-LFD. In our study, IPT results showed a higher positivity rate than ELISA and Ab-LFD, with the latter two showing similar sensitivity and a significant high correlation. Ag-LFD can help to quickly identify infected pigs; consequently, stables or sub-units with infected pigs can be immediately confined. However, due to the lower sensitivity compared to standard laboratory tests, LFDs can only be a complementary tool in outbreak investigations on farms where ASF has already been confirmed, but not for outbreak confirmation or for demonstration of the disease freedom.

According to EU legal requirements, all pigs on the affected holding must be culled, although derogations for culling are theoretically possible (EC, 2019). Application of derogation of killing of animals is a very complex question, where several epidemiological and also financial aspects have to be evaluated. A veterinary risk management that takes into account the characteristics of the disease, the husbandry system, biosecurity, and contacts between animal groups has to be considered within other measures (Costard et al., 2022). An important precondition is to obtain a clear picture of virus circulation within the farm. If the disease is detected early, there is a probability that large parts of the farm are not yet affected. In this study, we have demonstrated how it might be feasible to get an overview of the ASF virus spread in affected farms. Farm A, due to its small size and categorization results would not be a farm where partial culling could be envisaged. In farm B, derogation from culling could not be applied as all stables were affected. In farm C, several stables appeared not to be affected yet by ASF and therefore partial culling of the affected units only and closely monitoring the situation in the other units by constantly testing sick animals might have been an option, if biosecurity and legal requirements were met to the best of knowledge.

5 | CONCLUSIONS

The targeted selection of pigs to be sampled based on a careful clinical inspection followed by LFD testing, as well as plotting the results in the sketch of the farm is efficient and gives an initial rapid overview of the spread of the virus within the farm.

Targeted sampling of sick and dead animals is highly effective for identifying infected animals. The sampling strategy we have presented allows efficient and targeted identification of units where ASF virus is actively circulating.

The LFD test proved to be suitable for use in the field as part of outbreak investigations when samples from clinically suspect and dead animals are examined. Due to its limited sensitivity, the LFD test neither can be used as a diagnostic tool for primary detection and confirmation of disease nor for demonstrating freedom from infection.

It should be further investigated and discussed how the derogation from culling could be implemented in practice to save the animals in non-infected units of large farms and to continue operation of the business.

ACKNOWLEDGEMENTS

We thank the farm owners, their veterinarians, and workers from the investigated farms for their support and collaboration during the outbreak investigation. The authors acknowledge Svetlana Cvetkova and Laura Krivko for the careful laboratory work. Furthermore, we are grateful for the critical and careful review by autonomous reviewer. LFD and publication were funded by the FLI ASF research network.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Kristīne Lambergā, Klaus Depner, and Laura Zani. *Methodology:* Kristīne Lambergā, Klaus Depner, Edvīns Oļševskis, Mārtiņš Seržants, Anja Globig, and Sandra Blome. *Farm investigations:* Kristīne Lambergā, Edvīns Oļševskis, and Mārtiņš Seržants. *Laboratory investigations:* Santa Ansonska and Žanete Šteingolde. *Formal analysis, data curation, and graphs:* Kristīne Lambergā, Anja Globig, Laura Zani, and Arvo Viltrop. *Writing—original draft preparation:* Kristīne Lambergā. *Writing and editing:* Anja Globig, Klaus Depner, Laura Zani, and Santa Ansonska. *Review:* Sandra Blome, Edvīns Oļševskis, Mārtiņš Seržants. *Supervision:* Arvo Viltrop, Aivars Bērziņš, and Klaus Depner. All authors have read and agreed to the published version of the manuscript.

ETHICS STATEMENT

The outbreak investigation was based on the national and EU legislation and all ethical requirements were followed in the study.

DATA AVAILABILITY STATEMENT

The original data used for the analysis can be obtained from the author after approval from the responsible institution in Latvia.

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REFERENCES

- Addinsoft. (2019). *Xlstat statistical and data analysis solution*, Addinsoft.
- Berthe, F. (2020). The global impact of ASF. *OIE Bull* <https://doi.org/10.20506/bull.2020.1.3119>
- Blome, S., Franzke, K., & Beer, M. (2020). African swine fever—A review of current knowledge. *Virus Research*, 287, 198099. <https://doi.org/10.1016/j.virusres.2020.198099>
- Busch, F., Haumont, C., Penrith, M.-L., Laddomada, A., Dietze, K., Globig, A., Guberti, V., Zani, L., & Depner, K. (2021). Evidence-based African swine fever policies: Do we address virus and host adequately? *Frontiers in Veterinary Science*, 8, 637487. <https://doi.org/10.3389/fvets.2021.637487>
- Cappai, S., Loi, F., Coccollone, A., Cocco, M., Falconi, C., Dettori, G., Feliziani, F., Sanna, M. L., Oggiano, A., & Rolesu, S. (2017). Evaluation of a commercial field test to detect african swine fever. *Journal of Wildlife Diseases*. <https://doi.org/10.7589/2016-05-112>
- Carlson, J., Zani, L., Schwaiger, T., Nurmoja, I., Viltrop, A., Vilem, A., Beer, M., & Blome, S. (2017). Simplifying sampling for African swine fever surveillance: Assessment of antibody and pathogen detection from blood swabs. *Transboundary and Emerging Diseases*, 65, e165–e172. <https://doi.org/10.1111/tbed.12706>
- Chenais, E., Depner, K., Guberti, V., Dietze, K., Viltrop, A., & Ståhl, K. (2019). Epidemiological considerations on African swine fever in Europe 2014–2018. *Porcine Health Management*, 5(1), 6. <https://doi.org/10.1186/s40813-018-0109-2>
- Costard, S., Perez, A. M., Zagmutt, F. J., Pouzou, J. G., & Groenendaal, H. (2022). Partitioning, a novel approach to mitigate the risk and impact of African Swine Fever in affected areas. *Frontiers in Veterinary Science*, 8, 812876. <https://doi.org/10.3389/fvets.2021.812876>
- Deutschmann, P., Pikalo, J., Beer, M., & Blome, S. (2021). Lateral flow assays for the detection of African swine fever virus antigen are not fit for field diagnosis of wild boar carcasses. *Transboundary and Emerging Diseases*. <https://doi.org/10.1111/tbed.14248>
- EC. (2002). "Council directive 2002/60/EC of 27 June 2002 laying down specific provisions for the control of African swine fever and amending Directive 92/119/EEC as regards Teschen disease and African swine fever." Council of the European Union.
- EC. (2003). "Commission decision of 26 May 2003 approving an African swine fever diagnostic manual (2003/422/EC)." The Commission of the European Communities.
- EC. (2016). "Regulation (EU) 2016/429 OF the european parliament and of the council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law') Article 57." Council and Parliament of the European Union.
- EC. (2018). "Working document Strategic approach to the management of African Swine Fever for the EU".
- EC. (2019). "Commission delegated regulation (EU) 2020/687 of 17 December 2019 supplementing Regulation (EU) 2016/429 of the European Parliament and the Council, as regards rules for the prevention and control of certain listed diseases." The European Commission.
- EFSA. (2020). Epidemiological analyses of African swine fever in the European Union (November 2018 to October 2019). *European Food Safety Authority*, 18(1), e05996. <https://doi.org/10.2903/j.efsa.2020.5996>
- EFSA. (2021). Scientific Opinion on the assessment of the control measures of the category A diseases of Animal Health Law: African Swine Fever. *EFSA Journal*, 19(1), e06402. <https://doi.org/10.2903/j.efsa.2021.6402>
- European Union Reference Laboratory for ASF. (2018). "Standard operating procedure for the detection of antibodies against African swine fever by indirect immunoperoxidase technique".
- Fernández-Pinero, J., Gallardo, C., Elizalde, M., Robles, A., Gómez, C., Bishop, R., Heath, L., Couacy-Hymann, E., Fasina, F. O., Pelayo, V., Soler, A., & Arias, M. (2013). Molecular diagnosis of African Swine Fever by a new real-time PCR using universal probe library. *Transboundary and Emerging Diseases*, 60(1), 48–58. <https://doi.org/10.1111/j.1865-1682.2012.01317.x>
- Gallardo, C., Fernandez-Pinero, J., & Arias, M. (2019). African swine fever (ASF) diagnosis, an essential tool in the epidemiological investigation. *Virus Research*, 271, 197676. <https://doi.org/10.1016/j.virusres.2019.197676>
- Gallardo, C., Soler, A., Nurmoja, I., Cano-Gómez, C., Cvetkova, S., Frant, M., Woźniakowski, G., Simón, A., Pérez, C., Nieto, R., & Arias, M. (2021). Dynamics of African swine fever virus (ASFV) infection in domestic pigs infected with virulent, moderate virulent and attenuated genotype II ASFV European isolates. *Transboundary and Emerging Diseases*, 58, 2826–2841. <https://doi.org/10.1111/tbed.14222>
- Gallardo, C., Soler, A., Rodze, I., Nieto, R., Cano-Gómez, C., Fernandez-Pinero, J., & Arias, M. (2019). Attenuated and non-haemadsorbing (non-HAD) genotype II African swine fever virus (ASFV) isolated in Europe, Latvia 2017. *Transboundary and Emerging Diseases*, 66, 1399–1404. <https://doi.org/10.1111/tbed.13132>
- Gonzales, W., Moreno, C., Duran, U., Henao, N., Bencosme, M., Lora, P., Reyes, R., Núñez, R., De Gracia, A., & Perez, A. M. (2021). African swine fever in the Dominican Republic. *Transboundary and Emerging Diseases*, 68, 3018–3019.
- Hoffmann, B., Depner, K., Schirrmeier, H., & Beer, M. (2006). A universal heterologous internal control system for duplex real-time RT-PCR assays used in a detection system for pestiviruses. *Journal of Virological Methods*, 136(1–2), 200–209. <https://doi.org/10.1016/j.jviromet.2006.05.020>
- Lamberga, K., Olševskis, E., Seržants, M., Bērziņš, A., Viltrop, A., & Depner, K. (2020). African swine fever in two large commercial pig farms in Latvia—Estimation of the high risk period and virus spread within the farm. *Veterinary Sciences*, 7(3), 105. <https://doi.org/10.3390/vetsci7030105>
- Olševskis, E., Schulz, K., Staubach, C., Seržants, M., Lamberga, K., Pūle, D., Ozoliņš, J., Conraths, F. J., & Sauter-Louis, C. (2020). African swine fever in Latvian wild boar—A step closer to elimination. *Transboundary and Emerging Diseases*, 67(6), 2615–2629. <https://doi.org/10.1111/tbed.13611>
- Pikalo, J., Deutschmann, P., Fischer, M., Roszyk, H., Beer, M., & Blome, S. (2021). African swine fever laboratory diagnosis—Lessons learned from recent animal trials. *Pathogens*, 10(2), 177. <https://doi.org/10.3390/pathogens10020177>
- Pikalo, J., Schoder, M. E., Sehl, J., Breithaupt, A., Tignon, M., Cay, A. B., Gager, A. M., Fischer, M., Beer, M., & Blome, S. (2020). The African swine fever virus isolate Belgium 2018/1 shows high virulence in European wild boar. *Transboundary and Emerging Diseases*, 67(4), 1654–1659. <https://doi.org/10.1111/tbed.13503>
- Plowright, W., Thomson, G. R., & Neser, J. A. (1994). African swine fever. In J. A. W., Coetzer, G. R., Thomson, & R. C. Tustin (Eds.), *Infectious diseases of livestock, with special reference to southern Africa* (vol. 1, pp. 568–599). Oxford University Press.
- Sastre, P., Gallardo, C., Monedero, A., Ruiz, T., Arias, M., Sanz, A., & Rueda, P. (2016). Development of a novel lateral flow assay for detection of African swine fever in blood. *BMC Veterinary Research*, 12, 206. <https://doi.org/10.1186/s12917-016-0831-4>
- Sergeant, E. S. G. (2018). Epitools epidemiological calculators. Ausvet. <http://epitools.ausvet.com.au>
- Viltrop, A., Boinas, F., Depner, K., Jori, F., Kolbasov, D., Laddomada, A., Ståhl, K., & Chenais, E. (2021). African swine fever epidemiology, surveillance and control. In L. Iacolina, M.-L. Penrith, S. Bellini, E. Chenais, F. Jori, M. Montoya, K. Stahl, & D. Gavier-Widen (Eds.), *Understanding and com-*

batting African swine fever. Wageningen Academic Publishers, 230-263.

<https://doi.org/10.3920/978-90-8686-910-7>

World Organization for Animal Health (OIE). (2021). *Global Situation of African swine fever. Report No. 47: 2016 –2020* [https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/ASF/Report_47_Global_situation_ASF.pdf]

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Lamberg, K., Depner, K., Zani, L., Oļševskis, E., Seržants, M., Ansonska, S., Šteingolde, Ž., Bērziņš, A., Viltrop, A., Blome, S., & Globig, A. (2022). A practical guide for strategic and efficient sampling in African swine fever-affected pig farms. *Transboundary and Emerging Diseases*, 1–10. <https://doi.org/10.1111/tbed.14582>

IV

Communication

African Swine Fever Outbreak Investigations—The Significance of Disease-Related Anecdotal Information Coming from Laypersons

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Citation: Lambergā, K.; Ardelean, F.; Blome, S.; Busauskas, P.; Djuric, B.; Globig, A.; Guberti, V.; Miteva, A.; Olševskis, E.; Seržants, M.; et al. African Swine Fever Outbreak Investigations—The Significance of Disease-Related Anecdotal Information Coming from Laypersons. *Pathogens* **2022**, *11*, 702. <https://doi.org/10.3390/pathogens11060702>

Academic Editor: Lillianne Ganges

Received: 25 May 2022

Accepted: 14 June 2022

Published: 17 June 2022

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Abstract: Veterinarians who have conducted numerous investigations of African swine fever outbreaks in pig farms in various European countries over the years shared their experiences during a workshop in Germany in early 2020. One focus was on the so-called “anecdotal information” obtained from farmers, farm workers or other lay people during the outbreak investigations. Discussions revolved around how to correctly interpret and classify such information and how the subjective character of the statements can influence follow-up examinations. The statements of the lay persons were grouped into three categories according to their plausibility: (i) statements that were plausible and prompted further investigation, (ii) statements that were not plausible and could therefore be ignored, and (iii) statements that were rather implausible but should not be ignored completely. The easiest to deal with were statements that could be classified without doubt as important and very plausible and statements that were not plausible at all. Particularly difficult to assess were statements that had a certain plausibility and could not be immediately dismissed out of hand. We aim to show that during outbreak investigations, one is confronted with human subjective stories that are difficult to interpret but still important to understand the overall picture. Here, we present and briefly discuss an arbitrary selection of reports made by lay persons during outbreak investigations.

Keywords: ASF; outbreak investigation; anamneses; subjectivity; laypersons

1. Introduction

The current African swine fever (ASF) epidemic in Europe started in Georgia when a genotype II African swine fever virus (ASFV) was detected in 2007. From there, it spread to mainland Europe where it affected the wild boar population as well as the domestic pig

sector. Thousands of outbreaks have been reported in small farms, mainly in Central and Eastern Europe, but also in huge commercial farms with thousands of pigs [1–5].

An epidemiological investigation is mandatory in the case of ASF and other listed diseases according to EU legislation [6,7]. In the event of an ASF outbreak in a domestic pig farm, a timely and thorough epidemiological investigation has to be carried out by the competent authority to find out the likely origin of the virus, to estimate the time that the disease has been on the holding prior to notification and to obtain information on the potential spread of the virus to other holdings. To clarify all these crucial aspects, it is extremely important to reconstruct the course of the disease in the herd. This process of working up the case and searching for the reasons how and when the pathogen entered and/or left the farm usually starts with an anamnesis. Farm owners, farm workers, farm veterinarians, business operators and other people familiar with the events on the farm are asked to give their opinions and insights into the case. In most cases, these are lay persons who have no particular epidemiological knowledge about ASF. Nevertheless, they provide valuable information that can help to clarify the circumstances that led to the outbreak.

This short communication is based on an ASF workshop in Germany in early 2020, summarizing the experiences of veterinarians, mainly epidemiologists and disease managers, who have conducted outbreak investigations in several European countries during the past years and thereby gathered valuable information and experience. One focus of the workshop was on the so-called “anecdotal information” obtained from laymen during the outbreak investigations, particularly during anamnesis. In our context, we defined anecdotal information as observations or views that were directly linked to an ASF outbreak by the reporters, but were not based on scientific evidence according to the investigators. In particular, the subjective nature of such reports and their possible influence on further investigations was discussed.

We present and briefly discuss an arbitrary selection of statements collected during outbreak investigations in Central and Eastern Europe over the last six years.

2. Material and Methods

2.1. Statements and Opinions from Laypersons

During the ASF outbreak investigations conducted in Central and Eastern European countries and Balkans (Estonia, Latvia, Lithuania, Romania, Bulgaria and Serbia) since 2014, a wide range of opinions have been obtained from lay persons. These are the personal views of the affected farmers, farm workers and other lay persons regarding how and when the pathogen entered the farm and how the disease spread and manifested. They contain the interviewee’s personal subjective views on the case.

Most of the outbreaks occurred in countries and regions where local wild boar populations were also affected by ASF and served as virus reservoirs for the domestic pig outbreaks [8].

2.2. Grouping of Statements

The statements and opinions were grouped into three categories according to their plausibility regarding the course of ASF:

- Statements that are plausible and need to be followed up by further investigations;
- Statements that are not plausible and can therefore be ignored;
- Statements that are rather implausible but should not be ignored completely.

The plausibility classification was made based on generally accepted knowledge on ASF as described in the current textbooks, publications and documents of the international organizations and institutions (OIE, FAO, EFSA and EU Commission) as well as the scientific literature on ASF. This includes knowledge about the route of transmission, contagiousness, susceptibility, incubation period, clinical course, convalescence, diagnosis and epidemiology.

3. Results

The arbitrarily selected statements and opinions as well as the grouping according to plausibility are shown in Table 1.

4. Discussion

Epidemiological outbreak investigations are mostly carried out by a team of experts and usually start with the anamnesis. The collection of the case history is followed by the physical inspection of the farm with special attention to the farm's biosecurity and the clinical examination of the animals in the different farm units (stables). In the course of these investigations, findings accumulate, and initial speculations are confirmed or discarded. A repeated critical analysis after each step of the examination with meaningful inclusion of the anamnestic data can lead to a preliminary hypothesis about the course of ASF on the farm. Thus, it should be noted that the reconstruction of the infection course is initiated and supported by the anamnestic findings, which might be a mix of objective and subjective information. Much of this information is obtained from people outside the field who have no expertise or experience with the disease. In most cases, these are the owners themselves or employees or other lay people. Often, statements are anecdotal information and not based on biological, proven evidence. In the worst case, such information can become a source of fake news [9]. Therefore, investigators should always be aware that some of the information they receive is subjective and sometimes misleading. Nor can it be ruled out that reports will always be truthful. Reporters could attempt to exonerate themselves from blame if certain requirements (e.g., concerning farm biosecurity) were not followed. This requires that the interviewer has the necessary disease-related knowledge and expertise to interpret and classify correctly the information received. The interviewer has to be very familiar with the clinical course and epidemiology of the disease.

The easiest to cope with are statements that can be classified without doubt as important and very plausible and statements that are not plausible at all. Statements that have a certain plausibility and cannot be immediately dismissed are particularly difficult to assess.

ASF is an infectious disease that does not spread via droplets or air. However, it has been shown that over very short distances, transmission can also take place via the air [10]. A susceptible animal becomes infected mainly parenterally (e.g., via a tick bite or infected needle) or oronasally; other transmission routes become implausible [11,12]. Other cases need a deeper reflection. For example, statement Nr. 5 (Table 1) which is reported in a similar way more frequently would require that the grandfather would have stepped in wild boar droppings or carcasses contaminated with ASF virus in the forest and this contaminated shoe would then have to get into the pigsty where pigs chew on it. Such a scenario cannot be completely ruled out, of course, but it is very unlikely. Yet, following such a statement, there is a danger of losing sight of other transmission possibilities, which are much more likely, and thus being steered onto a wrong track. On the other hand, in a Lithuanian case control study "activities in the forest" were positively associated with ASF outbreaks in the affected pig herds [13].

Statements that could possibly be plausible must be given special attention, because here there is the greatest danger of being misled, i.e., example 7. It was assumed that the disease was introduced by rats in the course of rodent control measures on the neighboring farm. Here, there is both a spatial and temporal connection to the ASF-positive farm where the rodent control took place. However, it is neither known nor proven that rats can transmit ASF. Nevertheless, this finding should lead to further investigation of other potential transmission possibilities from the neighboring farm.

We do not want to exclude the possibility that the statements interpreted and classified by us might have been assessed differently by other experts. Rather, we want to highlight the subjective character and also point out that it is relatively easy to be misled and possibly steer further investigations in the wrong directions. However, the more comprehensive and up to date the interviewer's expertise on ASF is, the more likely it is that subjective information will be correctly understood and interpreted. The examples we have listed, which have crystallized from extensive experience over many years, are intended to provide

the reader with an incentive for their own interpretation and reflection. Furthermore, we aim to show that during outbreak investigations, one is confronted with subjective perceptions that are not in line with the purely scientific disease-related logic.

Taking the outbreak history aims at understanding the problem to be dealt with and guiding the farm inspection and clinical examinations. Good communication skills and a systematic but flexible approach are needed to gather as much useful information as possible. There are procedures throughout the examination process that can be very helpful. For example, these include questionnaires that ensure that no important aspects of the disease and its epidemiology are omitted. The way in which interviews are conducted and the order in which they are conducted is also helpful and important. For example, talking to farmworkers first, whenever possible, helps to find out what they have to say without being influenced by the farmer's version. The manner of speaking must be adapted, taking the interlocutor's knowledge and language proficiency into consideration. It is necessary to listen actively to what is being said and to give feedback in one's own words to confirm that what one has understood is correct. At this stage, the examiner should stick to the raw version of the history, which means not interpreting words and substituting them with medical terms as, e.g., "haemorrhagic lesions" instead of "bleedings in the skin." This allows the examiner to stay open-minded and avoid missing essential information. Targeted questions can then be asked for more in-depth details, but the risk of obtaining biased or suggested answers must be considered. Furthermore, it should be taken into consideration that the owners of the farm and/or the farmworker are under stress and want to present themselves in the best light. They will (un)intentionally try to avoid giving the interviewer the impression that their actions—no matter whether illegal or not—are responsible for disease introduction.

For a targeted collection of valid information, knowledge from communication and social science, psychology and biological/veterinary knowledge about ASF must be combined.

Epidemiological investigations, especially in backyard holdings in village settings, remain difficult. The ways of ASFV introduction and spread are not fully understood or seen; in particular, the ways of spread from backyard to backyard are still unclear, but human activities (farmers in contact, butchers and veterinarians) do play a major role [5,14].

Table 1. Statements and opinions about ASF collected from lay persons during the outbreak investigations and grouped according to their plausibility.

Nr.	Statement	Comments	Plausibility *
1	Two days ago, we bought three piglets from a farm located in an ASF restricted area. These piglets have brought the virus into our holding and infected our pigs which died yesterday.	Incubation period is longer than one day [15].	-
2	My neighbor is a hunter. He hunted wild boar in Eastern Europe last summer. He might have brought the virus with him that infected my pigs.	Time gap too long [15].	-
3	We never feed kitchen scraps from restaurants or unknown sources, only scraps from our own kitchen.	Swill feeding is a known risk factor [16].	+

Table 1. Cont.

Nr.	Statement	Comments	Plausibility *
4	The window at the end of the barn was broken for over a week until we replaced it. I assume that the virus entered through the broken window.	No evidence of airborne transmission [17].	-
5	Grandfather goes to the forest every week to pick mushrooms. On Sundays he visits us for lunch. Maybe he brought the virus on his shoes.	Quite a contrived scenario, very unlikely [17].	-
6	Three weeks ago, workers came to us to repair the water supply in the stables. The same day, they were previously on a farm that tested positive for ASF.	Within high-risk period, possibly gap in biosecurity [16].	+
7	The neighboring farm which was tested ASF positive last week has done rodent control two weeks ago. After that we saw many rats. Maybe the rats, brought the virus to our farm.	Mammals and flies unlikely to be mechanical vectors [18].	?
8	Crows come from the forest and sit on the roof of our stables. They bring the virus from the forest.	Minor risk of ravens carrying meat pieces to stables [19,20].	?
9	Three months ago, most of the farm workers were replaced by new employees.	This could be a gap in biosecurity (lack of knowledge) [3].	+
10	In fact, we were able to prove that our farm was free of ASF. We tested 10% of the animals by Antibody-ELISA two days before the outbreak and all tested animals were negative.	Usually it takes more than 10 days until antibodies are detected [15].	-
11	We have repaired all the fences and renovated all the buildings. The virus cannot enter our farm.	Biosecurity includes management practices as well not only physical barriers [3,16].	-
12	In recent weeks, pigs have been dying in neighboring villages; there is nothing I can do.	Backyard scenario often seen in Central and Eastern Europe [5,14].	+
13	Wild boar come very close to the farm. They contaminate the environment.	Direct or indirect contact of wild boar with domestic pigs cannot be excluded [21].	+
14	My daughter's boyfriend is from the farm where there was an outbreak 5 weeks ago. They have had regular contact, although I am against this relationship. Now he has brought us the virus.	Direct or indirect contact cannot be excluded in combination with insufficient biosecurity [16].	?

Table 1. Cont.

Nr.	Statement	Comments	Plausibility *
15	My farm is located on the main road; all animal transporters and rendering vehicles pass by here. The virus came from the tires of one such truck.	Direct or indirect contact cannot be excluded in combination with insufficient biosecurity [22].	?
16	The veterinarian came here last week and vaccinated my pigs, few days later the first animals became sick. He brought the virus.	Iatrogenic transmission cannot be excluded [22].	+
18	A few days ago, the pigs got new bedding material. It was harvested from a field not far from here.	Transmission through the bedding material cannot be excluded [23].	+
19	The week before the pigs died, it was very hot and there were a lot of blood sucking insects around. They could have brought in the virus.	Tabanids and vectors could be mechanical short-distance vectors but do not act as true vectors [22].	?
20	I found a bone in the bedding material. This could be the source of the introduction.	Transmission through the contaminated bedding material cannot be excluded [23].	+
21	My neighbour is a hunter. His dog often comes to our garden. The dog has brought the virus to us.	Vertebrates other than pigs are not known as active vectors, but they pose a minor risk for mechanical transmission. Some dogs have a strong instinct to bury their food and if they can freely run around, they can bring a piece of a wild boar to the farm and contaminate something from where or with what the virus could be carried to the pigs [20].	?
22	I visited an infected pig farm and saw a hedgehog in the pen. Maybe he brought the virus into the stable.	Hedgehogs are not known as active or passive vectors [20].	-
23	In the infected pigsty there were many stable flies. They were everywhere—flying around, sitting on the surface of the pens and on the pigs, eating the feed in the trough. The windows were not protected with nets. Maybe the flies brought the virus from the nearby forest.	Tabanids and vectors could be mechanical short-distance vectors but do not act as true vector [22].	?

Table 1. Cont.

Nr.	Statement	Comments	Plausibility *
24	Near each entrance to the barn are disinfecting mats for shoes. A wheelbarrow is used to clean the pens from pig manure. However, it is not possible to clean and disinfect the wheelbarrow after each use (between the barn and manure storage). Probably the virus was introduced into the barn with the wheelbarrow.	Low contagiousness, would require a high viral load on the wheelbarrow, but cannot be completely ruled out.	?
25	Due to very hot weather conditions all doors and windows of the stable were left open day and night for couple of weeks. During two days last week there were very strong winds and I think that ASF virus was brought into the stable by these winds, because pigs near the door and window were the first showing disease symptoms.	No evidence of airborne transmission by winds.	-
26	The price of pork is so cheap on the other side of the border, and I have heard that many pigs die. But people from abroad come to us to buy cheap alcohol and cigarettes.	Panic sales of pigs due to illness may lead to drop in meat price and higher purchasing activities.	+
27	Only my guard dogs, which are in front of the pigsty, get meat scraps, but not my pigs.	Dog could bring the meat (bones) to the pigs.	?
28	The manure truck removed pig manure from the storage basin recently. The manure was disposed on the nearby fields as a fertilizer.	Contamination of farmyards by vehicles, particularly through dirty tires, cross contamination possible [16].	?
29	Pigs walk outside from one pen to another, following their own path.	Direct or indirect contact with infected pigs or material (contaminated environment) Outdoor keeping requires additional biosecurity [8].	?
30	Last month, construction work was carried out in the stables, but it involved employees of a specialized company who had no contact with pigs.	Gaps in biosecurity cannot be excluded [3,16].	?
31	Only one horse is kept in the stable where the ASF-infected sow lived.	Other animals in the barn pose a biosecurity risk, e.g., if the horse goes frequently in and out.	?

*: (-) Implausible, makes no sense, can be neglected; (?) rather implausible—should not be ignored entirely, but eventually further investigated; (+) Plausible, makes sense, needs to be taken seriously.

Author Contributions: Conceptualization, K.L., F.A., S.B., P.B., B.D., A.G., V.G., A.M., E.O., M.S., A.V., L.Z., A.Z. and K.D.; writing—original draft preparation; K.L., K.D., A.G. and A.V.; methodology, K.L., A.G. and K.D.; review and editing; A.V. and A.G.; visualization, K.D. and K.L. All authors have read and agreed to the published version of the manuscript.

Funding: This work received no external funding.

Institutional Review Board Statement: Not relevant to this study.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank Eveliina Breukers for helping with writing and editing the text. A very special thanks goes to Mary-Lou Penrith for the many valuable comments and the extensive English revisions of the manuscript. The authors are grateful for the reviewers comments and suggestions.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. European Food Safety Authority (EFSA); Anette, B.; Anette, B.; Theodora, C.V.; Klaus, D.; Daniel, D.; Vittorio, G.; Georgina, H.; Daniela, K.; Annick, L.; et al. Epidemiological analyses of African swine fever in the European Union (November 2018 to October 2019). *EFSA J.* **2020**, *18*, e05996.
2. Nurmoja, I.; Mõtus, K.; Kristian, M.; Niine, T.; Schulz, K.; Depner, K.; Viltrop, A. Epidemiological analysis of the 2015–2017 African swine fever outbreaks in Estonia. *Prev. Veter. Med.* **2018**, *181*, 104556. [CrossRef] [PubMed]
3. Chenais, E.; Depner, K.; Guberti, V.; Dietze, K.; Viltrop, A.; Ståhl, K. Epidemiological considerations on African swine fever in Europe 2014–2018. *Porc. Health Manag.* **2019**, *5*, 6. [CrossRef] [PubMed]
4. Lamberg, K.; Olšovskis, E.; Seržants, M.; Bērziņš, A.; Viltrop, A.; Depner, K. African Swine Fever in Two Large Commercial Pig Farms in LATVIA—Estimation of the High Risk Period and Virus Spread within the Farm. *Veter. Sci.* **2020**, *7*, 105. [CrossRef] [PubMed]
5. Ardelean, F.; Globig, A.; Năvălici, A.I.G.; Blome, S.; Dietze, K.; Depner, K.; Zani, L. The course of African swine fever in Romanian backyard holdings—A case report. *Veter. Med. Sci.* **2021**, *7*, 2273–2279. [CrossRef]
6. European Union. Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on Transmissible Animal Diseases and Amending and Repealing Certain Acts in the Area of Animal Health ('Animal Health Law') Article 57. 2016/429/Council and Parliament of the European Union, 2016. *Off. J. Eur. Union* **2016**, *L 84*, 1–208.
7. European Union. Commission Delegated Regulation (EU) 2020/687 of 17 December 2019 supplementing Regulation (EU) 2016/429 of the European Parliament and the Council, as Regards Rules for the Prevention and Control of Certain Listed Diseases. 2020/687/EC, 2020. *Off. J. Eur. Union* **2020**, *L 174*, 64–139.
8. EFSA Panel on Animal Health and Welfare (AHAW); Nielsen, S.S.; Alvarez, J.; Bicout, D.J.; Calistri, P.; Canali, E.; Drewe, J.A.; Garin-Bastuji, B.; Rojas, J.L.G.; Herskin, M.; et al. African swine fever and outdoor farming of pigs. *EFSA J.* **2021**, *19*, e06639. [CrossRef]
9. Trotta, A.; Marinaro, M.; Cavalli, A.; Cordisco, M.; Piperis, A.; Buonavoglia, C.; Corrente, M. African Swine Fever—How to Unravel Fake News in Veterinary Medicine. *Animals* **2022**, *12*, 656. [CrossRef]
10. Olesen, A.S.; Lohse, L.; Boklund, A.; Halasa, T.; Gallardo, C.; Pejsak, Z.; Belsham, G.J.; Rasmussen, T.B.; Bøtner, A. Transmission of African swine fever virus from infected pigs by direct contact and aerosol routes. *Veter. Microbiol.* **2017**, *211*, 92–102. [CrossRef]
11. Beltrán-Alcrudo, D.; Arias, M.; Gallardo, C.; Kramer, S.; Penrith, M.L. *African Swine Fever: Detection and Diagnosis—A Manual for Veterinarians*; FAO: Rome, Italy, 2017. Available online: <http://www.fao.org/ag/againfo/resources/en/publications.html> (accessed on 8 June 2022).
12. Viltrop, A.; Boinas, F.; Depner, K.; Jori, F.; Kolbasov, D.; Laddomada, A.; Ståhl, K.; Chenais, E. African Swine Fever Epidemiology, Surveillance and Control. In *Understanding and Combatting African Swine Fever*; Iacolina, L., Penriths, M.-L., Bellini, S., Chenais, E., Ferran, J., Montoya, M., Stahl, K., Gaviera-Widen, D., Eds.; Wageningen Academic Publisher: Wageningen, The Netherlands, 2021. [CrossRef]
13. Malakauskas, A.; Schulz, K.; Kukanauskaitė, I.; Masiulis, M.; Conraths, F.J.; Sauter-Louis, C. African Swine Fever Outbreaks in Lithuanian Domestic Pigs in 2019. *Animals* **2022**, *12*, 115. [CrossRef] [PubMed]
14. Zani, L.; Dietze, K.; Dimova, Z.; Forth, J.H.; Denev, D.; Depner, K.; Alexandrov, T. African Swine Fever in a Bulgarian Backyard Farm—A Case Report. *Veter. Sci.* **2019**, *6*, 94. [CrossRef] [PubMed]
15. Blome, S.; Gabriel, C.; Beer, M. Pathogenesis of African swine fever in domestic pigs and European wild boar. *Virus Res.* **2013**, *173*, 122–130. [CrossRef] [PubMed]
16. Bellini, S.; Rutili, D.; Guberti, V. Preventive measures aimed at minimizing the risk of African swine fever virus spread in pig farming systems. *Acta Veter. Scand.* **2016**, *58*, 82. [CrossRef] [PubMed]

17. Guinat, C.; Gogin, A.; Blome, S.; Keil, G.; Pollin, R.; Pfeiffer, D.; Dixon, L. Transmission routes of African swine fever virus to domestic pigs: Current knowledge and future research directions. *Veter. Rec.* **2016**, *178*, 262–267. [[CrossRef](#)] [[PubMed](#)]
18. Forth, J.H.; Amendt, J.; Blome, S.; Depner, K.; Kampen, H. Evaluation of blowfly larvae (Diptera: Calliphoridae) as possible reservoirs and mechanical vectors of African swine fever virus. *Transbound. Emerg. Dis.* **2017**, *65*, e210–e213. [[CrossRef](#)]
19. Probst, C.; Globig, A.; Knoll, B.; Conraths, F.J.; Depner, K. Behaviour of free ranging wild boar towards their dead fellows: Potential implications for the transmission of African swine fever. *R. Soc. Open Sci.* **2017**, *4*, 170054. [[CrossRef](#)]
20. Probst, C.; Gethmann, J.; Amler, S.; Globig, A.; Knoll, B.; Conraths, F.J. The potential role of scavengers in spreading African swine fever among wild boar. *Sci. Rep.* **2019**, *9*, 11450. [[CrossRef](#)]
21. Sauter-Louis, C.; Conraths, F.J.; Probst, C.; Blohm, U.; Schulz, K.; Sehl, J.; Fischer, M.; Forth, J.H.; Zani, L.; Depner, K.; et al. African Swine Fever in Wild Boar in Europe—A Review. *Viruses* **2021**, *13*, 1717. [[CrossRef](#)]
22. Blome, S.; Franzke, K.; Beer, M. African swine fever—A review of current knowledge. *Virus Res.* **2020**, *287*, 198099. [[CrossRef](#)]
23. European Food Safety Authority (EFSA); Mosbach-Schulz, O.; Christoph, E.; Gervelmeyer, A. Expert Knowledge Elicitation to Assess the Ability of Matrices to Transmit African Swine Fever Virus. *EFSA J.* **2021**, *18*, 7103E. [[CrossRef](#)]