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Epidemiological analysis of the 2015–2017 African swine fever outbreaks in Estonia



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ABSTRACT

African swine fever (ASF) was first detected in the Estonian wild boar population in September 2014, while the first domestic pig farm was affected in July 2015. In the present study, we aimed to analyse, retrospectively, the epidemiology of the disease in all 26 outbreaks in domestic pig herds that occurred in Estonia during the period 2015–2017. Formal interviews were conducted to estimate the high-risk period for every farm, and to identify the possible origin of the ASF virus and the mode of virus introduction. Furthermore, the clinical manifestation of the disease as well as the course of the disease within the farm were investigated. Survival analysis was used to calculate herd incidence and to estimate outbreak risk. A hierarchical Bayesian space–time model was used to analyse the associations between outbreaks and ASF occurrence in wild boar. The spatial and temporal distribution of outbreaks was analysed to characterise the ASF epidemic in the Estonian domestic pig population from 2015 to 2017.

The estimated high-risk period varied from seven to 20 days with a median of 11 days. On most of the affected farms, the first clinical signs were mild and not specific to ASF despite the high virulence of the circulating virus. Morbidity and mortality were often limited to a single pen or unit of the farm. The highest mortality (29.7%) was seen on backyard farms with 1–10 pigs and the lowest (0.7%) on large commercial farms (>1000 pigs). The spread of the virus within affected farms has been slow and the contagiousness of the virus has been relatively low. Farms of all sizes and types have been at risk, including large commercial farms operating at a high biosecurity level. In none of the affected farms could the specific route of introduction be verified. However, the findings suggested that virus introduction occurred via indirect transmission routes due to insufficient biosecurity. The total herd incidence of outbreaks was similar across all three years, being 2.4% in 2015 and 2016, and 2.0% in 2017. All outbreaks occurred from June to September, during the warmest period of the year. The results suggest that the increase in ASF cases in local wild boar populations is the main risk factor leading to the infection of farms; 88% of outbreaks occurred in areas where ASF virus was detected in wild boar prior to the outbreak, within a radius of 15 km from the outbreak farm.

1. Introduction

Due to its serious impact on animal health and the pig industry, African swine fever (ASF) is considered one of the most important and dangerous viral diseases of pigs and wild boar. Highly virulent and lethal ASF virus strains from genotype II have been circulating in Eastern Europe since 2007, and in EU countries from 2014 (Blome et al., 2012; Gallardo et al., 2015b; OIE and WAHID, 2017). However,

not enough reliable and comprehensive epidemiological field data about domestic pig outbreaks is available. Although in recent decades, different epidemiological data from ASF endemic countries in Africa (Fasina et al., 2012; Penrith et al., 2013), the Italian island of Sardinia (Mur et al., 2018), as well as from the Iberian Peninsula (1960–1995) have been collected and made available, these results are often not valid for Eastern and Northern Europe. The genotype of the virus, and climatic, socio-economic and environmental conditions, as well as the

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Table 1
Number of detected ASF cases in wild boar and ASF outbreaks in domestic pig herds in Estonia from 1st September 2014 to 31st December 2017.

County	2014 ^a		2015		2016		2017	
	WB ^b cases	DP ^c outbreaks	WB cases	DP outbreaks	WB cases	DP outbreaks	WB cases	DP outbreaks
Harju	0	0	0	0	46	0	87	0
Hiiu	0	0	0	0	0	0	0	0
Ida-Viru	4	0	36	0	40	0	14	0
Jõgeva	0	0	60	2	192	3	15	0
Järva	0	0	102	1	117	1	9	0
Lääne	0	0	0	0	58	0	119	1
Lääne-Viru	0	0	91	1	198	1	64	0
Põlva	0	0	233	0	190	0	14	0
Pärnu	0	0	27	0	95	0	87	1
Rapla	0	0	6	0	203	0	90	0
Saare	0	0	0	0	98	1	305	1
Tartu	0	0	124	2	192	0	40	0
Valga	13	0	124	4	24	0	8	0
Viljandi	47	0	174	5	61	0	9	0
Võru	9	0	118	2	56	0	6	0
Total	73	0	1095	17	1570	6	867	3

^a From 1st September.

^b Wild boar.

^c Domestic pig.

structure of the pig industry and farming traditions are considerably different from the aforementioned countries and regions. In Estonia, pig production is highly industrialised and concentrated on large farms, whereas the backyard sector and the number of smallholders became relatively small in number and significance during the last decade. Pigs are kept predominantly inside in weatherproof facilities and keeping them outdoors has been the exception rather than the rule.

The first case of ASF in Estonia was diagnosed in a wild boar found dead near the Latvian border at the beginning of September 2014. In the Latvian wild boar population, ASF had already been present since June 2014 (OIE and WAHID, 2017; Olševskis et al., 2016). In the following years, the virus spread through the entire wild boar population in Estonia, leaving only some islands free of infection. The first ASF outbreak in domestic pigs in Estonia occurred in July 2015 and was followed by 16 outbreaks during the following nine weeks. Six outbreaks were notified in 2016 and three in 2017. An overview of Estonian ASF outbreaks in domestic pig herds and wild boar cases is given in Table 1 (see also Fig. 2).

The aim of the present study was to analyse, retrospectively, the epidemiology of ASF in domestic pigs, based on data from all Estonian outbreak farms. More specifically (i) to estimate the high-risk period and mortality risk, (ii) to analyse the characteristics of the affected herds, (iii) to clarify clinical manifestation of the disease as well as spread of the virus within the farms, (iv) to assess the virus transmission and introduction pathways, (v) to estimate herd incidence and outbreak risks, (vi) to assess temporal and spatial patterns of outbreaks, and (vii) to analyse associations between the occurrence of ASF in wild boar and domestic pig outbreaks.

2. Materials and methods

2.1. ASF outbreak detection

An outbreak farm was defined as a holding having an individual identification number in the National Animal Register (NAR) and meeting the criteria of infected herd as defined in Council Directive 2002/60/EC (European Commission, 2002). All ASF outbreaks were confirmed by virus genome detection in accordance with the EU diagnostic manual (European Commission, 2003). Tissue and blood samples were collected from all or selected dead or sick animals, depending on the clinical course of the disease on the farm in question. The laboratory analyses were performed at the Estonian Veterinary and Food

Laboratory, which is also the national reference laboratory for ASF (NRL).

The ASF virus genome was detected by real-time PCR according to the protocol published by Tignon et al. (2011). In addition, the presence of ASF-virus-specific antibodies was analysed using a commercial blocking ELISA (INGEZIM, PPA COMPAC K3, INGENASA, Madrid, Spain) and/or indirect immunoperoxidase technique (IPT) provided by the European Union reference laboratory for ASF (Gallardo et al., 2015a; European Unión Laboratory for African Swine Fever et al., 2014).

2.2. Outbreak investigations

Epidemiological investigations were conducted on all farms in which an ASF outbreak had been reported (18 farms in 2015, six farms in 2016 and three farms in 2017). However, a positive diagnosis of ASF was not confirmed in follow-up investigations of one of the herds in 2015, where all 15 pigs tested after culling were found to be negative for ASF.

Epidemiological investigations were conducted either by the local veterinary officers responsible for management of the outbreaks or by the epidemiology team of the Estonian University of Life Sciences in compliance with Council Directive 2002/60/EC (European Commission, 2002). In principle, epidemiological enquiries dealt with the following: (i) the length of time that the ASF virus may have existed on the holding before the disease was notified or suspected, (ii) the possible origin of the ASF virus at the holding and mode of introduction, (iii) the identification of other holdings at which pigs may have become infected from the same source.

Formal interviews using a structured questionnaire were conducted with farm managers, farm veterinarians and farm workers, focussing on farm management, herd data, animal movements, vehicle movements, feeding and bedding management, biosecurity measures and human activities, all of which might have facilitated virus introduction and spread. Furthermore, investigations were conducted focussing on clinical and pathological data and laboratory findings.

2.3. Biosecurity level of outbreak farms

The level of farm biosecurity was judged by a group of three experts as a consensus judgment based on interview data and from observations made during farm visits. The first step involved evaluating farms based

Table 2
Basic criteria for assessment of farm biosecurity level in ASF outbreak herds in Estonia, 2015–2017.

Criteria	Biosecurity level				
	Compliant				Non-compliant
	very high	high	moderate	low	very low
Indoor keeping ^a	+	+	+	+	One or more requirements not fulfilled
Fence surrounding the farm boundary ^b	+	+	+	+	
Disinfection barriers at entry points to the farm boundary for vehicles and humans	+	+	+	+/- ^c	
Disinfection barriers at entrances to farm buildings for humans and vehicles	+	+	+	+/-	
No swill and/or grass feeding	+	+	+	+	
No other farm and/or pet animals in pigsties	+	+	+	+	
Number of deficiencies in biosecurity procedures ^d	0	1	2	> 3	

^a One outdoor farm had special permission to keep pigs in a double-fenced area and was not automatically classified as “very low” – assessment was based on evaluation of all aspects of biosecurity.

^b Farms without a fence were not automatically classified as “very low” – assessment was based on evaluation of all aspects of biosecurity.

^c Partly fulfilled.

^d Functional infrastructure and procedures for disinfection; adequate procedures for entry of animals, humans, vehicles, equipment and materials; secure storage and handling of feed, and bedding material; existence of biosecurity plan.

on their compliance to basic biosecurity requirements enforced by national legislation, and classifying them as compliant or non-compliant (Teataja, 1999, 2004). In the second step, the herds were divided into five categories based on their biosecurity level as shown in Table 2.

2.4. High-risk period of outbreak farms

The length of time that ASF virus may have existed on a farm before it was suspected (high-risk period: HRP) was estimated based on mortality data, and clinical and laboratory findings. In cases where antibody-positive animals (detected by ELISA test) were found in an infected herd, it was concluded that the virus had been circulating in the herd for at least two weeks. In cases where sampled animals were only virus-positive, the time of virus circulation was considered to be one week or less. By combining mortality data, and clinical and laboratory findings, the HRP was established.

2.5. Pig herd data

A database on pig herds in Estonia for the period 2015–2017 was compiled using the information available from the NAR of the Estonian Agricultural Registers and Information Board and from the Veterinary and Food Board (VFB). According to Estonian law, all pig herds must be registered in the NAR and the number of animals in the herd must be reported by owners at least once a year by 1st May. However, during the second half of the years 2015 and 2016, the VFB conducted an inspection of all pig holdings and updated the NAR database with actual number of pigs in herds at the time of inspection. Where needed, the VFB added the holdings not yet registered in the NAR to the database or removed those holdings that no longer kept pigs. The final database included all farms and households that had kept pigs during the year of observation; the total number of pigs in a herd was counted as the largest number registered in one of the source databases (NAR or VFB).

An epidemiological unit was defined as a group of pigs kept in one

building or area (one outdoor herd) and having an individual identification number in the NAR. One owner may have one or several production units (herds) registered in the NAR. Herds belonging to the same owner were considered as connected herds (epidemiological units).

Holdings were grouped into four size categories according to the total number of pigs (piglets, weaners, growers, fatteners, gilts, sows and boars) in an epidemiological unit: 1–10 pigs (G1); 11–100 pigs (G2); 101–1000 pigs (G3); > 1000 pigs (G4). G1 holdings were classified as backyard or non-commercial farms where pigs were kept mainly for the farmers own consumption. G2–G4 holdings were classified as commercial farms.

The herd type (farrow-to-finish, multiplier, fatterer or grower) was identified based on the information available from the NAR. Herds consisting of only breeding animals and piglets (up to weaning age) were considered to be multiplier herds, herds with fatteners or growers were classified as fattening herds, and herds with all categories of pigs as farrow-to-finish herds.

The type of pigs kept on a farm (domestic pigs, wild boar, or crosses), as well as the location of the farm (including the coordinates), were taken from source datasets and included in the final database.

The total number of herds and pigs in different herd-size categories are presented in Table 3.

2.6. Wild boar ASF surveillance and hunting data

ASF surveillance data for wild boar from September 2014 until the end of 2017, including date and location (coordinates) of each ASF case, were obtained from the VFB. For the year 2015, data on ASF wild boar cases in Latvia were drawn from the Animal Diseases Notification System database (ADNS, 2017). For 2016 and 2017, Latvian ASF cases were not relevant for the analyses as all Estonian outbreaks in domestic pig farms occurred further away from the Latvian border.

The date and location of the closest wild boar case(s) to each

Table 3
Total number of pig herds and pigs in Estonia in the period 2015–2017.

Herd-size category	2015		2016		2017	
	No. of herds	No. of pigs	No. of herds	No. of pigs	No. of herds	No. of pigs
G1 (1–10)	488	1626	94	418	25	83
G2 (11–100)	94	2560	54	1665	37	735
G3 (101–1000)	37	15,034	29	12,498	24	7516
G4 (> 1000)	82	360,307	71	320,511	67	278,572
Total	701	379,527	248	335,092	153	286,906

outbreak farm were identified. The Euclidean distance between each affected farm and the closest wild boar case within a year before the outbreak was recorded, to characterise the infection pressure from wild boar.

Wild boar hunting data, as well as data regarding number of hunters, feeding sites and hunting hounds, were provided by the Estonian Environment Agency (Nature department) and based on regular reports submitted by regional hunting societies to the Environmental Board.

2.7. Statistical analysis and maps

2.7.1. Herd incidence and outbreak risk estimates

Survival analysis was used to calculate herd incidences. The outbreak risk estimates were based on incidence values.

The dataset included all pig farms recorded in source databases in 2015, 2016 and 2017. The observation period started from 1st January each year for those herds that were in the database. The date of start of pig keeping in new herds registered during the year of observation was not known, and such herds were not included in the analysis of the respective year. The observation period lasted either until the day that production ceased (removal of pigs from the farm), the end of the year (right censoring), or until the outbreak of ASF.

The data were declared as survival-time data by specifying the start of the observation period as the 'enter' option in the 'stset' command in Stata MP14*. The event of interest was the outbreak of ASF in a domestic pig herd and was specified as the 'failure' option in the 'stset' command. Incidence rate, together with 95% confidence intervals, was calculated for each of the study years as well as for the period between 1st January 2015 until 31st December 2017 using the 'strate' command.

A Cox proportional hazard random-effect model was applied to detect significant differences in ASF infection hazard across farm types, herd-size categories and the three study years. A Cox regression model ('stcox' command in STATA*) was applied to detect the significance of the association between variables and the event of interest. The model specified a Breslow method for handling ties, and also included county as a random effect in the 'shared' option.

Variables significantly associated with the event of interest ($p < 0.05$) were retained in the multivariable model. Akaike information criterion (AIC) values were used to compare the models in terms of their quality (Dohoo et al., 2009).

The assumption of proportional hazards was checked graphically by creating log-log plots of survival, and by a statistical test using Schoenfeld residuals (Dohoo et al., 2009).

2.7.2. Mortality calculations

Mortality risk (cumulative incidence) was calculated for the following: (i) for each outbreak herd, and (ii) for affected groups within the herd for the period including the HRP and the timespan from notification to culling. The affected group was defined as a physically separated unit of a building containing one type of pig (sows, fatteners, weaners etc.).

2.7.3. Spatio-temporal analysis

A hierarchical Bayesian spatio-temporal model (Varewyck et al., 2017) was used to assess the association between the occurrence of ASF cases in wild boar and ASF outbreaks in domestic pigs. No additional time or space-time interaction effects were included in the model; thus, priors were considered to be uninformative. Temporal resolution of the model was set at one month. Spatial resolution for the analysis was based on hunting district (an area allocated to one hunting club for hunting, $n = 344$) as this was the lowest spatial unit for which covariate data was available. Areas that shared boundaries were considered to be neighbouring, and the model assumed dependency of values between them. One hunting district (334EE–Naissaar) was dropped from the spatio-temporal analysis as it did not have any observations. The implications of this exclusion were considered minimal

as it is a small islet off the northern coast, with no direct connections to any other hunting districts.

The response variable was 'ASF outbreak in domestic pigs in hunting district' (set as binary). Covariates included by month were: 'total no. of ASF PCR-positive wild boar' (from September 2014 to November 2017), and 'total no. of wild boar hunted' (from March 2015 to November 2017). Covariates included by year (2014–2017) were: 'total no. of hunters', 'total no. of wild boar feeding sites', and 'total no. of hunting hounds'. These latter three covariates were chosen as they were expected to reflect hunting intensity in a hunting district. The model was checked for convergence.

2.7.4. Maps

Descriptive maps were generated using ArcGIS ArcMap 10.3.1 (ESRI, Redlands, CA, USA).

3. Results

3.1. Reporting and laboratory findings

ASF was immediately suspected on 12 out of the 26 farms, while on the other farms the first suspicion was feed poisoning ($n = 7$), erysipelas ($n = 3$), pneumonia ($n = 3$), salmonellosis ($n = 1$) and heat or stress ($n = 2$). The reason for reporting was sickness ($n = 19$) or death ($n = 7$) of one or several animals. In addition to outbreak farms, ASF was suspected and samples were submitted to the NRL for analyses from 18 other farms in 2015, from 28 other farms in 2016 and from 38 other farms in 2017.

On all outbreak farms, PCR-positive animals were detected. In addition, on seven farms, animals with ASF-virus-specific antibodies were detected by ELISA. All antibody-positive animals were also PCR-positive.

The estimated HRP varied from seven to 20 days with a median of 11 days (Fig. 1).

3.2. Characteristics of affected farms

The number of outbreaks across farms of different type and size categories is shown in Table 4.

Twenty-four outbreaks were classified as primary outbreaks, while two outbreaks were considered to be secondary outbreaks due to close contact with infected herds (common ownership and movements of farm workers, vehicles and equipment between farms). There was no movement of animals between these connected outbreak farms during the high-risk period.

3.3. Clinical signs and virus spread within farms

The first clinical signs in pigs were often mild and not specific to ASF. Cases of a severe course of the disease (excluding sudden deaths)

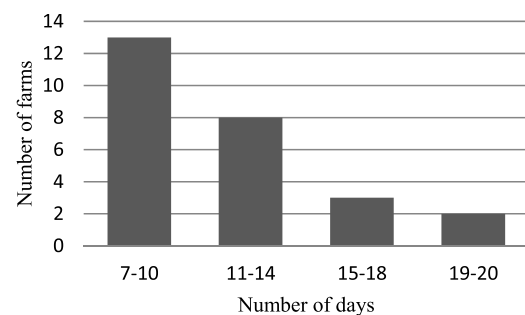


Fig. 1. Length of estimated high-risk period (the length of time that ASF virus may have existed on the farm before it was suspected) on 26 pig farms affected by ASF in Estonia, 2015–2017.

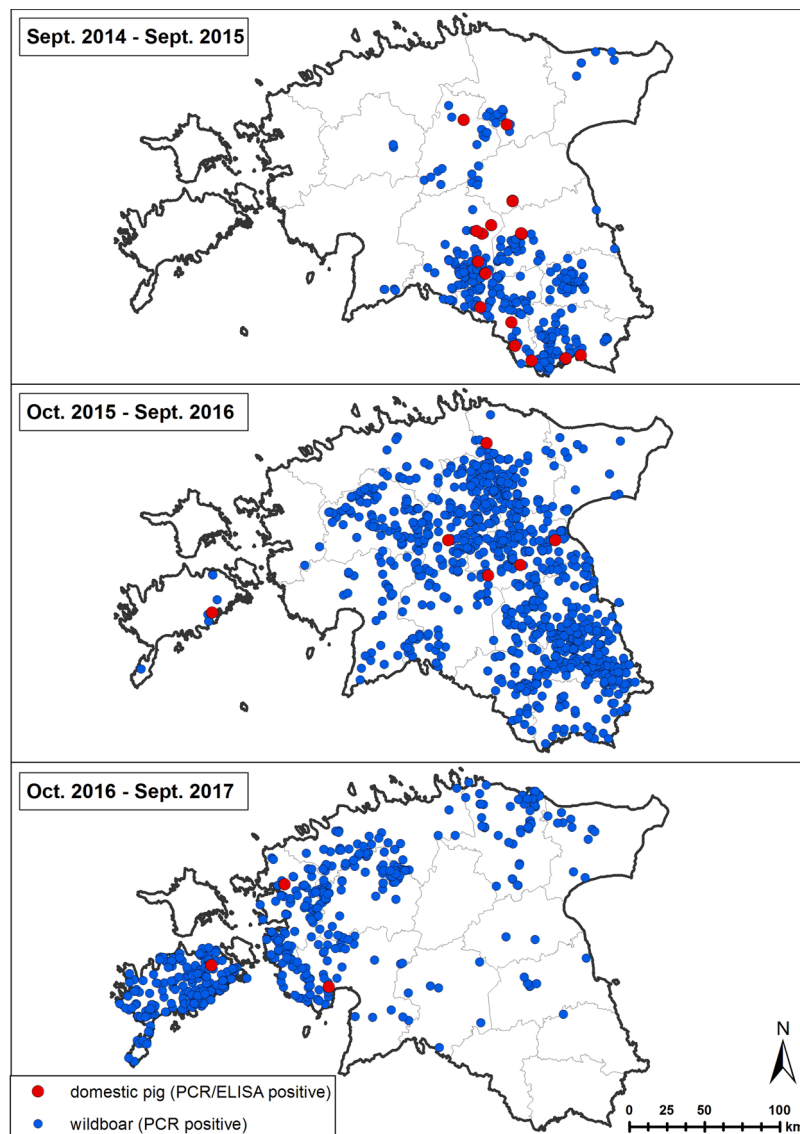


Fig. 2. Location of ASF domestic pig outbreak farms and virus-positive wild boar cases in Estonia in 2015, 2016 and 2017.

were recorded on 13 farms, often after longer circulation of the virus on the farm. On nine out of 12 farms where sows were kept, morbidity occurred firstly among pregnant or nursing sows. Skin haemorrhages or cyanosis were reported in pigs on 11 farms and sudden death on 14 farms, often occurring in a few animals only. A summary of recorded clinical signs in pigs on affected farms before and after reporting is given in Table 5.

In Table 6, the observed mortality estimates are presented. The average mortality was strongly dependent on the herd size, being the lowest in the largest herd-size category (0.7%) and the highest in the smallest one (29.7%).

Table 4
Distribution of Estonian ASF-positive domestic pig farms across herd type and size, 2015–2017.

Production type	Herd-size category (no. of pigs)				Total
	G1 (1–10)	G2 (11–100)	G3 (101–1000)	G4 (>1000)	
Multiplier	0	0	1	2	3
Farrow-to-finish	1	1	3 ^a	5	10
Fattening	7	0	1	5	13
Total	8	1	5	12	26

^a Two herds with crosses of wild boar and domestic pigs (one kept outdoors) and one organic pig farm.

Table 5
Clinical signs in pigs recorded before and after reporting on 26 ASF outbreak farms in Estonia, 2015–2017.

Clinical manifestation	No. of farms
Loss of appetite	19
Listlessness	19
Sudden death without prior signs in animal	14
Skin haemorrhages or cyanosis	11
Fever ^a	10
Recumbency	10
Incoordination	7
Abortions	5
Respiratory disorders	5
Other ^b	5

^a On six farms, fever was not detected; on 10 farms, temperature was not measured.

^b Vomiting (n = 2); decrease in milk yield of sows (n = 1); diarrhoea (n = 1); blood in urine (n = 1).

contaminated feed, grass, clothing, vehicles, other farm animals or pets on the farm, and kitchen waste). The cause of virus introduction for these herds was defined as “lack of/insufficient biosecurity measures”.

For commercial herds, possible pathways of virus introduction were identified more specifically by the epidemiology team who analysed the data collected during outbreak investigations. The results of the analysis are presented in Table 7.

It appears from the presented data that on all affected commercial farms the virus was introduced by indirect transmission routes. On the majority of commercial farms (n = 11), the virus was most likely introduced by means of contaminated fomites (vehicles, people, tools) as a result of errors in execution of biosecurity procedures.

The biosecurity levels of affected farms across herd-size categories are shown in Table 8.

The biosecurity measures required by national legislation as described in Table 2, at least at a minimum level, were in place for 13 (50%) outbreak herds. In 10 herds (38%), the measures were implemented at least at a moderate level, and in three outbreak herds (12%) a high or very high biosecurity level was in place.

The biosecurity level on all eight non-commercial (G1) farms was low or very low. On commercial farms (G2–G4), the biosecurity level was generally higher. Biosecurity level of six (33%) commercial farms was estimated as very low because of multiple deficiencies in the fulfilment of biosecurity requirements presented in Table 2.

3.5. Herd incidence

The data on occurrence of outbreaks, as well as the cumulative herd incidences (presented as outbreak risk estimates), for the years 2015 and 2016 per farm type and size category are given in Tables 9 and 10. In 2017, all outbreaks occurred in G4 herds (outbreak risk = 4.5%, 95% CI 1.5; 12.4), and the overall outbreak risk in all herd-size categories

Table 6
Estimated ASF mortality in affected domestic pig herds in Estonia, 2015–2017.

Herd-size category	n	Mortality in the herd			Mortality in the affected group		
		Average	Min	Max	Average	Min	Max
G1 (1–10)	8	29.7%	0.0%	100.0% ^a	NA	NA	NA
G2 (11–100)	1	25.0% ^b	NA	NA	NA	NA	NA
G3 (101–1000)	5	7.5%	0.4%	25.0%	13.8%	3.8%	25.0% ^c
G4 (>1000)	12	0.7%	0.04%	2.5%	7.2%	0.1%	43.6% ^d

NA – not applicable as pigs were kept in one group.

^a Mortality in a backyard farm with one pig.

^b At the moment of outbreak there were four pigs on the farm.

^c Herd of 126 crosses kept in one group.

^d Mortality in a group of 39 nursing sows.

was 2.0% (95% CI 0.7; 5.6).

The total herd incidence rates per year and for the whole three-year period obtained from survival analysis are presented in Table 11.

The overall yearly incidence rates did not differ significantly ($p > 0.05$) from each other.

In a univariable Cox proportional hazard random-effect model (including county as a random effect), the multiplier and farrow-to-finish herds had a significantly higher hazard of experiencing an outbreak compared to fattening herds in 2015 (data not presented). In the model that included the data from three years (2015–2017), a similar trend could be observed although the association was not statistically significant ($p = 0.064$). Including the variable ‘year’ in the model did not improve the model fit. Thus, only the variable ‘herd-size category’, adjusted for the random effect ‘county’, was included in the final model. Compared to the two smaller herd-size categories (G1, G2), larger herds (G3, G4) had a significantly higher risk of becoming infected with the ASF virus (Table 12).

3.6. Spatial and temporal distribution of outbreak farms

The geographical locations of outbreak farms changed during the epidemic. As shown in Fig. 2, domestic pig outbreaks appeared in those areas where ASF virus was circulating actively in the wild boar population.

Of 26 outbreaks, 23 occurred in regions where the disease was also present in the wild boar population within a radius of 15 km from the affected farm. The distances between the outbreak farm and the nearest case of ASF in wild boar within a year before an outbreak are shown in Fig. 3. In ten cases, the closest wild boar case was found less than one month before the outbreak, in six cases between one and four months before the outbreak, and in seven cases over four months before the outbreak.

All ASF outbreaks were detected during the warmest period of the year, between June and September. Most of the outbreaks (81%) were detected in July and August (See Fig. 4).

3.7. Results of the hierarchical Bayesian spatio-temporal model

The results of the model analysis are presented in Table 13.

The results of the analysis indicate a significant positive association with the total number of ASF-positive wild boar detected per month in a hunting district. The total number of wild boar hunted, number of hunters, feeding sites and hunting hounds in a hunting district were not significantly associated with outbreaks in domestic pigs.

4. Discussion

4.1. Reporting and laboratory findings

ASF occurrence on Estonian domestic pig farms was generally

Table 7

Most probable pathways of ASF virus introduction to commercial pig farms in Estonia, 2015–2017.

Introduction pathways	Herd-size category (no. of pigs)			Total
	G2 (11–100)	G3 (101–1000)	G4 (>1000)	
Multiple errors in execution of biosecurity procedures (introduction by fomites)	1	0	4	5
Inadequate disinfection of vehicles	0	0	2	2
Minor errors in execution of biosecurity procedures (introduction by fomites)	0	0	2	2
Movement of people or vehicle from an infected farm (secondary outbreak)	0	1	1	2
Contamination of cereal feed during storage or processing	0	3	2	5
Feeding of grass	0	1	0	1
Contamination of bedding material	0	0	1	1
Total	1	5	12	18

Table 8

Biosecurity levels of Estonian ASF outbreak farms according to herd size, 2015–2017.

Herd-size category (no. of pigs)	very high	high	moderate	low	very low
G1 (1–10)	0	0	0	1	7
G2 (11–100)	0	0	0	0	1
G3 (101–1000)	0	0	1	0	4
G4 (>1000)	2	1	6	2	1
Total	2	1	7	3	13

reported within the first week after the appearance of clinical signs, therefore at a relatively early stage of the outbreak. This was confirmed by the fact that no seroconverting (antibody ELISA positive) animals were found in most herds and the spread of the disease within farms was limited. In seven cases, reporting was delayed for two weeks after appearance of the first disease signs in animals. In these herds, ASF antibody ELISA positive pigs were present. However, all these animals were PCR-positive as well, which indicates that the virus should not have been present in the herd for more than four weeks (Nurmoja et al., 2017; Gallardo et al., 2018; Zani et al., 2018). The speed of reporting was not dependent on whether the herd was commercial or not.

In more than half of the outbreak herds, diseases other than ASF were suspected at first. This can mainly be explained by non-specific signs of ASF at the beginning of the outbreak, particularly due to a lack of characteristic pathological post-mortem findings (data not presented).

4.2. Characteristics of affected farms

Outbreaks occurred in herds of all production types and size categories. The proportion of herds with breeding animals among outbreak farms (50%) exceeded the proportion of these herds in the general population (28%), and there was a trend in the data towards a higher

risk for outbreaks in herds with breeding animals. This may be explained by differences in the management of breeding pigs compared to growers and fatteners (more human interaction with breeding pigs). Furthermore, pregnant and nursing sows may be more susceptible to the virus due to immune suppression, and so lower doses of the virus might be able to initiate the infection. Sows in heat may also attract male wild boar (including infected ones), and as a consequence the surrounding environment of breeding farms may become more contaminated with the virus, increasing the likelihood of transmission with fomites onto the farms.

The number of ASF outbreaks in commercial herds exceeded the number of outbreaks in backyard farms. This can partly be explained by the rapid reduction of backyard pig holdings due to strict biosecurity requirements, which are equal for all pig farms in Estonia. This brought the number of backyard pig farms down from 696 in 2014, to 25 by 2017. On the other hand, it may also indicate that large commercial farms are more exposed to the virus due to more frequent and intensive contact with the external environment through movement of people and vehicles.

4.3. Clinical findings and spread of the virus on farms

Although ASF is described as a severe, haemorrhagic disease that causes up to 100% morbidity in naive pig herds and can result in very high mortality (Sánchez-Vizcaíno et al., 2009; Costard et al., 2013), under field conditions we often found ASF cases with mild clinical signs. Severe clinical signs, as well as the haemorrhagic form of the disease, were seldom observed, and often limited to a few animals only. This can be explained by the relatively early detection of outbreaks, as most were reported within seven days of the first observation of disease signs. A severe clinical course and higher morbidity were seen in pregnant or nursing sows, or in the case of longer virus circulation on a farm.

The spread of the virus within affected herds was generally slow,

Table 9

Number of ASF outbreaks and cumulative herd incidence (outbreak risk) in different farm types and herd-size categories in Estonia in 2015.

Production type	Herd-size category					Total n herds/ n outbreaks	Outbreak risk (CI 95%)
	G1 n herds/ n outbreaks	G2 n herds/ n outbreaks	G3 n herds/ n outbreaks	G4 n herds/ n outbreaks	Total		
Multiplier	18/ 0	11/ 0	1/ 1	5/ 2	35/ 3	8.6% (3.0–22.4)	
Farrow-to-finish	13/ 0	44/ 1	22/ 3	31/ 3	110/ 7	6.4% (3.1–12.6)	
Fattening	456/ 4	39/ 0	13/ 1	46/ 2	556/ 7	1.3% (0.6–2.6)	
Total	488/ 4	95/ 1	36/ 5	82/ 7	701/ 17	2.4% (1.5–3.8)	
Outbreak risk (CI 95%):	0.8% (0.3–2.1)	1.1% (0.2–5.7)	13.9% (6.1–28.7)	8.5% (4.2–16.6)	2.4% (1.5–3.8)		

Table 10
Number of ASF outbreaks and cumulative herd incidence (outbreak risk) in different farm types and herd-size categories in 2016.

Production type	Herd-size category					Outbreak risk (CI 95%)
	G1 n herds/ n outbreaks	G2 n herds/ n outbreaks	G3 n herds/ n outbreaks	G4 n herds/ n outbreaks	Total n herds/ n outbreaks	
Multiplier	8/ 0	9/ 0	1/ 0	3/ 0	21/ 0	0.0% NC
Farrow-to-finish	6/ 1	24/ 0	17/ 0	28/ 1	75/ 2	2.7% (0.7–9.2)
Fattening	80/ 3	21/ 0	11/ 0	40/ 1	152/ 4	2.6% (1.0–6.6)
Total	94/ 4	54/ 0	29/ 0	71/ 2	248/ 6	2.4% (1.1–5.2)
Outbreak risk (CI 95%):	4.2% (1.7–10.4)	0.0% NC	0.0% NC	2.8% (0.8–9.7)	2.4% (1.4–5.2)	

Table 11
The herd incidence rates of ASF outbreaks among domestic pig herds in Estonia for the years 2015–2017.

Year	No. of outbreaks	No. of herd-years	Incidence rate (outbreaks per 100 herd-years)	95 % Confidence interval
2015	17	646.7	2.6	1.6–4.2
2016	6	229.8	2.6	1.2–5.8
2017	3	140.2	2.1	0.7–6.6
2015–2017	26	1016.7	2.6	1.7–3.8

Table 12
The results of the Cox proportional hazard random-effect model showing the effect of herd size on the incidence of ASF outbreaks in Estonian domestic pig herds in the period 2015–2017. ‘County’ was included as a random variable.

Herd-size category (no. of pigs)	N ^a (no. of outbreaks)	Hazard ratio (HR)	P-value	95% Confidence interval for HR
G1 (1–10)	607 (8)	1	X	X
G2 (11–100)	185 (1)	0.36	0.342	0.05–2.92
G3 (101–1000)	90 (5)	4.22	0.013	1.36–13.14
G4 (>1000)	220 (12)	4.31	0.002	1.72–10.80

Wald Chi squared = 14.71 (p = 0.002).

^a Number of herds after splitting the observation period into three years.

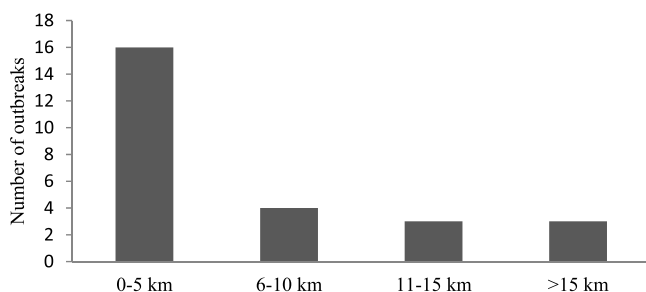


Fig. 3. The distance between domestic pig outbreak farms and the closest tested ASF-positive wild boar case within a year before an outbreak in Estonia, 2015–2017.

meaning that the contagiousness of the virus was low. Even in affected pens, some pigs were still ASF-virus-negative at the time of reporting, and in most outbreaks the infection was detected only in one unit or even in one pen. Similar findings were reported by Olšovskis et al. (2016) in Latvia.

The estimates of mortality risk reported here are arbitrary as the time-periods for calculation of the mortality risk for every affected herd

differed considerably (reporting 0–14 days from first symptoms, culling 1–3 days after reporting). Nevertheless, in the two largest farm-size categories (G3, G4), the herd-level and production-unit-level mortality risks were generally low. This indicates that in larger herds (G3, G4) the monitoring of general mortality is not suitable for early detection of an ASF outbreak. In smaller herds (G1, G2), the average mortality risk was considerably higher, as every case of death influenced the risk estimate markedly. However, the case fatality rate can be considered high, as most of the affected pigs died 1–5 days after the appearance of the first clinical signs, which means that an ASF epidemic may result in high mortality if there is enough time for the virus to spread within the herd.

Nevertheless, in affected and endangered regions, every sudden death of a pig with an unclear cause should be considered a possible case of ASF, and “high mortality” should not be expected at the start of an outbreak.

4.4. Probable routes of virus entry into farms and biosecurity level of the outbreak farms

Based on the collected epidemiological information, the introduction of the virus into domestic pig herds is likely to have occurred mainly by indirect transmission routes. None of the outbreaks could be linked to the introduction of infected pigs. Direct contact with potentially infected wild boar could not be completely excluded in two herds – one outdoor farm of crosses with double fencing, and one organic farm using a single fence with a walking area connected to the barn. However, even in these herds, direct contact was considered unlikely. The fencing of the outdoor farm was checked during the outbreak investigation and no damage was discovered. The organic farm was located in an open area (no forest nearby) and no direct signs or evidence of wild boar entering the farm could be identified.

Feeding of contaminated swill has generally been considered one of the main risk factors for indirect transmission of ASF (FAO, 2013; Gogin et al., 2013; Oganessian et al., 2013). In Estonia, the feeding of swill to pigs is illegal and could be excluded as a route of virus introduction on all affected commercial farms. On backyard farms, the feeding of kitchens leftovers could not be excluded. However, swill feeding was not considered the main possible route of virus introduction, as the owners mainly consumed pig meat from their own pigs. Introduction of the virus to these farms with purchased meat products (ham, sausages etc.) from local shops would assume hidden circulation of the virus in Estonia or contamination of imported products. This was considered unlikely. According to the interview results, none of the farmers or farm workers had contacts with affected non-EU countries. Thus, the introduction of contaminated pig meat or products from these countries to outbreak farms was also considered unlikely. Another possible source of infection is contaminated wild boar meat. Limited circulation and use of uncontrolled wild boar meat cannot be excluded in Estonia.

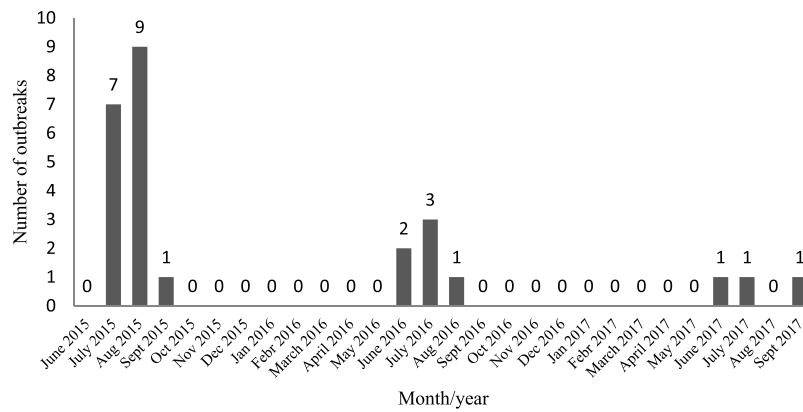


Fig. 4. Occurrence of ASF outbreaks in Estonia from June 2015 to September 2017.

Table 13

Fixed estimated parameters of the hierarchical Bayesian spatio-temporal model on a natural logarithmic scale.

Variable	Mean	SD	Prediction interval (quantile)		
			2.5%	50%	97.5%
Intercept	-6.775	0.41	-7.598	-6.764	-6.012
No. of wild boar hunted (monthly)	-0.024	0.026	-0.081	-0.022	0.022
No of ASF PCR-positive wild boar detected (monthly)	0.132^{a,b}	0.058	0.002	0.138	0.230
No. of hunters in a district (yearly)	0.012	0.009	-0.006	0.012	0.029
No. of feeding sites (yearly)	0.015	0.024	-0.036	0.016	0.058
No. of hunting hounds (yearly)	0.015	0.067	-0.122	0.017	0.141

^a Prediction intervals in bold indicate statistically significant parameters.

^b Mean effect of ASF-positive wild boar detection in a hunting district on the occurrence of a domestic pig outbreak on the territory of a hunting district was estimated to be 0.132. It means that for a one unit increase in ASF-positive wild boar detection the log odds of having a domestic pig outbreak increases by 0.132 (95% prediction interval = 0.002–0.230).

However, evidence of the use of wild boar meat in affected backyard herds could not be established except for in one case, where the owner was a hunter. Thus, most likely, the virus has entered affected herds by means of contaminated fomites – clothing, vehicles, feed and bedding material – due to inadequate biosecurity measures or errors in the implementation of these measures.

For most outbreaks, there was no single obvious cause or event that could be linked with the introduction of the virus. In most affected backyard farms, there were several biosecurity gaps at the time (e.g. lack of functional disinfection barriers, no separation of inside and outside zones, pet access or housing other farm animals together with pigs, feeding grass to pigs, unsafe storage of bedding material and feed etc.). It is difficult to single out one particular cause. In commercial herds, which followed relatively high biosecurity protocols, the route of virus introduction was difficult to trace. Seemingly minor errors in the implementation of (generally adequate) disinfection procedures must have led to the introduction of the virus.

The majority of outbreaks occurred on farms with either a low or very low biosecurity level. However, looking at commercial farms separately, it appears that those farms with at least a moderate biosecurity level experienced outbreaks to the same extent as those with low and very low biosecurity levels. It is generally assumed that low biosecurity level farms are at higher risk of introduction of infections. Based on available data, it was not possible to estimate whether herds with a low biosecurity level were at higher risk or not as information about the distribution of biosecurity levels for the whole population is lacking. However, assuming that the biosecurity level is in general higher on commercial farms than on backyard farms, our data on herd incidence do not support the general opinion that a higher biosecurity level ensures a lower risk of ASF introduction (see below). This may mean that the biosecurity measures applied so far (physical and

disinfection barriers) are not fully effective in protecting against the incursion of ASF virus.

4.5. Herd incidence

The herd incidence estimates are dependent on the accuracy of reporting. The observed herd incidence risk was significantly higher in the group of commercial herds in years 2015 and 2017 and did not differ significantly from the incidence risk in non-commercial (backyard) herds in 2016. One may question whether the reporting in the group of backyard farms was as good as for commercial farms or not. Considering the availability of veterinary services in Estonia (there are veterinarians available for every animal keeper), and the usual habits of smallholders to invite a veterinarian to check diseased animals, we would assume, at worst, only a slightly lower level of reporting in backyard herds compared to commercial farms. Surveillance (including serological and PCR testing) of herds located in restriction zones (areas where infection in wild boar or domestic pigs has been detected) has not revealed any case of undetected infection in domestic pigs (data not shown).

The observed herd incidence risk in commercial herds (G2–G4) decreased significantly in 2016 and 2017, compared to 2015. This is likely the result of improvements in biosecurity measures on farms, and more stringent surveillance by the veterinary authorities regarding the fulfilment of legal requirements on biosecurity. Interestingly, the total herd incidence across all herds did not change significantly. However, we might expect that there was some reporting bias for the group of backyard herds (G1) in 2015 as the owners might have not recognised or reported the disease if it was limited to the sudden death of just one or two pigs.

4.6. Spatial and temporal distribution of outbreak farms and associations between ASF outbreak farms and wild boar

Similarly to Latvia in 2014 (Olševskis et al., 2016), the vast majority of outbreaks in Estonian domestic pigs occurred in areas where ASF had been found in wild boar prior to detection of the virus in domestic herds. In 23 outbreaks, the virus had been circulating among wild boar within a radius of 15 km from the affected farm, and in 16 outbreaks, within a radius of 5 km from the affected farm. On the island of Saaremaa, the infection was first discovered in a domestic pig herd. However, a couple of days after the reporting of this case in domestic pigs, two infected wild boar carcasses were found 3 km and 10 km respectively from the outbreak farm. The age of these carcasses indicates that the virus was present in the wild boar population for some time before the outbreak in domestic pigs occurred.

According to the spatio-temporal analysis, the occurrence of outbreaks in domestic pigs was associated with the intensity of the infection in the wild boar population – the outbreaks occurred in areas where there were more virus-positive (as detected by PCR) cases in wild boar registered prior to the outbreak. There was no significant association with hunting intensity; this might be since there is minimal interaction between hunters and pig producers.

The introduction of ASF virus into domestic herds has been strictly seasonal in Estonia and associated with the warmest period of the year – June to September. Most of the outbreaks (81%) were detected in July and August. A similar seasonal trend has also been observed in other infected EU countries (Olševskis et al., 2016; EFSA (European Food Safety Authority) et al., 2017). One explanation for this seasonality might be that during the summer months, contact between farms (people and vehicles) and the wild boar in the surrounding environment is much more frequent because of the seasonal nature of field work. The high-risk period for introduction of the virus into domestic pig herds coincides with the harvest period, when wild boar also move to feed in the fields. This is also the period when wild boar density is highest (period after breeding season), and additionally, the number of infected wild boar is also at its highest, which indicates infection pressure. All these factors may increase the probability of transmission via contaminated fomites.

The high season of ASF outbreaks in domestic herds also coincides with the high season of blood-sucking insects in Estonia, suggesting their potential role in transmitting the virus from wild boar to pigs. However, there is very little scientific evidence regarding the capacity of mechanical insect vectors to transmit the ASF virus. Furthermore, if this was to be an important transmission route, many more outbreaks should be expected in domestic herds, as should a faster spread of the infection within herds. Nevertheless, the role of insect vectors in transmission of the virus is still not clear and needs further investigation.

5. Conclusions

The results of this study suggest that the presence of ASF virus in wild boar populations is the main risk for domestic pig farms becoming infected. Farms of all sizes and types are at risk, including large commercial farms operating at a high biosecurity level. Farms with breeding animals seem to be at higher risk of becoming infected. Despite the high virulence of the circulating virus strain, the clinical manifestation of the disease has initially been unspecific and mild in most herds. The spread of the virus within farms has been slow, and the contagiousness of the virus has been relatively low.

Conflict of interests

The authors of this research paper have no financial or personal interests that could have influenced this paper.

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