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African swine fever virus (ASFV) in Poland: Prevalence in a wild boar population (2017–2018)

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Abstract: African swine fever (ASF) was first described in 1921 in Kenya. The latest epidemic of ASF started in 2007 in Georgia. The virus was introduced to Poland in 2014. Since the beginning of the epidemics, the National Veterinary Research Institute in Pulawy (NVRI) has been testing wild boar samples from restricted areas and other parts of Poland to conduct passive and active surveillance for ASFV in these groups of animals. The aim of this study was to summarise the last two years of the ASF epidemiological status in Poland and the attempt to find disease patterns in the wild boar population. The period between 2017 and 2018 brought a massive number of new ASF cases in Poland. The number of ASF-positive wild boars jumped from 91 in 2016 to 1 140 in 2017 (approximately a 12 × increase), and 2018 was even worse, with the disease affecting 4 083 animals (2 435 cases; one case could even be 10 animals or more if they are found in one place next to each other). The percentage of positive wild boars found dead (passive surveillance) in the restricted area increased in 2018 to 73.1% from 70.8% in 2017. The chance of obtaining positive results in this group was six times higher in December and 4.5 times higher in January than in August and September. The percentage of positive wild boars detected through active surveillance reached 1.5% in 2018. The data suggested that, not only in Poland, but also in other ASF-affected countries, during the epizootic stage of the disease spread the most important measure is an effective passive surveillance of dead wild boars especially, in the winter season rather than in the summer.

Keywords: ASF; season; passive surveillance; active surveillance; dead animals; hunted animals

African swine fever (ASF) is not a recent epidemiological problem in swine production. The disease was first identified in pigs in Kenya in 1921 (Montgomery 1921). The African swine fever virus (ASFV) is a large dsDNA unique member of the *Asfaviridae* family infecting pigs, wild boars and other members of the *Suidae* family. The ASFV can also infect soft ticks of the *Ornithodoros* species as an insect-borne vector for the virus (Sanchez-Vizcaino et al. 2012; Wozniakowski et al. 2016).

The mortality reaches 100% at the initial stage of the disease (Sanchez-Vizcaino et al. 2012). The virus is highly resistant to degradation and may remain infectious, even after using meat preservation techniques such as freezing and smoking. The ASFV in wild boar carcases may contami-

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nate the environment and other wild boars as well (Zakaryan and Revilla 2016; Pejsak et al. 2018; Chenais et al. 2019).

Serious economic losses were observed during the first virus introductions into Europe in 1957 and 1960 (Portugal, genotype I). During these epidemic waves, the virus reached Portugal, Spain, France, Italy, Malta, the USSR, Belgium and the Netherlands. The outbreaks of ASF occurred mainly in domestic swine populations. Since 1999, continental Europe has been ASF-free (although the virus remained present in Sardinia, Italy) (Davies et al. 2015; Iglesias et al. 2017).

The second wave of the ASFV resulted from the virus being introduced to Georgia in 2007. The virus came from either South-East Africa or Madagascar (genotype II) and quickly spread to Armenia, Azerbaijan, Iran and the Russian Federation. The ASF wave later reached Belarus and spread further to the Ukraine, Poland and the Baltic States (Rahimi et al. 2010; Wozniakowski et al. 2016). The spread of the disease has not been stopped and it continues to reach new countries, including the Czech Republic, Moldova, Romania, Bulgaria, Belgium, and recently, Slovakia and Serbia. The ASFV broke into a new territory upon entering Asia through China (Pejsak et al. 2018; Wang et al. 2018; OIE 2019; Schulz et al. 2019a).

In Central and Eastern Europe, wild boars represent the main reservoir of the ASFV (Pejsak et al. 2018). Outbreaks in the domestic swine population in Poland and in the Baltic States mainly appear in areas where the virus is present in the local wild boars (Pejsak et al. 2018; Podgorski and Smietanka 2018; Pikalo et al. 2019). However, they are not the only cause of ASFV – weak biosecurity measures (or a lack thereof) may also have an impact on the safety of pig herds. In Romania, Bulgaria, Slovakia and Serbia, the number of outbreaks in domestic pig populations is larger than in the wild boar population, showing that wild swine are not the only source of ASFV (ADNS 2019; Schulz et al. 2019b).

However, the absence of the disease in the nearest area increases the safety of the herd. For these reasons, the surveillance of ASFV in wild boar populations is important and can help in securing and preparing the farms, pig herds and the economy against the disease (Costard et al. 2015; Juardo et al. 2018; Nurmoja et al. 2018; Podgorski and Smietanka 2018; Schulz et al. 2019a).

Current ASF situation in Poland

Wild boars are common forest animals in Poland. Their population has significantly increased over the last years, resulting in damage to the agricultural production and affecting the spread of the ASFV. The latest data published by the Polish government (Statistics Poland – SP), however, suggest that the number of wild boars in Poland is lower than the total number of hunted animals, which is connected to the calculation of wild boars that are conducted before the breeding period (SP 2018). These animals are a source of the disease in the European environment (Chenais et al. 2018). The spread of ASF in wild boars is density dependent: the higher the number of animals, the greater the probability of the disease transmission. The carcasses of the affected wild boars may even remain infectious for several months (Podgorski and Smietanka 2018). The relationship between the wild boar density and the occurrence of ASF cases in 2017 and 2018 is shown in Figure 1. The official data regarding the wild boar population in Poland state a total of 215.7 thousand individuals in 2017, and only 87.9 thousand in the beginning of 2018 (the calculations were conducted by Polish hunters before the breeding period). During the hunting season of 2017–2018, there were 341.411 thousand wild boars hunted in Poland (SP 2018). The goal of the Polish government and General Veterinary Inspectorate (GVI) is to reduce the boar population down to 0.1 animals/km^2 (GVI 2019).

According to European Union legislation, Poland is separated into four zones due to the occurrence of ASFV. In Zone 0 (the "safe zone"), ASFV is not present and restrictions related to this disease are limited. Zone I (the "protected zone") denotes an area in which the virus is not present, but the hunting of wild boars and the collection of carcases is intensive. In this zone, there are some restrictions on the swine production, but it is still limited. Zone I is designed 20 km wide from zone II and III. In Zone II (the "restricted zone"), the virus is present in the wild boar population, but remains undetectable in the swine population. Zone III (the "hazardous zone") is an area in which the ASFV was detected in pig farms. Due to the evolution of the epidemiological situation after an ASFV-positive case in a given area, the national veterinary authorities, in cooperation with European Union specialists, has introduced new ASF zones.

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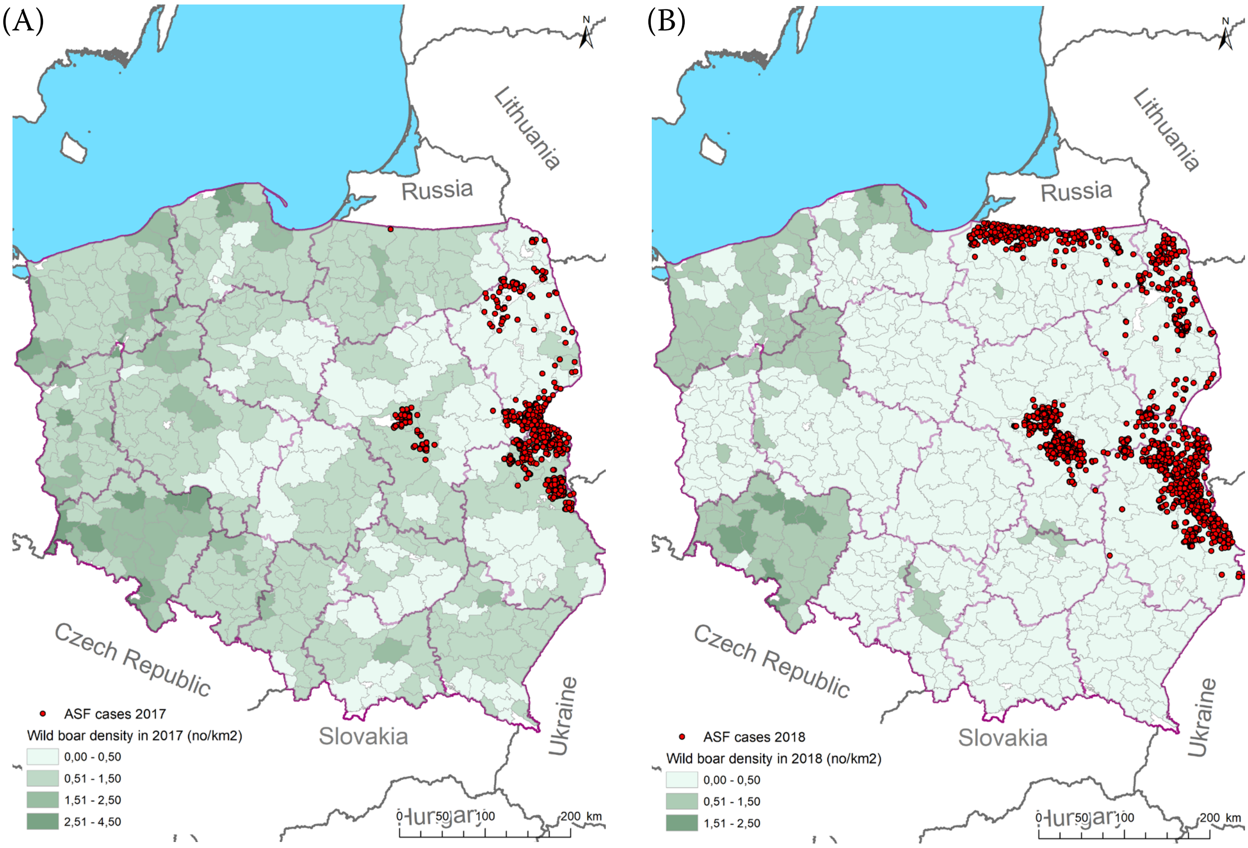

Figure 1. The distribution of the ASF-positive wild boars in Poland in 2017 (A) and 2018 (B); the maps include the wild boar density

The virus has expanded from previous zones and has appeared in completely new places such as the Warsaw area in 2017 and northern Poland (near the Russian border) in 2018 (Smietanka et al. 2016; Pautienus et al. 2018; GVI 2019).

MATERIAL AND METHODS

The objective of this study was to analyse the dynamics of the ASF spread in the wild boar population in Poland. An additional advantage of the conducted study is to track the changes in the movement of the virus in the environment by analysing field samples from the whole country over the last two years (2017–2018) using statistical methods.

Wild boars which were found dead were sampled throughout the country irrespective of the ASF status in the area (passive surveillance; Zone 0, I, II, III). However, the samples from the hunted wild boars (active surveillance; Zone I, II, III) changed several times depending on the ASF status of the affected area, as the size and shape of the restriction zones were continuously being updated according to the most recent epidemiological situation. These changes were due to the updates of the European Commission Implementing Decision 2014/709/EU (Nurmoja et al. 2017). Figure 2 shows the changes in the ASF zones between 2017 and 2018.

All the stages of the analyses, from the preparation of the samples and the DNA extraction to the molecular/serological analyses, were conducted in a biosafety level 3 (BSL-3) laboratory environment by qualified technicians and supervisors.

The samples used for the diagnostic testing include the blood, bone marrow and various tissues (e.g., tonsils, spleen, kidneys, lungs). All the samples were collected by local veterinary facilities (through ASFV monitoring programmes in Poland) and were analysed for the presence of ASFV DNA or host antibodies which target ASFV using molecular and serological methods.

Prior to the analyses, the tissue samples were homogenised in a phosphate-buffered saline (PBS) solution. 200 µl of material was used for the DNA extraction using a QIAamp DNA Mini Kit, following

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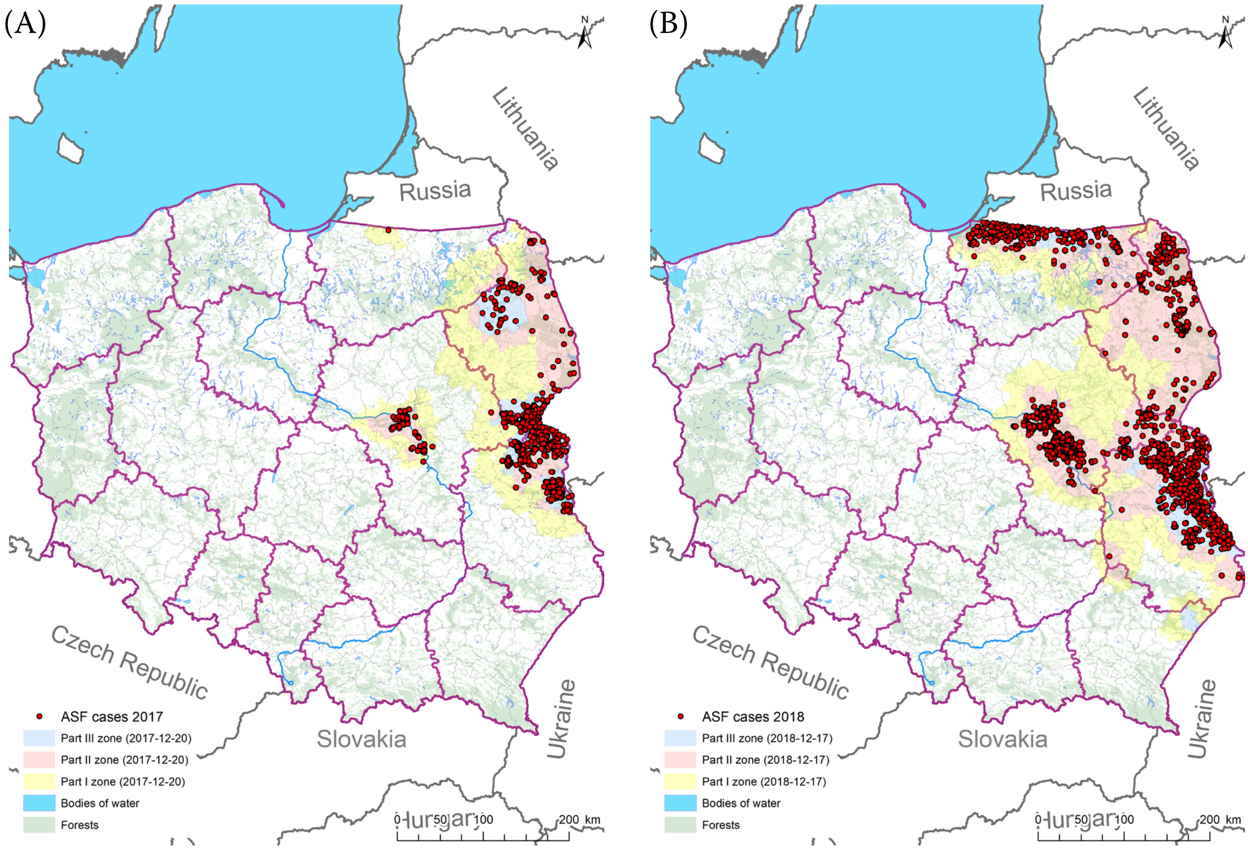

Figure 2. The distribution of the ASF-positive wild boars in Poland in 2017 (A) and 2018 (B) with the ASF zones (the zones designated at the end of the subsequent years); the maps include the forests

the manufacturer's procedures (QIAGEN, Germany). The positive control used in the isolation process was the ASFV reference material, which was kindly provided by the European Union Reference Laboratory (EURL) for-ASF (CISA-INIA, Valdeolmos, Spain).

To detect the presence of ASFV genetic material, a real-time PCR (polymerase chain reaction) method was used as previously described (Fernandez-Pinero et al. 2013). Briefly, the process was conducted in eight 0.2-ml optical tubes in three varying real-time PCR thermocyclers (Applied Biosystems 7500 and QuantStudio 5, Applied Biosystems, USA; Rotor Gene, QIAGEN, Germany). The primers ASF-VP72-F and ASF-VP72-R and the probe UPL162 used in this method were complementary to the ASFV conserved sequence VP72. The thermocycler conditions were consistent with protocol described by Fernandez-Pinero (2013).

The real-time PCR was conducted using a FastStart Universal Probe Master (ROX) kit (Roche Applied Science, Switzerland) in a final volume of 20 µl. Each reaction contained a 2×-concentrated Master PCR Mix, 0.4 µM each of the ASF-VP72-F and ASF-VP72-R primers and 0.2 µM of the UPL162 probe. The thermocycler parameters used are as follows: 10 min at 95 °C (initial denaturation), 40 cycles at 95 °C for 10 s (exact denaturation), and 58 °C for 30 s (primer annealing and PCR product elongation). The fluorescence signal was collected during the primer-annealing and elongation step of each cycle using the FAM (6-Carboxyfluorescein) channel (excitation λ = 495 nm, emission λ = 520 nm). A fluorescent curve with a threshold cycle value (Ct) lower than 37 was considered a positive result (Wozniakowski et al. 2016).

Before the serological analyses were conducted, the blood samples [without EDTA (ethylenediaminetetraacetic acid)] were centrifuged at 95–214 × *g* to isolate and obtain the serum. To determinate the presence of antibodies against ASFV, two enzyme-linked immunosorbent assays (ELISA) were carried out (Ingezim PPA COMPAC, Ingenasa, Spain; IDVet Indirect Screening test, IDVet, France). The method was applied according to the manufacturer's protocol. The criteria of validation for positive, negative and ambiguous

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results obtained using the kit were consistent with the manufacturer's guidelines.

In the case of positive and ambiguous results obtained from the ELISA test, a secondary confirmation test was conducted by means of an indirect immunoperoxidase technique (IPT), which is approximately 100 times more sensitive than an ELISA test. The principle is the same as an ELISA, but the result is observed under reverse-field microscopy without the use of spectrophotometry. The elements of the test were delivered by the EURL (European Union Reference Laboratory) for ASF (CISA-INIA, Valdeolmos, Spain), and the overall procedure was standardised by the EURL team.

In the passive and active surveillance using the molecular and/or serological assays as a positive result, the presence of specific virus DNA and/or antibodies has been considered.

Analysis of the ASF prevalence in the wild boars in 2017 and 2018, individually for each month and overall for the 2017–2018 period in total, was conducted separately in the following groups:

- passive surveillance (found dead), zones II and III;
- active surveillance (hunted), zones II and III;
- passive surveillance (found dead), zones I and 0;
- active surveillance (hunted), zones I and 0.

In addition, an ASF prevalence analysis was carried out separately between the wild boars with the different status (found dead, hunted) for the following groups:

- zones II and III, the entire period of 2017–2018;
- zones II and III, 2017;
- zones II and III, 2018;
- zones 0 and I, the entire period of 2017–2018;
- zones 0 and I, 2017;
- zones 0 and I, 2018.

The analyses were carried out using logistic regression models. One such model is a mathematical formula that we can use to describe the impact of several variables (x_1, x_2, ..., x_n) on the dichotomous variable Y, which has two values (in our case: positive/negative):

$$P(Y = 1|x_1, x_2, \dots, x_n) = \frac{e^{(\beta_0 + \sum_{i=1}^{n} \beta_i x_i)}}{1 + e^{(\beta_0 + \sum_{i=1}^{n} \beta_i x_i)}} \quad (1)$$

where:

β_i – the regression coefficient for i = 0, ..., n;

x_i – the independent variables (measurable or qualitative) for i = 1,2, ... n.

We received the ratings of the coefficients using the maximum likelihood method. The significance of the individual variables has been calculated using the t-test or Wald's statistics. The fitness of the model to the data using LR (likelihood ratio) statistics has also been determined.

The odds ratios (ORs) were calculated along with 95% confidence intervals.

The described relationships were demonstrated statistically at the adopted level of significance α = 0.05.

In the month-to-month comparative analysis between 2017 and 2018, where the logistic regression could not be applied due to the poor matrix conditions due to the zero subgroups, a chi-square independence test with appropriately selected corrections was used: Yates, Fisher or V-square.

The statistical calculations were performed using TIBCO Software Inc. (2017) Statistica v13 (data analysis software system). In order to show the geographical distribution of the ASF cases in connection with the wild boar density and the forests in Poland, ArcGIS 10.4.1 (ESRI) was used. Figure 3 and 4 were created by the use of Microsoft Office Excel 2016.

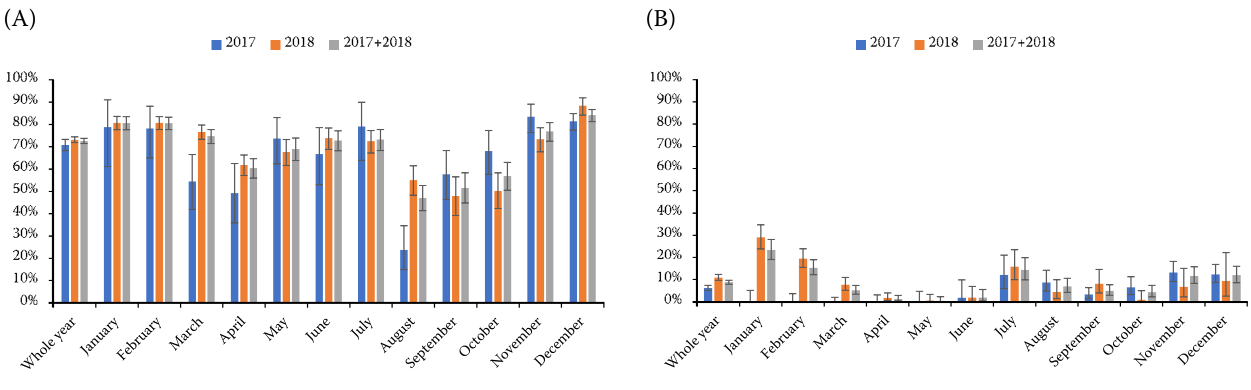

Figure 3. The monthly distribution of the passive surveillance (found dead), zone II and III (A), zone 0 and I (B)

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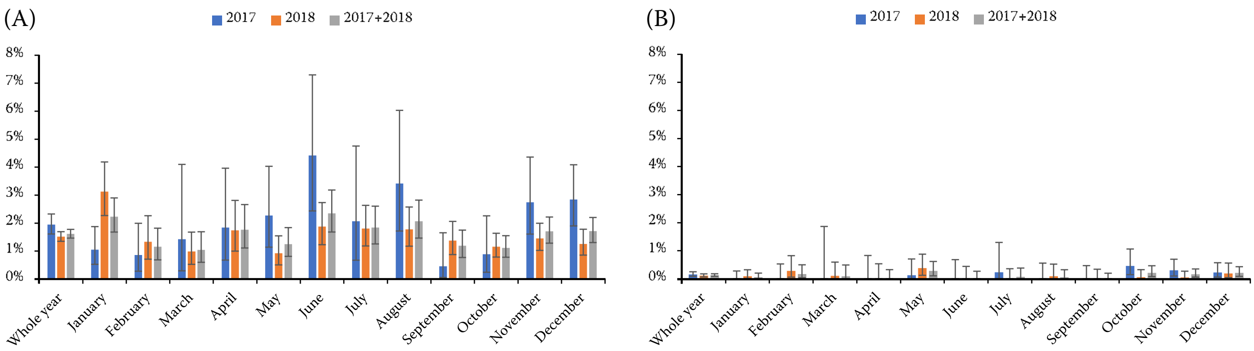

Figure 4. The monthly distribution of the active surveillance (hunted), zone II and III (A), zone 0 and I (B)

Table 1. Passive surveillance (wild boars found dead)

Year/month	Zone II and III			Zone 0 and I		
	negative	positive	total	negative	positive	total
2017	362	879	1 241	1 653	109	1 762
January	7	26	33	70	0	70
February	12	43	55	99	0	99
March	31	37	68	181	0	181
April	30	29	59	116	0	116
May	20	56	76	76	0	76
June	19	38	57	53	1	54
July	9	34	43	73	10	83
August	61	19	80	146	14	160
September	36	49	85	235	8	243
October	30	64	94	159	11	170
November	24	121	145	204	31	235
December	83	363	446	241	34	275
2018	1 242	3 383	4 625	1 925	236	2 161
January	133	558	691	203	83	286
February	146	613	759	298	72	370
March	165	542	707	335	28	363
April	173	280	453	282	5	287
May	85	178	263	164	1	165
June	90	254	344	100	2	102
July	87	229	316	106	20	126
August	105	128	233	109	5	114
September	72	66	138	112	10	122
October	80	81	161	108	1	109
November	73	201	274	69	5	74
December	33	253	286	39	4	43
2017 + 2018	1 604	4 262	5 866	3 578	345	3 923
January	140	584	724	273	83	356
February	158	656	814	397	72	469
March	196	579	775	516	28	544
April	203	309	512	398	5	403
May	105	234	339	240	1	241
June	109	292	401	153	3	156
July	96	263	359	179	30	209
August	166	147	313	255	19	274
September	108	115	223	347	18	365
October	110	145	255	267	12	279
November	97	322	419	273	36	309
December	116	616	732	280	38	318

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RESULTS

Passive surveillance (found dead)

In 2018, there were 3 383 ASF-positive animals (73.1% of all the tested animals) which is almost four times higher than the numbers in 2017 (879 = 70.8% of all the tested animals) in zone II and III (Table 1, Figure 3).

The logistic regression model has shown that the year (2017 vs. 2018) showed that the month had a significant influence on the level of prevalence (*P* model significance test < 0.000 1) in zone II and III. The chance of obtaining positive results in January and February was over 4.5 times higher, and in December was almost 6 times higher than in August (reference month). In December 2018, the odds of obtaining a positive result were almost four times greater than in December 2017 (Table 2).

The chance of obtaining a positive result in 2018 was significantly higher by almost 2-fold than in 2017 in the group of animals found dead in zone 0 and I. The model shows that the month

Table 2. The results of the logistic regression models

Significance assessment of model (*P*-value of LR test)	Independent variable	Coefficient (β_i)	Std. error	*P*-value (Wald)	Odds ratio	Confidence OR – 95%	Confidence OR + 95%
Model for the passive surveillance (found dead) of ASF in zone II and III: impact of the year on the result (2018 vs. 2017)							
0.11	Absolute term (β_0)	0.887 14	0.062 81	< 0.001	2.43	2.15	2.75
	2018	0.114 89	0.071 36	0.107	1.12	0.98	1.29
Model for the passive surveillance (found dead) of ASF in zone II and III in period 2017–2018: impact of the month on the result (reference month: August)							
< 0.001	Absolute term (β_0)	−0.121 57	0.109 26	0.266	0.89	0.71	1.1
	January	1.549 82	0.144 12	< 0.001	4.71	3.55	6.25
	February	1.545 12	0.140 66	< 0.001	4.69	3.56	6.18
	March	1.204 74	0.137	< 0.001	3.34	2.55	4.36
	April	0.541 69	0.142	< 0.001	1.72	1.3	2.27
	May	0.922 93	0.160 27	< 0.001	2.52	1.84	3.45
	June	1.106 95	0.156 64	< 0.001	3.03	2.23	4.11
	July	1.129 35	0.161 55	< 0.001	3.09	2.25	4.25
	September	0.184 35	0.173 03	0.287	1.2	0,86	1.69
	October	0.397 8	0.166 98	0.017	1.49	1.07	2.07
	November	1.321 39	0.159 27	< 0.001	3.75	2.74	5.12
	December	1.791 21	0.148 86	< 0.001	6	4.48	8.03
Model for the passive surveillance (found dead) of ASF in zone II and III in period 2017–2018: impact of the year on the result in January (January 2018 vs. January 2017)							
0.78	Absolute term (β_0)	1.312 19	0.426 39	0.002	3.71	1.61	8.58
	January 2018	0.121 82	0.437 25	0.78	1.13	0.48	2.67
Model for the passive surveillance (found dead) of ASF in zone II and III in period 2017–2018: impact of the year on the result in February (February 2018 vs. February 2017)							
0.64	Absolute term (β_0)	1.276 29	0.325 16	< 0.001	3.58	1.89	6.78
	February 2018	0.158 46	0.337 71	0.639	1.17	0.6	2.27
Model for the passive surveillance (found dead) of ASF in zone II and III in period 2017–2018: impact of the year on the result in March (March 2018 vs. March 2017)							
< 0.001	Absolute term (β_0)	0.176 93	0.242 86	0.466	1.19	0.74	1.92
	March 2018	1.012 39	0.258 61	< 0.001	2.75	1.66	4.57

Table 2 to be continued

Significance assessment of model (P-value of LR test)	Independent variable	Coefficient (β_i)	Std. error	P-value (Wald)	Odds ratio	Confidence OR – 95%	Confidence OR + 95%
Model for the passive surveillance (found dead) of ASF in zone II and III in period 2017–2018: impact of the year on the result in April (April 2018 vs. April 2017)							
0.06	Absolute term (β_0)	−0.033 9	0.283 61	0.905	0.97	0.55	1.69
	April 2018	0.515 4	0.299 85	0.086	1.67	0.93	3.02
Model for the passive surveillance (found dead) of ASF in zone II and III in period 2017–2018: impact of the year on the result in May (May 2018 vs. May 2017)							
0.31	Absolute term (β_0)	1.029 62	0.260 63	< 0.001	2.8	1.68	4.68
	May 2018	−0.290 49	0.292 14	0.32	0.75	0.42	1.33
Model for the passive surveillance (found dead) of ASF in zone II and III in period 2017–2018: impact of the year on the result in June (June 2018 vs. June 2017)							
0.27	Absolute term (β_0)	0.693 15	0.280 98	0.013	2	1.15	3.47
	June 2018	0.344 38	0.306 57	0.261	1.41	0.77	2.58
Model for the passive surveillance (found dead) of ASF in zone II and III in period 2017–2018: impact of the year on the result in July (July 2018 vs. July 2017)							
0.35	Absolute term (β_0)	1.329 14	0.374 11	< 0.001	3.78	1.81	7.89
	July 2018	−0.361 32	0.395 27	0.361	0.7	0.32	1.52
Model for the passive surveillance (found dead) of ASF in zone II and III in period 2017–2018: impact of the year on the result in August (August 2018 vs. August 2017)							
< 0.001	Absolute term (β_0)	−1.166 44	0.262 73	< 0.001	0.31	0.19	0.52
	July 2018	1.364 51	0.293 88	< 0.001	3.91	2.2	6.98
Model for the passive surveillance (found dead) of ASF in zone II and III in period 2017–2018: impact of the year on the result in September (September 2018 vs. September 2017)							
0.15	Absolute term (β_0)	0.308 3	0.219 59	0.16	1.36	0.88	2.1
	September 2018	−0.395 31	0.278	0.155	0.67	0.39	1.17
Model for the passive surveillance (found dead) of ASF in zone II and III in period 2017–2018: impact of the year on the result in October (October 2018 vs. October 2017)							
0.006	Absolute term (β_0)	0.012 42	0.159 2	0.938	1.01	0.74	1.39
	October 2018	0.745 26	0.272 59	0.006	2.11	1.23	3.6
Model for the passive surveillance (found dead) of ASF in zone II and III in period 2017–2018: impact of the year on the result in November (November 2018 vs. November 2017)							
0.02	Absolute term (β_0)	1.012 85	0.136 65	< 0.001	2.75	2.11	3.6
	November 2018	0.604 89	0.261 93	0.021	1.83	1.09	3.06
Model for the passive surveillance (found dead) of ASF in zone II and III in period 2017–2018: impact of the year on the result in December (December 2018 vs. December 2017)							
0.01	Absolute term (β_0)	1.475 56	0.121 67	< 0.001	4.37	3.44	5.55
	December 2018	0.561 32	0.221 49	0.011	1.75	1.14	2.71
Model for the passive surveillance (found dead) of ASF in zone 0 and I: impact of the year on the result (2018 vs. 2017)							
< 0.001	Absolute term (β_0)	−2.719	0.098 921	< 0.001	0.066	0.05	0.08
	2018	0.620 15	0.120 6	< 0.001	1.86	1.47	2.36

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Table 2 to be continued

Significance assessment of model (*P*-value of LR test)	Independent variable	Coefficient (β_i)	Std. error	*P*-value (Wald)	Odds ratio	Confidence OR – 95%	Confidence OR + 95%
Model for the passive surveillance (found dead) of ASF in zone 0 and I in period 2017–2018: impact of the month on the result (reference month: May)							
< 0.001	Absolute term (β_0)	−5.480 64	1.002 06	< 0.001	0.004	0.001	0.03
	January	4.290 01	1.009 71	< 0.001	72.97	10.07	528.28
	February	3.773 37	1.010 04	< 0.001	43.53	6.01	315.33
	March	2.566 74	1.020 47	0.012	13.02	1.76	96.3
	April	1.103 63	1.098 14	0.32	3.02	0.35	25.96
	June	1.548 81	1.159 19	0.18	4.71	0.49	45.67
	July	3.694 45	1.021 08	< 0.001	40.22	5.43	297.78
	August	2.883 81	1.029 71	0.005	17.88	2.38	134.64
	September	2.521 69	1.030 5	0.014	12.45	1.65	93.88
	October	2.378 3	1.044 39	0.022	10.79	1.39	83.59
	November	3.454 69	1.017 42	< 0.001	31.65	4.31	232.62
	December	3.483 44	1.016 67	< 0.001	32.57	4.44	239.05
Model for the passive surveillance (found dead) of ASF in zone 0 and I in period 2017–2018: impact of the year on the result in June (June 2018 vs. June 2017)							
0.96	Absolute term (β_0)	−3.970 29	1.014 4	< 0.001	0.02	0.003	0.14
	June 2018	0.058 26	1.245 35	0.963	1.06	0.09	12.41
Model for the passive surveillance (found dead) of ASF in zone 0 and I in period 2017–2018: impact of the year on the result in July (July 2018 vs. July 2017)							
0.44	Absolute term (β_0)	−1.987 87	0.337 31	< 0.001	0.14	0.07	0.27
	July 2018	0.320 16	0.416 27	0.441	1.38	0.61	3.13
Model for the passive surveillance (found dead) of ASF in zone 0 and I in period 2017–2018: impact of the year on the result in August (August 2018 vs. August 2017)							
0.15	Absolute term (β_0)	−2.344 55	0.279 8	< 0.001	0.1	0.06	0.17
	August 2018	−0.737 36	0.536 15	0.169	0.48	0.17	1.37
Model for the passive surveillance (found dead) of ASF in zone 0 and I in period 2017–2018: impact of the year on the result in September (September 2018 vs. September 2017)							
0.048	Absolute term (β_0)	−3.380 14	0.359 55	< 0.001	0.03	0.02	0.07
	September 2018	0.964 23	0.488 02	0.048	2.62	1.01	6.85
Model for the passive surveillance (found dead) of ASF in zone 0 and I in period 2017–2018: impact of the year on the result in October (October 2017 vs. October 2018)							
0.056	Absolute term (β_0)	−4.682 13	1.004 76	< 0.001	0.01	0.001	0.07
	October 2017	2.011 12	1.051 85	0.056	7.47	0.94	59.25
Model for the passive surveillance (found dead) of ASF in zone 0 and I in period 2017–2018: impact of the year on the result in November (November 2017 vs. November 2018)							
0.11	Absolute term (β_0)	−2.624 67	0.463 15	< 0.001	0.07	0.03	0.18
	November 2017	0.740 54	0.501 63	0.14	2.1	0.78	5.63
Model for the passive surveillance (found dead) of ASF in zone 0 and I in period 2017–2018: impact of the year on the result in December (December 2017 vs. December 2018)							
0.55	Absolute term (β_0)	−2.277 27	0.525 01	< 0.001	0.1	0.04	0.29
	December 2017	0.318 83	0.556 02	0.57	1.38	0.46	4.11

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Table 2 to be continued

Significance assessment of model (P-value of LR test)	Independent variable	Coefficient (β_i)	Std. error	P-value (Wald)	Odds ratio	Confidence OR – 95%	Confidence OR + 95%
Model for the active surveillance (hunted) of ASF in zone II and III: impact of the year on the result (2018 vs. 2017)							
0.02	Absolute term (β_0)	−4.175 31	0.058 17	< 0.001	0.02	0.01	0.02
	2018	0.254 95	0.109 91	0.02	1.29	1.04	1.6
Model for the active surveillance (hunted) of ASF in zone II and III in period 2017–2018: impact of the month on the result (reference month: March)							
0.02	Absolute term (β_0)	−4.550 58	0.221 99	< 0.001	0.01	0.01	0.02
	January	0.770 19	0.260 86	0.003	2.16	1.3	3.6
	February	0.100 77	0.274 9	0.714	1.11	0.65	1.9
	April	0.532 56	0.310 38	0.086	1.7	0.93	3.13
	May	0.181 13	0.320 08	0.571	1.2	0.64	2.25
	June	0.822 48	0.273 81	0.003	2.28	1.33	3.89
	July	0.575 43	0.289 03	0.047	1.78	1.01	3.13
	August	0.690 96	0.277 55	0.013	2	1.16	3.44
	September	0.130 29	0.299 51	0.664	1.14	0.63	2.05
	October	0.067 74	0.259 03	0.794	1.07	0.64	1.78
	November	0.495 9	0.260 6	0.057	1.64	0.99	2.74
	December	0.499 83	0.256 61	0.052	1.65	1	2.73
Model for the active surveillance (hunted) of ASF in zone II and III in period 2017–2018: impact of the year on the result in January (January 2018 vs. January 2017)							
0.001	Absolute term (β_0)	−4.543 3	0.303 17	< 0.001	0.01	0.01	0.02
	January 2018	1.109 31	0.340 42	0.001	3.03	1.56	5.91
Model for the active surveillance (hunted) of ASF in zone II and III in period 2017–2018: impact of the year on the result in February (February 2018 vs. February 2017)							
0.39	Absolute term (β_0)	−4.748 41	0.449 14	< 0.001	0.01	0.004	0.02
	February 2018	0.442 26	0.528 78	0.4	1.55	0.55	4.39
Model for the active surveillance (hunted) of ASF in zone II and III in period 2017–2018: impact of the year on the result in March (March 2018 vs. March 2017)							
0.58	Absolute term (β_0)	−4.238 93	0.581 44	< 0.001	0.01	0.01	0.05
	March 2018	−0.371 61	0.644 7	0.564	0.69	0.2	2.44
Model for the active surveillance (hunted) of ASF in zone II and III in period 2017–2018: impact of the year on the result in April (April 2018 vs. April 2017)							
0.91	Absolute term (β_0)	−3.976 56	0.432 59	< 0.001	0.02	0.01	0.04
	April 2018	−0.056 57	0.516 2	0.913	0.95	0.34	2.6
Model for the active surveillance (hunted) of ASF in zone II and III in period 2017–2018: impact of the year on the result in May (May 2017 vs. May 2018)							
0.02	Absolute term (β_0)	−4.675 5	0.268 54	< 0.001	0.01	0.01	0.02
	May 2017	0.914 3	0.406 33	0.024	2.5	1.13	5.54
Model for the active surveillance (hunted) of ASF in zone II and III in period 2017–2018: impact of the year on the result in June (June 2017 vs. June 2018)							
0.009	Absolute term (β_0)	−3.957 88	0.198	< 0.001	0.02	0.01	0.03
	June 2017	0.883 21	0.337 52	0.009	2.42	1.25	4.69

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Table 2 to be continued

Significance assessment of model (P-value of LR test)	Independent variable	Coefficient (β_i)	Std. error	P-value (Wald)	Odds ratio	Confidence OR – 95%	Confidence OR + 95%
Model for the active surveillance (hunted) of ASF in zone II and III in period 2017–2018: impact of the year on the result in July (July 2017 vs. July 2018)							
0.78	Absolute term (β_0)	−3.996 08	0.197 93	< 0.001	0.02	0.01	0.03
	July 2017	0.137 46	0.493 27	0.781	1.15	0.44	3.02
Model for the active surveillance (hunted) of ASF in zone II and III in period 2017–2018: impact of the year on the result in August (August 2017 vs. August 2018)							
0.08	Absolute term (β_0)	−4.012 04	0.194 2	< 0.001	0.02	0.01	0.03
	August 2017	0.670 14	0.363 1	0.065	1.95	0.96	3.98
Model for the active surveillance (hunted) of ASF in zone II and III in period 2017–2018: impact of the year on the result in September (September 2018 vs. September 2017)							
0.08	Absolute term (β_0)	−5.375 28	0.708 26	< 0.001	0.01	0.001	0.02
	September 2018	1.104 67	0.738 51	0.135	3.02	0.71	12.85
Model for the active surveillance (hunted) of ASF in zone II and III in period 2017–2018: impact of the year on the result in October (October 2018 vs. October 2017)							
0.61	Absolute term (β_0)	−4.714 03	0.499 54	< 0.001	0.01	0.003	0.02
	October 2018	0.265 32	0.530 19	0.617	1.3	0.46	3.69
Model for the active surveillance (hunted) of ASF in zone II and III in period 2017–2018: impact of the year on the result in November (November 2017 vs. November 2018)							
0.03	Absolute term (β_0)	−4.217 92	0.165 62	< 0.001	0.02	0.01	0.02
	November 2017	0.650 87	0.296 49	0.028	1.92	1.07	3.43
Model for the active surveillance (hunted) of ASF in zone II and III in period 2017–2018: impact of the year on the result in December (December 2017 vs. December 2018)							
0.002	Absolute term (β_0)	−4.362 89	0.180 76	< 0.001	0.01	0.01	0.02
	December 2017	0.832 34	0.263 48	0.002	2.3	1.37	3.85
Model for the active surveillance (hunted) of ASF in zone 0 and I: impact of the year on the result (2017 vs. 2018)							
0.33	Absolute term (β_0)	−6.822 13	0.243 35	< 0.001	0.001	0.001	0.002
	2017	0.344 39	0.350 34	0.326	1.41	0.71	2.8
Model for the active surveillance (hunted) of ASF in zone 0 and I in period 2017–2018: impact of the month on the result (reference months: January, April, June and September count together)							
0.008	Absolute term (β_0)	−8.287 91	0.711 04	< 0.001	0.0003	0.0001	0.001
	February	1.915 72	0.916 11	0.037	6.79	1.13	40.91
	March	1.256 16	1.225 17	0.305	3.51	0.32	38.77
	May	2.425 22	0.820 06	0.003	11.31	2.27	56.41
	July	1.003 77	1.227 82	0.414	2.73	0.25	30.28
	August	0.834 92	1.242 55	0.502	2.31	0.2	26.32
	October	2.140 15	0.820 22	0.009	8.5	1.7	42.43
	November	1.854 97	0.820 1	0.024	6.39	1.28	31.89
	December	2.118 89	0.804 95	0.008	8.32	1.72	40.31
Model for the active surveillance (hunted) of ASF in zone 0 and I in period 2017–2018: impact of the year on the result in May (May 2018 vs. May 2017)							
0.27	Absolute term (β_0)	−6.668 23	1.000 78	< 0.001	0.001	0.0002	0.01
	May 2018	1.090 01	1.096 28	0.32	2.97	0.347	25.53

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Table 2 to be continued

Significance assessment of model (*P*-value of LR test)	Independent variable	Coefficient (β_i)	Std. error	*P*-value (Wald)	Odds ratio	Confidence OR – 95%	Confidence OR + 95%
Model for the active surveillance (hunted) of ASF in zone 0 and I in period 2017–2018: impact of the year on the result in October (October 2017 vs. October 2018)							
0.06	Absolute term (β_0)	−7.446 59	1.000 43	< 0.001	0.001	0.0001	0.004
	October 2017	2.060 26	1.095 95	0.06	7.85	0.92	67.3
Model for the active surveillance (hunted) of ASF in zone 0 and I in period 2017–2018: impact of the year on the result in November (November 2017 vs. November 2018)							
0.1	Absolute term (β_0)	−7.631 92	1.000 58	< 0.001	0.001	0.0001	0.003
	November 2017	1.821 38	1.096 02	0.097	6.18	0.72	53
Model for the active surveillance (hunted) of ASF in zone 0 and I in period 2017–2018: impact of the year on the result in December (December 2017 vs. December 2018)							
0.84	Absolute term (β_0)	−6.253 83	0.573	< 0.001	0.002	0.001	0.01
	December 2017	0.153 51	0.752 99	0.839	1.17	0.27	5.1
Model for the surveillance in zone II and III in period 2017–2018: impact of the kind of surveillance on the result (found dead and traffic accident vs. hunted)							
	Absolute term (β_0)	−4.110 09	0.049 38	< 0.001	0.02	0.02	0.02
< 0.001	Found dead	5.087 33	0.057 42	< 0.001	161.96	144.72	181.25
	Car accident	1.693 65	0.135 82	< 0.001	5.44	4.17	7.1
Model for the surveillance in zone II and III in 2017: impact of the kind of surveillance on the result (found dead and traffic accident vs. hunted)							
	Absolute term (β_0)	−3.920 36	0.093 37	< 0.001	0.02	0.02	0.02
< 0.001	Found dead	4.807 51	0.112 34	< 0.001	122.43	98.23	152.59
	Car accident	0.836 93	0.427 79	0.051	2.31	1	5.34
Model for the surveillance in zone II and III in 2018: impact of the kind of surveillance on the result (found dead and traffic accident vs. hunted)							
	Absolute term (β_0)	−4.175 32	0.058 19	< 0.001	0.02	0.01	0.02
< 0.001	Found dead	5.177 36	0.066 99	< 0.001	177.21	155.41	202.08
	Car accident	1.855 12	0.145 27	< 0.001	6.39	4.81	8.5
Model for the surveillance in zone 0 and I in period 2017–2018: impact of the kind of surveillance on the result (found dead and traffic accident vs. hunted)							
	Absolute term (β_0)	−6.669 85	0.173 04	< 0.001	0.001	0.001	0.002
< 0.001	Found dead	4.330 83	0.181 95	< 0.001	76.01	53.21	108.579
	Car accident	0.578 35	0.263 63	0.028	1.78	1.06	2.99
Model for the surveillance in zone 0 and I in 2017: impact of the kind of surveillance on the result (found dead and traffic accident vs. hunted)							
	Absolute term (β_0)	−6.477 74	0.251 46	< 0.001	0.002	0.001	0.003
< 0.001	Found dead	3.758 74	0.270 14	< 0.001	42.89	25.26	72.84
	Car accident	0.413 42	0.377 67	0.274	1.51	0.72	3.17
Model for the surveillance in zone 0 and I in 2018: impact of the kind of surveillance on the result (found dead and traffic accident vs. hunted)							
	Absolute term (β_0)	−6.822 13	0.245 3	< 0.001	0.001	0.001	0.002
< 0.001	Found dead	4.723 29	0.254 75	< 0.001	112.54	68.3	185.42
	Car accident	0.699 44	0.397 07	0.079	2.01	0.92	4.38

OR = odds ratio

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has a significant influence on the level of prevalence (*P* model significance test < 0.000 1).

In that model, the chance of obtaining positive results in January was almost 73 times, in February 43.5 times, in July 40 times, in December 32.5 times higher than in May (reference month).

The OR results are collected in Table 2. Table 1 shows the monthly distribution of the positive results in these two groups of wild boars.

Active surveillance (hunted)

The number of ASF-positive results in the groups of hunted animals reached 300 in 2018 in zones II and III (1.5%), and these numbers were almost 3 times greater than in the previous years (117; 1.9%) (Table 3).

The chance of a positive result in 2017 (zone II and III) was significantly higher, by 1.3 times, than

Table 3. Active surveillance (hunted animals)

Year/month	Zone II and III			Zone 0 and I		
	negative	positive	total	negative	positive	total
2017	5 899	117	6 016	10 408	16	10 424
January	1 034	11	1 045	1 329	0	1 329
February	577	5	582	697	0	697
March	208	3	211	196	0	196
April	320	6	326	443	0	443
May	473	11	484	787	1	788
June	303	14	317	539	0	539
July	237	5	242	427	1	428
August	311	11	322	660	0	660
September	432	2	434	785	0	785
October	446	4	450	1 092	5	1 097
November	602	17	619	1 669	5	1 674
December	956	28	984	1784	4	1 788
2018	19 518	300	19 818	15 605	17	15 622
January	1 333	43	1 376	2 247	2	2 249
February	964	13	977	1 059	3	1 062
March	1 307	13	1 320	936	1	937
April	903	16	919	689	0	689
May	1 502	14	1 516	1 323	5	1 328
June	1 361	26	1 387	836	0	836
July	1 414	26	1 440	1 030	0	1 030
August	1 492	27	1 519	1 065	1	1 066
September	1 646	23	1 669	1 083	0	1 083
October	2 651	31	2 682	1 714	1	1 715
November	2 512	37	2 549	2 063	1	2 064
December	2 433	31	2 464	1 560	3	1 563
2017 + 2018	25 417	417	25 834	26 013	33	26 046
January	2 367	54	2 421	3 576	2	3 578
February	1 541	18	1 559	1 756	3	1 759
March	1 515	16	1 531	1 132	1	1 133
April	1 223	22	1 245	1 132	0	1 132
May	1 975	25	2 000	2 110	6	2 116
June	1 664	40	1 704	1 375	0	1 375
July	1 651	31	1 682	1 457	1	1 458
August	1 803	38	1 841	1 725	1	1 726
September	2 078	25	2 103	1 868	0	1 868
October	3 097	35	3 132	2 806	6	2 812
November	3 114	54	3 168	3 732	6	3 738
December	3 389	59	3 448	3 344	7	3 351

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in 2018 in the group of hunted animals. The model shows that the month plays a significant influence on the level of prevalence (P = 0.002). In January, June, July and August, the percentage of the positive results was significantly higher than the reference month of March, in which the prevalence was the lowest. In addition, the chance of obtaining a positive result in January 2018 was 3 times higher than in January 2017 (Table 2).

The year (2017 vs. 2018) had no significant impact on the prevalence (P = 0.33) in the group of hunted animals in zones 0 and I.

The model shows that the month plays a significant influence on the level of the prevalence (P model significance test = 0.02). Only the months of March, July and August did not differ significantly from the reference months of January, April, June and September together (in April, June and September there were no ASF cases) in which the prevalence was the lowest (Table 2).

Positive results in zones II and III areas acc. to the 2014/709/EU decision

The chance to obtain positive results in the samples from the dead wild boars was almost 162 times higher in comparison to the hunted animals during the 2017–2018 time period. In 2017, the chance was over 122 times, and in 2018 was over 177 times higher, respectively (Table 2).

Positive results in zones 0 and I areas acc. to the 2014/709/EU decision

The chance of obtaining positive results in the wild boars that were found dead was 76 times higher than among the hunted animals during the 2017–2018 period. In 2017, the chance was almost 43 times higher than in the hunted animals (Table 2).

The last two years (2017–2018) of the active surveillance and the passive surveillance show that the ASFV in Poland has been noted in new territories (Figures 1 and 2).

DISCUSSION

Poland has been struggling with ASF since 2014 when the first ASF-positive wild boar was found near the Belarusian border (800 m). From 2014 until the end of 2016, 188 cases of ASFV were reported in the wild boar populations of Poland, as well as 23 outbreaks in the domestic pig populations. The disease was restricted to only three provinces: Podlaskie, Mazowieckie and Lubelskie (Pejsak et al. 2018; Podgorski and Smietanka 2018; GVI 2019).

In 2017, the number of ASF cases reached a new peak. The introduction of the ASFV to the Warsaw area was the first big turning point in 2017. The first cases of ASF in this area were detected in the Legionowo district on November 17, 2017. The first ASF-positive wild boar was found dead, and the second was found injured due to a car accident. Other ASF-positive wild boars were also found in the suburbs of West Warsaw, Piaseczno, Nowy Dwor Mazowiecki, and directly in Warsaw, the capital city of Poland (GVI 2019).

The virus reached the Warminsko-Mazurskie province for the first time on November 21, 2017. Since then, the virus has been present in four Polish provinces (GVI 2019).

In 2018, the total number of cases increased to 3 347 by the end of December (2 435 of which were from a single year). The total number of ASFV-positive wild boars were even higher in 2018 than in all the previous years combined. A similar situation has occurred in Latvia, Lithuania, and Estonia, indicating that the virus is very hard to eradicate from the environment (Nurmoja et al. 2018; Pautienius et al. 2018; Schulz et al. 2019a).

The percentage of ASF-positive wild boars that were found dead in the restricted area increased to 73.1% (the highest percentage in the history of ASF in Poland; Table 1). Most of these cases were observed in the winter months, when the carcases were easier to find in the forest area. According to the statistical analyses, it is proven that the chance of identification of an ASF-positive dead wild boar within the infected area was 6 times higher in December and 4.5 times higher in January than in August and in September. A decrease in the positive results during the summer could be related to the higher plant growth in the fields (i.e., maize), as well as an increase in the density of the leaves on the bushes and trees where wild boars can hide before death. The Latvian researchers observed an increased number of ASF-positive wild boars in the winter season of 2015; however, they proved that the monthly distribution is a random effect (Schulz et al. 2019b). Scientists connected with the Estonian results ob-

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served a decrease in the number of ASF-positive wild boars in the last few years in the Baltic States (Nurmoja et al. 2018; Schulz et al. 2019b). The situation in Lithuania was observed to be similar to that in Poland. Most of the positive results (molecular and/or serological) occurred during the autumn and winter seasons. Passive surveillance in the winter in Lithuania reach 83.23% (95% CI 80.30–86.15%, 521 positive results from 626 analysed in total) (Pautienus et al. 2018) and was similar to the results observed in Poland (83.4% in November, 81.4% in December). The percentage of ASF-positive wild boars detected through the active surveillance did not look significant (1.47% in 2018), but the number of animals is exceedingly high, reaching a value of 320. The active surveillance in Lithuania was also towards the lower end from 2014–2017 (0.45%) (Pautienus et al. 2018). What is alarming, most of the positive results obtained from the active surveillance were only seropositive, which may or may not indicate that the animals could possibly be passive disease carriers. The role of seropositive animals in the spread of ASF is still unclear and needs more studies (Magdla et al. 2016).

The passive and active surveillance showed a significant increase in the number of positive cases, forecasting that the upcoming years could be even more difficult in terms of the eradication of the disease. The continuous monitoring of wild boar populations is a very important tool in the ASF prevention, as reported by Estonian researchers (Nurmoja et al. 2018). The knowledge from the epidemiological data regarding this disease (the chance of obtaining a positive result in a given month) may assist the veterinary authorities in the preparation of the stakeholders and pig holdings for ASF.

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Conflict of interest

The authors declare no conflict of interest.

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