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African Swine Fever Virus (ASFV) in Poland in 2019—Wild Boars: Searching Pattern

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Abstract: African swine fever (ASF) was introduced to Poland in 2014. Despite the implementation of preventive actions focused on the reduction of wild boar populations and the introduction of biosecurity rules in domestic pig farms, the disease has been continuously spreading to new areas. The aim of this paper was to analyze the dynamics of ASFV spread in wild boar populations in Poland and to summarize the 2019 epidemiological situation. Using a logistic regression model, it has been shown that there is a significant correlation between the month, ASF affected area and ASF prevalence among wild boars. According to EU definitions, Part II and Part III zones had a total of 3065 (65.2%) ASF-positive death wild boars. In addition, there were 36 post-accident (road-killed) wild boars (2.6%) and 612 hunted animals (1.5%) in this area. These results showed the importance of passive surveillance and its advantages overactive surveillance in ASF control and prevention. The data indicated a greater chance of a positive result in the winter months (January, February, March) than in reference September, where the ASF prevalence was the lowest. This observation confirms the preliminary theory about the seasonality of the disease in wild boar populations and its connection with winter.

Keywords: African swine fever (ASF); prevalence; wild boars; surveillance; seasonality correlation; ASF regionalization

1. Introduction

African swine fever (ASF) is an infectious and fatal swine disease affecting wild boar, domestic pigs, and other free-ranging representatives of the Suidae family found throughout numerous regions of Africa. ASF is caused by the African swine fever virus (ASFV). In a domestic pig, ASFV infection kills almost 100% of infected animals within 4–9 days of exposure to the virus [1]. With this high mortality rate, the appearance of ASF on pig farms around the world had devastating impacts on the global pork trade and caused great economic losses [2].

ASF first appeared in Western and Central Europe in the 1970s and 1980s in the domestic pig population and the strains from that time fall into genotype I. In 2007, a few years after the initial wave of the disease that had hit the western and central countries of the European continent (including Spain, Portugal, Italy, Malta, Belgium, The Netherlands and even the USSR), the virus was transferred from Africa to Georgia (genotype II). The most likely source of the virus were contaminated pig by-products or pigs from Southeast
Africa or Madagascar, where the virus is endemic (genotype II). Few years later, ASF has been reported in Baltic States, i.e., Lithuania, Latvia, Estonia, and finally in Poland [3,4]. In February 2014, the disease spread to Poland through Belarus [5]. Three years later, in 2017, ASF cases were notified in Czech Republic and Romania. By then, the global situation with ASF had changed. The virus reached Asia, including China (the world’s largest pig producer), Vietnam, Cambodia, Laos, Philippines, Indonesia and other European countries (Belgium, Bulgaria, Romania, Greece, Germany) and is still present in the environment [6–8]. Two countries which won the struggle with the second wave of ASF are the Czech Republic and Belgium. In the Czech Republic, the last positive case was confirmed on 15 April 2018, and as of 19 April 2019, the country is officially ASF-free. Similarly, at the beginning of November 2020, Belgium applied for an ASF exemption to the World Organization for Animal Health (OIE). Joint forces of government and scientists led to the eradication of the disease in the wild boar population within one and two years of the virus introduction, respectively [8,9]. It was crucial to implement appropriate measures to prevent further spread of the virus, such as wild boar hunting by specially trained hunters and police snipers, installation of electric and scent fences in high-risk areas, and implementation of controls on farms for biosecurity rules [10].

Since there is still no success in obtaining an effective vaccine or dedicated veterinary treatment against ASF, several sanitary measures, which were implemented as a reaction to the growing number of outbreaks and cases in affected countries, are being recommended. All activities leading to the control and eradication of ASF in the European Union (EU) are based on Council Directive 2002/60/EC [3], therefore all ASF outbreaks and cases are subjected to notification in the EU and OIE. These activities mainly include early detection of the disease, efficient laboratory diagnosis, and proper sanitary measures [11]. Given the virological diagnosis, the OIE recommends the use of genome detection by polymerase chain reaction (PCR), detection of viral antigens or antibodies against ASFV using enzyme-linked immunosorbent assay (ELISA) or the immunofluorescence assay (IFA) and virus isolation (VI). Applications of laboratory assays specific for ASF are important taking into account the differential diagnosis and the similarity of clinical ASF signs to other swine diseases such as classical swine fever (CSF), porcine reproductive and respiratory syndrome (PRRS), erysipelas or porcine dermatitis and nephropathy syndrome (PDNS) caused by the porcine circovirus 2 (PCV2) [10].

In 2019, there was a gradual expansion of ASF into new regions of the EU. There is a tendency of ASF spread towards South-Western direction with parallel observation of different stages of the epidemic. Within the affected countries, there are now non-affected areas; recently affected areas where ASF has been introduced into affected areas; expanding areas and recent areas with long-term ASF infection, including areas where ASF seems to be eradicated [12].

Various routes of ASFV transmission are known depending on the geographic region. The most important role in ASF transmission in Europe is played by wild boars and human-mediated transmission to domestic pig holdings. The virus is transmitted directly between infected and susceptible animals or indirectly by contaminated byproducts including pork, meat, pigswill, contaminated feed, pig carcasses, or other contaminated fomites [13,14]. Despite the well-documented routes of ASF transmission with the implementation of control measures, some outbreaks have been reported in pig farms where biosecurity measures have been fully implemented, but the source of the virus is still unknown [15,16]. Because of this fact, in the current situation, one of the priorities to eliminate ASF is to understand the mechanisms of infection as well as disease transmission and distribution among wild boars, which play a key role in virus transmission throughout Eurasia [1].

Due to the presence of ASF in Poland, in accordance with the regulation of the Minister of Agriculture and Rural Development of 6 May 2015, the biosecurity program was implemented just one year after the first infection of wild boars in Eastern Poland has been discovered [17]. Since then, the biosecurity legislations have been updated many times according to the ASF regionalization as well as by the Commission implementing Decision
2014/709/the EU and later updated by EU government [4,18]. One of the decisions included in the program was to designate areas within the country subject to various restrictions. In the context of the epizootic situation in a specific area, four different ASF zones were introduced. These areas included: hazard area (Part III), around the epicenter of the recent ASF outbreak in domestic pigs; restricted area (Part II) with confirmed cases in wild boars; protective area (Part I) which is the buffer area; and the ASF free area (Part 0) [18]. In Poland, despite the implementation of preventive actions consisting of reduction of wild boar population and implementation of biosecurity measures in pig holdings, the disease has been spreading continuously to new areas [3]. In November 2019, the first ASF case was confirmed in Lubuskie voivodship in western Poland [19,20]. Due to the very large distance from the nearest case (approx. 300 km) located in the Mazowieckie voivodeship, the virus could probably have been transmitted by illegal human activity [21]. The situation was alarming as the region is close to the German border with almost 30 million pigs, the largest production in the EU. Moreover, the neighboring voivodship is situated in the area with the highest pig density, which poses a serious threat to domestic animal production based on pork husbandry [22]. An additional complication is related to the number of wild boars in the area adjacent to the ASF cases confirmed in western Poland [23].

In response to the epizootic situation in western Poland, the German authorities decided to build a solid, electric and movable fence to prevent further expansion of infected wild boars. Unfortunately, these actions turned out to be not fully effective. At the beginning of September 2020, the German ASF Reference Laboratory located at the Friedrich-Loeffler Institute reported the first confirmed case of ASF to the OIE. An infected wild boar was found in Brandenburg state, about 7 km away from the infected area established in Poland (distance to the nearest confirmed positive wild boar). Immediate actions were implemented, including the establishment of three zones: the core zone, the infected area covering three administration districts, and the buffer zone. Appropriate measures have been implemented in these zones to prevent further spread of the disease [7].

The purpose of the presented study was to examine, analyze, and summarize the dynamics of ASF spread in Poland among wild boar populations in 2019, which resulted in a large number of new cases of the disease. To formulate this thesis, changes in the spread of the virus to new areas were tracked, mainly by the laboratory analysis of wild boar samples (found dead; road-killed; hunted) from all over the country throughout the year (2019). To track the possible role of natural wild boar movement and human activity in relation to ASF, the obtained results were analyzed and summarized using statistical methods.

2. Materials and Methods

All stages of the laboratory examinations were carried out in the biosafety level 3 laboratory (BSL-3) by competent team.

The analyzed materials were represented by field samples from wild boars collected from all over Poland (Part 0, I, II, III). The samples included different tissues including blood, bone marrow, tonsils, spleen, kidneys, or lungs. The sampling was conducted by the authorized veterinary officer (accordingly to the ASF monitoring program) and were analyzed for the presence of genetic ASFV material (DNA) using molecular (real-time PCR) or antibody presence against ASFV by serological methods (ELISA and immunoperoxidase assay—IPT).

Before the analyses, tissue materials were homogenized in phosphate-buffered saline (PBS) yielding a 10% homogenate. Next, the prepared tissue lysate was used for genetic material extraction using QIAamp DNA Mini Kit (manual extraction; QIAGEN, Hilden, Germany) or QIAcube HT system (automatic extraction) following the producer’s recommendations (Indical, Leipzig, Germany). Thanks to the courtesy of the European Union Reference Laboratory (EURL) for ASF (CISA-INIA, Valdeolmos, Spain), the refer-
ence ASFV isolate representing genotype II was used as the control of DNA extraction in further analyses.

To determine the ASFV’ DNA presence real-time PCR (qPCR) method was used [24]. The process was conducted in plastic tubes in one of four types of thermocyclers (Applied Biosystems 7500, Applied Biosystems, Waltham, MA, USA; QuantStudio™ 5, ThermoFisher Scientific, Waltham, MA, USA; Rotor-Gene Q, QIAGEN, Hilden, Germany; LightCycler 480, Roche, Basel, Switzerland).

The qPCR was performed using one of the three kits: Virotype (QIAGEN, Gemany), IDGene African Swine Fever Duplex (IDVet, Grabels, France) or with the procedure reported by the Fernández-Pinero (2013) [24]. A fluorescent signal with a threshold cycle value (Ct) below 37.0 was considered as positive.

Before the serological analyses, the serum was obtained from blood samples (without ethylenediaminetetraacetic acid—EDTA) after centrifugation (896–1514 × g). To determine the serological status of each sample, the enzyme-linked immunosorbent assay (ELISA) was conducted (ID Screen® African Swine Fever Indirect, IDVet Innovative diagnostic, Grabels, France; INgezim PPA COMPAC, Inmunologia Y Genetica Aplicada Sa—Ingenasa, Madrid, Spain). The procedure was carried out according to the producer’s manual. The basis for validation of the sample status (positive, negative, doubtful) were analyzed in accordance with the producer’s instructions.

All positive and doubtful results from the ELISA test were verified by the indirect immuno-peroxidase technique (IPT). The procedure is more sensitive than the standard ELISA. The concept of the method is similar to the ELISA, but the read-out of the result is conducted in the reverse-field microscope, not by the spectrophotometer. The reagents of the IPT test were provided by the EURL for the ASF and the protocol was standardized by the EURL researchers.

As a positive result in the calculation of the surveillance (passive and active), we were considering every positive result obtained in qPCR (ASFV DNA) and/or IPT (anti-ASFV antibody).

Examination of the ASF occurrence frequency in wild boars during 2019 was conducted separately for every month, individually in each category:

- passive surveillance (found dead), parts II-III (areas acc. to 2014/709/EU decision);
- passive surveillance (road-killed), parts II-III (areas acc. to 2014/709/EU decision);
- active surveillance (hunted), parts II-III (areas acc. to 2014/709/EU decision);
- passive surveillance (found dead), part I-0 (areas acc. to 2014/709/EU decision);
- active surveillance (hunted), parts I-0 (areas acc. to 2014/709/EU decision);
- passive surveillance (road-killed), parts I-0 (areas acc. to 2014/709/EU decision).

Furthermore, examination of ASF prevalence was carried out individually for wild boars found in different conditions (found dead, road-killed, hunted) in the following categories:

- parts II-III;
- parts 0-I.

A comprehensive model for the 2019 year was also constructed, analyzing all factors taken simultaneously—months, wild boar status, and the zone from which the sample was collected.

The statistical tests were conducted with the application of logistic regression models. This type of model is a mathematical formula which can be used to report the effect of several variables \((x_1, x_2, \ldots, x_n)\) on the dichotomous variable \(Y\), which has one of two possible rates (in this case: positive or negative):

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

where:

\(\beta_i\)—regression coefficient for \(i = 0, \ldots, n\),
x_i—are independent variables (measurable or qualitative) for i = 1, 2, ..., n.

We obtained the ratings of the coefficients using the maximum likelihood method. The significance of independent variables was estimated using the Wald’s statistics. The fit of the model to the data was also determined using the likelihood ratio (LR statistics).

Odds ratios (ORs) were determined with 95% confidence intervals.

The demonstrations of the reported connections were expressed statistically at the accepted level of significance $\alpha = 0.05$ (logistic regression model).

All statistical estimations were calculated using TIBCO Software Inc. (Palo Alto, CA, USA) Statistica (data analysis software system), version 13 (2017). The monthly distribution of ASF positive wild boars were made in Excel 2016 (Microsoft, Redmond, WA, USA). The geographical distribution of ASF cases were made in ArcGIS 10.4.1 (ESRI, Redlands, CA, USA).

3. Results

All tested wild boars (82,459) were divided into three main groups: found dead (6468); road-killed (10,262) and hunted (65,729). Found dead wild boars were the animals which carcasses were found in the environment after the natural death, caused mainly by the ASF. Road-killed wild boars were the animals killed accidently by cars. Hunted animals used in the active surveillance were shoot by hunters (animals without symptoms of ASF—active surveillance). In total, 3830 ASF-positive and 78,629 ASF-negative wild boars were analyzed in this study.

Monthly distribution of positive results for each group of wild boars is presented in Figure 1. Most of ASF-positive wild boars are located in the group of found dead animals in each zone. In Part II-III, the highest number of ASF affected animals were confirmed in January. In part 0-I the highest number of ASF positive wild boars was in August. Detailed results are collected in Supplementary Materials (Tables S1–S3).

All ASF cases in 2019 were presented in Figure 2. Figure 2a shows to the ASF cases in relation the wild boar density. In 2019, the wild boar density in ASF affected areas was approximately 0.5 wild boar/km$^2$. Figure 2b has shown the ASF cases in connection with ASF Parts (0, I, II, III) at the end of 2019.

![Figure 1](a)
3.1. Passive Surveillance of ASF in Wild Boar Populations (Found Dead)

In 2019, it was confirmed 3065 ASF-positive wild boars in Poland, in Parts II-III (65.2% of all tested animals).

The logistic regression model showed that the month had an important effect on the prevalence level ($p < 0.0001$) in Parts II-III. It was observed that all months were significantly

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Figure 2. ASF cases in wild boar populations in Poland during the 2019 year. (a) map shows wild boar density, while (b) map presents ASF zones (at the end of the year 2019) and forests.
different from September (the reference month—the lowest prevalence). The chance of getting positive results was the highest in the winter months (January—13 times; February, 10.5 times; March 9 times) than in the mentioned September (Table 1).

Table 1. Results logistic regression models. Passive surveillance model (found dead).

| Significance Assessment of Model (p Value of LR 1 Test) | Independent Variable | Coefficient (βi) | Std. 2 Error | p Value (Wald) | Odds Ratio | Confidence OR 3 − 95% | Confidence OR 3 + 95% |
|-------------------------------------------------------|----------------------|------------------|--------------|---------------|------------|----------------------|----------------------|
| Passive surveillance model (found dead) of ASF in Parts II-III in 2019—influence of the month on the result (reference month: September) | Absolute term (β0) | −1.05711 | 0.18180 | p < 0.0001 | 0.34746 | 0.24329 | 0.49623 |
| January | 2.57172 | 0.21813 | 0 | 13.08827 | 8.53422 | 20.07247 |
| February | 2.36645 | 0.21169 | p < 0.0001 | 10.65944 | 7.03874 | 16.14261 |
| March | 2.18922 | 0.20665 | p < 0.0001 | 8.92825 | 5.95412 | 13.38798 |
| April | 1.91084 | 0.20557 | p < 0.0001 | 6.75877 | 4.51691 | 10.11334 |
| May | 1.96901 | 0.20807 | p < 0.0001 | 7.16361 | 4.76409 | 10.7717 |
| June | 1.29659 | 0.23257 | p < 0.0001 | 3.65682 | 2.31785 | 5.76927 |
| July | 1.42924 | 0.21655 | p < 0.0001 | 4.17553 | 2.73112 | 6.38385 |
| August | 1.25709 | 0.21661 | p < 0.0001 | 3.51517 | 2.29891 | 5.37491 |
| October | 0.50740 | 0.21616 | p < 0.0001 | 1.66096 | 1.08723 | 2.53747 |
| November | 1.28299 | 0.21200 | p < 0.0001 | 3.60742 | 2.37600 | 5.47705 |
| December | 1.61809 | 0.20133 | p < 0.0001 | 5.04344 | 3.39870 | 7.48411 |
| Passive surveillance model (found dead) of ASF in Parts 0-I in 2019—influence of the month on the result (reference month: January, February, April, September and October combine) | Absolute term (β0) | −5.50262 | 0.57870 | p < 0.0001 | 0.00408 | 0.00131 | 0.01268 |
| March | 1.68858 | 0.82181 | 0.04006 | 5.41177 | 1.07978 | 27.12343 |
| May | 3.08472 | 0.67508 | p < 0.0001 | 21.86139 | 5.81638 | 82.16792 |
| June | 1.58064 | 0.91911 | 0.08565 | 4.85809 | 0.80090 | 29.46818 |
| July | 2.70941 | 0.71524 | 0.00016 | 15.02041 | 3.69362 | 61.08176 |
| August | 3.49286 | 0.64960 | p < 0.0001 | 32.87973 | 9.19623 | 117.5565 |
| November | 3.45880 | 0.61639 | p < 0.0001 | 31.77893 | 9.48654 | 106.4562 |
| December | 3.022 | 0.62593 | p < 0.0001 | 20.53451 | 6.01630 | 70.09733 |

1 LR—logistic regression; 2 Std.—standard; 3 OR—odds ratio.

The chance of getting a positive result in the group of wild boars found dead (carcasses) in the Parts 0-I was the highest in August (33 times) and in November (32 times) than in the reference months (January, February, April, September, October taken together—the lowest prevalence) (Table 1).

3.2. Passive Surveillance of ASF in Wild Boar Populations (Road-Killed)

The total number of ASF-positive wild boars in the group of traffic accidents in Parts II-III reached 36 (2.6%). In Parts 0-I it was 12 (0.1%).

Logistic regression model showed that it was the highest in January (p = 0.0019) in Parts II-III. In Part 0-I the chance of getting positive results in November was the highest (14 times higher than in October). In the remaining months, no positive result in wild boars was obtained (Table 2).

3.3. Active Surveillance of ASF in Wild Boar Populations (Hunted)

In 2019, it was 612 (1.5%) in Parts II-III and 25 (0.1%) in Parts 0-I ASF-positive results in the category of hunted wild boars.

The model (p = 0.01) showed that in Parts II-III the chance of getting a ASF-positive result in that group was twice higher in January, August, September, November, and December than in reference (the lowest prevalence). In Parts 0-I the prevalence was the highest in August (Table 3).
Table 2. Results logistic regression models. Passive surveillance model (road-killed).

| Significance Assessment of Model (p Value of LR \(^1\) Test) | Independent Variable | Coefficient (\(\beta_i\)) | Std. \(^2\) Error | \(p\) Value (Wald) | Odds Ratio | Confidence OR \(^3\) – 95% | Confidence OR \(^3\) + 95% |
|-----------------------------------------------------------|----------------------|--------------------------|-------------------|------------------|------------|------------------|------------------|
| Passive surveillance model (road-killed) of ASF in Parts II-III in 2019—influence of the month on the result (reference month: January) | Absolute term (\(\beta_0\)) | −2.02443 | 0.40221 | \(< 0.0001\) | 0.13207 | 0.06000 | 0.29071 |
| | February | −0.57831 | 0.65595 | 0.37813 | 0.56085 | 0.15489 | 2.03087 |
| | March | −0.86599 | 0.71670 | 0.22713 | 0.42063 | 0.10311 | 1.71591 |
| | April | −0.80884 | 0.71753 | 0.25984 | 0.44538 | 0.10900 | 1.81983 |
| | May | −1.28980 | 0.82466 | 0.11804 | 0.27532 | 0.05461 | 1.38808 |
| | June | −1.02014 | 0.82801 | 0.21814 | 0.36054 | 0.07105 | 1.82970 |
| | July | −1.08914 | 0.82705 | 0.18810 | 0.33651 | 0.06643 | 1.70454 |
| | August | −2.46425 | 1.08310 | 0.02305 | 0.08507 | 0.01016 | 0.71210 |
| | September | −21.68095 | 2378.285 | 0.99273 | \(< 0.0001\) | 0 | 0 |
| | October | −1.83283 | 0.57621 | 0.0015 | 0.15996 | 0.05165 | 0.49535 |
| | November | −2.41433 | 0.70647 | 0.0006 | 0.08943 | 0.02237 | 0.35756 |
| | December | −2.46800 | 0.70636 | 0.0004 | 0.08475 | 0.02120 | 0.33880 |
| Passive surveillance model (road-killed) of ASF in Parts 0-I in 2019—influence of the month on the result (reference month: October) | Absolute term (\(\beta_0\)) | −7.32778 | 1.00070 | \(< 0.0001\) | 0.00066 | 0.0009 | 0.00468 |
| | November | 2.63144 | 1.04523 | 0.01188 | 13.89378 | 1.78931 | 107.8835 |

\(^1\) LR—logistic regression; \(^2\) Std.—standard; \(^3\) OR—odds ratio.

Table 3. Results logistic regression models. Active surveillance model (hunted).

| Significance Assessment of Model (p Value of LR \(^1\) Test) | Independent Variable | Coefficient (\(\beta_i\)) | Std. \(^2\) Error | \(p\) Value (Wald) | Odds Ratio | Confidence OR \(^3\) – 95% | Confidence OR \(^3\) + 95% |
|-----------------------------------------------------------|----------------------|--------------------------|-------------------|------------------|------------|------------------|------------------|
| Active surveillance model (hunted) of ASF in Parts II-III in 2019—influence of the month on the result (reference month: May) | Absolute term (\(\beta_0\)) | −4.73884 | 0.24241 | 0 | 0.00875 | 0.00544 | 0.01407 |
| | January | 0.81408 | 0.26623 | 0.00223 | 2.25709 | 1.33940 | 3.80354 |
| | February | 0.26434 | 0.35073 | 0.45105 | 1.30256 | 0.65498 | 2.59041 |
| | March | 0.39574 | 0.33693 | 0.24019 | 1.48549 | 0.76743 | 2.87540 |
| | April | 0.43176 | 0.30396 | 0.15549 | 1.53997 | 0.84669 | 2.79430 |
| | June | 0.20551 | 0.33693 | 0.24019 | 1.48549 | 0.76743 | 2.87540 |
| | July | 0.39574 | 0.33693 | 0.24019 | 1.48549 | 0.76743 | 2.87540 |
| | August | 0.60490 | 0.28435 | 0.03340 | 1.83108 | 1.04868 | 3.29721 |
| | September | 0.63386 | 0.26966 | 0.01875 | 1.88487 | 1.11102 | 3.19773 |
| | October | 0.44049 | 0.25995 | 0.09017 | 1.55347 | 0.93328 | 2.58850 |
| | November | 0.61648 | 0.26635 | 0.02065 | 1.85239 | 1.09897 | 3.12232 |
| | December | 0.61788 | 0.26062 | 0.01775 | 1.85499 | 1.12977 | 3.09174 |
| Active surveillance model (hunted) of ASF in Parts 0-I in 2019—influence of the month on the result (reference months: August) | Absolute term (\(\beta_0\)) | −5.28899 | 0.37995 | 0 | 0.00505 | 0.00240 | 0.01062 |
| | January | −2.79927 | 1.07221 | 0.00904 | 0.06086 | 0.00744 | 0.49778 |
| | June | −1.66661 | 1.06840 | 0.11881 | 0.18889 | 0.02327 | 1.53538 |
| | July | −0.69059 | 0.69091 | 0.34016 | 0.51734 | 0.13354 | 2.0042 |
| | November | −0.89722 | 0.53862 | 0.09578 | 0.40770 | 0.14185 | 1.17180 |
| | December | −1.20603 | 0.55866 | 0.03088 | 0.29939 | 0.10015 | 0.89495 |

\(^1\) LR—logistic regression; \(^2\) Std.—standard; \(^3\) OR—odds ratio.

3.4. ASF-Positive Results in Part II-III (Areas Acc. to 2014/709/EU Decision)

The model indicated the significance of the animal status for prevalence (\(p < 0.0001\)). The chance to obtain positive results in samples from wild boar carcasses (found dead) was 126 times higher compared to the group of hunted boars in the analyzed period. In addition,
the chance to obtain positive results in road-killed category was twice bigger than in the group of hunted animals (Table 4).

Table 4. Results logistic regression models. Surveillance model in Parts II-III in 2019.

| Significance Assessment of Model (p Value of LR Test) | Independent Variable | Coefficient ($\beta_i$) | Std. Error | $p$ Value (Wald) | Odds Ratio | Confidence OR $^3$ – 95% | Confidence OR $^3$ + 95% |
|-----------------------------------------------------|----------------------|--------------------------|------------|----------------|------------|-------------------|-------------------|
| Surveillance model in Parts II-III in 2019—influence of the type of surveillance on the result (found dead + road-killed vs. hunted) | Absolute term ($\beta_0$) | −4.20815 | 0.04074 | 0 | 0.01487 | 0.01373 | 0.01611 |
| Found dead | 4.83717 | 0.05097 | 0 | 126.1116 | 114.1189 | 139.3646 |
| Road-killed | 0.58529 | 0.17522 | 0.00083 | 1.79552 | 1.27355 | 2.53141 |

1 LR—logistic regression; 2 Std.—standard; 3 OR—odds ratio.

3.5. ASF-Positive Results in Part 0-I (Areas Acc. to 2014/709/EU Decision)

As in the previous case, the model showed the importance of the animal status for the prevalence ($p < 0.0001$). The chance to obtain ASF-positive results in samples from animal carcasses (found dead) was 45 times bigger in comparison to hunted boars, however the difference between hunted and road-killed animals was not noticed (Table 5).

Table 5. Results logistic regression models. Surveillance model in Parts 0-I in 2019.

| Significance Assessment of Model (p Value of LR Test) | Independent Variable | Coefficient ($\beta_i$) | Std. Error | $p$ Value (Wald) | Odds Ratio | Confidence OR $^3$ – 95% | Confidence OR $^3$ + 95% |
|-----------------------------------------------------|----------------------|--------------------------|------------|----------------|------------|-------------------|-------------------|
| Surveillance model in Parts 0-I in 2019—influence of the type of surveillance on the result (found dead + road-killed vs. hunted) | Absolute term ($\beta_0$) | −6.86468 | 0.21685 | 0 | 0.00104 | 0.00068 | 0.00160 |
| Found dead | 3.81482 | 0.24513 | 0 | 45.36845 | 28.0599 | 73.35367 |
| Road-killed | 0.26097 | 0.43431 | 0.54793 | 1.29818 | 0.55415 | 3.04119 |

1 LR—logistic regression; 2 Std.—standard; 3 OR—odds ratio.

3.6. Comprehensive Model

The comprehensive model of logistic regression showed the significance of the month, ASF zone and wild boars’ status to prevalence ($p < 0.0001$). The lowest prevalence was noticed in September (reference month). The chance to obtain a positive result was the highest in winter months: January (4 times), February and March (3.5 times) than in reference month (Table 6).

Parts II and III were significantly different from the reference Part I, where the prevalence was clearly lower (protection zone). Similarly, in Part 0, the prevalence was significantly lower than in Part 1 ($p < 0.0001$) (Table 6).

The chance to obtain positive results in Part II was 11 times higher than in Part I, in Part III 20 times higher than in Part I, and in Part 0 6 times lower than in Part I ($p < 0.0001$) (Table 6).

The model also showed that the prevalence of both found dead and post-accident animals was significantly bigger than among the reference group of hunted ones. In the group of found dead wild boars, the chance of a positive result was 118 times bigger than in the group of hunted boars ($p = 0$). In the case of post-accident wild boars, the chance of a positive result was more than 2 times higher than among hunted boars ($p < 0.0001$) (Table 6).
Table 6. Results of logistic regression models. Comprehensive model.

| Significance Assessment of the Model (p Value of LR 1 Test) | Independent Variable | Coefficient ($\beta_i$) | Std. 2 Error | $p$ Value (Wald) | Odds Ratio | Confidence OR 3 – 95% | Confidence OR 3 + 95% |
|----------------------------------------------------------|----------------------|-------------------------|--------------|-----------------|-----------|---------------------|---------------------|
| <0.0001                                                  | Absolute term ($\beta_0$) | −7.62727 | 0.11215 | 0 | 0.00049 | 0.00037 | 0.00065 |
|                                                          | January               | 1.41178 | 0.11212 | 0 | 4.10323 | 3.29282 | 5.11311 |
|                                                          | February              | 1.28645 | 0.11910 | $p < 0.0001$ | 3.61992 | 2.86560 | 4.57279 |
|                                                          | March                 | 1.30786 | 0.12078 | $p < 0.0001$ | 3.69824 | 2.91795 | 4.68719 |
|                                                          | April                 | 1.04541 | 0.11949 | $p < 0.0001$ | 2.84457 | 2.25011 | 3.59609 |
|                                                          | May                   | 1.06977 | 0.12014 | $p < 0.0001$ | 2.91470 | 2.30262 | 3.68949 |
|                                                          | June                  | 0.58602 | 0.15095 | 0.000104 | 1.79684 | 1.33626 | 2.41616 |
|                                                          | July                  | 0.57061 | 0.13341 | $p < 0.0001$ | 1.76934 | 1.36187 | 2.29873 |
|                                                          | August                | 0.60239 | 0.12991 | $p < 0.0001$ | 1.82647 | 1.41552 | 2.35673 |
|                                                          | October               | 0.08943 | 0.08318 | 0.28233 | 1.09355 | 0.92889 | 1.28740 |
|                                                          | November              | 0.81967 | 0.11439 | $p < 0.0001$ | 2.26976 | 1.81349 | 2.84083 |
|                                                          | December              | 0.84847 | 0.10738 | $p < 0.0001$ | 2.33607 | 1.89231 | 2.88391 |
|                                                          | Part 0                | −1.79986 | 0.21278 | $p < 0.0001$ | 0.16532 | 0.10890 | 0.25098 |
|                                                          | Part II               | 2.45275 | 0.11672 | 0 | 11.6204 | 9.24184 | 14.61071 |
|                                                          | Part III              | 3.02873 | 0.12497 | 0 | 20.6709 | 16.1761 | 26.41465 |
|                                                          | Found dead            | 4.76653 | 0.05199 | 0 | 117.5102 | 106.1143 | 130.1299 |
|                                                          | Road-killed           | 0.86516 | 0.15383 | $p < 0.0001$ | 2.37538 | 1.75654 | 3.21223 |

1 LR—logistic regression; 2 Std.—standard; 3 OR—odds ratio.

4. Discussion

As it was previously mentioned, Poland is combating with the disease since 2014. ASFV has been present in the country for almost seven years, and currently ASF zones cover half of the territory of Poland [17,18]. The alarming fact is that the number of ASF cases are still increasing despite the relatively low wild boar density in Poland, reaching 0.5 boar/km$^2$ in the ASF-affected area (Figure 2a), and is lower than two years before (up to 1.5 boar/km$^2$ in the ASF-affected area), when the number of ASF cases was much lower in Poland [4]. After the occurrence of each new case of ASF in wild boar populations or an outbreak in domestic pigs, parallel changes in ASF zoning are introduced (according to the 2014/709/EU decision) [18]. As a result, in Parts 0-4, there was a significant increase in the odds of obtaining positive ASF results in August and November in the category of boars found dead and killed on the roads, which was associated with the reintroduction of ASF to the affected areas. The observed peak in August (found dead group) was probably connected with the natural summer movement of wild boars [25,26], in that particular situation on the south of Poland to poviats: Zamojski, Biłgorański, and Rycki, which were ASF free zones until that moment [17]. As demonstrated by the team of Boklund (2018), the natural average speed of ASF spread in Poland and the Baltic states ranges from 8 to 17 km/year [27]. However, the peak incidence in November was associated with the outbreak of the disease in the Lubuskie voivodeship in western Poland (Figure 1), hundreds of kilometers from the last ASF cases [19]. A similar spread of the disease was observed in 2017, in November, when the disease arrived in Warsaw, also away from the last ASF outbreak [4]. In both situations of Warsaw district and Lubuskie voivodeship, the introduction of ASF might have been related to human activity [4,19] as it was in the case of ASF introduction to the Czech Republic [28]. In the case of ASF from Lubuskie, it was also taken into account that the passive monitoring of wild boars killed on the roads is essential, because the first animals with a positive ASF result from this area came from those killed on the roads [19].

The study performed by Johann et al., (2020) indicated that wild boars are mostly active during summer, which confirms the bigger chance of obtaining positive results in hunted animals during the summer period [26]. In our study, we calculated that in Parts II-III in August and in September there was a double chance of a positive result than in...
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spring (Table 4). Additionally, in Parts 0-I, the greatest chance of obtaining a positive result was in August, which also confirms the theory of summer wild boar movement [25,26]. A similar increase in the chance of obtaining a positive ASF result in winter in Parts II-III can be associated with the increase in the number of hunts in Poland after the discovery of new ASF cases in the Lubuskie and Wielkopolskie voivodeships [17,19].

Since the onset of the ASF epizootic, the number of sick wild boars in Poland has been growing every year [4,29], and this tendency is still confirmed by the presented results. However, a different situation is observed in the Baltic States. Contrary to Poland, the number of ASF-positive wild boars has been significantly decreased in Estonia (peaked 2016) and Latvia (peaked 2017) [29]. In Lithuania, there is still a growing number of ASF cases, but not as dynamic as in Poland [29,30].

Most of ASF positive results were recorded in passive surveillance in a group of found dead wild boars in Parts II-III. This observation was not surprising as most of the cases were concentrated in a group of dead animals in restricted areas. The same correlation was observed by other researchers, also in neighboring European countries [4,27–30]. The comprehensive model proved also that passive surveillance in found dead wild boars is more important than among the hunted ones (118 had a greater chance to obtain positive results in passive surveillance than in active surveillance). These data confirm the importance of collecting wild boar carcasses, which was also confirmed by other researchers [4,27,31]. In addition, in the group of road-killed animals, the chance of getting a positive result was two times bigger in comparison with active surveillance. This indicates that sampling wild boars from road accidents is also essential in ASF surveillance as it was supported by Schulz et al., (2020) [32] and Mazur-Panasiuk et al., (2020) [19].

The comprehensive model indicated that January, February, and March are the months with the highest chance of ASF positive results in the analyzed animals. Similarly, in passive surveillance (found dead) in Parts II and III during the same months, similar results were obtained. This indicates that the seasonality of the disease in the wild boar population is connected with the winter period. Likewise, other researchers observed a winter peak of positive results in Poland and the Baltic States and a summer peak that was not observed in this study [27,33]. In winter, when temperatures drop, the virus can persist for longer periods in a variety of materials and fomites, being contagious for susceptible wild boars for longer [27]. Additionally, food sources are limited in winter, which may lead to starvation of wild boars [27,34]. Another factor is the cold, which can weaken the wild boars and once again the low temperature protects the carcass in the environment [27].

Surveillance of ASF is essential for the early diagnosis of the disease and prevention of its further spread. The active and passive surveillance, including testing of wild boars from road-killed, is crucial to accomplish the efficient implementation of prevention measures. The knowledge of the connection between wild boar movement and ASF cases can enhance farm protection against disease. Knowing the disease pattern such as seasonality and the chance of a positive ASF result can help avoid future ASF cases in wild boars and outbreaks in domestic pigs in new areas currently free from the disease. This has a direct impact on the economics of pig production in Poland as well as in other affected countries.

Supplementary Materials: The following are available online at https://www.mdpi.com/2077-0472/11/1/45/s1, Table S1. Passive surveillance (wild boars found dead), Table S2. Passive surveillance (road-killed), Table S3. Active surveillance (hunted).

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