Original Article

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Environmental and Biosecurity-Related Risk Factors for African Swine Fever Outbreaks in Peninsular Malaysia

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Abstract

As African swine fever is a serious threat to the pig industry in Malaysia, scientifically analyzed information on the disease is vital for better control of the disease. The present study was conducted to determine the environmental and biosecurity-related risk factors for African swine fever outbreaks among domestic pig farms in Peninsular Malaysia. Data collected through a face-to-face interview with the pig farmers using a structured, close-ended questionnaire were analyzed using univariate and multivariate logistic regression. The final logistic regression model identified (1) presence of wild birds in the pig pens area (OR = 0.08; 95% CI, 0.01–0.74; $p = .03$), (2) presence of attractive crops/fruit trees surrounding the farm (OR = 4.00; 95% CI = 1.25–12.82; $p = 0.02$), (3) sharing of workers with other farms (OR = 6.11, 95% CI, 1.46–25.61; $p = .01$), (4) entry of visitor's vehicle into farm (OR = 0.14; 95% CI, 0.02–1.00; $p = .05$), (5) entry of feed truck into farm (OR = 5.45; 95% CI, 1.03–28.92; $p = .04$), (6) presence of biting insects such as flies, mosquitoes on pig's body which irritates the pigs (OR = 0.21; 95% CI, 0.06–0.80; $p = .02$), and (7) farm prone to rodent access and infestation (OR = 0.15; 95% CI, 0.03–0.79; $p = .03$) as the significant risk factors for African swine fever outbreaks in the studied farms. These findings highlight the need to strengthen on-farm biosecurity practices, farm management practices, and strict control of vehicle movement into the farm to prevent African swine fear outbreaks in the future.

Keywords: African swine fever, biosecurity, environmental

Introduction

African swine fever virus (ASFV), the causative agent of African swine fever (ASF) disease, is a large, double-stranded DNA virus in the *Asfarviridae* family. The virus causes hemorrhagic fever with high mortality rates, almost up to 100% in domestic pigs (Salguero, 2020). Currently, there is no efficient commercial vaccination against ASF. In order to stop the spread of the disease, the disease control plan primarily focuses on limiting animal movement, improving border control, preventing interaction between domestic and wild boar populations, improving sanitation and hygiene, and culling infected animals (Costard et al., 2009). The disease has a major impact on pig health and production and also poses a threat to the global pig and pork trade industry and food security (Nielsen et al., 2019). The rapid spread of ASF in the recent years since its introduction to Georgia in 2007 (Rowlands et al., 2008) has alarmed the industry players and the veterinary services for a constructive approach for prevention and control of the disease (Beaunée et al., 2023).

Malaysia heightened its biosecurity and imposed several preventive measures when ASF was detected in Northeast China at the Liaoning Province in August 2018. Several important measures to prevent the entry of ASF into Malaysia were implemented, including restrictions on pig and pork products from ASFV-infected countries and restrictions on the hand-carry or luggage carry of pork and pork products for own consumption (Salwahanim & Nazri, 2020). Department of Veterinary Services, Malaysia (DVS) also carried out clinical and serological surveillance for early detection of ASF, conducted risk communication and public awareness programs to share updates and information on ASF, and collaborated with relevant agencies such as the Malaysian Quarantine and Inspection Services Department and waste management operating companies at the entry ports throughout the country to heighten the border control inspection and proper waste management to curb the spread of the ASFV. Further to that, DVS also carried out an ASF outbreak simulation exercise to test Malaysia's readiness with important agencies such as the National Disaster Management Agencies, Malaysian Armed Forces, Malaysia Civil Défense Department, Royal Malaysia Police, and the Fire and Rescue Department of Malaysia (Salwahanim & Nazri, 2020).

Despite several efforts, the genotype II ASFV strain made its way into Malaysia and was detected for the first time in East Malaysia from organ samples of backyard domestic pigs in the state of Sabah in 2021; subsequently, the virus was also detected among domestic pigs in Sarawak (WOAH 2022; Khoo et al., 2021). The backyard domestic pig's organ samples were tested at the Veterinary Research Institute using the real-time polymerase chain reaction (RT-PCR) method. Phylogenetic analysis revealed that the 3 ASFV strains detected in the organ samples from Sabah belonged to p72 genotype II. And the partial analysis on the p72 showed that the 3 Sabah strains were 100% identical to each other and to ASFV strains from Indonesia, Vietnam, and China (Khoo et al., 2021; WOAH, 2022).

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In Peninsular Malaysia, the first case of ASF was detected in December 2021 among dead wild boar carcasses in the Batang Padang district, in the state of Perak. Following that, several ASFV cases were reported among wild boar carcasses found in the forest, natural parks, and oil palm plantations in the states of Melaka, Perak, Penang, Johor, Pahang, Terengganu, and Kelantan. In the state of Negeri Sembilan, ASFV was detected in a decayed carcass of a wild boar in a farm (WAHIS, 2023). Melaka was the first state to record ASF outbreaks among domestic pigs in December 2021, followed by Perak in March 2021 (WAHIS, 2023). The ASF outbreak in domestic pigs affected approximately 40,000 pigs in Melaka and 8488 pigs in Perak (Bernama, 2023). Even though the exact value of the economic losses is not published, it can be estimated that the outbreaks would have and may further cause significant financial impacts to the pig production industry if the spread of ASFV is not controlled, as the ex-farm value of pork in 2021 is approximately 3,459.12 million (DVS, 2022).

The key control measures applied by DVS to manage the ongoing outbreaks and prevent further spread of viruses include stamping out infected animals and susceptible animals surrounding the outbreak area, disinfection of the infected farms, live pig and pork product movement control into the country and within the country, and increased border inspection to prevent entry of pork products (WAHIS, 2023). For early detection of ASFV, surveillance is conducted throughout the country (WAHIS, 2023). The surveillance activities include the search and testing of dead wild boar carcasses; ante-mortem and post-mortem examinations during slaughter and screening of samples from slaughterhouses; and clinical surveillance in all the pig farms throughout the country (WAHIS, 2023). Further to that, the farmers were also advised to improve the biosecurity level of their farms (WAHIS, 2023).

An epidemiological investigation report by the authority in East Malaysia and Peninsular Malaysia hypothesized environmental contamination by wild boars, poor biosecurity practices, and live pig and pork product movements as the sources of ASFV transmission (WAHIS, 2023). To date, there is no published literature on the details of the epidemiology and risk factors of ASF in Malaysia. The only recent publication related to ASF in Malaysia is on the isolation and characterization of ASFV in pig organ samples from the state of Sabah in East Malaysia (Khoo et al., 2021).

Swill feeding, interaction with wild boar, and indirect transmission by individuals coming into contact with infected farms or using fresh grass or crops as feed were identified as the most likely modes of transmission to domestic farms in Lativia (Oļševskis et al., 2016). Another matched case-control study carried out in Romania revealed that the prevalence of wild boars and the close proximity of the farm to infected wild boars were important risk factors for ASF outbreaks in commercial and small-scale pig farms (Boklund et al., 2020). Meanwhile, among Asian countries, farm density and proximity, poor biosecurity practices, swill feeding practices, the movement of vehicles, and live pig transportation were regarded as important risks for the spread of ASF (Cheng & Ward, 2022; Thi et al., 2022).

As the risk factors for ASF incursion and spread differ substantially between countries and regions based on farm demographic, sociodemographic, human practices, and environmental factors (Bellini et al., 2021), it warrants a need for a study to identify the possible risk factors associated with the ASF outbreaks in Malaysia in spite of the hypothesized risk factors.

The aim of this study is to identify the possible environmental and biosecurity-related risk factors associated with the ASF outbreak among pig farms in Peninsular Malaysia. The findings of this analysis may provide enhanced insight to the Department X and the pig industry stakeholders on the exact risk factors associated with ASF transmission and spread into domestic pig farms.

Materials and Methods

Study Site and Selection of Farm

To determine the environmental and biosecurity-related risk factors associated with the ASF outbreak among pig farms in Peninsular Malaysia, a case–control study was conducted between August 2022 and October 2022. The states of Melaka and Perak in Peninsular Malaysia were selected as points of study for ASF outbreak that occurred only in these two states. The state of Melaka is located in the southern region of Peninsular Malaysia, facing the Strait of Malacca,while the state of Perak is located on the west coast of Peninsular Malaysia, as shown in (Figure 1.) Thirty farms in the state of Melaka and five farms in the state of Perak experienced ASF outbreaks between December 2021 and February 2022. All the outbreaks were caused by the ASFV genotype 2 strain, which was confirmed through the real-time polymerase chain reaction (RT-PCR) method (WAHIS, 2023).

The list of pig farms and records of ASF outbreaks in both states were obtained from Department of Veterinary Services, Malaysia. Based on the list, a total of 70 farms (cases N = 35, control N = 35) were included in this study. All 35 ASF-positive farms in the states of Melaka ($n = 30$ farms) and Perak ($n = 5$ farms) were selected as case farms. For each of the case farms, a control farm that is free from ASF outbreaks was selected from both states. The case and control farms were matched by farm type, farm system, breeding method, and vaccination status for Aujeszky's disease (AD), porcine reproductive and respiratory syndrome (PRRS), porcine circovirus type 2 (PCV2), classical swine fever (CSF), and foot-and-mouth disease (FMD). The standing pig population (SPP) is below 2000 heads in 29 case farms. Meanwhile, another six case farms have SPP between 2000 and 10,000 heads. All the studied farms are porker farm, which use artificial insemination breeding methods and operate within an open-house system. The mortality rate in all the case farms is above 50%, and following confirmation for ASF through clinical signs and the RT-PCR method, the remaining pigs were 100% culled.

Data Collection

Field Data Collection and Components of the Questionnaire

Data were collected from August to October 2022 through a face-to-face meet-up session with 70 pig farmers who are the owners of the farms included in this study (cases $N = 35$, control $N = 35$) using a structured, close-ended questionnaire. The questionnaire was developed based on Department of Veterinary Services, Malaysia's existing ASF clinical surveillance form, with additional questions on the potential risk factors associated with ASF transmission as reported in previous studies by de la Torre et al. (2022) and Boklund et al. (2020). Prior to the administration of the questionnaire, the farmers were met in groups, and the purpose of the questionnaire was discussed. Each farmer was then requested to fill in the questionnaire without

Figure 1.
Map Showing the Location of the State of Perak and Melaka in Peninsular Malaysia.

interference to avoid personal biases. Assistance was provided to the farmers when needed, for example, when they needed help in understanding the meaning of the questions. All procedures were explained to the farmers, and informed verbal consent was obtained from all the participating farmers prior to the administration of the questionnaire.

The questionnaire consisted of two parts, whereby part 1 contained information on farm demography and part 2 contained information on the potential risk factors associated with ASF transmission. Part 2 was divided into four sections: Section A: information on access to wild boar/wild animals, and wild birds; Section B: biosecurity practices; Section C: control of visitors and vehicle movement into farm; and Section D: presence and control of ticks, biting insects, and rodents. All the questions in Part 2 (Sections A–D) are dichotomous questions that require "yes" or "no" answers.

Statistical Analysis

Descriptive statistics were generated for the study farms in relation to the location of the farm (state), SPP of the farm, type of farm, farm system, breeding method, and vaccination program in farm.

Each of the potential risk factors listed in the questionnaire was coded as a dichotomous independent variable. The "yes" answers were regarded as 1 while the "no" answers were regarded as 0. The ASF status of the farm was regarded as the dependent variable and modeled as the function for the dichotomous risk factors using a logistic regression model as suggested by Hosmer and Lemeshow (2000). The risk factors associated with the ASF status of the farms were initially examined with univariable logistic regression. Variables associated with positive ASF status at $p < .1$ were included in the following multivariable logistic regression models. Variables significant in the univariable analysis were tested for collinearity using the variance inflation factor (VIF). Four multivariable conditional logistic regression models were developed using the backward (conditional) procedure with a selection threshold of $p \leq .05$ to reduce the number of variables in the model. The 4 multivariable models developed are for: [A] access to wild boar, wild animals, and wild birds; [B] biosecurity practices; [C] control of visitors and vehicle movement into the farm; and [D] presence and control of ticks, biting insects, and rodent. The overall fit of the final models was assessed using the Hosmer–Lemeshow goodness-of-fit test. The ability of the model to discriminate between ASF-positive and ASF-negative farm was assessed using the receiver operating characteristic (ROC) curve. Area under the curve (AUC) value between 0.7 and 0.8 was regarded as acceptable discrimination (Hosmer and Lemeshow, 2000). In the final models, the ORs, *p*-values, and 95% CI were reported to demonstrate the significance and strength of the association between ASF farm status and the studied risk factors. All statistical analyses were conducted with Statistical Package for Social Sciences Statistics software, version 26.

Results

Descriptive Statistics

A total of 70 farmers from the selected farms were included in this study at the following distributions: 35 case farms and 35 control farms, with the distribution of farms in the states of Melaka (30 case farms, 5 control farms) and Perak (5 case farms, 30 control farms). The response rate is 100%; all 70 farmers agreed to participate and responded to the questionnaire.

The descriptive summary of case and control farm demography characteristic are as shown in Table 1.

Risk Factors

Logistic Regression Model for Risk Factors Associated with Access of Wild Boar, Wild Animals and Wild Birds to the Farm with the ASF Status of the Farms

Table 2 presents the findings from the univariate logistic regression analysis. The risk factors related to access of wild boar, wild animals, and wild birds to the farm that were found to be significantly associated with the ASF status of the farm (*p*-value $< .1$) were the presence of wild boar roaming near the farm; presence of wild birds in the pig pens area; fencing surrounding the farm able to prevent entry of wild boar/wild animals into farm workers involved in or contact with wild animals/birds hunting activities.

The final multivariate conditional logistic regression model as shown in Table 3 identified that the risk factor 'presence of wild birds in the

Table 1.

Case and Control Farm Demography Characteristics

Variable	Cases (*n*)	Controls (*n*)
State		
Melaka	30	5
Perak	5	30
SPP (number of heads)		
<2000	29	29
>2000	6	6
Farm type		
Porker	35	35
Farm system		
Open	35	35
Breeding method		
Artificial insemination	35	35
Vaccination status		
AD, PRRS, PCV2, CSF, and FMD	35	35

Note: Case and control farms were matched by farm type, farm system, breeding method, and vaccination status for AD, PRRS, PCV2, CSF, and FMD.
AD = Aujeszky's disease; CSF = Classical swine fever; FMD = Foot-and-mouth disease; PCV2 = Porcine circovirus type 2; PRRS = Porcine reproductive and respiratory syndrome; SPP, standing pig population.

pig pens area' (OR = 0.08; 95% CI, 0.01–0.74; $p = .03$) most likely influences the occurrence of ASF in the pig farms.

The Hosmer–Lemeshow goodness of fit test showed that the model fit the data well ($\chi^2 = 0.199$, *df* 3, $p = .978$). The VIF value for the independent variables showed no important collinearities. The accuracy of the final model was assessed by the ROC method and showed good ability to distinguish between the ASF-positive farm and the ASF-negative farm with the AUC of 0.712 (Figure 2).

Logistic Regression Model for Risk Factors Associated with Biosecurity Practices in the Farm with the ASF Status of the Farms

Table 4 presents the findings from the univariate logistic regression analysis. The risk factors related to biosecurity practices on the farm that were found to be significantly associated with the ASF status of the farm ($p < .1$) were: the water used as drinking water for pigs is treated; the presence of attractive crops/fruit trees surrounding the farm; sharing of workers with other farms. The final multivariate conditional logistic regression model as shown in Table 5 identified that the risk factors "presence of attractive crops/fruit trees surrounding the farm" (OR = 4.00; 95% CI, 1.25–12.82; $p = .02$) and "sharing of workers with other farms" (OR = 6.11, 95% CI = 1.46–25.61; $p = .01$) most likely influence the occurrence of ASF in the pig farms. The Hosmer–Lemeshow goodness of fit test showed that the model fit the data well ($\chi^2 = 0.02$, *df* 1, $p = .886$). The VIF value for the independent variables showed no important collinearities. The accuracy of the final model was assessed by ROC method and showed good ability to distinguish between the ASF-positive farm and the ASF-negative farm with the AUC of 0.703 (Figure 3).

Table 2.

Univariate Logistic Regression Analysis of Risk Factors Related to Access of Wild Boar, Wild Animals and Wild Birds to the Farm with the African Swine Fever Status of the Farms

Variable/factors	Category	Cases (*n*)	Controls (*n*)	OR	95% CI	*p*
A1. Seen wild boar roaming near farm	Yes	5	13	3.54	1.10–11.41	.03*
	No	30	22	Ref	-	-
A2. Found wild boar body or remains in the vicinity of farm	Yes	4	7	1.94	0.51–7.33	.33
	No	31	28	Ref	-	-
A3. Seen wild animals roaming near farm	Yes	7	8	1.19	0.38–3.72	.77
	No	28	27	Ref	-	-
A4. Seen wild birds in pig pens area	Yes	8	1	0.09	0.01–0.84	.03*
	No	27	34	Ref	-	-
A5. Fencing in the farm able to prevent entry of wild birds into pig pens area	Yes	2	4	2.13	0.36–12.46	.40
	No	33	31	Ref	-	-
A6. Fencing surrounding farm able to prevent entry of wild boar/wild animals into farm	Yes	28	33	4.12	0.79–21.48	.09*
	No	7	2	Ref	-	-
A7. Worker involved or contact with wild boar hunting activities	Yes	3	4	1.38	0.29–6.66	.69
	No	32	31	Ref	-	-
A8. Worker involved or contact with wild animals/birds hunting activities	Yes	7	1	0.12	0.01–1.01	.05*
	No	28	34	Ref	-	-

Note: CI = Confidence interval; OR = Odds ratio; Ref = Reference.
*Statistically significant.

Table 3.

Multivariate Conditional Logistic Regression Analysis of Risk Factors Related to Access of Wild Boar, Wild Animals, and Wild Birds to the Farm with the African Swine Fever Status of the Farms

Variable/factors	Category	OR	95% CI	p
A4. Seen wild birds in pig pens area	Yes	0.08	0.01–0.74	.03*
	No	Ref	-	-
A6. Fencing surrounding farm able to prevent entry of wild boar/wild animals into farm	Yes	4.10	0.72–23.40	.11
	No	Ref	-	-
A8. Worker involved or contact with wild animals/birds hunting activities	Yes	0.12	0.01–1.06	.06
	No	Ref	-	-

Note: CI = Confidence interval; OR = Odds ratio; Ref = Reference.
*Statistically significant.

Logistic Regression Model for Risk Factors Associated with Visitors and Vehicles Movement into the Farm with the ASF Status of the Farms

Table 6 presents the findings from the univariate logistic regression analysis. The risk factors related to visitors and vehicle movement into the farm that were found to be significantly associated with the ASF status of the farm ($p < .1$) were: entry of visitors who have visited other farms on the same day; visitors required to wear disposable protective clothes when entering the farm; entry of visitors' vehicles into farm; and entry of feed trucks into the farm.

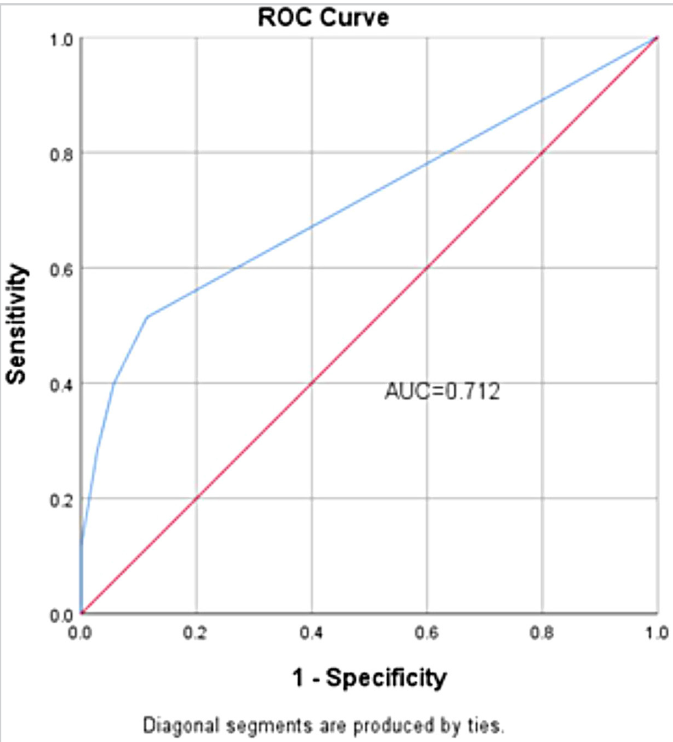

Figure 2.
Predicted Probabilities of Final Model Based on the Receiver Operating Characteristic (ROC) Method (the Area Under the Curve (AUC) = 0.712).

The final multivariate conditional logistic regression model as shown in Table 7 identified that the risk factors "entry of visitor's vehicle into farm" (OR = 0.14; 95% CI, 0.02–1.00; $p = .05$) and "entry of feed truck into farm" (OR = 5.45; 95% CI, 1.03–28.92; $p = .04$) most likely influence the occurrence of ASF in the pig farms. The Hosmer–Lemeshow goodness of fit test showed that the model fit the data well ($\chi^2 = 0.609$, *df* 3, $p = .894$). The VIF value for the independent variables showed no important collinearities. The accuracy of the final model was assessed by the ROC method and showed good ability to distinguish between the ASF-positive farm and ASF-negative farm with the AUC of 0.715 (Figure 4).

Logistic Regression Model for Risk Factors Associated with Presence and Control of Ticks, Biting Insects, and Rodents in the Farm with the ASF Status of the Farms

Table 8 presents the findings from the univariate logistic regression analysis. The risk factors related to the presence and control of ticks, biting insects, and rodents in the farm that were found to be significantly associated with the ASF status of the farm ($p < .1$) were the presence of biting insects such as flies and mosquitoes on the pig's body, which to the extent irritates the pigs; insecticides sprayed routinely at the farm; and the farm prone to rodent access and infestation. The final multivariate conditional logistic regression model, as shown in Table 9, identified that the risk factors "presence of biting insects such as flies and mosquitoes on pig's body, which to the extent irritates the pigs" (OR = 0.21; 95% CI, 0.06–0.80; $p = .02$) and "farm prone to rodent access and infestation" (OR = 0.15; 95% CI, 0.03–0.79; $p = .03$) most likely influence the occurrence of ASF in the pig farms. The Hosmer–Lemeshow goodness of fit test showed that the model fit the data well ($\chi^2 = 0.163$, *df* 2, $p = .922$). The VIF value for the independent variables showed no important collinearities. The accuracy of the final model was assessed by the ROC method and showed good ability to distinguish between the ASF-positive farm and the ASF-negative farm with the AUC of 0.700 (Figure 5).

Discussion

This study identified the farm-level risk factors related to environmental and biosecurity practices associated with ASF outbreaks among pig farms in Peninsular Malaysia. The significant risk factors identified were (1) presence of wild birds in pig pens area, (2) presence of attractive crops/fruit trees surrounding the farm, (3) sharing of workers with other farms, (4) entry of visitor's vehicle into farm, (5) entry of feed truck into farm, (6) presence of biting insects such as flies, mosquitoes on pig's body which to the extent irritates the pigs; and[7] farm prone to rodent access and infestation.

As evidenced in the study by Fasina et al. (2012), which indicated that direct and indirect contact with wild birds and rats is the risk factor contributing to ASF infection at the farm level in Nigeria, this study identified the presence of wild birds in the pig pen area and farms prone to rodent access and infestation as the factors significantly associated with ASF outbreaks in the pig farms. As most of the pig farms in Peninsular Malaysia adopt an open house system, it is apt to give importance on the access of wild birds, especially scavenging birds, into the pig pen area of the farm, which may cause contamination of the pig feeds with ASF-infected materials. This is because the study by Probst et al. (2019) evidenced that there is a high probability of ASF-infected materials being transferred into farms from ASF-infected wild boars' carcasses from nearby forests

Table 4.

Univariable Logistic Regression Analysis of Risk Factors Related to Biosecurity Practices in the Farm with the African Swine Fever Status of the Farms

Variable/factors	Category	Cases (*n*)	Controls (*n*)	OR	95% CI	*p*
B1. Use only commercial feed	Yes	31	30	0.77	0.19–3.16	.72
	No	4	5	Ref	-	-
B2. Use only commercially bought ingredients for self-milled feed	Yes	35	35	1.00	0.00	1.00
	No	0	0	NA	-	-
B3. Never used or mixed kitchen waste or catering waste in feed	Yes	35	35	1.00	0.00	1.00
	No	0	0	NA	-	-
B4. Water used as drinking water for pigs are treated	Yes	4	11	3.55	1.02–12.55	.04*
	No	31	24	Ref	-	-
B5. Vehicle dip/spray at the farm entrance is functional at all times	Yes	34	35	1.00	0.00	1.00
	No	1	0	NA	-	-
B6. Presence of attractive crops/fruit trees surrounding the farm	Yes	21	29	3.22	1.06–9.77	.04*
	No	14	6	Ref	-	-
B7. No other animals kept in the pig shed	Yes	35	35	1.00	0.00	1.00
	No	0	0	NA	-	-
B8. Boar from other farm is not used for reproduction	Yes	35	35	1.00	0.00	1.00
	No	0	0	NA	-	-
B9. All in-all out system practiced at all stage of production	Yes	29	33	3.41	0.64–18.25	.15
	No	6	2	Ref	-	-
B10. Workers shared with other farms	Yes	24	32	4.89	1.23–19.47	.02*
	No	11	3	Ref	-	-
B11. Workers change to new/different clothes when enter farm	Yes	4	3	0.73	0.15–3.52	.69
	No	31	32	Ref	-	-
B12. Workers change to new/different boots when enter farm	Yes	35	35	1.00	0.00	1.00
	No	0	0	NA	-	-
B13. Farm has quarantine area	Yes	27	31	2.29	0.62–8.48	.21
	No	8	4	Ref	-	-
B14. No new pigs introduced into farm 60 days prior ASF outbreak in the state	Yes	35	35	1.00	0.00	1.00
	No	0	0	NA	-	-
B15.Manure and farm waste treated prior discharge	Yes	33	30	0.36	0.06–2.02	.25
	No	2	5	Ref	-	-
B16. Farm routinely cleaned using disinfectant solutions	Yes	16	15	0.89	0.35	.81
	No	19	20	Ref	-	-
B17.Farm thoroughly disinfected after an outbreak/disease	Yes	35	35	1.00	0.00	1.00
	No	0	0	NA	-	-
B18.Carcass of dead pigs disposed by burial or burning method	Yes	35	35	1.00	0.00	1.00
	No	0	0	NA	-	-

Note: CI = Confidence interval; OR = Odds ratio; Ref = Reference; NA = Not available.
*Statistically significant.

or from other domestic pig farms by the scavenging birds as the research on scavenging activities on wild boar carcasses on its natural habitat in Germany detected several species of birds, such as the common raven, common buzzard, white-tailed eagle, and hooded crow scavenging on wild boar carcasses. Several studies have proved that the introduction of ASFV into pigs raised in closed or confined environments was preventable during ASF outbreaks (Aliro et al., 2022; Barnes et al., 2020; Bisimwa et al., 2021; Brown et al., 2018). Thus, to manage the ASF transmission risk, the adoption of closed pig houses under the modern pig farming system is vital to minimize the risk of ASFV-infected material entering the farms.

Meanwhile, even though the majority of the farms in this study have rodent control programs applied in their farms, the factor of

Table 5.

Multivariate Conditional Logistic Regression Analysis of Risk Factors Related to Biosecurity Practices in the Farm with the African Swine Fever Status of the Farms

Variable/factors	Category	OR	95% CI	p
B4. Water used as drinking water for pigs are treated	Yes	2.81	0.70–11.27	.144
	No	Ref	-	-
B6. Presence of attractive crops/ fruit trees surrounding the farm	Yes	4.00	1.25–12.82	.02*
	No	Ref	-	-
B10. Workers shared with other farms	Yes	6.11	1.46–25.61	.01*
	No	Ref	-	-

Note: CI = Confidence interval; OR = Odds ratio; Ref = Reference.*Statistically significant.

farms prone to rodent access and infestation appeared to be significantly associated with the ASF outbreaks. Rodent control programs in livestock farms in Malaysia is one of the requirements under the Malaysian Good Agricultural Practices (MyGap) program. However, currently, the program is on voluntary basis, and it is not mandatory for the pig farms to adopt the program. Thus, it is possible that rodent control is carried out inconsistently, which may have led to sudden fluctuations in the rodent population. The study by Fasina et al. (2012) highlighted that the sudden increase in rodent populations may increase the risk of disease transmission as the rodents may transfer ASFV-infected remnants between farms.

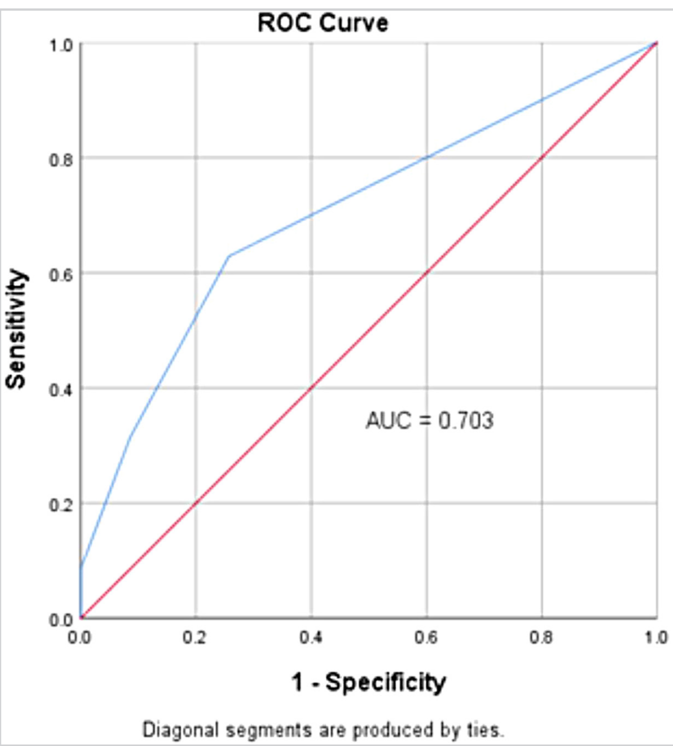

Figure 3.

Predicted Probabilities of Final Model Based on the Receiver Operating Characteristic (ROC) Method (the area under the curve (AUC) = 0.703).

The presence of attractive crops/fruit trees surrounding the farm was identified as one of the significant risk factors associated with the occurrence of ASF outbreaks. In Malaysia, it is a common practice to utilize the areas surrounding livestock farms by planting crops, vegetables, and fruits; hence, the attractive crops and fruit trees surrounding the farms may attract the entry of wild boar, wild animals, and birds, which may transmit the virus to naïve pigs in the farm. In fact, EFSA (2014) has reported that domestic pigs in Latvia and Lithuania may have been exposed to the ASF virus through fresh grass and seeds contaminated with wild boar feces. At present, in Malaysia, there are no rules or laws that dictate the distance of pig farm locations from crop cultivation farms or fruit orchards. Thus, this finding highlights the need for a practical preventive measure to mitigate the risk of cross-contamination associated with the presence of crops or fruit trees farming around livestock farm, which is common practice in the country.

Moreover, the study also found that sharing workers with other farms was significantly associated with the ASF outbreak. It is clear and logical that the sharing of workers may intensify the transmission of the virus from one farm to another. This is because factors such as unchanged clothing or shoes inbetween farms may serve as a mediator for the spread of the virus between farms. Numerous ASF outbreaks that occurred in major commercial farms in Russia and Lithuania were caused by contact with contaminated fomites as a result of improper disinfection of clothing and boots (Gogin et al., 2013; Gonzales et al., 2021; Oganesyan et al., 2013). However, in Peninsular Malaysia, due to the ownership of more than one farm and to reduce labor costs, the practice of sharing pig farm workers will continue for the foreseeable future, especially among small- to medium-scale farms. It was noted that the risk factors identified in this study are closely related to inadequate biosecurity practices, which are commonly reported as the main factor for ASF outbreaks in countries such as the Dominican Republic and South Africa (Amar et al., 2021; Gonzales et al., 2021; Mutua & Dione, 2021). Thus, increased awareness on the risk of workers sharing practices and the importance of adhering to strict biosecurity measures in between farm-to-farm movement is crucial to minimizing the risk of ASF virus transmission into naïve farms (Chenais et al., 2022; Dione et al., 2020).

As has been discussed in previous studies, this study also identified the factors of entry of visitors' vehicles into farms and entry of feed trucks into farms to be significantly associated with the ASF outbreaks. In the majority of the study farms, the vehicle parking and unloading spaces are located within the farm area. Hence, there is a high probability of the ASF virus being transmitted to the farm through infected vehicles, the equipment of the vehicle, and the driver's shoe due to the resilient characteristic of the ASF virus, which enables it to last for several days in the environment, especially in organic material (Bellini et al., 2016). Madec et al. (2010) recommended that every farm establish appropriate feed and pig loading area in the farm that prevents the entry of trucks, including the drivers, into the farm by any means. The European Commission, in its regulation for ASF, has stated that entry of vehicles into farms shall be restricted and regulated strictly, with a record of the movement of vehicles and people into the farm (Duc et al., 2022). In Malaysia, restrictions and movement control on pig farms are enforced by the authority in the event of an outbreak to control the transmission of disease. However, under normal circumstances, movement into

Table 6.
Univariate Logistic Regression Analysis of Risk Factors Related to Visitors and Vehicles Movement into the Farm with the African Swine Fever Status of the Farms

Variable/factors	Category	Cases (*n*)	Controls (*n*)	OR	95% CI	*p*
C1. Entry of visitors who have visited other farms on the same day	Yes	34	27	0.09	0.01–0.84	.03*
	No	1	8	Ref	-	-
C2. Visitors wear new clean boots/disposable shoe cover when entering farm	Yes	31	29	0.62	0.16–2.44	.49
	No	4	6	Ref	-	-
C3. Visitors required to wear disposable protective clothes when entering farm	Yes	27	33	4.89	0.96–24.97	.06*
	No	8	2	Ref	-	-
C4. Entry of visitor's vehicle into farm	Yes	33	27	0.21	0.04–1.05	.05*
	No	2	8	Ref	-	-
C5. Entry of feed truck into farm	Yes	25	32	4.27	1.06–17.17	.04*
	No	10	3	Ref	-	-
C6. Entry of butcher or pig catcher's lorry into farm	Yes	31	29	0.37	0.10–1.35	.13
	No	4	6	Ref	-	-

Note: CI = Confidence interval; OR = Odds ratio; Ref = Reference.
*Statistically significant.

farms are regulated by the farm owner itself. This may provide room for a higher risk of disease transmission between farms due to the uncontrolled movement of vehicles into farms.

The presence of biting insects such as flies and mosquitoes on the pig's body, which to the extent irritates the pigs, was also significantly associated with the ASF outbreak farms. Even though soft ticks of the genus *Ornithodoros* were commonly regarded as the major biological vector for ASF virus transmission among pigs and wild boar (Gallardo et al., 2015), there are studies that have indicated that ASF virus may also be transferred to pigs by biting flies like *Stomoxys calcitrans* during their feeding cycle (Mellor et al., 1987; Saegerman et al., 2021) or through ingestion of infected flies (Olesen et al., 2017; Olesen et al., 2018). The possibility of transmission is further supported by the persistence of high virus titers in the flies for up to 2 days (Baldacchino et al., 2013) and the detection of ASF virus DNA traces in flies and mosquitoes' samples in a pilot investigation carried out by Herm et al. (2020) in an ASF outbreak farm in Estonia. Therefore, this indicates that the vector control program to control the population of mosquitoes and insects on pig farms is necessary. This is because the tropical weather in Malaysia and the monsoon seasons with higher rainfall frequencies are conducive to the breeding and maturation cycles of insects like flies

Table 7.
Multivariate Conditional Logistic Regression Analysis of Risk Factors Related to Visitors and Vehicles Movement into the Farm with the African Swine Fever Status of the Farms

Variable/factors	Category	OR	95% CI	*p*
C1. Entry of visitors who have visited other farms on the same day	Yes	0.16	0.02-1.44	.10
	No	Ref	-	-
C4. Entry of visitor's vehicle into farm	Yes	0.14	0.02-1.00	.05*
	No	Ref	-	-
C5. Entry of feed truck into farm	Yes	5.45	1.03-28.92	.04*
	No	Ref	-	-

Note: CI = Confidence interval; OR = Odds ratio; Ref = Reference.
*Statistically significant.

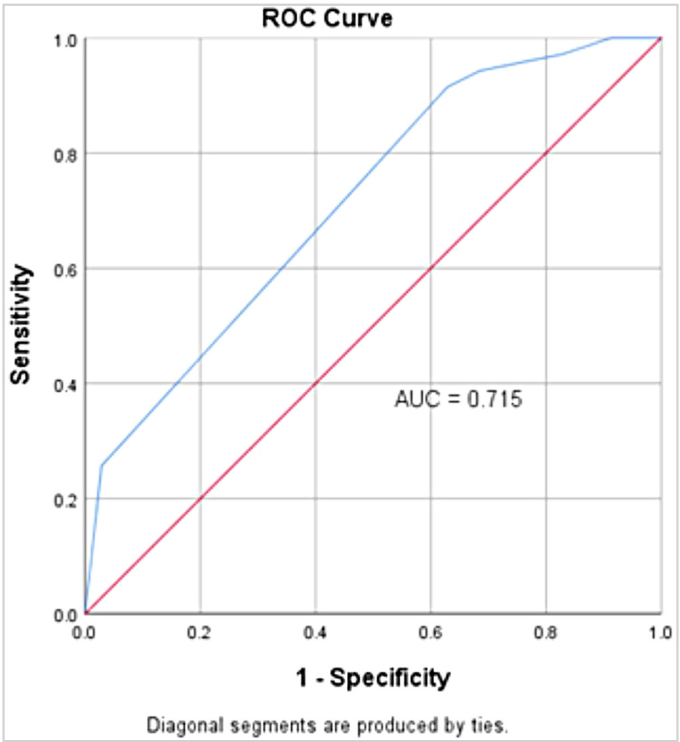

Figure 4.
Predicted Probabilities of Final Model Based on the Receiver Operating Characteristic (ROC) Method (the Area Under the Curve (AUC) = 0.715).

Table 8.
Univariate Logistic Regression Analysis of Risk Factors Related to Presence and Control of Ticks, Biting Insects, and Rodents in the Farm with the African Swine Fever Status of the Farms

Variable/factors	Category	Cases (*n*)	Controls (*n*)	OR	95% CI	*p*
D1. Pigs free from tick infestation	Yes	34	32	0.314	0.31-3.17	.32
	No	1	3	Ref	-	-
D2. Tick control treatment carried out routinely in farm	Yes	30	31	1.29	0.32-5.28	.72
	No	5	4	Ref	-	-
D3. Presence of biting insects such as flies, mosquitoes on pig's body which to the extent irritates the pigs	Yes	13	4	0.22	0.06-0.76	.02*
	No	22	31	Ref	-	-
D4. Insecticide sprayed routinely at farm	Yes	28	33	4.13	0.79-21.48	.09*
	No	7	2	Ref	-	-
D5. Farm prone to rodent access and infestation	Yes	33	25	0.15	0.03-0.75	.02*
	No	2	10	Ref	-	-
D6. Presence of rodent control program in farm	Yes	34	33	0.48	0.04-5.61	.56
	No	1	2	Ref	-	-

Note: CI = Confidence interval; OR = Odds ratio; Ref = Reference.
*Statistically significant.

and mosquitoes (Gopalsamy et al., 2021; Semelbauer et al., 2018; Wee et al., 2013). Further to that, it is important to note that most of the ASF outbreaks in Peninsular Malaysia occurred during the monsoon seasons, which favored the increase of mosquitoes and fly populations due to higher rainfall and a lower environmental temperature. Thus, this finding warrants a need to carry out a detailed vector study on the significant function of insects in the spread of ASF among pig farms in tropical climate countries like Malaysia.

The environmental risk factors, which are the presence of wild birds in the pig pens area and the presence of attractive crops or fruit trees surrounding the farm identified in the present study, can be regarded as mitigable factors with high financial implications. This is because transitioning from an open to a closed house system, which is the best option to prevent access to wild birds, including wild animals, to reduce the risk of disease transmission or cross-contamination between herds, farms, and wild animals, will incur additional costs to the farmers. On the other hand, clearance of attractive crops or fruit trees surrounding the farm may cause losses as the crop yields might be another source of income for the farmers.

Meanwhile, the biosecurity practices related risk factors, which are the sharing of workers with other farms, entry of visitors' vehicles

Table 9.
Multivariate Conditional Logistic Regression Analysis of Risk Factors Related to Presence and Control of Ticks, Biting Insects, and Rodents in the Farm with the African Swine Fever Status of the Farms

Variable/factors	Category	OR	95% CI	*p*
D3. Presence of biting insects such as flies, mosquitoes on pig's body which to the extent irritates the pigs	Yes	0.21	0.06-0.80	.02*
	No	Ref	-	-
D5. Farm prone to rodent access and infestation	Yes	0.15	0.03-0.79	.03*
	No	Ref	-	-

Note: CI = Confidence interval; OR = Odds ratio; Ref = Reference.
*Statistically significant.

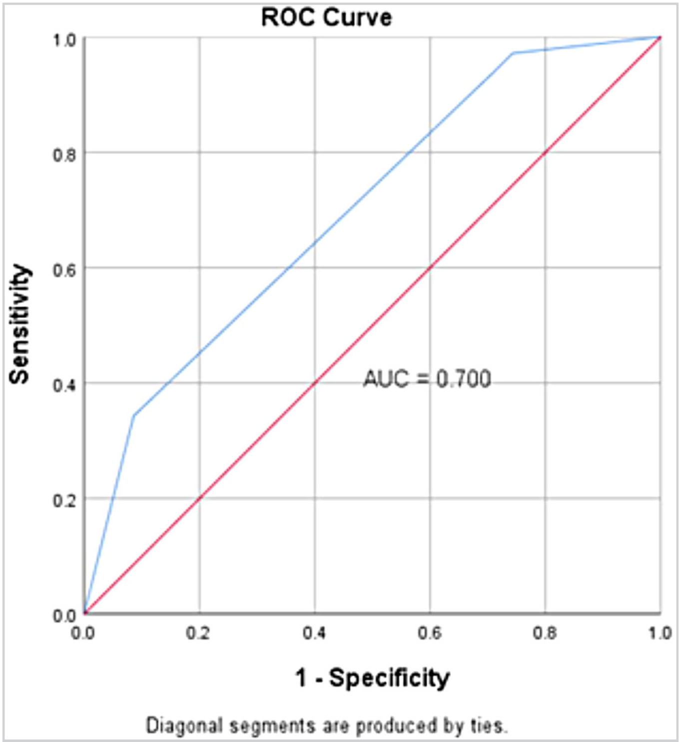

Figure 5.
Predicted Probabilities of Final Model Based on the Receiver Operating Characteristic (ROC) Method (the Area Under the Curve (AUC) = 0.700).

into farms, entry of feed trucks into farms, the presence of biting insects such as flies, and mosquitoes on pigs' bodies which to the extent irritate the pigs, and farms prone to rodent access and infestation, can be regarded as mitigable risk factors with reasonable financial implications. If possible, it is best to avoid sharing workers between farms of the same owner or company unless very stringent biosecurity measures could be adhered to by the workers during the movement between farms. In addition, total restriction of vehicle entry into the farms is recommended to reduce the risk of contact and transmission of the ASF virus through contaminated vehicles, drivers, and its associated materials. Cleaning of the farm and the surroundings should also be carried out routinely in addition to the vector control program to clear the potential breeding grounds of flies or mosquitoes.

To limit the confounding bias in the present study, the case and control farms were matched in terms of farm type, farm system, breeding method, and vaccination status for AD, PRRS, PCV2, CSF, and FMD. In addition, an effort was made to include all the farms in Peninsular Malaysia that had ASF outbreaks during the study period; thus, all the farms that had outbreaks in the states of Melaka and Perak were selected as case farms. Self-report bias was possible in this study as farmers may tend to provide "socially acceptable" answers to the questions in the questionnaire rather than their actual practices. However, where possible, we counter-checked with the representative of the respective state pig association and Department of Veterinary Services officers, who are familiar with the farms. In addition, to avoid misconceptions or different interpretations of the questions in the questionnaire, a pre-administration group discussion was held with the group of farmers, and each of the close-ended questions was well described before the farmers were requested to fill in the questionnaire. Recall bias is considered negligible, as most of the questions in the questionnaire are straightforward on static aspects and practices carried out on a daily basis.

Conclusion

The risk factors identified in this study are mostly related to inadequate biosecurity practices. The low level of biosecurity could be one of the reasons that led to the incursion of ASF into the domestic pig farms in the states of Perak and Melaka within a short period of time. Thus, it is crucial to implement good biosecurity to prevent contact between infected pigs and ASFV-contaminated materials with naïve pigs, which causes ASF outbreaks in domestic farms. As an early step, the adoption of MyGap system, which directly heightens the biosecurity system in livestock farms, shall be made mandatory for all the pig farms to minimize ASF risk. Additionally, adaptation of the modern pig farming system, which includes the conversion of open houses to closed houses, shall be expedited to not only manage the ASF risk but also the risk of other swine diseases. Therefore, to enhance and improve the implementation of good biosecurity practices in the domestic pig farms, good engagement between the authority, and the farmers is important. Proposal and implementation of new systems or measures, such as the MyGap system and the modern pig farming system, to manage and minimize ASF risk in a resource-constrained condition, especially in the midst or post-ASF outbreak period, might not be well accepted by the pig farmers or the industry. However, it can be achieved holistically through continuous awareness campaigns, engagement, and cooperation between the authority, farmers, stakeholders, and the private sector to ensure the new system or measures not only address the ASF risk but also contribute to productivity along the pig value chain.

Data Availability Statement: Availability of data analyzed during this study is available from the correspondence author on reasonable request.

Ethics Committee Approval: Ethical committee approval was received from the Committee for Research Involving Human Subject of Putra Malaysia University (Approval no: JKEUPM-2022-288, Date: July 2, 2022).

Informed Consent: Verbal informed consent was obtained from the participants who agreed to take part in the study.

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