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Studies on risk assessment of transboundary swine diseases in Japan

日本における越境性豚伝染病のリスク評価に関する研究

Satoshi Ito

Notes

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Abbreviations

ASF:	African swine fever
ASFV:	African swine fever virus
CI:	Confidence interval
CSF:	Classical swine fever
CSFV:	Classical wine fever virus
DP:	Domestic pig
EU:	European Union
FMD:	Foot-and-mouth disease
GLOBCOVER:	Global land cover vegetation
IPPAP:	Illegal pork products being brought in international air passengers' luggage
JAQS:	Japan Animal Quarantine Service
JPPA:	Japan Pork Producers Association
LSL:	Labor Standard Law
MAFF:	Ministry of Agriculture, Forestry and Fisheries
MLIT:	Ministry of Land, Infrastructure, Transport and Tourism
OIE:	World Organisation for Animal Health
PPAP:	Pork products being brought in international air passengers' luggage
QAH:	Quality of available habitat
SDE:	Standard Deviational Ellipse
VLA:	Veterinary Laboratories Agency
WAHIS:	World Animal Health Information database interface
WB:	Wild boar

Preface

Classical swine fever (CSF) is a contagious viral swine disease which can cause enormous economic loss in the pig industry worldwide [1]. The disease is caused by infection with CSF virus (CSFV), which belongs to the genus *Pestivirus* (family *Flaviviridae*). Infected animals exhibit various symptoms and lesions, which vary in severity and may result in high mortality depending on the acute and chronic forms of the disease [2]. In the last decade, several outbreaks of CSF have been reported worldwide: 14 in Asia, 5 in Europe, 4 in Latin America, 3 in the Caribbean, and 1 in Africa [3].

African swine fever (ASF), which is caused by infection with ASF virus (ASFV) belongs to the *Asfarviridae* family, is a highly contagious hemorrhagic viral disease that infects domestic and wild pigs; like CSF, it can result in substantial economic losses in pig production [4]. At present, an approved vaccine with high efficacy does not exist for ASF; therefore, where the introduction of ASF is possible, detection and culling practices must be implemented even more thoroughly than in CSF cases. Clinical signs and mortality rates vary according to the virulence of ASF and the types/species of pig infected [4]. In recent years, the prevalence of ASF has increased globally, with outbreaks reported in sub-Saharan Africa, Central and Eastern Europe, and Asia. In 2007, ASF was transmitted to Georgia, potentially via contaminated food waste used as pig feed [5], from where it spread to the European Union (EU) in 2014, with outbreaks reported in nine EU territories. After the first confirmed ASF outbreak in China in 2018, the disease spread rapidly inside and outside of the territory; currently, in Asia, ASF outbreaks have been reported in Cambodia, China, Democratic People's Republic of Korea, Hong Kong, Indonesia, Lao People's Democratic Republic, Myanmar, the Philippines, Republic of

Korea, Timor-Leste, and Vietnam [6,7].

Territories with land borders tend to suffer from transboundary animal diseases, whereas Japan, an island territory with well-established quarantine measures, has seen a limited number of disease outbreaks. However, foot-and-mouth disease (FMD) emerged in Miyazaki prefecture in 2010 and severely damaged livestock production. The results of phylogenetic analyses indicated that the pathogenic virus isolated in Miyazaki prefecture was closely related to viruses isolated in nearby territories [8]; thus, the disease apparently entered Japan through the movement of people or infected materials from these affected territories. In Japan, the outbreaks were contained in 3 months using a preventive vaccination and the disposal of approximately 290,000 animals [8].

Eight years after the last outbreak of FMD, CSF reemerged in Japan in September 2018; this was the first CSF outbreak since the World Organization for Animal Health gave Japan CSF-free status in 2007 [10]. The first CSF outbreak ever reported in Japan occurred in 1888 and subsequent outbreaks occurred repeatedly for more than a century [9]. Following the use of a live vaccine in Japan, the number of CSF infections decreased substantially, and the last CSF outbreak (before 2018) occurred in 1992. The 2018 spread of CSF in Japan had not been eradicated even one year after the initial disease notification due to the disease being spread mainly by wild boar; the infection continued despite an oral vaccination of wild boar was applied in the first 6 months after the initial notification [11]. During the outbreak, more than 150,000 pigs were slaughtered, and the preventive vaccination for domestic pigs was applied one year after the initial disease notification [11]. The results of phylogenetic analyses revealed that the strain of CSFV isolated in Japan in 2018 showed the highest homology with strains isolated in China and Mongolia in 2014 and 2015 [10], respectively. Therefore, similar to the outbreak of FMD

in 2010, the CSF outbreak in Japan in 2018 was likely to have been introduced from nearby affected territories.

The number of foreign tourists entering Japan increases every year, with tourists from Asia accounting for a large proportion of visitors to Japan [12]. Concurrent with the increase in foreign visitors to Japan, there has been a proportional increase in the number of confiscated animal products that may potentially contain infectious pathogens. In 2018, more than 85% of the animal products confiscated in Japan originated from other Asian territories [13]. Given that ASF has spread rapidly across the world, it is possible that ASFV-contaminated products could have been brought into Japan in large numbers. To prevent the introduction of contagious diseases from other territories, the Japanese Animal Quarantine Service has strengthened border controls by imposing penalty charges, increasing the number of detection dogs, and increasing the number of flights investigated; nevertheless, the risk of contamination remains because incoming travelers and imported commodities cannot all be investigated with 100% confidence.

The present thesis consists of three chapters. In Chapter I, a spatio-temporal analysis was conducted to obtain epidemiological information on the current epidemic of CSF in Japan. Our findings will help to assess the potential risk of disease spread among pig species, including domestic pigs and wild boar, and assist in implementing control measures effectively. In Chapter II, a quantitative assessment of the risk of ASF virus (ASFV) being introduced into Japan via pork products brought in air passengers' luggage was conducted. Identification of ASFV risks such as origin territories and destination airports in Japan will be useful for improving the risk management schemes and monitoring systems that aim to prevent the introduction of ASFV into Japan. In Chapter III, the risk of domestic pigs and wild boars in prefectures of Japan being exposed to

ASFV via pork products brought in air passengers' luggage was assessed. The results could be useful for reviewing the current risk reduction activities applicable for ASFV exposure in susceptible animals. The foci of this thesis are the current control measures used in Japan for contagious diseases in animals, which are investigated by epidemiological analysis of the CSF outbreak, and the efficacy of risk assessment for these diseases, which was investigated using a risk assessment for ASF.

Chapter I

Role of wild boar in the spread of Classical swine fever in Japan

Introduction

CSF is caused by infection with the CSFV, which belongs to the genus *Pestivirus*, family *Flaviviridae*. CSF is described by the World Organisation for Animal Health as a highly contagious febrile disease with potential for high mortality that causes enormous economic loss in the pig industry worldwide [9]. CSFV is a positive-sense, single-stranded RNA virus with a genome of approximately 12.3 kb, comprising one large open reading frame that encodes a polyprotein and flanked by 5'-untranslated region (5'-UTR) and 3'-untranslated region [14]. During virus replication, the polyprotein is processed by cellular and viral proteases into four structural and nine nonstructural proteins [15]. Outbreaks of CSF have been reported over the past decade in Asia (Bhutan, Cambodia, China, India, Indonesia, Republic of Korea, Lao PDR, Mongolia, Myanmar, Nepal, the Philippines, Thailand, Timor-Leste, and Vietnam), Europe (Latvia, Lithuania, the Russian Federation, Serbia, and Ukraine), Africa (Madagascar), the Caribbean (the Dominican Republic, Guatemala, and Haiti), and Latin America (Bolivia, Colombia, Ecuador, and Peru) [16]. Based on the amino acid sequence of the 5'-UTR and E2 which is one of the structural regions of the protein, CSFVs are classified into three genotypes (1, 2, and 3), and several subgenotypes (1.1–1.4, 2.1–2.3, and 3.1–3, and 3.1-3.4) [17,18]. The virulence of CSFV is categorized via a clinical score into highly virulent, moderately virulent, low virulent, and avirulent [19,20]. Although the CSFV genotype 2.1b isolated from Republic of Korea was highly virulent, the same genotype isolated in Mongolia was moderately virulent [21,22]. Moreover, the recently classified CSFV genotype 2.1d from China was moderately virulent compared to different variants and antigenicity from field strains identified in China in the past [23].

No notifications of CSF were reported in Japan since 1992, and the territory had an 11-year stretch of CSF-free status defined by the World Organisation for Animal Health (OIE) Terrestrial Animal Health Code since 2007. However, CSF reemerged in Japan in September 2018 in Gifu prefecture, which is located in the central part of the main island of Japan. Phylogenetic analysis revealed that the CSFV strain isolated in Japan in 2018 showed the highest identity in the complete E2 gene sequence with Chinese strains isolated between 2011 and 2015, and in the partial 5'-UTR sequence with strains isolated in China and Mongolia in 2014 and 2015 [10].

By the end of August 2019, a total of 39 CSFV outbreaks on pig farms in four prefectures and 1,071 cases in wild boar in seven prefectures have been reported [24]. Despite the implementation of intensive responses, including movement bans of domestic pigs, surveillance, and oral immunization of wild boar, new notifications of CSF cases in both wild boar and domestic pigs were being reported continually [25]. This might indicate that the pathogenic viruses were widely prevalent and persisted in wildlife around the affected area. As the Eurasian wild boar is also susceptible to CSFV, the circulation and persistence of CSFV among food animals and wildlife makes it difficult to carry out effective control measures for eradicating it in affected areas. Due to contact with infected animals and feeds contaminated with contagious pathogens in garbage dumped on the human sphere, naïve wild boar populations are often infected with CSFV [21,26-36]. Before the 1990s, CSF cases in wild boar were rare concerns as infection was detected rapidly due to the high virulence of circulating strains. However, disease detection appears delayed in the current epidemic due to infection with more moderately virulent strains [37]. As a consequence, there have been serious outbreaks of CSF in the wild boar population in Germany. During an outbreak of CSF in Germany from 1993 to

1998, an epidemiological field investigation confirmed that 59% of the primary cases in domestic pigs could be attributed to either direct or indirect contact with infected wild boar [29]. Virus characteristics and population size both can be considered critical factors for the persistence of CSFV, especially in wild boar populations [37]. It has been suggested that CSFV would be self-limiting within one year in populations of 2,000 wild boar, whereas it will persist and become endemic in a larger population [38]. In addition, the population density of wild boar has also been suggested as being a potential factor for the persistence of CSF, because more frequent turnover occurs in dense populations, which provides faster renewal of susceptible piglets that increases the chance that the virus will persist in the population [37]. Once the contagious viruses are transmitted to wildlife, specific control measures for wild boar will be needed to eradicate CSF in the affected area and to contain it more effectively.

The present study conducted a spatio-temporal analysis to obtain epidemiological information on current epidemics of CSF in Japan. Based on the official CSF reports on domestic pig farms and wild boar, notified in Gifu prefecture from September 2018 to June 2019, we assessed the direction of the spread of the disease and identified areas with high densities of notifications. In addition, to identify spatio-temporal aggregation of notifications and to characterize land cover vegetation in areas of disease aggregation, a clustering analysis was conducted, and obtained clusters were then overlapped with quality habitat map. The obtained information can be used to develop more effective disease control measures for application in both domestic pigs and wild boar.

Material and Methods

Data and data sources

Epidemiological data for the periods from September 9, 2018, to June 25, 2019, were provided by the Gifu Prefectural Government, which provided the dates and coordinates (latitude and longitude) of the notifications of CSF in domestic pigs and wild boar. A total of 743 CSF notifications, 16 outbreaks on domestic pig farms, and 727 cases in wild boar were confirmed by RT-PCR and/or ELISA tests in the laboratory [25]. As we focused on local transmission of CSFV, notifications of CSF in slaughterhouses or in facilities through which CSF-affected pigs had been transported were removed from the current study. Notifications of CSF in wild boar reported on the same day and location were regarded as one case.

Standard deviational ellipse analysis

Standard deviational ellipse (SDE) analysis is a tool that provides the orientation and shape of a distribution, as well as its location, and dispersion or concentration of the data [39]. It requires a single point that is used to define the standard deviational ellipse. The analysis was conducted to describe the trend and spatial characteristics of CSF notifications in the study area in ArcGIS 10.6.1 software (ESRI Inc., Redlands, CA, USA) following an approach similar to Fonseca et al. and Lu et al. [40,41]. The ratio (R) of the long and short axes was used to identify the degree of clustering (R>1) or dispersion (R = 1) [40,41]. To analyze temporal changes of CSF notifications, the study period was divided into three stages: (i) September to December 2018 (4 months), (ii) January to March 2019 (3 months), and (iii) April to June 2019 (3 months).

Multi-distance spatial cluster analysis

A multi-distance spatial cluster analysis tool in ArcGIS software version 10.6.1 was used to identify the maximum distance of the relationships between CSF notifications according to the guide on the manufacture's website [42]. In brief, the tool uses a common transformation of Ripley's k function, wherein the expected result with a random set of events is equal to the input distance. The transformation *L(d)* is given by the following formula:

$$\mathrm{L(d)} = \sqrt{\frac{A\sum_{i=1}^{N}\sum_{j=1,j\neq i}^{N}k(i,j)}{\pi N(N-1)}}$$

where *A* is the area, *N* is the number of events, *d* is the distance, and *k(i, j)* is the weight, in which it is 1 when the distance between *i* and *j* is less than or equal to *d* and it is 0 when the distance between *i* and *j* is greater than *d*. To analyze the spatial pattern of CSF notifications, Observed K values were compared to the Expected K values of a completely random spatial distribution of CSF notifications with 999 simulations, which is equal to confidence levels of 99.9%.

The Diff K values contain the Observed K values minus the Expected K values. In the present analysis, the Expected K values that yield the highest Diff K values were applied as the maximum distance for relationships between notifications of CSF outbreaks in Gifu prefecture.

Kernel density estimation analysis

Kernel density estimation is a non-parametric estimator for describing the spatial extent of a series of events [43]. In the current study, the kernel density tool was applied to explore the influence of the CSF notifications in the study area by calculating the density of CSF notifications in ArcGIS 10.6.1. A radius of 23 km based on results obtained from Ripley's k function, was applied as the maximum distance for significant

spatial association between CSF notifications. Kernel density estimation was divided into five categories according to the equal interval method.

Space-time cluster analysis

A space-time permutation technique was applied to examine the presence of space-time clusters in Gifu prefecture. The upper limit on the geographical size of the cluster was set as 23 km, the minimum time aggregation as 7 days, and the maximum temporal cluster size as 50% of the total study period (default setting) [44]. Monte Carlo process was implemented using 999 replications to test for the presence of candidate clusters ($P < 0.05$). Analyses were conducted in SaTScan software v9.6 (Kulldorff, Boston, MA, USA) [45].

Quality of available habitat (QAH) within space-time cluster area

CSF notifications within significant space-time clusters were overlaid on a QAH map to characterize land cover vegetation in areas of disease aggregation. The QAH map developed by Bosch et al. [46] is a cartographic tool previously suggested as a potential tool for managing ASF. Briefly, it is a standardized distribution map based on global land cover vegetation (GLOBCOVER) that quantifies QAH for wild boar [47]. The QAH map provides seven levels of QAH, namely (i) 0, “absent”; (ii) 0.1, “unsuitable”; (iii) 0.5, “worst suitable area”; (iv) 1, “suitable areas for food or shelter”; (v) 1.5, “suitable areas for food and shelter, but used mainly for one or the other”; (vi) 1.75, “suitable areas for food and shelter, but mainly used for food”; and (vii) 2, “suitable areas for both food and shelter.” In addition, the QAH map also differentiates between landscapes such as natural (mainly QAHs 2 and 1.5) and agricultural landscapes (QAHs 1.75 and 1), among others.

Results

Standard deviational ellipse analysis

A standard deviational ellipse analysis was applied to describe the directional trend and dispersion of CSF notifications in the study area throughout the study period. The study covered the period between September 2018 and June 2019, which was divided into three stages (September–December, January–March, and April–June). Figure 1 illustrates standard deviational ellipses and CSF notifications between September 2018 and June 2019 (Figure 1). To indicate the potential explanation for the directional trend of the CSF outbreaks, the ellipses were overlaid on a map of snowfall area in Gifu prefecture obtained from the National Land Information Division, Ministry of Land, Infrastructure, Transport and Tourism [48]. The findings showed that CSF notifications appeared to move northeast while spreading along the border of the snowfall area.

Multi-distance spatial cluster analysis

The multi-distance spatial cluster analysis was applied to explore the maximum distance between cases of CSF notifications. The results indicated that 23 km was the maximum distance of the significant spatial association between CSF notifications in Gifu prefecture. The obtained maximum distance was used in the subsequent analyses.

Kernel density estimation analysis

The kernel density estimation analysis was applied to describe the spatial distribution of the CSF notifications. The analysis showed that the highest density of CSF notifications was located in the southern part of Gifu prefecture (Figure 2) with further expansion to the east. Among the 16 CSF-positive farms, 37.5% were located in areas with very high or high density of notifications, 31.25% in areas of medium density and

31.25% in areas of low density. Moreover, most of the non-affected domestic farms were located in areas with very low density of notifications (80%), followed by areas with low density (20%). The analysis revealed that CSF-positive farms were located in areas with higher density of notifications, whereas the non-affected farms tended to locate in areas with low density.

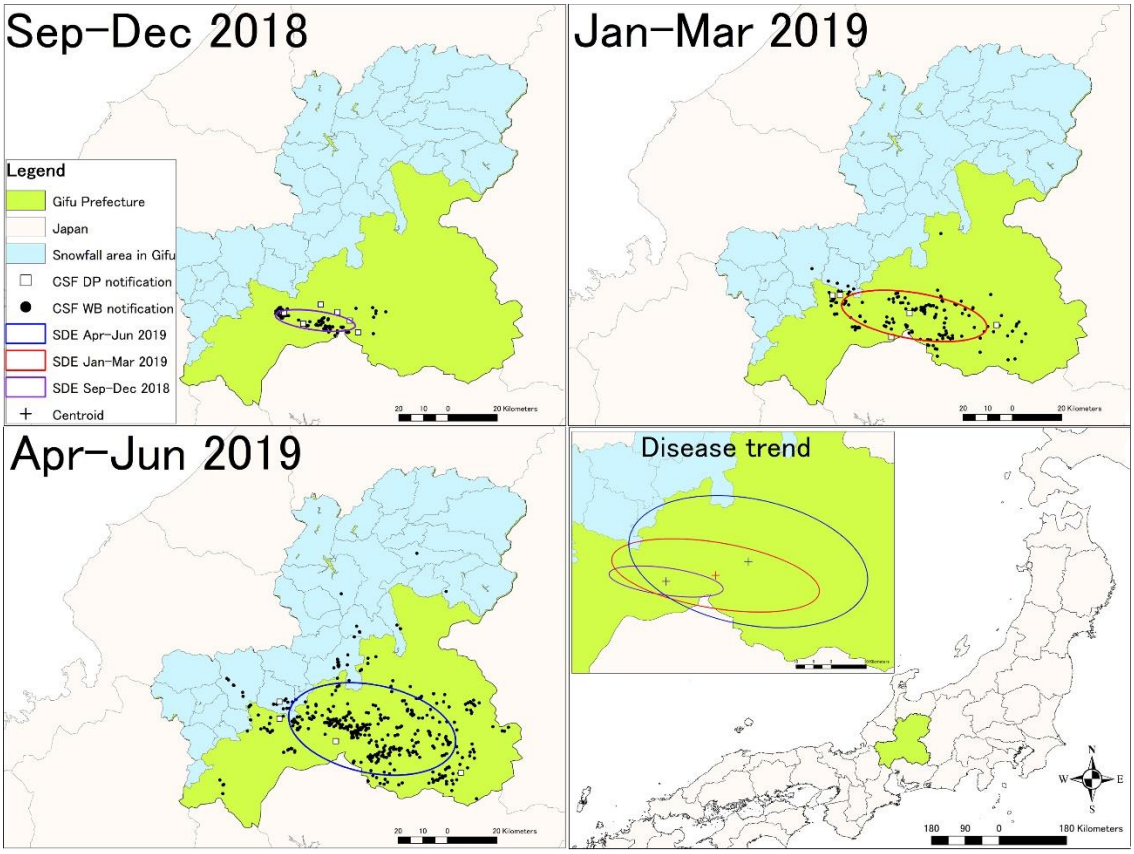


Figure 1. Directional distribution of CSF notifications from September 2018 to June 2019. Standard deviational ellipses (SDEs) identified between September and December 2018, between January and March 2019, and between April and June 2019. Ellipses were overlaid with CSF notifications distinguishing domestic pig (DP) (square) and wild boar (WB) (circle). Ellipses with centroids were combined to indicate the directional trend of the CSF outbreaks.

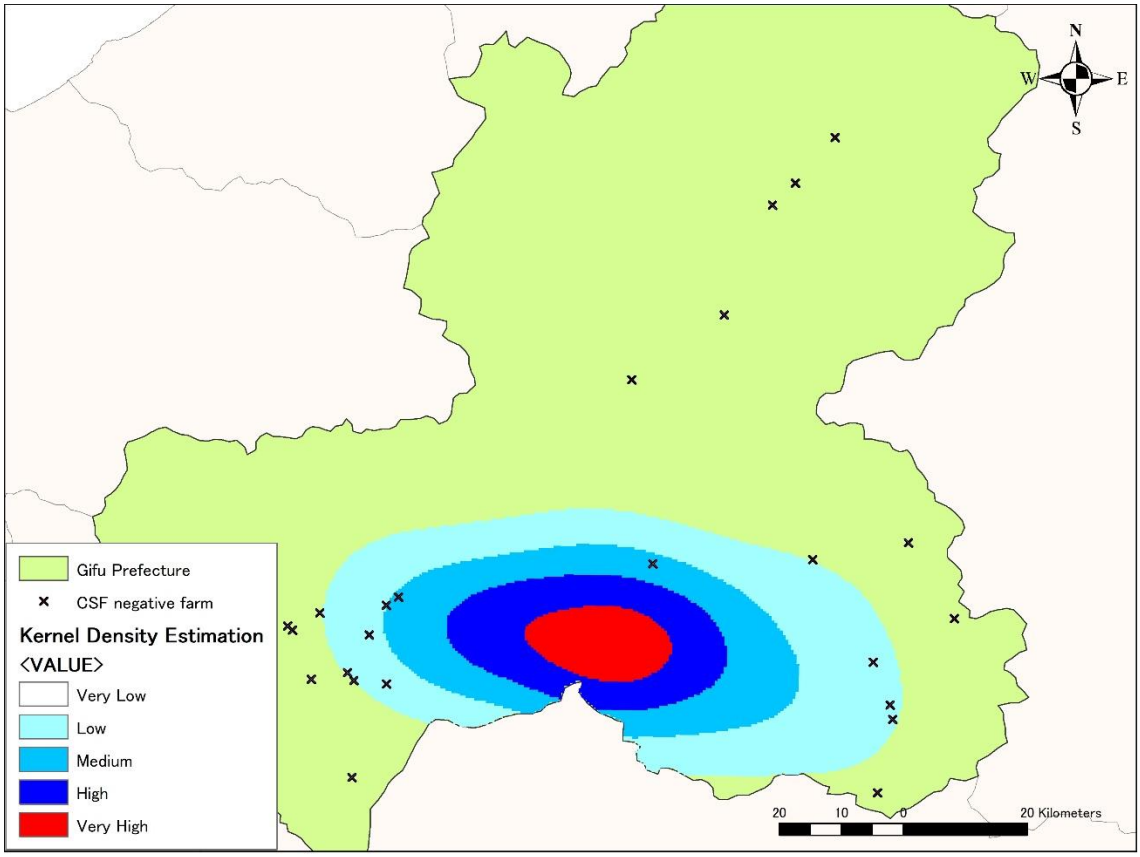


Figure 2. Density of CSF notifications in Gifu prefecture. The heat map illustrates the estimated kernel density of CSF notifications (notifications/km^2) from very high (red) to very low (transparent). Each coloured area indicates the density of CSF notifications per square kilometer: very high (>0.400), high (0.300–0.399), medium (0.200–0.299), low (0.100–0.199), and very low (<0.100). The highest density of CSF notifications was located in the southern part of Gifu prefecture. A very low density of CSF notifications was located in other areas of the prefecture. Locations of CSF-negative pig farms are represented by crosses.

Space-time cluster analysis

The space-time permutation analysis was applied to analyze the space-time patterns of the CSF notifications. The analysis identified two significant space-time clusters ($P < 0.05$) in Gifu prefecture during the study period. Cluster 1, which had a radius of 12.12 km, covered September 9, 2018, to January 13, 2019, and contained 83 notifications, including 4 outbreaks on domestic pig farms. Cluster 2 had a radius of 19.79 km, spanning the period from February 11, 2019, to May 19, 2019, and contained 198 notifications, including three outbreaks in domestic pigs (Table 1).

QAH within space-time cluster area

In order to characterize the land cover vegetation within two significant space-time clusters, the clusters were overlaid with a QAH map. The results showed different patterns between cluster 1 and cluster 2 (Figure 3). In cluster 1, 50.6% of CSF notifications were reported in areas at QAH 1, while 31.3% were reported in areas at QAH 1.5 and 18.1% were reported in areas at QAH 2 (Table 2). In cluster 2, 22.7% of CSF notifications were reported in areas at QAH 1, 52.5% were reported in areas at QAH 1.5, 2.5% were reported in areas at QAH 1.75, and 22.2% were reported in areas at QAH 2 (Table 2).

The CSF notifications within clusters 1 and 2 occurred within habitats that included rainfed croplands (QAH 1), a closed (>40%) needle-leaved evergreen forest (>5 m) (QAH 1.5), a mosaic of cropland (50%–70%) and vegetation (grassland/shrubland/forest) (20%–50%) (QAH 1.75), a mosaic of vegetation (grassland/shrubland/forest) (50%–70%) and cropland (20%–50%) (QAH 2), closed (>40%) broadleaved deciduous forest (>5 m) (QAH 2), and closed to open (>15%) mixed broadleaved and needle-leaved forest (>5 m) (QAH 2).

Although different patterns of land cover vegetation were observed between clusters 1 and 2, nearly 50% of CSF notifications within cluster 1 and more than 75% within cluster 2 were notified in QAH 1.5–2, which provides the greatest opportunities for food and shelter for wild boar.

Table 1. Observed and expected notifications, duration, start and end dates, and radius of each space-time cluster detected ($P < 0.05$) in CSF notifications in Gifu prefecture.

Cluster	Observed notifications	Expected notifications	Duration (Days)	Start date	End date	Radius (km)
1	83	17.34	124	2018/9/9	2019/1/13	12.12
2	198	131.87	98	2019/2/11	2019/5/19	19.79

Table 2. Quality of availability habitats (QAH) of CSF notifications within the two identified space-time clusters.

QAH Category	Land Cover	Cluster 1			Cluster 2		
		DP (*n*)	WB (*n*)	Total (*n*) (%)	DP (*n*)	WB (*n*)	Total (*n*) (%)
1.0	Rainfed croplands	4	38	42 (50.6)	0	45	45 (22.7)
1.5	Closed (>40%) needleleaved evergreen forest (>5m)	0	26	26 (31.3)	3	101	104 (52.5)
1.75	Mosaic cropland (50-70%) / vegetation (grassland/shrubland/forest) (20-50%)	0	0	0 (0.0)	0	5	5 (2.5)
2.0	Mosaic vegetation (grassland/shrubland/forest) (50-70%) / cropland (20-50%)	0	0	0 (0.0)	0	14	14 (7.1)
2.0	Closed (>40%) broadleaved deciduous forest (>5m)	0	1	1 (1.2)	0	0	0 (0.0)
2.0	Closed to open (>15%) mixed broadleaved and needleleaved forest (>5m)	0	14	14 (16.9)	0	30	30 (15.2)
	Total	4	79	83 (100.0)	3	195	198 (100.0)

DP: domestic pig. WB: wild boar. *n*: the number of notifications

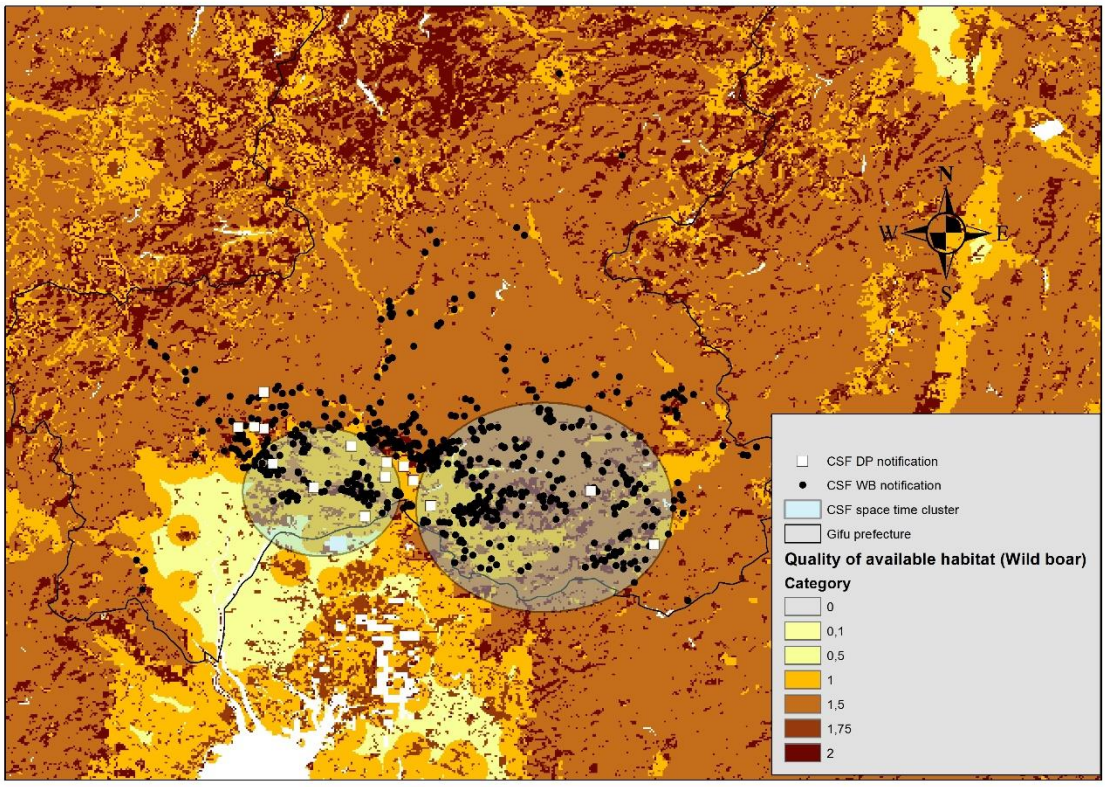


Figure 3. Locations of the significant space-time clusters of CSF notifications ($P < 0.05$) in Gifu prefecture overlaid on a map of the quality of available habitat (QAH) levels for wild boar. Graduated colors indicate the quality of habitat availability from darker colors (areas with better quality of habitat availability) to lighter colors (areas with worse-quality of habitat availability).

Discussion

From 2018 until August 2019, all notifications of CSF outbreaks in Japan have been made in Gifu prefecture as well as in the surrounding four prefectures. A total of 1,110 notifications had been reported, so far, with 1,071 affecting wild boar and 39 affecting domestic pig farms. The continuous notification of CSF in the area might have been attributed to wide spread of the virus within wild boar populations favored by free animal movements, as well as to the emergence of epidemiologically related domestic pig farms. To prevent the disease spreading in wild boar, control measures including (i) fencing to restrict animal movements, (ii) hunting activities for active monitoring and to reduce susceptible populations, and (iii) disseminating baits for oral immunization, were implemented. However, the efficacy of these strategies has not been confirmed. Therefore, we conducted a spatio-temporal analysis to obtain epidemiological information of the spread of CSF in Gifu prefecture. Results from this analysis could help to increase our understanding of the current CSF epidemic and to contribute strategies for the containment of the disease in domestic pigs and wild boar.

Japan is an island territory which has achieved the status of freedom from several contagious animal diseases by implementing adequate control measures that took advantage of the territory's geography. Nevertheless, Japan has imported outbreaks of contagious animal diseases from neighboring territories. In 2010, there was an outbreak of FMD in Miyazaki prefecture in the southern part of Japan, which caused extensive losses in animal husbandry [8]. According to the high degree of sequence homology between an original virus isolated in Japan and viruses that were circulating widely in East Asia, it was suspected that the FMD virus might have been introduced via movement

of people or commodities from East Asia [8]. The high homology of genetic sequences between the CSFV isolated in Japan and viruses prevailing in China suggests that the infectious CSFV may have been introduced from China [10]. Potential factors that could have contributed to disease introduction include easy access from the international airport to the affected area, which has regular and direct flights from China, and the relatively high population density of Chinese people in the affected area.

In the present study, standard deviational ellipse analysis was conducted to measure the standard distance of CSF notifications. Shifting the centroids of identified ellipses indicated that the disease notification has spread in a northeast direction. Overlaying the three identified ellipses with a map of snowfall area in Gifu prefecture revealed that the disease spread along the border of the snowfall area. In the south of Gifu prefecture, there is a widespread area of flat land with field crops or animal farms, residential areas, and forests surrounded by mountains to the north. As suggested by other authors [49,50], wild boar do not move to the snowfall or high mountain areas. Therefore, mountains could have acted as an effective geographical barrier to limit wild boar movements and guide the direction of the spread of CSF.

Another concern regarding the spread of the disease is the potential for it to jump to remote areas. During the epidemic, CSFV infections were confirmed on 7 farms that were geographically distant from, but epidemiologically linked, to the farms affected by CSFV (i.e., run by the same owner, supported by the same husbandry company, etc.) [25]. Given the potential for transmission of the virus between pigs on any farms or from wild boar near that farm, the epidemiologically related farms may further expand the spread of disease. This “disordered” spread of disease could affect the accuracy of spatio-temporal analysis by overestimating the maximum distance of significant spatial

association between notifications. During the FMD epidemic in Miyazaki, the disease was confirmed 70 km away from the zone of movement restriction, which could have been caused by vehicle transportation [8]. Unexpected occurrences of disease in epidemiologically related farms would require reviewing farm biosecurity measures, as well as disease monitoring protocols.

In the present study, the results of the multi-distance spatial cluster analysis revealed that the maximum distance of relationship between CSF notifications was 23 km. Because of the small number of CSF outbreaks on domestic pig farms, we estimated the maximum distance of the relationship between notifications of domestic pigs and wild boar. This assumption could have influenced our estimated distance resulting in overestimation due to long distance spread observed on domestic pig farms. Nevertheless, similar approaches have studied another transboundary animal disease, ASF, which shares hosts and most of the transmission mechanisms with CSF [44,51,52]. When comparing our results with other studies, the estimated distance (23 km) was similar to that obtained for notifications of ASF in domestic pigs (15 km) and wild boar (25 km) in Sardinia [44]. This finding may be useful for setting the range of effective surveillance and control zones in the affected area.

The application of cluster analysis to identify areas with significant spatio-temporal aggregation of the ASF outbreaks in Sardinia from 2004 to 2013 indicated four clusters, the largest of which had a radius of 30 km [52]. This does not correspond with the results of another report that identified one cluster with a radius of 3 km in the same area [44]. As discussed in Iglesias et al. methodological differences could have led to the discrepancy [44]. In present study, because of the small number of CSF outbreaks in pig farms, we could not identify the maximum distance for the relationship between

notifications of CSF in pigs alone, but we were able to do it by considering pigs and wild boar together. The discordance between the findings of the two spatio-temporal analyses in Sardinia may suggest that by using mixed data for two species in the present study, we may have overestimated the distance of the spread of disease compared to true distance of transmission in each of the two species. However, we believe that this uncertainty would be acceptable for setting the monitoring area with high efficacy. Thus, these findings may be useful for setting the range of an effective surveillance and control zone. Data on wild boar cases consisted of animals found dead and/or captured during surveillance activities. Many of wild boar were captured during active surveillance activities by setting traps and conducting hunting activities. Considering that most of the reported wild boar cases were located close to human habitats, the wild boar capture area may have been biased. Therefore, the disease could be wider spread in the area than what has been reported in official notifications, and the identified clusters could have had a shorter radius. Ideally, active virologic surveys should be intensively implemented to decrease the reporting biases by providing more samples to detect low levels of prevalence [53,54]. The Gifu Animal Health Administration has authorized hunting activities to reduce the number of susceptible, as well as potentially infected, individuals. Hunters are a critical group for implementing population control and proper disposal of wild boar carcasses.

According to the investigative report of the affected farms, there were some factors that might have increased the risk of CSFV introduction into affected farms, including (i) improper preparedness against invasion of wild or small animals into farms; (ii) imperfect clothing and boot changes in farms and pig pens, or disinfection of those materials; and (iii) inadequate vehicle disinfection [25]. To prevent contact among each

of the hosts, in addition to raising awareness of disease among farmers and hunters, it is important to improve biosecurity measures in pig farms against CSFV as well as other infectious diseases.

Finally, we analyzed the QAH level of areas within the two identified clusters to characterize land cover vegetation in areas of disease aggregation. According to Bosch et al, a QAH 1 level corresponded to suitable areas for food or shelter for wild boar (mainly agricultural landscapes) [46]. In cluster 1, 50.6% of CSF notifications were reported in areas at QAH 1, whereas in cluster 2, 22.7% of CSF notifications were reported in areas at QAH 1. Considering that frequent direct and indirect contact is likely to occur between both hosts, contagious viruses in wild boar could be transmitted to pigs in the farms due to insufficient biosecurity in the affected farms since wild boar was the suspected source of infection on 80% of affected domestic pig farms in Gifu prefecture during the studied epidemic [25,54]. On the other hand, almost 50% of CSF notifications within cluster 1 and over 75% within cluster 2 were associated with QAH 1.5–2, which mainly corresponded to natural landscapes. These natural areas provided the greatest opportunities for food and shelter for wild boar. In the case of ASF, it has been reported that wild boar can transmit the disease efficiently at local levels within their own population [44,46]. Furthermore, De la Torre et al. suggested that the spread of ASF in Europe was driven by contact between animals from different populations that moved short distances [55]. Although ASF is caused by another virus, given that wild boar play an important role in both diseases, it is plausible to assume that CSF also could have expanded through contact between individual wild boar. Therefore, it would be critical to control wild boar populations and manage wild boar carcasses adequately from the environment to reduce habitat contamination.

Interestingly, the QAH map could also identify routes of CSF introduction or spread, mediated by wild boar, through vegetation or travel corridors. Travel corridors are either unbroken vegetation corridors or patches of habitat that enable animals to travel securely from one habitat to another [46]. These patches of habitat and vegetation corridors could be used as strategic points of vaccination where oral baits could be placed. In Gifu prefecture, the vegetation is composed mainly of broadleaved evergreen and broadleaved deciduous forests, which provide suitable habitat for wild boar [56,57]. Given that the composition of the vegetation in Gifu prefecture is common throughout Japan, it is likely that the disease could spread similarly to other prefectures.

It should be noted that vegetation types and wild boar behavior could vary among geographical features. For example, mountains usually have gentle slopes in Germany, whereas Japanese mountains tend to have precipitous slopes [40]. These topographical differences may require different approaches for control of wild boar populations.

Almost one year has passed since the first notification of the CSF outbreak in Japan, and the spread of the disease has been confirmed mainly in wild boar. Fortunately, CSF outbreaks on domestic pig farms have been limited. Nevertheless, the potential risk of CSF introduction on farms could be high due to limited biosecurity, high number of wild boar cases in the area, and difficulties in implementing disease control measures in wildlife [25]. The results from this study provide information on the current epidemic, which may help improve current approaches for controlling CSF in Japan. Information on the direction and distance of disease spread could help with the implementation of control measures by modifying the area for control and surveillance zones or identifying specific locations for increasing efforts of oral immunization.

Given the potential risk of the ASF introduction from neighboring territories, we

should summarize and disseminate the lessons learned from the current CSF outbreak to achieve the protection of ASF invasion or rapid containment of its occurrence even if it occurred.

Summary

Since September 2018, nearly 900 notifications of CSF have been reported in Gifu prefecture (Japan) affecting domestic pig and wild boar by the end of August 2019. To determine the epidemiological characteristics of its spread, a spatio-temporal analysis was performed using actual field data on the current epidemic. The spatial study, based on standard deviational ellipses of official CSF notifications, showed that the disease likely spread to the northeast part of the prefecture. A maximum significant spatial association estimated between CSF notifications was 23 km by the multi-distance spatial cluster analysis. A space-time permutation analysis identified two significant clusters with an approximate radius of 12 and 20 km and 124 and 98 days of duration, respectively. When the area of the identified clusters was overlaid on a map of habitat quality, approximately 82% and 75% of CSF notifications, respectively, were found in areas with potential contact between domestic pigs and wild boar. The obtained results provide information on the current CSF epidemic which is mainly driven by wild boar cases with sporadic outbreaks on pig farms. These findings will help implement control measures in Gifu prefecture.

Chapter II

Quantitative risk assessment of African swine fever virus introduction to Japan via pork products brought in air passengers' luggage

Introduction

ASF is one of the most important transmissible infectious diseases in animal health and is caused by infection with the ASFV. ASF is classified as a List A disease in the Old Classification of Diseases Notifiable to the OIE, indicating that it can potentially have serious socio-economic and public health consequences and is of major importance in the international trade of animals and animal products [58].

Direct contact with contaminated fluids or excretion is the main route of ASF spread on a farm, on the other hand, indirect contact with contaminated fomites and feed on infective pork products are other important routes of disease spread over long distances [55,59]. There is currently no effective vaccine against ASFV and it is known to be highly resistant to various environmental conditions–for instance, the viral infectivity can persist for longer than 1,000 days in frozen meat, 110 days in chilled meat, and 1 month in contaminated pig pens [59]. Therefore, early detection and culling and the restriction of pig movements in infected areas are critical to controlling this disease [60].

In recent years, ASF has become prevalent in many parts of the world, including sub-Saharan Africa, Central and Eastern Europe, and Asia. In 2007, ASF was transmitted to Georgia through the Poti Sea Port, potentially via contaminated food waste that was used as pig feed [5], from where it spread to neighboring territories, such as Azerbaijan, Armenia, and the Russian Federation. Since then, wild boar and domestic pig populations in these territories have been infected with ASFV, as well as in Belarus and Ukraine. In 2014, the disease spread to the north and west, reaching nine different EU territories: Lithuania (2014), Poland (2014), Latvia (2014), Estonia (2014), Czech Republic (2017), Romania (2017), Hungary (2018), Bulgaria (2018), and Belgium (2018) [61].

In August 2018, the first notification of an ASF outbreak in East Asia was reported on a domestic pig farm in Liaoning Province, China. Genetic analysis based on the partial sequence of the ASFV p72 gene indicated that the outbreak isolate, ASFV-SY18, belonged to the genotype II group and had an identical nucleotide sequence to the Georgian (Georgia 2007/1), Russian (Krasnodar 2012 and Irkutsk 2017), and Estonian (Estonia 2014) isolates [62], suggesting that it was most likely introduced from epidemic areas in Europe. Since then, more than 130 outbreaks have been reported in China and approximately 1,000,000 domestic pigs have been slaughtered as at May 23, 2019 [63]. However, disease control has not yet been achieved, so the biggest concern to neighboring territories is the potential spread of ASF by human movement and trading with China, which have caused recent outbreaks in Vietnam [64]. Likewise, 30 provinces in Vietnam have reported the presence of ASF and more than 1,300,000 domestic pigs have been culled. Moreover, a positive sample from a domestic pig collected in a slaughterhouse in Hong Kong was recently found [65,66].

There are various potential ASFV introduction pathways into disease-free territories. For disease-free territories, legal or illegal importation of live pigs or pork products by vehicles are considered as important pathways [67]. Furthermore, territories sharing border with ASF-affected territories have potential risk that movement of ASF affected wild boar introduces the disease into their territories [55]. Considering that Japan is an island territory which does not share border with any territories and quarantine measures for live animal import at ports of entry are established, there are a negligible risk of ASFV introduction into Japan via vehicle or wild boar movements or via live pig imports, but might be a potential risk via pork products. The number of foreign tourists to Japan increases each year, with tourists from the Asian region accounting for a large

proportion of these visitors [68]. The number of confiscated animal products at airports is proportional to the number of foreign visitors, and in 2016, more than 80% of the confiscated animal products originated from Asian region, with 42% coming from China, 11.4% from Taiwan, and 10.1% from Vietnam [69]. Pork products contaminated with ASFV that were being illegally carried in international passengers' luggage have been detected at border controls in several ASF-free territories, including Republic of Korea, Taiwan, Thailand, Australia, and Japan [70-74]. As of April 2, 2019, two isolations of ASFV from pork products brought from mainland China into Japan were reported [74], indicating that passengers' luggage could be a potential route of ASFV introduction to this territory. The Japan Animal Quarantine Service (JAQS) plays an important role in preventing the incursion of animal diseases [75]. However, it is no easy task to confiscate all animal products that are illegally brought into airports. Therefore, limitations of the current quarantine systems, as well as a failure to detected illegally transported animal products could exacerbate the potential risk of foreign animal diseases being introduced to Japan.

Japanese authorities are concerned about the economic losses and animal health impacts that would result from the arrival of animal transboundary infectious diseases. In 2010, there was an outbreak of foot and mouth disease in Miyazaki prefecture, Japan, which affected a total of 292 farms and resulted in nearly 290,000 animals being culled (including vaccinated animals) [76]. This outbreak took 3 months to be contained and had an estimated economic impact of approximately 235 billion JPY [77]. Considering that there are 9,189,000 head of pigs in Japan [78], an outbreak of ASF would cause serious losses to the swine industry. Furthermore, it may also affect wild boar populations, which are considered to have played an important role in the spread of this disease in Europe

[79]. The Biodiversity Center of Japan estimated that there are approximately 220,000 to 1,200,000 wild boars living across a broad area of Japan [80], indicating that it would require tremendous amounts of money and time to eradicate the disease from Japan should it spread to these populations.

The aim of the present study was to quantitatively evaluate the risk of ASFV introduction to Japan via pork products being brought in international air passengers' luggage (PPAP). The results from the evaluation and spatial characterization of the risk of ASFV introduction could be used to improve the effectivity of preventive measures already in place. The Japanese quarantine system relies heavily on border controls at ports of entry. Therefore, this study will help to focus available resources on specific airports and flights according to the estimated risk of ASFV entry.

Materials and Methods

A quantitative stochastic risk assessment model was developed to estimate the annual probability of ASFV introduction to each international airport in Japan via PPAP based on information obtained from relevant websites, public databases, published literature, and expert opinion. The risk model was developed using @RISK 7.6 (Palisade Corporation, Newfield, NY, USA) in Microsoft Excel 2013® and 10,000 iterations were run using the Latin hypercube sampling method. In the current analysis, 214 international commercial flights traveling from 47 origin territories to 31 destination airports in Japan were considered as potential routes of ASFV introduction to Japan via PPAP, information on which was obtained for the period August 2015 to October 2017 from the Ministry of Land, Infrastructure, Transport and Tourism (MLIT) database [81]. As PPAP include both legal and illegal pork products, the legal importation of pork products to Japan requires specific certification of inspection provided by the Government of the territory of origin [82], we assumed that legal pork products would not be contaminated with ASFV. Therefore, we focused only on the risk of illegal PPAP (IPPAP) in the current analysis. A dataset on confiscated items was obtained from the JAQS database [69], and information about ASF disease status and number of susceptible pigs in each territory of origin was obtained from the OIE-WAHIS (World Animal Health Information database interface) as at April 5, 2019 [61] and FAOSTAT 2018 [83], respectively.

Model structure

The current risk model was developed following an approach similar to [84], who assessed the risk of ASFV and CSFV introduction to the U.S. via swine products carried in air passengers' luggage. In this quantitative model, the risk of introduction

followed a binomial process according to the formula:

$$\mathrm{P}(x \geq 1) = \sum 1 - (1 - Pio)^{V_od}$$

where $V_{_od}$ is the assumed weight (kg) of PPAP brought into each destination Japanese airport (d) by international commercial flights from each origin territory (o), P_{io} is the estimated probability of at least 1 kg of PPAP being contaminated with ASFV from each origin territory, and $\sum$ refers to the probability of entry considering all of the different international commercial flights. Thus, the model consists of two main components: (a) the quantity of PPAP brought into Japan in kg and (b) the probability of these PPAP being contaminated with ASFV. All input values, parameterizations, and references are presented in Tables 3 and 4 and are explained in the following sections. The structure of model is described in Figure 4.

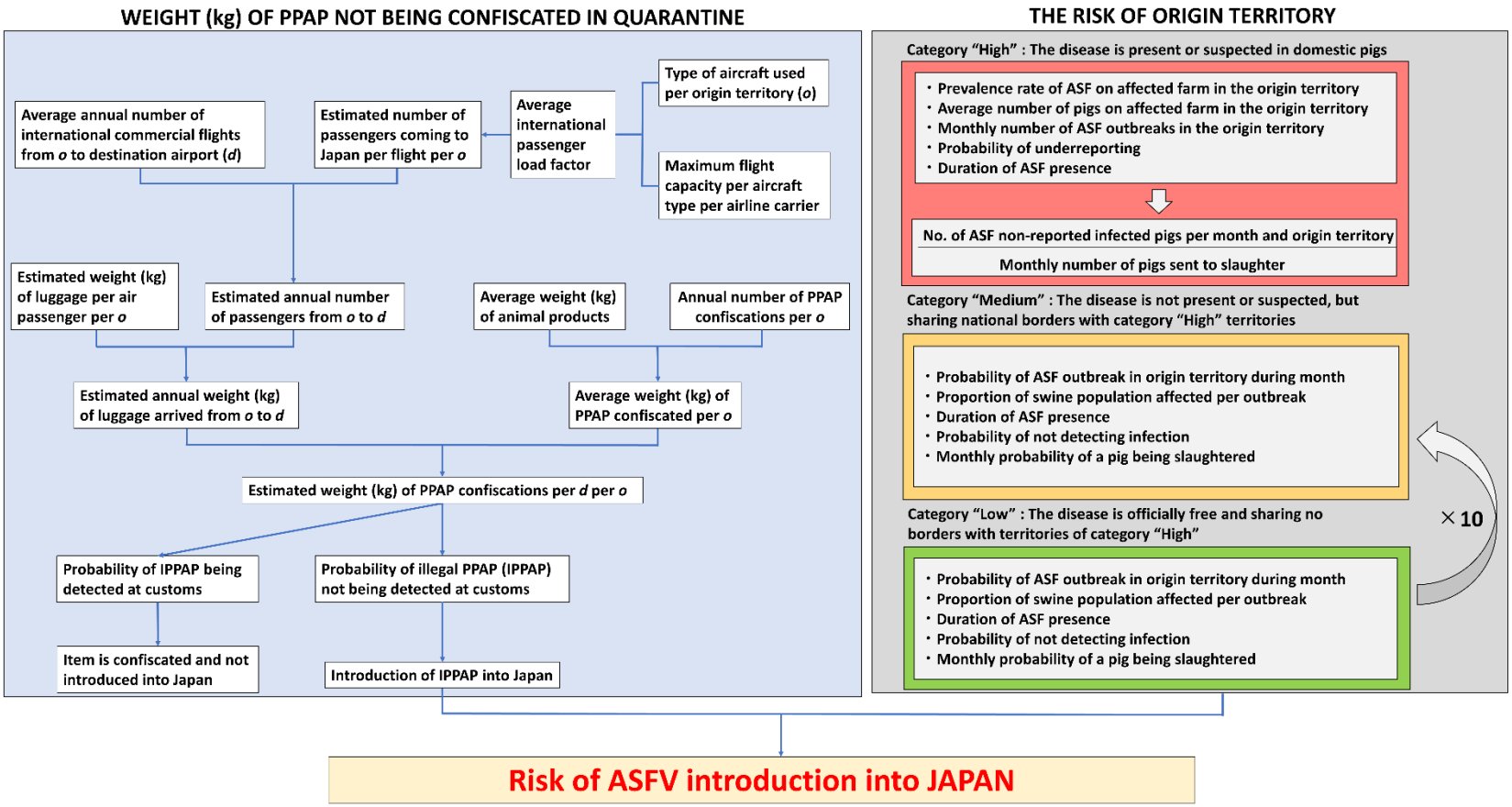


Figure 4. The structure of the model to estimate the quantity of pork products brought in air passengers' luggage (PPAP) in kg which is introduced into Japan. The origin territories are classified as three categories of high, medium and low risk considering the situation of ASF.

Table 3. Description and parameterization of model inputs for estimation of the quantity (kg) of illegal pork products brought in air passenger's luggage (IPPAP) introduce into Japan per origin territory and destination airport

Notation	Definition	Parameterization	Values	Reference/ Source
$N_{f\text{-}ann_od}$	Annual number of international commercial flights coming to Japan per origin territory (*o*), per destination airport (*d*)	Normal (μ ,α)		[85]
C_{p_a}	Capacity of air passengers per aircraft type	(min, max)		[86]
$A_{_o}$	Type of aircraft used per *o*			[87]
C_{f_o}	Flight capacity coming to Japan per *o*	Normal (μ ,α)		Assumption based on fourteen airline carriers [88-102]
LF	Average international passenger load factor			[103]
N_{p_of}	Number of passengers coming to Japan per *o*, per flight (*f*)		C_{f_o}*LF	
$N_{p\text{-}ann_od}$	Annual number of passengers coming to Japan per *o*, per *d*		$N_{f\text{-}ann_od}$*N_{p_of}	
V_{lal_po}	Weight (kg) of luggage allowance per air passenger, per *o*	Pert (min, most likely, max)		Checked luggage allowance of 14 most important airline carriers listed above
$V_{l\text{-}ann_od}$	Annual weight (kg) of luggage coming to Japan per *o*, per *d*		$N_{p\text{-}ann_od}$*V_{lal_po}	
$V_{l\text{-}ann_o}$	Annual weight (kg) of luggage coming from *o* to Japan		$N_{p\text{-}ann_o}$*V_{lal_po}	
Pro_{vl_od}	Proportion of weight (kg) of luggage from each *o* to each *d*		$V_{l\text{-}ann_od}$/$V_{l\text{-}ann_o}$	
V_c	Individual weight of each confiscated animal product	Mean		[104]

$N_{c\text{-}ann_o}$	Annual number of confiscated pork products brought in international passengers' luggage per *o*	Mean		[105]
$V_{c\text{-}ann_o}$	Annual weight (kg) of confiscated PPAP per *o*		V_c*$N_{c\text{-}ann_o}$	
$V_{c\text{-}ann_od}$	Annual weight (kg) of IPPAP per *o*, per *d*		$V_{c\text{-}ann_o}$*Pro_{vl_od}	
H_{non}	Probability of non-detection of ASFV contaminated IPPAP at airport quarantine	Triang (min, most likely, max)	Triang (0.2, 0.5, 0.9)	[84]
$V_{_od}$	Annual weight (kg) of IPPAP introduction into Japan without detection at quarantine per *o*, per *d*		$V_{c\text{-}ann_od}$*H_{non}/(1-H_{non})	

Table 4. Description and parameterization of model inputs for estimation of the probability of PPAP being contaminated with ASFV per origin territories (*o*), per month (*m*)

Notation	Definition	Parameterization	Values	Reference/ Source
N_{dp_o}	Domestic pig population in *o*	NA	Data on 2017	[106]
Pro_{slt_m}	Proportion of annual domestic pig population slaughtered for pork product per *m*	Pert (min, most likely, max)	Pert (0.1, 0.18, 0.25)	[106]
N_{slt_om}	Number of domestic pigs slaughtered for pork products in *o* per *m*	Pert (min, most likely, max)	N_{dp_o}*Pro_{slt_m}	
Pre_{v_o}	Prevalence rate of ASF on affected farms in *o*		Mean prevalence rate of ASF on affected farms in *o*	[6]
N_{af_o}	Average number of pigs on affected farm in *o*			[6]
N_{asf_om}	Monthly number of ASF outbreaks in *o*			[6]
H_{under}	Probability of notification underreporting	Pert (min, most likely, max)	Pert (0.2, 0.4, 0.6)	[107]
T_{inf}	Duration of ASF infection in months	Pert (min, most likely, max)	Pert (3, 11, 30)	Modified [107]
N_{nasf_m}	Potential number of nonreported ASFV infected domestic pigs per *m*		(Pre_{v_o}*N_{af_o}*N_{asf_om}*T_{inf})/H_{under}	
Pro_{imp_m}	Proportion of pigs improperly slaughtered for pork product per *m*	Uniform (min, max)	min = Pro_{slt_m}, max = 1	Modified [106]
$H_{slt\text{-}high_m}$	Probability of ASFV infected domestic pigs slaughtered in *m* in origin territory of "High" risk	Beta (α_1, α_2)	$\alpha_1 = N_{nasf}$*Pro_{imp_m}+1, $\alpha_2 = N_{slt_om}$ - (N_{nasf}*Pro_{imp_m})+1	
Pro_{asf}	Proportion of domestic pig population affected by ASF outbreak	Pert (min, most likely, max)	Pert (0, 0.002, 0.02)	

H_{med}	Probability of occurrence of an outbreak of ASF in origin territory of "Medium" risk	Pert (min, most likely, max)	Pert (0, 0.0022, 0.022)	Modified [107]
$H_{slt\text{-}med_m}$	Probability of ASFV infected domestic pigs slaughtered in *m* in origin territory of "Medium" risk	Pert (min, most likely, max)	$Pro_{asf}*H_{med}*T_{inf}*H_{under}$	
H_{low}	Probability of occurrence of an outbreak of ASF in origin territory of "Low" risk	Pert (min, most likely, max)	Pert (0, 0.00022, 0.0022)	Modified [107]
$H_{slt\text{-}low_m}$	Probability of ASFV infected domestic pigs slaughtered in *m* in origin territory of "Low" risk	Pert (min, most likely, max)	$Pro_{asf}*H_{low}*T_{inf}*H_{under}$	

Annual number of passengers coming to Japan ($N_{\text{p-ann_}od}$)

The annual number of passengers from each origin territory and at each destination airport ($N_{\text{p-ann_}od}$) was estimated by multiplying the annual number of international commercial flights coming to Japan per origin territory and destination airport ($N_{\text{f-ann_}od}$) by the number of passengers coming to Japan per origin territory and flight (f) ($N_{\text{p_}of}$). First, the value of $N_{\text{f-ann_}od}$ during the period from August 2015 to October 2017 was parameterized using a normal distribution. The value of $N_{\text{p_}of}$ was then estimated as the product of the flight capacity per origin territory ($C_{\text{f_}o}$) and the average international passenger load factor (i.e., seat occupancy) in 2017, which was obtained from the MLIT database [108]. The value of $C_{\text{f_}o}$ was estimated by obtaining information on the aircraft types that traveled between each origin territory and the seven most important Japanese airports (Narita International Airport, Kansai International Airport, Tokyo International Airport, Chubu Centrair International Airport, Fukuoka Airport, Naha Airport, and New Chitose Airport), which represented 94.7% of the total international commercial flights, from the travel booking website Expedia.com [87]. Information on the flight capacity for each aircraft type and airline carrier was then obtained from the SeatGuru website [86]. In particular, data were obtained for the 14 most important airline carriers, which represented 63% of the total international commercial flights to Japan from August 2015 to October 2017 and included Japan Airlines [99], All Nippon Airways [89], Cathay Pacific Airways [91], Eva Air [98], Air China [88], China Airlines [92], China Eastern Airlines [93], China Southern Airlines [94], Korean Air [101], Asiana Airlines [90], Delta Air Lines [95], United Airlines [102], Jeju Air [100], and Peach Aviation [109]. The value of $C_{\text{f_}o}$ was then parameterized using the normal distribution based on the estimated minimum, mean, and maximum flight capacities per

flight, which were estimated from the aircraft type and flight capacity data.

Weight of luggage allowance ($V_{\text{l-ann_}od}$)

The annual weight (kg) of luggage arriving in Japan per origin territory and destination airport ($V_{\text{l-ann_}od}$) was estimated as the product of the weight (kg) of luggage allowance per air passenger (p) per origin territory ($V_{\text{lal_}po}$) and the annual number of passengers to Japan per origin territory and destination airport ($N_{\text{p-ann_}od}$). The value of $V_{\text{lal_}po}$ was estimated at the regional level based on the free checked luggage allowance in economy class for the 14 most important airline carriers listed above. Since flights between the African continent and Japan were operated by either Ethiopian Airlines or EgyptAir, the weight of air passengers' luggage from the African region was estimated based on the free checked luggage allowance in economy class for both companies [96,97]. This resulted in the estimation of a minimum value, which corresponded to the weight of hand luggage alone, and a maximum value, which corresponded to the total weight of the hand luggage and the maximum free luggage allowance for each origin territory. The median values of these estimated free luggage allowances were then used as the most probable values. The value of $V_{\text{lal_}po}$ was parameterized using a pert distribution with minimum, most likely, and maximum values. The obtained data were used to estimate the proportion of the weight of air passengers' luggage that came from each origin territory to each destination airport ($\text{Pro}_{\text{vl_}od}$).

Annual weight of IPPAP ($V_{\text{c-ann_}od}$)

Under the current JAQS regulation, pork products that have specific certification of inspection by the Government of the territory of origin are allowed to be imported into Japan. Therefore, it was assumed that only IPPAP were potentially contaminated with ASFV. The annual weight of confiscated PPAP (i.e., IPPAP) coming from each origin

territory to each destination airport ($V_{\text{c-ann_}od}$) was estimated from information obtained from the JAQS database [69]. Annual information on the mean weight confiscation was available by referring the JAQS database. Information on weight was aggregated as animal products without pointing out any differences in weight between them. Therefore, it was assumed that the individual weight of PPAP was equivalent to the individual weight of confiscated animal products. Moreover, data on the number of confiscated pork products that were brought in by airline passengers and ship passengers per year (PPAP and PPSP, respectively) was available and public [110]. However, there was not distinction between PPAP and PPSP. So, the annual number of confiscated pork products brought in by international passengers per origin territory ($N_{\text{c-ann_}o}$) was considered as the annual number of confiscated PPAP per origin territory in the current analysis. Thus, the annual weight of confiscated PPAP per origin territory ($V_{\text{c-ann_}o}$) was estimated as the product of the individual weight of each confiscated animal product (V_{c}) and $N_{\text{c-ann_}o}$ [69,110]. $N_{\text{c-ann_}od}$ was estimated by multiplying the value of $\text{Pro}_{\text{vl_}od}$ by the value of $V_{\text{c-ann_}o}$. $N_{\text{c-ann_}od}$ and parameterized using a pert distribution.

Weight of IPPAP being missed at border controls ($V_{_od}$)

In the current analysis, we assumed that IPPAP could only enter airports in Japan when the products were missed at border controls at the destination airport. Unfortunately, we were unable to obtain any data or expert opinion that would allow us to investigate the probability of the non-detection of IPPAP at border controls in Japan (H_{non}), so this was modeled with a triangular distribution that had minimum, most likely, and maximum values of 0.2, 0.5, and 0.9, respectively, which were obtained from [84]. The weight of IPPAP that had been missed at border controls per origin territory and destination airport ($V_{_od}$) was then estimated from the obtained probability.

Probability of PPAP being contaminated with ASFV

To estimate the probability that PPAP could be contaminated with ASFV, an approach was developed following the methods of [84] and Veterinary Laboratories Agency (VLA) [107]. As mentioned above, although PPAP includes both legal and illegal pork products, only IPPAP was considered to have the potential of being contaminated with ASFV.

A total of 47 origin territories that have direct or indirect flights to Japan were classified into three risk categories based on information on their ASF disease status contained in the OIE-WAHIS as at April 5, 2019 [6]: "High" where the disease is present or suspected in pig species; "Medium" where a territory shares a national border with at least one territory in the "High" category; and "Low" for all other territories that officially have no ASF outbreaks and do not share borders with territories in the "High" category. For each of these categories, it was assumed that one kg of PPAP that was contaminated with ASFV indicated that at least one domestic pig was infected in the origin territory.

For each territory in the "High" category, the probability of ASFV infection was modeled using a beta distribution with consideration of the potential number of non-reported infected domestic pigs in each origin territory per month ($N_{\text{nasf_}m}$) and the number of slaughtered domestic pigs in each origin territory per month ($N_{\text{slt_}om}$). $N_{\text{nasf_}m}$ was estimated from the prevalence rate of ASF on affected farms in the origin territory ($\text{Prev}_{_o}$), the size of ASF-affected farms in the origin territory ($N_{\text{af_}o}$), the average number of outbreaks per month in the origin territory ($N_{\text{asf_}om}$), the duration of the infection (T_{inf}), and the probability of notification underreporting (H_{under}). The number of non-reported infected domestic pigs slaughtered per month in each origin territory was estimated as the product of $N_{\text{nasf_}m}$ and the proportion of pigs that were improperly slaughtered per month

(Pro_{imp_m}). Since the probability of slaughtering pigs may be greatly influenced by the farmer's attitude, economic condition, and other social factors, a conservative approach was applied whereby Pro_{imp_m} was modeled using a uniform distribution with the mean value of the Pro_{slt_m} distribution as a minimum and 100% as the maximum (indicating the worst-case scenario where all pigs would be slaughtered if farmers suspected the disease). The number of pigs slaughtered per month (N_{slt_om}) was estimated by considering the annual pig census in each origin territory (N_{dp_o}) and the proportion of pigs slaughtered per month (Pro_{slt_m}).

The probability of ASFV infection in the "Medium" and "Low" risk categories ($H_{slt\text{-}med_m}$ and $H_{slt\text{-}low_m}$, respectively) was estimated by multiplying the probability of an outbreak occurring in the origin territory (H_{med} and H_{low}, respectively) by the average size of an ASF outbreak (Pro_{asf}), the duration of the infection (T_{inf}), the probability of outbreaks being undetected, and the proportion of pigs going to the slaughterhouse that month. The probability of outbreak occurrence was set 10 times higher for territories in the "Medium" category than for those in the "Low" category based on expert opinion considering the geographical closeness to territories in the "High" category.

Specific standards were applied to some origin territories due to their unique situations. In the case of Ethiopia, no data on ASF outbreaks were available even though the disease is suspected there [111], so the median value of the probability of infection for the other affected African territories was used. In the case of Italy, the risk was set at "Medium" even though ASF outbreaks have been reported there for a long time because the disease is confined to the island of Sardinia and has only escaped from there once in the last 40 years. Taiwan, Hong Kong, Macau, and mainland China (China) were recognized as origin territories based on the territory classification that is used in the OIE-

WAHIS [111]. Finally, in the case of official international commercial indirect flights [81], the probability of infection was estimated as the combination of the probability of ASFV infection in the origin territory and the connecting territory.

Sensitivity analysis

In order to identify the most influential parameters to model outputs, Spearman correlation coefficients (ρ_i) between each input and the annual probability of ASFV entry into Japan were calculated. Inputs with $\rho_i \geq 0.4$ that contributed > 9% to the variance of the output were identified as the most influential parameters for the model and were analyzed in detail using the advanced sensitivity analysis tool @RISK 7.6, with 10,000 iterations for each scenario. A total of 10 scenarios were assessed for each selected parameter by changing the base values in 10 consecutive steps from a minimum of 50% reduction to a maximum of 50% increase.

Results

The overall mean annual probability of ASFV introduction to Japan via PPAP was found to be 0.941 with a 95% confidence interval (CI) of 0.661–1.000, which approximately corresponds to one introduction every 1.06 years.

At the flight level, the highest risk of ASFV entry was the direct flight between Mongolia and Narita International Airport (0.864; 95% CI, 0.434–1.000), followed by Vietnam and Narita (0.485; 0.104–0.955), China and Kansai (0.413; 0.08–0.903), and China and Narita (0.313; 0.052–0.779) (Table 5).

In particular, the risk was clustered around flights from several territories in the "High" category (Mongolia, China, and Vietnam) that were traveling to the four largest airports (Narita, Kansai, Chubu, and Tokyo). Among the indirect flights, the flight from France to Narita via the Russian Federation was found to have the highest risk (0.014; 95% CI, 0.001–0.043), but none of the indirect flights were in the top 10 highest risk flights.

At the origin territory level, the top 10 highest risk territories included six Asian territories, three European territories, and one African territory. Mongolia was the highest risk territory, with a risk of 0.864 (95% CI, 0.434–1.000), followed by China (0.697; 0.223–0.999), Vietnam (0.662; 0.196–0.998), the Russian Federation (0.136; 0.018–0.401), Cambodia (0.073; 0.009–0.226), and Ethiopia (0.002; 0.0002–0.0050). Poland was also among the top 10 highest risk territories, where ASF is currently present. However, non-affected territories were also in this list, including Taiwan, Germany, and Thailand. Results are depicted in Figure 5 and presented in Table 6.

At the destination airport level, a high probability of ASFV introduction to Japan

via PPAP was clustered within the four largest airports in Japan, with Narita having the highest risk (0.905; 95% CI, 0.537–1.000), followed by Kansai (0.496, 0.109–0.961), Tokyo (0.389; 0.072–0.879), and Chubu (0.338; 0.058–0.816). Results are depicted in Figure 6 and presented in Table 7.

Table 5. The risk result of ASFV introduction to Japan via pork product brought in air passenger's luggage per international flight

Flight: Origin / Airport	Risk value of ASFV introduction into Japan [95% confidence interval]
Mongolia / Narita	0.864 [0.434 - 1.000]
Vietnam / Narita	0.485 [0.104 - 0.955]
China / Kansai	0.413 [0.079 - 0.903]
China / Narita	0.313 [0.052 - 0.779]
Vietnam / Tokyo	0.272 [0.043 - 0.708]
China / Chubu	0.236 [0.035 - 0.637]
Vietnam / Kansai	0.217 [0.032 - 0.596]
China / Tokyo	0.211 [0.030 - 0.583]
Vietnam / Chubu	0.164 [0.022 - 0.471]
the Russian Federation / Narita	0.120 [0.015 - 0.356]
China / Fukuoka	0.120 [0.015 - 0.355]
Vietnam / Fukuoka	0.087 [0.011 - 0.262]
Cambodia / Narita	0.073 [0.009 - 0.226]
China / Naha	0.063 [0.007 - 0.192]
China / Shizuoka	0.048 [0.006 - 0.147]
China / New Chitose	0.039 [0.005 - 0.121]

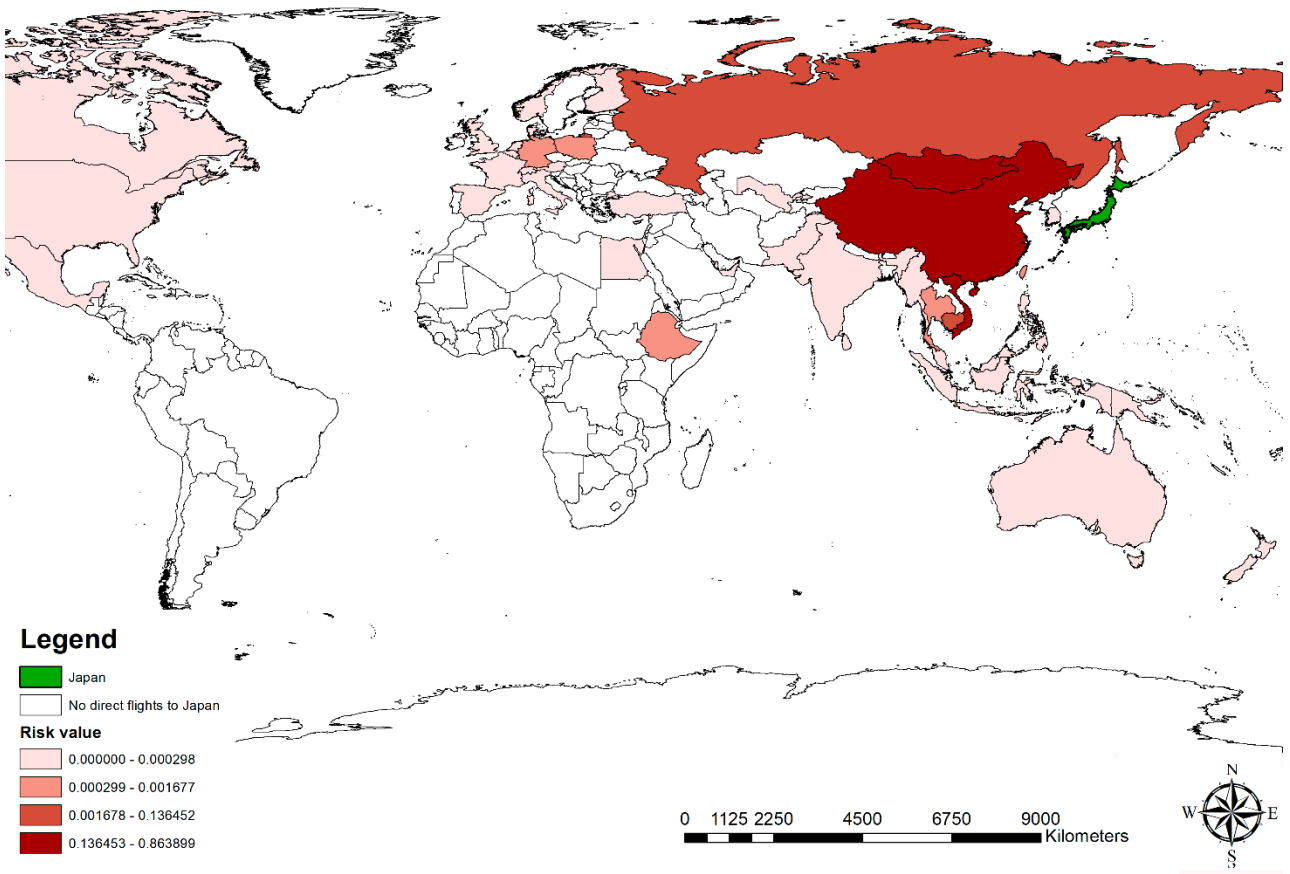


Figure 5. The risk results of ASFV introduction into Japan via pork products brought in air passengers' luggage at origin territory level. The graduated color map represents the annual risk from the highest (darker) to the lowest (lighter).

Table 6. The risk result of ASFV introduction from origin territories into Japan via pork product brought in air passenger's luggage

Territory of origin	Risk value of ASFV introduction into Japan [95% confidence interval]
Mongolia	0.864 [0.434 - 1.000]
China	0.697 [0.223 - 0.999]
Vietnam	0.662 [0.196 - 0.998]
the Russian Federation	0.136 [0.018 - 0.401]
Cambodia	0.073 [0.009 - 0.226]
Ethiopia	0.002 [2×10^{-4} - 0.005]
Taiwan	0.001 [2×10^{-5} - 0.006]
Poland	0.001 [1×10^{-4} - 0.004]
Germany	0.001 [1×10^{-5} - 0.003]
Thailand	0.001 [1×10^{-5} - 0.003]

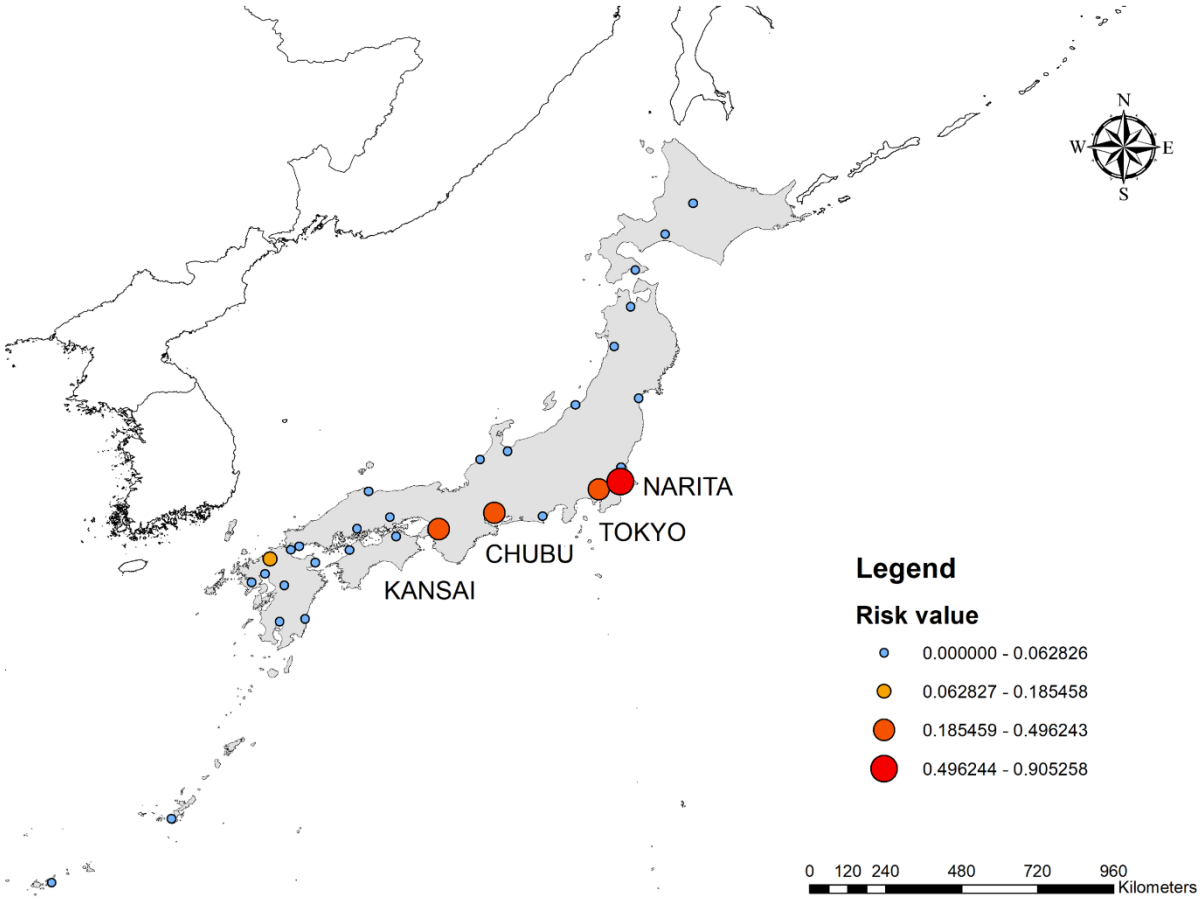


Figure 6. The risk results of ASFV introduction into Japan via pork products brought in air passengers' luggage at airport level. Airports were represented by colored circles from the highest (larger) to the lowest (smaller) at airport level.

Table 7. The risk result of ASFV introduction into Japan via pork product brought in air passenger's luggage per airport

Airport	Risk value of ASFV introduction into Japan [95% confidence interval]
Narita	0.905 [0.537 - 1.000]
Kansai	0.496 [0.109 - 0.961]
Tokyo	0.389 [0.072 - 0.879]
Chubu	0.338 [0.058 - 0.816]
Fukuoka	0.185 [0.026 - 0.524]
Naha	0.063 [0.008 - 0.192]
New Chitose	0.057 [0.007 - 0.175]
Shizuoka	0.048 [0.006 - 0.147]

Sensitivity analysis

Examination of the Spearman correlation coefficients indicated that the probability of PPAP not being detected at border controls, the probability of ASF infection in Mongolia, and the volume of PPAP coming into Japan were highly correlated input parameters in the model. The advanced sensitivity analysis revealed that the probability of PPAP not being detected at border controls was the most influential parameter in the model, followed by the volume of PPAP coming into Japan and the probability of ASF infection in Mongolia (Figure 7).

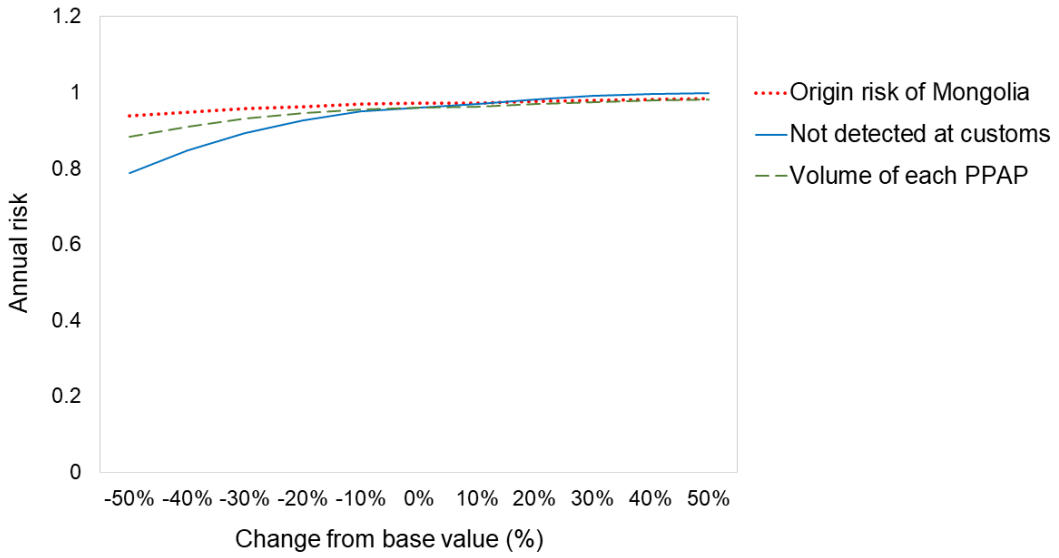


Figure 7. Results of the advanced sensitivity analysis for the risk of ASFV introduction via into Japan via pork products brought in air passengers' luggage. Horizontal axis shows the percentage change of the selected three input parameters against the annual risk in the vertical axis.

Discussion

Several studies have assessed the import risk of diseases such as rabies and bovine spongiform encephalopathy being introduced to Japan [112,113]. However, this is the first study to quantify the risk of ASFV introduction to Japan via PPAP. This information is urgently needed as although Japan has never been affected by ASF, outbreaks have now been reported in the neighboring territories of China, Mongolia, Vietnam, Cambodia, and Hong Kong [6]. A similar study has also been carried out in Europe and the U.S. following the introduction of this disease into the Caucasus region in 2007 [67,84,114-116].

The number of detected cases of ASFV-contaminated pork products in Asian airports has increased in proportion to the number of ASF outbreaks in this region, and these products have mainly been brought in by air passengers traveling from epidemic areas. Therefore, considering the risk of ASFV entry to Japan from neighboring epidemic areas, routine quarantine measures will definitely play a critical role in preventing the introduction of this disease. Passengers who are boarding international flights are usually informed in advance about the ban on bringing animal products to Japan without official certification, and once they arrive at their destination port, passengers are obliged to fill out a questionnaire that includes their flight number and the contents of their luggage. Voluntary luggage inspections are conducted by quarantine officers based on this questionnaire as well as their impression of the passengers. Detection dogs are also engaged in finding potential contrabands, including drugs and prohibited animal products in passengers' luggage at major international airports [117]. However, it is almost impossible to find all animal products that have been illegally brought into airports, so

limitations on current quarantine capacities as well as the failure to detect illegally transported animal products will have a large effect on the risk of animal disease introduction. Consequently, the identification of flights, origin territories, and destination airports that are at high risk of ASFV introduction will help to improve the current surveillance system and allow a monitoring system to be developed for conducting risk-based surveillance, such as preparing a specific quarantine system for particular territories of origin.

The availability and quality of data are always major limitations for any risk assessment. Ideally, information on the number of air passengers arriving in Japan at each airport from each origin territory would be used to accurately assess the volume of air passengers' luggage. However, the data used in this study were not categorized in great detail. Consequently, the number of passengers per origin territory and aircraft type were estimated using an approximation algorithm based on data that were available for 14 airline carriers. Since information on the size of the passengers' hand luggage and the proportion of pork products in the passengers' hand luggage were not available for this risk assessment, we assumed the total volume of IPPAP based on the number of confiscated pork products per origin territory [110]. This assumption also considered the range of the free luggage allowance for passengers at a regional level because the acceptable size of free luggage varies between different regions within origin territories. The data on confiscated pork products, which are accounted for both data of PPAP and one of PPSP and are not separated. However, since 97.5% of foreign visitors travel to Japan by airplane [118], it seemed safe to assume that most of these pork products were brought in by air passengers.

The Ministry of Agriculture, Forestry and Fisheries reported 36 incidences of

ASFV detection from pork products in Japanese airports during the period from August 2018 to May 2019 [74]. Based on these data, our finding that Mongolia, China, and Vietnam are the highest risk territories for ASFV introduction to Japan appears to be appropriate. However, our model may have underestimated the risk of the annual probability of ASFV introduction at the level of the origin territory, as 32 cases of ASFV detection from PPAP were reported from China in 9 months, whereas our model indicated that the risk for China was 0.697 (95% CI, 0.223–0.999), which approximately corresponds to one ASFV introduction into Japanese airports every 1.43 years. While our analysis considered the underreporting probability of ASF in the origin territory, we were unable to fully account for inadequate behavior by farmers due to a lack of reliable data sources. Some pig farmers tend to put pigs that are infected with ASFV on sale as they are afraid of suffering losses, which may greatly increase the risk of pork products being contaminated with ASFV in epidemic areas, as evidenced by the case of Hong Kong [65]. Therefore, additional data are required to fill these gaps and conduct a more accurate risk assessment.

In the current analysis, Mongolia was identified as the highest risk origin territory for ASFV introduction to Japan. This is likely due to the large proportion of domestic pigs in Mongolia that have been affected by ASF since the first outbreak was notified [119]. However, there may be a huge gap in the pig census data for Mongolia, as the Food and Agriculture Organization of the United Nations database reported 31,870 head of pigs in Mongolia in 2017 [83], whereas Enkhbold et al. [21] assumed approximately 150,000 head. Therefore, the final risk posed by Mongolia could have been overestimated. However, as Mongolia is geographically close to Japan and has the highest risk, more accurate data are required to accurately assess this risk.

China had the second highest risk despite having the greatest number of ASFV-contaminated PPAP detections. This difference between the results of our assessment and the reported ASFV detections in Japanese airports may have been caused by the underestimation of several factors, such as the probability of PPAP not being detected at border controls, the number of confiscated pork products, and the number of ASF outbreaks and affected animals. Therefore, given that detected ASFV-contaminated pork products were brought in by air passengers from various parts of China, the risk for this territory should be reassessed at a regional level.

Vietnam had the third highest risk of introducing ASFV to Japan. This result may have been greatly influenced by the fact that Vietnam has the largest number of ASF outbreaks per month and the highest prevalence rate of ASF in affected farms among the ASF-affected territories, as well as the large number of PPAP that originated from this territory.

The Russian Federation was the fourth highest risk origin territory, despite the disease having been present there for a long period of time and having affected a large number of domestic pigs. This might be because the average number of ASF outbreaks per month and the prevalence of ASF in affected farms were lower than in China, Vietnam, and Mongolia. Following this was Cambodia, as the fifth highest risk territory, which reflected the fact that although only one ASF outbreak has been reported in Cambodia to the OIE as at April 5, 2019, it is geographically close to Vietnam, so the potential risk of a further outbreak should not be overlooked. Ethiopia was the sixth highest risk territory of origin. While no official notification of ASF has been reported to the OIE from Ethiopia, ASFV was detected in tissue samples collected from domestic pigs in this territory from 2011 to 2014 [120], so it was classified as an ASF-affected territory and its risk was

assessed based on the disease situation of other ASF-affected African territories. Therefore, further information is required to more accurately assess the risk from Ethiopia.

Remarkably, Taiwan was found to pose a higher risk than Poland, which is an ASF-affected territory, and similarly Germany was considered a higher risk than ASF-affected Belgium. Categorization of these ASF-free territories as higher risk territories of ASFV introduction to Japan would be owing to three potential parameters: the high number of PPAP originating from these territories, the number of visitors from these territories to Japan, and the geographical distance between these territories and ASF-affected territories. On the other hand, the risk from Belgium may have been lower due to no ASF outbreak having occurred in the domestic pig population there (it has only been detected among wild boars and our model did not include products originating from wildlife) and the low number of visitors to Japan.

In terms of the destination airport, the risk was clustered around the four largest airports in Japan: Narita, Kansai, Tokyo, and Chubu Centrair. This result may have been highly influenced by the volume of air passengers' hand luggage arriving from high risk territories–indeed, Narita International Airport has the largest volume of air passengers' hand luggage, followed by Kansai, Tokyo, and Chubu Centrair, which corresponds with the order of risk for these airports.

While limitations to the quality and accessibility of data always pose challenges in any risk assessment, our study revealed the potential risk of ASFV introduction at the flight, origin territory, and destination airport levels, which will be useful for improving risk management activities and monitoring systems to prevent the introduction of ASFV into Japan. However, it should also be noted that half of the pork that is consumed in Japan is imported [121], and these products are mainly transported by ship freight or air

cargo for commercial purposes. In addition, live pigs are imported to Japan from overseas, mainly for breeding purposes [122]. Therefore, these also represent potential ASFV introduction pathways into Japan. These pathways were not evaluated in the present study due to a lack of reliable information, so further assessment is required to estimate these risks more accurately.

Summary

The spread of ASF has reached pandemic levels over the last decade, and outbreaks of this disease in China, Mongolia, Vietnam, and Cambodia in 2018 and 2019 could accelerate its transmission to neighboring Asian territories. Thus, the risk that the ASFV will be introduced to disease-free territories increases each year. Since Japan is an island nation, the most likely way in which ASFV would be introduced is via PPAP. Therefore, in the present study, we assessed the risk of ASFV introduction to Japan via PPAP. For the purposes of this analysis, we considered 214 international commercial flights traveling from 47 origin territories to 31 destination airports as potential routes of ASFV introduction via PPAP. The risk was estimated quantitatively through a stochastic model that considered the volume of air passengers' luggage, the amount of confiscated pork products that were carried in air passengers' luggage, and the disease status of the origin territory. The overall mean annual probability of ASFV introduction to Japan via PPAP was found to be 0.941 [95% CI, 0.661–1.000], which approximately corresponds to one introduction every 1.06 years. At the origin territory level, Mongolia was led as the highest risk territory, with a risk of 0.864 (95% CI, 0.434–1.000), followed by China (0.697; 0.223–0.999), Vietnam (0.662; 0.196–0.998), and the Russian Federation (0.136; 0.018–0.401). At the destination airport level, Narita International Airport had the highest risk (0.905; 0.537–1.000), followed by Kansai International Airport (0.496; 0.109–0.961), Tokyo International Airport (0.389; 0.072–0.879), and Chubu Centrair International Airport (0.338; 0.058–0.816). This information will help improve risk management activities and monitoring systems to prevent the introduction of ASFV to Japan.

Chapter III

Risk assessment of African swine fever virus exposure to pigs and wild boar in Japan via pork products brought in air passengers' luggage

Introduction

ASF is a highly haemorrhagic and devastating disease in swine caused by the ASFV. Susceptible hosts among pig species, soft ticks, and infectious materials play essential roles in the transmission of the disease [4]. In addition to direct contact with these hosts, indirect contact with materials contaminated with ASFV is one of the crucial causes of ASF outbreaks, which may occur even in a distant place. Detection and culling of infected animals or those suspected of infection and restriction of animal movements are the fundamental countermeasures for the control of contagious diseases, including ASF. The illegal trade in animal or pork products and the movement of ASF-affected wild boar play important roles in the transmission of the disease to ASF-free territories. None of the effective vaccines against ASFV have been commercialised to date, despite the high mortality rate among affected animals, and therefore control measures against ASFV rely heavily on preventing the introduction of the disease into ASF-free territories by means of strict border controls.

After ASF was introduced into Georgia in 2007, the disease immediately spread to the Caucasian region and then Eastern European territories mainly by the movement of wild boar or suspected illegal animal transportation [55]. In August 2018, the first ASF outbreak was reported in China. The results of a partial sequence of the ASFV p72 gene indicated that the outbreak isolate, ASFV-SY18, belonged to the genotype II group and had an identical nucleotide sequence to the Georgian (Georgia 2007/1), Russian (Krasnodar 2012 and Irkutsk 2017), and Estonian (Estonia 2014) isolates (Zhou et al., 2018) [62], meaning that ASF was potentially transmitted from those European territories where the disease is epidemic. Since then, ASF outbreaks have been confirmed in a total

of 11 territories in the Asian region including Cambodia, China, Democratic People's Republic of Korea, Hong Kong, Indonesia, Laos, Myanmar, the Philippines, Republic of Korea, Timor-Leste, and Vietnam [123].

Japan had remained free from several contagious animal diseases through the implementation of strict border controls at ports of entry. However, the recent introduction of transboundary animal diseases such as FMD in 2010 and CSF in 2018 indicates that contagious viruses can be introduced from infected areas [8,19]. As cases of CSF in susceptible animals have continued to be notified despite vigorous efforts at disease control [124], it is assumed that the introduction of ASFV would cause a similar, or perhaps even worse, situation since no effective vaccines are available for ASF.

In a previous study, the risks of the introduction of ASFV to Japan via PPAP were evaluated [125]. The detection of ASFV genes in PPAPs confiscated at Japanese airports has been continually reported since the first ASF outbreak was notified in China [126]. According to a report from the JAQS, by the end of November 2019, ASFV genes had been detected in a total of 83 confiscated PPAPs two of which ASFV was isolated from [126]. Considering this, the probability that ASFVs will be introduced into Japan via PPAP and transmitted to susceptible pig species is not negligible.

The aim of the present study is to evaluate the risk of ASFV exposure to susceptible animals in Japan via PPAP by a combination of quantitative and semi-quantitative methods. The obtained results could be useful for establishing or improving control measures against the introduction and spread of ASFV, and for reviewing current risk management activities in the field.

Materials and Methods

Concept of the risk assessment model

Two quantitative and stochastic models and a semi-quantitative model were developed to assess the risk of ASFV exposure to pigs via PPAP from abroad in each prefecture in Japan. The data used in the risk assessment were obtained from relevant websites, public databases, published literature and experts in animal husbandry. Two quantitative and stochastic risk assessment models were built to estimate the annual probability of ASFV exposure to domestic pigs that could be attributed to foreign job trainees or to foreign tourists. The risk of ASFV exposure to wild boar via PPAP was assessed semi-quantitatively since there were some difficulties in terms of data accessibility. In the present study, air passengers coming to Japan with PPAPs from 47 origin territories with commercial routes to Japan were regarded as potential contributors to the exposure of ASFV to pigs in each of the 47 prefectures of Japan. Flight information from August 2016 to October 2018 was obtained from the MLIT database [103]. Since legal pork products were not assumed to be contaminated with ASFV, in the present study we focused only on the risk of IPPAPs [125]. Datasets on confiscated pork products, some of which contained ASFV genes, were obtained from the JAQS database [105,126]. Information about ASF disease status and the number of susceptible pigs in each territory of origin was obtained from the OIE–WAHIS as at November 30, 2019 [61] and FAOSTAT 2018 [106], respectively. The risk model was developed using @RISK 7.6 (Palisade Corporation, Newfield, NY, USA) in Microsoft Excel (Microsoft, Redmond, WA, USA) and 10,000 iterations were run using the Latin hypercube sampling method.

Structure of the quantitative risk assessment model for domestic pigs

Two quantitative stochastic models, one for foreign job trainees and the other for foreign tourists, were built to assess the annual probability of ASFV exposure to domestic pig in the 47 prefectures of Japan via PPAP. In the present study, it is assumed that all domestic pigs in a farm have been exposed to ASFV once the virus has reached it. Each quantitative model followed a binomial process according to the formula below:

$$\mathrm{P}(x \geq 1) = \sum 1 - (1 - P_{\mathrm{asf}_o})^{V_od}$$

where $V_{_od}$ is the assumed weight (kg) of PPAP brought into pig farms in each prefecture (d) by visitors from each origin territory (o), $P_{\text{asf-}o}$ is the estimated probability of at least 1 kg of PPAP being contaminated with ASFV from each origin territory, and $\sum$ refers to the probability of ASFV being introduced to pig farms considering all visitors to Japan. Thus, the model consists of two main components: (a) the quantity of PPAP to which domestic pig is exposed and (b) the probability of PPAP being contaminated with ASFV. All input values, parameterisations, and references are presented in Table 8 and are described in the following sections. The outline of the scenarios is described in Figure 8.

Table 8. Description and parameterization of model inputs for estimation of the risk of ASFV exposure to domestic pig in prefectures of Japan

Notation	Definition	Parameterization	Values	Reference/ Source
V_{enter_vo}	Weight of PPAP (kg) being missed at quarantine per visitor per origin territory		V_{miss_o} / N_{all_o}	
V_{miss_o}	Annual weight of PPAP (kg) being missed at quarantine per origin territory		$P_{miss} * V_{conf_o}$	
N_{all_o}	Annual number of visitors coming to Japan per origin territory	Normal (μ ,α)	$N_{qall_o} * (N_{all} / N_{qall})$	
N_{all}	Annual number of visitors coming to Japan			[127]
N_{qall}	Total number of tourists come to Japan during the period of survey			[85]
N_{qall_o}	Total number of tourists come to Japan per origin territory during the period of survey			[85]
P_{miss}	Probability of PPAP not detected at border controls in Japan	Triang (min, most likely, max)	Triang (0.2, 0.5, 0.9)	[84]
V_{conf_o}	Weight of PPAP (kg) confiscated at quarantine per origin territory		$N_{conf_o} * V_{ppap}$	
N_{conf_o}	Annual number of PPAP confiscated at quarantine per origin territory			[105]
V_{ppap}	Weight of PPAP (kg) per item	Triang (min, most likely, max)	Triang (0.04, 1.1, 9.7)	[126]
N_{jt_od}	Adjusted annual number of foreign job trainees working in pig farms per origin territory per prefecture		$(N_{qjt_od} / N_{qjt_d}) * N_{jt_d}$	
N_{qjt_od}	Number of foreign job trainees per origin territory per prefecture in JPPA report in 2017			[128]
N_{qjt_d}	Number of foreign job trainees per prefecture in JPPA report in 2017			[128]
W_{fjt_d}	Proportion of farms accepting foreign job trainees per prefecture		N_{qfjt_d} / N_{qf_d}	
W_{fjt_r}	Proportion of farms accepting foreign job trainees per region		N_{qfjt_r} / N_{qf_r}	
N_{qfjt_d}	Obtained number of farms accepting foreign job trainees per prefecture in the JPPA report in 2017			
N_{qf_d}	Total number of questionnaires answered farms per prefecture in the JPPA report in 2017			
N_{qfjt_r}	Obtained number of farms accepting foreign job trainees per region in the JPPA report in 2017			
N_{qf_r}	Total number of questionnaires answered farms per region in the JPPA report in 2017			

Mod-W_{fjt_d}	Modified proportion of farms accepting foreign job trainees	Uniform (min, max)	min, max = Between W_{fjt_d} and W_{fjt_r}	
N_{fjt_d}	Estimated number of farms accepting foreign job trainees per prefecture		$Mod\text{-}W_{fjt_d} * N_{f_d}$	
N_{f_d}	Number of pig farms per prefecture			[129]
N_{jt_f}	Number of foreign job trainees per farm	Mean	N_{jt} / N_{fjt}	
N_{jt}	Total number of foreign job trainees			
N_{fjt}	Total number of farms accepting foreign job trainees			
N_{jt_d}	Expected number of foreign job trainees per prefecture		$N_{jt_f} * N_{fjt_d}$	
V_{jt_od}	Annual weight of PPAP (kg) brought into Japan by foreign job trainees per origin territory per destination prefecture		$N_{jt_od} * V_{enter_vo}$	
W_{left}	Proportion of meat leftover per family		0.03	[130]
N_{vis}	Number of days foreign job trainees visit pig farm		313	[131]
P_{ent_od}	Probability of leftover PPAP reach to pig farms		$P_{bs_d} * P_{bhv_o}$	
P_{bs_d}	Level of farm biosecurity per prefecture	Triang (min, most likely, max)	Different values among prefectures	[132]
I_{life_o}	Quality of life index			[133]
P_{bhv_o}	Potential risk of worker's behavior per origin territory		$I_{life_Japan} / I_{life_o}$	[133]
P_{asf_o}	Probability of 1 kg of PPAP being contaminated with ASFV in origin territory			[84,125]
N_{frd_od}	Annual number of visitors coming to Japan to see their friends or family per origin territory per destination prefecture	Normal (μ ,α)	$N_{all_od} * (N_{qfrd_od} / N_{qall_od})$	
N_{qall_od}	Total number of tourists coming to Japan per origin territory per destination prefecture during the period of survey			[85]
N_{all_od}	Annual number of tourists per origin territory per destination prefecture	Normal (μ ,α)	$N_{qall_od} * (N_{all} / N_{qall})$	

P_{pass_d}	Probability of tourists delivering PPAP to pig farmers			$(N_{pf_d} * N_{fami_d}) / N_{pop_d}$	
N_{pf_d}	Number of pig farmers per prefecture			$N_{emp_s} * N_{f_s}$	
N_{pop_d}	Total population per prefecture				[134]
N_{emp_s}	Number of employees per farm size		Uniform (min, max)		[132]
N_{f_sd}	Number of pig farms per farm size per prefecture				[129]
N_{fami_d}	Estimated number of family members per prefecture		Pert (min, most likely, max)	(1, different values per prefecture, 5)	[135]
N_{pass_od}	Annual number of tourists delivering PPAP to pig farmers			$N_{frd_od} * P_{pass_d}$	
V_{pass_od}	Annual weight of PPAP (kg) delivering to pig farmers			$N_{pass_od} * V_{enter_vo}$	
N_{vis}	Number of days farmers visit pig farm			Same value as foreign job trainees	[131]

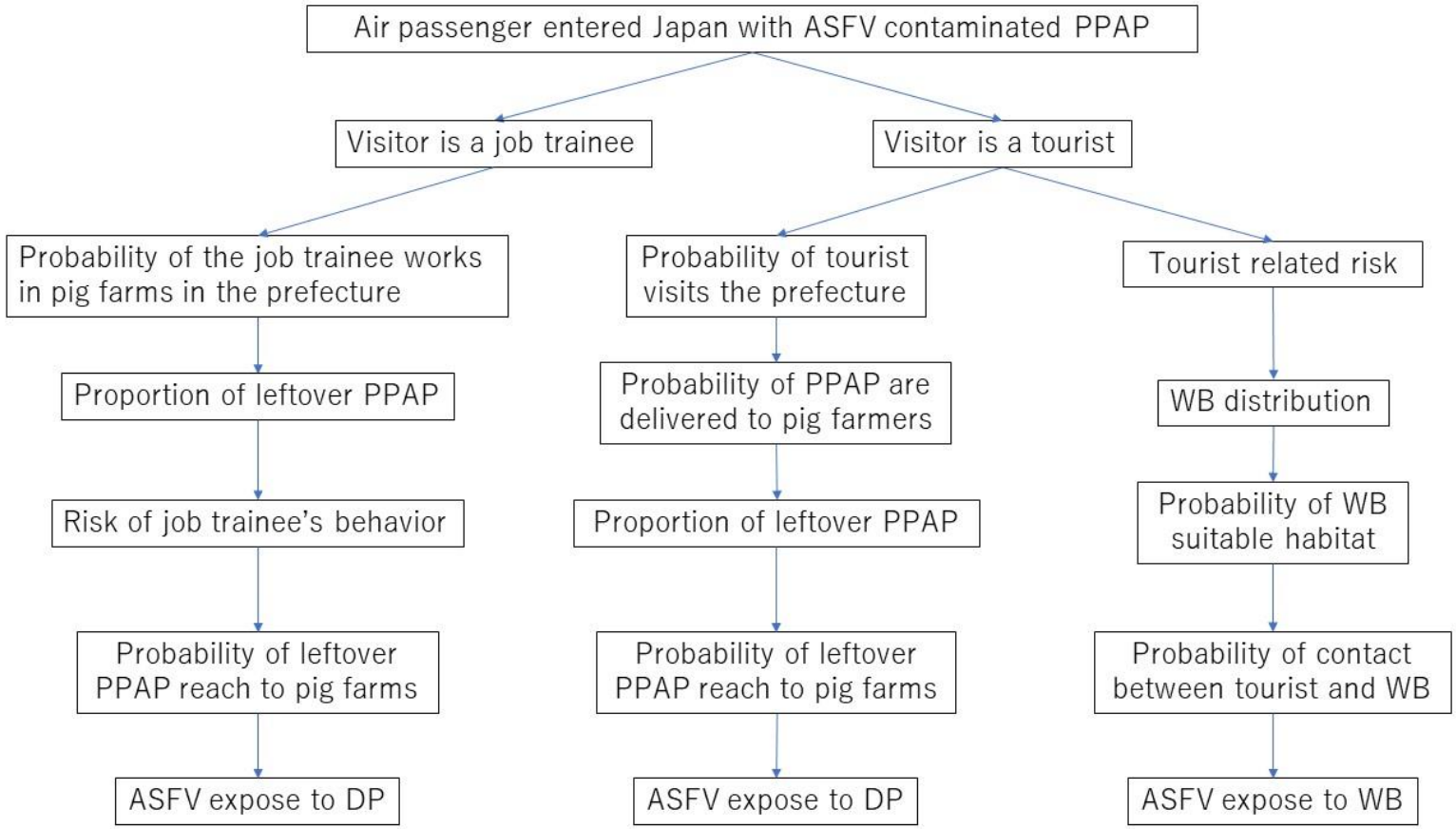


Figure 8. The flowchart shows the structure of the models to estimate the risk of ASFV exposure to susceptible animals in each prefecture of Japan via PPAP. Foreign job trainees and foreign tourists coming to Japan could have risk of ASFV exposure to domestic pig (DP). Foreign tourists coming for sightseeing could have risk of ASFV exposure to wild boar (WB).

Weight of PPAPs introduced into Japan (V_{enter_vo})

In the present study, PPAPs missed at the border by JAQS were regarded as the products that carried the risk of exposing susceptible animals to ASFV. The weight of PPAP (kg) not being detected by border controls per visitor per origin territory (V_{enter_vo}) was calculated. The value of V_{enter_vo} was calculated by dividing the annual weight of PPAP (kg) being missed by border controls per territory (V_{miss_o}) by the annual number of visitors coming to Japan from the given territory (N_{all_o}). The value of V_{miss_o} was calculated by multiplying the probability of PPAP not being detected by border controls (P_{miss}) by the weight of PPAP (kg) confiscated per origin territory (V_{conf_o}). None of the data or expert opinions that would have allowed us to determine the value of P_{miss} were available; therefore, this was set as a triangular distribution that had minimum, most likely and maximum values of 0.2, 0.5, and 0.9, respectively, following the approach used in previous studies [84,125]. The value of V_{conf_o} was calculated by the multiplying the annual number of PPAPs confiscated by border controls per territory (N_{conf_o}) by the weight of PPAP (kg) per item (V_{ppap}). The data on the annual number of PPAPs confiscated by border controls per territory was obtained from the JAQS database [105]. As information on the weight of pork products confiscated at Japanese international airports in which ASFV genes were detected for the period of October 2018 to September 2019 was available from the JAQS database, the value of V_{ppap} was set as a triangular distribution that had minimum, most likely and maximum values of 0.04, 1.1, and 9.7, respectively [126].

The value of N_{all_o} was estimated using data from the air passengers' survey for the period from 2015 to 2017 [85]. The survey includes information on the origin

territories of tourists, their destination prefecture and the purpose of their visits. An annual number of tourists per territory was estimated based on the available data. Information on tourist origins is not directly referred to in the present study. However, the annual number of tourists per territory was calculated using the formula below. The annual number of air passengers coming to Japan (N_{all}) was available from the Statistics on Legal Migrants [127]:

$$N_{\mathrm{all_}o} = N_{\mathrm{qall_}o} * (N_{\mathrm{all}} / N_{\mathrm{qall}})$$

where $N_{\mathrm{qall_}o}$ and N_{qall} are the numbers of tourists coming to Japan per territory and in total, respectively. The number $N_{\mathrm{all_}o}$ was assumed to follow a normal distribution.

Number of foreign job trainees working in pig farms ($N_{\mathrm{jt_}od}$)

The adjusted annual number of foreign job trainees working in pig farms per origin territory per prefecture ($N_{\mathrm{jt_}od}$) was estimated using the 2017 pig farming survey of Japan (JPPA report 2017) [128]. Although the information on the number of foreign job trainees per territory was provided by farmers in the survey ($N_{\mathrm{qjt_}od}$), not all pig farmers in Japan participated in the survey. Therefore, the value of $N_{\mathrm{jt_}od}$ was estimated in the present study by the value of $N_{\mathrm{qjt_}od}$. First, a proportion of farms accepting foreign job trainees was calculated by dividing the obtained number of farms accepting foreign job trainees ($N_{q\mathrm{fjt_}d}$ or $N_{q\mathrm{fjt_}r}$) by the total number of farms participating in the survey ($N_{\mathrm{qf_}d}$ or $N_{\mathrm{qf_}r}$) at prefecture level ($W_{\mathrm{fjt_}d}$) and region level ($W_{\mathrm{fjt_}r}$) according to the 2017 JPPA report. These values were used to calculate the adjusted proportion of farms accepting foreign job trainees ($Mod\text{-}W_{\mathrm{fjt_}d}$). The value of Mod-$W_{\mathrm{fjt_}d}$ was set as a uniform distribution between $W_{\mathrm{fjt_}d}$ and $W_{\mathrm{fjt_}r}$. Secondly, the estimated number of farms accepting foreign job trainees per prefecture ($N_{\mathrm{fjt_}d}$) was calculated by multiplying the value of $Mod\text{-}W_{\mathrm{fjt_}d}$ by the number of pig farms per prefecture ($N_{\mathrm{f_}d}$) obtained from the 2017 Statistical Survey on Livestock

[129]. The number of foreign job trainees per farm (N_{jt_f}) was also calculated by dividing the total number of foreign job trainees (N_{jt}) by the total number of the farms accepting foreign job trainees (N_{fjt}). The expected number of foreign job trainees per prefecture (N_{jt_d}) was calculated by multiplying the value of N_{jt_f} by the value of N_{fjt_d}. Finally, the value of N_{jt_od} was calculated using the following formula:

$$N_{jt_od} = (N_{qjt_od} / N_{qjt_d}) * N_{jt_d}$$

where N_{qjt_d} is the total number of foreign job trainees per prefecture in the 2017 JPPA report [128].

Annual weight of PPAPs brought into Japan by foreign job trainees (V_{jt_od})

The annual weight of PPAPs brought into Japan by foreign job trainees per origin territory per destination prefecture (V_{jt_od}) was calculated by multiplying the value of N_{jt_od} by the value of V_{enter_vo}.

Proportion of leftover PPAPs (W_{left})

In the present study, we assumed that leftover PPAP, that is, PPAP not eaten, could reach pig farms and infect susceptible animals. The data on the proportion of meat left over per family was used for estimating the proportion of leftover PPAPs [130]. The average proportion of meat leftover per family (W_{left}) was set as 0.03.

Number of visit days to pig farms by foreign job trainees (N_{vis})

Throughout the year, foreign job trainees have many opportunities to visit farms, and each visit carries the potential risk that contaminated PPAP is introduced to farms. In general, the number of working days is stipulated by the Labor Standard Law (LSL) [131]. According to the LSL, workers need to take at least one day off per week, indicating that there are approximately 313 days per year on which foreign job trainees could visit farms.

That is why the figure for the number of days foreign job trainees spend visiting pig farms per year (N_{vis}) was set as 313 in the present study.

Probability of leftover PPAPs reaching pig farms (P_{ent_od})

It was generally assumed that the risk of PPAP being brought onto farms would be affected by farms' biosecurity measures and workers' behaviour. Farm biosecurity measures are usually established by employers, whereas employee behaviour is influenced by personal factors. According to these assumptions, the probability of leftover PPAPs reaching pig farms (P_{ent_od}) was calculated as the product of the level of farm biosecurity per prefecture (P_{bs_d}) and the potential risks arising from workers' behaviour (P_{bhv_o}).

The level of farm biosecurity

The value of P_{bs_d} was estimated by the 2016 JPPA report [132]. In the report, farmers replied to several questions about the level of biosecurity on their farms with 'yes' (score of 1) or 'no' (score of 0), so the total score of farmers' responses could be assumed to represent an index of farm biosecurity. Although the questionnaire was prepared taking into consideration animal hygiene standards [136], it was not designed to assess measures to control ASF specifically, but swine diseases more generally. To obtain specific information relating to ASF control, each of the questions was weighted based on expert opinion. In the present study, we convened a total of 12 specialists with extensive field experience and knowledge of farm biosecurity relating to ASF. Six were invited from Spain, given that this territory has experienced outbreaks of ASF; the other six were from Japan, the territory serving as the research object. The 12 experts were asked to grade the importance of each question from zero (not important) to five (very important). The final weight of each question was determined using the median values of the expert responses.

The degree of consensus among the experts was measured using the coefficient of variation [137], which is the rate of the standard deviation to the mean. The results from the experts' opinions are shown in Table 9.

The weighted total score of farm biosecurity per farm was calculated by the weighted score of each question and farmers' responses. The value of P_{bs_d} was then set as a triangular distribution that had minimum, most likely and maximum values per prefecture.

Table 9. The expert opinion ranges of importance of farm biosecurity for ASFV

Description	Median	Mean	SD	CV
Section 1 : Entrance and exit of farm visitor				
Set up a sign board to indicate its restriction area	3.5	3.7	1.11	0.30
Shower in / Shower out	5	4.3	0.94	0.22
Exchange clothes for farm	5	4.8	0.60	0.13
Exchange shoes for farm	5	4.8	0.60	0.13
Step-in disinfection tank for farm	4	3.8	0.99	0.26
Exchange clothes for pigpen	3.5	3.8	1.07	0.28
Exchange shoes for pigpen	4.5	4.0	1.15	0.29
Step-in disinfection tank for pig pen	3	3.4	1.11	0.33
Record of visitor's information	4.5	3.8	1.53	0.41
Movement restriction of the human who visit overseas	5	4.7	0.62	0.13
Section 2 : Quarantine for pig introduction				
Quarantine at isolation facility during certain period	5	3.5	0.87	0.25
Section 3 : Manual for Introduction of materials				
Materials are stored in a warehouse for certain period and disinfected before introduction	5	3.5	1.22	0.35
Section 4 : Entrance and exit of the vehicles				
Disinfection of vehicles before entrance	5	4.9	0.28	0.06
Appropriate disinfection of vehicles for pig transportation	5	5.0	0.00	0.00
Set the gate for disinfection	5	4.2	1.14	0.27
Set the power sprayer for disinfection	5	4.6	0.76	0.17
Dissemination of sprinkle lime for disinfection	4	3.8	0.83	0.22
Frequent check of appropriateness of disinfection	5	4.8	0.43	0.09
Section 5 : Prevention for wild animal intrusion				
Appropriate maintenance of pig farm	5	4.5	0.76	0.17
Setting of wire-mesh fence	5	4.9	0.28	0.06
Invasion prevention for wild birds	5	4.8	0.43	0.09
Invasion prevention for small animals	5	4.4	0.86	0.20

SD, standard deviation; CV, coefficient of variation

Estimation of employee's attitude

It was assumed that employees' behaviour would be influenced by the lifestyle and culture of their territory of origin. As quality of life can be associated with social compliance, and a low level of social compliance might cause illegal or unethical activities such as illegal trade in animals or prohibited swill feeding, a quality of life index per territory was used as a proxy indicator to quantify the potential risk per territory arising from workers' behaviour (P_{bhv_o}) [133]. The quality of life index used was an estimation of overall quality of life using an empirical formula that considered eight indices relating to human life [138].

Probability of PPAP contamination with ASFV (P_{asf_o})

To estimate the probability that PPAP was contaminated with ASFV (P_{asf_o}), the same approach as in a previous study was applied [84,125]. Briefly, a total of 47 origin territories that had direct or indirect flights to Japan were classified into three risk categories based on information about the level of ASF in each territory as at November 30, 2019 [6]: 'High' where the disease was present or suspected in pig species; 'Medium' where a territory shared a national border with at least one territory in the 'High' category; and 'Low' for all other territories that had no officially recorded ASF outbreaks and did not share borders with territories in the 'High' category. For each of these categories, it was assumed that 1 kg of PPAP contaminated with ASFV indicated that at least one domestic pig was infected in the territory. Further detail of the risk calculation method is available from Ito et al. [125].

Number of foreigners as tourists (N_{frd_od})

In the present study, tourists coming to Japan to see their friends or family were also assumed to have the potential to introduce ASF to pig farms. As described above, the

coverage of the survey of air passengers' movements [85] was limited to a certain period, and therefore the annual number of tourists coming to Japan to see their friends or family (N_{frd_od}) was estimated following the approach mentioned previously. The annual number of tourists per origin territory per destination prefecture was calculated according to the following formula:

$$N_{all_od} = N_{qall_od} * (N_{all} / N_{qall})$$

where N_{qall_od} is the total number of tourists coming to Japan per territory per prefecture during the period of the survey. The obtained value of N_{all_od} was assumed to follow a normal distribution.

The value of N_{frd_od} was calculated using the following formula:

$$N_{frd_od} = N_{all_od} * (N_{qfrd_od} / N_{qall_od})$$

where N_{qfrd_od} is the number of tourists coming to Japan to see their friends or family per territory per destination prefecture during the period of the survey. The obtained value of N_{frd_od} was assumed to follow a normal distribution.

Probability of tourists providing PPAP to pig farmers (P_{pass_d})

In the present study, we assumed that tourists' PPAPs could reach pig farms only when their friends or families lived very close to pig farms. We assumed the worst scenario to be PPAPs delivered to pig farmers directly. The probability of tourists providing PPAPs to pig farmers (P_{pass_d}) was assumed to be same as the probability of tourists meeting pig farmers in the present study. Therefore, the value of P_{pass_d} was calculated by dividing the number of pig farmers per prefecture (N_{pf_d}) by the total population per prefecture (N_{pop_d}). The number of the pig farmers per prefecture was not available in the present study, so the value of N_{pf_d} was estimated based on the 2016 JPPA report and the 2017 Statistical Survey of Livestock [129]. The number of employees per

farm size (N_{emp_s}) was obtained from the 2016 JPPA report and then set as a uniform distribution that had minimum and maximum values [132]. The size of pig farm was classified into seven grades according to the number of domestic pigs fed in the farm: 1–99, 100–299, 300–499, 500–999, 1000–1999, 2000–2999, and more than 3000 animals according to the survey [129]. The obtained value of N_{emp_s} was then multiplied by the number of pig farms per farm size per prefecture (N_{f_sd}) to calculate the number of pig farmers per prefecture. It was also expected that PPAPs could be brought into pig farms after they were delivered by tourists to each of the farm members. To calculate the total number of potential recipients of PPAPs from tourists, the obtained value of N_{pf_d} was multiplied by the number of family members per prefecture (N_{fami_d}) using the data set from the Outline of Health, Labour and Welfare Statistics [135]. The value of N_{fami_d} was set as a pert distribution of that had the minimum, most likely and maximum values of one, different values per prefecture, five, respectively. Finally, the value of P_{pass_d} was calculated using the following formula:

$$P_{pass_d} = (N_{pf_d} * N_{fami_d}) / N_{pop_d}$$

Annual weight of PPAPs (kg) delivered to pig farmers (V_{pass_od})

First, the annual number of tourists delivering PPAPs to pig farmers (N_{pass_od}) was calculated by multiplying the value of N_{frd_od} by the value of P_{pass_d}. The annual weight of PPAPs given to pig farmers (V_{pass_od}) was then estimated as the product of the value of N_{pass_od} and the value of V_{enter_vo}.

Proportion of leftover PPAP (W_{left})

The proportion of leftover PPAP, which was not eaten by pig farmers, was assumed to be the same as in the case of foreign job trainees. That is, the average proportion of meat left over per family (W_{left}) was set as 0.03.

Number of days farmers visit pig farms (N_{vis})

Although no apparent restriction on working days was set for farmers in the LSL [131], it is likely that they have days off during the week. Therefore, the same methodology was also applied to calculate the probability of uneaten PPAP reaching pig farms (P_{ent_od}) and the probability of PPAP being contaminated with ASFV. The value for the number of days that farmers visit pig farms (N_{vis}) was the same as the one used in relation to foreign job trainees.

Annual probability of domestic pigs' exposure to ASFV via PPAP (R_{dp_od})

Finally, the overall annual risk of domestic pig exposure to ASFV from PPAP (R_{dp_od}) was calculated using the following formula:

$$R_{dp_od} = P_{dp\text{-}jt_od} + P_{dp\text{-}ft_od}$$

where $P_{dp\text{-}jt_od}$ and $P_{dp\text{-}ft_od}$ are the annual probabilities of domestic pigs' exposure to ASFV caused by foreign job trainees and foreign tourists, respectively.

Structure of the semi-quantitative risk assessment model

The semi-quantitative stochastic model was built to assess the risk of ASFV transmission to wild boar in the 47 prefectures of Japan arising from PPAP. The present approach was similar to the one used to assess the risk of ASF introduction into the EU [55,67,114,115]. To compare the risk of transmission from each origin territory to each of the 47 prefectures of Japan, we set the final index which would be introduced from the several risk estimators to be assigned throughout the present method. Twelve risk sub-estimators were collated and combined into five risk estimators: (1) origin territory risk, (2) tourist-related risk, (3) wild boar distribution, (4) probability of wild boar habitat, and (5) probability of direct or indirect contact between tourists and wild boar. These estimators were divided into ranks, and then all five ranked scores were combined to

obtain the relative risk value for each prefecture from each territory. The semi-quantitative model was constructed in Microsoft Excel (Microsoft, Redmond, WA, USA). In each parameter, from territory to destination prefecture, a score from 1 to 5 was assigned based on natural breaks in the data adjusted by the Jenks natural breaks classification method [139] using the Real Statistics Resource Pack [140]. The overall risk was defined as the product of the various risk estimators incorporated in the model. Details of the model inputs and references are presented in Table 10 and are described in the following paragraphs.

Table 10. Description and parameterization of model inputs for estimation of the risk of ASFV exposure to wild boar (WB) in prefectures of Japan

Notation	Definition	Parameterization	Values	Reference / Source
R_{asf_o}	Risk of origin territory	Jenks natural breaks (1-5)	P_{asf_o}	
R_{visit_od}	Tourists related risk per origin territory per destination prefecture	Jenks natural breaks (1-5)	V_{nature_od}	
N_{sight_od}	Annual number of tourists coming to Japan for sightseeing per origin territory per destination prefecture		$N_{all_od} * (N_{qsight_od} / N_{qall_od})$	
N_{qsight_od}	Number of tourists coming to Japan for sightseeing per origin territory per destination prefecture during the period of survey			[85]
W_{nature_o}	Proportion of tourists visiting natural area per origin territory	Mean		[141]
N_{nature_od}	Annual number of tourists visiting natural area per origin territory per destination prefecture		$N_{sight_od} * W_{nature_o}$	
V_{nature_od}	Annual weight of PPAP (kg) arrived at natural area in destination prefecture from origin territory		$N_{nature_od} * V_{enter_vo}$	
R_{prsc_d}	WB distribution per prefecture	Jenks natural breaks (1-5)	$W_{prsc_d} * D_{capt_d}$	
W_{prsc_d}	Proportion of mesh with WB presence per prefecture		$N_{mesh\text{-}wb_d} / N_{mesh\text{-}all_d}$	
$N_{mesh\text{-}wb_d}$	Number of 5km^2 meshes with WB presence per prefecture			[142]
$N_{mesh\text{-}all_d}$	Total number of 5km^2 meshes per prefecture			[142]
$_{capt_d}$	Density of captured WB per WB habitat area per prefecture		N_{capt_d} / A_{habit_d}	
N_{capt_d}	Annual number of captured WB per prefecture	Mean		[143]
A_{habit_d}	Area for WB habitat per prefecture (km^2)			[46]
R_{area_d}	Probability of WB suitable habitat per prefecture	Jenks natural breaks (1-5)	W_{habit_d}	
W_{habit_d}	Proportion of WB suitable habitat area in the total area of the prefecture		$A_{habit_d} * A_{all_d}$	
$A_{suit\text{-}habit_d}$	Area for wild boar suitable habitat per prefecture (km^2)			[46]
A_{all_d}	Total area of the prefecture (km^2)			[144]

$R_{contact_d}$	Probability of direct or indirect contact between tourists and WB per prefecture	Jenks natural breaks (1-5)	$W_{qah1_d} * N_{bus\text{-}over_d} * N_{road\text{-}over_d} * N_{train\text{-}over_d} * N_{tores\text{-}over_d} * W_{npark_d} * N_{hunt_d}$	
W_{qah1_d}	Proportion of area with QAH levels of 1 in the total area of the prefecture		A_{qah1_d} / A_{all_d}	
A_{qah1_d}	Area with QAH levels of 1 (km^2) per prefecture			[46]
$N_{bus\text{-}over_d}$	Number of bus stops overlapped with WB habitat area per square kilometer per prefecture			[46,48]
$N_{road\text{-}over_d}$	Number of road stations overlapped with WB habitat area per square kilometer per prefecture			[46,48]
$N_{train\text{-}over_d}$	Number of train stations overlapped with WB habitat area per square kilometer per prefecture			[46,48]
$N_{tores\text{-}over_d}$	Number of tourist resource points overlapped with WB habitat area per square kilometer per prefecture			[46,48]
W_{npark_d}	Proportion of natural park area in the prefecture		A_{nature_d} / A_{all_d}	
A_{nature_d}	Area of natural park per prefecture (km^2)			[48]
N_{hunt_d}	Number of hunters per prefecture	Mean		[143]

Origin territory risk (R_{asf_o})

In the present study, the origin territory risk (R_{asf_o}) was regarded as equal to the probability of the PPAP being contaminated with ASFV in the given territory (P_{asf_o}). The value of P_{asf_o} was calculated by the approach used above. Briefly, territories that had direct or indirect flights to Japan were classified into three risk categories of 'High', 'Medium' or 'Low' as described above. For territories in the 'High' category, the territory risk was set as a beta distribution of the potential number of non-reported ASF-infected domestic pigs in each territory per month and the estimated number of slaughtered domestic pigs in each territory per month. For territories in the 'Medium' or 'Low' risk categories, the territory risk was estimated by considering the probability of an outbreak occurring in the territory, the average size of an ASF outbreak, the duration of the infection, the probability of outbreaks being undetected and the proportion of pigs to be slaughtered every month. Further detail of the risk calculation method is available from the previous study [84,125]. The obtained value of P_{asf_o} was then ranked from 1 to 5 based on the Jenks natural breaks classification (R_{asf_o}).

Tourist-related risk (R_{visit_od})

In the present study, tourists visiting Japan for sightseeing purposes (N_{sight}) were assumed to carry the potential risk of exposing wild boar to ASFV via PPAP. Tourists may come into contact with wild boar in the natural areas of wild boar inhabit. First, the annual number of tourists coming to Japan for sightseeing purposes per origin territory per destination prefecture (N_{sight_od}) was calculated using a similar approach to that already mentioned above. The value of N_{sight_od} was calculated using the following formula:

$$N_{sight_od} = N_{all_od} * (N_{qsight_od} / N_{qall_od})$$

where N_{qsight_od} is the number of tourists coming to Japan per territory per

destination prefecture during the period of the survey.

The mean proportion of tourists visiting natural areas per territory (W_{nature_o}) was estimated based on a survey by the Japan Tourism Agency [141]. The annual number of tourists visiting natural areas per territory per destination prefecture (N_{nature_od}) was estimated by multiplying the value of N_{sight_od} by the value of W_{nature_o}. The annual weight of PPAP arriving in the natural area in each destination prefecture from each origin territory (V_{nature_od}) was calculated by multiplying the value of N_{nature_od} by the value of V_{enter_vo}, which had already been estimated above. The obtained value of V_{nature_od} was then ranked from 1 to 5 based on Jenks natural breaks (R_{visit_od}).

Wild boar distribution (R_{prsc_d})

The wild boar distribution per destination prefecture (R_{prsc_d}) was estimated based on two risk sub-estimators: the proportion of 5 km^2 meshes with a wild boar presence per prefecture (W_{prsc_d}) and the density of captured wild boar per wild boar habitable area per prefecture (D_{capt_d}). The annual number of captured wild boar per prefecture (N_{capt_d}) was used for the risk calculation since no estimation of the wild boar population had been conducted at prefecture level in Japan. The value of W_{prsc_d} was calculated by dividing the number of 5 km^2 meshes with a wild boar presence per prefecture ($N_{mesh\text{-}wb_d}$) by the total number of 5 km^2 meshes per prefecture ($N_{mesh\text{-}all_d}$). The information on the number of 5 km^2 meshes with a wild boar presence was obtained from a survey by the Biodiversity Center of Japan, Ministry of the Environment [142]. The value of D_{capt_d} was calculated by dividing the annual number of captured wild boar per prefecture (N_{capt_d}) by the habitable area for wild boar per prefecture (km^2) (A_{habit_d}). The value of N_{capt_d} was obtained from MOE statistics [143]. The value of A_{habit_d} was calculated based on a QAH map, which is a cartographic tool previously suggested as a potential tool for managing ASF

[46]. Briefly, it is a standardised distribution map based on GLOBCOVER that quantifies the QAH for wild boar. The QAH map provided seven levels of QAH, namely 0, 'absent'; 0.1, 'unsuitable'; 0.5, 'worst suitable area'; 1, 'suitable areas for food or shelter'; 1.5, 'suitable areas for food and shelter, but used mainly for one or the other'; 1.75, 'suitable areas for food and shelter, but mainly used for food'; and 2, 'suitable areas for both food and shelter'. In the present study, areas with QAH levels greater than 1 were regarded as habitable areas for wild boar and areas with QAH levels greater than 1.5 were regarded as areas suitable for wild boar. The obtained values of W_{prsc_d} and D_{capt_d} were converted to an adjusted risk rank on a scale of 1 to 5 using Jenks natural breaks, respectively. The value of R_{prsc_d} was then calculated as the product of the values of W_{prsc_d} and D_{capt_d}.

Probability of wild boar suitable habitat (R_{area_d})

The probability that there would be suitable habitat for wild boar in each prefecture (R_{area_d}) was estimated based on the proportion of the wild boar suitable habitat area (QAH levels of more than 1.5) in the total area of each prefecture (W_{habit_d}). The value of W_{habit_d} was calculated by dividing the area of the wild boar suitable habitat ($A_{suit\text{-}habit_d}$) by the total area of the prefecture (km^2) (A_{all_d}) obtained from the Geospatial Information Authority of Japan database [144]. The obtained value was converted to an adjusted risk rank on a scale of 1 to 5 using Jenks natural breaks (R_{area_d}).

Risk of direct or indirect contact between tourists and wild boar ($R_{contact_d}$)

The probability of direct or indirect contact between tourists and wild boar per prefecture ($R_{contact_d}$) was calculated based on seven risk sub-estimators: the proportion of the area with QAH levels of 1 in the total area of the prefecture (W_{qah1_d}); the number of bus stops, road stations, train stations, and tourist resource points overlapping with the wild boar habitat area per square kilometre per prefecture ($N_{bus\text{-}over_d}$, $N_{road\text{-}over_d}$, $N_{train\text{-}over_d}$,

and $N_{\text{tores-over_}d}$, respectively); the proportion of the prefecture covered by natural parks ($W_{\text{npark_}d}$); and the number of hunters per prefecture ($N_{\text{hunt_}d}$). According to Bosch et al., an area with QAH level of 1 to two km buffer area is the place where frequent contact between domestic pig and wild boar is likely to occur, suggesting that, due to the frequent emergence of wild boar in areas of human habitation, the risk of contact between tourists and wild boar should be at a certain level. The value of $W_{\text{qah1_}d}$ was calculated by dividing the area with QAH levels of 1 (km^2) ($A_{\text{qah1_}d}$) by the value of $A_{\text{all_}d}$. The value of $A_{\text{qah1_}d}$ was calculated based on the QAH map as applied above.

The values of $N_{\text{bus-over_}d}$, $N_{\text{road-over_}d}$, $N_{\text{train-over_}d}$, and $N_{\text{tores-over_}d}$ were included as risk sub-estimators since it was expected that an increase in the number of tourists could increase the risk of contact between tourists and wild boar. First, the number of bus stops per prefecture was obtained from the MLIT database [145]. The obtained data was overlaid on the QAH map to calculate the value of $N_{\text{bus-over_}d}$ in ArcGIS 10.6.1 software (ESRI Inc., Redlands, CA, USA). The same method was applied to calculate the value of $N_{\text{road-over_}d}$, $N_{\text{train-over_}d}$, and $N_{\text{tores-over_}d}$.

Since natural parks are intended not only to protect the landscape but also to contribute to maintaining biodiversity, a higher risk of contact between tourists and wild boar was estimated for these areas. The value of $W_{\text{npark_}d}$ was calculated by dividing the area of the natural park per prefecture (km^2) ($A_{\text{nature_}d}$) by the value of $A_{\text{all_}d}$. The information on the area of the natural park per prefecture (km^2) was obtained from the MLIT database [145].

The number of hunters per prefecture ($N_{\text{hunt_}d}$) was also included as one of the risk sub-estimators since hunters should be familiar with wild animal habitats and have the potential to lead tourists to areas inhabited by wild boar. The information on the

number of hunters was obtained from MOE statistics [143].

Each of the obtained risk sub-estimators was converted to an adjusted risk rank on a scale of 1 to 5 using Jenks natural breaks. The value of $R_{contact_d}$ was calculated as the product of seven risk sub-estimators, and then it was ranged from 1 to 5 based on Jenks natural breaks again.

Finally, the risk of ASFV transmission from each origin territory to wild boar in each prefecture of Japan via PPAP (R_{wb_od}) was calculated semi-quantitatively using the following formula:

$$R\text{wb}_od = \sum_n R\text{asf}_o * R\text{visit}_od * R\text{prsc}_d * R\text{area}_d * R\text{contact}_d$$

Risk of ASFV exposure to susceptible animals in prefectures of Japan (R_{total_od})

The risks of domestic pig and wild boar exposure to ASFV were collated to estimate the overall risk per territory per destination prefecture. As the risk of domestic pig exposure to ASFV was calculated quantitatively, the risks of transmission by foreign job trainees and foreign tourists were summed up, and then the risk score from 1 to 5 was assigned based on Jenks natural breaks. The overall relative risk of ASFV transmission to susceptible animals in prefectures of Japan (R_{total_od}) was calculated using the following formula:

$$R_{total_od} = R_{dp_od} * R_{wb_od}$$

Results

Risks to domestic pigs posed by foreign job trainees

The overall mean annual probability of domestic pig exposure to ASFV via PPAP by foreign job trainees was assumed to be 0.435 (95% CI: 0.035–0.966), which approximately corresponds to one introduction every 2.3 years.

In terms of the highest risk territory, the top five highest risk territories were in Asia (Table 11). Vietnam was the highest risk territory, with a risk of 0.432 (95% CI; 0.035–0.965), followed by the Philippines with a risk of 0.011 (95% CI; 0.000–0.043) and by Cambodia with a risk of 0.001 (95% CI; 0.000–0.003).

In terms of the destination prefecture, the top three highest risk prefectures were located in the Kanto region, which is located in the eastern part of the main island of Japan, namely Chiba prefecture with a risk of 0.082 (95% CI; 0.005–0.288), Ibaraki prefecture with a risk of 0.068 (95% CI; 0.004–0.234) and Saitama prefecture with a risk of 0.058 (95% CI; 0.003–0.201) (Table 12).

Table 11. The risk result of ASFV exposure to domestic pig in prefectures of Japan via PPAP by foreign job trainees per origin territory

Territory of origin	Risk value [95% confidence interval]
Vietnam	0.432 [0.035 - 0.965]
the Philippines	0.011 [0.000 - 0.043]
Cambodia	8×10^{-4} [0.000 - 0.003]
China	3×10^{-4} [0.000 - 0.001]
Mongolia	2×10^{-5} [0.000 - 6×10^{-5}]
Myanmar	1×10^{-5} [0.000 - 4×10^{-5}]
Thailand	1×10^{-6} [0.000 - 5×10^{-6}]
Indonesia	9×10^{-9} [0.000 - 0.000]
Sri Lanka	4×10^{-9} [0.000 - 8×10^{-9}]

Table 12. The risk result of ASFV exposure to domestic pig in prefectures of Japan via PPAP by foreign job trainees per prefecture

Destination prefecture	Risk value [95% confidence interval]
Chiba	0.082 [0.005 - 0.286]
Ibaraki	0.068 [0.004 - 0.234]
Saitama	0.058 [0.003 - 0.201]
Miyazaki	0.045 [0.002 - 0.160]
Iwate	0.043 [0.001 - 0.155]
Aichi	4×10^{-2} [1×10^{-3} - 0.150]
Gunma	4×10^{-2} [8×10^{-4} - 0.147]
Hokkaido	5×10^{-2} [8×10^{-4} - 0.147]
Kanagawa	4×10^{-2} [1×10^{-3} - 0.135]
Ehime	3×10^{-2} [0.000 - 0.127]

The risk to domestic pigs posed by foreign tourists

The overall mean annual probability of domestic pig exposure to ASFV via PPAP by foreign job trainees was assumed to be 0.050 with a 95% CI of 0.000–0.214, which approximately corresponds to one introduction every 20 years.

In terms of origin territory, the top five territories with the highest risk were territories in Asia and Europe (Table 13). Vietnam was the territory with the highest risk of 0.042 (95% CI; 0.000–0.179), followed by the Philippines with a risk of 0.007 (95% CI; 0.000–0.026), China with a risk of 0.001 (95% CI; 0.000–0.005) and the Russian Federation with a risk of 0.0003 (95% CI; 0.000–4.95E-04).

In terms of the destination prefecture, the risk was dispersed around the whole of Japan. Chiba prefecture was at the highest risk at 0.012 (95% CI; 0.00–0.053), followed by Aichi prefecture with a risk of 0.010 (95% CI; 0.000–0.041), Nagasaki prefecture with a risk of 0.005 (95% CI; 0.000–0.019), and Oita prefecture with a risk of 0.005 (95% CI; 0.000–0.017) (Table 14).

The risks to domestic pig posed by foreign job trainees and foreign tourists were summed up to calculate the total risks to domestic pig, and then a risk score from 1 to 5 was assigned based on Jenks natural breaks (Figure 9). The obtained results showed that prefectures with high risks were located in the Kanto region. Chiba prefecture was found to be the prefecture at highest risk (5), followed by Ibaraki (4) and Saitama (4) prefectures.

In the present study, the level of farm biosecurity in each prefecture was scored from 0 to 1 as one of the parameters to calculate the overall risk of domestic pig exposure to ASFV. The obtained results indicated that the levels of farm biosecurity were widely

different among prefectures. The scores were distributed between the highest score of 0.73 and the lowest score of 0.20, with an average score of 0.49.

Table 13. The risk result of ASFV exposure to domestic pig in prefectures of Japan via PPAP by foreign tourists per origin territory

Territory of origin	Risk value [95% confidence interval]
Vietnam	0.042 [0.000 - 0.179]
the Philippines	0.007 [0.000 - 0.026]
China	0.001 [0.000 - 0.005]
the Russian Federation	3×10^{-4} [0.000 - 5×10^{-4}]
Cambodia	3×10^{-4} [0.000 - 6×10^{-4}]
Republic of Korea	2×10^{-4} [0.000 - 6×10^{-4}]
Poland	8×10^{-6} [0.000 - 0.000]
Myanmar	3×10^{-6} [0.000 - 3×10^{-6}]
Germany	7×10^{-7} [0.000 - 2×10^{-6}]
Italy	4×10^{-7} [0.000 - 2×10^{-7}]

Table 14. The risk result of ASFV exposure to domestic pig in prefectures of Japan via PPAP by foreign tourists per prefecture

Destination prefecture	Risk value [95% confidence interval]
Chiba	0.012 [0.000 - 0.053]
Aichi	0.010 [0.000 - 0.041]
Nagasaki	0.005 [0.000 - 0.019]
Oita	0.005 [0.000 - 0.017]
Hokkaido	4×10^{-3} [0.000 - 1×10^{-2}]
Yamanashi	4×10^{-3} [0.000 - 1×10^{-2}]
Mie	2×10^{-3} [0.000 - 7×10^{-3}]
Miyazaki	2×10^{-3} [0.000 - 7×10^{-3}]
Ishikawa	2×10^{-3} [0.000 - 6×10^{-3}]
Saitama	1×10^{-3} [0.000 - 3×10^{-3}]

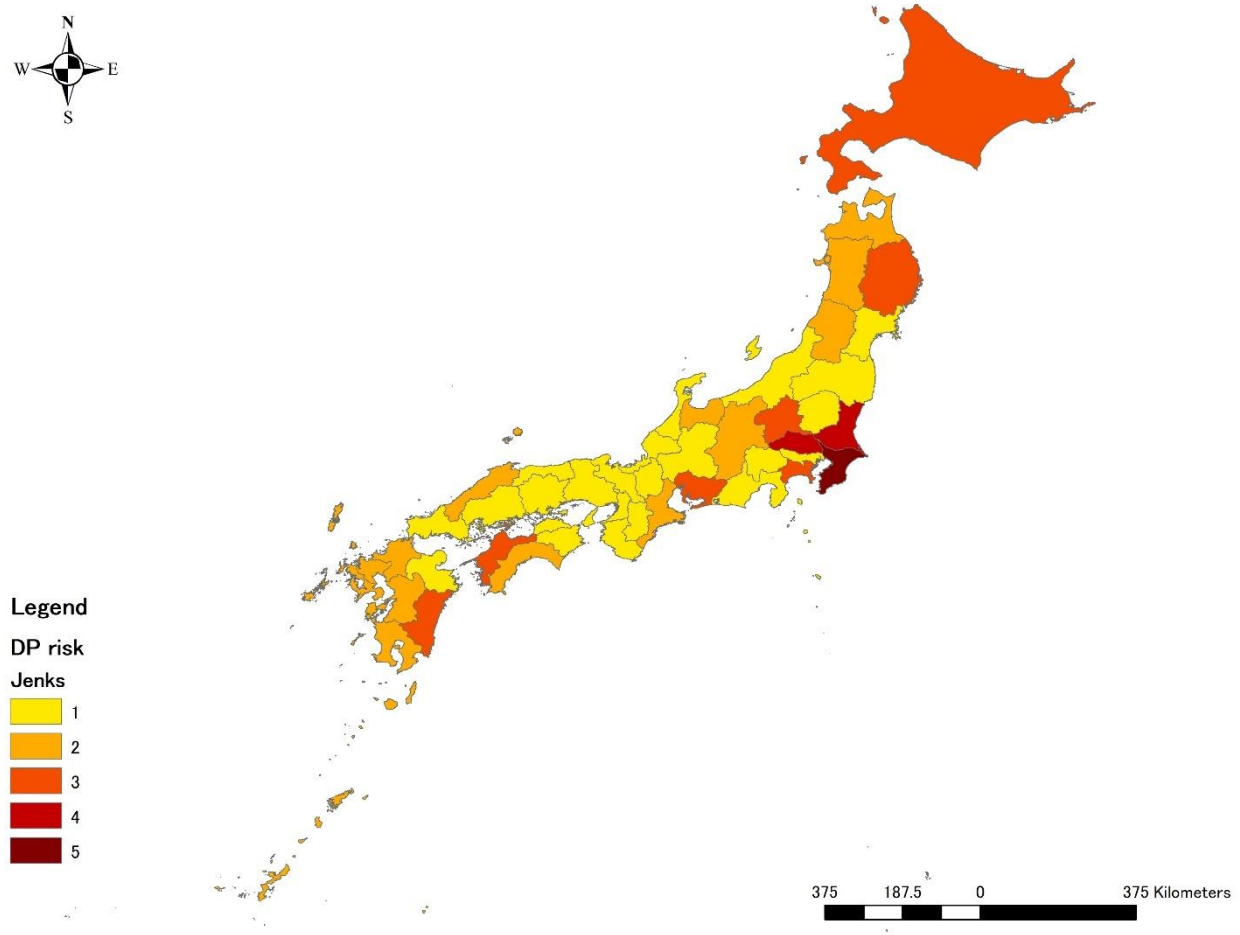


Figure 9. The risk results of ASFV exposure to domestic pig via pork products brought in air passengers' luggage at prefecture level. The graduated color map represents the relative risk from the highest (darker) to the lowest (lighter).

Risks to wild boar

In terms of the origin territory, the final output indicated that the highest risk of wild boar exposure to ASFV via PPAP came from Vietnam (5), followed by Cambodia (4), the Philippines (4), the Russian Federation (4), China (3), Ethiopia (3), and Republic of Korea (3) (Table 15). It is understandable that territories in the Asian region should present the highest risk of exposure to ASFV for wild boar in Japan, although the Russian Federation and Ethiopia were ranked in the top seven highest risk territories. The other territories were assigned a score of 1.

In terms of destination prefectures, prefectures in the Kyushu region, which is an island located in the western part of Japan, were likely to be categorised as high risk (Figure 10). The two prefectures of Fukuoka and Oita in Kyushu had the highest risk (5), followed by Hyogo (4) and Nagasaki (4). For the other prefectures, eight prefectures were assigned the score of 3, 16 prefectures scored 2, and the remaining 19 prefectures scored 1. Overall, the western part of Japan was likely to be at high risk of wild boar exposure to ASFV whereas the eastern and northern parts of Japan were likely to be at low risk of it.

Overall risk

The risks of domestic pig and wild boar exposure to ASFV were collated to obtain the overall risk. The obtained results indicated that susceptible animals in Fukuoka prefecture have the highest risk of exposure to ASFV (7), followed by Aichi (6), Chiba (6), Ehime (6), Miyazaki (6), Nagasaki (6) and Oita (6) prefectures (Figure 11). Six prefectures were assigned a score of 5, and nine prefectures were assigned a score of 4. The remaining 25 prefectures were assigned scores of less than 3.

Table 15. The risk result of ASFV exposure to wild boar via PPAP per origin territory

Territory of origin	Jenks score
Vietnam	5
Cambodia	4
the Philippines	4
the Russian Federation	4
China	3
Ethiopia	3
Republic of Korea	3

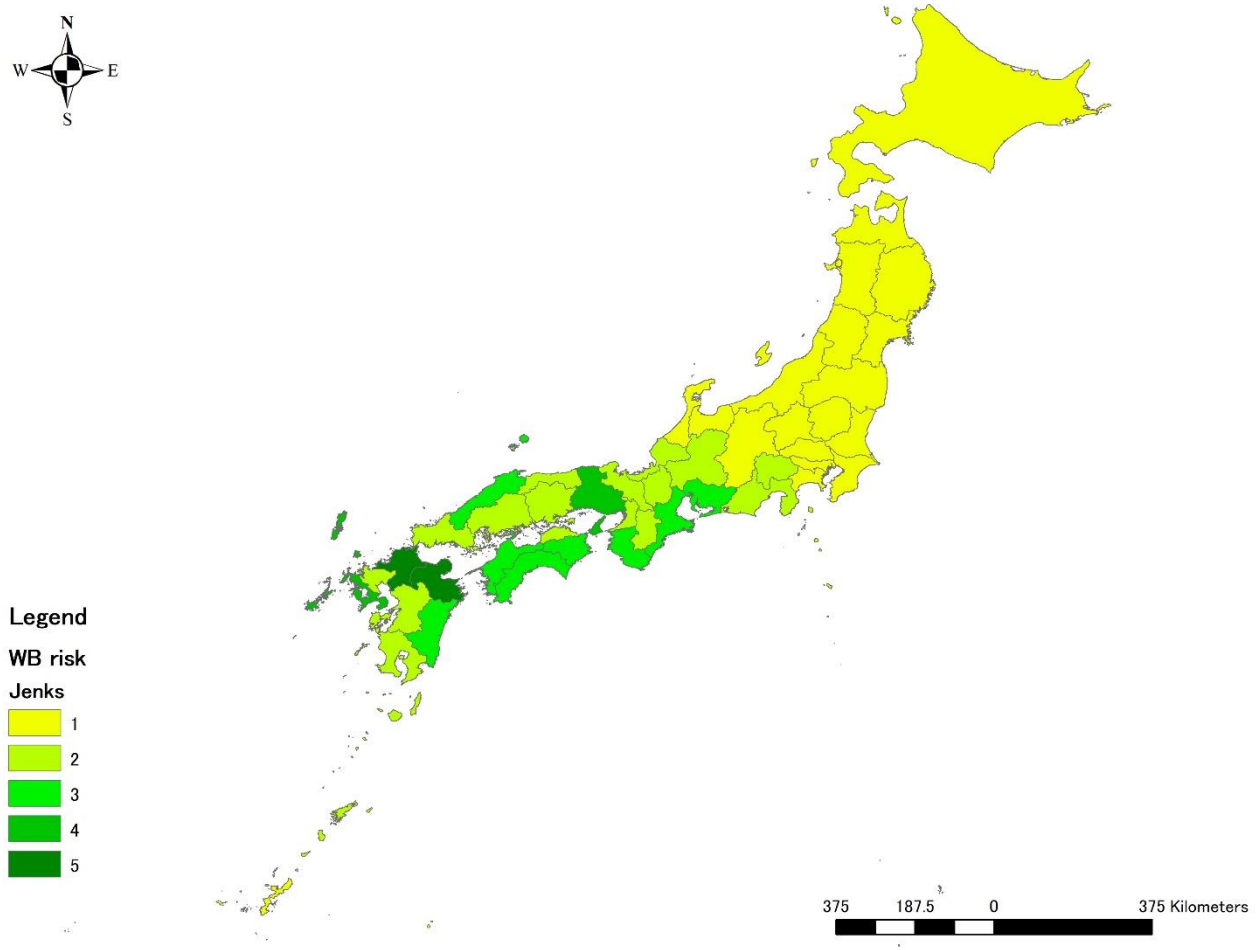


Figure 10. The risk results of ASFV exposure to wild boar via pork products brought in air passengers' luggage at prefecture level. The graduated color map represents the relative risk from the highest (darker) to the lowest (lighter).

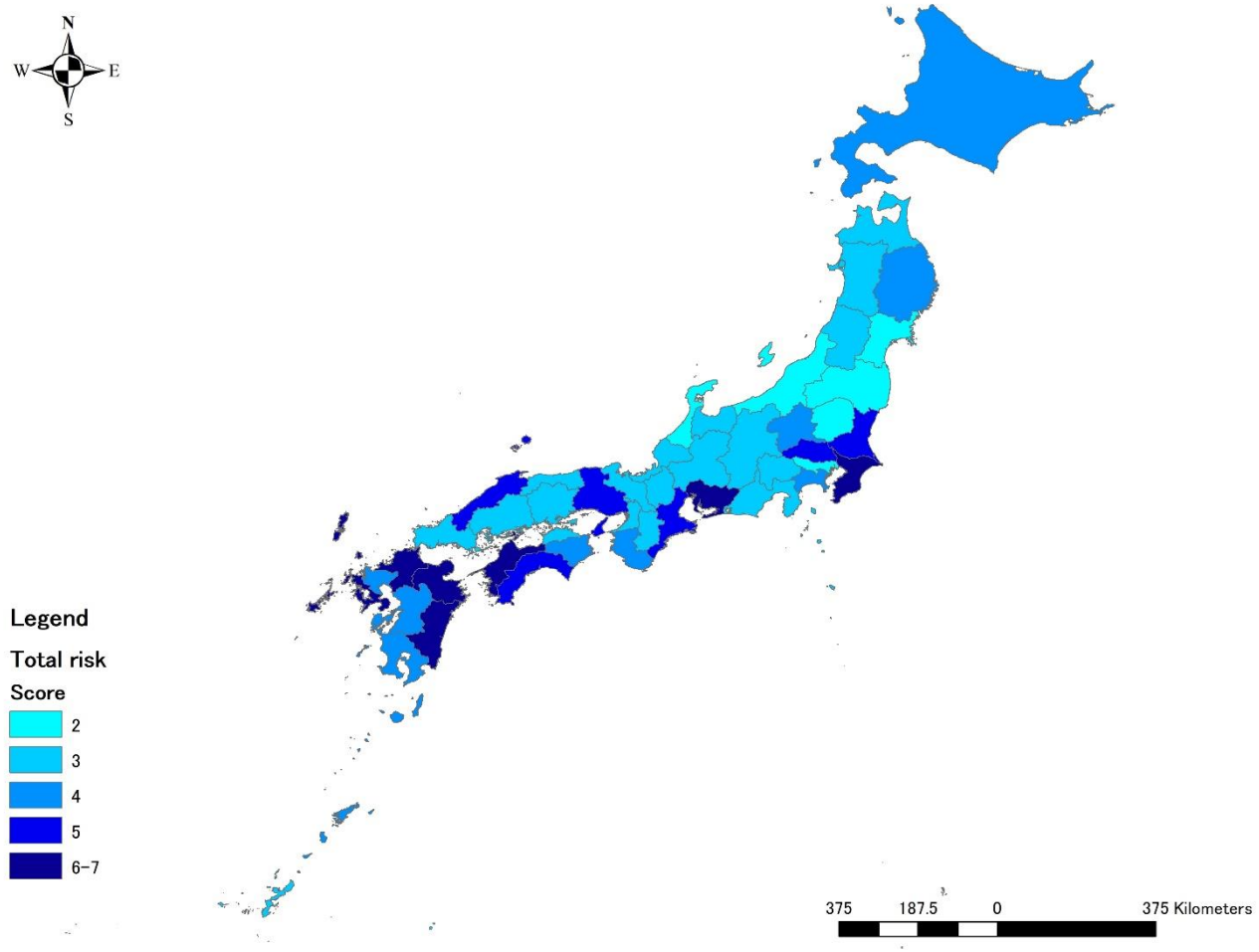


Figure 11. The risk results of ASFV exposure to susceptible animals via pork products brought in air passengers' luggage at prefecture level. The graduated color map represents the relative risk from the highest (darker) to the lowest (lighter).

Discussion

Several studies have assessed the risk of ASF introduction through various pathways [55,67,84,114-116]. However, epidemic ASF conditions require exposure risk assessments since the detection of ASFV-contaminated items has been reported in several territories [70-73]. Although the higher risk of disease in airports in Japan and origin territories were identified in the previous study [125], taking into consideration that the number of ASF outbreaks and affected areas in Asia have been continually increasing, it was necessary to conduct this in-depth study to protect Japan from ASF outbreaks. A total of 83 confiscations of PPAPs contaminated with ASFV were reported in Japan's 12 airports up to the end of November 2019, demonstrating that contagious pathogens have already reached the border of Japan and have the potential to penetrate inside the nation. Given this situation, JAQS has strengthened border controls by imposing penalty charges, increasing the number of detection dogs, and increasing the number of flights to be investigated [146]. However, it is difficult to investigate PPAPs of all passengers arriving in Japan, so the risk of ASFV being introduced into Japan is not negligible. Therefore, prevention of domestic ASF outbreaks depends on the effectiveness of preventive measures by competent authorities. Actually, under Japanese regulations, each of the prefectures is responsible for containing contagious animal diseases. Risk assessment at prefecture level should help to identify the necessary measures for prevention of and reaction to ASF outbreaks once they occur.

In the present study, the risk of exposure to ASFV via PPAP among susceptible animals in the prefectures of Japan was analysed in relation to three pathways. This was the first study to quantify the risk of foreign job trainees, even though the accessible data

was limited. The obtained results indicated that foreign job trainees from two territories present more than 99% of the total risk; the highest risk territory, Vietnam, accounted for 97% of the total risk, followed by the Philippines (2.5%). These findings can be attributed to several factors: the probability of PPAP contaminated with ASFV arriving from these territories, the average weight (kg) of PPAP not confiscated per person per territory, and the number of job trainees working in Japan per territory per prefecture. According to the 2017 JPPA report, more than 36% of foreign job trainees come from Vietnam, followed by China (21.9%) and the Philippines (11.7%). Although job trainees from China were the second-highest in number, the overall risk derived from Chinese job trainees was lower than those from the Philippines and Cambodia in the present study. Chinese visitors had the highest number of confiscated pork products, but the average weight of the PPAPs per person from China was lower than that from Vietnam, the Philippines and Cambodia. In addition to these factors, the current level of ASF infection in each territory also contributed to the results. In China, the first ASF outbreak was confirmed in August 2018; since then, 163 outbreaks had been notified and about 1.2 million pigs had been culled as at December 5, 2019 [7]. However, an enormous number of ASF outbreaks have been reported in Vietnam as well. Since the first ASF outbreak was confirmed on February 19, 2019, almost 8500 outbreaks had been detected and more than 5.9 million pigs had been culled in Vietnam by November 10, 2019 [7,147].

In terms of the prefectures at risk, all three prefectures with the highest risk were located in the Kanto region. According to the JPPA report, about 35% of farms in the Kanto region accept foreign job trainees, whereas in the Kyushu region, where the largest number of pigs are reared, only 3.6% of the farms accept foreign job trainees [128]. As not all farmers participated in the survey, the risk rank would change if all information

about foreign job trainees was available.

The risk of domestic pig exposure to ASFV via PPAP carried by foreign tourists was also evaluated in the present study. The obtained results indicated that the risk of the origin territory is more widely dispersed compared with the results of the scenario for foreign job trainees. Although 95% of the risks were derived from Vietnam (81%) and the Philippines (14%), European territories including the Russian Federation, Poland, Germany and Italy were listed in the top ten highest risk territories. The current ASF epidemic in Asia has given Japan warning of the considerable threat of ASF from the Asian region; however, the obtained results indicate that the risk of ASF transmission from Europe should not be ignored.

It was demonstrated that 90% of the total risk of domestic pig exposure to ASFV caused by foreign tourists was concentrated in the top ten highest risk prefectures; more than 50% of the entire risk was concentrated in the top three highest risk prefectures of Chiba (23%), Aichi (18%) and Nagasaki (10%). Tourists coming to Japan to see their friends or family may deliver PPAP to pig farmers. Therefore, the risk is likely to be influenced by the number of visitors coming to see friends or family and the proportion of pig farmers per total population in each prefecture.

In the present study, the annual risk of domestic pig exposure to ASFV via PPAP carried by foreign job trainees was 8.7 times higher than that presented by foreign tourists. It is generally held that the risk attributed to foreign job trainees is higher than that attributed to tourists. Our current approach successfully demonstrated the potential risk posed by foreign job trainees. According to our assumptions, the risk of ASFV introduction onto farms should be influenced by the level of farm biosecurity and workers' behaviour. Even if employees are educated in farm biosecurity by employers, the virus

could be introduced onto farms if workers do not follow instructions.

The management of farm biosecurity is the most basic but most important factor for the prevention of the disease. We adjusted the questionnaire survey regarding farm biosecurity to address more specifically the risk of introduction of ASFV in order to clarify the importance of each quarantine measure by gathering expert opinions. In the questionnaire, some of the questions were consistently scored as important by our experts, whereas opinion varied with regard to other questions such as the recording of visitors' details. Most of the experts agreed with the importance of the disinfection of vehicles and countermeasures against the invasion of wild animals, whereas there were various opinions about human movements on farms. The obtained results will be useful for reviewing farm biosecurity in each prefecture.

The risk of wild boar exposure to ASFV via PPAP was also evaluated as one of the scenarios in the current study. It is generally held that continuous ASF outbreaks in Europe can be attributed to the movement of affected wild boar as well as human movements [4]. Taking into consideration the fact that wild boar played an essential role in the Japanese CSF outbreak in 2018 [124], it can be easily assumed that ASF could have also been widely prevalent in Japan through successful contact among wild boar. The obtained results showed that the risk of wild boar exposure to ASF tends to be clustered in the western part of Japan; prefectures with a higher risk are centred in the Kyushu region. These results can be attributed to the probability of the existence of suitable habitat areas for wild boar and the density of captured wild boar per wild boar habitat area (km^2) as well as the high number of tourists potentially having contact with wild boar. As the largest number of pigs are in the Kyushu region, tremendous damage to the swine industry may be caused if ASF becomes prevalent, as was the case in the FMD outbreak in

Miyazaki in 2009 [8]. In contrast to the western part of Japan, almost all prefectures in the eastern and northern parts of Japan were assigned a score of 1. In terms of the risk presented by wild boar distribution, a total of five prefectures were assigned a score of 4 to 5, but none of the prefectures in the eastern part of Japan were included. A similar trend was observed in the probabilities relating to wild boar suitable areas and potential contact between tourists and wild boar. Although some prefectures in the eastern part of Japan were assigned relatively high scores according to some risk estimators, none of these prefectures was assigned a high score in other risk estimators, and therefore the overall risk score of prefectures in the eastern part of Japan was 1.

The results of the overall risk assessment demonstrated that Fukuoka prefecture had the highest score of 7, followed by Aichi (6), Chiba (6), Ehime (6), Miyazaki (6), Nagasaki (6) and Oita (6) prefectures, although the risk of domestic pig and wild boar infection cannot be compared directly due to the implementation of different approaches. Higher-risk prefectures tended to be clustered in the Kyushu region (Fukuoka, Miyazaki, Oita and Nagasaki), where the risks for wild boar tended to be higher than that for domestic pig, except for in Miyazaki prefecture. The prefectures of Aichi, Ehime and Miyazaki were assigned the same risk score of 3 in both the domestic pig and wild boar scenarios. Chiba prefecture had the highest score in the domestic pig scenario, but the lowest score was assigned for the risk to wild boar, whereas the results for Oita prefecture were the opposite. Even though the total score was the same, it is noteworthy that the characteristics of the potential ASF risks in each prefecture varied. The obtained results indicate that it is essential to understand the characteristics of the potential ASF risk in each prefecture and the effectiveness of control measures against the introduction of ASFV planned by each prefecture.

Summary

In recent years, ASF has become prevalent in many areas, including Asia. After notification of the first ASF outbreak in China in 2018, the disease spread rapidly to surrounding territories, and therefore it should be an urgent and critical matter for Asian territories, including Japan, to prepare for and ideally prevent the introduction of ASF. Repeated detection of ASFV genes in PPAP and confiscated at Japanese airports indicates that PPAP is one of the most likely risk pathways for the introduction of ASFV into Japan. In the present study, the risk of ASFV exposure to domestic pigs and wild boar in the 47 prefectures of Japan via three different PPAP pathways was assessed. Two quantitative and stochastic risk assessment models were built to estimate the annual probability of ASFV exposure to domestic pigs, which could be attributed to foreign job trainees or to foreign tourists. A semi-quantitative stochastic model was built to assess the risk of ASFV exposure to wild boar. The overall mean annual probability of ASFV exposure to domestic pig via PPAP carried by foreign job trainees was 0.435 [95% CI: 0.035–0.966], which corresponds to approximately one introduction every 2.3 years. The overall mean annual probability of ASFV exposure to domestic pig via PPAP carried by foreign tourists was 0.050 [95% CI: 0.000–0.214] which corresponds to approximately one introduction every 20 years. The overall risk of ASFV transmission to domestic pig was highest in the Kanto region. The risk of ASFV exposure to wild boar was generally higher in the western part of Japan; prefectures in Kyushu region were at especially high risk, whereas the risks in the eastern and northern parts of Japan were low. The results of the overall risk assessment demonstrated that the risk was dispersed around the entire nation of Japan, and the characteristics of the potential ASF risk in each prefecture were varied. The

obtained results could be useful for launching or improving effective control measures to prevent the introduction of ASFV, and for reviewing the current risk reduction activities in the field.

Conclusion

CSF is caused by infection with the CSFV, which belongs to the genus *Pestivirus*, family *Flaviviridae*. CSF is a highly contagious febrile disease with potential for high mortality that causes enormous economic loss in the pig industry worldwide [9].
After re-emergence of the CSF in Japan 2018, the diseases have transmitted continuously in both domestic pig and wild boar population, nevertheless of the vigorous effort to address the disease control.

The results of the spatio-temporal analysis showed that the disease likely spread to the northeast part of the prefecture. A maximum significant spatial association estimated between CSF notifications was 23 km by the multi-distance spatial cluster analysis. A space-time permutation analysis identified two significant clusters with an approximate radius of 12 and 20 km and 124 and 98 days of duration, respectively. When the area of the identified clusters was overlaid on a map of habitat quality, approximately 82% and 75% of CSF notifications, respectively, were found in areas with potential contact between domestic pigs and wild boar. These findings will help implement control measures in the affected area.

It has already well known that there are effective vaccines against CSFV but no approved vaccines against ASFV is available. The histories of transboundary animal disease outbreaks in Japan and the current ASF epidemic situation in Asia have warned Japan against the considerable threat of ASF spread from affected territories. Given that wild boar play an important role in both CSF and ASF, it is plausible to assume that similar or even worth epidemic situation could be caused once the ASF outbreak occurred in Japan.

In the present study, we assessed the risk of ASFV introduction to Japan via PPAP, which is one of the most likely risk pathways for the introduction of ASFV into Japan. For the purposes of this analysis, we considered 214 international commercial flights traveling from 47 origin territories to 31 destination airports as potential routes of ASFV introduction via PPAP. The risk was estimated quantitatively through a stochastic model that considered the volume of air passengers' luggage, the amount of confiscated pork products that were carried in air passengers' luggage, and the disease status of the origin territory. The overall mean annual probability of ASFV introduction to Japan via PPAP was found to be 0.941 [95% CI, 0.661–1.000], which approximately corresponds to one introduction every 1.06 years. At the origin territory level, Mongolia was led as the highest risk territory, with a risk of 0.864 (95% CI, 0.434–1.000), followed by China (0.697; 0.223–0.999), Vietnam (0.662; 0.196–0.998), and the Russian Federation (0.136; 0.018–0.401). At the destination airport level, Narita International Airport had the highest risk (0.905; 0.537–1.000), followed by Kansai International Airport (0.496; 0.109–0.961), Tokyo International Airport (0.389; 0.072–0.879), and Chubu Centrair International Airport (0.338; 0.058–0.816). This information will help improve risk management activities and monitoring systems to prevent the introduction of ASFV to Japan.

Repeated detection of ASFV genes from PPAP confiscated at Japanese airports indicates that PPAP is one of the most likely risk pathways for the introduction of ASFV into Japan. In the present study, the risk of ASFV exposure to domestic pigs and wild boar in the 47 prefectures of Japan via three different PPAP pathways was assessed. Two quantitative and stochastic risk assessment models were built to estimate the annual probability of ASFV exposure to domestic pigs, which could be attributed to foreign job trainees or to foreign tourists. A semi-quantitative stochastic model was built to assess the

risk of ASFV exposure to wild boar. The overall mean annual probability of ASFV exposure to domestic pig via PPAP carried by foreign job trainees was 0.435 [95% CI: 0.035–0.966], which corresponds to approximately one introduction every 2.3 years. The overall mean annual probability of ASFV exposure to domestic pig via PPAP carried by foreign tourists was 0.050 [95% CI: 0.000–0.214] which corresponds to approximately one introduction every 20 years. The overall risk of ASFV transmission to domestic pig was highest in the Kanto region. The risk of ASFV exposure to wild boar was generally higher in the western part of Japan; prefectures in Kyushu region were at especially high risk, whereas the risks in the eastern and northern parts of Japan were low. The results of the overall risk assessment demonstrated that the risk was dispersed around the entire nation of Japan, and the characteristics of the potential ASF risk in each prefecture were varied. The obtained results could be useful for launching or improving effective control measures to prevent the introduction of ASFV, and for reviewing the current risk reduction activities in the field.

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Summary in Japanese (和文要旨)

CSF（豚コレラ）はフラビウイルス科ペスチウイルス属 CSF ウイルスによって引き起こされる家畜伝染病で、感受性動物である豚及びイノシシに高い致死率と強い伝染力をもたらす。2018 年 9 月に日本国内で 26 年ぶりとなる CSF の発生が確認されたが、迅速な終息には至らず、発生から 1 年 3 ヶ月経過しても家畜豚・野生イノシシへの感染が報告されている。本研究では、日本国内における CSF 発生拡大に関する疫学的知見を得るために発生報告情報を用いて時空間疫学解析を実施した。分布指向性分析の結果、本疾病の北東方向への拡大が示唆された。また、Ripley の K 関数法を用いた解析では、各発生間での最大関連距離は 23km と算出された。推定イノシシ最適生息域と時空間置換モデルクラスター解析の結果を重ね合わせたところ、クラスターは野生イノシシと家畜豚の接触が想定される地域に形成された。本解析で得られた情報は今後の CSF 感染拡大防止策を検討するうえで有益であると考えられる。

CSF と同様に越境性動物感染症に分類され、疫学的にも CSF と類似している ASF(アフリカ豚コレラ)は、アスファウイルス科アスフィウイルス属 ASF ウイルス(ASFV)によって引き起こされる豚及びイノシシに感染する家畜伝染病である。2018 年より流行地域であるアフリカ・欧州諸国からアジア地域へと感染が急速に拡大していることから、日本への侵入が危惧されている疾病であり、ASF に対して有効なワクチンが開発されていないことから、一度我が国への侵入を許せば畜産産業に甚大な被害が及ぶことが想定される。そこで本研究では、ASFV 侵入経路の 1 つとして監視すべき重要な経路である国際航空旅客手荷物由来の豚肉製品(PPAP)を介した ASFV の日本への侵入リスクと、国内侵入後の感受性動物への曝露リスクを出発国別かつ都道府県別に評価した。侵入リスク評価について、本研究では 47 の国と地域から日本国内 31 の国際空港に就航している 214 航空路線を対象とした。確率論的モデルを用い、旅客の手荷物重量、各出発国由来の豚肉製品押収品量および出

発国の ASF 流行状況を考慮して定量的にリスクを評価した。定量的リスク評価の結果、PPAPを介したASFVの日本への侵入リスクは0.941 (95%信頼区間 0.661,1.000)と導かれ、この結果は ASFV が PPAP を介して 1.06 年に 1 回日本へ侵入する可能性があることを示している。また感受性分析の結果、検疫での違法持ち込み豚肉製品の見逃し率、PPAP の重量が結果に大きな影響を及ぼしていることがわかった。

次に、上記の国及び地域から日本を訪れた外国人による PPAP を介し、国内感受性動物への曝露リスク評価を都道府県別に実施した。PPAP を介して ASFV が家畜豚に曝露する経路として、外国人技能実習生及び外国人観光客を介した経路を想定し、これらの経路について確率論的モデルを用いて定量的に暴露リスクを評価した。また、PPAP を介して ASFV が野生イノシシに曝露する経路として、観光地を訪れた外国人観光客を介した経路を想定し、本経路については確率論的モデルを用いた半定量的リスク評価を実施した。解析の結果、外国人技能実習生による家畜豚への ASFV 曝露リスクは 0.435 (95%信頼区間 0.035,0.966)と導かれ、2.3 年に 1 回 ASFV が家畜豚に曝露する可能性が示された。また、外国人観光客を介した家畜豚への ASFV 曝露リスクは 0.050 (95%信頼区間 0.000,0.214)と導かれ、これは 20 年に 1 回 ASFV が家畜豚に曝露する可能性が示された。家畜豚への ASFV 曝露リスクを都道府県毎に評価したところ、関東地方にリスクの高い県が集中していた。野生イノシシへの ASFV 曝露リスク評価では、北・東日本と比べて西日本におけるリスクが全体的に高く、特に九州地方にリスクの高い県が集中していた。得られた結果は、PPAP を介した感受性動物への ASFV 曝露リスクは日本全国に広がっており、危惧すべき曝露経路も各都道府県で異なることを示唆していた。本研究で得られた結果は、リスク分析に基づく高リスク地域における ASFV 侵入対策の実施に有用であると考えられる。