

GOPEN ACCESS

Citation: Sugiura K, Lei Z, Holley C, Haga T (2020) Assessing the risk of ASFV entry into Japan through pork products illegally brought in by air passengers from China and fed to pigs in Japan. PLoS ONE 15(5): e0232132. https://doi.org/ 10.1371/journal.pone.0232132

Editor: Grzegorz Woźniakowski, Panstwowy Instytut Weterynaryjny - Panstwowy Instytut Badawczy w Pulawach, POLAND

Received: February 6, 2020

Accepted: April 7, 2020

Published: May 5, 2020

Copyright: © 2020 Sugiura et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: KS, TH Ken-163 The Ito Foundation https://www.itokinen-zaidan.or.jp/ The funder had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

RESEARCH ARTICLE

Assessing the risk of ASFV entry into Japan through pork products illegally brought in by air passengers from China and fed to pigs in Japan

Katsuaki Sugiura^{1*}, Zhihao Lei¹, Caitlin Holley², Takeshi Haga³

1 Department of Global Agricultural Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan, 2 Regional Representation for Asian and Pacific, World Organisation for Animal Health (OIE), Bunkyo-ku, Tokyo, Japan, 3 Department of Veterinary Medical Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

* aksugiur@mail.ecc.u-tokyo.ac.jp

Abstract

A risk assessment was conducted to assess the risk of ASFV entry into Japan through pork products illegally brought in by air passengers from China and fed to pigs in Japan. Scenario tree modelling was used with the following entry and exposure pathway considered to be the most likely route of ASF entry: an ASFV infected pork product is illegally brought into Japan by air travellers from China; this pork product is then used in a restaurant where scrap waste is recycled for animal feed and subsequently fed to pigs without being heattreated. Input parameter values were based on surveys conducted by the authors, scientific data gathered from the literature and official data published by government agencies. The annual probability of ASFV entry into Japan via this pathway was predicted to be 0.20 (90% prediction interval: 0.00–0.90). The wide prediction interval was mainly caused by the uncertainty regarding the dose response relation of ASFV, followed by the probability of an ASF infected pig dying on affected farms, the loading of ASFV in an infected pig and the probability of an illegally imported pork product being heat-treated in China and used in restaurants. The results of scenario analysis revealed that the annual probability of ASFV entry into Japan will increase with an increase in the number of ASF affected farms in China. The probability of ASFV entry will increase substantially even if only a small proportion of Ecofeed is not heat-treated during the production process. The probability will decrease if an increased proportion of farms that feed swill apply heat-treatment before feeding swill to their pigs. These findings indicate that stringent application of heat-treatment of Ecofeed and swill is key to protecting the Japanese pig industry from the introduction of ASFV.

Introduction

African swine fever (ASF) is a highly contagious disease affecting pigs. It is caused by African swine fever virus (ASFV), which belongs to the genus Asfivirus of the Asfaviridae family [1].

ASF can spread through direct or indirect contact and causes high mortality. The ASFV persists for a long time in the environment a in a variety of pig products. Wild boar can harbor the virus and ASF may become endemic with or without an added transmission cycle through Ornithodoros ticks [2].

Traditionally ASF was confined to Africa, with occasional incursions into other regions until in 2007 when ASF was introduced into Georgia, a Caucasus country [3]. From Georgia, the disease spread to neighboring countries, including Armenia, the Russian Federation in 2007, and Azerbaijan in 2008. From the Caucasus, the disease continued northward and westward in Ukraine and Belarus in 2013, in Poland, Latvia, Estonia and Lithuania in 2014, and in Czech Republic and Romania in 2017, with additional outbreaks in the east of the Russian Federation in domestic pig and wild boar populations [3, 4]. In March 2017, ASF was reported in Irkutsk, Russian Federation, approx. 1,000 km from the border with China [5].

ASF appeared in China, the world's largest pig producer and pig meat consumer, with the first outbreak reported on a farm near Shenyang City in Liaoning Province on 3 August 2018 [6, 7]. The pigs on this farm had been fed table scraps and developed acute clinical disease around mid-June 2018 [7]. Since then, ASF spread in China, with 163 outbreaks reported in 22 provinces, 5 autonomous regions and 5 municipalities as of 16 January 2020 [8]. The epidemic of ASF in China constitutes an important threat to Japan. The outbreaks reported in Vietnam, Mongolia, Cambodia, Hong Kong SAR, Democratic People's Republic of Korea, Laos, Myanmar, the Philippines, Republic of Korea, Timor-Leste and Indonesia at the time of writing of this paper further highlights the risk of spread of ASF to Japan [8].

Japan has a geographical advantage of being isolated by the sea and thus the risk of introduction of ASF through importation of live pigs or pork products by land transportation and movements of wild boar is practically null. However, Japan is subject to considerable international movements of aircrafts carrying increasing numbers of travelers: a total of 26.3 million foreign visitors traveled to Japan by aircraft in 2018, accounting for 92% of all foreign visitors. Of this, 5.8 million were from China, accounting for 22% of the foreign air travelers [9].

The increasing number of travelers coming to Japan might also increase the number of incidences of illegal importation of pig products into Japan, thus increasing the probability of introduction of ASF into Japan. In 2018, 42,280 cases of illegal importation of pork products by Chinese travelers were detected by detector dogs and/or interrogation by the customs officers at the airports, accounting for 45% of all confiscated products [10].

In response to the increasing risk of introduction of ASF, the Japanese government has tightened quarantine operations at airports and seaports, especially for travelers from China, by

- strengthening measures and penalties against illegal importation of meat and meat products by air passengers [11];
- raising the awareness by the use of posters and inflight announcements [12];
- stringent application of penalties for travelers attempting to illegally import meat and meat products [12]; and
- increasing the number of detector dogs at the airports [13].

The possibility of animal products infected with ASFV being introduced into Japan has been identified previously as one of the highest risks for the entry of ASF into Japan [14]. Given the importance of the pig industry in Japan and the current situation of ASF in China, this study aimed at assessing the risk of ASFV entry into Japan through pig products illegally brought in by air passengers from China and fed to pigs in Japan through swill feeding.

Materials and methods

Model development

Model structure. We developed a quantitative stochastic risk assessment model. The model structure is shown in Figs 1 and 2. First we estimated the total amount of infected pig products illegally imported by air travelers from China into Japan ($W_release$). From this, we then estimated the amount of infected pork scraps originating from pork products illegally imported by air travelers from China and being fed to pigs in Japan as swill without being heat-treated ($W_exposure$). We multiplied this with the infectious load of infected pork product ($L_infected pork$), to estimate the infectious load of ASFV fed to pigs without being heat-treated ($L_exposure$). Using this load and dose response relation, we assessed the risk of at least one pig becoming infected with ASFV ($P_introduction$).

Total amount of infected pig products illegally imported by air travelers from China into Japan (g) ($W_{release}$). We assumed that infected pig products are brought from China into Japan by air travelers when:

- the pig used for the production of pork products in China is infected with ASFV;
- the pork products derived from infected pig are not heat-treated in China and
- the pork products are illegally brought in by air travelers from China.

Based on this assumption, the *W_release* can be modelled:

 $W_release = P_slaughter \times (1-P_heat-treated in China) \times N_air travelers \times P_illegal Model 1 importation \times W_pork product per traveler$

where *P_slaughter* is the prevalence of ASF infected pigs slaughtered for consumption in China; *P_heat-treated in China* is the probability that the pork product brought in illegally is heat-treated in China; *N_air travelers* is the number of air travelers that visit Japan annually; *P_illegal importation* is the probability that an air traveler from China illegally brings in pork products; and *W_pork product per traveler* is the average amount of pork product illegally brought in by air travelers from China.

Of these variables, *P_slaughter* was estimated using the model developed by VLA [15]:

$$P_slaughter = \frac{N_affected_farms \times H \times t \times (1 - d)}{N_pigs \times P_notfification}$$

where *P_affected farms* is the number of affected farms per year; *H* is the average herd size; *t* is the duration of infection in years; *d* is the probability an infected animal dies; *N_pigs* is the pig population in China; and *P_notification* is the probability of notification.

Amount of infected pork waste originating from pork products illegally brought in by air travelers from China and fed to pigs in Japan as swill without being heat-treated (g) ($W_exposure$). We assumed that pigs in Japan are exposed to infected pig products illegally imported by air travelers when:

- the infected pig products illegally brought in were used in restaurants;
- the infected pork products are not consumed in restaurants;
- the waste from the restaurants is recycled for feed; and
- the recycled waste is used for production of Ecofeed without being properly heat-treated or
- the recycled waste is fed to pigs without being heat-treated.



Based on this assumption, *W_exposure* can be modelled:

$$\begin{split} W_exposure &= W_release \times P_restaurant use \times P_waste \times P_recycle \times P_recycle for feed \times \\ (P_recycle for ecofeed \times (1 - P_ecofeed heat - treated) + (1 - P_recycle for ecofeed) \times \\ (1 - P_swill heat - treated on farm)) \end{split}$$

where *P_restaurant use* is the proportion of *W_release* used at restaurants; *P_waste* is the proportion of the pork product waste to the pork products used in the restaurants; *P_recycle* is the proportion of the amount of pork product waste recycled to the amount of the pork product waste produced in restaurants; *P_recycle for feed* is the proportion of recycled pork product waste used for feed to the amount of pork product waste recycled; *P_recycle for ecofeed* is the proportion of the amount of pork product waste recycled for Ecofeed to the amount of pork product waste recycled for Ecofeed to the amount of pork product waste recycled for Ecofeed to the amount of pork product waste recycled for Ecofeed to the amount of pork product waste recycled for Ecofeed is the probability that pork product waste recycled for Ecofeed is heat-treated; and *P_swill heat-treated on farm* is the probability that pork product waste recycled for feed and used as swill is heat-treated before being fed to pigs.

Ecofeed is feed produced from food waste under the Law for the Promotion of Recycling of Food Waste (Law No.116, 2000) to increase the self-sufficiency of feed ingredients. There are 367 registered Ecofeed plants as of May 2017 with an annual production of 1,220,000 metric tons in terms of TDN [16].

P_restaurant use was modeled as:

$P_{restautrant use} = Prob_{restaurant} \times Ratio_{restaurant}$

where *Prob_restaurant* is the probability that the pork product illegally imported is used for restaurant; and *Ratio_restaurant* is the ratio between the average weight of pork products destined for restaurants and the average weight of pork products brought in by air travelers.



Fig 2. Model structure for estimating the amount of infected pork scraps fed to pigs as swill without being heat-treated (W_exposure).

In our model, we assumed that infected pork products that is consumed personally in households will present no risk. This is a reasonable assumption as food waste from Japanese households is incinerated without being recycled for other uses including use as animal feed [17].

Infectious load of ASFV fed to pigs without being heat-treated (*L_exposure*). L_exposure was calculated as the product of

$$Lexposure = W_exposure \times Linfected pork$$
 Model 3

where *L_infected pork* is the loading of ASFV in an pig infected with ASF.

Probability that a pig becomes infected (*P_infection***).** Probability of a pig becoming infected through consumption of infected pork product waste (*P_infection*) was calculated using an exponential dose-response curve based on the dose-response data by McVicar [18].

$$P_{-infection} = 1 - \exp(-c \times Lexposure/n)$$
Model 4

where *c* is a dose reponse coefficient; and *n* is the number of pigs that consume infected pork products without being heat-treated.

Risk of at least one pig becoming infected (*P_introduction*). P_introduction was calculated by:

$$P_{\text{introduction}} = 1 - (1 - P_{\text{infection}})^n \qquad \text{Model 5}$$

Inserting Model 4 into Model 5,

$$P_{introduction} = 1 - (1 - (1 - \exp(-c \times Lexposure/n))^{n}$$

= 1 - exp(-c × Lexposure) Model 6

Thus, the variable *n* disappears, i.e. *P_introduction* does not depend on the value of *n*.

Input variables

List of input variables used in this study is shown in Table 1.

Number of ASF affected farms in China per year (*P_affected farms*). *N_affected farms* is estimated to be 56 based on the number of outbreaks reported to the OIE by the Chinese government during the year 2019: during this period 57 outbreaks were reported [19], with one only involving wild boar.

Average pig herd size (H). Because there is no official information available regarding the average pig herd size in China, *H* was assumed to be 1,644 based on the number of susceptible animals that were killed or died on ASF affected farms reported to the OIE during 2019 [19].

Duration of infection in years (*t***).** *t* was assumed to be 230/365 = 0.63 as assumed by VLA [15].

Probability an infected animal dies (*d***).** *d* was assumed to be Uniform (0.5, 1) as assumed by VLA [15].

Pig population in China (*N_pigs***).** *N_pigs* was estimated to be 441,589,000 based on China Animal Husbandry Yearbook 2018 [20].

Probability of notification (*P_notification*). There is no official information available on the level of underreporting of ASF outbreaks in China. Under the Chinese compensation system for ASF affected farms provincial authorities are responsible for compensation and if there are insufficient funds or for other reasons, there is a chance outbreaks are not reported [21]. *P_notification* was assumed to be 0.4 as assumed by VLA [15] with some uncertainty and modeled as Pert(0.2, 0.4, 0.6).

Probability that the pork product brought in illegally is heat-treated in China (*P_heat-treated in China*). *P_heat-treated in China* was modeled as Beta (69–2+1, 2+1) based on the result of testing of pork products illegally imported by air travelers from China and confiscated by Japanese Animal Quarantine Service (AQS) from 1 October 2018 to 18 February 2020: 69 products tested ASF positive with PCR, indicating that they are made from ASF infected pigs. From two of these PCR positive products, ASFV was isolated, suggesting that they were not properly heat-treated during the processing procedure in China [22].

Number of air travelers from China that visit Japan annually (*N_air travelers*). *N_air travelers* was estimated to be 5,952,745 based on the Immigration Statistics 2018 issued by the Ministry of Justice [9].

Probability that an air traveler from China illegally brings in pork products (*P_illegal importation*). *P_illegal importation* was modelled as Beta (7+1, 248–7+1), based on the result of a survey conducted in 2019: of 248 selected air travelers arriving in Japan from China by air, seven had illegally brought in pork products [23].

Variables	Description		
L_exposure	Infectious load of ASFV fed to pigs without being heat-treated (HAD50s)		
L_infected pork	Infectious load of infected pork product (HAD50s) in an ASF infected pig		
N_air travelers	Number of air travelers that visit Japan annually		
N_pigs	Pig population in China		
P_affected farms	Number of affected farms per year		
P_ecofeed heat-treated	Probability that pork product waste recycled for Ecofeed is heat-treated		
P_heat-treated in China	Probability that the pork product brought in illegally is heat-treated in China		
P_illegal importation	Probability that an air traveler from China illegally brings in pork products		
P_infection	Probability of a pig becoming infected through consumption of infected pork product waste		
P_introduction	Probability of at least one pig becoming infected		
P_notification	Probability of notification		
P_recycle	Proportion of the amount of pork product waste recycled to the amount of the pork product waste produced in restaurants		
P_recycle for ecofeed	Proportion of the amount of pork product waste recycled for Ecofeed to the amount of pork product waste recycle for feed		
P_recycle for feed	Proportion of recycled pork product waste used for feed to the amount of pork product waste recycled		
P_restaurant use	Proportion of W_release used in restaurants		
P_slaughter	Prevalence of ASF infected pigs shipped for slaughter in China		
P_swill heat-treated on farm	Probability that pork product waste recycled for feed used as swill is heat-treated before being fed to pigs		
P_waste	Proportion of the pork product waste to the pork products used in restaurants		
Prob_restaurant	Probability that the pork product illegally imported is used in restaurants		
Ratio_restaurant	Ratio between the average weight of pork products destined for restaurants and the average weight of pork products brought in by air travelers		
W_exposure	Amount of infected pork scraps originating from pork products illegally brought in by air travelers from China and fed to pigs in Japan as swill without being heat-treated (g)		
W_pork product per traveler	Average amount of pork product illegally brought in by air travelers from China (g)		
W_release	Total amount of infected pork products illegally imported by air travelers from China into Japan (g)		
c	Dose response coefficient		
d	Probability an infected animal dies		
Н	Average herd size of pig farms in China		
n	Number of pigs that are fed with infected pork products without being heat-treated		
t	Duration of infection (year)		

Table 1. An alphabetical list of variables used in the model.

Average amount of pork product illegally brought in by air travelers from China (*W_pork product per traveler*). The weight of pork products brought in illegally by traveler (g) was modeled as Lognormal (834, 1,139), based on the distribution of weights of the 69 pork products confiscated and tested positive for ASF by AQS [22]. *W_pork product per traveler* was estimated to be 834g by taking the average of this weight distribution (Fig 3).

Probability that the pork product illegally imported is used for restaurant (*Prob_res-taurant***).** *Prob_restaurant use* was modeled as Beta (5+1, 69–5+1) based on the data on the weights of 69 pork products confiscated and tested positive for ASF by AQS [22]: of the 69 pork products tested PCR positive, five weighed over 2kg and were assumed to be destined for restaurant use [22]. This is based on the assumption that pork products weighing more than





2kg would be used in restaurants and those weighing less than 2kg for personal consumption [15].

Ratio between the average weight of pork products destined for restaurants and the average weight of pork products brought in by air travelers (*Ratio_restaurant***).** *Ratio_restaurant* was estimated to be 4.27 by taking the ratio between the average of Lognormal (834, 1,139) and the average of the fraction over 2kg of this distribution (3,560g). This is based on the assumption that pork products weighing more than 2kg are used in restaurants and those weighing less than 2kg for personal consumption [15].

Proportion of the pork product waste to the pork products used in the restaurants (*P_waste*). *P_waste* was estimated to be 0.013 based on the results of a survey by the Ministry of Agriculture, Forestry and Fisheries (MAFF) [24]. According to the results of this survey 1.3% of meat and meat products used in restaurants are discarded as waste.

Proportion of the amount of pork product waste recycled to the amount of the pork product waste produced in restaurants (*P_recycle***).** *P_recycle* was modeled as Normal (0.2087, 0.2087×0.049) based on the results of a survey by the MAFF [24]. According to the results of this survey, 20.87% of food waste from restaurants was recycled for some use, with a relative precision of 4.9%.

Proportion of recycled pork product waste used for feed to the amount of pork product waste recycled (*P_recycle for feed***).** *P_recycle for feed* was estimated to be 0.2118 based on the results of a survey by the MAFF [24]. According to the results of this survey 21.18% of food waste from restaurants recycled was used for animal feed.

Proportion of the amount of recycled pork products used for production of Ecofeed to the amount of pork product waste recycled for feed (*P_recycle for ecofeed***).** *P_recycled for ecofeed* was estimated to be 0.929 based on data from survey results of 114 selected pig farms in Japan conducted by the Japan Pig Producers Association in 2018 [25]. According to the results of this survey, 88.9% of feed materials used in pig farms in 2018 was commercial compound feed, 1.2% rice, 9.2% Ecofeed and 0.7% other materials. We assumed that the latter two are of materials of food waste origin.

Probability that pork product waste recycled for feed used the production of Ecofeed is heat-treated (*P_ecofeed heat-treated***).** *P_ecofeed heat-treated* was assumed to be 1 in our baseline model, because according to the guidelines issued by the Food and Agricultural Materials Inspection Center in 2006, the heat-treatment condition of either 70 degree Celsius for 30 minutes or 80 degree Celsius for 3 minutes is required in the production of Ecofeed [26]. This heat-treatment condition meets the recommended criteria for inactivation of ASFV [26].

Probability that pork product waste recycled for feed used as swill is heat-treated before being fed to pigs (*P_swill heat-treated on farm***)**. *P_swill heat-treated on farm* was modelled as Beta (7+1, 12–7+1). According to a questionnaire survey of 578 pig farmers who are members of JPPA in Japan conducted by the authors in 2018, 12 farmers were feeding swill containing meat products, of which seven were practicing heat-treatment before feeding swill [27].

Loading of ASFV in an pig infected with ASF (*L_infected pork***).** We assumed that pork products from infected pigs have a virus loading equivalent to that of muscle from an infected pig. This was modeled as 10^Uniform (3.8, 5.0) HAD50s based on the findings by Petrini et al. (2019) [28]. The ASFV infectivity titers of different muscles of infected pigs at slaughter were between 10^3.8–10^5.0 HAD50s per ml or gram [28].

Coefficient for the exponential dose response curve (*c***).** Many studies have been conducted to determine the infectious dose of ASFV. Early studies indicated a minimum dose of 10^5 HAD50s of ASFV KWH/12 was required to cause infection when administered orally in milk [29]. More recently, Howey et al. (2013) demonstrated, based on an inoculation experiment using a highly virulent Malawi strain, that 10^2 HAD50s did not induce infection but 10^4 HAD50s and 10^5 HAD50s were sufficient to cause infection in 100% of the pigs [30]. Pietschmann et al. (2015) demonstrated that as low as 3 and 25 hemadsorption units (HAU) of ASFV Armenia 2008, when delivered in 2 mL of splenic suspension, caused infection in wild boar, with administration of 3 HAU infecting only the weakest animals [31]. Niederwerder et al. (2019) estimated, based on a sequential adaptive experiment using ASF strain Georgia 2007, that ID50 was $10^{1.0}$ and $10^{6.8}$ TCID50 (equivalent to HAD50 [32]) for liquid and feed respectively [33]. Based on these recent findings, we modeled the coefficient *c* in Model 6 as 10^{10} Uuniform (-4.155, -1.558): the dose response model with coefficient *c* of this distribution assumes any doses between 25 HADs and 10^{4} HADs as a median infectious dose with an equal probability (Fig 4).

Model implementation

The model was developed in @Risk Version 7.6 (Palisade, Ithaca, New York) within Microsoft ® Excel 2013, and was run with 10000 iterations using Latin Hybercube sampling for each simulation. Results of model outputs are presented as: Mean (5th percentile, 95th percentile).

Sensitivity analysis

To assess the effect of uncertainty in the current model, sensitivity analysis was performed using Spearman's correlation coefficient to rank all model input parameters according to their contributions to the variance of model output *P_introduction*.

Scenario analysis

Scenario analysis was performed to assess the effect of changes in selected input variables summarized in <u>Table 2</u>. A two-way scenario analysis was also performed to assess the effect of simultaneous changes of *P_ecofeed heat-treated* and *P_swill heat-treated on farm*.

Results

Risk quantification

The probability of at least one pig becoming infected with ASFV in one year was predicted to be 0.20 (90% PI: 0.00-0.90) (Fig 5).

Sensitivity analysis

The results of the sensitivity analysis are shown in Fig 6. The most correlated parameters were the dose response coefficient (c), followed by the probability that infected pigs die on affected



Fig 4. Dose response curves depicting the model *P_infection* = 1-exp(-*c* x dose (HAD50s)), where *c* = 10^Uuniform (-4.155, -1.558). This dose response model assumes any doses between 25 HAD50s and 10^3 HAD50s as a median infectious dose. 30 dose response curves randomly selected from the model are shown to provide a visual image of the distribution.

https://doi.org/10.1371/journal.pone.0232132.g004

Input variable	Scenario	Value	Comments
N_affected farms	1	56 (baseline)	To assess the effect of increased number of outbreaks in China
	2	2 fold increase	
	3	3 fold increase	
	4	5 fold increase	
P_illegal import	1	1%	To assess the effect of an increased probability of illegal importation of pork products by air travelers from China
	2	Beta (8, 242) (baseline)	
	3	5%	
	4	10%	
P_swill heat-treated on farm	1	Beta (68, 3) (baseline)	To assess the effect of an decreased proportion of farms practicing heat-treatment before feeding swill to pigs
	2	75%	
	3	90%	
	4	99%	
P_ecofeed heat-treated	1	100% (baseline)	To assess the effect of some proportion of Ecofeed not properly heat-treated
	2	99%	
	3	95%	
	4	90%	

Table 2. List of scenarios that were tested in scenario analysis and the modified input variables under each scenario.

https://doi.org/10.1371/journal.pone.0232132.t002



Fig 5. Distribution of the probability of at least one pig becoming infected by consuming non heat-heated swill originating from pork products illegally imported by air travelers from China (*P_introduction*).

https://doi.org/10.1371/journal.pone.0232132.g005



Fig 6. Tornado graph illustrating the results of sensitivity analysis. All model input parameters are ranked by Spearman's correlation coefficient according to their contribution to the variance of model output *P_introduction*. The nine most correlated input parameters are shown.

farms (*d*), the infectious load of ASFV in an infected pig (*L_infected pork*), the probability that the pork product is heat-treated in China (*P_heat-treated in China*) and the probability that the infected pork product illegally imported is used in restaurants (*Prob_restaurant use*).

Scenario analysis

The effect of different scenarios on the probability of ASF introduction ($P_{introduction}$) was investigated and results are shown in Fig 7. From this analysis, it was concluded that an increase in the number of outbreaks in China will increase the risk of ASF introduction, with $P_{introduction}$ rising to 0.51 (0.01–1.00) if the number of outbreaks increase ten fold (Fig 7A).

P_introduction will decrease to 0.10 (0.00–0.52) with the *P_illegal importation* of 1%. Contrarily, *P_introducction* will increase to 0.35 (0.04–1.00) with the *P_illegal importation* of 10% (Fig 7B).

P_introduction will substantially increase if a proportion of Ecofeed is not properly heat-treated during the production process: *P_introduction* will increase to 0.23 (0.00–0.96), 0.31 (0.00–1.00) and 0.38 (0.01–1.00) if 1%, 5% and 10% of Ecofeed is not properly heat-treated respectively (Fig 7C).

P_introduction will decrease to 0.15 (0.00–0.76), 0.08 (0.00–0.43) and 0.01 (0.00–0.05) as the proportion of swill feeding pig farms that apply heat-treatment increases to 75%, 90% and 99% respectively (Fig 7D).

The result of two-way scenario analysis assessing the effect of simultaneous changes of $P_ecofeed$ heat-treated and P_swill heat-treated on farm is shown in Fig 8. The change of $P_eco-feed$ from the baseline value of 1 has a more significant effect on $P_introduction$ than change of P_swill heat-treated on farm from its baseline value.



Fig 7. Scenario analysis depicting the effects of tested scenarios on the probability of at least one pig becoming infected by consuming untreated swill originating from pork products illegally imported by air travelers from China (*P_introduction*). For each box-whisker plot, the line in the box indicates the mean; the length of the box indicates the inter-quartile range; the whiskers indicate the 5th percentile and the 95th percentile respectively. For b and d, fixed values of 0.028 and 0.57 were used as the baseline respectively.

Discussion

The risk of ASF introduction into Japan through the illegal importation of pork products by air travelers from China and swill feeding identified in this study is relatively high and accompanied with a large degree of uncertainty. The uncertainty associated with the risk of introduction is mainly due to the dose response relation of ASFV, followed by the probability of an infected pig dying on affected farms, loading of ASVF in an infected pig, probability of an illegally imported pork product being heat-treated in China and used in restaurants (Fig 6), indicating that there is a need to collect more data on these parameters to reduce the uncertainty of prediction.

Illegal importation or smuggling of meat and meat products is a serious issue which could greatly compromise a country's import regime in preventing the introduction of diseases. Its effect on the risk of entry of ASF and other TADs has been evaluated in various QRA [34–40]. Most recently, Jurado et al. (2019) and Ito et al. (2019) assessed the risk of ASF introduction into the US and Japan respectively through smuggling of pork products in air passenger



Fig 8. Two-way scenario analysis depicting the effects of *P_ecofeed heat-treated* and *P_swill heat-treated on farm* on the mean value of *P_introduction*. The red X indicates the mean value of *P_introduction* with *P_ecofeed heat-treated* and *P_swill heat-treated on farm* taking the baseline values (1.0 and Beta (8, 6) respectively).

luggage [36, 37]. They both calculated the risk using two main inputs without considering the exposure assessment, namely, i) number of prohibited swine products of air passengers confiscated at airports and ii) number of air passengers arriving in the US and Japan via international commercial flights.

Although not totally comparable because of the methodological difference, the risk calculated in our study was lower than the risk calculated previously by Ito et al.: they predicted that the annual risk of ASFV introduction from China into Japan via pork product brought in in air passenger's luggage is 0.67 (95% PI: 0.223–0.999) [37]. This is mostly because that they only considered the release risk in assessing the risk of introduction while in our current model we took account of both release and exposure risk.

The impact of illegal importation of pork products was assessed in the current model by changing the probability of illegal importation. The result indicates that the risk of ASF introduction into Japan would increase 1.8 times if the probability of illegal importation increases from the current level to 10% (Fig 7B). This analysis is essential in highlighting the importance of minimization of illegal imports of pork products by air travelers through awareness raising campaign and use of detector dogs at the airports targeting air travelers visiting Japan from ASF affected countries.

In our baseline model we assumed that infected pork product waste used for the production of Ecofeed presents no risk, because the required heat-treatment condition of 70 degree Celsius for 30 minutes is considered to be sufficient to inactivate ASFV. However, although ASFV is shown to be inactivated by heating at 56°C/70 minutes or 60°C/20 minutes [41], depending on the components that constitute the swill there might be a chance that ASFV is not

inactivated even with this heat-treatment condition. Meat and swill cannot be compared because their water/fat content is very different, and with a diversity of materials that can be present in swill, uniform heating may not occur and some materials could potentially protect the virus [42]. The World Organisation for Animal Health (OIE) recommends a higher heat-treatment condition, i.e. 90° C for at least 60 minutes, with continuous stirring; or 121° C for at least 10 minutes at an absolute pressure of 3 bar [43]. The quality management programmes practiced by the Ecofeed plants should be monitored to ensure that heat-treatment conditions prescribed by the guidelines are observed [26]. Depending on the type of food waste used for Ecofeed, the MAFF should even consider amending the guidelines to strengthen the heat-treatment requirements, in line with the recommended procedures for the inactivation of ASFV in swill by the OIE. This should be reiterated as the result of our two-way scenario analysis indicated that even a small change of *P_ecofeed heat-treated* from 1 will result in a more significant rise in *P_production* than a change of *P_swill heat-treated on farm* (Fig 8).

The result of our scenario analysis revealed that with an increased proportion of pig farms practicing heat-treatment of swill before feeding to pigs, the risk of ASF introduction can be effectively reduced. The swill feeding practice in pig farms should be regularly monitored to ensure that consistent heat-treatment procedures are practiced, or this practice should be banned when the *W_release* is high, to protect Japanese pig industry from the introduction of ASF.

In our model, we assumed that infected pork products that are not heat-treated have the same infectious load as that of muscle of an infected pig at slaughter [28], because no data were available regarding the change in infectious load of ASFV in non-heat-treated or improperly heat-treated pork products. With this assumption, our model might be over-estimating the risk of ASF entry into Japan.

With the limited amount of data on some of the input variables, the result of our risk assessment was accompanied with a large degree of uncertainty. Nevertheless, it has given us insight into what measures should be taken to help protect the Japanese pig industry from the introduction of ASF and identified what additional data are needed to conduct risk assessment with a greater degree of certainty.

In this study, only travelers from China were considered. The risk posed by travelers from other ASF infected countries both in Asia and other regions was not considered. Considering the number of travelers arriving in Japan from China compared to other countries, travelers from China pose the highest risk as suggested in our previous study [15]. However, as ASF has been spreading to other Asian countries and other regions, it would be worthwhile conducting the risk assessment of ASF entry to Japan from other countries with additional information.

Supporting information

S1 File. ASF risk assessment model. (XLSX)

Acknowledgments

The authors would like to thank officials of the Ministry of Agriculture, Forestry and Fisheries of Japan for helping them locate the data required for this risk assessment.

Author Contributions

Conceptualization: Katsuaki Sugiura.

Data curation: Zhihao Lei.

Formal analysis: Katsuaki Sugiura.

Funding acquisition: Katsuaki Sugiura, Takeshi Haga.

Project administration: Katsuaki Sugiura.

Supervision: Katsuaki Sugiura.

Validation: Katsuaki Sugiura, Takeshi Haga.

Writing - original draft: Katsuaki Sugiura.

Writing – review & editing: Caitlin Holley.

References

- Alonso C, Borca M, Dixon L, Revilla Y, Rodriguez F, Escribano JM, et al. ICTV Virus Taxonomy Profile: Asfarviridae. Journal of General Virology. 2018; 99: 613–614. https://doi.org/10.1099/jgv.0.001049 PMID: 29565243
- Plowright W, Thomson GR, Neser JA. African swine fever. In: Coetzer JAW, Thomson GR, Tutsin RC, editors. Infectious diseases of livestock, with special reference to southern Africa, vol 1, 1st edn. Cape Town: Oxford University Press; 1994. pp. 567–599.
- Sánchez–Vizcaíno JM, Mur L, Martínez–López B. African Swine Fever: An Epidemiological Update. Transbound Emerg Dis. 2012; 59:27–35. <u>https://doi.org/10.1111/j.1865-1682.2011.01293.x</u> PMID: 22225967
- European Food Safety Authority (EFSA). Scientific opinion on African swine fever. EFSA Journal. 2010; 8:1556.
- Kolbasov D, Titov I, Tsybanov S, Gogin A, Malogolovkin A. African Swine Fever Virus, Siberia, Russia, 2017. Emerging Infectious Diseases. 2018; 24: 796–798. https://doi.org/10.3201/eid2404.171238 PMID: 29553323
- OIE. African swine fever, China, Immediate Notification (03/08/2018). [cited 2019 December 17] Available from: http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer= MapFullEventReport&reportid=27568
- 7. Zhou X, Li N, Luo Y, Liu Y, Miao F, Chen T, et al. Emergence of African Swine Fever in China, 2018. Transbound Emerg Dis. 2018;1–3. https://doi.org/10.1111/tbed.13004 PMID: 30153367
- Ministry of Justice (MOJ). Immigration Control Statistics in 2018-Number of Foreigners Entering Japan by Port and Nationality. [Cited 2020 January 25] Available from: https://www.e-stat.go.jp/stat-search/ files?page=1&layout=datalist&toukei=00250011&tstat=000001012480&cycle=7&year=20180&month= 0&tclass1=000001012481
- 9. OIE. Situational updates of ASF in Asia and the Pacific. [cited 2020 January 25] Available from: https:// rr-asia.oie.int/en/projects/asf/situational-updates-of-asf-in-asia-and-the-pacific/-10.pdf
- Animal Quarantine Service (AQS), 2019. Number and quantity of animal products imported illegally as portable goods and seizured at the Animal Quarantine Service (top 10 countries). <u>http://www.maff.go.jp/aqs/tokei/attach/pdf/toukei-6.pdf</u>
- 11. Ministry of Agriculture, Forestry and Fisheries (MAFF). Bringing animal products into Japan from overseas. [Cited 2020 January 25] Available from: http://www.maff.go.jp/aqs/english/product/import.html
- 12. Ministry of Agriculture, Forestry and Fisheries (MAFF). Outbreak of African swine fever in Asia. [Cited 2020 January 25] Available from: http://www.maff.go.jp/j/syouan/douei/attach/pdf/asf-783.pdf
- 13. Ministry of Agriculture, Forestry and Fisheries (MAFF). Quarantine detector dogs. [Cited 2020 January 25] Available from: http://www.maff.go.jp/aqs/job/detectordog.html
- Sugiura K, Haga T, A rapid risk assessment of African swine fever introduction and spread in Japan based on expert opinions. J Vet Med Sci. 2018; 80: 1743–1746. <u>https://doi.org/10.1292/jvms.18-0543</u> PMID: 30282884
- 15. Veterinary Laboratories Agency (VLA). Risk Assessment for the Import of Contaminated Meat and Meat Products into Great Britain and the Subsequent Exposure of GB Livestock. Defra Publications, Crown Copyright, 2004. [Cited 2020 January 25] Available from: http://webarchive.nationalarchives.gov.uk/20061209024841/http://www.defra.gov.uk/animalh/illegali/reports/index.htm
- Ministry of Agriculture, Forestry and Fisheries (MAFF). Current Situation of Ecofeed (September 2019). [Cited 2020 January 25] Available from: http://www.maff.go.jp/j/chikusan/sinko/lin/l_siryo/attach/pdf/ ecofeed-66.pdf

- Ministry of Agriculture, Forestry and Fisheries (MAFF). Agriculture, Forestry and Fisheries Statistics-Survey of the Current Situation of Recycling of Food Waste in 2017 (29 March 2019). [Cited 2020 January 25] Available from: http://www.maff.go.jp/j/council/seisaku/syokusan/recycle/h31_1/attach/pdf/ index
- McVicar JWV. Quantitative aspects of the transmission of African swine fever. American Journal of Veterinary Research 1984; 45: 1535–1541. PMID: 6476567
- OIE. WAHIS. [Cited 2020 January 25] Available from: https://www.oie.int/wahis_2/public/wahid.php/ Countryinformation/Countryreports
- 20. China Animal Husbandry Yearbook Editorial Department. China Animal Husbandry Yearbook 2018. China Agriculture Publishing; 2019.
- ter Beek V. ASF China: Pig herd shrinks 26%; stricter checks announced. PigProgress. 2019 July 17 [Cited 2019 December 17] Available from: https://www.pigprogress.net/Health/Articles/2019/7/ASF-China-Pig-herd-shrinks-26-stricter-checks-announced-451827E/
- 22. Animal Quarantine Service (AQS). Positive cases for the presence of genetic materials of ASFV detected in pork products brought in by passengers from China and other Asian countries as of 21 February 2020. https://www.maff.go.jp/aqs/topix/attach/pdf/asf2018-60.pdf
- Lei Z, Haga T, Obara H, Sekiyama H, Sekiguchi S, Hombu A, et al. A questionnaire survey of the illegal importation of pork products by air travelers into Japan from China and exploration of causal factors. Prev Vet Med 2020; 177:104947. https://doi.org/10.1016/j.prevetmed.2020.104947 PMID: 32203815
- 24. Ministry of Agriculture, Forestry and Fisheries (MAFF). Food loss statistics survey and restaurant survey (Fiscal Year 2015). [Cited 2020 January 25] Available from: http://www.maff.go.jp/j/tokei/kouhyou/syokuhin_loss/
- 25. Japan Pig Producers Association (JPPA). Report on Actual Situation of Pig Production in Japan in 2018 (Result of Nationwide Questionnaire Survey). Japan Pig Producers Association, Tokyo. [Cited 2020 January 25] Available from: http://www.alpa.or.jp/wp/wp-content/uploads/2019/05/h30_Pig_farming. pdf
- 26. Ministry of Agriculture, Forestry and Fisheries (MAFF). Guidelines for Safety Assurance of Feed produced by recycling of food waste (issued on 30 August 2006 by Director-General of the Consumers Protection Bureau). [Cited 2020 January 25] Available from: http://www.famic.go.jp/ffis/feed/tuti/18_6074. html
- 27. Foundation Ito. Report of the Research Grant Project on Meat for the fiscal year 2018; 36: 229-344.
- Petrini S, Feliziani F, Casciari C, Giammarioli M, Torresi C, De Mia GM. Survival of African swine fever virus (ASFV) in various traditional Italian dry-cured meat products. Prev Vet Med. 2019; 162:126–130. https://doi.org/10.1016/j.prevetmed.2018.11.013 PMID: 30621891
- Greig A. Pathogenesis of African swine fever in pigs naturally exposed to the disease. J Comp Pathol. 1972; 82: 73–9. https://doi.org/10.1016/0021-9975(72)90028-x PMID: 4553010
- 30. Howey EB, O'Donnell V, de Carvalho Ferreira HC, Borca MV, Arzt J. Pathogenesis of highly virulent African swine fever virus in domestic pigs exposed via intraoropharyngeal, intranasopharyngeal, and intramuscular inoculation, and by direct contact with infected pigs. Virus Res. 2013; 178: 328–39. https://doi.org/10.1016/j.virusres.2013.09.024 PMID: 24076499
- Pietschmann J, Guinat C, Beer M, Pronin V, Tauscher K, Petrov A, et al. Course and transmission characteristics of oral low-dose infection of domestic pigs and European wild boar with a Caucasian African swine fever virus isolate. Arch Virol. 2015; 160: 1657–67. https://doi.org/10.1007/s00705-015-2430-2 PMID: 25916610
- 32. Davies K, Goatley LC, Guinat C, Netherton CL, Gubbins S, Dixon LK, et al. Survival of African Swine Fever Virus in Excretions from Pigs Experimentally Infected with the Georgia 2007/1 Isolate. Transbound Emerg Dis. 2017; 64:425–431. https://doi.org/10.1111/tbed.12381 PMID: 26104842
- Niederwerder MC, Stoian AMM, Rowland RRR, Dritz SS, Petrovan V, Constance LA, et al. Infectious Dose of African Swine Fever Virus When Consumed Naturally in Liquid or Feed. Emerg Infect Dis. 2019; 25:891–897. https://doi.org/10.3201/eid2505.181495 PMID: 30761988
- Costard S, Jones BA, Martinez–Lopez B, Mur L, de la Torre A, Martinez M, et al. Introduction of African Swine Fever into the European Union through Illegal Importation of Pork and Pork Products. PLoS ONE. 2013; 8:e61104. https://doi.org/10.1371/journal.pone.0061104 PMID: 23613795
- 35. Hartnett E, Adkin A, Seaman M, Cooper J, Watson E, Coburn H, et al. A quantitative assessment of the risks from illegally imported meat contaminated with foot and mouth disease virus to Great Britain. Risk Anal. 2007; 27: 187–202. https://doi.org/10.1111/j.1539-6924.2006.00869.x PMID: 17362409
- 36. Jurado C, Mur L, Aguirreburualde MSP, Cadenasfernández E, Martínez-López B, Sánchez-Vizcaíno JM1, et al. Risk of African swine fever virus introduction into the United States through smuggling of

pork in air passenger luggage Scientific Report Natureresearch. 2019; 9: 14423 <u>https://doi.org/10.</u> 1038/s41598-019-50403-w

- Ito S, Jurado C, Sánchez-Vizcaíno JM, Isoda N. Quantitative risk assessment of African swine fever virus introduction to Japan via pork products brought in air passengers' luggage. Transbound Emerg Dis. 2019 Nov 6. https://doi.org/10.1111/tbed.13414 PMID: 31692238
- Shih TH, Chou CC, Morley RS. Monte Carlo simulation of animal-product violations incurred by air passengers at an international airport in Taiwan. Prev Vet Med. 2005; 68: 115–122. <u>https://doi.org/10.1016/j.prevetmed.2004.11.010</u> PMID: 15820111
- Lin XW, Chiang CT, Shih TH, Jiang YN, Chou CC. Foot-and-mouth disease entrance assessment model through air passenger violations. Risk Anal. 2009; 29:601–611. https://doi.org/10.1111/j.1539-6924.2008.01183.x PMID: 19144072
- Lai JM, Hwang YT, Chou CC. Modeling exotic highly pathogenic avian influenza virus entrance risk through air passenger violations. Risk Anal. 2012; 32:1093–1103. <u>https://doi.org/10.1111/j.1539-6924</u>. 2011.01740.x PMID: 22150450
- World Organisation for Animal Health (OIE). Technical disease cards -African swine fever. [Cited 2020 January 25] Available from: https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/ AFRICAN%20SWINE%20FEVER.pdf
- 42. World Organisation for Animal Health (OIE). Report Of The Meeting of The OIE Terrestrial Animal Health Standards Commission Paris, 10–19 Semtember 2019. [Cited 2020 January 25] Available from: https://www.oie.int/fileadmin/Home/eng/Internationa_Standard_Setting/docs/pdf/A_TAHSC_Feb_2019_Report.pdf
- **43.** World Organisation for Animal Health (OIE). Terrestrial Animal Health Code (2019)-Article 15.1.22. Procedures for the inactivation of ASFV in swill. [Cited 2020 January 25] Available from: https://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_asf.htm