UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE VETERINARIA

TESIS DOCTORAL

Nuevas estrategias para el control y erradicación de la peste porcina africana

New strategies for the control and eradication of African swine fever

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

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MEMORIA DE TESIS DOCTORAL

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de la Facultad de Veterinaria de la Universidad Complutense de Madrid, como autor/a de la tesis presentada para la obtención del título de Doctor y titulada:

Nuevas estrategias para el control y erradicación de la peste porcina africana

y dirigida por: José Manuel Sánchez-Vizcaíno Rodríguez
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A mi abuelo,

“Un buen científico debe tener curiosidad, ilusión, perseverancia y unas metas claras, aunque estas pueden variar a lo largo de la vida.”

Vicente J. Arán Redó

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Guau, miau, miau, miau.

ÍNDICE

LISTADO DE ABREVIATURAS

AIC: Akaike's Information Criterion.

APHIS: Animal and Plant Health Inspection Service.

ASF: African Swine Fever.

ASFV: African Swine Fever Virus.

CBP: Customs and Border Protection.

CE: Comisión Europea.

CI: Confidence Inverval.

COST: European Cooperation in Science and Technology.

CSF: Classical Swine Fever.

CSFV: Classical Swine Fever Virus.

DEFRA: Department of Environment, Food and Rural Affairs.

DG SANTE: Dirección General de Salud y Seguridad Alimentaria.

EEC: European Economic Community.

ELISA: Enzyme-Linked Immunosorbent Assay.

EOVR: Regional Veterinary Epidemiological Observatory.

EU: European Union.

EEUU: Estados Unidos de América.

FAD: Foreign Animal Disease.

FAO: Food and Agriculture Organization of the United Nations.

FAOSTAT: Food and Agriculture Organization of the United Nations Statistics.

HAD: haemadsorption/hemadsorción.

IATA: International Air Transport Association.

IZS: Istituto Zooprofilattico Sperimentale della Sardegna.

MGF: Multigene Families.

OIE: Organización Mundial de Sanidad Animal.

PCR: Polymerase Chain Reaction.

PPA: Peste Porcina Africana.

PPC: Peste Porcina Clásica.

PSPAP: Prohibited Swine Products Carried by Air Passengers.

RPC: República Popular China.

UE: Unión Europea:

UPL: Universal Probe Library.

US: United States.

USDA: United States Department of Agriculture.

VPPA: Virus de la Peste Porcina Africana.

VPPC: Virus de la Peste Porcina Clásica.

WADS: Agricultural Quarantine Activity Work Accomplishment.

WAHIS: World Animal Health Information Database.

RESUMEN

La peste porcina africana (PPA) es una de las enfermedades infecciosas, de declaración obligatoria, con mayor repercusión sanitaria y económica de cuantas afectan al ganado porcino. En la actualidad, su importancia es aún mayor si cabe, con países afectados en África, Asia y Europa. Esta tesis doctoral titulada “Nuevas estrategias para el control y erradicación de la peste porcina africana” ha tenido como principal objetivo proporcionar nuevas herramientas y conocimientos epidemiológicos para la prevención, control y erradicación de la PPA en el contexto mundial.

Para ello, han sido explorados los aspectos más relevantes de la enfermedad y sus riesgos asociados a tres escenarios diferenciados: un escenario endémico usando como modelo Cerdeña, un escenario epidémico centrado en el sector porcino de la Unión Europea (UE) y un escenario libre representado por los Estados Unidos de América (EEUU). Los resultados de esta tesis han sido recogidos en cinco artículos científicos publicados en revistas indexadas.

A su vez, el objetivo principal de esta tesis doctoral fue desagregado en cuatro objetivos específicos. El primer objetivo pretendía identificar las principales lagunas de conocimiento en relación a la PPA, sugiriendo mediante una evaluación de expertos cuáles de ellas son prioritarias. Los resultados de este estudio identificaron un total de treinta y seis prioridades, diecinueve de ellas clasificadas con importancia mayor, once con importancia media y seis con importancia menor relacionadas con las características del virus, hospedadores naturales, formas clínicas, epidemiología, impacto socio-económico, respuesta inmune, prevención, detección y control, y diagnóstico y vacuna.

El segundo objetivo se basó en la identificación de los factores de riesgo que favorecen la ocurrencia de la PPA en Cerdeña. Para ello, se evaluaron un total de 28 variables. Los resultados obtenidos identificaron un total de nueve factores de riesgo relacionados con la falta de profesionalización de las granjas, la cría de animales de *“brado”* y la presencia de jabalíes. Además, se recomendaron medidas de control concretas a implementar en el plan de erradicación actual, a fin de mitigar los factores de riesgo señalados.

En el tercer objetivo se identificaron las medidas aplicables en granjas de tipo comercial, no comercial y extensiva para evitar la entrada y difusión de la PPA en la UE. Para ello se realizó una revisión sistemática de la literatura disponible (científica y no científica), con una posterior evaluación de las mismas, por parte de un panel de expertos en PPA. La identificación de animales y registros de la granja, la prohibición de alimentar a los animales con restos alimenticios y la estabulación permanente se consideraron tres medidas clave en los tres tipos de granjas. Todos los expertos coincidieron en que la medida preventiva más importante para granjas no comerciales y extensivas es mejorar el acceso de estas a los servicios veterinarios.

Finalmente, en el cuarto objetivo se realizó una evaluación probabilística del riesgo de entrada de PPA en EEUU a través de viajeros procedentes de vuelos internacionales. Los resultados mostraron que China (formalmente República Popular China, RPC), Hong Kong, Rusia (formalmente Federación de Rusia) y Polonia fueron las regiones de origen representando un mayor riesgo.

Por tanto, los resultados de la presente tesis doctoral aportan soluciones a problemas actuales, habiendo sido ya utilizados por las autoridades competentes sardas o suscitando interés en asociaciones de productores de EEUU. Además, se espera que

los resultados y conclusiones sirvan de base para futuras investigaciones en nuevos escenarios como es el asiático, a fin de conseguir el control y erradicación de la PPA.

ABSTRACT

African swine fever (ASF) is one of the notifiable infectious diseases of swine that causes greater sanitary and economic impact. Currently, its relevance is increasing due to disease presence in Africa, Asia and Europe. This doctoral thesis is entitled “New strategies to control and eradicate African swine fever”, and aims at developing new tools and epidemiological knowledge to prevent, control and eradicate ASF globally.

Thus, the most relevant characteristics of the disease and its related risks were explored within the framework of three different scenarios: endemic scenario focused on Sardinia, epidemic scenario regarding the European Union (EU) pig industry and free scenario represented by the United States (US). Results from this doctoral thesis have been published in international peer reviewed journals as five scientific articles.

In addition, four specific objectives were achieved.

First, to identify the main gaps of knowledge with regard to ASF and evaluated by experts as high, medium or low priorities. The obtained results identified thirty six gaps, nineteen of them were classified as high priorities, eleven as medium and six as low. These priorities were related to virus characteristics, natural hosts, clinical forms, epidemiology, socio-economic impact, immune response, prevention, detection and control, and diagnosis and vaccine.

Second, to identify risk factors favouring ASF endemicity in Sardinia. Thus, 28 variables were assessed. A total of nine risk factors were identified which were related to lack of professionalism of the pig sector, cultural practices (such as *“brado”* animals) and wild boar presence. Moreover, specific strategies were suggested in order to mitigate the identified risk factors.

Third, to identify available preventive measures to avoid the spread of ASF on domestic pig farms (commercial, non-commercial and outdoor farms) from the EU. To do so, a systematic review was conducted by analysing the published scientific and non-scientific literature. The identified measures were also assess by an ASF expert panel. Among others, the identification of animals and farm records, enforcement of the ban on swill feeding and containment of pigs to not allow contact with pigs from other farms, feral pigs or wild boar or their products were identified as relevant measures on any type of pig farms. In addition, all experts agreed that the most important preventive measure on non-commercial and outdoor farm was to improve access to veterinary services.

Fourth, to assess the risk of entry of ASFV into the US by prohibited pork products carried in air passengers' luggage (PSPAP). The obtained results showed that China, Hong Kong, the Russian Federation and Poland were the origin regions that represented the highest risk.

Therefore, this doctoral thesis have tried to give solutions to the current situation. Indeed, results presented have been already used by Sardinian authorities and several American swine associations have shown their interest in these results. Moreover, it is expected that results and conclusions of this doctoral thesis could help to initiate other studies in new scenarios such as the Asian one in order to control and eradicate ASF.

INTRODUCCIÓN

La PPA es una de las enfermedades más preocupantes para el sector porcino en el contexto actual. Se trata de una enfermedad incluida en la lista de enfermedades notificables a la Organización Mundial de Sanidad Animal (OIE), así como de declaración obligatoria a la UE y autoridades nacionales, en concreto al Ministerio de Agricultura, Pesca y Alimentación. Por tanto, su notificación conlleva una serie de medidas sanitarias y restricciones comerciales que repercuten de manera directa e indirecta en la economía de los territorios afectados.

Desde 2007, la PPA comenzó a circular por Europa continental afectando a países como Georgia, Armenia, Rusia, Azerbaiyán, Ucrania y Bielorrusia. Esta tesis doctoral se inició en 2014, en un momento de preocupación creciente, en el que la UE notificó los primeros casos de PPA. Pese a los esfuerzos realizados para controlar esta enfermedad en el territorio comunitario, la situación actual es, aún si cabe, más preocupante. Nueve países de la UE, en concreto Estonia, Letonia, Lituania, Polonia, Hungría, Bulgaria, República Checa, Rumanía y Bélgica, han notificado PPA en sus territorios, junto a otros países no comunitarios como Moldavia. Además, en verano de 2018, China notificó la presencia de esta enfermedad, seguida de otros países asiáticos como Vietnam, Mongolia, Camboya, Corea del Norte y Laos. La entrada de la enfermedad en el continente asiático ha generado una gran preocupación, debido a la importancia de China en la producción mundial y es que más del 50% de la población porcina total se encuentra en este país. Así pues, esta tesis doctoral se finaliza en un momento crítico tratando de proporcionar nuevas herramientas y conocimientos epidemiológicos para la prevención, control y erradicación de la PPA en el contexto mundial.

A lo largo de la introducción se revisarán las principales características de esta enfermedad, la situación epidemiológica actual y las herramientas de las que se dispone para prevenir y luchar frente a ella.

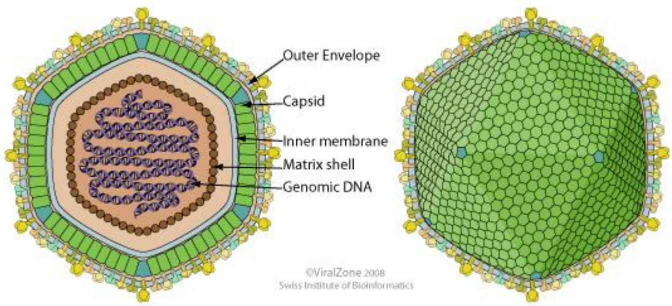
1. Peste porcina africana

1.1. Agente etiológico

La PPA está causada por un virus ADN, el virus de la PPA (VPPA), único miembro de la familia Asfarviridae (1). Se trata de un virus de gran tamaño y complejidad estructural, lo cual hace que en la actualidad parte de su composición aún sea desconocida (2). El genoma del VPPA es una única molécula de ADN de entre 170 y 193 kpb, la cual contiene más de 150 marcos de lectura (3). Dicho genoma, codifica un gran número de enzimas, factores de transcripción viral, más de 100 proteínas estructurales, así como proteínas implicadas en la modulación de la respuesta inmune del hospedador (3).

Su complejidad estructural radica en la existencia de cuatro capas concéntricas: el núcleo central, la envuelta del núcleo, la membrana interna y una cápside icosaédrica. Además, el virus adquiere una capa extracelular obtenida de las células a las que infecta (ver Figura 1) (4). Todo ello, confiere al VPPA una gran resistencia ante condiciones ambientales adversas y distintos valores de pH, especialmente en condiciones de alcalinidad. Su resistencia se puede ver incrementada en presencia de materia orgánica, hasta 7 días a pH 13,4 y durante horas a pH inferior a 4 (5). Además, en carne congelada, refrigerada o cruda, el virus es capaz de sobrevivir durante semanas o incluso meses (6).

Figura 1: Ilustración de un virión del VPPA en el que se detalla el genoma viral (*"genomic DNA"*), envuelta del núcleo (*"matrix shell"*), membrana interna (*"inner membrane"*), cápside (*"capsid"*) y membrana externa (*"outer envelope"*). *Fuente: ViralZone 2008, Swiss Institute of Bioinformatics.*



La estructura genómica del VPPA consiste en una región conservada central de unas 125 kpb y dos regiones terminales variables, las cuales confieren variabilidad en longitud entre aislados (3). Ciertas regiones del genoma del virus son empleadas para la realización de estudios de epidemiología molecular. Actualmente, la secuenciación parcial del gen que codifica la proteína viral p72 (proteína de mayor antigenicidad de la cápside viral), permite diferenciar los aislados por genotipos, aunque esta diferenciación no se traduce en variaciones en patogenicidad o inmunogenicidad (7). Hasta la fecha, han sido descritos 24 genotipos diferentes, concentrándose la mayor variabilidad en el este y sur del continente africano (8-10). Además, existen otras regiones del genoma que permiten una diferenciación más precisa entre aislados muy similares, como son la región codificante de las proteínas p54, p30 o CD2v, el análisis de la región variable central del gen B602L o la región intergénica ubicada entre los genes I73R y I329L, ubicados en el extremo terminal derecho del genoma del virus (11, 12).

1.2. Hospedadores susceptibles y ciclos epidemiológicos

El rango de hospedadores susceptibles a la infección por el VPPA pertenece en exclusiva a la familia Suidae, entre los que se encuentran el cerdo doméstico, jabalí, facóquero, potamóquero e hialóquero. Además, cabe destacar el papel de las garrapatas blandas del género *Ornithodoros,* las cuales actúan como vector de la enfermedad (13). Existen varios ciclos epidemiológicos de la enfermedad en los que se relacionan los distintos hospedadores: suidos silvestres africanos, cerdos domésticos y/o jabalíes; vectores biológicos: *O. moubata* (continente africano) y *O. erraticus* (Península Ibérica); y el medio, incluyendo en este último cualquier fómite que pudiera actuar como vector mecánico: vehículos, instrumental, ropa, calzado, etc. (14).

En el ciclo selvático se ven implicados suidos silvestres africanos, principalmente facóqueros, y garrapatas, los cuales actúan como reservorio de la enfermedad. Los facóqueros infectados no desarrollan sintomatología clínica, aunque sí replicación viral y niveles detectables de viremia en animales jóvenes y adultos. Las viremias en animales adultos raramente exceden las 10^2 unidades hemadsorbentes por mililitro (HAD/ml) aunque las seroprevalencias oscilan en función de las regiones de estudio (15). En animales neonatos los niveles de viremia exceden las 10^2-10^3 HAD/ml, siendo esta suficiente para infectar al vector. Las garrapatas que participan en este ciclo pertenecen al complejo *O. moubata* en el cual se ha observado replicación viral, así como transmisión sexual, transovárica y transestádica. Todo ello favorece la presencia del virus durante largos periodos de tiempo, incluso en ausencia de hospedadores vertebrados infectados (16). Este ciclo epidemiológico ha sido observado en zonas del sur y este del continente africano (17). La implicación de otros suidos silvestres africanos como los potamóqueros o hialóqueros parece menos relevante en la epidemiología de la PPA aunque se requieren más estudios para elucidar realmente sus roles e importancia en el escenario africano (15, 18). Del mismo modo, sería deseable dilucidar el rol de otros suidos silvestres ya que podrían ser resistentes a la infección como ocurre con el pecarí americano (familia Tayassuidae) (19).

Por otro lado, existe un ciclo similar al anteriormente mencionado donde participan cerdos domésticos y garrapatas. Este ciclo ha sido descrito en África y la Península Ibérica (20). En este escenario, las formas extensivas de producción de cerdo doméstico en áreas del suroeste de España y Portugal, sufrieron la transmisión del VPPA mediada por otra especie de garrapata, en concreto *O. erraticus*. A diferencia del complejo *O. moubata*, en esta especie únicamente existe transmisión transestádica (21). Además, tanto en la Península Ibérica como en la isla de Cerdeña (endémica desde 1978) también participa el jabalí euroasiático como hospedador susceptible a la infección por el VPPA. La transmisión entre hospedadores domésticos y silvestres se ve favorecida cuando las medidas de bioseguridad son reducidas (22). Sin embargo, de acuerdo con la opinión de varios autores, el rol de los jabalíes en el mantenimiento de la infección sería limitado ya que en ausencia de brotes en cerdo doméstico la infección en jabalí parecía ser eliminada (23, 24). En los países afectados de la UE, más del 90% de las notificaciones oficiales de PPA son atribuídas a casos en jabalíes (25). Algunos autores han denominado este ciclo como jabalí-hábitat, en el que participan jabalíes, su medio y carcasas procedentes de animales infectados con el VPPA (26).

Finalmente, el ciclo doméstico está caracterizado por la participación de cerdos domésticos y sus productos. Este ciclo epidemiológico adquiere especial relevancia en áreas donde la producción porcina aún se desarrolla en condiciones de bioseguridad escasas, como son las granjas familiares y las granjas de traspatio. De hecho, en África, Europa del Este y Asia, el mantenimiento de este ciclo ha sido identificado como una de las principales causas de dificultad en el control de la enfermedad (17, 27, 28).

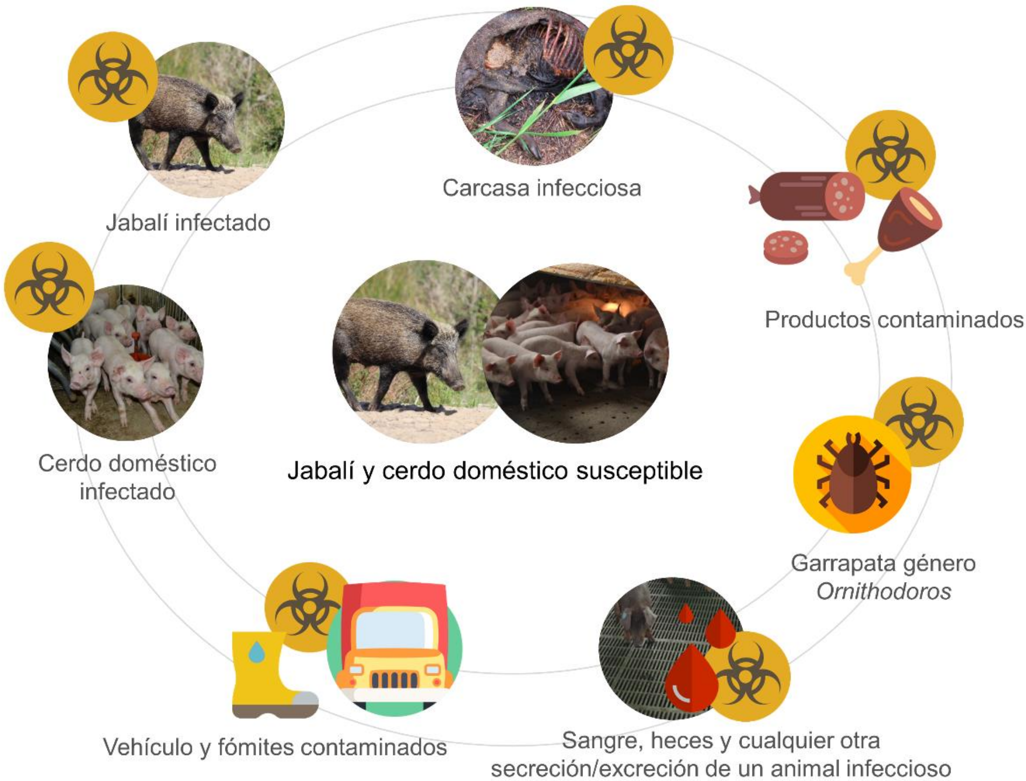
1.3. Mecanismos de transmisión

Considerando la variedad de hospedadores implicados en los ciclos epidemiológicos de la PPA, existen varios mecanismos de transmisión. I) por contacto directo, con exposición oro-nasal o a través de abrasiones en la piel, con sangre y con excreciones y/o secreciones, incluyendo orina, heces y saliva procedente de animales infectados (29). La sangre adquiere especial relevancia debido a las altas concentraciones de virus en sangre en animales con viremias tempranas (30).

II) Mediante ingestión de material contaminado, ya sean residuos alimenticios, pienso elaborado a partir de productos del cerdo contaminado, agua y restos de carcasas de animales infectados (13). La ingestión de moscas (*Stomoxys calcitrans*) alimentadas con sangre infecciosa, también ha sido identificada como otra posible vía de transmisión del virus (31). Además, ha sido descrita la transmisión por *S. calcitrans* al actuar como vector mecánico en condiciones experimentales (32).

III) La PPA puede ser transmitida por la picadura de garrapatas del género *Ornithodoros* infectadas con el VPPA (21). IV) Por último, la transmisión mediante contacto indirecto con superficies contaminadas incluyendo vehículos, calzado, ropa, así como utensilios de matanza o herramientas de las propias granjas constituye otra fuente de transmisión (ver Figura 2) (29). Esta vía se ve mediada por la actividad humana y facilitada por la gran resistencia del virus en el ambiente.

Figura 2: Mecanismos de transmisión de la PPA (elaboración propia). *Fuente: iconos diseñados por Freepik en www.flaticon.com, imagen de jabalí cortesía de JA Barasona, imagen carcasa infecciosa de Z Peksak y T Stadejek en www.pig333.com, imágenes de cerdos propias.*



1.4. Respuesta inmunitaria y opciones terapéuticas: tratamiento y vacuna

El VPPA posee más de cincuenta proteínas con capacidad antigénica, sin embargo los anticuerpos sintetizados no neutralizan por completo al virus (33). Por tanto, los hospedadores susceptibles (*i.e.* cerdos domésticos y/o jabalíes) que no presentan cuadros clínicos hiperagudos son capaces de desarrollar inmunidad de tipo humoral y celular frente a la infección por el VPPA (34). Un porcentaje variable de estos animales sobrevive a la infección, mostrando viremias intermitentes y altos títulos de anticuerpos (35-37).

En la actualidad no existe tratamiento ni vacuna frente a este virus, por lo que la presencia de anticuerpos frente al VPPA siempre será sinónimo de infección. Por ello, resulta crucial establecer un diagnóstico virológico y serológico en paralelo con el fin de identificar aspectos clave como es el tiempo que la enfermedad lleva circulando en el escenario sometido a estudio o la presencia de animales supervivientes (38).

Se han invertido numerosos esfuerzos en la búsqueda de una vacuna siguiendo estrategias diferentes como el uso de vacunas inactivadas, vivas atenuadas, recombinantes o vacunas de subunidades, entre otras. Sin embargo, pese a que algunos candidatos vacunales indujeron inmunidad, todavía existían problemas de protección, seguridad y aparición de efectos adversos (34).

En cuanto a las opciones de tratamiento, se ha propuesto la utilización de antivirales como una alternativa de control hasta la obtención de una vacuna comercial. Varios prototipos han sido descritos aunque pueden distinguirse dos grandes grupos. Por un lado, antivirales que actúan directamente sobre el ciclo de replicación viral y por otro lado, antivirales encaminados a actuar sobre las células diana del virus evitando procesos de adhesión, endocitosis, etc. La eficacia de estos tratamientos antivirales ha sido probada en condiciones *in vitro*, sin embargo, todavía se requieren estudios *in vivo* (39).

1.5. Formas clínicas, sintomatología y lesiones

El periodo de incubación oscila entre 3-19 días, siendo la presentación clínica variable en función de la virulencia del aislado, las características del hospedador afectado y la ruta de infección (34). La sintomatología clínica y lesiones observadas en animales infectados no son patognomónicas aunque se caracterizan por ser lesiones de tipo hemorrágico. Por ello, es necesario recurrir al diagnóstico de la enfermedad mediante técnicas directas e indirectas, así como realizar un diagnóstico diferencial con otras enfermedades hemorrágicas como son la peste porcina clásica (PPC), el mal rojo o procesos septicémicos como la salmonelosis o estreptococias (13).

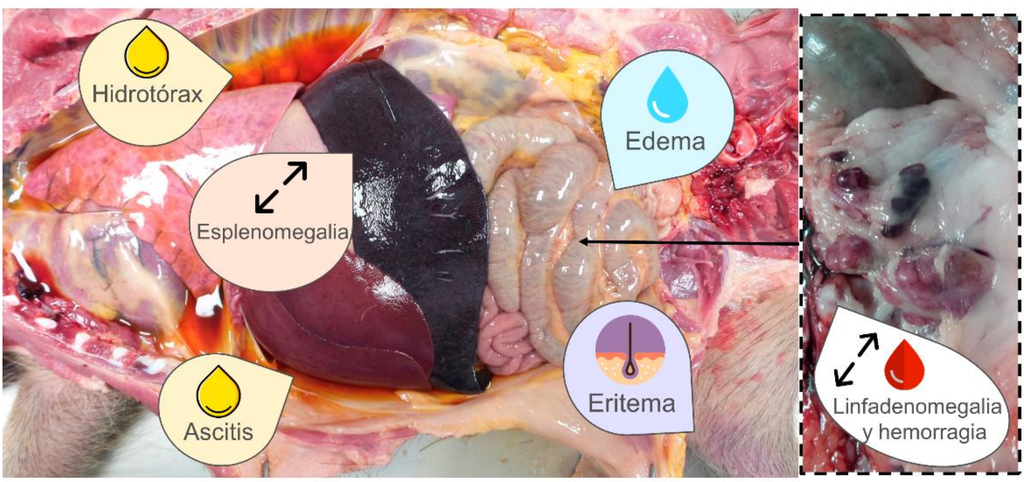
Los signos clínicos más frecuentes tanto en cerdos domésticos como en jabalíes son fiebre, anorexia y modificación en la actividad de los animales, mostrando desde estados letárgicos hasta postración (40). Externamente, los animales pueden mostrar lesiones eritematosas y hemorrágicas en la piel, especialmente en regiones distales de extremidades y orejas (13, 41). No obstante, en cuadros hiperagudos, los animales pueden mostrar letargia y muerte sin ninguna otra sintomatología adicional.

Los hallazgos macroscópicos más habituales incluyen lesiones hemorrágicas en un número variable de órganos como piel, riñones, vejiga de la orina, pulmón, corazón o hígado. Estas lesiones de tipo hemorrágico son también frecuentes en nodos linfáticos, donde suele acompañarse de una hiperplasia de los mismos. Además, puede observarse esplenomegalia con efusiones hemorrágicas en cavidad torácica, pericárdica y abdominal (ver Figura 3) (13, 42). La Tabla 1 describe las formas clínicas descritas en la infección con el VPPA en función de la virulencia del aislado viral causante, detallando las tasas de mortalidad más frecuentes, sintomatología y lesiones (externas y hallazgos en necropsia).

Tabla 1: Diferencias en las formas clínicas, tasas de mortalidad (M), sintomatología (S) y lesiones (L) observadas en la infección por el VPPA en función de la virulencia del aislado viral.

Formas clínicas	Virulencia	M (%)	Sintomatología y lesiones
Hiperaguda	Alta	100%	**S:** fiebre alta, pérdida de apetito, taquipnea, inactividad y eritema con muerte en 1-4 días post-infección **L:** sin signos evidentes
Aguda	Alta - Moderada	100% - 70%	**S:** fiebre alta, letargia, debilidad, postración y eritema **L:** esplenomegalia hiperémica, lesiones hemorrágicas en linfonodos, riñones y corazón entre otros órganos
Subaguda	Moderada	70% – 30%	**S:** temperatura fluctuante de alta a moderada durante 2-3 semanas. Cuadro clínico más leve **L:** esplenomegalia, hemorragias petequiales y aborto en hembras gestantes
Crónica	Moderada - Baja	-	**S:** temperatura fluctuante **L:** focos necróticos en piel y sintomatología respiratoria

Figura 3: Lesiones frecuentes observadas en la infección por un aislado virulento del VPPA en jabalí. *Fuente: iconos diseñados por Freepik en www.flaticon.com, imagen del grupo SUAT-VISAVET.*



2. Escenarios epidemiológicos

Desde el descubrimiento de la PPA a principios del siglo veinte, su distribución ha variado considerablemente a lo largo de la historia. Quizás la época de mayor incidencia y expansión se registró entre las décadas de los años 60 y 80 cuando la PPA alcanzó el continente europeo. Por aquel entonces, la enfermedad se expandió afectando a países como Portugal, España, Francia, Italia, Malta, Bélgica y Holanda. Además, durante esta misma época la PPA alcanzó el continente americano, afectando a territorios como Brasil, República Dominicana, Haití y Cuba (43). Todos los países anteriormente mencionados fueron capaces de erradicar la enfermedad salvo la isla italiana de Cerdeña, donde hoy en día la enfermedad aún persiste.

La situación actual está alcanzando niveles de expansión comparables a escenarios históricos, con tres continentes afectados: África, Europa y Asia. A día de hoy, la PPA está ampliamente distribuida por el continente africano y desde el año 2007, en Europa continental de nuevo (44). Además, tras la expansión en Rusia hacia territorios orientales limítrofes con Mongolia en marzo de 2017, en agosto de 2018, la PPA fue notificada por primera vez en la historia en China donde está describiendo un proceso de expansión constante (25).

Por ello, en la actualidad podemos distinguir dos escenarios bien diferenciados.

2.1. Escenarios endémicos

África: la PPA está presente en al menos 35 países subsaharianos de forma endémica, donde han sido descritos hasta 24 genotipos distintos (7-10, 17, 25). El censo porcino en este territorio representa cerca del 5% de la población mundial, la cual se ha triplicado en las últimas décadas (45). Este incremento en la población porcina se ha visto acompañado por un aumento en la incidencia de la enfermedad. De acuerdo con la información oficial de la OIE, se notificaron más de 5.000 brotes en cerdos domésticos entre 2005-2018 (17, 25). Sin embargo, varios autores surgieren que estas cifras serían aún mayores si se tiene en cuenta información publicada en fuentes extraoficiales (17).

Debido a la existencia de diferentes ciclos epidemiológicos dentro del continente africano, la incidencia y prevalencia de la enfermedad muestran cierta heterogeneidad en sus distintos territorios (46). Si bien, poco más de un 10% de los brotes especifican la fuente de infección, de estos casi el 90% se debe a la transmisión a través del ciclo doméstico mediado por movimiento de cerdos infectados o sus productos, los cuales son utilizados a su vez para alimentar a otros animales susceptibles. En aquellos brotes en los que el origen de la infección se atribuye a poblaciones silvestres, la principal hipótesis se basa en el acercamiento de cerdos domésticos en condiciones de libertad a zonas protegidas donde habitan poblaciones de facóqueros (17).

Por tanto, el nivel de riesgo vendrá determinado en gran medida por el modo en el que son estabuladas las poblaciones domésticas de cerdos (47).

Cerdeña: la PPA ha estado presente en la isla italiana de Cerdeña desde 1978 afectando a cerdos domésticos y jabalíes, donde es considerada una enfermedad endémica (48). Desde su introducción, las autoridades de la isla han implementado varios programas de control, erradicación y vigilancia sin resultados satisfactorios. Por ello, se han hipotetizado varios factores como causantes de la persistencia de la enfermedad en la isla. Si bien la ausencia de garrapatas (*O. erraticus*)

(49) fue reafirmada en un estudio realizado entre 2013 y 2014 (50), otras prácticas, como es el continuo mantenimiento de cerdos en extensivo, sin registro oficial, ni control veterinario (conocidos como cerdos de *“brado”* en sardo), así como las bajas condiciones de bioseguridad en granja, fueron identificados como factores de riesgo para la PPA (48, 51).

La presencia de la PPA en la isla no ha seguido una tendencia temporal clara, con picos en la incidencia en 1992, 1995, 2004, 2005 y 2013 (48). Espacialmente, la PPA consolidó su presencia en las provincias de Nuoro y Ogliastra, ubicadas en zonas centro-orientales de la isla, con notificaciones esporádicas fuera de esta zona. Sin embargo, con el último plan de erradicación 2015-2018, ha podido consolidarse una tendencia clara en el descenso de brotes en granjas de cerdo doméstico. A modo de ilustración, el último brote notificado en granja data de septiembre de 2018. La prevalencia en las poblaciones silvestres ha seguido esta misma tendencia con una reducción en los positivos a serología desde 10,44% en la campaña de caza 2012-2013 hasta 3,80% en la campaña 2017-2018 (52).

2.2. Escenarios epidémicos

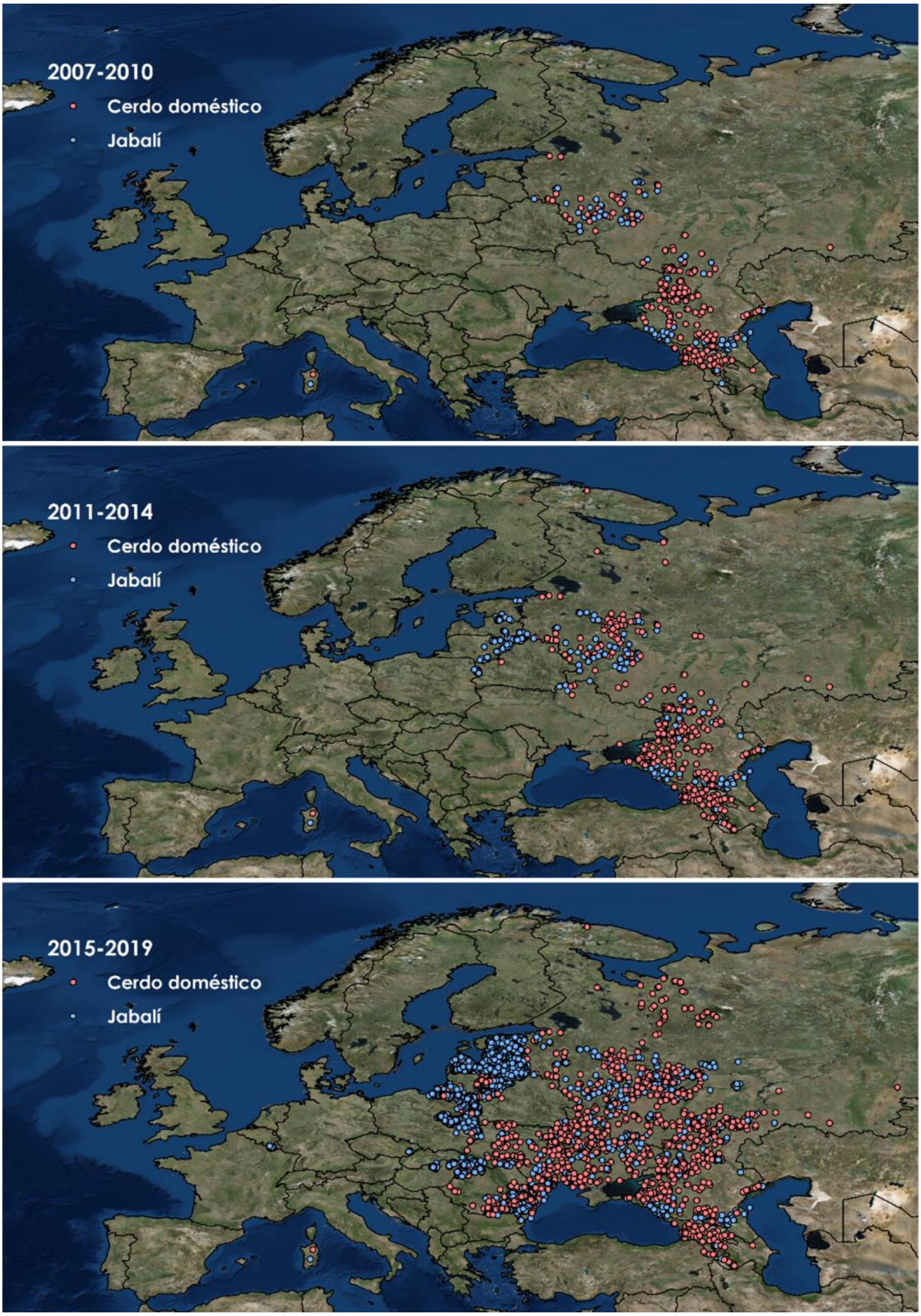
Europa: la epidemia actual comenzó en el año 2007, cuando la PPA entró a través de Georgia. Desde allí, la enfermedad comenzó su progresión hacia el resto de países del Cáucaso incluyendo Armenia, Rusia y Azerbaiyán (53). Inicialmente el único hospedador afectado fue el cerdo doméstico, sin embargo con la notificación de casos en jabalí en Rusia, se inició una expansión progresiva hacia zonas septentrionales del país (25). En 2010, varios brotes en zonas alejadas de las áreas iniciales de infección, apuntaron a la posible implicación de actividades humanas en la difusión de la enfermedad. Fue en 2012 cuando la PPA se notificó de forma reiterada cerca de Moscú, incrementando el riesgo por proximidad en países fronterizos, incluyendo áreas orientales de la UE (44). Entre 2012 y 2013, Ucrania y Bielorrusia notificaron PPA en sus territorios, seguidas por cuatro países de la UE: Lituania, Polonia, Letonia y Estonia, en 2014. A estos se les unió Moldavia (2016), Rumanía (2017), República Checa (2017), Bulgaria (2018), Hungría (2018) y Bélgica (2018). Al igual que ocurrió en Rusia, la difusión a larga distancia hasta países como República Checa, Hungría y Bélgica podría haberse debido a actividades humanas (26).

En todos estos países se han visto afectados jabalíes y cerdos domésticos, sin embargo la proporción de infección por hospedador difiere por territorio. En la UE, a excepción de Rumanía, el hospedador mayoritariamente afectado es el jabalí, al cual se le ha atribuido por encima del 90% de las notificaciones. A los casos en jabalí se le unen casos esporádicos en granjas de cerdo, siendo estas en su mayoría granjas de baja bioseguridad. Por el contrario, en los países del Este y Rumanía, la PPA afecta mayoritariamente a granjas de cerdo doméstico de tamaño variable (25).

Si bien este escenario ha sido clasificado como epidémico en el contexto de la población porcina doméstica, lo cierto es que determinadas áreas y poblaciones de jabalíes se encuentran en la actualidad infectadas de forma endémica (27, 44, 54).

La Figura 4 muestra la distribución de las notificaciones de PPA desde 2017 hasta junio de 2019, distinguiendo entre brotes en granjas de cerdo doméstico y casos en jabalíes.

Figura 4: Notificaciones de PPA a la OIE desde 2007 hasta junio de 2019 distinguiendo entre brotes en granja y casos en jabalí. *Fuente: imagen cortesía de E Cadenas-Fernández.*

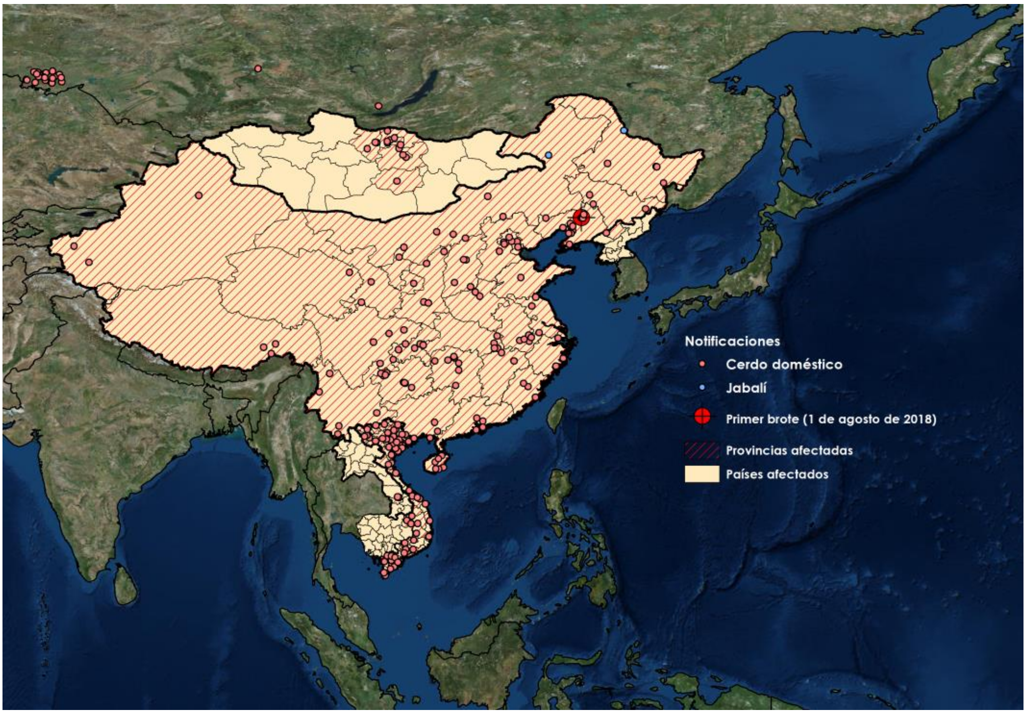


Asia: por primera vez en la historia, China notificó a la OIE la presencia de PPA en una granja de cerdo doméstico, en agosto de 2018 (55). Los estudios genéticos del aislado causante del primer brote mostraron similitudes con los aislados circulantes en Rusia y la UE (56, 57). La posible fuente de infección que se barajó fue la alimentación de animales con restos alimenticios contaminados (58). Tras la primera notificación, la enfermedad ha sido notificada en 28 unidades administrativas diferentes incluyendo provincias, municipalidades y regiones autónomas, con 118 brotes en granjas (a fecha 12/04/2019) y alrededor de 1 millón de animales sacrificados debido a las medidas de control impuestas. Además, en noviembre de 2018, un jabalí infectado con un aislado ligeramente diferente a los previamente descritos fue encontrado en la provincia de Jilin, donde también han sido notificados brotes en cerdo doméstico (59).

Otros cuatro países asiáticos también han notificado PPA en sus territorios, en concreto Mongolia, Vietnam, Camboya, Corea del Norte, así como la región administrativa especial de Hong Kong de la RPC, Laos, Myanmar y Filipinas en 2019. En Mongolia, tras la aplicación de medidas de control como el sacrificio obligatorio, alrededor del 10% de su población total porcina ha sido reducida. En Vietnam, desde la entrada de la PPA en febrero de 2019, 23 provincias distintas han sido afectadas con más de 100 brotes en granjas. Camboya y Corea del Norte notificaron en abril y mayo de 2019 la presencia de PPA en granjas de cerdo doméstico con baja bioseguridad (60). Las notificaciones de Hong Kong se atribuyeron a resultados positivos detectados en un matadero de la región, cuyas muestras procedían de cerdos domésticos infectados, importados desde China (61, 62).

La Figura 5 muestra la distribución de las notificaciones de PPA desde 2017 hasta junio de 2019, distinguiendo entre brotes en granjas de cerdo doméstico y casos en jabalíes.

Figura 5: Notificaciones de PPA a la OIE desde el 1 de agosto de 2018 (primer brote) hasta la actualidad distinguiendo entre brotes en granja y casos en jabalí. *Fuente: imagen cortesía de E Cadenas-Fernández.*



3. Herramientas de control y erradicación

La presencia de PPA debe ser notificada de forma obligatoria a las autoridades nacionales competentes, autoridades supranacionales como es la UE (en el caso de España y otros Estados Miembros) e internacionalmente a la OIE. Esta notificación pone en marcha una cadena de actuaciones a nivel local, nacional e internacional, entre las que se encuentra el potencial establecimiento de barreras al comercio. Por ello, es crucial llevar a cabo una detección, confirmación y comunicación temprana, así como el establecimiento rápido y efectivo de medidas para el control y erradicación de la enfermedad. Del mismo modo, en áreas libres la única herramienta disponible para salvaguardar la sanidad del territorio es el establecimiento de medidas preventivas. Dichas medidas también formarán parte de la estrategia de control en áreas afectadas, con el fin de evitar la difusión de la enfermedad.

En líneas generales, las estrategias de prevención se enfocarán a la prohibición de entrada tanto de animales vivos como de productos de origen porcino procedentes de áreas infectadas. Estas adquieren especial relevancia en el contexto de globalización actual debido al continuo flujo de personas, equipajes y mercancías, donde podrían movilizarse productos de origen porcino potencialmente contaminados. Por ello, los estudios de análisis de riesgo, así como las actividades de vigilancia y el correcto manejo y destrucción de desperdicios alimentarios constituyen herramientas clave de prevención. Otro aspecto fundamental relacionado con la prevención son las campañas de formación e información a todos los integrantes de la cadena de producción, incluyendo a los consumidores, acerca de las actividades que suponen riesgos para la industria y las consecuencias que tendría la introducción de una enfermedad de este tipo.

Cuando los mecanismos de prevención fallan y existe un contacto efectivo entre el VPPA y un animal susceptible, será necesario establecer mecanismos de control. En España, como parte del Plan Coordinado Estatal de Alerta Sanitaria Veterinaria, existe un manual práctico de operaciones (63) en el que se describe de forma detallada las actividades a realizar por los servicios veterinarios oficiales en caso de sospecha y confirmación. Entre las estrategias de control planteadas en este manual se puede destacar:

Inmovilización de la granja bajo control oficial hasta la confirmación de la sospecha.

En España, para la confirmación de la sospecha y diagnóstico de la enfermedad se procederá al envío de muestras a los Laboratorios Oficiales de Diagnóstico de las Comunidades Autónomas afectadas, desde donde se remitirán al Laboratorio Oficial Nacional designado para esta enfermedad; para la PPA, se trata del Laboratorio Central de Veterinaria ubicado en Algete, Madrid.

Tras la confirmación del foco, se procederá al sacrificio inmediato de todos los animales susceptibles de la explotación afectada y destrucción de los cadáveres de forma adecuada.

Establecimiento de estrictas medidas de bioseguridad en relación a la contención del foco, tratamientos de limpieza, desinfección y desinsectación de instalaciones, materiales y vehículos.

Zonificación de las áreas afectadas, estableciendo zonas de control y zonas de vigilancia de 3 km y 10 km, respectivamente, así como compartimentalización territorial para el control de movimientos.

Movimientos controlados tanto de animales vivos como de sus productos desde granjas ubicadas en zonas bajo control y vigilancia. También se controlará el movimiento de desechos relacionados, tales como purines u otros materiales potencialmente contaminados.

Además, se realizarán tareas de estudio, vigilancia y control en las poblaciones de jabalíes adyacentes.

Del mismo modo, se estudiará la presencia de vectores en la zona.

Una herramienta fundamental en el control y erradicación de enfermedades en medicina veterinaria es el empleo de vacunas. Desafortunadamente, como ya ha sido mencionado con anterioridad, todavía no existe una vacuna comercial frente a este virus.

JUSTIFICACIÓN Y OBJETIVOS

En el contexto actual la PPA representa la principal amenaza para el sector porcino, afectando a países cuyos censos representan el 77% de la población porcina mundial. Desde que la enfermedad entrase en el continente europeo en 2007, han sido numerosos los estudios en los que se ha intentado dar respuesta a cuestiones aún desconocidas de la enfermedad. Pese a los esfuerzos empleados en materia de prevención, control y erradicación, los resultados han sido muy limitados. El deterioro de la situación epidemiológica no solo en Europa, si no la expansión de la PPA al continente asiático, ha puesto en jaque a países líderes en el sector porcino como son Estados Unidos, España o Alemania. En estos países, así como en territorios todavía libres, la necesidad de establecer mecanismos de prevención basados en riesgo es fundamental para frenar la expansión de la PPA. Además, en territorios afectados es vital el establecimiento de medidas de control y erradicación efectivas.

Así pues, el **objetivo principal** de esta tesis ha sido proporcionar nuevas herramientas y conocimientos epidemiológicos para la prevención, control y erradicación de la PPA en el contexto mundial. Inicialmente, se planteó un primer objetivo, ***objetivo 1***: identificar las principales lagunas de conocimiento en relación a la PPA, sugiriendo mediante una evaluación de expertos cuáles de ellas son prioridades para un mejor control de la enfermedad.

La identificación y evaluación de estas prioridades reflejó la necesidad de establecer unos objetivos específicos, vertebrados en torno a tres escenarios epidemiológicos:

Escenario endémico, utilizando como modelo la isla de Cerdeña donde la enfermedad está presente desde 1978.

Escenario epidémico, siendo objeto de estudio el sector porcino doméstico de la UE, segundo productor mundial por detrás de China.

Escenario libre, centrado en Estados Unidos, donde el correcto funcionamiento de los sistemas de prevención y vigilancia determinará el futuro del sector porcino norteamericano.

Encuadrados en dichos escenarios epidemiológicos se plantearon los siguientes objetivos específicos:

Objetivo 2: identificar los factores de riesgo que explican la persistencia y endemismo de la PPA en la isla de Cerdeña. De acuerdo con los factores de riesgo identificados, se propondrán medidas suplementarias a las ya implantadas en el programa de control y erradicación vigente, con el fin de mitigarlos.

Objetivo 3: identificar las medidas disponibles para prevenir la introducción y difusión de la PPA en las granjas de cerdo doméstico de la UE a través de una revisión sistemática. Además, se evaluará mediante opinión de expertos la relevancia de cada medida identificada dependiendo de los sistemas productivos presente en el escenario de la UE.

Objetivo 4: evaluación del riesgo de entrada del VPPA en Estados Unidos a través de productos de origen porcino transportados ilegalmente en el equipaje de viajeros procedentes de vuelos internacionales. Debido al cambio drástico experimentado en la distribución de la enfermedad, con la notificación de PPA en China, este objetivo fue dividido en dos sub-objetivos.

Sub-objetivo 4.1: evaluación del riesgo de entrada de PPA y peste porcina clásica con información epidemiológica disponible hasta julio de 2016.

Sub-objetivo 4.2: reevaluación del riesgo de entrada de PPA en Estados Unidos con información epidemiológica hasta febrero de 2019.

CHAPTER 1

OBJECTIVE 1. To review and identify the main gaps of knowledge regarding ASF, suggesting which of them are priorities to better improve disease control based on expert opinion.

Main scientific publication of objective 1.

Arias M, **Jurado C**, Gallardo C, Fernandez-Pinero J, Sanchez-Vizcaino JM. **Gaps in African swine fever: analysis and priorities.** Transbound Emerg Dis. 2017;65(Suppl 1):235-247. DOI: 10.1111/tbed.12695.

Related scientific contributions:

Other scientific publications not included in this doctoral thesis:

Guinat C, Vergne T, **Jurado C**, Sanchez-Vizcaino JM, Dixon L, Pfeiffer DU. **Effectiveness and practicality of control strategies for African swine fever: what do we really know?** Vet Rec. 2016;180(4):97. DOI: 10.1136/vr.103992.

Scientific dissemination articles:

Jurado C, Sanchez-Vizcaino JM. **ASF: the biggest threat to the world's swine industry. What can we do?** Pig333. 2018 [Available at https://www.pig333.com/articles/asf-the-biggest-threat-to-the-worldE2%80%9-9s-swine-industry_14135/].

Jurado C, Sanchez-Vizcaino JM. **Cursos: gestión de crisis sanitarias en el ganado porcino.** SUIS, Nº 150, 151 y 152. 2018.

Jurado C, Cadenas-Fernandez E, Sanchez-Vizcaino JM. **La peste porcina africana, la mayor amenaza para del sector porcino mundial**. SUIS, Nº 156. 2019.

Book chapters:

Sanchez-Vizcaino JM, **Jurado C**, Mur L. **Capítulo 57. Familia Asfarviridae.** Editors: Stanchi NO, Copes JA, Echevarria MG, Gatti EMM, Gentilini ER, Larser A, Leotta GA, Martino PE, Moredo FA, Reinoso EH. Microbiología Veterinaria. INTER-Médica. Buenos Aires, República Argentina. 2018. ISBN 978-950-555-474-4.

RESUMEN DE LOS RESULTADOS DEL CAPÍTULO 1

La notificación de PPA da lugar a graves consecuencias sanitarias, sociales y económicas si se compara con cualquier otra enfermedad del ganado porcino. Aunque la PPA se describió por primera vez en 1921 y ha afectado a más de cincuenta países en África, Europa y América del Sur, varios aspectos relacionados con su patogénesis, capacidad de evasión inmunológica y epidemiología siguen siendo inciertos. Algunas de estas lagunas de conocimiento podrían ser cuestiones clave para descifrar por qué los hospedadores no pueden desarrollar anticuerpos que neutralicen completamente la infección o, incluso, podrían ayudar a obtener una vacuna eficaz contra el agente etiológico de esta enfermedad. Por lo tanto, este objetivo y su publicación científica relacionada revisan las características principales del VPPA, su epidemiología molecular, hospedadores naturales, características clínicas, epidemiología y control. También identifica y prioriza, en base a la opinión de expertos, estas lagunas de conocimiento desde un punto de vista horizontal abarcando campos como la biología molecular, epidemiología, prevención, diagnóstico y desarrollo de vacunas.

Para ello, se invitó a los expertos pertenecientes al Laboratorio de Referencia en PPA de la OIE y al Laboratorio de Referencia en PPA de la UE, a clasificar las lagunas de conocimiento identificadas como prioridades de "menor", "media" o "mayor" importancia. Es importante resaltar que en el momento en que se llevó a cabo este objetivo, la PPA solo estaba presente en África y Europa. Por lo tanto, las prioridades identificadas en aquel momento podrían haber pasado por alto aspectos de interés en relación a las nuevas áreas afectadas.

De acuerdo con los resultados obtenidos, se identificaron un total de 36 prioridades durante el proceso de revisión, 19 de ellas se clasificaron como prioridades de importancia mayor, 11 con importancia media y 6 con importancia menor. Las prioridades de gran importancia se relacionaron con la necesidad de comprender el papel de ciertos segmentos del genoma del VPPA, caracterizando los aislados circulantes en África y Europa e identificando los factores del virus que determinan su persistencia en los distintos hospedadores y los resultados clínicos de la infección. Otra de las prioridades identificadas se relacionó con la importancia de diseñar tecnologías de modelización para establecer medidas de control basado en riesgo. Las prioridades de importancia media se relacionaron con la identificación de marcadores genómicos de virulencia, la realización de estudios de análisis de riesgo para evaluar la posible introducción y propagación de la PPA en regiones expuestas a un mayor riesgo o la implementación de actividades de vigilancia. Finalmente, las prioridades pertenecientes a la categoría de menor importancia se relacionaron principalmente con las garrapatas del género *Ornithodoros*, en relación a la evaluación de su presencia, características ecológicas y su papel en cada escenario.

Por tanto, para concluir este objetivo, esta revisión ha tratado de promover la investigación de la PPA en diferentes campos a fin de avanzar hacia una comprensión más completa de esta enfermedad, mejorar su control y promover su erradicación en los territorios afectados. Dado que el escenario asiático no se tuvo en cuenta, sería necesario realizar una revaluación de las prioridades en investigación en este nuevo territorio ya que una mejor comprensión de las características de la industria porcina en China y Vietnam podría ayudar a diseñar medidas de control más efectivas.

Gaps in African swine fever: analysis and priorities

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Summary

African swine fever (ASF) causes greater sanitary, social and economic impacts on swine herds than many other swine diseases. Although ASF was first described in 1921 and it has affected more than fifty countries in Africa, Europe and South America, several key issues about its pathogenesis, immune evasion and epidemiology remain uncertain. This article reviews the main characteristics of the causative virus, its molecular epidemiology, natural hosts, clinical features, epidemiology and control worldwide. It also identifies and prioritises gaps in ASF from a horizontal point of view encompassing fields including molecular biology, epidemiology, prevention, diagnosis and vaccine development. The purpose of this review is to promote ASF research and enhance its control.

Keywords

African swine fever, diagnostics, disease control, emerging diseases, gaps analysis, potential vaccines, priorities.

1. Introduction

African swine fever (ASF) is an infectious disease of swine, notifiable to the World Organisation of Animal Health (OIE). It causes greater sanitary, social and economic impacts than many other animal diseases because the occurrence of ASF is sufficient to trigger regional, national and international trade restrictions. ASF affects domestic and wild suids of all breeds and ages. Fortunately, it is not a zoonotic disease, which limits its impact on public health.

Currently, no vaccine or treatment against ASF is available, and control strategies depend mainly on early disease detection through rapid field suspicion and laboratory diagnosis followed by implementation of strict sanitary measures (41, 64). A reliable laboratory diagnosis is performed by using virus and antibody detection techniques that allow the identification of infected animals, including survivors as potential virus carriers.

ASF is present in Africa and Europe, where it shows different epidemiological patterns and scenarios. On the African continent, the disease has been recognised in 28 countries (25); and in Europe, ASF has been endemic on the Italian island of Sardinia since 1978. In 2007 ASF reached Eastern Europe from East Africa. Since then, ASF has spread from the Caucasus region (Georgia, Azerbaijan and Armenia) to the Russian Federation (2007), Ukraine (2012), Belarus (2013), Estonia (2014), Latvia (2014), Lithuania (2014), Poland (2014) and Moldova (2016), where it has affected domestic pigs and wild boar (25, 44, 64-66). The disease is currently endemic in some parts of Eastern Europe (27).

Transboundary movement of this disease has been historically related to the single introduction of contaminated pork or pork products used to pig feed (41). In contrast, current ASF movements in Europe, especially in the European Union affected states, are driven by the movement of free-ranging infected wild boar, which can move the disease through natural corridors (67-69). Nevertheless, other routes of ASF introduction and spread have been reported and are present in Eastern Europe such as the illegal movement of infected pigs or the use of contaminated pork products for feeding pigs (27, 70, 71).

The aims of this review are to provide an overview of current ASF epidemiology and control strategies, point out important gaps in disease control and suggest priorities for filling those gaps through ASF research and policy.

2. Material and methods

Firstly, a comprehensive review of the published scientific literature was conducted to identify gaps and priorities regarding ASF. Then, gaps and priorities were classified based on expert opinion. The group of experts belonged to the OIE-ASF Reference Laboratory, the FAO-ASF Reference Centre and the European Union ASF Reference Laboratory (five experts) with proved expertise and experience on ASF. Experts were invited to rank each gap and priority as high, medium and low importance. Finally, mode value was used for the final score of each gap.

3. Results and discussion

1.1. ASF virus characteristics

ASF virus (ASFV) is a complex, large, icosahedral multi-enveloped DNA virus, classified as the only member of the family Asfarviridae, genus Asfivirus (1). ASFV genome encodes a significant number of viral enzymes, viral transcription factors, and immune homologues among others. The viral particle contains fifty four structural proteins. Nearly a hundred proteins have been identified on the target cells during ASFV infection, particularly in pig macrophages (3). Both, structural and infection-related proteins can regulate, inhibit and modulate essential and non-essential mechanisms affecting virus replication, virus particle production and apoptosis. Some of them are based on the inhibition of host transcription factors, the interferon response or several immune cell subsets, to evade host immune system (72, 73).

ASFV genome consists of a conserved central region of about 125 kb and two variable ends encoding five multigene families (MGFs); these variable ends account for the variable size of the genome (170-193 kb) among virus isolates (3, 4). Several MGFs help determine virulence of isolates as well as viral replication in soft ticks. Concretely, deletion of certain MGFs has given rise to attenuated phenotype isolates that has been shown to induce protection against virulent challenges (74). Deletion of MGFs genes also reduced viral replication and generalisation of infection in infected ticks (75). Whether MGFs also help the virus generate antigenic variability and thereby evade the immune response remains uncertain. Likewise, which genes in MGFs may be related to host protection has not been fully identified.

ASFV classification is based on molecular epidemiology, which has proven useful for tracking virus spread. The current approach is based at a first step on partial sequencing of the B646L gene encoding the p72 protein. This can differentiate up to 23 genotypes (9, 10), as recently, a new genotype XXIII was described in Ethiopia (9), suggesting that more ASFV genotypes could remain to be discovered in Africa. Thus further biological and molecular characterisation of isolates currently circulating within Africa and Europe should be a priority. Closely related ASFV isolates can be distinguished through sequence analysis of tandem repeats in the central variable region within the B602L gene (11) or the intergenic region between the I73R and I329L genes at the right end of the genome (69). Several other gene regions such as the E183L encoding p54 protein, the CP204L encoding p30 protein and the protein encoded by the EP402R gene (CD2v), have been proved as useful tools to analyse ASFVs from different locations to track the virus spread (11, 12, 76). The genetic characterisation approach is not related to biological properties. More research would be needed to identify new genetic markers for ASFV, including those involved in the evolution of circulating ASFV isolates, especially in endemic regions. In addition, new genetic markers intricate in virulence would be very useful for control strategies. The genetic characterisation of MGF virulence genes to cluster/group ASFV isolates based on virulence factors could be a potential interesting area of research.

1.2. ASF in natural hosts

Suids are the animal hosts naturally infected by ASFV: domestic pigs, European wild boar and feral pigs of all ages and breeds are susceptible to infection. These animals, when infected, may show a variety of clinical presentations: peracute, acute, subacute, chronic and subclinical (77-79). In contrast, wild African suids such as warthogs (*Phacochoerus aethiopicus*), bush pigs (*Potamochoerus porcus*) and giant forest hogs (*Hylochoerus meinertzhageni*), develop asymptomatic infections, allowing them to act as true ASFV reservoirs in Africa (47, 80).

Several studies in East Africa have revealed a complex epidemiological situation in which local breeds of domestic pig seem to show greater tolerance to ASFV, that favours endemicity and spread of the disease (38, 81-83). In addition, virus evolution towards moderate virulent forms could be also contributing for the presence of asymptomatic pigs acting as virus carriers (79). The molecular factors in wild African suids determining whether ASFV infection will be asymptomatic remain unknown. The host factors that determine clinical outcomes of infection also determine susceptibility, resistance (the ability to limit the pathogen load) and tolerance (the ability to limit the impact of the pathogen on host health) to ASFV infection should be priorities for future research.

ASFV also replicates in the soft ticks of the *Ornithodoros* genus. *O. moubata* complex in East and South Africa and *O. erraticus* on the Iberian Peninsula are biological vectors and reservoirs of ASFV (18, 84, 85). *O. moubata* shows trans-stadial, transovarial and sexual ASFV transmission (86), while only trans-stadial transmission has been observed with *O. erraticus* (6, 16). In the absence of viraemic hosts, *Ornithodoros* ticks can allow ASFV infection to persist for more than 5 years (20). In West Africa, ASFV has been detected in *O. sonrai* ticks, yet they seem to play a limited role in ASF epidemiology (87). So far, all *Ornithodoros* species experimentally tested seem able to transmit ASFV, including *O. moubata, O. porcinus, O. erraticus, O. coriaceus. O. turicata* and *O. savignyi* (6, 18, 88-90). Other *Ornithodoros* species have been already identified along different ecological settings from the United States and Latin America (91). The detailed geographical distribution of *Ornithodoros* ticks is not well understood making it difficult to assess the potential role of soft ticks in current ASF scenarios. The role of soft ticks in virus transmission, persistence and dissemination is not yet well understood and needs to be clarified, especially in Europe.

1.3. Clinical forms of ASF

The ASF incubation period usually ranges from 3 to 19 days. ASF is not associated with pathognomonic lesions, so clinical signs may be similar to other haemorrhagic diseases such as classical swine fever, salmonellosis or erysipelas. The clinical form of ASF depends on isolate virulence, host species and breed, and routes of infection (13, 30, 92). Identifying virulence factors and pathogenesis mechanisms would improve our understanding of different clinical forms of ASF, facilitating a better diagnosis recognition and potentially early detection on farms and in the field. For example, genomic markers related to ASFV virulence need to be identified and fully characterised. That would allow to design better and more appropriate diagnostic strategies, according the clinical symptoms to be expected in the infected animals, improving surveillance and control programs.

Highly virulent isolates usually induce acute ASF, which in naïve animals is associated with mortality as high as 100% within 4-9 days post-infection. Acute ASF is characterised by high fever followed by moderate anorexia, lethargy, weakness, decubitus and erythema. Congestive-haemorrhagic signs and functional failures of internal organs can be observed. Internal lesions are usually related to hyperaemic splenomegaly and haemorrhages in a large number of organs and tissues (13).

Moderately virulent isolates may produce acute and subacute forms (78, 93). These clinical presentations have been reported in endemic areas such as Eastern Europe, Sardinia or the Iberian Peninsula (48, 94, 95). Subacute ASF is associated with fluctuating temperature for two or three weeks and clinical signs similar to those of the acute form but less severe (13, 96). Mortality rates range from 30% to 70%, usually after 20 days post-infection. Other isolates can induce subclinical or even unapparent forms, resulting in intermittent viraemia, seroconversion and lower mortality rates (77, 92, 97-99).

Unapparent ASF is usually reported in endemic scenarios, in which clinical signs are mild or even absent. Unapparent and recovered pigs should be identified through detection of specific antibodies and ASFV antigens or genome. Such animals should be studied as potential carriers to detect changes in the virulence of circulating isolates and assess the role of those animals in transmitting and maintaining the disease. Animal experiments using ASFV isolates from recovered animals would allow a better knowledge about the ability of these virus isolates to be transmitted by different routes, its presence and persistence in excretions and tissues, a deeper characterisation of the carrier state or the potential clinical activation of unapparent infections. Chronic forms of ASF have been reported mainly in Spain (94), Portugal (100) and Latin American countries (96) infected with isolates coming from the Iberian Peninsula. Infected animals show necrotic skin lesions as well as respiratory symptoms (98-100). These lesions have been also observed in two recent experimental infections with moderately virulent ASFV isolates from Eastern Europe (101, 102).

1.4. Immune response to infection

During ASFV infection, the protective immune response includes both cellular and humoral immunity (103). Pigs that do not die within the first days of infection produce high levels of specific antibodies against ASFV, which are detectable for long periods of time but that are not fully neutralising (41). Nevertheless, some protection related to antibody-mediated immunity is observed. Passive transfer of sera from ASFV-infected and recovered pigs, partially protected pigs against homologous ASFV challenge infection and the potential fatal consequences of infection by delaying the onset of the ASF clinical signs and reducing the levels of viremia (104-106). The antibodies may also protect the host through antibody-dependent cytotoxicity (107). So far, at least fifty viral proteins have been identified as immunogenic (33, 108), but how these proteins elicit an effective immune response in surviving animals remains unknown.

Wild African suids show tolerance to ASFV via unknown mechanisms.

Understanding how ASFV can persist in hosts is needed. Such persistence could involve immune cells targeted by the virus for replication, particularly macrophages (109). A recent study conducted by Franzoni *et al.* (110) showed that virulent isolates have evolved mechanisms to counteract activated macrophage response promoting viral survival, dissemination in the host and pathogenesis. More detailed characterisation of interactions between ASFV and macrophages and other cells in the host may provide new insights into how to induce a protective immune response. Such work should also examine the potential roles of MGFs.

1.5. ASF epidemiology

ASFV can be transmitted through direct or indirect contact between infected animals, pork products or contaminated fomites (*e.g.* clothing, vehicles, boots) and susceptible animals. Healthy animals may be directly infected through contact with blood, secretions, faeces and excretions from infected animals. Recently, some studies have been carried to better understand ASFV shedding patterns (111-115). These studies have provided information on ASFV excretion through oropharyngeal, oral, for at least 70 days, and through nasal and rectal swabs among others, but only with regard to domestic pigs. In addition to this, these studies evaluated shedding patterns when animals were infected through three routes of direct inoculation (intramuscular, intranasopharyngeal and introaoropharingeal) or through direct contact with inoculated animals. However, no information on ASFV shedding and kinetics after infection via consumption of contaminated pork or cannibalism are available. Therefore, a more detailed understanding of virus shedding patterns and kinetics evolving domestic pigs and wild boar are still needed.

Historically, ASF introductions into free distantly located areas have been driven by indirect transmission via animal consumption of contaminated pork or pork products (13). ASFV can also be transmitted through the bite of soft ticks. Contaminated vehicles are also a potential way of introduction of ASF into free areas (13). The resistance of ASFV to various environmental conditions favours its spread (6), which can also be promoted by poor farming practices, swill feeding, and slaughtering on the farm.

Overall, ASF epidemiology depends on the host (domestic pigs, wild boar, wild suids), presence of ticks and type of pig production (indoor, outdoor). So far, three transmission models have been observed in affected countries (13). The first and most complex model was observed in East and South Africa, where domestic pigs, wild suids and ticks cohabit. The second model was observed on the Iberian Peninsula, where wild boar, outdoor domestic pigs and ticks are involved. The third model is present in currently affected European areas, which contain infected wild boar and/or domestic pigs but no soft ticks. However, the presence of *Ornithodoros* ticks in Eastern Europe cannot be completely discarded since several researchers reported the presence of these ticks between the 1930s and the 1960s (116). Elucidating the respective roles of host, vector and environment under the different conditions of each epidemiological scenario should be a key research priority.

ASF is present in 28 sub-Saharan African countries, where it affects domestic and wild populations (25). In April 2007, ASF was introduced from East Africa into the Republic of Georgia, from where it spread to Armenia, Azerbaijan and the Russian Federation (44). After several years of continuous outbreaks, two endemic regions in the Russian Federation are now recognised (27). As a result of the situation in Eastern Europe, ASFV was introduced into neighbouring countries such as Ukraine and Belarus, mainly by free-ranging wild boar. In January 2014, ASF cases in wild boar were reported within parts of the European Union (EU) bordering with Belarus. Since then, ASF cases in wild boar and outbreaks in domestic pigs have been reported in four EU countries: Lithuania, Poland, Estonia and Latvia. In 2016, other European state, Moldova, became infected (25). The current situation poses a threat to pig production and economies of affected and neighbouring countries.

The current situation in the EU and some Eastern European countries shows several characteristics not observed in previous epidemics. First, multiple viral introductions through movements of infected free-ranging wild boar have taken place in the affected areas. Second, wild boar is the most severely affected host, giving it an important role in ASF spread and maintenance (67). Third, the combination of pig farms located in areas suitable for wild boar as well as the existence of low biosecurity measures, especially on backyard farms, may have facilitated contacts between both hosts and thereby promoted ASF transmission.

These novel characteristics of the current ASF situation reflect the need for control and eradication measures that take into consideration the interactions among hosts, pathogen and environment in each epidemiological scenario. The role of wild boar in virus transmission, maintenance and dissemination in Eastern Europe requires further investigation, as does the role of wild African reservoirs in disease transmission under different conditions. Although some studies referred that wild boar avoided feeding on conspecifics (animals of the same species) suffering from illness (117), the presence of infected wild boar carcasses in the field has been already identified as cause of ASFV maintenance in the environment and spread due to scavenging behaviours among wild boar population (118, 119). Studies are needed that better understand this fact as well as examine neighbourhood transmission in densely populated areas and transmission between pigs and wild boar. Whether or not soft ticks are present in Eastern Europe, Sardinia and Northern Europe should be determined definitively, and, if present, their role in ASF maintenance and transmission should be clarified in Northern European scenarios. A better understanding of the seasonal cycle of these soft ticks, and how climate affects it, should also be a priority.

Finally, in order to reduce ASF spread due to human factors, communication campaigns and training courses should be organised to raise the awareness of hunters, farmers and field veterinarians.

1.6. Socio-economic impact

ASF is not a zoonotic disease, but it has serious socio-economic impact, especially in countries that export live pigs, pork and/or products, as well as in countries where these products are important sources of protein. ASF directly affects the economies of affected countries because its notification triggers control measures (“stamping out” policies) as well as national and international trade restrictions on animals and pork products. These measures include export restrictions, control of animal movements and their products, and animal quarantine (35).

Preventive measures and early detection (including suspicion and diagnosis) are the best way to reduce or eliminate the socio-economic impact of ASF. Epidemiological and qualitative/quantitative risk assessments are needed to identify routes of introduction-transmission and regions at greatest risk (risk mapping). The results of these assessments should then be used to focus preventive measures and surveillance activities on certain areas. Disease modelling technologies, such as Be-FAST (120), InterSpread (121), NAADSM (122) DTU-DADS (123) software or the modelling approaches developed by Barongo *et al.* (124) or Verge *et al.* (125), among others, have been used to model animal disease and control options in different scenarios. Incorporating wild animals, vectors and human factors into these modelling algorithms should be a priority for future work.

Funding from the EU has been provided to Estonia, Latvia, Lithuania and Poland to strengthen their preparedness against ASF and to enhance protective measures, though the amount of funding is not known officially. Cost-benefit analyses based on the current EU scenario are needed in order to evaluate preventive costs, disease-controlling efforts made so far and optimise future control measures.

1.7. Prevention, detection and control

Preventive measures are crucial for avoiding the introduction of infectious diseases into herds and their subsequent spread. The feasibility and efficacy of prevention and control measures depend on farm location (suitable or not for wild boar), sort of farm (confined, outdoor or backyards), type of production (for instance breeding or fattening farms), animal movements, sanitary status of animals to be replaced, and farm biosecurity standards. Biosecurity can be improved by erecting physical barriers, such as internal and external fences; installing bird nets; creating quarantine facilities for animals and changing facilities for workers and visitors; running pest-control programmes; erecting sanitary enclosures; disposing safely of manure; following good farming practices; and washing and disinfecting transport vehicles (35, 118).

There is no a single recipe for preventing ASF. Success depends on many parameters in the epidemiological situation, such as whether the affected population is domestic and/or wild, and whether vectors are present. Success also depends on current legislation, economic resources and logistical aspects. Countries at higher risk should be aware of the characteristics of the isolates circulating in neighbouring areas, as well as which host populations are affected.

Farmers and farm staff need to be aware of both exotic and common infectious diseases, and they should be familiar with preventive measures that can block disease entrance. Some risk factors associated with ASF introduction are poor farming practices, poor training of farm personnel, lack of communication and awareness, lack of motivation for following regulations, poor record-keeping on the farm, and no audit of biosecurity-related activities (35, 38, 126).

The efficacy of preventive and control measures depends on early suspicion and identification of suspected disease, early diagnosis of disease, identification of subacute/unapparent infected animals, basic biosecurity on pig holdings (fences and bird nets), identification of individual animals, updated census and animal movement records and control of soft ticks (if present) (35, 127). Preventing contact between wild boar and domestic pigs is crucial, particularly in the EU. Farms should be located far from areas suitable for wild boar, especially backyard farms and farms with poor biosecurity. Pigs in infected areas should be confined (instead of held outdoors) in order to prevent them from coming into contact with wild boar or pigs from other farms, as well as to prevent scavenging activities. Control failures may be caused by cultural practices (48), trade of infected products and the taboo of throwing away food observed in some cultures (128).

Every country should have a contingency plan and early warning system in place in the event of ASF entrance. Any delay in outbreak response and implementation of control measures can result in greater viral contamination of the environment and promote disease spread (118). Field veterinarians and the relevant authorities should be aware of, and trained in, how to detect the various clinical forms of ASF. Highly virulent ASFV isolates are associated with more evident clinical forms and should therefore be easier to detect by passive surveillance. In contrast, passive surveillance may not be sufficient for early disease detection in the case of moderately virulent ASFV isolates or infection of wild boar or wild suids. In these cases, additional control measures should be implemented. For instance, areas with infected wild boar should be monitored through a combination of passive surveillance of dead wild boar and active surveillance in areas at highest risk. This is because discovering wild boar carcasses is not an easy task; they are usually eaten by other animals or hidden under vegetation or snow. A priority is to develop new, non-invasive methods to sample wild populations, particularly given the current situation in Northern Europe.

1.8. ASF diagnosis and potential vaccines

So far, neither a vaccine nor treatment against ASF is available. Therefore, control strategies are based initially on early disease detection based on rapid suspicion, identification and diagnosis of suspected cases, followed by implementation of strict sanitary measures (38, 41, 64).

A wide range of laboratory tests is available to detect ASFV genome, antigens or antibodies against the virus. Since there is no vaccine against ASF, antibody presence is always indicative of infection. ASF infection produces long-term viraemia and antibody response can be detected from the first week of infection for up to months or even years (41). Serological diagnosis should be performed in parallel with viral diagnosis because animals with subacute or unapparent ASF possess antibodies but may show only intermittent viraemia (64, 98). Serological tests were particularly important, for example, during ASF eradication on the Iberian Peninsula and in Brazil (35, 129). Thus, both virus and antibody detection are crucial for full understanding of the epidemiological situation and the roles of infected animals in disease maintenance and spread. Certain ASF diagnostic tools may be more appropriate depending on whether the area is ASF-free or already affected by the disease (see Table 1 at the end of the publication). Because of the emergence of several new valuable ASF diagnostic tests in Europe over the last decade, international reference laboratories should collaborate to develop an updated diagnostic manual listing all validated tests.

While several reliable commercial kits for viral genome, antigen and antibody detection have become available in recent years, commercial confirmatory serological tests are still lacking and should be a priority for future work. Another gap is the lack of cell lines that can replace primary cell cultures for ASFV isolation, which would help standardise isolation techniques.

Detection of ASFV in ticks can be achieved based on virus isolation or PCR (130, 131). Several ELISA tests have been developed to detect swine exposed to *Ornithodoros* ticks which presumably have antibodies against salivary glands of *O. erraticus* and/or *O. moubata* (50, 132, 133). At the moment, these techniques usually involve “in-house” procedures. A priority should be to develop standardised approaches for more reliable assessment of epidemiological situations.

New technologies including lateral flow devices (pen-side tests) and portable PCR machines that allow rapid diagnosis have been recently developed (134, 135). A deeper validation under field conditions should be encouraged. At the same time, non-invasive sampling methods are lacking, which are especially important for ASF control in Northern Europe. Samples obtained through non-invasive sampling methods such as oral fluid and faeces allow ASFV and anti-ASFV antibodies detection (115, 136-139). Commercial tests based on oral fluid are already available for porcine reproductive and respiratory syndrome as well as sampling guidelines for oral fluid-based survey on grouped-housed animals (140). However, standardised methods for sampling and testing ASF on such matrices (oral fluid and faeces) need still to be developed and validated for domestic pig and wild swine populations.

Vaccine development remains a major gap in ASF control and eradication. Efforts to develop a vaccine for ASFV based on inactivated virus as well as viral proteins and peptides have been hindered by the genetic complexity of ASFV, virus-host interactions and technical difficulties (see Table 2 at the end of the publication). For example, inactivated and subunit virus vaccines can induce antibody responses, but these do not confer strong protection (Table 2). Live attenuated vaccines can confer protection against homologous, but not heterologous, viral challenge in surviving pigs (80, 97, 141). Several studies have suggested the key role for the innate immunity and natural killer cells (99, 142) as well as the cytotoxic activity by CD8 T-cells (103, 143, 144). Current vaccine development efforts and priorities include strategies to stimulate both antibody response and cytotoxic activity by T-cells. Side effects, virus persistence, doses and other safety parameters are some gaps related to vaccine development that need to be filled. Improvements in the current and new vaccine candidates will require more extensive analysis of viral genes that should be deleted to build more effective deletion mutants. Another priority is to clarify the roles of specific viral genes in the infection cycle regarding immune evasion and infection control. It will also require further study of ASF pathogenesis and interferon-mediated induction. Optimised delivery systems that can induce a protective immune response are needed. Another important issue is the availability of cell lines that can propagate the virus at high scale to help drive vaccine research, optimisation and manufacture. In parallel with vaccine development, efforts should be initiated to develop accompanying DIVA tests.

4. Conclusion

Although ASF was first described nearly a century ago, numerous gaps remain in our understanding of its epidemiology and pathogenesis. These main gaps in ASF have been identified and prioritised throughout this article (see Table 3 at the end of the publication). Virulence genes and genes related to host protection and immune evasion are largely unknown. Likewise, the role of multigene families is antigenic variability and evasion of immune response is uncertain. At the same time, factors in the host that determine viral persistence and infection outcomes remain to be elucidated, and interactions between ASFV and wild African suids, which are tolerant to ASFV infection, need to be clarified. Such studies will provide a more complete understanding of ASF pathogenesis and potential host protection. Moreover, biological and molecular characterisation of circulating isolates in Europe and Africa are needed to identify and understand the evolution of existing isolates, especially in endemic regions.

ASF is known for its complex epidemiology, involving different transmission models via domestic and wild swine populations as well as vectors. The specific role of different hosts, vectors and environmental factors in disease propagation need to be clarified for the different epidemiological scenarios. For example, the Northern European scenario, in which infected wild boar drive disease transmission, spread and maintenance, needs to be investigated further. Gaps in sanitary control of wild boar populations make ASF control difficult. Disease modelling technologies including wild boar, human activities and vector data are needed to implement control actions based on risk. In addition, reassessing routes of introduction and transmission to identify regions most at risk and raising awareness among hunters, farmers and veterinarians should be priorities for ASF control. Advances in non-invasive sampling are required in order to facilitate surveillance in affected areas, and current and future tests need to be optimised, harmonised and validated for non-invasive matrices. The availability of a commercial confirmatory serological test and cell lines for replacing primary cell cultures are priorities for future work. Ultimately, ASF prevention and control could benefit tremendously from an ASFV vaccine, but despite some advances, a safe, effective vaccine is still lacking.

Table 1: African swine fever recommended diagnostic tests.

Detection	Activity	ASF-infected area	ASF-free area	References
Virus	Surveillance	PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits[a]) Antigen detection commercial kit[b]	PCR (Taqman probe, UPL probe or conventional and commercials kits[a]) Antigen detection commercial kit[b]	(145-147)
	Suspicion	PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits[a]) Pen-side test (useful in field)	PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits[a]) Pen-side test (useful in field) Direct immunofluorescence (acute forms)	(145-148)
	Outbreak	PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits[a])	PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits[a]) Virus isolation-Haemadsorption test	(141, 145-147)
Antibody	Surveillance	ELISA (OIE, commercial kits[c]) Immunoblotting, Immunofluorescence and Immunoperoxidase (confirmation/ tissue analysis)	ELISA (OIE, commercial kits[c]) Immunoperoxidase, Immunofluorescence and Immunoblotting (confirmation)	(64, 149-151)
	Suspicion	ELISA (OIE, commercial kits[c]) Pen-side test (useful in field) Immunoblotting, Immunofluorescence and Immunoperoxidase (confirmation/ tissue analysis)	ELISA (OIE, commercial kits[c]) Pen-side test (useful in field) Immunoperoxidase Immunofluorescence and Immunoblotting (confirmation)	(64, 149-151)
	Outbreak	ELISA (OIE, commercial kits[c]) Pen-side test (useful in field) Immunoperoxidase, Immunofluorescence and Immunoblotting (confirmation/tissue analysis)	ELISA (OIE, commercial kits[c]) Pen-side test (useful in field) Immunoperoxidase, Immunofluorescence and Immunoblotting (confirmation)	(64, 149-151)

[a] PCR Commercial Kits currently validated: INgene q PPA, INGENASA. 11.PPA.K.5TX/Q; Tetracore TC-9017-064; Virotype ASFV PCR Kit, QIAGEN; LSI VetMAX™ Thermo Fisher Scientific.

[b] Antigen ELISA INGEZIM PPA K2 (INGENASA) and Ag Penside tests useful for field: (INGENASA).

[c] Commercial ELISA tests for antibody detection: INGEZIM PPA COMPAC K3 (INGENASA); ID Screen®, ID-VET; SVANOVIR® ASFV-Ab: SVANOVIR® and Penside test: Ab PPA-CROM (INGENASA).

Table 2: General approaches to develop vaccine candidates for African swine fever.

Vaccine type candidate	Protection	Side effects/ residual virulence after challenge	References
Live attenuated candidates based on passages in bone marrow cells	Partial and/or full protection	Yes	(100)
Inactivated virus	No	Not applicable	(152-155)
Recombinant proteins/peptides	No, or delay in the onset of the disease	Not applicable	(106, 108, 156-158)
DNA vaccine candidates	No, or delay in the onset of the disease	Not applicable	(156, 158-160)
Viral vectored vaccines	Ongoing	Not applicable	(161)
Naturally attenuated virus isolates	Partial and/or full protection. Protection against homologous and heterologous virus challenge	Yes	(83, 92, 99, 162, 163)
Live attenuated candidates based on deletion mutants from virulent ASF virus isolates	Partial and/or full protection against homologous virus and heterologous virus challenge	Yes	(72, 74, 164)
Live attenuated candidates based on deletion mutants from attenuated virus isolates	Full against homologous virus and partial protection against heterologous virus challenge	Yes	(79)

Table 3: Prioritised gaps for African swine fever.

Field	Gap	Prioritisation
ASFV	Role of multigene families in antigenic variability and evasion of immune response	H
	Genes related to host protection	H
	Biological and molecular characterisation of currently circulating isolates in Europe and Africa	H
	Understanding the evolution of circulating isolates (especially in endemic regions)	M
ASF in natural hosts	Host factors that determine the different clinical forms (susceptibility, tolerance and resistance)	H
	Geographical distribution of *Ornithodoros* ticks	L
	Role of *Ornithodoros* ticks in the current scenarios	L
ASF clinical forms	Studies on subclinical and unapparent animals to assess their role in transmitting and maintaining the disease	H
	Genome markers related to the virulence of ASFV isolates	M
ASF epidemiology	Shedding kinetic parameters	L
	Role of host, vector and environment under different conditions of each epidemiological scenarios	M
	Role of wild boars in transmission, maintenance and dissemination in eastern Europe	H
	The role of reservoirs in the transmission of the disease	M
	Studies on neighbourhood transmission in densely populated areas	M
	Transmission studies between pigs and wild boars	H
	Seasonal cycle of *Ornithodoros* ticks linked to climate	L
Socio-economic impact	Risk assessment to identify routes of introduction–transmission and regions most at risk	M
	Disease modelling technologies to implement control actions based on risk	H
	Cost-benefit studies to evaluate efforts made to control ASF	M
Immune response	Role of viral proteins in inducing effective immune mechanisms in surviving animals	H
	Identify interactions between wild African suids (asymptomatic infections) and ASFV	M
	Mechanisms of viral persistence in the host	H
	Interactions between ASFV, macrophages and other cells in host	M
Prevention, detection and control	Raise awareness among hunters, farmers and veterinarians	H
	Take measures to ensure farm location far from suitable wild boar areas. In affected areas promote confinement.	L
	Early warning systems, contingency plans, and control measures ready	H
	Implemented surveillance activities based on the risk of potential exposure, introduction and spread	M
Diagnosis and vaccines	Non-invasive sampling methodologies for wild boars	H
	Optimization, harmonization and validation of tests using non-invasive samples for domestic pigs and wild boar	H
	Commercial confirmatory serological tests	H
	Cell lines for replacing primary cell cultures	H
	Standardisation and validation of techniques for *Ornithodoros* ticks	L
	Update a diagnosis manual for ASF	H
	Research on vaccine candidates: new types and strategies.	M
	Studies on existing live attenuated vaccine candidates need further investigation on side effects, virus persistence, doses and other parameters of safety.	H
	Knowledge on mechanisms to evade immune response, induce protection and pathogenicity	H

H: High, Medium: M, Low: L.

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DISCONTOOL SUPPLEMENT

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Gaps in African swine fever: Analysis and priorities

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Summary

African swine fever (ASF) causes greater sanitary, social and economic impacts on swine herds than many other swine diseases. Although ASF was first described in 1921 and it has affected more than fifty countries in Africa, Europe and South America, several key issues about its pathogenesis, immune evasion and epidemiology remain uncertain. This article reviews the main characteristics of the causative virus, its molecular epidemiology, natural hosts, clinical features, epidemiology and control worldwide. It also identifies and prioritizes gaps in ASF from a horizontal point of view encompassing fields including molecular biology, epidemiology, prevention, diagnosis and vaccine development. The purpose of this review is to promote ASF research and enhance its control.

KEYWORDS

African swine fever, diagnostics, disease control, emerging diseases, gaps analysis, potential vaccines, priorities

1 | INTRODUCTION

African swine fever (ASF) is an infectious disease of swine, notifiable to the World Organisation of Animal Health (OIE). It causes greater sanitary, social and economic impacts than many other animal diseases because the occurrence of ASF is sufficient to trigger regional, national and international trade restrictions. ASF affects domestic and wild suids of all breeds and ages. Fortunately, it is not a zoonotic disease, which limits its impact on public health. Currently, no vaccine or treatment against ASF is available, and control strategies depend mainly on early disease detection through rapid field suspicion and laboratory diagnosis followed by implementation of strict sanitary measures (Gallardo, Nieto, et al., 2015; Sánchez-Vizcaíno & Arias, 2012). A reliable laboratory diagnosis is performed using virus and antibody detection techniques that allow the identification of infected animals, including survivors as potential virus carriers.

ASF is present in Africa and Europe, where it shows different epidemiological patterns and scenarios. On the African continent, the disease has been recognized in 28 countries (World Organisation for Animal Health, Wahid Database (OIE WAHID) Interface, 2017); and in Europe, ASF has been endemic on the Italian island of Sardinia since 1978. In 2007, ASF reached eastern Europe from East Africa. Since then, ASF has spread from the Caucasus region (Georgia, Azerbaijan and Armenia) to the Russian Federation (2007), Ukraine (2012), Belarus (2013), Estonia (2014), Latvia (2014), Lithuania (2014), Poland (2014) and Moldova (2016), where it has affected domestic pigs and wild boar (Bosch, Iglesias, Muñoz, & De la Torre, 2016; EFSA, 2015; Gallardo, Nieto, et al., 2015; Sánchez-Vizcaíno, Mur, & Martínez-López, 2013; World Organisation for Animal Health, Wahid Database (OIE WAHID) Interface, 2017). The disease is currently endemic in some parts of eastern Europe (Gogin, Gerasimov, Malogolovkin, & Kolbasov, 2013). Transboundary movement of this disease has been historically related to the single introduction of contaminated pork or pork products used to pig feed (Sánchez-Vizcaíno & Arias, 2012). In contrast, current ASF movements in Europe, especially in the European Union affected states, are driven by the movement of free-ranging infected wild boar, which can move the disease through natural corridors (Bosch, Rodríguez, et al., 2016; De la Torre et al., 2015; Gallardo et al., 2014). Nevertheless, other routes of ASF introduction and spread have been reported and are present in eastern Europe such as the illegal movement of infected pigs or the use of contaminated pork products for feeding pigs (Gogin et al., 2013; Oganesyan et al., 2013; Vergne, Gogin, & Pfeiffer, 2015).

The aims of this review are to provide an overview of current ASF epidemiology and control strategies, point out important gaps in disease control and suggest priorities for filling those gaps through ASF research and policy (Table 3).

2 | METHODS

Firstly, a comprehensive review of the published scientific literature was conducted to identify gaps and priorities regarding ASF. Then, gaps and priorities were classified based on expert opinion. The group of experts belonged to the OIE-ASF Reference Laboratory, the FAO-ASF Reference Centre and the European Union ASF Reference Laboratory (five experts) with proved expertise and experience on ASF. Experts were invited to rank each gap and priority as high, medium and low importance. Finally, mode value was used for the final score of each gap.

3 | ASF VIRUS CHARACTERISTICS

ASF virus (ASFV) is a complex, large, icosahedral multi-enveloped DNA virus, classified as the only member of the family Asfarviridae, genus Asfivirus (Dixon et al., 2005). ASFV genome encodes a significant number of viral enzymes, viral transcription factors and immune homologues among others. The viral particle contains 54 structural proteins. Nearly, a hundred proteins have been identified on the target cells during ASFV infection, particularly in pig macrophages (Dixon, Chapman, Netherton, & Upton, 2013). Both, structural and infection-related proteins can regulate, inhibit and modulate essential and non-essential mechanisms affecting virus replication, virus particle production and apoptosis. Some of them are based on the inhibition of host transcription factors, the interferon response or several immune cell subsets, to evade host immune system (Reis, Netherton, & Dixon, 2017; Sánchez, Quintas, Nogal, Castelló, & Revilla, 2013).

ASFV genome consists of a conserved central region of about 125 kb and two variable ends encoding five multigene families (MGFs); these variable ends account for the variable size of the genome (170–193 kb) among virus isolates (Dixon et al., 2013; Salas & Andrés, 2013). Several MGFs help determine virulence of isolates as well as viral replication in soft ticks. Concretely, deletion of certain MGFs has given rise to attenuated phenotype isolates that have been shown to induce protection against virulent challenges (O'Donnell et al., 2016). Deletion of MGFs genes also reduced viral replication and generalization of infection in infected ticks (Burrage, Lu, Neilan, Rock, & Zsak, 2004). Whether MGFs also help the virus generate antigenic variability and thereby evade the immune response remains uncertain. Likewise, which genes in MGFs may be related to host protection has not been fully identified.

ASFV classification is based on molecular epidemiology, which has proven useful for tracking virus spread. The current approach is based at a first step on partial sequencing of the B646L gene encoding the p72 protein. This can differentiate up to 23 genotypes (Achenbach et al., 2016; Boshoff, Bastos, Gerber, & Vosloo, 2007), as recently, a new genotype XXIII was described in Ethiopia (Achenbach et al., 2016), suggesting that more ASFV genotypes could remain to be discovered in Africa. Thus further biological and molecular characterization of isolates currently circulating within Africa and Europe should be a priority. Closely related ASFV isolates can be distinguished through sequence analysis of tandem repeats in the central variable region within the B602L gene (Gallardo et al., 2009) or the intergenic region between the I73R and I329L genes at the right end of the genome (Gallardo et al., 2014). Several other gene regions, such as the E183L encoding p54 protein, the CP204L encoding p30 protein and the protein encoded by the EP402R gene (CD2v), have been proved as useful tools to analyse ASFVs from different locations to track the virus spread (Gallardo et al., 2009; Gallardo et al., 2011; Sanna et al., 2017). The genetic characterization approach is not related to biological properties. More research would be needed to identify new genetic markers for ASFV, including those involved in the evolution of circulating ASFV isolates, especially in endemic regions. In addition, new genetic markers intricate in virulence would be very useful for control strategies. The genetic characterization of MGF virulence genes to cluster/group ASFV isolates based on virulence factors could be a potential interesting area of research.

4 | ASF IN NATURAL HOSTS

Suids are the animal hosts naturally infected by ASFV: domestic pigs, European wild boar and feral pigs of all ages and breeds are susceptible to infection. These animals, when infected, may show a variety of clinical presentations: peracute, acute, subacute, chronic and subclinical (Gallardo, Soler, Nieto, et al., 2015; Mebus, McVicar, & Dardiri, 1983; Pan & Hess, 1984). In contrast, wild African suids such as warthogs (*Phacochoerus aethiopicus*), bush pigs (*Potamochoerus porcus*) and giant forest hogs (*Hylochoerus meinertzhageni*) develop asymptomatic infections, allowing them to act as true ASFV reservoirs in Africa (Detray, 1957; Penrith & Vosloo, 2009;). Several studies in East Africa have revealed a complex epidemiological situation in which local breeds of domestic pig seem to show greater tolerance to ASFV that favours endemicity and spread of the disease (Atuhaire et al., 2013; Gallardo, De la Torre, et al., 2015; Gallardo, Nieto, et al., 2012; Uttenthal et al., 2013). In addition, virus evolution towards moderate virulent forms could be also contributing for the presence of asymptomatic pigs acting as virus carriers (Gallardo et al., 2016). The molecular factors in wild African suids determining whether ASFV infection will be asymptomatic remain unknown. The host factors that determine clinical outcomes of infection, susceptibility, resistance (the ability to limit the pathogen load) and tolerance (the ability to limit the impact of the pathogen on host health) to ASFV infection should be the priorities for future research.

ASFV also replicates in the soft ticks of the *Ornithodoros* genus. *Ornithodoros moubata* complex in East and South Africa and *O.*

erraticus on the Iberian Peninsula are biological vectors and reservoirs of ASFV (Jori et al., 2013; Oleaga-Pérez, Pérez-Sánchez, & Encinas-Grandes, 1990; Pérez-Sánchez, Astigarraga, Oleaga-Pérez, & Encinas-Grandes, 1994). *Ornithodoros moubata* shows trans-stadial, transovarial and sexual ASFV transmission (Plowright, Perry, & Peirce, 1970), while only trans-stadial transmission has been observed with *O. erraticus* (EFSA, 2010; Plowright, Thomson, & Neser, 1994). In the absence of viraemic hosts, *Ornithodoros* ticks can allow ASFV infection to persist for more than 5 years (Boinas, Wilson, Hutchings, Martins, & Dixon, 2011). In West Africa, ASFV has been detected in *O. sonrai* ticks, yet they seem to play a limited role in ASF epidemiology (Vial et al., 2007). So far, all *Ornithodoros* species experimentally tested seem able to transmit ASFV, including *O. moubata*, *O. porcinus*, *O. erraticus*, *O. coriaceus*. *Ornithodoros turicata* and *O. savignyi* (EFSA, 2010; Groocock, Hess, & Gladney, 1980; Hess, Endris, Haslett, Monahan, & McCoy, 1987; Jori et al., 2013; Mellor & Wilkinson, 1985). Other *Ornithodoros* species have been already identified along different ecological settings from the United States and Latin America (Donaldson et al., 2016). The detailed geographical distribution of *Ornithodoros* ticks is not well understood, making it difficult to assess the potential role of soft ticks in current ASF scenarios. The role of soft ticks in virus transmission, persistence and dissemination is not yet well understood and needs to be clarified, especially in Europe.

5 | CLINICAL FORMS OF ASF

The ASF incubation period usually ranges from 3 to 19 days. ASF is not associated with pathognomonic lesions, so clinical signs may be similar to other haemorrhagic diseases such as classical swine fever, salmonellosis or erysipelas. The clinical form of ASF depends on isolate virulence, host species and breed, and routes of infection (Guinat et al., 2016; Sánchez-Cordón et al., 2017; Sánchez-Vizcaíno, Mur, Gómez-Villamandos, & Carrasco, 2015). Identifying virulence factors and pathogenesis mechanisms would improve our understanding of different clinical forms of ASF, facilitating a better diagnosis recognition and potentially early detection on farms and in the field. For example, genomic markers related to ASFV virulence need to be identified and fully characterized that would allow to design better and more appropriate diagnostic strategies, according to the clinical symptoms to be expected in the infected animals, thereby improving surveillance and control programs.

Highly virulent isolates usually induce acute ASF, which in naïve animals is associated with mortality as high as 100% within 4-9 days post-infection. Acute ASF is characterized by high fever followed by moderate anorexia, lethargy, weakness, decubitus and erythema. Congestive-haemorrhagic signs and functional failures of internal organs can be observed. Internal lesions are usually related to hyperaemic splenomegaly and haemorrhages in a large number of organs and tissues (Sánchez-Vizcaíno et al., 2015).

Moderately virulent isolates may produce acute and subacute forms (Gómez-Villamandos, Bautista, Sánchez-Cordón, & Carrasco, 2013; Pan & Hess, 1984). These clinical presentations have been reported in endemic areas such as eastern Europe, Sardinia or the Iberian Peninsula (Mur, Atzeni, et al., 2016; Mur, Igolkin, et al., 2016; Sánchez-Botija, 1982). Subacute ASF is associated with fluctuating temperature for 2 or 3 weeks and clinical signs similar to those of the acute form but less severe (Mebus & Dardiri, 1979; Mebus et al., 1983; Sánchez-Vizcaíno et al., 2015). Mortality rates range from 30% to 70%, usually after 20 days post-infection. Other isolates can induce subclinical or even unapparent forms, resulting in intermittent viraemia, seroconversion and lower mortality rates (Gallardo, Soler, Nieto, et al., 2015; Leitão et al., 2001; Mebus & Dardiri, 1980; Mebus et al., 1983; Sánchez-Cordón et al., 2017). Unapparent ASF is usually reported in endemic scenarios, in which clinical signs are mild or even absent. Unapparent and recovered pigs should be identified through detection of specific antibodies and ASFV antigens or genome. Such animals should be studied as potential carriers to detect changes in the virulence of circulating isolates and assess the role of those animals in transmitting and maintaining the disease. Animal experiments using ASFV isolates from recovered animals would allow a better knowledge about the ability of these virus isolates to be transmitted by different routes, its presence and persistence in excretions and tissues, a deeper characterization of the carrier state or the potential clinical activation of unapparent infections. Chronic forms of ASF have been reported mainly in Spain (Sánchez-Botija, 1982), Portugal (Petisca, 1965) and Latin American countries (Mebus & Dardiri, 1979) infected with isolates coming from the Iberian Peninsula. Infected animals show necrotic skin lesions as well as respiratory symptoms (Gallardo, Soler, Nieto, et al., 2015; Leitão et al., 2001; Petisca, 1965). These lesions have been also observed in two recent experimental infections with moderately virulent ASFV isolates from eastern Europe (Gallardo et al., 2016; Nurmoja et al., 2017).

6 | IMMUNE RESPONSE TO INFECTION

During ASFV infection, the protective immune response includes both cellular and humoral immunity (Takamatsu et al., 2013). Pigs that do not die within the first days of infection produce high levels of specific antibodies against ASFV, which are detectable for long periods of time but that are not fully neutralizing (Sánchez-Vizcaíno & Arias, 2012). Nevertheless, some protection related to antibody-mediated immunity is observed. Passive transfer of sera from ASFV-infected and recovered pigs partially protected pigs against parental homologous ASFV challenge infection and the potential fatal consequences of infection by delaying the onset of the ASF clinical signs and reducing the levels of viraemia (Onisk et al., 1994; Ruiz-Gonzalvo, Rodríguez, & Escribano, 1996; Schlafer, Mebus, & McVicar, 1984). The antibodies may also protect the host through antibody-dependent cytotoxicity (Wardley, Norley, Wilkinson, & Williams, 1985). So far, at least fifty viral proteins have been identified as immunogenic (Gallardo, Blanco, Rodríguez, Carrascosa, & Sánchez-Vizcaíno, 2006; Neilan et al., 2004), but how these proteins elicit an effective immune response in surviving animals remains unknown.

Wild African suids show tolerance to ASFV via unknown mechanisms.

Understanding how ASFV can persist in hosts is needed. Such persistence could involve immune cells targeted by the virus for replication, particularly macrophages (Mínguez, Rueda, Domínguez, & Sánchez-Vizcaíno, 1988). A recent study conducted by Franzoni et al. (2017) showed that virulent isolates have evolved mechanisms to counteract activated macrophage response promoting viral survival, dissemination in the host and pathogenesis. More detailed characterization of interactions between ASFV and macrophages and other cells in the host may provide new insights into how to induce a protective immune response. Such work should also examine the potential roles of MGFs.

7 | ASF EPIDEMIOLOGY

ASFV can be transmitted through direct or indirect contact between infected animals, pork products or contaminated fomites (e.g., clothing, vehicles, boots) and susceptible animals. Healthy animals may be directly infected through contact with blood, secretions, faeces and excretions from infected animals. Recently, some studies have been carried to better understand ASFV shedding patterns (Davies et al., 2017; De Carvalho Ferreira, Weesendorp, Quak, Stegeman, & Loeffen, 2013; De Carvalho Ferreira et al., 2012; Guinat et al., 2014; Howey, O'Donnell, De Carvalho Ferreira, Borca, & Arzt, 2013). These studies have provided information on ASFV excretion through oropharyngeal, oral, for at least 70 days, and through nasal and rectal swabs among others, but only with regard to domestic pigs. In addition to this, these studies evaluated shedding patterns when animals were infected through three routes of direct inoculation (intramuscular, intranasopharyngeal and intra-oropharyngeal) or through direct contact with inoculated animals. However, no information on ASFV shedding and kinetics after infection via consumption of contaminated pork or cannibalism is available. Therefore, a more detailed understanding of virus shedding patterns and kinetics evolving domestic pigs and wild boar is still needed.

Historically, ASF introductions into free distantly located areas have been driven by indirect transmission via animal consumption of contaminated pork or pork products (Sánchez-Vizcaíno et al., 2015). ASFV can also be transmitted through the bite of soft ticks. Contaminated vehicles are also a potential way of introduction of ASF into free areas (Sánchez-Vizcaíno et al., 2015). The resistance of ASFV to various environmental conditions favours its spread (EFSA, 2010), which can also be promoted by poor farming practices, swill feeding and slaughtering on the farm.

Overall, ASF epidemiology depends on the host (domestic pigs, wild boar, wild suids), presence of ticks and type of pig production (indoor, outdoor). So far, three transmission models have been observed in affected countries (Sánchez-Vizcaíno et al., 2015). The first and most complex model was observed in East and South Africa, where domestic pigs, wild suids and ticks cohabit. The second model was observed on the Iberian Peninsula, where wild boar, outdoor domestic pigs and ticks are involved. The third model is present in currently affected European areas, which contain infected wild boar and/or domestic pigs but no soft ticks. However, the presence of *Ornithodoros* ticks in eastern Europe cannot be completely discarded as several researchers reported the presence of these ticks between the 1930s and the 1960s (Vial, 2009). Elucidating the respective roles of host, vector and environment under the different conditions of each epidemiological scenario should be a key research priority.

ASF is present in 28 sub-Saharan African countries, where it affects domestic and wild populations (OIE WAHID, 2017). In April 2007, ASF was introduced from East Africa into the Republic of Georgia, from where it spread to Armenia, Azerbaijan and the Russian Federation (Sánchez-Vizcaíno et al., 2013). After several years of continuous outbreaks, two endemic regions in the Russian Federation are now recognized (Gogin et al., 2013). As a result of the situation in eastern Europe, ASFV was introduced into neighbouring countries such as Ukraine and Belarus, mainly by free-ranging wild boar. In January 2014, ASF cases in wild boar were reported within parts of the European Union (EU) bordering with Belarus. Since then, ASF cases in wild boar and outbreaks in domestic pigs have been reported in four EU countries: Lithuania, Poland, Estonia and Latvia. In 2016, the other European state, Moldova, became infected (World Organisation for Animal Health, Wahid Database (OIE WAHID) Interface, 2017). The current situation poses a threat to pig production and economies of affected and neighbouring countries.

The current situation in the EU and some eastern European countries shows several characteristics not observed in previous epidemics. First, multiple viral introductions through movements of infected free-ranging wild boar have taken place in the affected areas. Second, wild boar is the most severely affected host, giving it an important role in ASF spread and maintenance (Bosch, Rodríguez, et al., 2016). Third, the combination of pig farms located in areas suitable for wild boar as well as the existence of low biosecurity measures, especially on backyard farms, may have facilitated contacts between both hosts and thereby promoted ASF transmission.

These novel characteristics of the current ASF situation reflect the need for control and eradication measures that take into consideration the interactions among hosts, pathogen and environment in each epidemiological scenario. The role of wild boar in virus transmission, maintenance and dissemination in eastern Europe requires further investigation, as does the role of wild African reservoirs in disease transmission under different conditions. Although some studies referred that wild boar avoided feeding on conspecifics (animals of the same species) suffering from illness (Selva, Jedrzejewska, Jedrzejewski, & Wajrak, 2005), the presence of infected wild boar carcasses in the field has been already identified as cause of ASFV maintenance in the environment and spread due to scavenging behaviours among wild boar population (Bellini, Rutili, & Guberti, 2016; Olševskis et al., 2016). Studies are needed that better understand this fact as well as examine neighbourhood transmission in densely populated areas and transmission between pigs and wild boar. Whether soft ticks are present in eastern Europe, Sardinia and northern Europe should be determined definitively, and, if present, their role in ASF maintenance and transmission should be clarified in northern European scenarios. A better understanding of the seasonal cycle of these soft ticks, and how climate affects it, should also be a priority.

Finally, to reduce ASF spread due to human factors, communication campaigns and training courses should be organized to raise the awareness of hunters, farmers and field veterinarians.

8 | SOCIO-ECONOMIC IMPACT

ASF is not a zoonotic disease, but it has serious socio-economic impact, especially in countries that export live pigs, pork and/or products, as well as in countries where these products are important sources of protein. ASF directly affects the economies of affected countries because its notification triggers control measures ("stamping out" policies) as well as national and international trade restrictions on animals and pork products. These measures include export restrictions, control of animal movements and their products, and animal quarantine (Arias & Sánchez-Vizcaíno, 2002).

Preventive measures and early detection (including suspicion and diagnosis) are the best way to reduce or eliminate the socio-economic impact of ASF. Epidemiological and qualitative/quantitative risk assessments are needed to identify routes of introduction–transmission and regions at greatest risk (risk mapping). The results of these assessments should then be used to focus preventive measures and surveillance activities on certain areas. Disease modelling technologies, such as Be-FAST (Ivorra, Martínez-López, Sánchez-Vizcaíno, & Ramos, 2014), InterSpread (Stevenson et al., 2013), NAADSM (NAADSM Development team, 2008) DTU-DADS (Halasa et al., 2016) software or the modelling approaches developed by Barongo et al. (2016) or Vergne, Korennoy, Combelles, Gogin, and Pfeiffer (2016), among others, have been used to model animal disease and control options in different scenarios. Incorporating wild animals, vectors and human factors into these modelling algorithms should be a priority for future work.

Funding from the EU has been provided to Estonia, Latvia, Lithuania and Poland to strengthen their preparedness against ASF and to enhance protective measures, although the amount of funding is not known officially. Cost-benefit analyses based on the current EU scenario are needed to evaluate preventive costs, disease-controlling efforts made so far and optimize future control measures.

9 | PREVENTION, DETECTION AND CONTROL

Preventive measures are crucial for avoiding the introduction of infectious diseases into herds and their subsequent spread. The feasibility and efficacy of prevention and control measures depend on farm location (suitable or not for wild boar), sort of farm (confined, outdoor or backyards), type of production (for instance breeding or fattening farms), animal movements, sanitary status of animals to be replaced and farm biosecurity standards. Biosecurity can be improved by erecting physical barriers, such as internal and external fences; installing bird nets; creating quarantine facilities for animals and changing facilities for workers and visitors; running pest-control programmes; erecting sanitary enclosures; disposing safely of manure; following good farming practices; and washing and disinfecting transport vehicles (Arias & Sánchez-Vizcaíno, 2002; Bellini et al., 2016).

There is no a single recipe for preventing ASF. Success depends on many parameters in the epidemiological situation, such as whether the affected population is domestic and/or wild, and whether vectors are present. Success also depends on current legislation, economic resources and logistical aspects. Countries at higher risk should be aware of the characteristics of the isolates circulating in neighbouring areas, as well as which host populations are affected.

Farmers and farm staff need to be aware of both exotic and common infectious diseases, and they should be familiar with preventive measures that can block disease entrance. Some risk factors associated with ASF introduction are poor farming practices, poor training of farm personnel, lack of communication and awareness, lack of motivation for following regulations, poor record-keeping on the farm and no audit of biosecurity-related activities (Arias & Sánchez-Vizcaíno, 2002; Dione, Ouma, Opio, Kawuma, & Pezo, 2016; Gallardo, De la Torre, et al., 2015).

The efficacy of preventive and control measures depends on early suspicion and identification of suspected disease, early diagnosis of disease, identification of subacute/unapparent infected animals, basic biosecurity on pig holdings (fences and bird nets), identification of individual animals, updated census and animal movement records and control of soft ticks (if present) (Arias & Sánchez-Vizcaíno, 2002; Guinat et al., 2016). Preventing contact between wild boar and domestic pigs is crucial, particularly in the EU. Farms should be located far from areas suitable for wild boar, especially backyard farms and farms with poor biosecurity. Pigs in infected areas should be confined (instead of held outdoors) in order to prevent them from coming into contact with wild boar or pigs from other farms, as well as to prevent scavenging activities. Control failures may be caused by cultural practices (Mur, Atzeni, et al., 2016), trade of infected products and the taboo of throwing away food observed in some cultures (Chenais et al., 2015).

Every country should have a contingency plan and early warning system in place in the event of ASF entrance. Any delay in outbreak response and implementation of control measures can result in greater viral contamination of the environment and promote disease spread (Bellini et al., 2016). Field veterinarians and the relevant authorities should be aware of, and trained in, how to detect the various clinical forms of ASF. Highly virulent ASFV isolates are associated with more evident clinical forms and should therefore be easier to detect by passive surveillance. In contrast, passive surveillance may not be sufficient for early disease detection in the case of moderately virulent ASFV isolates or infection of wild boar or wild suids. In these cases, additional control measures should be implemented. For instance, areas with infected wild boar should be monitored through a combination of passive surveillance of dead wild boar and active surveillance in areas at highest risk. This is because discovering wild boar carcasses is not an easy task; they are usually eaten by other animals or hidden under vegetation or snow. A priority is to develop new, non-invasive methods to sample wild populations, particularly given the current situation in northern Europe.

10 | ASF DIAGNOSIS AND POTENTIAL VACCINES

So far, neither a vaccine nor treatment against ASF is available. Therefore, control strategies are based initially on early disease detection based on rapid suspicion, identification and diagnosis of suspected cases, followed by implementation of strict sanitary measures (Gallardo, De la Torre, et al., 2015; Gallardo, Nieto, et al., 2015; Sánchez-Vizcaíno & Arias, 2012).

A wide range of laboratory tests is available to detect ASFV genome, antigens or antibodies against the virus. As there is no vaccine against ASF, antibody presence is always indicative of infection. ASF infection produces long-term viraemia, and antibody response can be detected from the first week of infection for up to months or even years (Sánchez-Vizcaíno & Arias, 2012). Serological diagnosis should be performed in parallel with viral diagnosis because animals with subacute or unapparent ASF possess antibodies but may show only intermittent viraemia (Gallardo, Nieto, et al., 2015; Gallardo, Soler, Nieto, et al., 2015). Serological tests were particularly important, for example during ASF eradication on the Iberian Peninsula and in Brazil (Arias & Sánchez-Vizcaíno, 2002; De Paula Lyra, Saraiva, Hermida Lage, & Samarcos, 1986). Thus, both virus and antibody detection are crucial for full understanding of the epidemiological situation and the roles of infected animals in disease maintenance and spread. Certain ASF diagnostic tools may be more appropriate depending on whether the area is ASF-free or already affected by the disease (see Table 1). Because of the emergence of several new valuable ASF diagnostic tests in Europe over the last decade, international reference laboratories should collaborate to develop an updated diagnostic manual listing all validated tests.

TABLE 1 African swine fever recommended diagnostic tests

Detection	Activity	ASF-infected area	ASF-free area	References
Virus	Surveillance	PCR (OIE Taqman probe, UPL probe or conventional,, and commercial kits[a]) Antigen detection commercial kit[b]	PCR (Taqman probe, UPL probe or conventional and commercials kits[a]) Antigen detection commercial kit[b]	Agüero et al. (2003), Fernández-Pinero et al. (2013), King et al. (2003)
	Suspicion	PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits[a]) Pen-side test (useful in field)	PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits[a]) Pen-side test (useful in field) Direct immunofluorescence (acute forms)	Agüero et al. (2003), Bool, Ordas, and Sánchez-Botija (1969), Fernández-Pinero et al. (2013), King et al. (2003)
	Outbreak	PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits[a])	PCR (OIE Taqman probe, UPL probe or conventional, and commercial kits[a]) Virus isolation-Haemadsorption test	Agüero et al. (2003), Fernández-Pinero et al. (2013), King et al. (2003), Malmquist and Hay (1960)
Antibody	Surveillance	ELISA (OIE, commercial kits[c]) Immunoblotting, Immunofluorescence and Immunoperoxidase (confirmation/ tissue analysis)	ELISA (OIE, commercial kits[c]) Immunoperoxidase, Immunofluorescence and Immunoblotting (confirmation)	Gallardo et al. (2013), Gallardo, Nieto, et al. (2015), Pastor, Laviada, Sánchez-Vizcaíno, and Escribano (1989), Sánchez-Vizcaíno, Tabarés, Salvador, and Sánchez-Botija (1982)
	Suspicion	ELISA (OIE, commercial kits[c]) Pen-side test (useful in field) Immunoblotting, Immunofluorescence and Immunoperoxidase (confirmation/ tissue analysis)	ELISA (OIE, commercial kits[c]) Pen-side test (useful in field) Immunoperoxidase Immunofluorescence and Immunoblotting (confirmation)	Gallardo et al. (2013), Gallardo, Nieto, et al. (2015), Pastor et al. (1989), Sánchez-Vizcaíno et al. (1982)
	Outbreak	ELISA (OIE, commercial kits[c]) Pen-side test (useful in field) Immunoperoxidase, Immunofluorescence and Immunoblotting (confirmation/tissue analysis)	ELISA (OIE, commercial kits[c]) Pen-side test (useful in field) Immunoperoxidase, Immunofluorescence and Immunoblotting (confirmation)	Gallardo et al. (2013), Gallardo, Nieto, et al. (2015), Pastor et al. (1989), Sánchez-Vizcaíno et al. (1982)

[a]PCR Commercial Kits currently validated: INgene q PPA, INGENASA. 11.PPA.K.5TX/Q; Tetracore TC-9017-064; Virotype ASFV PCR Kit, QIAGEN; LSI VetMAX™ Thermo Fisher Scientific.
[b]Antigen ELISA INGEZIM PPA K2 (INGENASA) and Ag pen-side tests useful for field: (INGENASA).
[c]Commercial ELISA tests for antibody detection: INGEZIM PPA COMPAC K3 (INGENASA); ID Screen, ID-VET; SVANOVIR ASFV-Ab: SVANOVIR and pen-side tests: Ab PPA-CROM (INGENASA).

While several reliable commercial kits for viral genome, antigen and antibody detection have become available in recent years, commercial confirmatory serological tests are still lacking and should be a priority for future work. Another gap is the lack of cell lines that can replace primary cell cultures for ASFV isolation, which would help standardize isolation techniques.

Detection of ASFV in ticks can be achieved based on virus isolation or PCR (Basto et al., 2006; Oura, Edwards, & Batten, 2013). Several ELISA tests have been developed to detect swine exposed to *Ornithodoros* ticks, which presumably have antibodies against salivary glands of *O. erraticus* and/or *O. moubata* (Baranda, Pérez-Sánchez, Oleaga, Manzano, & Encinas-Grandes, 2000; Díaz-Martín, Manzano-Román, Siles-Lucas, Oleaga, & Pérez-Sánchez, 2011; Mur, Iscaro, et al., 2017). At the moment, these techniques usually involve "in-house" procedures. A priority should be to develop standardized approaches for more reliable assessment of epidemiological situations.

New technologies including lateral flow devices (pen-side tests) and portable PCR machines that allow rapid diagnosis have been recently developed (Sastre, Gallardo, et al., 2016; Sastre, Pérez, et al., 2016). A deeper validation under field conditions should be encouraged. At the same time, non-invasive sampling methods are lacking, which are especially important for ASF control in northern Europe. Samples obtained through non-invasive sampling methods such as oral fluid and faeces allow ASFV and anti-ASFV antibodies detection (Davies et al., 2017; De Carvalho Ferreira, Weesendorp, Quak, Stegeman, & Loeffen, 2014; Giménez-Lirola et al., 2016; Mur et al., 2013; Nieto-Pelegrín, Rivera-Arroyo, & Sánchez-Vizcaíno, 2015). Commercial tests based on oral fluid are already available for porcine reproductive and respiratory syndrome as well as sampling guidelines for oral fluid-based survey on grouped-housed animals (Rotolo et al., 2017). However, standardized methods for sampling and testing ASF on such matrices (oral fluid and faeces) need still to be developed and validated for domestic pig and wild swine populations.

Vaccine development remains a major gap in ASF control and eradication. Efforts to develop a vaccine for ASFV based on inactivated virus as well as viral proteins and peptides have been hindered by the genetic complexity of ASFV, virus–host interactions and technical difficulties (see Table 2). For example, inactivated and subunit virus vaccines can induce antibody responses, but these do not confer strong protection (Table 2). Live attenuated vaccines can confer protection against homologous, but not heterologous, viral challenge in surviving pigs (Detray, 1957; Malmquist, 1963; Mebus & Dardiri, 1980). Several studies have suggested the key role for the innate immunity and natural killer cells (Correia, Ventura, & Parkhouse, 2013; Leitão et al.,2001) as well as the cytotoxic activity by CD8 T-cells (Oura, Denyer, Takamatsu, & Parkhouse, 2005; Martins, Lawman, Scholl, Mebus, & Lunney, 1993; Takamatsu et al., 2013). Current vaccine development efforts and priorities include strategies to stimulate both antibody response and cytotoxic activity by T cells. Side effects, virus persistence, doses and other safety parameters are some gaps related to vaccine development that need to be filled. Improvements in the current and new vaccine candidates will require more extensive analysis of viral genes that

TABLE 2 General approaches to develop vaccine candidates for African swine fever

Vaccine type candidate	Protection	Side effects/ residual virulence after challenge	References
Live attenuated candidates based on passages in bone marrow cells	Partial and/or full protection	Yes	Petisca (1965)
Inactivated virus	No	Not applicable	Blome, Gabriel, and Beer (2014), Bommeli, Kihm, and Ehrensperger (1981), Mebus (1988), Stone and Hess (1967)
Recombinant proteins/peptides	No, or delay in the onset of the disease	Not applicable	Argilaguet et al. (2013), Burmakina et al. (2016), Neilan et al. (2004), Revilla et al. (2016), Ruiz-Gonzalvo et al. (1996)
DNA vaccine candidates	No, or delay in the onset of the disease	Not applicable	Argilaguet et al. (2011, 2012), Lacasta et al. (2014), Revilla et al. (2016)
Viral vectored vaccines	Ongoing	Not applicable	Lokhandwala et al. (2016)
Naturally attenuated virus isolates	Partial and/or full protection. Protection against homologous and heterologous virus challenge	Yes	Boinas, Hutchings, Dixon, and Wilkinson (2004), Gallardo, Soler, et al. (2012), King et al. (2011), Leitão et al. (2001), Sánchez-Cordón et al. (2016)
Live attenuated candidates based on deletion mutants from virulent ASF virus isolates	Partial and/or full protection against homologous virus and heterologous virus challenge	Yes	O'Donnell et al. (2016), Reis et al. (2016), Rodríguez (2015)
Live attenuated candidates based on deletion mutants from attenuated virus isolates	Full against homologous virus and partial protection against heterologous virus challenge	Yes	Gallardo, Soler, Carrascosa, et al. (2015)

should be deleted to build more effective deletion mutants. Another priority is to clarify the roles of specific viral genes in the infection cycle regarding immune evasion and infection control. It will also require further study of ASF pathogenesis and interferon-mediated induction. Optimized delivery systems that can induce a protective immune response are needed. Another important issue is the availability of cell lines that can propagate the virus at high scale to help drive vaccine research, optimization and manufacture. In parallel with vaccine development, efforts should be initiated to develop accompanying DIVA tests.

TABLE 3 prioritized gaps for African swine fever

Field	Gap	Prioritisation
ASFV	Role of multigene families in antigenic variability and evasion of immune response	H
	Genes related to host protection	H
	Biological and molecular characterisation of currently circulating isolates in Europe and Africa	H
	Understanding the evolution of circulating isolates (especially in endemic regions)	M
ASF in natural hosts	Host factors that determine the different clinical forms (susceptibility, tolerance and resistance)	H
	Geographical distribution of *Ornithodoros* ticks	L
	Role of *Ornithodoros* ticks in the current scenarios	L
ASF clinical forms	Studies on subclinical and unapparent animals to assess their role in transmitting and maintaining the disease	H
	Genome markers related to the virulence of ASFV isolates	M
ASF epidemiology	Shedding kinetic parameters	L
	Role of host, vector and environment under different conditions of each epidemiological scenarios	M
	Role of wild boars in transmission, maintenance and dissemination in eastern Europe	H
	The role of reservoirs in the transmission of the disease	M
	Studies on neighbourhood transmission in densely populated areas	M
	Transmission studies between pigs and wild boars	H
	Seasonal cycle of *Ornithodoros* ticks linked to climate	L
Socio-economic impact	Risk assessment to identify routes of introduction transmission and regions most at risk	M
	Disease modelling technologies to implement control actions based on risk	H
	Cost-benefit studies to evaluate efforts made to control ASF	M
Immune response	Role of viral proteins in inducing effective immune mechanisms in surviving animals	H
	Identify interactions between wild African suids (asymptomatic infections) and ASFV	M
	Mechanisms of viral persistence in the host	H
	Interactions between ASFV, macrophages and other cells in host	M
Prevention, detection and control	Raise awareness among hunters, farmers and veterinarians	H
	Take measures to ensure farm location far from suitable wild boar areas. In affected areas promote confinement.	L
	Early warning systems, contingency plans, and control measures ready	H
	Implemented surveillance activities based on the risk of potential exposure, introduction and spread	M
Diagnosis and vaccines	Non-invasive sampling methodologies for wild boars	H
	Optimization, harmonization and validation of tests using non-invasive samples for domestic pigs and wild boar	H
	Commercial confirmatory serological tests	H
	Cell lines for replacing primary cell cultures	H
	Standardisation and validation of techniques for *Ornithodoros* ticks	L
	Update a diagnosis manual for ASF	H
	Research on vaccine candidates: new types and strategies.	M
	Studies on existing live attenuated vaccine candidates need further investigation on side effects, virus persistence, doses and other parameters of safety.	H
	Knowledge on mechanisms to evade immune response, induce protection and pathogenicity	H

H, high; M, medium; L, low.

11 | CONCLUSION

Although ASF was first described nearly a century ago, numerous gaps remain in our understanding of its epidemiology and pathogenesis. These main gaps in ASF have been identified and prioritized throughout this article (see Table 3). Virulence genes and genes related to host protection and immune evasion are largely unknown. Likewise, the role of multigene families is antigenic variability, and evasion of immune response is uncertain. At the same time, factors in the host that determine viral persistence and infection outcomes remain to be elucidated, and interactions between ASFV and wild African suids, which are tolerant to ASFV infection, need to be clarified. Such studies will provide a more complete understanding of ASF pathogenesis and potential host protection. Moreover, biological and molecular characterization of circulating isolates in Europe and Africa are needed to identify and understand the evolution of existing isolates, especially in endemic regions.

ASF is known for its complex epidemiology, involving different transmission models via domestic and wild swine populations as well as vectors. The specific role of different hosts, vectors and environmental factors in disease propagation needs to be clarified for the different epidemiological scenarios. For example, the northern European scenario, in which infected wild boar drive disease transmission, spread and maintenance, needs to be investigated further. Gaps in sanitary control of wild boar populations make ASF control difficult. Disease modelling technologies including wild boar, human activities and vector data are needed to implement control actions based on risk. In addition, reassessing routes of introduction and transmission to identify regions most at risk and raising awareness among hunters, farmers and veterinarians should be the priorities for ASF control. Advances in non-invasive sampling are required in order to facilitate surveillance in affected areas, and current and future tests need to be optimized, harmonized and validated for non-invasive matrices. The availability of a commercial confirmatory serological test and cell lines for replacing primary cell cultures is the priorities for future work. Ultimately, ASF prevention and control could benefit tremendously from an ASFV vaccine, but despite some advances, a safe, effective vaccine is still lacking.

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CHAPTER 2

OBJECTIVE 2. To identify the risk factors that explain why ASF has persisted in Sardinia and where these risk factors are present. In addition, according to the obtained results, supplementary measures that could be implemented in the current eradication plan are suggested.

Main scientific publication of objective 2.

Jurado C, Fernandez-Carrion E, Mur L, Rolesu S, Laddomada A, Sanchez-Vizcaino JM. **Why is African swine fever still present in Sardinia?** Transbound Emerg Dis. 2017;65(2):557-566. DOI: 10.1111/tbed.12740.

Related scientific contributions:

Scientific publications not included in this doctoral thesis:

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RESUMEN DE LOS RESULTADOS DEL CAPÍTULO 2

La PPA está presente en la isla de Cerdeña desde 1978. Desde su entrada, han sido varios los planes implantados a fin de controlar y erradicar la enfermedad de la isla. La persistencia de la PPA en este territorio se ha atribuido tradicionalmente a la presencia de cerdos domésticos, no registrados, mantenidos en sistemas extensivos ilegales. Dichos animales, también conocidos como *"brado*", suelen ser mantenidos en condiciones extensivas, en áreas comunales y sin control veterinario. En 2015, se lanzó un nuevo programa de erradicación cofinanciado por la UE. Algunas de las novedades de este programa de control incluyeron: i) proporcionar información a los ganaderos, cazadores y la población en general; ii) combatir la presencia de *“brado”* y cerdos no registrados; iii) mejorar la bioseguridad en las granjas; y iv) fortalecer el control de las explotaciones porcinas a través de certificaciones sanitarias. Así, el establecimiento del programa de erradicación más reciente ha permitido obtener información epidemiológica más completa y precisa.

Desde el inicio de este último plan de erradicación, la Universidad Complutense de Madrid (UCM) y el Gobierno de la Región Autónoma de Cerdeña comenzaron un programa de colaboración. A través de esta colaboración, estos nuevos datos pudieron ser empleados para la realización de estudios epidemiológicos. Por ello, el objetivo 2 de esta tesis doctoral, se centró en la identificación de los factores de riesgo que favorecen la endemicidad de la PPA en Cerdeña. Para ello, se evaluaron un total de 28 variables agrupadas en seis categorías: granja, jabalí, *“brado”*, medio ambiente, movimiento y humano.

En lugar de utilizar divisiones administrativas como unidad de análisis, la isla se dividió en polígonos de forma y tamaño similares, definidos en base a la distribución de granjas de cerdo doméstico y jabalí. Se crearon un total de 360 polígonos con una superficie media de 64,65 km^2, el 62,27% de los cuales fueron positivos a la presencia de virus y/o anticuerpos.

Respecto a las variables estudiadas, nueve de ellas fueron identificadas como factores de riesgo significativos, seis de los cuales no habían sido descritos previamente para este escenario. En concreto, los tres factores de riesgo más significativos fueron el número de granjas de tamaño mediano, la presencia de animales de *“brado”* y la combinación de la densidad estimada de jabalí y la altitud. Espacialmente, estos tres factores de riesgo se ubicaron de forma simultánea en regiones ubicadas en la parte oriental y central de la isla. Curiosamente, estas áreas pertenecían a provincias donde la PPA ha permanecido de forma recurrente durante largos periodos de tiempo.

Estos resultados respaldan varias medidas de control propuestas por las autoridades sardas, como es la prohibición del *"brado"* y la necesidad de promover la profesionalización del sector mediante el aumento de la bioseguridad en las granjas o la mejora de los sistemas de registro e identificación de animales. De hecho, estudios publicados en 2019 han corroborado la importancia de los animales de *"brado"* como fuente del VPPA en la isla.

Además, se propusieron medidas de control centradas en los factores de riesgo identificados, como es el control rápido de los casos en jabalí y sus poblaciones, la reducción del número de granjas familiares, el aumento de los niveles de bioseguridad en granja y la mejora de las prácticas ganaderas junto con campañas de concienciación y sensibilización entre los ganaderos de la zona.

La implementación de medidas concretas frente a los factores de riesgo identificados podría ayudar a erradicar la PPA de Cerdeña, siempre que las medidas se puedan aplicar de manera efectiva y uniforme en toda la isla, con el apoyo de todos los miembros implicados.

Why is African swine fever still present in Sardinia?

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Summary

African swine fever (ASF) is an infectious disease of swine that has been present in Sardinia since 1978. Soon after introduction of the disease, several control and eradication programmes were established with limited success. Some researchers attributed the persistence of the disease in central and eastern areas to certain socio-economic factors, the existence of some local and traditional farming practices (*i.e.* unregistered free-ranging pigs known as brado animals) and the high density of wild boar in the region. In the past, scarcity of swine data in Sardinia complicated the evaluation and study of ASF on the island. More complete, accurate and reliable information on pig farms has become available as a result of the most recent eradication programmes. Here, we perform statistical modelling based on these data and the known distribution of domestic pig and wild boar to identify the main risk factors that have caused ASF persistence in Sardinia. Our results categorised, identified and quantified nine significant risk factors, six of which have not been previously described. The most significant factors were the number of medium-sized farms, the presence of brado animals and the combination of estimated wild boar density and mean altitude above sea level. Based on these factors, we identified regions in eastern and central

Sardinia to be at greatest risk of ASF persistence; these regions are also where the disease has traditionally been endemic. Based on these risk factors, we propose specific control measures aimed at mitigating such risks and eradicating ASF from the island.

Keywords

Control measures, epidemiology, eradication, risk factors.

1. Introduction

African swine fever (ASF) is an infectious disease of swine, notifiable to the World Organisation for Animal Health (OIE). It has been present on the Italian island of Sardinia since 1978 (48). In March of that year, ASF virus (ASFV) was introduced into southern Sardinia, most likely from the Iberian Peninsula. This introduction presumably occurred through ASFV-contaminated waste, containing raw pork or pork products that was subsequently used to feed pigs (51). ASF affects swine of all ages and breeds, although clinical forms can vary depending on the isolate, dose, route of infection and affected host (13). The disease continues to affect domestic pigs as well as wild boar in Sardinia (23, 48). *Ornithodoros* ticks, a well-known ASFV vector, have not been found so far on the island (21, 50), even though they have been found in other Mediterranean areas such as Spain and Portugal (20, 85).

Early after introduction of the disease on the island, several control and eradication programmes have been established with limited success. As a result, periods of relative stability (1995-2004, 2006-2011) have alternated with epidemic waves (2004-2005, 2013-2014). In 1982, four years after ASF introduction, the first eradication programme began with funding from the European Economic Community (EEC) and the Italian government. This plan aimed to enhance active surveillance and effective control of outbreaks following traditional stamping out policies in order to optimise early detection of the disease. As a result of these efforts, ASF has been eradicated from southern Sardinia, but it has remained endemic in central and eastern regions of Barbagia, Baronia and Ogliastra (165). Some researchers have attributed the persistence of the disease in these areas to certain socio-economic factors, the high density of wild boar in the region, and the traditional farming practice of grazing free-range herds (brado animals) on communal pastures without any veterinary controls (23, 51, 166).

In the 1990s, the EEC provided the island with further funding aimed at eliminating the disease. Efforts seemed to bring it under control from 1995 onwards, as numbers of notifications decreased. However, between 2004 and 2005, ASF outbreaks occurred in some previously unaffected territories of the island, such as the western province of Carbonia Iglesias, and the number of notifications increased from 11 in 2003 to 243 in 2004 and 201 in 2005 (48). Additional control measures approved by the European Commission (167) reduced the numbers of outbreaks from 2006 to mid-2011, after which a steady number of notifications were reported, some of them outside the traditional endemic region. Since then, several eradication programmes have been launched in Sardinia (168-170) focusing on promoting farm professionalism, increasing farm biosecurity, and strengthening veterinary monitoring and reporting by pig farms. These measures also prohibited brado practices, which earlier studies had singled out as the main limitation to ASF eradication (23, 51, 166).

The current eradication programme [regional programme cofounded by the European Union in 2015 (171)], implemented from 2015, aims to eradicate ASF definitively from the island. It stipulates measures aimed at i) providing information to farmers, hunters and the general population; ii) fighting the presence of brado and unregistered pigs; iii) improving biosecurity on farms; and iv) strengthening the control on pig farms through sanitary status qualifications (168).

Controlling and ultimately eradicating ASF from the island is complex for several reasons, including the co-existence of several animal populations (registered domestic pigs, unregistered domestic pigs, brado animals and wild boar) and socio-economic factors, as several epidemiological studies have pointed out (48, 166, 172, 173). These studies as well as the Italian National Animal Husbandry Statistics Registry reveal a scarcity or even total absence of pig data essential for epidemiological analyses, which has complicated ASF evaluation in Sardinia.

Efforts during implementation of the most recent eradication programmes on the island have yielded more complete, accurate and reliable information on pig farms. Now, through a collaboration between the Government of the Autonomous Region of Sardinia and Complutense University of Madrid (UCM), these data can be used for the first time to perform detailed epidemiological analyses.

The present study, then, draws on relatively complete data from 2010 to 2016 in order to identify the risk factors most likely to explain the persistence of ASF in Sardinia as well as regions where such risk factors are present. Moreover, on the basis of these results, we propose supplementary measures that may be implemented as part of the current eradication plan in order to mitigate risk factors and contribute to the final eradication of ASF from the island.

2. Materials and methods

2.1. Data sources

Epidemiological data from 2010 to 2016 were provided by the Regional Veterinary Epidemiological Observatory-Istituto Zooprofilattico Sperimentale della Sardegna (EOVR-IZS). This data included information covering the period from 2010 to July 2015 related to ASF occurrence including notified cases on pig farms as well as serology and virology laboratory results. The data also included information on ASF-susceptible populations on the island, including wild boar and domestic pigs (registered farms, census on pig farms, registered incoming and outgoing movements and sacrifices for self-consumption). In addition, data covering the period from 2012 to December 2016 included information on brado pig sightings, defined as domestic pigs not registered or counted in censuses but found free-ranging on pastures or as carcasses in the field. Altitude data were obtained from the Regional Geographical Service (174).

2.2. Delineation of the study area by tessellation

Traditionally, epidemiological studies of animal diseases have relied on political-administrative units such as municipalities and provinces (175), but such units do not accurately reflect environmental, ecological or biological realities. Epidemiological characteristics such as surface terrain, ecosystems, livestock density and wildlife populations can vary substantially within the same administrative unit, which would be masked in an analysis across units, leading to an incomplete or inaccurate model. In order to reduce the risk of such ecological bias, the spatial units of analysis in the present work were based not on administrative units but on tiles of similar shape and size defined according to the distribution of ASF-susceptible domestic pig farms and wild boar.

The wild boar distribution density map provided by EOVR-IZS and based on Rolesu *et al.* (176) was transformed into a raster layer of 100 x 100 m through spatial kernel density estimation in ArcMap 10.3 (ESRI®). Another raster layer of 100 x 100 m showing the distribution of pig farms was generated by applying a point density function (radius, 2 km) to EOVR-IZS data. Local maxima points in both layers were computed using a focal statistic function with radius 2.5 km. This radius was selected to obtain a number of polygons similar to the amount of municipalities, 360 vs 376. Finally, tessellation was performed using Thiessen polygons to obtain Voronoi polygons of similar size strategically centred at local maxima of wild boar and domestic pig populations. These polygons served as the spatial unit in all subsequent analyses.

2.3. Risk factor selection

A list of potential risk factors associated with ASF persistence on the island was generated based on the literature (23, 48, 51, 166, 173, 176, 177), experts opinion sessions at more than 15 meetings between specialists from UCM, EOVR-IZS and the Regione della Sardegna authorities between 2014-2016, and discussions with private-sector and government veterinarians from Nuoro and Lanusei in April 2015. A final list of 28 candidate variables was generated and grouped into six risk categories (farm, movements, wild boar, brado, environment and humans) for which data were available at the Voronoi polygon level (see Table 1 at the end of the publication).

Data in the farm risk category included information related to swine census and production characteristics that could be relevant to risk of spread of infectious diseases.

Based on census data, farms were categorised as small (< 5 animals), medium (5-30 animals) and large (> 30 animals). From 2010 onwards, pig premises have been classified according to Sardinian regulations as breeding, fattening or family farms (165, 168-170, 178). Family farms have a maximum of 4 animals (not breeders), and animal movements to other premises are forbidden. Family farms failing to meet at least one these conditions during the study period were re-classified as fattening farms. Breeding farms were sub-categorised as having an open or closed cycle, depending on whether breeders could be shared with, or sold to, other farms. Farms that were registered as having an open and closed cycle at the same time were categorised in the present study as open in accord with the principle of maximum risk.

Farms were classified according to management practices as intensive/confined or semi-extensive, with limited access of animals to outdoor terrain. Farms that reported both types of management were classified as semi-extensive in accord with the principle of maximum risk.

The following risk variables were included in the animal movements risk category: i) number of farms reporting incoming movements, ii) number of farms reporting movements to slaughterhouses, iii) number of farms reporting movements for self-consumption, iv) number of movements reported between farms, v) number of recorded shipments to slaughterhouses, and vi) number of self-consumption slaughters reported on each farm.

The wild boar risk category was analysed on the basis of the wild boar density layer provided by EOVR-IZS. The *"brado"* risk category included information on sightings of free-ranging pigs and domestic pig carcasses reported by forestry officials since 2012. The mean altitude per polygon was included within the environment risk category.

Finally, a human risk category was included to assess the impact of poor farming practices. These practices included i) non-compliance with regulations during the annual census and the presence of non-professionalised premises, such as ii) farms with a census of ≤ 4 animals, iii) farms with multiple pens and mixed systems (simultaneously open and closed cycles and/or intensive and semi-extensive management practices) and iv) farms reporting movements to themselves.

In order to be included in models, variables were assigned binary values of 0 or 1 if their actual value was, respectively, above or equal the median or no greater than the median based on Voronoi polygons. The exception was the *"brado"* risk category, for which the variable was assigned a value of 1 if any individual or carcass was reported, and 0 otherwise.

2.4. Statistical models

The significance of the six risk categories outlined in section 2.3 was assessed using negative binomial multivariable regression. The response variable in this model was the total number of ASF-positive farms in each Voronoi polygon (either by presence of antibodies and/or virus). Considering that no vaccine is available, the presence of antibodies against ASFV always means previous contact with the virus. Therefore, farms were classified as ASF-positive in the present study if any animals on that farm tested positive to ASFV (by PCR) and/or antibody presence (screening by ELISA and confirming by Western blotting) during any routine checks, such as during surveillance and control campaigns, before shipment to slaughterhouses or when slaughtered for self-consumption. A total of 862 positive records reported between 2010 and July-2015 were included in our analyses.

Modeling was performed using the MASS package (179) in R software (180). Variables were added to the model according to a stepwise forward selection procedure; highly correlated variables were not included in the model simultaneously in order to avoid multicollinearity effects. Best-fit models were selected based on the lowest Akaike's Information Criterion (AIC) (181) when all included variables showed significance at 95% ($p < 0.05$). The best-fit model provided the final set of risk factors, for which the impact on ASF persistence was assessed using median regression coefficients and 95% confidence intervals (95% CI).

2.5. Risk factor distribution map

For visualising purposes, the modelling results were displayed by calculating a final risk factor score per Voronoi polygon. This final score was simply the sum of each risk regression coefficient computed by the best-fit model. Scores were mapped using ArcMap 10.3 (ESRI®) and Jenks' natural break classification method, in which a cut-off value was calculated for each of the five risk categories (from 1 to 5) (182).

3. Results

3.1. Delineation of the study area by tessellation

Voronoi tessellation divided Sardinia into 360 polygons (Figure 1) which had an average surface of 64.65 km^2 and 62.27% of which were ASF-positive based on the presence of virus and/or antibody (Figure 2).

Figure 1: (A) Sardinia depicted with municipalities boundaries and B) after tessellation into Voronoi polygons. Superimposed on the polygon representation are (C) farm density in blue and (D) wild boar density in red. Black dots indicate local maxima of wild boar and domestic pig density.

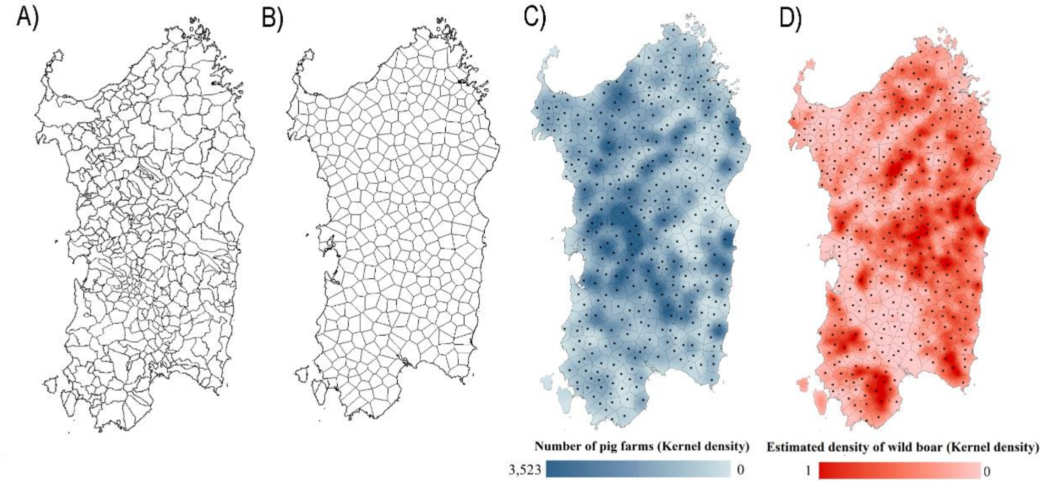
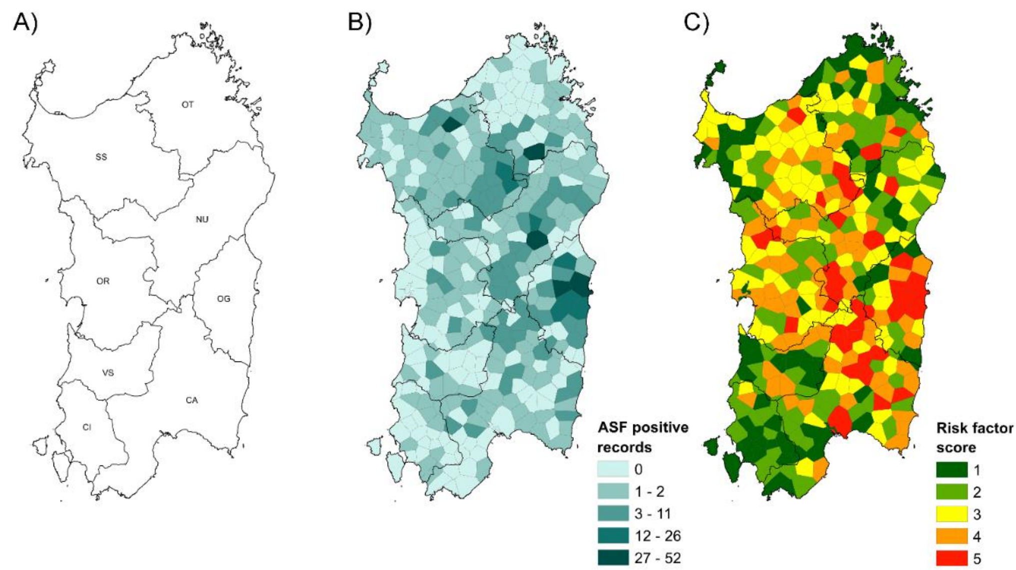


Figure 2: **(**A) Sardinia divided into provinces and (B-C) Voronoi polygons. Also shown are (B) the number of African swine fever (ASF)-positive farms based on positive records (PCR, Western blot or ELISA tests) per polygon and (C) final risk scores per polygon were calculated based on data from 2010 to 2016. Jenks' natural breaks algorithm was used to calculate a cut-off value for each of the five risk categories. CA, Cagliari; CI, Carbonia Iglesias; NU, Nuoro; OG, Ogliastra; OR, Oristano; OT, Oblia Tempio; SS, Sassari; VS, Medio Campidano.



3.2. Statistical models

The best-fit model (AIC = 1,217) identified the interaction between estimated wild boar density and mean altitude above sea level as significantly associated with ASF. Also associated with ASF in the model were eight individual variables (Table 2, shown at the end of the publication): number of medium-size farms, presence of *"brado"* animals, number of family farms, number of farms reporting outgoing movements, number of farms reporting self-consumption activities; the number of not-censused farms and number of open-cycle farms and number of semi-extensive farms. Main effects namely estimated wild boar density and altitude where also included in the model together with its interaction (wild boar x altitude) and the rest of significant variables. In that case, the main effects were no significant plus obtained regression coefficients were very similar to the ones presented here. However, the AIC value for that model was higher than the obtained for the best-fit model (AIC = 1,220.7). Therefore, the selected model identified eight individual variables and one interaction as risk factors significantly related to ASF presence (183).

The number of medium farms was the most significant risk factor (1.04, 95% CI 0.74-1.33), and it correlated with number of farms (coefficient, 0.742). In other words, areas with a high density of medium-sized farms were at greater risk of being infected by ASF than areas with a low density of such farms. Estimated wild boar density and altitude above sea level on their own were significant in the model (data not shown), while their combination was one of the most significant risk factors for ASF persistence (0.88, 95% CI 0.61-1.16), and it yielded the best-fit model ($AIC_{\text{wild boar x altitude}}$ = 1,127 vs $AIC_{\text{wild boar and altitude separately}}$ = 1,229). The presence of *"brado"* or free-ranging pigs was significantly related to ASF (0.7, 95% CI 0.44-0.98), which is consistent with the significant relationship between ASF and the number of semi-extensive farms (0.27, 95% CI 0.01-0.55). Such farms provide animals with access to outdoor ranging, facilitating contact with wild boar, *“brado”* and pigs from other premises. The number of outgoing movements to other pig premises was also identified as a significant factor (0.36, 95% CI 0.09-0.63), as was the number of farms not censused (0.35, 95% CI 0.09-0.62).

3.3. Risk factor distribution map

Figure 2 shows the final risk factor score for each polygon. Over the total number of polygons, 110 (30.6%) were at highest risk, with risk scores of 4-5; 89 (24.7%) were at medium risk, with a score of 3; and 161 (44.7%) were at lowest risk, with scores of 1-2. Regions at highest risk (red and orange categories) were located mainly around the central and eastern parts of the island within Nuoro and Ogliastra provinces. Highest-risk polygons also occurred in not traditional endemic areas such as in the south of Sassari and north of Cagliari.

4. Discussion

The goal of this study was to understand why ASF is still present in Sardinia. Modelling based on the most complete epidemiological data so far on ASF-susceptible animal populations on the island identified nine significant risk factors for ASF occurrence: number of medium-sized farms, the combination of estimated wild boar density and mean altitude above sea level, presence of *“brado”* animals, number of family farms, number of farms reporting outgoing movements, number of farms reporting self-consumption activities, number of not censused farms, number of open-cycle breeding farms and number of semi-extensive farms. Six of these factors have not previously been linked to ASF occurrence.

These results support several control measures proposed by Sardinian authorities (165, 168, 169, 178), such as the prohibition on *“brado”* and the need to promote farm professionalism through increasing farm biosecurity or strengthening reporting by pig farms, among others. However, the results also highlight several risk factors that have yet to be adequately addressed in control and eradication efforts. These factors include open-cycle breeding farms, outgoing movements of pigs and self-consumption slaughtering practices. Based on our results, supplementary measures are needed to address these factors in order to achieve ASF eradication.

Our analysis considered ASF-positive farms to be those showing positive results based on virological assay and/or antibody assays. This approach is similar to that used in some older studies, such as the work of Mannelli *et al.* (51) on the relationship between husbandry method and ASF seropositivity, but it contrasts with several recent studies that only considered virus detection (166, 173). We believe that taking into account both virological and antibody testing provides a more accurate picture of the disease. The presence of antibodies always indicates infection because no ASFV vaccine is available (41), so taking into account these laboratory results may detect cases that give false negative results in virological assays, such as persistently infected animals with weak or intermittent viraemia but high antibody titre (35, 184, 185). This approach may be particularly important for endemic scenarios such as in Sardinia, where subacute or even unapparent forms may be present (41, 186). Indeed, serological results played an important role in the eradication of ASF from the Iberian Peninsula, where it had been endemic for more than 20 years (35), and from Brazil (187). In these scenarios, serological testing was key for certifying farms and areas as ASF-free.

A second methodological advantage of our study over many previous ones is that we divided up the study area not according to administrative units but rather according to the distribution of pig farms and wild boar density, which we believe provides a more rational basis for analysing the disease. This leads to more homogeneous units of analysis and reduces the ecological bias associated with the use of administrative units (188). On the other hand, this approach may complicate the implementation of control measures, since the affected areas may involve several administrative jurisdictions at once.

The present study draws on the most complete data ever compiled on farms and ASF-susceptible populations in Sardinia. Therefore it addresses the data deficiencies pointed out previously by Martinez-Lopez *et al.* (166) (data from 2009) or Mur *et al.* (173) (data from 2012). In particular, the study draws on data collected since 2012, when reporting requirements and guidelines were implemented and led to substantial improvement in data quality, particularly in annual census reports, registration of animal movements (increased 200% since 2012) and reports on slaughters for self-consumption and sacrifices at slaughterhouses. Nevertheless, there are still some deficiencies remaining in data collection and completeness that should still be addressed, such as the number of animals born and slaughtered, annual census reports and biosecurity on farms.

The combination of estimated wild boar density and altitude was one of the most significant risk factors for ASF in our study. The altitude variable was included in our study as it was one previously described risk factor for ASF by Martinez-Lopez *et al.* (166). In that work, altitude served as a proxy for areas potentially occupied by *“brado”* animals. This assumption was supported by previous publications about ASF in Sardinia (23, 48, 51). Based on our results, a significant interaction between the estimated wild boar density and altitude increased the risk of ASF occurrence. This interaction could point out at an increased ratio of transmission/spread of ASFV due to contacts between wild boar and *“brado”* animals. Moreover, the role of altitude influencing wild boar over ASF occurrence might be explained by two facts. On the one hand, it could be expected to find higher densities of wild boar in elevated areas where there is less/little human pressure. On the other hand, those areas could potentially have more available resources and shelter for animals that would positively influence inter-specific contacts (189). Therefore, the observation that these two variables together are linked to ASF supports the idea that an external source of ASFV, such as infected *“brado”* animals, is required in order for the wild boar population to maintain the disease (23, 176, 177, 190). The significance of altitude may also be explained in part by the suggestion that highlands are usually remote, inaccessible, colder areas where hardy viruses such as ASFV can persist for long periods (166). This risk factor should be particularly useful for assessing ASF risk in areas with small or family farms with poor or no biosecurity and in wild boar-suitable areas with semi-extensive farms. Although previous studies have concluded that wild boar are unlikely to play a crucial role in ASF epidemiology in Sardinia, all have recommended that wild boar be taken into account when designing eradication programmes (23, 51, 166, 177, 191).

In contrast to wild boar, *“brado”* animals are considered the primary ASFV reservoirs in Sardinia. For example, several studies have identified them as a major contributor to disease persistence in Nuoro province (23, 48, 51). Official reports on *“brado”* sightings have been collected since 2012, and analysis of these data indicate that this now-prohibited local practice significantly increases risk of ASF, consistent with previous work (173). These results highlight the need to regulate illegal pig practices in Sardinia and support several measures in the current eradication programme.

Dealing with *“brado”* practices will be challenging because of the strong socio-political dimension. Current regulations require that unregistered animals found to be free-ranging must be reported and subsequently slaughtered. Unfortunately, such slaughter is not uniformly performed across the island, in large part because locals do not understand why authorities want to slaughter their animals. Since the implementation of the current eradication programme, locals in Desulo and other areas (192, 193) have protested on several occasions, refusing to allow veterinarians or police to confiscate their animals. Desulo is in Nuoro province, where ASF has historically been endemic and where *“brado”* practices are quite popular. Sardinian authorities have established a social advisory committee in an effort to deal with this situation, but up to date it remains unsolved.

The number of medium-sized farms (average census of 5-30 animals) emerged as a significant risk factor for ASF persistence on the island. This reflects the correlation between the number of medium-sized farms and areas with high farm density. These results are consistent with previous work (166, 173), although we go further by identifying medium-sized farms as a metric that may be useful for assessing risk and focusing control and eradication efforts. This highlights the way in which biosecurity and farming practices in areas of high farm density can strongly determine the transmission of infectious diseases.

The number of slaughters for self-consumption, evaluated for the first time in this work, emerged as another significant risk factor associated with ASF persistence. Martinez-Lopez *et al.* (166) examined the number of farms reporting slaughters for self-consumption, but this variable was not found to be significantly associated with ASF occurrence. This apparent discrepancy may be explained because slaughters for self-consumption are highly prevalent, with approximately 70% of farms in Sardinia reporting them. By focusing on the number of slaughters rather than the number of farms, we were able to identify areas at lower or higher risk of ASF persistence. Our results are consistent with the risk involved in this practice: it may expose animals on the same farm to potentially infected blood and contaminated materials; the slaughterer may process animals from different farms and may fail to clean and disinfect clothing and ensure adequate biosecurity to prevent contamination; and insects and flies, which are abundant during the spring and summer when this slaughtering is most common, can touch blood and contaminated material and act as mechanical vectors to spread the disease over short distances (32, 194). In addition, slaughters for self-consumption are a social activity, where family and friends reunite and celebrate together, sharing food and pork products that they bring back to their houses, facilitating the potential spread of the disease.

Our results identified several additional risk factors significantly associated with ASF persistence in Sardinia: number of semi-extensive farms, number of movements between farms, number of family farms, open-cycle breeding premises and the presence of non-censused farms. All these factors imply lower biosecurity levels, lack of regulatory compliance, and the entrance of animals, vehicles and people from other premises. Thus, all may have contributed to the explosive occurrence of the disease between late spring and late summer in 2013. Semi-extensive farms can favour transmission among pigs, wild boar and *“brado”* animals because they give animals access to outdoor ranging. Fences must be correctly established to prevent potential contacts, especially in areas suitable for wild boar or zones were *“brado”* animals have been spotted.

The improvement in reporting of animal movements in Sardinia since 2012 allowed us to confirm that such movements are associated with ASF risk, fulfilling a research gap noted previously Mur *et al.* (173). Our findings contrast with those of Martinez-Lopez at al. (166), who concluded that farms with movements coming from other pig premises can protect against ASF. This discrepancy may be explained by differences in the data level (movements vs farms) and response variable (polygons vs farms), by the tightening of regulations since the previous study, and by the present study's reliance on both virological and serological tests for defining ASF positivity.

The number of family farms was associated with ASF persistence in our study. Such farms may be at higher risk of infection because they are owned by non-professional producers, who may be more likely to follow poor farming practices. Moreover, the biosecurity on such farms is usually poor or nearly absent (48). The low professionalism of the sector means that breeding farms, which account for approximately 94% of Sardinian pig farms, tend to use open cycles. For lack of boars, farmers usually share male breeders with their neighbours and most farms do not perform artificial insemination on a regular basis. Sharing breeders and semen is one of the routes of ASF infection (195). Our results suggest that this route may contribute substantially to ASF transmission between nearby breeding farms.

Finally, the number of non-censused farms was significantly related to ASF. Such farms, even if they had not emerged as significant in the model, require attention because they do not follow current census reporting regulations. This variable, within the human risk category, served to take into account risky local practices such as non-compliance with census reporting. It is likely that there are other aspects of the social reality in Sardinia that we did not adequately model here and that may affect risk of ASF occurrence. It may be challenging to analyse such social factors completely given the resistance of the local population to some control and eradication measures, such as the slaughter of *"brado"* animals. For example, we found it quite difficult to collect data regarding farm productivity, slaughterer visits, and degree of involvement of farmers with current farming regulations or the reporting system.

The final risk factor map (Figure 2) showed polygons at the highest risk (score, 4-5) within every Sardinian province. Around 90% of polygons at the maximum risk (score, 5) overlapped with ASF-positive areas. Other polygons showed maximum risk even though the area was ASF-negative, such as in southern Cagliari or Oristano. In these cases, the risk may be due to the high density of medium-sized and open-cycle breeding farms, together with other significant risks such as wild boar density or *“brado”* presence. Most polygons with a risk score of 5 lie within the areas where ASF has traditionally been endemic: Nuoro, Ogliastra and northern Cagliari. The present analysis is the first clear risk factor identification in these areas of the island, since previous studies could not identified them adequately for lack of data (166, 173).

Our results lead us to propose several control measures to supplement what is already being done in the current eradication programme, in order to increase the likelihood of achieving programme goals.

First, the importance of wild boar for ASF occurrence means that additional control measures should target this population, such as rapid control of cases, rapid removal of infectious carcasses from the environment, and targeted reduction of wild boar populations by allowing the hunting of adult and sub-adult females or banning the feeding of animals. Awareness campaigns covering the basics of ASF and hunting practices should be directed at hunters. In areas with high density of wild boar, the biosecurity of semi-extensive farms should be increased to reduce potential contact with wild boar and free-ranging pigs. New construction of semi-extensive farms should be forbidden in areas with high density of wild boar or notified cases. Efforts to eliminate *"brado"* practices should be reinforced in light of the clear evidence of their negative effects on ASF control and eradication.

Second, we recommend reducing the number of family farms, raising biosecurity levels on remaining farms, improving farming practices as well as increasing knowledge, concern and awareness among pig owners. Monitoring of family farms, medium-sized farms and open-cycle breeding farms should be strengthened to allow more effective oversight of productivity rates and animal movements. Establishing a continuous census may promote farm professionalism and help veterinary authorities monitor pig farming practices. Improving the reporting of animal movements would facilitate the estimation of illegal trade and reduce the lack of farm transparency and non-compliance with the law.

Third, protocols applied during slaughters for self-consumption should address the appropriate roles of slaughterer, visitors, buyers and assistants; and they should emphasise the importance of cleaning and disinfecting slaughtering tools. This factor alone may substantially reduce risk of ASF spread, since 83% of seropositive farms in the present study were engaged in slaughters for self-consumption.

Fourth, measures should be implemented to increase biosecurity during animal movements, such as through strict protocols for vehicles entering a farm (*e.g.* cleaning and disinfecting vehicles), quarantine procedures for animals arriving from other farms and ensure health status of purchased animals.

Fifth, we suggest efforts to identify and penalise farms that do not perform annual censuses. This contravenes current regulations and severely impedes official efforts to eradicate ASF in Sardinia.

5. Conclusions

The most complete and accurate data so far available on farms, wild boar and *“brado”* animals in Sardinia from 2010 to 2016 were analysed statistically. This identified nine factors associated with risk of ASF occurrence, three of which were previously identified (number of medium-sized farms, presence of *“brado”* animals, and the combination of estimated wild boar density and altitude), and another six that are novel (number of family farms, number of farms reporting outgoing movements, number of farms reporting movements for self-consumption, number of non-censused farms, number of open-cycle breeding farms and number of semi-extensive farms). While several of these risk factors have been and are being addressed in eradication programmes on the island, some are not and so need to be addressed in supplementary measures. Comprehensive interventions to address all risk factors is likely to eradicate ASF from Sardinia, as long as measures can be applied effectively and uniformly across the island and as long as the social dimension of the measures (such as conflict over *“brado”* practices) can be addressed.

Table 1: List of variables included in the negative binomial multivariable regression model as potential risk factors for African swine fever (ASF) occurrence in Sardinia between 2010 and 2016. Median and rank values are per Voronoi polygon.

#	Variable	Risk type	Median	Range
1	The number of farms	Farm	45	[0-336]
2	The number of fattening farms	Farm	0	[0-9]
3	The number of breeding farms	Farm	42	[1-302]
4	The number of family farms	Farm	2	[0-52]
5	The number of small farms	Farm	25	[0-280]
6	The number of medium farms	Farm	15	[0-85]
7	The number of large farms	Farm	1	[0-22]
8	The number of intensive farms	Farm	23	[0-334]
9	The number of semi-extensive farms	Farm	6	[0-162]
10	The number of open cycle breeding farms	Farm	7	[0-94]
11	The number of closed cycle breeding farms	Farm	26	[0-34]
12	The number of farms that did not report the type of productive cycle	Farm	2	[0-49]
13	The number of farms that reported incoming movements	Movement	1	[0-22]

14	The number of farms that reported movements for self-consumption	Movement	23	[0-204]
15	The number of farms that reported movements to slaughterhouses	Movement	3.5	[0-60]
16	The number of farms that reported outgoing movements	Movement	2	[0-29]
17	Total number of incoming movements reported	Movement	1.5	[0-207]
18	Total number of movements reported for self-consumption	Movement	91.5	[0-1,016]
19	Total number of movements reported to slaughterhouses	Movement	28	[0-5,299]
20	Total number of outgoing movements reported	Movement	3	[0-249]
21	Estimated wild boar density (heads/km^2)	Wild boar	0.22	[0-1]
22	Presence of *“brado”* animals	*“Brado”*	NA	[0-1]
23	Altitude (metres above sea level)	Environment	342.3	[0-1,175]
24	The number of farms that sent animals to themselves	Human	1	[0-13]
25	The number of non-professionalised farms	Human	30	[0-303]

26	The number of farms with multiple pens	Human	6	[0-89]
27	The number of not-censused farms	Human	2	[0-46]
28	The number of farms that reported fewer than one annual-census	Human	10.5	[0-131]

NA: not applicable.

Table 2: Risk factor coefficients for the best-fit negative binomial multivariable regression model based on data between 2010 and 2016.

#	Variable	Regression Coefficient	
		Median	95% Confidence Interval
4	The number of family farms	0.44	[0.15 – 0.73]
6	The number of medium farms	1.04	[0.74 – 1.33]
9	The number of semi-extensive farms	0.27	[0.01 – 0.55]
10	The number of open cycle breeding farms	0.34	[0.06 – 0.62]
14	The number of farms that reported movements for self-consumption	0.36	[0.09 – 0.63]
16	The number of farms that reported outgoing movements	0.36	[0.09 – 0.63]
21/23	Estimated wild boar density x Altitude	0.88	[0.61 – 1.16]
22	Presence of *“brado”* animals	0.7	[0.44 – 0.98]
27	The number of not-censused farms	0.35	[0.09 – 0.62]

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ORIGINAL ARTICLE

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Why is African swine fever still present in Sardinia?

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Summary

African swine fever (ASF) is an infectious disease of swine that has been present in Sardinia since 1978. Soon after introduction of the disease, several control and eradication programmes were established with limited success. Some researchers attributed the persistence of the disease in central and eastern areas to certain socio-economic factors, the existence of some local and traditional farming practices (i.e., unregistered free-ranging pigs known as brado animals) and the high density of wild boar in the region. In the past, scarcity of swine data in Sardinia complicated the evaluation and study of ASF on the island. More complete, accurate and reliable information on pig farms has become available as a result of the most recent eradication programmes. Here, we perform statistical modelling based on these data and the known distribution of domestic pig and wild boar to identify the main risk factors that have caused ASF persistence in Sardinia. Our results categorized, identified and quantified nine significant risk factors, six of which have not been previously described. The most significant factors were the number of medium-sized farms, the presence of brado animals and the combination of estimated wild boar density and mean altitude above sea level. Based on these factors, we identified regions in eastern and central Sardinia to be at greatest risk of ASF persistence; these regions are also where the disease has traditionally been endemic. Based on these risk factors, we propose specific control measures aimed at mitigating such risks and eradicating ASF from the island.

KEYWORDS
control measures, epidemiology, eradication, risk factors

1 | INTRODUCTION

African swine fever (ASF) is an infectious disease of swine, notifiable to the World Organisation for Animal Health (OIE). It has been present on the Italian island of Sardinia since 1978 (Mur, Atzeni et al., 2016). In March of that year, ASF virus (ASFV) was introduced into southern Sardinia, most likely from the Iberian Peninsula. This introduction presumably occurred through ASFV-contaminated waste, containing raw pork or pork products that were subsequently used to feed pigs (Mannelli et al., 1997). ASF affects swine of all ages and breeds, although clinical forms can vary depending on the isolate, dose, route of infection and affected host (Sánchez-Vizcaíno, Mur, Gómez-Villamandos, & Carrasco, 2015). The disease continues to affect domestic pigs as well as wild boar in Sardinia (Laddomada et al., 1994; Mur, Atzeni et al., 2016). Ornithodoros ticks, a well-known ASFV vector, have not been found so far on the island (Mur, Iscaro et al., 2016; Sánchez-Vizcaíno, Mur, Bastos, & Penrith, 2015), even though they have been found in other Mediterranean areas such as Spain and Portugal (Boinas, Wilson, Hutchings, Martins, & Dixon, 2011; Pérez-Sánchez, Astigarraga, Oleaga-Pérez, & Encinas-Grandes, 1994).

Early after introduction of the disease on the island, several control and eradication programmes have been established with limited success. As a result, periods of relative stability (1995–2004, 2006–2011) have alternated with epidemic waves (2004–2005, 2013–2014). In 1982, 4 years after ASF introduction, the first eradication programme began with funding from the European Economic Community (EEC) and the Italian government. This plan aimed to enhance active surveillance and effective control of outbreaks following traditional stamping out policies in order to optimize early detection of the disease. As a result of these efforts, ASF has been eradicated from southern Sardinia, but it has remained endemic in central and eastern regions of Barbagia, Baronia and Ogliastra (Regione Autonoma della Sardegna, 2009). Some researchers have attributed the persistence of the disease in these areas to certain socio-economic factors, the high density of wild boar in the region and the traditional farming practice of grazing free-range herds (brado animals) on communal pastures without any veterinary controls (Laddomada et al., 1994; Mannelli et al., 1997; Martínez-López et al., 2015).

In the 1990s, the EEC provided the island with further funding aimed at eliminating the disease. Efforts seemed to bring it under control from 1995 onwards, as numbers of notifications decreased. However, between 2004 and 2005, ASF outbreaks occurred in some previously unaffected territories of the island, such as the western province of Carbonia Iglesias, and the number of notifications increased from 11 in 2003 to 243 in 2004 and 201 in 2005 (Mur, Atzeni et al., 2016). Additional control measures approved by the European Commission (Commission, 2005) reduced the numbers of outbreaks from 2006 to mid-2011, after which a steady number of notifications were reported, some of them outside the traditional endemic region. Since then, several eradication programmes have been launched in Sardinia (Regione Autonoma della Sardegna, 2012a,b, 2014) focusing on promoting farm professionalism, increasing farm biosecurity and strengthening veterinary monitoring and reporting by pig farms. These measures also prohibited brado practices, which earlier studies had singled out as the main limitation to ASF eradication (Laddomada et al., 1994; Mannelli et al., 1997; Mur, Atzeni et al., 2016).

The current eradication programme [regional programme cofounded by the European Union in 2015 (Regione Autonoma della Sardegna, 2017)], implemented from 2015, aims to eradicate ASF definitively from the island. It stipulates measures aimed at (i) providing information to farmers, hunters and the general population; (ii) fighting the presence of brado and unregistered pigs; (iii) improving biosecurity on farms; and (iv) strengthening the control on pig farms through sanitary status qualifications (Regione Autonoma della Sardegna, 2014).

Controlling and ultimately eradicating ASF from the island is complex for several reasons, including the co-existence of several animal populations (registered domestic pigs, unregistered domestic pigs, brado animals and wild boar) and socio-economic factors, as several epidemiological studies have pointed out (Cappai, Rolesu, Coccollone, Laddomada, & Loi, 2017; Martínez-López et al., 2015; Mur, Atzeni et al., 2016; Mur et al., 2017). These studies as well as the Italian National Animal Husbandry Statistics Registry reveal a scarcity or even total absence of pig data essential for epidemiological analyses, which have complicated ASF evaluation in Sardinia.

Efforts during implementation of the most recent eradication programmes on the island have yielded more complete, accurate and reliable information on pig farms. Now, through a collaboration between the Government of the Autonomous Region of Sardinia and Complutense University of Madrid (UCM), these data can be used for the first time to perform detailed epidemiological analyses.

This study, then, draws on relatively complete data from 2010 to 2016 in order to identify the risk factors most likely to explain the persistence of ASF in Sardinia as well as regions where such risk factors are present. Moreover, on the basis of these results, we propose supplementary measures that may be implemented as part of the current eradication plan in order to mitigate risk factors and contribute to the final eradication of ASF from the island.

2 | MATERIALS AND METHODS

2.1 | Data sources

Epidemiological data from 2010 to 2016 were provided by the Regional Veterinary Epidemiological Observatory-Istituto Zooprofilattico Sperimentale della Sardegna (EOVR-IZS). These data included information covering the period from 2010 to July 2015 related to ASF occurrence including notified cases on pig farms as well as serology and virology laboratory results. The data also included information on ASF-susceptible populations on the island, including wild boar and domestic pigs (registered farms, census on pig farms, registered incoming and outgoing movements and slaughters for self-consumption). In addition, data covering the period from 2012 to December 2016 included information on brado pig sightings, defined as domestic pigs not registered or counted in censuses but found free-ranging on pastures or as carcasses in the field. Altitude data were obtained from the Regional Geographical Service (Servizio Informativo e Cartografico Regionale, 2011).

2.2 | Delineation of the study area by tessellation

Traditionally, epidemiological studies of animal diseases have relied on political administrative units such as municipalities and provinces (Arnold, Diamond, & Wakefield, 2000), but such units do not accurately reflect environmental, ecological or biological realities. Epidemiological characteristics such as surface terrain, ecosystems, livestock density and wildlife populations can vary substantially within the same administrative unit, which would be masked in an analysis across units, leading to an incomplete or inaccurate model. In order to reduce the risk of such ecological bias, the spatial units of analysis in this work were based not on administrative units but on tiles of similar shape and size defined according to the distribution of ASF-susceptible domestic pig farms and wild boar.

The wild boar distribution density map provided by EOVR-IZS and based on Rolesu et al. (2007) was transformed into a raster layer of 100 × 100 m through spatial kernel density estimation in ArcMap 10.3 (ESRI®). Another raster layer of 100 × 100 m showing the distribution of pig farms was generated by applying a point-density function (radius, 2 km) to EOVR-IZS data. Local maxima points in both layers were computed using a focal statistic function with radius 2.5 km. This radius was selected to obtain a number of polygons similar to the amount of municipalities, 360 versus 376. Finally, tessellation was performed using Thiessen polygons to obtain Voronoi polygons of similar size strategically centred at local maxima of wild boar and domestic pig populations. These polygons served as the spatial unit in all subsequent analyses.

2.3 | Risk factor selection

A list of potential risk factors associated with ASF persistence on the island was generated based on the literature (Iglesias, Rodríguez, Feliziani, Rolesu, & De la Torre, 2017; Laddomada et al., 1994; Mannelli et al., 1997; Martínez-López et al., 2015; Mur, Atzeni et al., 2016; Mur et al., 2017; Rolesu et al., 2007), experts opinion sessions at more than 15 meetings between specialists from UCM, EOVR-IZS and the Regione della Sardegna authorities between 2014 and 2016, and discussions with private-sector and government veterinarians from Nuoro and Lanusei in April 2015. A final list of 28 candidate variables was generated and grouped into six risk categories (farm, movements, wild boar, brado, environment and humans) for which data were available at the Voronoi polygon level (Table 1).

Data in the farm risk category included information related to swine census and production characteristics that could be relevant to risk of spread of infectious diseases. Based on census data, farms were categorized as small (<5 animals), medium (5–30 animals) and large (>30 animals). From 2010 onwards, pig premises have been classified according to Sardinian regulations as breeding, fattening or family farms (Regione Autonoma della Sardegna, 2009, 2010, 2011, 2012a,b, 2014). Family farms have a maximum of four animals (not breeders), and animal movements to other premises are forbidden. Family farms failing to meet at least one these conditions during the study period were reclassified as fattening farms. Breeding farms were subcategorized as having an open or closed cycle, depending on whether breeders could be shared with, or sold to, other farms. Farms that were registered as having an open and closed cycle at the same time were categorized in this study as open in accord with the principle of maximum risk.

Farms were classified according to management practices as intensive/confined or semi-extensive, with limited access of animals to outdoor terrain. Farms that reported both types of management were classified as semi-extensive in accord with the principle of maximum risk.

The following risk variables were included in the animal movements risk category: (i) number of farms reporting incoming movements, (ii) number of farms reporting movements to slaughterhouses, (iii) number of farms reporting movements for self-consumption, (iv) number of movements reported between farms, (v) number of recorded shipments to slaughterhouses, and (vi) number of self-consumption slaughters reported on each farm.

The wild boar risk category was analysed on the basis of the wild boar density layer provided by EOVR-IZS. The brado risk category included information on sightings of free-ranging pigs and domestic pig carcasses reported by forestry officials since 2012. The mean altitude per polygon was included within the environment risk category.

Finally, a human risk category was included to assess the impact of poor farming practices. These practices included (i) non-compliance with regulations during the annual census and the presence of non-professionalized premises, such as (ii) farms with a census of ≤4 animals, (iii) farms with multiple pens and mixed systems (simultaneously open and closed cycles and/or intensive and semi-extensive management practices) and (iv) farms reporting movements to themselves.

To be included in models, variables were assigned binary values of 0 or 1 if their actual value was, respectively, above or equal the median or no greater than the median based on Voronoi polygons. The exception was the brado risk category, for which the variable was assigned a value of 1 if any individual or carcass was reported, and 0 otherwise.

2.4 | Statistical models

The significance of the six risk categories outlined in section 2.3 was assessed using negative binomial multivariable regression. The response variable in this model was the total number of ASF-positive farms in each Voronoi polygon (either by the presence of antibodies and/or virus). Considering that no vaccine is available, the presence of antibodies against ASFV always means previous contact with the virus. Therefore, farms were classified as ASF-positive in this study if any animals on that farm tested positive to ASFV (by PCR) and/or antibody presence (screening by ELISA and confirming by Western blotting) during any routine checks, such as during surveillance and control campaigns, before shipment to slaughterhouses or when slaughtered for self-consumption. A total of 862 positive records reported between 2010 and July 2015 were included in our analyses.

Modelling was performed using the MASS package (Venables & Ripley, 2002) in R software (R Development Core Team, 2015). Variables were added to the model according to a stepwise forward selection procedure; highly correlated variables were not included in the model simultaneously in order to avoid multicollinearity effects. Best-fit models were selected based on the lowest Akaike's information criterion (AIC) (Burnham & Anderson, 2002) when all included variables showed significance at 95% ($p < .05$). The best-fit model provided the final set of risk factors, for which the impact on ASF persistence was assessed using median regression coefficients and 95% confidence intervals (95% CI).

2.5 | Risk factor distribution map

For visualizing purposes, the modelling results were displayed by calculating a final risk factor score per Voronoi polygon. This final score was simply the sum of each risk regression coefficient computed by the best-fit model. Scores were mapped using ArcMap 10.3 (ESRI®) and Jenks' natural break classification method, in which a cut-off

TABLE 1 List of variables included in the negative binomial multivariable regression model as potential risk factors for African swine fever (ASF) occurrence in Sardinia between 2010 and 2016. Median and rank values are per Voronoi polygon

#	Variable	Risk type	Median	Range
1	The number of farms	Farm	45	0 336
2	The number of fattening farms	Farm	0	0 9
3	The number of breeding farms	Farm	42	1 302
4	The number of family farms	Farm	2	0 52
5	The number of small farms	Farm	25	0 280
6	The number of medium farms	Farm	15	0 85
7	The number of large farms	Farm	1	0 22
8	The number of intensive farms	Farm	23	0 334
9	The number of semi-extensive farms	Farm	6	0 162
10	The number of open-cycle breeding farms	Farm	7	0 94
11	The number of closed-cycle breeding farms	Farm	26	0 34
12	The number of farms that did not report the type of productive cycle	Farm	2	0 49
13	The number of farms that reported incoming movements	Movement	1	0 22
14	The number of farms that reported movements for self-consumption	Movement	23	0 204
15	The number of farms that reported movements to slaughterhouses	Movement	3.5	0 60
16	The number of farms that reported outgoing movements	Movement	2	0 29
17	Total number of incoming movements reported	Movement	1.5	0 207
18	Total number of movements reported for self-consumption	Movement	91.5	0 1,016
19	Total number of movements reported to slaughterhouses	Movement	28	0 5,299
20	Total number of outgoing movements reported	Movement	3	0 249
21	Estimated wild boar density (heads/km2)	Wild boar	0.22	0 1
22	Presence of brado animals	Brado	NA	0 1
23	Altitude (metres above sea level)	Environment	342.3	0 1,175
24	The number of farms that sent animals to themselves	Human	1	0 13
25	The number of non-professionalised farms	Human	30	0 303
26	The number of farms with multiple pens	Human	6	0 89
27	The number of non-censused farms	Human	2	0 46
28	The number of farms that reported fewer than one annual census	Human	10.5	0 131

value was calculated for each of the five risk categories (from 1 to 5; Jenks, 1967).

3 | RESULTS

3.1 | Delineation of the study area by tessellation

Voronoi tessellation divided Sardinia into 360 polygons (Figure 1) which had an average surface of 64.65 km^2 and 62.27% of which were ASF-positive based on the presence of virus and/or antibody (Figure 2).

3.2 | Statistical models

The best-fit model (AIC = 1,217) identified the interaction between estimated wild boar density and mean altitude above sea level as significantly associated with ASF. Also associated with ASF in the model were eight individual variables (Table 2): number of medium-size farms, the presence of brado animals, number of family farms, number of farms reporting outgoing movements, number of farms reporting self-consumption activities, the number of non-censused farms, number of open-cycle farms and number of semi-extensive farms. Main effects, namely estimated wild boar density and altitude, were also included in the model together with its interaction (wild boar × altitude) and the rest of significant variables. In that case, the main effects were no significant plus obtained regression coefficients were very similar to the ones presented here. However, the AIC value for that model was higher than the obtained for the best-fit model (AIC = 1220.7). Therefore, the selected model identified eight individual variables and one interaction as risk factors significantly related to ASF presence (Cleves, Gutiérrez, Gould, & Marchenko, 2008).

The number of medium farms was the most significant risk factor (1.04, 95% CI 0.74–1.33), and it correlated with number of farms (coefficient, 0.742). In other words, areas with a high density of medium-sized farms were at greater risk of being infected by ASF than areas with a low density of such farms. Estimated wild boar

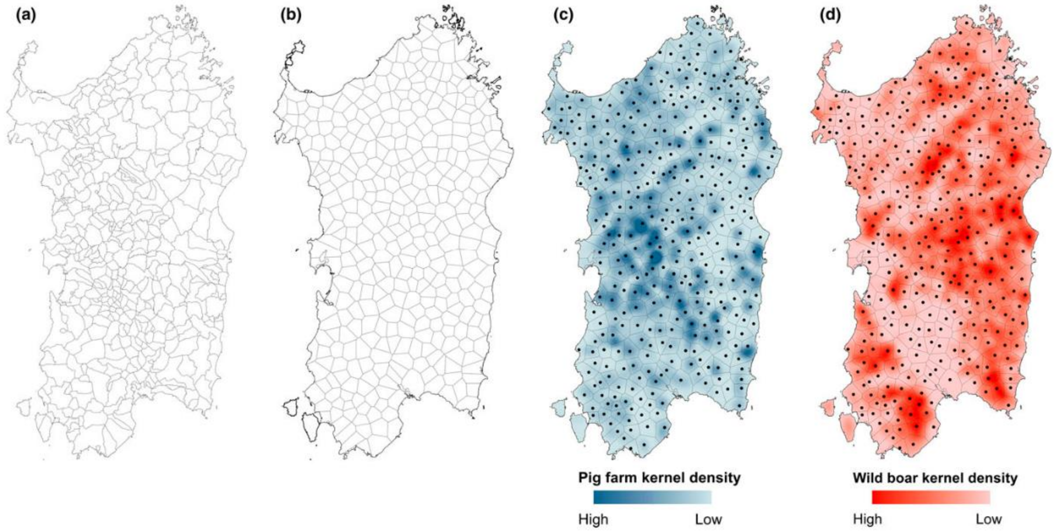

FIGURE 1 (a) Sardinia depicted with municipalities boundaries and (b) after tessellation into Voronoi polygons. Superimposed on the polygon representation are (c) farm density in blue and (d) wild boar density in red. Black dots indicate local maxima of wild boar and pig farm density

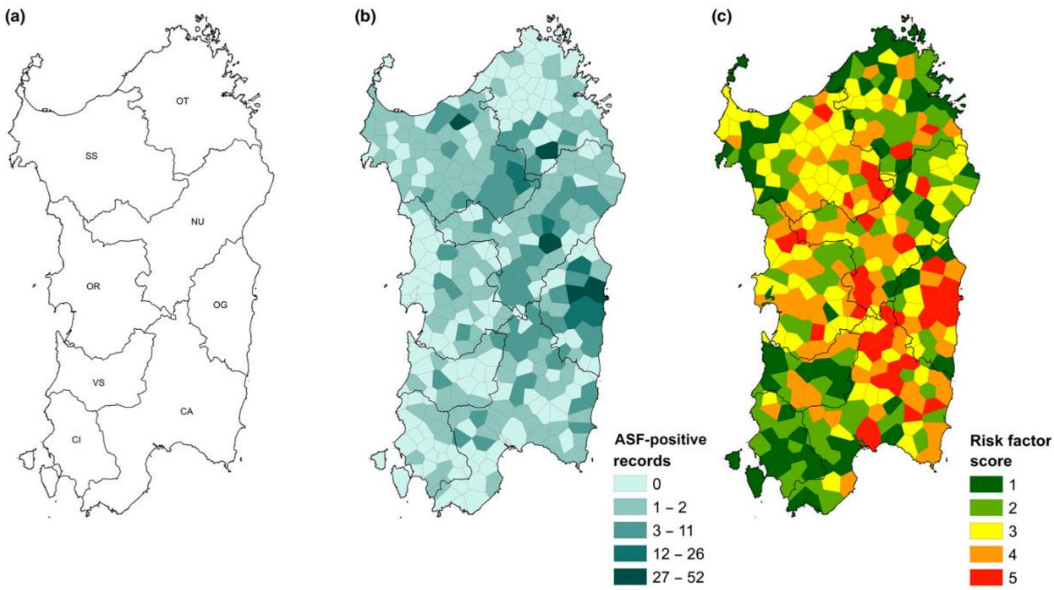

FIGURE 2 (a) Sardinia divided into provinces and (b–c) Voronoi polygons. Also shown are (b) the number of African swine fever (ASF)-positive farms based on positive records (PCR, Western blot or ELISA tests) per polygon and (c) final risk factor scores per polygon were calculated based on data from 2010 to 2016. Jenks' natural breaks algorithm was used to calculate a cut-off value for each of the five risk categories. CA, Cagliari; CI, Carbonia Iglesias; NU, Nuoro; OG, Ogliastra; OR, Oristano; OT, Oblia Tempio; SS, Sassari; VS, Medio Campidano

density and altitude above sea level on their own were significant in the model (data not shown), while their combination was one of the most significant risk factors for ASF persistence (0.88, 95% CI 0.61–1.16), and it yielded the best-fit model (AICwild boar × altitude = 1,127 versus AICwild boar and altitude separately = 1,229). The presence of brado or free-ranging pigs was significantly related

TABLE 2 Risk factor coefficients for the best-fit negative binomial multivariable regression model based on data between 2010 and 2016

#	Variable	Regression coefficient	
		Median	95% Confidence interval
4	The number of family farms	0.44	0.15 0.73
6	The number of medium farms	1.04	0.74 1.33
9	The number of semi-extensive farms	0.27	0.01 0.55
10	The number of open-cycle breeding farms	0.34	0.06 0.62
14	The number of farms that reported movements for self-consumption	0.36	0.09 0.63
16	The number of farms that reported outgoing movements	0.36	0.09 0.63
21/23	Estimated wild boar density × Altitude	0.88	0.61 1.16
22	Presence of brado animals	0.7	0.44 0.98
27	The number of non-censused farms	0.35	0.09 0.62

to ASF (0.7, 95% CI 0.44–0.98), which is consistent with the significant relationship between ASF and the number of semi-extensive farms (0.27, 95% CI 0.01–0.55). Such farms provide animals with access to outdoor ranging, facilitating contact with wild boar, brado and pigs from other premises. The number of outgoing movements to other pig premises was also identified as a significant factor (0.36, 95% CI 0.09–0.63), as was the number of farms not censused (0.35, 95% CI 0.09–0.62).

3.3 | Risk factor distribution map

Figure 2 shows the final risk factor score for each polygon. Over the total number of polygons, 110 (30.6%) were at highest risk, with risk scores of 4–5; 89 (24.7%) were at medium risk, with a score of 3; and 161 (44.7%) were at lowest risk, with scores of 1–2. Regions at highest risk (red and orange categories) were located mainly around the central and eastern parts of the island within Nuoro and Ogliastra provinces. Highest-risk polygons also occurred in non-traditional endemic areas such as in the south of Sassari and north of Cagliari.

4 | DISCUSSION

The goal of this study was to understand why ASF is still present in Sardinia. Modelling based on the most complete epidemiological data so far on ASF-susceptible animal populations on the island identified nine significant risk factors for ASF occurrence: number of medium-sized farms, the combination of estimated wild boar density and mean altitude above sea level, presence of brado animals, number of family farms, number of farms reporting outgoing movements, number of farms reporting self-consumption activities, number of non-censused farms, number of open-cycle breeding farms and number of semi-extensive farms. Six of these factors have not previously been linked to ASF occurrence.

These results support several control measures proposed by Sardinian authorities (Regione Autonoma della Sardegna, 2009, 2010, 2011, 2012a,b, 2014), such as the prohibition on brado and the need to promote farm professionalism through increasing farm biosecurity or strengthening reporting by pig farms, among others. However, the results also highlight several risk factors that have yet to be adequately addressed in control and eradication efforts. These factors include open-cycle breeding farms, outgoing movements of pigs and self-consumption slaughtering practices. Based on our results, supplementary measures are needed to address these factors to achieve ASF eradication.

Our analysis considered ASF-positive farms to be those showing positive results based on virological assay and/or antibody assays. This approach is similar to that used in some older studies, such as the work of Mannelli et al. (1997) on the relationship between husbandry method and ASF seropositivity, but it contrasts with several recent studies that only considered virus detection (Martínez-López et al., 2015; Mur et al., 2017). We believe that taking into account both virological and antibody testing provides a more accurate picture of the disease. The presence of antibodies always indicates infection because no ASFV vaccine is available (Sánchez-Vizcaíno & Arias, 2012), so taking into account these laboratory results may detect cases that give false-negative results in virological assays, such as persistently infected animals with weak or intermittent viraemia but high antibody titre (Arias & Sánchez-Vizcaíno, 2002; Gallardo et al., 2017; Vigário, Castro-Portugal, Festas, & Vasco, 1983). This approach may be particularly important for endemic scenarios such as in Sardinia, where subacute or even unapparent forms may be present (Sánchez-Vizcaíno & Arias, 2012; Sánchez-Vizcaíno & Mur, 2013). Indeed, serological results played an important role in the eradication of ASF from the Iberian Peninsula, where it had been endemic for more than 20 years (Arias & Sánchez-Vizcaíno, 2002), and from Brazil (Lyra, 2006). In these scenarios, serological testing was key for certifying farms and areas as ASF-free.

A second methodological advantage of our study over many previous ones is that we divided up the study area not according to the administrative units but rather according to the distribution of pig farms and wild boar density, which we believe provides a more rational basis for analysing the disease. This leads to more homogeneous units of analysis and reduces the ecological bias associated with the use of administrative units (Okabe, Boots, Sugihara, & Chiu, 2000). On the other hand, this approach may complicate the implementation of control measures, as the affected areas may involve several administrative jurisdictions at once.

The present study draws on the most complete data ever compiled on farms and ASF-susceptible populations in Sardinia. Therefore, it addresses the data deficiencies pointed out previously by Martínez-López et al. (2015) (data from 2009) or Mur et al. (2017) (data from 2012). In particular, the study draws data collected since 2012, when reporting requirements, and guidelines were implemented and led to substantial improvement in data quality, particularly in annual census reports, registration of animal movements (increased 200% since 2012) and reports on slaughters for self-consumption and killing at slaughterhouses. Nevertheless, there are still some deficiencies remaining in data collection and completeness that should still be addressed, such as the number of animals born and slaughtered, annual census reports and biosecurity on farms.

The combination of estimated wild boar density and altitude was one of the most significant risk factors for ASF in our study. The altitude variable was included in our study as it was one previously described risk factor for ASF by Martínez-López et al. (2015). In that work, altitude served as a proxy for areas potentially occupied by brado animals. This assumption was supported by previous publications about ASF in Sardinia (Laddomada et al., 1994; Mannelli et al., 1997; Mur, Atzeni et al., 2016). Based on our results, a significant interaction between the estimated wild boar density and altitude increased the risk of ASF occurrence. This interaction could point out at an increased ratio of transmission/spread of ASFV due to contacts between wild boar and brado animals. Moreover, the role of altitude influencing wild boar over ASF occurrence might be explained by two facts. On the one hand, it could be expected to find higher densities of wild boar in elevated areas where there is less/little human pressure. On the other hand, those areas could potentially have more available resources and shelter for animals that would positively influence inter-specific contacts (Barasona et al., 2014). Therefore, the observation that these two variables together are linked to ASF supports the idea that an external source of ASFV, such as infected brado animals, is required in order for the wild boar population to maintain the disease (Iglesias et al., 2017; Laddomada et al., 1994; Mur et al., 2012; Rolesu et al., 2007). The significance of altitude may also be explained in part by the suggestion that highlands are usually remote, inaccessible, colder areas where hardy viruses such as ASFV can persist for long periods (Martínez-López et al., 2015). This risk factor should be particularly useful for assessing ASF risk in areas with small or family farms with poor or no biosecurity and in wild boar-suitable areas with semi-extensive farms. Although previous studies have concluded that wild boar are unlikely to play a crucial role in ASF epidemiology in Sardinia, all have recommended that wild boar be taken into account when designing eradication programmes (Iglesias et al., 2017; Laddomada et al., 1994; Mannelli et al., 1997, 1998; Martínez-López et al., 2015).

In contrast to wild boar, brado animals are considered the primary ASFV reservoirs in Sardinia. For example, several studies have identified them as a major contributor to disease persistence in Nuoro province (Laddomada et al., 1994; Mannelli et al., 1997; Mur, Atzeni et al., 2016). Official reports on brado sightings have been collected since 2012, and analysis of these data indicate that this now-prohibited local practice significantly increases risk of ASF, consistent with previous work (Mur et al., 2017). These results highlight the need to regulate illegal pig practices in Sardinia and support several measures in the current eradication programme.

Dealing with brado practices will be challenging because of the strong sociopolitical dimension. Current regulations require that unregistered animals found to be free-ranging must be reported and subsequently slaughtered. Unfortunately, such slaughter is not uniformly performed across the island, in large part because locals do not understand why authorities want to slaughter their animals. Since the implementation of the current eradication programme, locals in Desulo and other areas (INFOaut, 2016; L'Unione Sarda, 2016) have protested on several occasions, refusing to allow veterinarians or police to confiscate their animals. Desulo is in Nuoro province, where ASF has historically been endemic and where brado practices are quite popular. Sardinian authorities have established a social advisory committee in an effort to deal with this situation, but, up to date, it remains unsolved.

The number of medium-sized farms (average census of 5–30 animals) emerged as a significant risk factor for ASF persistence on the island. This reflects the correlation between the number of medium-sized farms and areas with high farm density. These results are consistent with previous work (Martínez-López et al., 2015; Mur et al., 2017), although we go further by identifying medium-sized farms as a metric that may be useful for assessing risk and focusing control and eradication efforts. This highlights the way in which biosecurity and farming practices in areas of high farm density can strongly determine the transmission of infectious diseases.

The number of slaughters for self-consumption, evaluated for the first time in this work, emerged as another significant risk factor associated with ASF persistence. Martínez-López et al. (2015) examined the number of farms reporting slaughters for self-consumption, but this variable was not found to be significantly associated with ASF occurrence. This apparent discrepancy may be explained because slaughters for self-consumption are highly prevalent, with approximately 70% of farms in Sardinia reporting them. By focusing on the number of slaughters rather than the number of farms, we were able to identify areas at lower or higher risk of ASF persistence. Our results are consistent with the risk involved in this practice: it may expose animals on the same farm to potentially infected blood and contaminated materials; the slaughterer may process animals from different farms and may fail to clean and disinfect clothing and ensure adequate biosecurity to prevent contamination; and insects and flies, which are abundant during the spring and summer when this slaughtering is most common, can touch blood and contaminated material and act as mechanical vectors to spread the disease over short distances (Baldacchino et al., 2013; Mellor, Kitching, & Wilkinson, 1987). In addition, slaughters for self-consumption are a social activity, where family and friends reunite and celebrate together, sharing food and pork products that they bring back to their houses, facilitating the potential spread of the disease.

Our results identified several additional risk factors significantly associated with ASF persistence in Sardinia: number of semi-extensive farms, number of movements between farms, number of family farms, open-cycle breeding premises and the presence of non-censused farms. All these factors imply lower biosecurity levels, lack of regulatory compliance and the entrance of animals, vehicles and people from other premises. Thus, all may have contributed to the explosive occurrence of the disease between late spring and late summer in 2013. Semi-extensive farms can favour transmission among pigs, wild boar and brado animals because they give animals access to outdoor ranging. Fences must be correctly established to prevent potential contacts, especially in areas suitable for wild boar or zones where brado animals have been spotted.

The improvement in reporting of animal movements in Sardinia since 2012 allowed us to confirm that such movements are associated with ASF risk, fulfilling a research gap noted previously Mur et al. (2017). Our findings contrast with those of Martínez-López et al. (2015), who concluded that farms with movements coming from other pig premises can protect against ASF. This discrepancy may be explained by differences in the data level (movements versus farms) and response variable (polygons versus farms), by the tightening of regulations since the previous study, and by the present study's reliance on both virological and serological tests for defining ASF positivity.

The number of family farms was associated with ASF persistence in our study. Such farms may be at higher risk of infection because they are owned by non-professional producers, who may be more likely to follow poor farming practices. Moreover, the biosecurity on such farms is usually poor or nearly absent (Mur, Atzeni et al., 2016). The low professionalism of the sector means that breeding farms, which account for approximately 94% of Sardinian pig farms, tend to use open cycles. For lack of boars, farmers usually share male breeders with their neighbours, and most farms do not perform artificial insemination on a regular basis. Sharing breeders and semen is one of the routes of ASF infection (Guérin & Pozzi, 2005). Our results suggest that this route may contribute substantially to ASF transmission between nearby breeding farms.

Finally, the number of non-censused farms was significantly related to ASF. Such farms, even if they had not emerged as significant in the model, require attention because they do not follow current census reporting regulations. This variable, within the human risk category, served to take into account risky local practices such as non-compliance with census reporting. It is likely that there are other aspects of the social reality in Sardinia that we did not adequately model here and that may affect risk of ASF occurrence. It may be challenging to analyse such social factors completely given the resistance of the local population to some control and eradication measures, such as the slaughter of brado animals. For example, we found it quite difficult to collect data regarding farm productivity, slaughterer visits and degree of involvement of farmers with current farming regulations or the reporting system.

The final risk factor map (Figure 2) showed polygons at the highest risk (score, 4–5) within every Sardinian province. Around 90% of polygons at the maximum risk (score, 5) overlapped with ASF-positive areas. Other polygons showed maximum risk even though the area was ASF-negative, such as in southern Cagliari or Oristano. In these cases, the risk may be due to the high density of medium-sized and open-cycle breeding farms, together with other significant risks such as wild boar density or brado presence. Most polygons with a risk score of 5 lie within the areas where ASF has traditionally been endemic: Nuoro, Ogliastra and northern Cagliari. The present analysis is the first clear risk factor identification in these areas of the island, as previous studies could not identified them adequately for lack of data (Martínez-López et al., 2015; Mur et al., 2017).

Our results lead us to propose several control measures to supplement what is already being done in the current eradication programme, in order to increase the likelihood of achieving programme goals.

First, the importance of wild boar for ASF occurrence means that additional control measures should target this population, such as rapid control of cases, rapid removal of infectious carcasses from the environment and targeted reduction in wild boar populations by allowing the hunting of adult and subadult females or banning the feeding of animals. Awareness campaigns covering the basics of ASF and hunting practices should be directed at hunters. In areas with high density of wild boar, the biosecurity of semi-extensive farms should be increased to reduce potential contact with wild boar and free-ranging pigs. New construction of semi-extensive farms should be forbidden in areas with high density of wild boar or notified cases. Efforts to eliminate brado practices should be reinforced in the light of the clear evidence of their negative effects on ASF control and eradication.

Second, we recommend reducing the number of family farms, raising biosecurity levels on remaining farms, improving farming practices as well as increasing knowledge, concern and awareness among pig owners. Monitoring of family farms, medium-sized farms and open-cycle breeding farms should be strengthened to allow more effective oversight of productivity rates and animal movements. Establishing a continuous census may promote farm professionalism and help veterinary authorities monitor pig farming practices. Improving the reporting of animal movements would facilitate the estimation of illegal trade and reduce the lack of farm transparency and non-compliance with the law.

Third, protocols applied during slaughters for self-consumption should address the appropriate roles of slaughterer, visitors, buyers and assistants; and they should emphasize the importance of cleaning and disinfecting slaughtering tools. This factor alone may substantially reduce risk of ASF spread, as 83% of seropositive farms in the present study were engaged in slaughters for self-consumption.

Fourth, measures should be implemented to increase biosecurity during animal movements, such as through strict protocols for vehicles entering a farm (e.g., cleaning and disinfecting vehicles), quarantine procedures for animals arriving from other farms and ensure health status of purchased animals.

Fifth, we suggest efforts to identify and penalize farms that do not perform annual censuses. This contravenes current regulations and severely impedes official efforts to eradicate ASF in Sardinia.

5 | CONCLUSIONS

The most complete and accurate data so far available on farms, wild boar and brado animals in Sardinia from 2010 to 2016 were analysed statistically. This identified nine factors associated with risk of ASF occurrence, three of which were previously identified (number of medium-sized farms, presence of brado animals and the combination of estimated wild boar density and altitude), and another six that are novel (number of family farms, number of farms reporting outgoing movements, number of farms reporting movements for self-consumption, number of non-censused farms, number of open-cycle breeding farms and number of semi-extensive farms). While several of these risk factors have been and are being addressed in eradication programmes on the island, some are not and so need to be addressed in supplementary measures. Comprehensive interventions to address all risk factors is likely to eradicate ASF from Sardinia, as long as measures can be applied effectively and uniformly across the island and as long as the social dimension of the measures (such as conflict over brado practices) can be addressed.

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AUTHOR CONTRIBUTION

CJ, JMSV, AL and LM identified the research question. CJ, EFC, LM and JMSV selected the methodology to be used. AL and SR provided the data needed to conduct this study and the valuable knowledge with regard to the Sardinian situation. EFC and CJ run the models and statistical analysis and summarized results. All authors contributed to the critical review of the results and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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CHAPTER 3

OBJECTIVE 3. To identify available measures to prevent the introduction and spread of ASF on domestic pig farms into the EU through a systematic review. Moreover, the relevance of each measure will be assessed by expert opinion depending on the pig farming systems present in the EU scenario.

Main scientific publication of objective 3.

Jurado C, Martinez-Aviles M, de la Torre A, Štukelj M, de Carvalho Ferreira HC, Cerioli M, et al. **Relevant measures to prevent the spread of African swine fever in the European Union domestic pig sector.** Front Vet Sci. 2018;5:77. DOI: 10.3389/fvets.2018.00077.

Related scientific contributions:

Scientific dissemination articles:

Martínez M, Iglesias I, Bosch J, **Jurado C**, Vicente J, Sanchez-Vizcaino JM, et al. **Protecting the extensive pig production system in Spain** (original title in Spanish: "Proteger al sector porcino extensivo en España"). Euroganadería.eu. 2017. [Available at http://www.euroganaderia.eu/ganaderia/reportajes/proteger-al-sector-porcino-extensivo-en-espana_3053_6_4564_0_1_in.html].

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Jurado C, Sanchez-Vizcaino JM, Martinez-Aviles M, de la Torre A, Bellini S. **Systematic review and assessment of measures to prevent the spread of**

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RESUMEN DE LOS RESULTADOS DEL CAPÍTULO 3

La UE es el segundo productor de porcino del mundo después de China. Desde que la PPA llegó al continente europeo en 2007, un sector clave de nuestra economía está siendo continuamente amenazado. En respuesta a esta situación, la UE desarrolló una de las principales piezas legislativas que proporcionan herramientas para el control de la PPA en la UE (Directiva del Consejo 2002/60/CE). A pesar de las medidas de control implementadas, la PPA ha sido notificada en varios Estados Miembro de la UE. Además, existen medidas específicas de regionalización basadas en una Decisión de Ejecución de la Comisión (2014/709/UE). Dentro de la Acción COST (de sus siglas en inglés *“European Cooperation in Science and Technology”*) 15116 titulada “entendiendo y combatiendo la peste porcina africana en Europa” (título original en inglés: *“understanding and combating African swine fever in Europe”*), se realizó una revisión sistemática para identificar las medidas disponibles para prevenir la introducción de la PPA en granjas de cerdo doméstico de la UE.

La estrategia de búsqueda incluyó palabras clave como "peste porcina africana", "medidas preventivas", "bioseguridad", "riesgo" y "granja de cerdos". Para garantizar la inclusión de otros documentos relevantes, como directrices técnicas, reglamentos u opiniones científicas, entre otros, la búsqueda de bibliografía también se realizó en Internet mediante un navegador común.

Después de aplicar los criterios de exclusión y realizar el proceso de revisión, se incluyeron 34 artículos científicos, 4 recomendaciones oficiales (es decir, información proveniente de autoridades gubernamentales), 4 informes, 5 opiniones científicas y 5 directrices técnicas.

Utilizando como base el documento de trabajo producido por la Dirección General de Salud y Seguridad Alimentaria (DG SANTE), las granjas domésticas de cerdos en la UE se clasifican en granjas comerciales, granjas no comerciales y granjas extensivas. Se identificaron un total de 37 medidas preventivas disponibles para granjas de cerdo doméstico. Posteriormente, se realizó una evaluación de la relevancia de las medidas identificadas dependiendo del sistema productivo presente. Así, 12 expertos en PPA pertenecientes a la UE evaluaron la importancia de cada medida.

Basado en las respuestas recibidas, la identificación de animales y registros de la granja, la prohibición de alimentar a los animales con restos alimenticios y la estabulación permanente de los cerdos a fin de no permitir los contactos directos o indirectos cerdo-cerdo y/o cerdo-jabalí, se consideraron tres medidas clave para prevenir la introducción de la PPA y su propagación en los tres tipos de granjas. Todos los expertos coincidieron en que la medida preventiva más importante para granjas no comerciales y extensivas es mejorar el acceso a los servicios veterinarios.

Algunos expertos sugirieron medidas preventivas adicionales, como es el uso de mosquiteras en las instalaciones donde se encuentran los animales, el establecimiento de programas de control de plagas o el cambio de calzado. Además, varios encuestados enfatizaron acerca de la importancia de algunas medidas ya incluidas en el cuestionario. Por ejemplo, establecer un vallado perimetral doble en granjas extensivas, programas de formación continuada de veterinarios y campañas de información para ganaderos, prestando especial atención a los signos clínicos y las rutas de transmisión de la PPA y evitar el uso de jeringas de forma compartida entre granjas.

Por tanto y a modo de conclusión, este estudio identifica medidas preventivas disponibles para los tres tipos de granjas de cerdo doméstico descritas en la UE. La implementación adecuada de estas medidas puede llevar a avances significativos en la prevención y el control de la PPA, y la posibilidad de contribuir a la erradicación de la PPA en el sector porcino de la UE.

Relevant measures to prevent the spread of African swine fever in the European Union domestic pig sector

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Summary

During the past decade, African swine fever (ASF) has spread from the Caucasus region to eastern European Union countries affecting domestic pig and wild boar populations. In order to avert ASF spread, mitigation measures targeting both populations have been established. However, despite these efforts, ASF has been reported in thirteen different countries (Georgia, Azerbaijan, Armenia, the Russian Federation, Ukraine, Belarus, Estonia, Latvia, Lithuania, Poland, Moldova, Czech Republic, and Romania). In the absence of an effective vaccine or treatment to ASF, introduction and spread of ASF onto domestic pig farms can only be prevented by strict compliance to control measures. This study systematically reviewed available measures to prevent the spread of ASF in the EU domestic pig sector distinguishing between commercial, non-commercial, and outdoor farms. The search was performed in PubMed and using a common browser. A total of 52 documents were selected for the final review process, which included scientific articles, reports, EU documents and official recommendations, among others. From this literature review, 37 measures were identified as preventive measures for the introduction and spread of ASF. Subsequently, these measures were assessed by ASF experts for their relevance in the mitigation of ASF spread on the three mentioned types of farms. All experts agreed that some of the important preventive measures for all three types of farms were: the identification of animals and farm records; strict enforcement of the ban on swill feeding; and containment of pigs, so as to not allow direct or indirect pig–pig and/or pig–wild boar contacts. Other important preventive measures for all farms were education of farmers, workers, and operators; no contact between farmers and farm staff and external pigs; appropriate removal of carcasses, slaughter residues, and food waste; proper disposal of manure and dead animals, and abstaining from hunting activities during the previous 48 h (allowing a 48 h interval between hunting and being in contact with domestic pigs). Finally, all experts identified that the important preventive measures for non-commercial and outdoor farms is to improve access of those farms to veterinarians and health services.

Keywords

Biosecurity, Europe, epidemiology, pig farm, preventive measures.

1. Introduction

African swine fever (ASF) is an infectious disease of swine notifiable in the European Union (EU) and to the World Organisation for Animal Health (OIE). Susceptible pigs can be infected by direct or indirect contact with infectious animals or their fluids, ingestion of contaminated animal feed, pork or pig products, or contact with contaminated surfaces or fomites (clothing, footwear, vehicles, farming tools, etc.) acting as mechanical vectors (30). In the southern and eastern parts of the African continent and the Iberian Peninsula, ASF can also be transmitted by biological vectors, infected soft ticks belonging to the *Ornithodoros* genus (41). No vaccine or treatment is available against ASF. Therefore, prevention and control of the disease is mainly based on the early detection of the disease by timely recognition in the field and efficient laboratory diagnosis, followed by the implementation of strict sanitary measures (38, 41, 196). Adequate implementation of sanitary measures will reduce the number of secondary outbreaks on domestic pig farms, which will decrease the potential contamination of the environment and, finally minimise the likelihood of infection in wild boar (118).

Since 1978 and until recently, the Italian island of Sardinia has been the only European ASF-infected area (48). However, in 2007, ASF was introduced into Georgia, from there it spread to neighbouring countries Azerbaijan and Armenia. As a result of the disease introduction and spread throughout the Russian Federation and Belarus, the EU strengthened its preparedness against ASF. Among the protection measures implemented by EU member countries bordering the Russian Federation were actions such as improving cleaning and disinfection of livestock vehicles, suspension of livestock markets, surveillance, enhanced biosecurity on farms, and awareness campaigns. Moreover, contingency plans were revised and the diagnostic capabilities of the EU labs were assured. However, ASF entered into four EU member countries in 2014, namely Lithuania, Poland, Latvia and Estonia; and in 2017, ASF was reported for the first time in Czech Republic and Romania (25). During this period, between January 2014 and December 2017, ASF outbreaks (occurrence of one or more ASF cases on a pig farm) were reported in over 250 farms, and more than 8,500 wild boar cases (an individual wild boar infected by ASF virus) were reported within the EU (25, 197-199). As a reaction to this large number of outbreaks and cases, the Community Veterinary

Emergency Team recommended several measures such as: i) focus surveillance on wild boar and domestic pigs, ii) implement control of animal movements, iii) safe disposal of wild boar carcasses, iv) avoid swill feeding practices, v) implement biosecurity on farms, vi) conduct awareness campaigns and finally, vii) review wild boar hunting practices (200). These measures were aimed at reducing the risk of spread of the disease to domestic pig farms and its transmission between wild boar populations. In contrast to what has been observed in non-EU European countries (*i.e.* the Russian Federation or Ukraine), in the EU scenario the number of infected farms has been comparatively lower, with wild boar being the most severely affected host (25, 198).

The main piece of legislation providing the tools for the control of ASF in the EU is the Council Directive 2002/60/EC (201), which establishes the minimum measures to be applied within the EU for the control of ASF. It includes the measures to be taken in the event of an outbreak of ASF on a pig holding and in cases where the disease is suspected or confirmed in feral pigs. The main objectives of controlling ASF in feral pigs are to reduce the risk of transmission to domestic pigs and to prevent it becoming endemic in the feral pig population (see definitions section) (198). The Directive lays down the measures to be taken in the infected area and the provisions to apply on the holdings of that area. All control and eradication measures applicable are based on classical disease control methods, which include surveillance, epidemiological investigation, tracing of pigs, and stamping out in infected holdings. These measures are applied in combination with strict quarantine and biosecurity measures on domestic pig holdings and animal movement control. The Directive also requires that Member States develop and implement plans for the eradication of the disease.

Moreover, specific regionalisation measures are laid down in Commission Implementing Decision 2014/709/EU (202). This Decision establishes animal health control measures on the movement, dispatch of pigs and certain pig products, and marking pig meat from the areas at risk of infection in order to prevent the spread of ASF to other areas of the Union. Affected Member States and territories are listed in different parts of the Annex to the Decision, the differentiation is made based on their epidemiological situation and level of risk. The Annex is divided into four parts, and territories that are listed in Part IV have a higher risk of spread of ASF than the ones listed in Part I. In determining the application of control measures on a certain commodity of a certain territory, the level of risk of that area and the type of commodity is taken into account. Indeed, in terms of risk of spread of ASF, movement of different porcine commodities pose different levels of risk. It is worth to mentioning that this Decision is also aimed at avoiding unnecessary disturbance to trade within the EU, as well as avoiding unjustified barriers to trade by third countries and the provisions that are set in this Decision are aligned with the OIE standards (200).

Bearing in mind all of the above, the aim of this study is to review described measures to prevent the introduction and further spread of ASF in the domestic pig sector focused on the EU scenario. An additional aim of this review was to assess the importance of these identified measures depending on the different pig farming systems (see materials and methods section). Adequate identification of relevant measures will allow for the creation of guidelines for pig producers to prevent the spread of ASF, which is one of the identified goals of the COST Action 15116 Understanding and combating African swine fever in Europe (ASF-STOP) supported by COST (European Cooperation in Science and Technology).

2. Materials and methods

2.1. Literature sources and search strategy

Following an approach similar to Rodriguez-Prieto *et al.* (203), the systematic review targeted preventive measures to avoid the spread of ASF in the domestic pig sector described in scientific publications, grey literature (materials produced by organisations outside the academic publishing channels), technical guidelines and international, national and regional regulations. The literature search was performed in March 2017, and supplemented with further search in December 2017 using PubMed database (http://www.ncbi.nlm.nih.gov/pubmed) for scientific articles. Scientific papers written in English (for reviewing convenience) between the last 39 years (1978 and 2017) were reviewed. A list of key words was combined into a Boolean query to identify titles and/or abstracts of documents of interest. The key words used (and any word containing the stem presented) were "African swine fever", "Preventive measure/s", "Biosecurity", "Risk", "Pig farm". The search terms applied were "African swine fever" AND [Preventive measure* OR Biosecurity OR Risk OR Pig farm]. To make sure other relevant documents such as technical guidelines, regulations, or scientific opinions, among others, were included, the literature search was performed following the same query on the internet using a common browser.

2.2. Definitions

'Control measures' are defined as the best/safest options to eliminate or reduce specific risks while 'preventive measures' are actions taken to avoid specific risks (204). As the glossary of the Terrestrial Animal Health code of the OIE states (205), 'biosecurity' means a set of management and physical measures designed to reduce the risk of introduction, establishment and spread of pathogenic agents to, from and within an animal population. On the other hand, 'risk' means the likelihood of the occurrence and the likely magnitude of the biological and economic consequences of an adverse event or effect to animal or human health (205).

Based on the working document SANTE/7113/2015-Rev 7 produced by the Directorate-General for Health and Food Safety (206) pig farming systems and subsequently, pig farms can be classified as: i) 'commercial farms' which refers to farms that sell pigs, send pigs to a slaughterhouse or move pig products off the holding, ii) 'outdoor pig farms' which refers to farms in which pigs are kept temporarily or permanently outdoor, and iii) 'non-commercial farms' which refers to farms where pigs are kept only for fattening for own consumption and neither pigs nor any of their products leave the holding. Elsewhere, this last type of farm is referred as 'family farms' (207) or 'backyard farms' (208). Commercial farms can be divided into multi-site farms which are holdings specialised on one production step (farrowing, nurseries or finishing) and on-site farms which are premises that produce all production steps (209). Moreover, 'feral pig' or 'free-ranging pig' means a pig which is not kept or bred on a holding according to the Council Directive 2002/60/EC (201). In Sardinia, free-ranging pigs are usually referred as 'brado' (168, 207).

2.3. Study selection

A two step-process was followed to select the literature relevant for the aim of this review. A primary exclusion criteria was applied when reading title and abstract of found literature (abstract when available): i) published before 1978; ii) not related to the theme of this review; iii) not related to the European scenario, iv) repeated document (already selected among retrieved results). If abstract were not available, the piece of literature would be kept for the next stage. Then, the full text of each selected piece of literature was screened. As a second exclusion criteria, documents v) which full text was not available; vi) no preventive measures were described; vii) described preventive measures were not focused on ASF or viii) information on the theme was insufficient, were excluded. The explained process was individually performed by three reviewers following the mentioned exclusion criteria in order to cross-check selected literature and resolve any disagreement.

2.4. Assessment of described preventive measures

A group of experts was invited to participate in an expert opinion session to assess the preventive measures identified in this review.

Participants belonging to the COST (European Cooperation in Science and Technology) action: 'Understanding and combating African swine fever in Europe' (ASF-STOP) supported by COST (COST Action 15116) (https://www.asf-stop.com/stsms/) were encouraged to suggest experts with relevant expertise in ASF prevention, ASF control and eradication, ASF epidemiology and the EU domestic pig sector.

Before starting the assessment, the list of measures were reviewed by authors to ensure measures were accurate and clear, as well as no measures were omitted. Table 2 summarises the list of preventive measures used in the expert opinion session (see Table 2 at the end of the publication). In total, 20 experts were invited to participate and contacted by email, 12 of them returned their responses.

Experts were asked to assess the relevance of each described preventive measure by answering yes or no to the closed question: “Is this measure important for commercial, non-commercial, and outdoor-farms?” ‘Importance’ was defined as the perceived need for each measure. Experts were asked to perform this assessment within the EU context. Moreover, experts were encouraged to suggest additional measures if they thought they were missing. Results were recorded in an Excel datasheet (Microsoft Corp. Redmond, Washington USA).

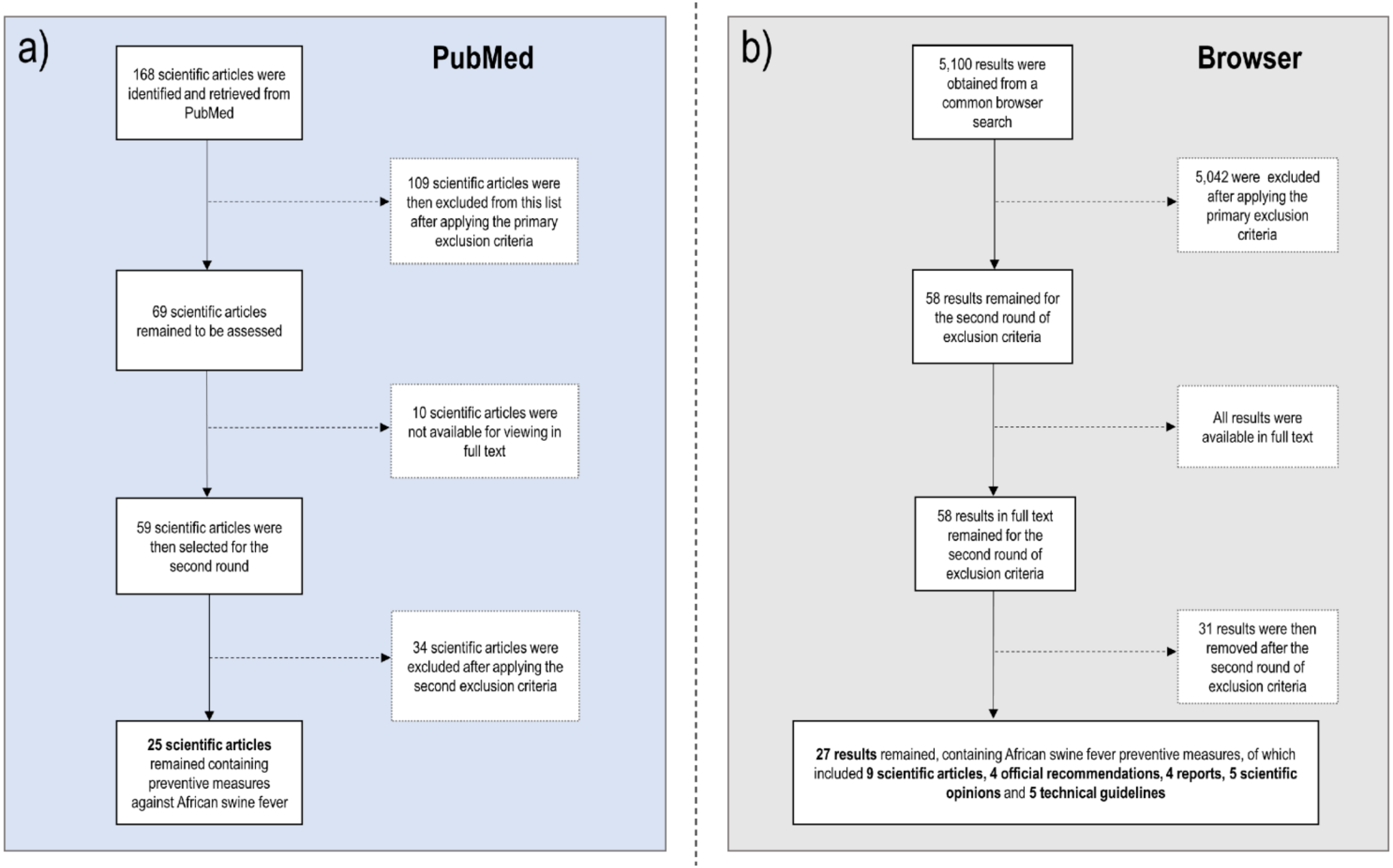
3. Results

3.1. Selection process

Figure 1 shows the literature selection process and Table 1 compiles the selected literature (see Table 1 at the end of the publication). The search made on PubMed database returned 168 scientific papers. After applying the primary exclusion criteria, 69 were selected for the second step of the review. However, the full text was not available for 10 of them. Therefore, 59 scientific articles were selected for the second screening round. The same search on a common browser returned 5,100 results of potential interest. By applying the primary exclusion criteria, 58 results were selected for the second round, all of them had available the full text.

After applying the second exclusion criteria and completion of the screening rounds, 34 articles (25 retrieved from PubMed and 9 retrieved from the browser search), 4 official recommendations (meaning information coming from governmental authorities), 4 reports, 5 scientific opinions and 5 technical guidelines were included in the review. The rest of the documents including reports, recommendations and guidelines were retrieved from the browser search.

Figure 1: Flowchart summarising the literature search selection process (A) on PubMed database and (B) on a common browser



3.2. Results from the systematic review

Preventive measures described hereinafter were obtained from the 52 pieces of literature selected during the systematic review. These measures were classified in four different groups: general prevented measures suggested for all types of farms (as some of them were common for commercial, non-commercial and outdoor farms), and three groups of suggested measures for each of the identified types of farms.

3.2.1. General preventive measures

The risk of introduction and exposure to ASF depends on the epidemiological characteristics of the country, area and type of farm (51, 71, 210-215). Pig production in Europe is highly heterogeneous with different biosecurity standards and productive levels (216, 217). Actions to prevent ASF introduction and spread should take into consideration the epidemiology of the disease, with especial focus on the virus resistance in the environment, routes of transmission and excretion as well as the characteristics of the farming systems in place (34, 43, 118, 218, 219). As no vaccine for ASF is available, prevention of ASF relies upon implementing strict biosecurity measures to avoid potential contact between domestic pigs and ASF virus (218, 220-222).

In the EU, movements of pigs or pig products coming from infected areas has been prohibited to prevent ASF spread (196, 201, 223). Moreover, the presence of infected wild boar in the area and its hunt constitutes an additional source of risk that cannot be discarded (67, 222). Minimum biosecurity requirements to apply during hunting in the affected territories have been proposed (118, 206, 224). First of all, hunters shall be authorised to hunt after receiving training on basic biosecurity practices. Hunted wild boar should be tested and only released after receiving negative results. Hunted animals should be moved to the dressing facilities in dedicated vehicles, private cars should be parked outside the hunting field. Dressing facilities would be used if they have tap water, electricity, freezers and waste water collection. Evisceration should be performed with gloves at the dressing facilities and hands gently washed with soap and water. Offal should be stored in proper containers in the dressing area and then, cleaned and disinfected. Clothing, footwear and hunting equipment should be cleaned and disinfect after each use (clothing washed at 60 °C).

Finally, contact with domestic pigs should be avoided, allowing a 48 hour interval between hunting and being in contact with domestic pigs. All of above the needs to be implemented together with education and training campaigns to get hunters involved in control strategies as much as possible (118). Thoen *et al.* (225) and Sanchez-Vizcaino *et al.* (226) also suggested that systems that wild boar can use as artificial feeding places (feeders, water holes, supplementary feeding of ungulates) should be avoided, as these systems can significantly increase wild boar abundance and spatial concentrations. However, it has been also suggested that this ban may be deemed effective only in regions where the habitat is unsuitable for wild boar and where feeding caused artificial population establishment (65).

The EU Commission has established minimum biosecurity requirements for commercial, non-commercial and outdoor farms (227). Health status and free-ASF certificates have to be checked before acquiring new animals (21, 206, 207, 209, 218, 227, 228). On breeding farms, semen (195, 223, 228), embryos or ova should come from free-ASFV certified farms (53, 201, 206, 229). Visits should be discouraged (228, 229), limiting access to the farm and animals, to workers and veterinarian services (47, 71, 118, 227). If visitors enter the farm, visits should be registered and visitors should follow strict biosecurity measures regarding footwear and clothing (21, 47, 53, 218, 228, 230). Farm staff should follow the same biosecurity procedures. Likewise, workers and owners should be aware and well trained with regard to ASF (34, 43, 44, 47, 53, 173, 227, 230, 231) as well as veterinarians and operators along the market chain (218). Moreover, farm staff must not have contact with animals from other pig premises nor own pigs (53, 118, 196, 206, 207). In addition to this, the Finnish Food Safety Authority recommends that farm staff should not directly enter the farm after visiting a farm abroad, they should wait at least for 48 hours (232).

Regarding physical barriers on farms, animals should be kept in a way that ensures that no direct, nor indirect contact occurs with wild boar, feral pigs, or domestic pigs coming from other premises (206, 227). Additionally, perimeter fences should delimitate the commercial holding to prevent such contact (206). On outdoor farms, fences will be preferably doubled (227), at least 1 metre apart (53), and proofed against wild boar and pigs (35, 206, 207, 209, 228, 230). Fences should be at least 2 metres high of which 50 cm should be under the ground (233).

In addition, as part of good farming practices, carcasses, discarded parts from slaughtered pigs and food waste should be disposed in accordance with Regulation (EC) No 1069/2009 (53, 218, 227, 234). Sharing equipment between holdings should be discouraged (6, 47, 53, 227, 228, 230), and footbaths should be used at the entrance of every unit where animals are held (47, 206, 227). Organic material should be removed from footwear prior to disinfecting (221). Animals must be checked at least once a day paying special attention to mortality rates and clinical signs compatible with ASF (228). Moreover, cleaning and disinfection protocols should be established and periodically performed on every farm facility, vehicle, and piece of equipment (6, 53, 206, 207, 220, 227, 229). Disinfectants effective against ASF virus include 2% caustic soda, 2% sodium hypochlorite, 0.3% formalin, 3% ortho-phenylphenol and iodine compounds, among others (218, 235). Organic material (faeces, feed, bedding materials) should be completely removed to maximise the efficacy of disinfection (221).

Moreover, regarding the location of pig farms, several scientific publications point out that farms should be located far from suitable wild boar areas and close to geographical barriers (such as mountains, rivers, etc.) (34, 65, 66, 207). Finally, Mellor *et al.* (194) observed experimental transmission of ASF through *Stomoxys calcitrans* flies. Therefore, given this potential role of stable flies as mechanical vectors, it has been suggested that sanitation, biological, and chemical controls should be applied to suppress stable flies. As an example, commercial and non-commercial farms could eliminate fly breeding sites in combination with placing insecticide-treated nets to reduce the potential risk posed by flies (32, 228).

Specific preventive measures based on biosecurity have been proposed depending on the type of farm: commercial, outdoor or non-commercial (206).

3.2.2. Specific measures focusing on commercial farms

Commercial farms are significantly larger in size and number of animals (209) and so, the economic and animal health impact of ASF is greater than on outdoor and non-commercial farms (219, 236).

Key measures to prevent the introduction of ASF on commercial farms are to establish clear clean/dirty areas for personnel including changing rooms and shower (6, 65, 71, 206, 209, 221), and to review logistical arrangement for entry of new animals. This measure will allow for the adequate identification of critical control points (206), which is particularly relevant since contaminated vehicles transporting pigs or carcasses are associated to a high risk of disease transmission (209, 213).

Several steps should be included when preparing a protocol for animal transport. First, farms should be designed to allow deliveries without entering the farm (118, 227, 228). If it is not possible, decontamination of vehicles is necessary before entering the farm (35, 218, 232). Employees involved in pig transport should not come in contact with farm workers nor with animals (118, 228). If other drivers (*i.e.* animal feed suppliers) need to enter the farm, footwear should be changed, cleaned and disinfected when entering the farm and again before getting into the vehicle (228). Moreover, parking areas should be designed to avoid cross-contamination between workers and farm vehicles. In case vehicles have to enter into the farm, loading and unloading areas should be placed at least 20 m away from animal facilities within the perimeter of the farm (233). Vehicles transporting pigs and other vehicles must be cleaned and disinfected before and after each use (228, 232). Returning trucks should be cleaned and disinfected at the farm where pigs are unloaded (228). In addition to this, the Danish regulation applies a 48 hour quarantine period before the next movement of animals (123). After that new animals should be kept in quarantine rooms (6, 27, 207, 220) between 14-30 days (21, 118, 209, 218, 228). Quarantine rooms should be located away from the main herd (228).

Furthermore, animals should be identified and all animal movements recorded (166, 173, 206, 228, 237); farm records should be ensured to easy track animals if an outbreak is reported; births and deaths, animal census, entry and exit of animals (live and dead), vehicles, visits, pest control, or cleaning and disinfection procedures should be properly registered in a farm record book (6, 35, 207). Moreover, internal audits or self-evaluation need to be periodically conducted to enforce biosecurity measures (206, 209). Furthermore, a set of rules on food for workers entering the farm should be clearly specify (71) and food should be restricted to eating rooms (206, 209) or not allowed (47, 229).

Finally, proper disposal of manure as well as dead animals and other removable material should be ensured (35, 118, 218). Containers and storage basins should accomplish with the minimum requirements for storage capacities recommended by the Best Available Techniques (238).

3.2.3. Specific measures focusing on non-commercial farms

Backyard farms are characterised by limited farming management practices and nearly absent biosecurity levels (6, 48, 118). This type of farm is common in countries such as Romania (239), Bulgaria (214), Poland (240) or Sardinia (Italy) (48, 173), among others. Non-commercial farms are built for own consumption purposes, investment is minimum and animals could be fed on kitchen leftovers (241). Depending on the country and local practices, pigs are allowed to move freely (without physical restrictions) during the day or even scavenge for days or months (23, 51, 239). Pig slaughtering is usually carried out on the farm, although it may be restricted to proper slaughterhouses if there are local regulations on this issue (207, 239).

Specific measures focusing on these farms have been proposed, swill feeding practices are not allowed (47, 53, 206, 228, 230, 231, 242), as ASF can be transmitted through ingestion of contaminated raw pork or pork products (13, 41, 118, 206). Pigs should be kept in animal facilities ensuring no contact with domestic pigs from other non-commercial farms, feral pigs, wild boar nor their products (6, 118, 206, 242). If there were infected wild boar in the area, the owner or the person in charge of taking care of the pigs should allow a 48 hour interval between hunting and being in contact with domestic pigs (206, 224) and should not use dogs during hunting (224). Any hunting equipment used as well as the dog's coat should be cleaned and disinfected (232). Effective disinfectants such as calcium hydrate (slaked lime), should be spread and renewed around the holding including its entrance (118). A veterinarian needs to supervise home slaughtering activities (170, 206). If a slaughterer comes to slaughter the animals, cleaned and disinfected clothing and footwear should be provided. Cleaning and disinfection protocols have to be applied after slaughtering on the facilities and to the slaughter tools (206, 207). The Directorate-General for Health and Food Safety and the Sardinian regulations agree that sows or boars cannot be held on non-commercial farms for mating purposes (170, 206) while Decision 830/2016 of the Romanian Government states that sows and boars might be present but they cannot be moved between holdings for matting purposes (242), movements from these farms are neither allowed in the Sardinian regulations (170). Furthermore, governments and institutions are encouraged to promote educational programs as well as improve access to health services on non-commercial farms (173, 196, 206). This measure is one of the novelties of the latest eradication program launched in Sardinia (168).

Moreover, the use of fresh fodder harvested in areas at risk for ASFV exposure should be avoided (65, 66, 206), as its consumption has been observed that could be related to ASF outbreaks in Eastern EU countries (65). If this is not possible, Directorate General for Health and Food Safety (206) recommends to perform treatments on grass or grains to inactivate ASFV or store them, out of reach of wild boar, for at least 30 days. In Estonia, according to the Regulation of the Minister of Agriculture No 179, it is forbidden to bring green fodder to the farm (230). Likewise, Directorate General for Health and Food Safety (206) recommends to avoid using straw as bedding material unless treated to inactivate ASFV or stored for at least 90 days (206). Additionally, the Estonian Veterinary and Food Board established as compulsory biosecurity rule, no exchange feed and bedding material with other farms (230). Field experiences showed that no additional cases were reported when non-commercial farm had feed from reliable sources and contact with infectious free-ranging pigs was prevented (6).

3.2.4. Specific measures focusing on outdoor farms

The number of outdoor farms is increasing in Europe due to a growing interest in organic farming systems (243), particularly from pork consumers due to animal welfare concerns. Simultaneously, veterinarians and pig producers have been urging for improvements in biosecurity, so as to avoid health threats (244). Depending on the country and local practices, outdoor pig production may vary from outdoor farms that implement several biosecurity measures (245), to free-ranging herds where biosecurity is absent (48).

Spain is a good example of a country with strict biosecurity standards for outdoor pig production. Regulations regarding biosecurity on outdoor pig farms (246) are a result of the presence of ASF for more than 30 years in the Iberian Peninsula (35). Applied control and preventive measures allowed to eradicate ASF from outdoor pig production and avoided new introductions on outdoor farms, despite the constant threat posed by the presence of infected wild boar and infectious *Ornithodoros* ticks in the surroundings (35, 196). In other areas such as Sardinia in Italy, pigs are allowed to range free in public forests during the day, for days or even months under no biosecurity measures (191). Free-range management practices in communal areas has been identified as a dangerous practice for the persistence and re-emergence of ASF in endemic areas like

Sardinia (48, 51, 207). During the free-ranging period, pigs might be in contact with wild boar and pigs belonging to different herds (23, 166, 177). For this reason, free-range management practices in communal areas or public forest with no biosecurity measures nor veterinary control have been banned (118, 168, 173, 207), such as in Sardinia since 2012 (168).

Bearing in mind the current situation in Eastern Europe, the EU Commission has banned outdoor keeping of pigs as the main strategy to avoid ASF spread (206, 230). Although prevention becomes challenging in outdoor and semi-extensive pig production (51), several preventive measures can be implemented to ensure biosecurity levels. For instance, the territories/fields where animals are allowed to range free should be fenced (double fenced, if it is possible) to avoid the entrance and direct contact with wild boar, feral pigs, and other domestic pigs, as well as people and vehicles (6, 118, 221). Sardinian regulations state farms should have perimeter barriers of at least 1.5 m high and wild boar proofed and fenced fields had a maximum extension of 3 hectares (170). Outdoor farms should be separated from other outdoor farms to reduce the risk of ASF introduction through direct or indirect contact (246). This minimum distance between farms will vary depending on national and local regulations. If pigs were free to roam within no fenced fields, distance would become irrelevant (209).

So far, *Ornithodoros* ticks have not been implicated in the transmission of ASF in Eastern nor Central Europe (13). In Sardinia, ticks have also not been identified as a major transmission source (50). Several preventive measures were described in Portugal and Spain were *O. erraticus* are present such as keeping traditional pig-housing facilities (typically, used in outdoor production), in good repair, otherwise it is recommended to fence them or destroy them if ticks are present (21, 35, 247). In case ticks are present, either chemical control with methylene bromide should be applied on the facilities, or treating pigs with an ivermectin treatment (247). If infected ticks were present in such constructions, it is not recommended to use the infested buildings (248) or keep these buildings empty for 6 years (201). Nevertheless, it should be considered that eradication of *O. erraticus* ticks is extremely difficult due to the long life of ticks, long survival without feeding, presence of accidental hosts and possibility of penetrating into cracks and surfaces not accessible to acaricides (248).

Table 2 compiles the general preventive measures and specific preventive measures for commercial, non-commercial, and outdoor farms described in this review (see Table 2 at the end of the publication).

3.3. Assessment of the importance of described preventive measures

A total of 12 experts participated in the assessment of the importance of identified preventive measures. All of them completed the questionnaire and therefore, their responses were included in the analysis. Around 3% of assessed measures (2.85%) were categorised as “not applicable” preventive measure.

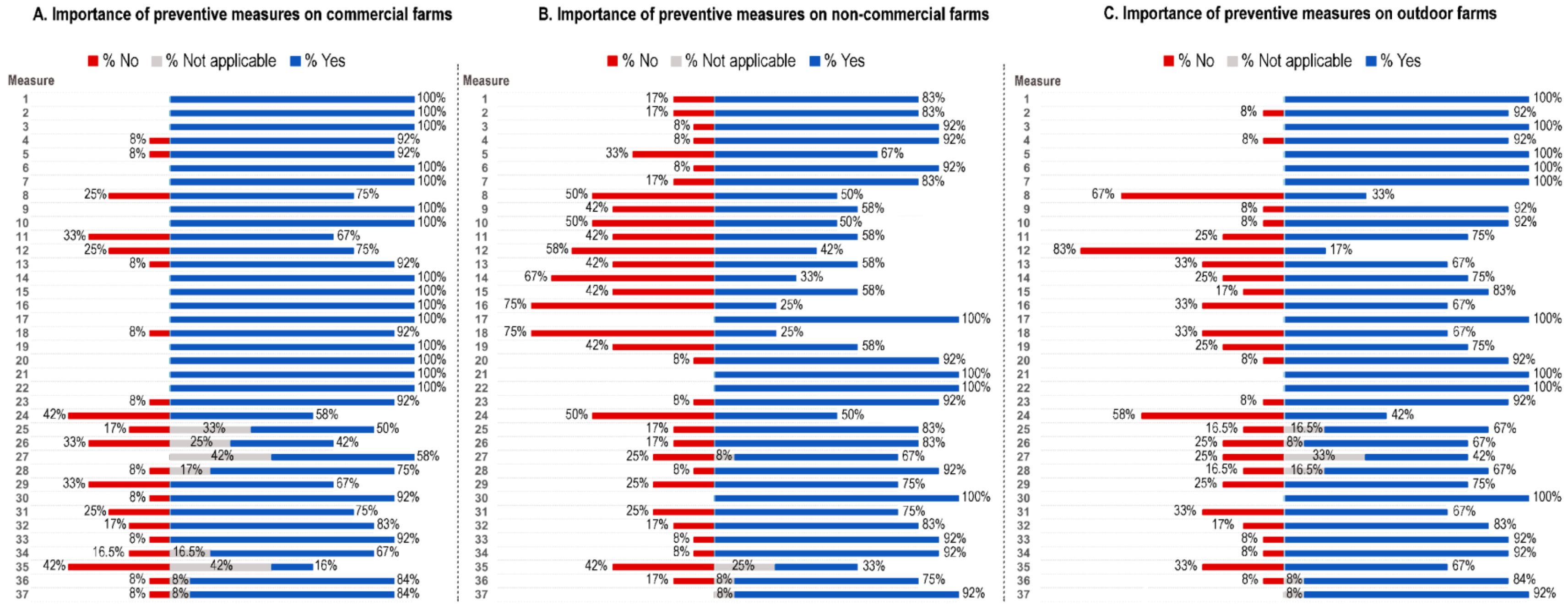
There was 100% agreement among experts (twelve experts out of twelve) that the identification of animals and farm records including animal movements; enforcement of the ban on swill feeding; and containment of pigs to not allow contact with pigs from other farms, feral pigs or wild boar or their products, were important preventive measures for the three types of farms (commercial, non-commercial and outdoor). Other important preventive measures identified for all farms were education of farmers, workers, and operators; no contact between farmers and farm staff and external pigs; appropriate removal of carcasses, slaughter residues and food waste; proper disposal of manure and dead animals; and a 48 hour (minimum) interval between hunting and being in contact with domestic pigs for all farm staff, particularly those who work in an infected wild boar area.

Moreover, all experts identified as important preventive measures for non-commercial and outdoor farms, to improve access of those farms to veterinarians and health services. Between 8 and 9 of experts considered that logistical arrangement for the entry and exit of animals including protocols regarding entrance of vehicles, loading areas and role of pig transporters; quarantine period for purchased animals and quarantine rooms; and internal audits and evaluations to enforce biosecurity measures, were not important preventive measures for non-commercial farms. In addition, 10 experts concluded that control measures against flies was not an important preventive measure on outdoor farms.

Additional preventive measures were suggested by some experts such as the use of nets on animal facilities; establishment of pest control programs on farms; use of carbonic dioxide traps to check the presence of *Ornithodoros* ticks and change of boots before entering the farm and units. Furthermore, several respondents wanted to emphasise the importance of measures already included in the questionnaire. For instance, establishment of double fencing perimeter on outdoor farms; education of swine veterinarians and farmers paying especial attention to clinical signs and transmission routes; and discouragement of using the same injection syringes and instruments on different farms unless thoroughly disinfected-sterilised.

Figure 2 summarises results obtained for preventive measures on commercial, non-commercial and outdoor farms.

Figure 2: Results of the assessment of identified preventive measures represented as percentage of yes (blue bars), no (red bars), and not applicable (grey bars) to (A) commercial farms, (B) non-commercial farms, and (C) outdoor farms. Listed preventive measures are described in Table 2



3. Discussion

In the absence of an effective vaccine, prevention is the main tool to avoid further spread of ASF or an endemic situation. Both the systematic literature review as well as the expert opinion elicitation, highlighted three main areas where preventive measures would be very relevant to halt ASF spread in the domestic pig population: 1) control of entries into the farm; 2) control of pigs' feed, and 3) improvement of health services and education.

The first main area of prevention encompasses both the movements associated to production as well as the potential spill-over from infected wild boar in the surrounding areas. Both have been major drivers of spread in the current ASF epidemic in Eastern EU, where the majority of ASF notifications in domestic pigs have occurred in backyard or small commercial farms with limited biosecurity (200). The identification of animals and the containment of pigs were also identified by the experts as important preventive measures for all type of holdings. Quarantine period for purchased animals in quarantine rooms was identified as a relevant measure for commercial farms by 12 experts. In agreement with this result, experts in Switzerland perceived that purchasing from farms with known disease status and health certificates as 5/5 for importance and effectiveness as a biosecurity measure to prevent the introduction of ASF onto pig farms (219). Interestingly, nine experts out of eleven did not consider this measure important on non-commercial farms. Although, the same number consider it important to check ASF-free certificates and health status before acquiring new animals. This may be explained because the feasibility of quarantine periods and establishment of quarantine rooms and procedure could be challenging on non-commercial farms where investment and facilities are minimum. This measure becomes particularly relevant when tackling the phenomenon of “emergency sale”, in which farmers from non-commercial holdings attempt to sell infected pigs to minimise their economic losses (240, 249-251). The latest working document elaborated by the Directorate General for Health and Food Safety (206), which contains the majority of measures reviewed in the systematic literature review, aim at the improvement of biosecurity measures dealing with the replacement of animals, facilities design and management practices, in particular in relation with cleaning and disinfection facilities, in such holdings. Very few outbreaks have led to secondary spread in the EU and there has been a significant progress in EU advice to improve preventive measures against ASF in non-commercial farms.

The Eastern EU scenario presents the additional challenge of spill-over from wild boar, where 95% of the ASF notifications have taken place (197) and which is playing a primary role in disease spread. However, additional measures were extracted during the review process (see Table 1 at the end of the publication). In Poland and Latvia, outbreak investigations carried out on several ASF positive farms determined that the most likely source of infection was wild boar (119, 222). Studies concluded that the poor biosecurity measures of affected holdings favoured transmission between wild boar and domestic pigs (119, 222). Consequently, the EU elaborated a guidance where minimum biosecurity measures on farms were defined and biosecurity was enhance to minimise the risk of spread from wild boar (206, 227). One of the suggested measures found in the literature is ‘to locate farms far from suitable wild boar areas and close to physical barriers’ (34, 65, 67, 207) since there is a disease interface where domestic pig and wild boar share location. Observations related to the wild boar - domestic pig interface indicated that all ASF notifications in domestic pig holdings were situated in areas with suitable wild boar habitat (65). Around 65% occurred in natural landscapes, the natural habitat for wild boar (66). The remaining 35% were located in mosaic agroforestry areas and buffer monoculture areas surrounding natural landscapes where agro-livestock activities are usually concentrated (66). In these areas, wild boars can receive, with minimal foraging, substantial amounts of protein from cultivated plants such as maize, wheat, barley, rapeseed and sunflower seeds (252). Farm location far from suitable wild boar areas and close to geographical barriers was classified as important by more than half of experts. As expected, such measures were relevant to more experts on outdoor farms (9 experts), followed by commercial (8 experts) and non-commercial holdings (7 experts). This slight difference might be explained because the likelihood of wild boar being in contact with pigs would be higher on outdoor farms (where biosecurity is intrinsically lower) than on commercial or non-commercial farms. Experts who declined to consider it important, refereed that this measure is almost unfeasible considering the ecological characteristics of the European continent. Moreover, some of the experts who considered it important, wanted to highlight that such a measure would only be applicable to new holdings.

Most experts (eleven out of twelve) recognised the importance of allowing a 48 hour interval between hunting and being in contact with domestic pigs if farmers and farm staff worked in an infected wild boar area. Although it is not the scope of this article to cover the control measures in wild boar, the management of wild boar populations and hunting practices in affected areas has an undeniable effect over the prevention of ASF at the interface with domestic pigs located in the same area. Such measures have included the reduction of wild boar densities (65, 253) and the immediate removal of infectious carcases (118). However, wild boar cases have continued being notified in the area suggesting that there is still room for improving the strategy.

The second main area of prevention deals with avoiding ASF transmission through the ingestion of contaminated food. Even if swill feeding is banned in the EU, all experts agreed that it was an important measure to prevent ASF spread. Other measures identified in this sense are rules on food entry for farm workers in commercial farms; proper disposal of manure and dead animals; avoiding the use of fresh fodder from areas at risk of ASF unless a treatment to inactivate potential ASF virus, has been applied; or avoid sharing feed between farms. Long distance ASF transmission has been associated to the disposal of infected waste, meat or meat products in wild boar habitat, for example, in the Czech Republic, where the closest ASF cases were about 400-500 km away. Moreover, evidences of domestic pigs and/or pig sub-products as source of infection are scarce but they have been suspected in a few cases, like in Romania. On July 31, 2017, Romania's Veterinary Authority confirmed the first detection of ASF in a backyard herd of domestic pigs. Romania's Veterinary Authority suspects that contaminated Ukrainian products are the likely source of the Romanian detection (254). Human mistakes, lack of knowledge on ASF transmission, or insufficient enforcement are the most common reasons to fail to comply with these measures, particularly for non-commercial farms, and are directly related to the third main area of ASF prevention: improvement of health services and education.

Better access to veterinary health services and educational programmes, with specific training on ASF identification and biosecurity measures, are essential tools to improve human-mediated prevention measures. More than 11 experts agreed with this idea, considering both measures important for non-commercial farms but also, for commercial and outdoor facilities. In the end, effectiveness of prevention depends on awareness, compliance and diligence of people dealing with disease control and good timing of implemented measures (70). The effectiveness of prevention is also influenced by socio-economic, cultural or traditional factors that will predispose the capability, attitudes or willingness of people involved in disease control to implement preventive strategies. The understanding of such factors is particularly critical for backyards and small farmers, since economic and resources restraints can more easily limit the achievement of the preventive measure objective (207, 249). Generally, the effectiveness of preventive measures will be related to how farmers perceive the importance of each measures as well as what measures they are actually implementing (6). Farmers and workers are at the forefront of implementing biosecurity measures on the farms to prevent the spread of diseases. The application of these measures heavily depends upon the attitude and knowledge they have with regard to biosecurity measures (255). A study carried out in Great Britain showed that English pig farmers had poor knowledge about ASF as well as limited concern about it (256). Vergne *et al.* (257) also highlighted that the reasons for lack of immediate reporting in suspected ASF cases in Germany, the Russian Federation and Bulgaria would be due to not knowing reporting procedures, fear that the report could have a negative impact on their reputation, and assuming they would be capable of handling the outbreak on their own. These studies (256-258) suggested that there is still room for improving farmers' knowledge to bridge the gap between authorities and farmers, and consequently help prevent the spread of ASF (217). Similarly, to be able to effectively influence farm workers, veterinarians and hunters' behaviour, it is essential to analyse the "at-risk" practices that depended on human behaviour which can perpetuate ASF spread, and find out measures tailored to each specific situation.

From the research side, efforts have been made to fill in gaps that make disease control and eradication difficult. A recent publication identified current gaps in ASF and prioritised them into high importance, medium importance, low importance (34). Highest importance was attributed to measures aimed at improving prevention and control of ASF, namely i) to raise awareness among hunters, farmers and veterinarians; and ii) to have adequate implementation of early warning systems, contingency plans, and control measures. Preventive measures of medium importance were iii) to implement surveillance activities based on the risk of potential exposure, introduction and spread. Measures of low importance were iv) to promote confinement of pigs in infected areas, and v) to establish regulations to ensure farms are located far from areas suitable for wild boar. Finally, with regard to the importance of wild boar in ASF epidemiology, more research should be focused on vi) increasing the availability of reliable population data, vii) understanding role of this host in disease maintenance and spread, and viii) developing non-invasive sampling methods (34, 226, 253). However, without an ASF vaccine, prevention of ASF becomes very challenging for the European pig sector. Despite advances, a safe and effective vaccine is still lacking. Thus, control and eradication of this disease still relies on rapid detection in field followed by the application of strict sanitary measures. Likewise, biosecurity is the only tool farms have to prevent the introduction of ASF. Therefore, joined efforts focusing on the domestic pig sector and wild boar need to be applied in parallel. This way, we will move forward to the final goal of eradicating ASF from the second largest world’s pork producer, the EU.

4. Conclusion

ASF is currently one of the major threats to the pig production in the EU. As there is no a vaccine against ASF, biosecurity is key to prevent its spread between and within domestic pig farms. This study identified thirty-seven preventive measures aimed at reducing the spread of ASF among domestic pigs. These measures were also assessed by ASF experts within the framework of the EU scenario. According to this expert panel, the most important preventive measures for commercial, non-commercial and outdoor farms were the identification of animals and farm records; enforcement of the ban on swill feeding; and containment of pigs to not allow contact with pigs from other farms, feral pigs or wild boar or their products. In addition to this, other measures were considered relevant in preventing ASF introduction, namely education of farmers, workers and operators; no contact between farmers, farm staff and external pigs; appropriate removal of carcasses, slaughter residues and food waste; proper disposal of manure and dead animals, and abstention from hunting activities for a period of 48 hours prior to any contact with domestic pigs. Finally, all experts considered important to facilitate and promote the access of veterinarians and health services to non-commercial and outdoor farms. Adequate implementation of these measures can lead to significant advances in ASF prevention and control, and possibility contributing to the eradication of ASF from the EU pig sector.

Table 1: Pieces of literature included in the review process.

ID	Title	Search	Type	Reference
1	African and classical swine fever: similarities, differences and epidemiological consequences	PubMed	Article	(223)
2	Why is African swine fever still present in Sardinia?	PubMed	Article	(207)
3	African swine fever in eastern Europe: the risk to the UK	PubMed	Article	(231)
4	Understanding African swine fever infection dynamics in Sardinia using a spatially explicit transmission model in domestic pig farms	PubMed	Article	(173)
5	Control of African swine fever epidemics in industrialised swine populations	PubMed	Article	(236)
6	Preventive measures aimed at minimising the risk of African swine fever virus spread in pig farming systems	PubMed	Article	(118)
7	Modelling African swine fever presence and reported abundance in the Russian Federation using national surveillance data from 2007 to 2014	PubMed	Article	(125)
8	English pig farmers' knowledge and behaviour towards African swine fever suspicion and reporting	PubMed	Article	(256)
9	Simulating the epidemiological and economic effects of an African swine fever epidemic in industrialised swine populations	PubMed	Article	(123)
10	A cartographic tool for managing African swine fever in Eurasia: mapping wild boar distribution based on the quality of available habitats	PubMed	Article	(66)
11	Transmission routes of African swine fever virus to domestic pigs: current knowledge and future research directions	PubMed	Article	(30)

12	Expert opinion on the perceived effectiveness and importance of on-farm biosecurity measures for cattle and swine farms in Switzerland	PubMed	Article	(219)
13	Spatio-temporal analysis of African swine fever in Sardinia (2012-2014): trends in domestic pigs and wild boar	PubMed	Article	(177)
14	Statistical exploration of local transmission routes for African swine fever in pigs in the Russian Federation, 2007-2014	PubMed	Article	(71)
15	Evaluation of the risk factors contributing to the African swine fever occurrence in Sardinia, Italy	PubMed	Article	(166)
16	Spatio-temporal modeling of the African swine fever epidemic in the Russian Federation, 2007-2012	PubMed	Article	(259)
17	Thirty-five-year presence of African swine fever in Sardinia: history, evolution and risk factors for disease maintenance	PubMed	Article	(48)
18	The medical and veterinary role of *Ornithodoros erraticus* complex ticks (Acari: Ixodida) on the Iberian Peninsula	PubMed	Article	(247)
19	Pig producers urged to review biosecurity as ASF and PED spread	PubMed	Article	(220)
20	African swine fever in the North Caucasus region and the Russian Federation in years 2007-2012	PubMed	Article	(27)
21	African swine fever (ASF): five years around Europe	PubMed	Article	(44)
22	African swine fever: an epidemiological update	PubMed	Article	(43)
23	Qualitative risk assessment in a data-scarce environment: a model to assess the impact of control measures on spread of African swine fever	PubMed	Article	(217)

24	Viruses in boar semen: detection and clinical as well as epidemiological consequences regarding disease transmission by artificial insemination	PubMed	Article	(195)
25	Temporal and spatial patterns of African swine fever in Sardinia	PubMed	Article	(191)
26	Do not bring African swine fever to Finland	Browser	Official recommendation	(232)
27	African swine fever facing Romania	Browser	Report	(242)
28	African swine fever	Browser	Official recommendation	(229)
29	African swine fever – Guidance	Browser	Official recommendation	(228)
30	Guidelines for the cost effective prevention and control of African swine fever	Browser	Report	(239)
31	African swine fever in Poland and Baltic countries	Browser	Report	(230)
32	Gaps in African swine fever: analysis and priorities	Browser	Article	(34)
33	African swine fever (ASF)	Browser	Article	(221)
34	African swine fever: new challenges and measures to prevent its spread	Browser	Article	(226)
35	African swine fever	Browser	Scientific opinion	(260)
36	Review of African swine fever: transmission, spread and control	Browser	Article	(47)
37	African swine fever: how can global spread be prevented?	Browser	Article	(196)
38	African swine fever	Browser	Scientific opinion	(65)
39	Epidemiological analyses of African swine fever in the Baltic States and Poland	Browser	Scientific opinion	(199)
40	Role of tick vectors in the epidemiology of Crimean-Congo haemorrhagic fever and African swine fever in Eurasia	Browser	Scientific opinion	(248)

41	African swine fever	Browser	Scientific opinion	(6)
42	Implementation of a regional training program on African swine fever as part of the cooperative biological engagement program across the Caucasus region	Browser	Article	(261)
43	African swine fever in the Caucasus	Browser	Report	(262)
44	African swine fever: detection and diagnosis. A manual for veterinarians	Browser	Technical guideline	(218)
45	African swine fever in wild boar in Europe: a notable challenge	Browser	Article	(253)
46	The costs of preventive activities for exotic contagious diseases-A Danish case study of foot and mouth disease and swine fever	Browser	Article	(263)
47	African swine fever strategy for Eastern part of the EU	Browser	Official recommendation	(206)
48	African swine fever in wild boar and African wild suids	Browser	Technical guideline	(224)
49	Transboundary and emerging viral infections of pigs in central and eastern Europe	Browser	Technical guideline	(264)
50	Guidelines on surveillance and control of African swine fever in feral pigs and preventive measures for pig holdings	Browser	Technical guideline	(227)
51	Good practices for biosecurity in the pig sector	Browser	Technical guideline	(209)
52	New insights into the role of ticks in African swine fever epidemiology	Browser	Article	(21)

Table 2: General measures to prevent African swine fever spread on domestic pig farms plus specific measures focused on commercial (CM), non-commercial (NCM) and outdoor holdings (OD). Results of the assessment of identified preventive measures represented as percentage of yes, not applicable (Na) and no.

ID	Preventive measures	Systematic literature review		Results of the assessment		
		Type	Reference	CM	NCM	OD
1	Check ASF-free certificates and health status before acquiring new animals as well as semen, ova or embryos on breeding farms	General (CM, NCM, OD)	(21, 53, 195, 201, 206, 207, 209, 218, 223, 227-229)	Y: 100%	Y: 83% N: 17%	Y: 100%
2	Limited farm visitation with proper register and establishment of biosecurity measures regarding footwear and clothing	General (CM, NCM, OD)	(21, 47, 53, 71, 118, 218, 227-230)	Y: 100%	Y: 83% N: 17%	Y: 92% N: 8%
3	Farmers/workers and operators education	General (CM, NCM, OD)	(37, 43, 44, 47, 53, 173, 218, 227, 230, 231)	Y: 100%	Y: 92% N: 8%	Y: 100%
4	Farmers/workers should not contact with external pigs	General (CM, NCM, OD)	(53, 118, 196, 206, 207)	Y: 92% N: 8%	Y: 92% N: 8%	Y: 92% N: 8%
5	Perimeter fences to prevent contacts with external pigs and wild boar	General (CM, NCM, OD)	(6, 35, 53, 206, 207, 209, 227, 228, 230, 233)	Y: 92% N: 8%	Y: 67% N: 33%	Y: 100%
6	Appropriate removal of carcasses, slaughter residues and food waste	General (CM, NCM, OD)	(53, 218, 227, 234)	Y: 100%	Y: 92% N: 8%	Y: 100%
7	Discouragement of sharing used equipment between holdings and/or units	General (CM, NCM, OD)	(6, 53, 227, 228, 230, 260)	Y: 100%	Y: 83% N: 17%	Y: 100%
8	Use of footbaths in entrance of units where animals are held	General (CM, NCM, OD)	(47, 118, 206, 218, 221, 227)	Y: 75% N: 25%	Y: 50% N: 50%	Y: 33% N: 67%
9	Daily health checks for clinical signs and mortality rates	General (CM, NCM, OD)	(228)	Y: 100%	Y: 58% N: 42%	Y: 92% N: 8%

10	Cleaning and disinfectant protocols for facilities, vehicles and equipment	General (CM, NCM, OD)	(6, 53, 206, 207, 209, 220, 221, 227, 229, 232)	Y: 100%	Y: 50% N: 50%	Y: 92% N: 8%
11	Farm location far from suitable wild boar areas and close to geographical barriers	General (CM, NCM, OD)	(34, 65, 66, 207)	Y: 67% N: 33%	Y: 58% N: 42%	Y: 75% N: 25%
12	Control measures against flies	General (CM, NCM, OD)	(32, 194, 228)	Y: 75% N: 25%	Y: 42% N: 58%	Y: 17% N: 83%
13	Establishing clean/dirty areas (including changing rooms and showers)	CM	(6, 71, 206, 209, 221)	Y: 92% N: 8%	Y: 58% N: 42%	Y: 67% N: 33%
14	Logistical arrangement for the entry and exit of animals including protocols regarding entrance of vehicles, loading areas, role of pig transporters, etc.	CM	(35, 118, 206, 209, 213, 218, 228, 232, 233)	Y: 100%	Y: 33% N: 67%	Y: 75% N: 25%
15	Cleaning and disinfection protocols for transport vehicles	CM	(123, 228, 232)	Y: 100%	Y: 58% N: 42%	Y: 83% N: 17%
16	Quarantine period for purchased animals and quarantine rooms	CM	(6, 21, 118, 207, 209, 218, 220, 228, 236)	Y: 100%	Y: 25% N: 75%	Y: 67% N: 33%
17	Identification of animals and farm records including animal movements	CM	(6, 35, 123, 166, 173, 206, 207, 228)	Y: 100%	Y: 100%	Y: 100%
18	Internal audits and evaluations to enforce biosecurity measures	CM	(206, 209)	Y: 92% N: 8%	Y: 25% N: 75%	Y: 67% N: 33%
19	Rules for food staff entering the farm (*i.e.* restricted to eating rooms or not allowed)	CM	(47, 206, 209, 229)	Y: 100%	Y: 58% N: 42%	Y: 75% N: 25%
20	Proper disposal of manure and dead animals	CM	(35, 118, 218, 238)	Y: 100%	Y: 92% N: 8%	Y: 92% N: 8%
21	Strict enforcement of the ban on swill feeding	NCM	(13, 43, 47, 53, 118, 206, 228, 230, 231, 242)	Y: 100%	Y: 100%	Y: 100%
22	Containment of pigs, do not allow contact with pigs from other farms, feral pigs or wild boar or their products	NCM	(6, 118, 206, 242)	Y: 100%	Y: 100%	Y: 100%
23	Farmers/farm staff should not have hunted, allowing a 48 hour interval between hunting	NCM	(206, 224)	Y: 92% N: 8%	Y: 92% N: 8%	Y: 92% N: 8%

	and being in contact with domestic pigs, if they work in an infected wild boar area					
24	Effective disinfection and cleaning of the surrounding of the holding including its entrance	NCM	(118)	Y: 58% N: 42%	Y: 50% N: 50%	Y: 42% N: 58%
25	Veterinary supervision prior and while home slaughtering	NCM	(170, 206)	Y: 50% Na: 33% N: 17%	Y: 83% N: 17%	Y: 67% Na: 16.5% N: 16.5%
26	Cleaning and disinfection protocols before and after home slaughter (regarding slaughtering tools, facilities, clothing and footwear, etc.)	NCM	(206, 207)	Y: 42% Na: 25% N: 33%	Y: 83% N: 17%	Y: 67% Na: 8% N: 25%
27	No sows or boars used for mating purposes held on non-commercial farm	NCM	(170, 206)	Y: 58% Na: 42%	Y: 67% Na: 8% N: 25%	Y: 42 Na: 33% N: 25%
28	No movements between/from non-commercial farms	NCM	(170, 242)	Y: 75% Na: 17% N: 8%	Y: 92% N: 8%	Y: 67% Na: 16.5% N: 16.5%
29	Avoid use of fresh fodder in areas at risk of exposure to ASF	NCM	(66, 206, 230, 231)	Y: 67% N: 33%	Y: 75% N: 25%	Y: 75% N: 25%
30	Promote educational programs through governmental training programmes and improve access to health services	NCM	(168, 173, 196, 206)	Y: 92% N: 8%	Y: 100%	Y: 100%
31	Treatment and storage (out of reach of wild boars) of grass or grains for at least 30 days or prohibit its use	NCM	(206, 230)	Y: 75% N: 25%	Y: 75% N: 25%	Y: 67% N: 33%
32	Avoid the use of straw bedding unless treated to inactivate ASF and stored for at least 90 days	NCM	(206)	Y: 83% N: 17%	Y: 83% N: 17%	Y: 83% N: 17%
33	No exchange of feed or bedding with other farms	NCM	(230)	Y: 92% N: 8%	Y: 92% N: 8%	Y: 92% N: 8%
34	Banning of free-range management on communal areas or public forests with no biosecurity measure	OD	(118, 166, 168, 173, 206, 207, 230)	Y: 67 Na: 16.5% N: 16.5%	Y: 92% N: 8%	Y: 92% N: 8%
35	Distance between outdoor farms (at least 1 km) to minimise the risk of ASF introduction through direct and indirect contact	OD	(246)	Y: 16% Na: 42% N: 42%	Y: 33% Na: 25% N: 42%	Y: 67% N: 33%

36	If they were *Ornithodoros* ticks avoid using traditional pig-housing facilities (usually made of wood and stones were ticks can be hidden)	OD	(21, 35, 247, 248)	Y: 84% Na: 8% N: 8%	Y: 75% Na: 8% N: 17%	Y: 84% Na: 8% N: 8%
37	Apply chemical control if ticks were present in traditional pig-housing facilities	OD	(247)	Y: 84% Na: 8% N: 8%	Y: 92% Na: 8%	Y: 92% Na: 8%

Yes: Y, No: N, Not applicable: Na.

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REVIEW
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Relevant Measures to Prevent the Spread of African Swine Fever in the European Union Domestic Pig Sector

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During the past decade, African swine fever (ASF) has spread from the Caucasus region to eastern European Union countries affecting domestic pig and wild boar populations. In order to avert ASF spread, mitigation measures targeting both populations have been established. However, despite these efforts, ASF has been reported in thirteen different countries (Georgia, Azerbaijan, Armenia, the Russian Federation, Ukraine, Belarus, Estonia, Latvia, Lithuania, Poland, Moldova, Czech Republic, and Romania). In the absence of an effective vaccine or treatment to ASF, introduction and spread of ASF onto domestic pig farms can only be prevented by strict compliance to control measures. This study systematically reviewed available measures to prevent the spread of ASF in the EU domestic pig sector distinguishing between commercial, non-commercial, and outdoor farms. The search was performed in PubMed and using a common browser. A total of 52 documents were selected for the final review process, which included scientific articles, reports, EU documents and official recommendations, among others. From this literature review, 37 measures were identified as preventive measures for the introduction and spread of ASF. Subsequently, these measures were assessed by ASF experts for their relevance in the mitigation of ASF spread on the three mentioned types of farms. All experts agreed that some of the important preventive measures for all three types of farms were: the identification of animals and farm records; strict enforcement of the ban on swill feeding; and containment of pigs, so as to not allow direct or indirect pig–pig and/or pig–wild boar contacts. Other important preventive measures for all farms were education of farmers, workers, and operators; no contact between farmers and farm staff and external pigs; appropriate removal of carcasses, slaughter residues, and food waste; proper disposal of manure and dead animals, and abstaining from hunting activities during the previous 48 h (allowing a 48 h interval between hunting and being in contact with domestic pigs). Finally, all experts identified that the important preventive measures for non-commercial and outdoor farms is to improve access of those farms to veterinarians and health services.

Keywords: biosecurity, Europe, epidemiology, pig farm, preventive measures

INTRODUCTION

African swine fever (ASF) is an infectious disease of swine notifiable in the European Union (EU) and to the World Organization for Animal Health (OIE). Susceptible pigs can be infected by direct or indirect contact with infectious animals or their fluids, ingestion of contaminated animal feed, pork, or pig products, or contact with contaminated surfaces or fomites (clothing, footwear, vehicles, farming tools, etc.) acting as mechanical vectors (1). In the southern and eastern parts of the African continent and the Iberian Peninsula, ASF can also be transmitted by biological vectors, infected soft ticks belonging to the *Ornithodoros* genus (2). No vaccine or treatment is available against ASF. Therefore, prevention and control of the disease is mainly based on the early detection of the disease by timely recognition in the field and efficient laboratory diagnosis, followed by the implementation of strict sanitary measures (2–4). Adequate implementation of sanitary measures will reduce the number of secondary outbreaks on domestic pig farms, which will decrease the potential contamination of the environment and, finally minimize the likelihood of infection in wild boar (5).

Since 1978 and until recently, the Italian island of Sardinia has been the only European ASF-infected area (6). However, in 2007, ASF was introduced into Georgia, from there it spread to neighboring countries Azerbaijan and Armenia. As a result of the disease introduction and spread throughout the Russian Federation and Belarus, the EU strengthened its preparedness against ASF. Among the protection measures implemented by EU member countries bordering the Russian Federation were actions such as improving cleaning and disinfection of livestock vehicles, suspension of livestock markets, surveillance, enhanced biosecurity on farms, and awareness campaigns. Moreover, contingency plans were revised and the diagnostic capabilities of the EU labs were assured. However, ASF entered into four EU member countries in 2014, namely Lithuania, Poland, Latvia, and Estonia; and in 2017, ASF was reported for the first time in Czech Republic and Romania (7). During this period, between January 2014 and December 2017, ASF outbreaks (occurrence of one or more ASF cases on a pig farm) were reported in over 250 farms, and more than 8,500 wild boar cases (an individual wild boar infected by ASF virus) were reported within the EU (7–10). As a reaction to this large number of outbreaks and cases, the Community Veterinary Emergency Team recommended several measures such as: (i) focus surveillance on wild boar and domestic pigs, (ii) implement control of animal movements, (iii) safe disposal of wild boar carcasses, (iv) avoid swill feeding practices, (v) implement biosecurity on farms, (vi) conduct awareness campaigns and finally, and (vii) review wild boar hunting practices (11). These measures were aimed at reducing the risk of spread of the disease to domestic pig farms and its transmission between wild boar populations. In contrast to what has been observed in non-EU European countries (i.e., the Russian Federation or Ukraine), in the EU scenario the number of infected farms has been comparatively lower, with wild boar being the most severely affected host (7, 8).

The main piece of legislation providing the tools for the control of ASF in the EU is the Council Directive 2002/60/EC (9), which establishes the minimum measures to be applied within the EU for the control of ASF. It includes the measures to be taken in the event of an outbreak of ASF on a pig holding and in cases where the disease is suspected or confirmed in feral pigs. The main objectives of controlling ASF in feral pigs are to reduce the risk of transmission to domestic pigs and to prevent it becoming endemic in the feral pig population (see Definitions) (9). The Directive lays down the measures to be taken in the infected area and the provisions to apply on the holdings of that area. All control and eradication measures applicable are based on classical disease control methods, which include surveillance, epidemiological investigation, tracing of pigs, and stamping out in infected holdings. These measures are applied in combination with strict quarantine and biosecurity measures on domestic pig holdings and animal movement control. The Directive also requires that Member States develop and implement plans for the eradication of the disease.

Moreover, specific regionalization measures are laid down in Commission Implementing Decision 2014/709/EU (10). This Decision establishes animal health control measures on the movement, dispatch of pigs and certain pig products, and marking pig meat from the areas at risk of infection in order to prevent the spread of ASF to other areas of the Union. Affected Member States and territories are listed in different parts of the Annex to the Decision, the differentiation is made based on their epidemiological situation and level of risk. The Annex is divided into four parts, and territories that are listed in Part IV have a higher risk of spread of ASF than the ones listed in Part I. In determining the application of control measures on a certain commodity of a certain territory, the level of risk of that area and the type of commodity is taken into account. Indeed, in terms of risk of spread of ASF, movement of different porcine commodities poses different levels of risk. It is worth to mentioning that this Decision is also aimed at avoiding unnecessary disturbance to trade within the EU, as well as avoiding unjustified barriers to trade by third countries and the provisions that are set in this Decision are aligned with the OIE standards (11).

Bearing in mind all of the above, the aim of this study is to review described measures to prevent the introduction and further spread of ASF in the domestic pig sector focused on the EU scenario. An additional aim of this review was to assess the importance of these identified measures depending on the different pig farming systems (see materials and methods section). Adequate identification of relevant measures will allow for the creation of guidelines for pig producers to prevent the spread of ASF, which is one of the identified goals of the COST Action 15116 Understanding and combating African swine fever in Europe (ASF-STOP) supported by COST (European Cooperation in Science and Technology).

MATERIALS AND METHODS

Literature Sources and Search Strategy

Following an approach similar to Rodríguez-Prieto et al. (12), the systematic review targeted preventive measures to avoid the spread of ASF in the domestic pig sector described in scientific publications, gray literature (materials produced by organizations outside the academic publishing channels), technical guidelines and international, national, and regional regulations. The literature search was performed in 3rd March 2017 and supplemented with further search in 14th December 2017 using PubMed database[1] for scientific articles. Scientific papers written in English (for reviewing convenience) between the last 39 years (1978 and 2017) were reviewed. A list of key words was combined into a Boolean query to identify titles and/or abstracts of documents of interest. The key words used (and any word containing the stem presented) were "African swine fever," "Preventive measure/s," "Biosecurity," "Risk," and "Pig farm." The search terms applied were "African swine fever" AND [Preventive measure* OR Biosecurity OR Risk OR Pig farm]. To make sure other relevant documents such as technical guidelines, regulations, or scientific opinions, among others, were included, the literature search was performed following the same query on the internet using a common browser.

Definitions

"*Control measures*" are defined as the best/safest options to eliminate or reduce specific risks, while "*preventive measures*" are actions taken to avoid specific risks (13). As the glossary of the Terrestrial Animal Health code of the OIE states (14), "*biosecurity*" means a set of management and physical measures designed to reduce the risk of introduction, establishment, and spread of pathogenic agents to, from and within an animal population. On the other hand, "*risk*" means the likelihood of the occurrence and the likely magnitude of the biological and economic consequences of an adverse event or effect to animal or human health (14).

Based on the working document SANTE/7113/2015-Rev 7 produced by the Directorate-General for Health and Food Safety (15) pig farming systems and subsequently, pig farms can be classified as: (i) "*commercial farms*" which refers to farms that sell pigs, send pigs to a slaughterhouse or move pig products off the holding, (ii) "*outdoor pig farms*" which refers to farms in which pigs are kept temporarily or permanently outdoor, and (iii) "*non-commercial farms*" which refers to farms where pigs are kept only for fattening for own consumption and neither pigs nor any of their products leave the holding. Elsewhere, this last type of farm is referred as "*family farms*" (16) or "*backyard farms*" (17). Commercial farms can be divided into multi-site farms which are holdings specialized on one production step (farrowing, nurseries, or finishing) and on-site farms which are premises that produce all production steps (18). Moreover, "*feral pig*" or "*free-ranging pig*" means a pig which is not kept or bred on a holding according to the Council Directive 2002/60/EC (19). In Sardinia, free-ranging pigs are usually referred as "*brado*" (16, 20).

Study Selection

A two step-process was followed to select the literature relevant for the aim of this review. A primary exclusion criteria was applied when reading title and abstract of found literature (abstract when available): (i) published before 1978; (ii) not related to the theme of this review; (iii) not related to the European scenario; and (iv) repeated document (already selected among retrieved results). If abstract were not available, the piece of literature would be kept for the next stage. Then, the full text of each selected piece of literature was screened. As a second exclusion criteria, documents (v) which full text was not available; (vi) no preventive measures were described; (vii) described preventive measures were not focused on ASF; or (viii) information on the theme was insufficient, were excluded. The explained process was individually performed by three reviewers following the mentioned exclusion criteria in order to cross-check selected literature and resolve any disagreement.

Assessment of Described Preventive Measures

A group of experts was invited to participate in an expert opinion session to assess the preventive measures identified in this review.

Participants belonging to the COST (European Cooperation in Science and Technology) action: "Understanding and combating African swine fever in Europe" (ASF-STOP) supported by COST (COST Action 15116)[2] were encouraged to suggest experts with relevant expertise in ASF prevention, ASF control and eradication, ASF epidemiology and the EU domestic pig sector.

Before starting the assessment, the list of measures were reviewed by authors to ensure measures were accurate and clear, as well as no measures were omitted. In total, 20 experts were invited to participate and contacted by email, 12 of them returned their responses.

Experts were asked to assess the relevance of each described preventive measure by answering yes or no to the closed question: "Is this measure important for commercial, non-commercial, and outdoor-farms?" "Importance" was defined as the perceived need for each measure. Experts were asked to perform this assessment within the EU context. Moreover, experts were encouraged to suggest additional measures if they thought they were missing. Results were recorded in an Excel datasheet (Microsoft Corp., Redmond, WA, USA).

RESULTS

Selection Process

Figure 1 shows the literature selection process and **Table 1** compiles the selected literature. The search made on PubMed database returned 168 scientific papers. After applying the primary exclusion criteria, 69 were selected for the second step of the review. However, the full text was not available for 10 of them. Therefore, 59 scientific articles were selected for the second screening round. The same search on a common browser returned 5,100 results of potential interest. By applying the primary exclusion criteria, 58 results were selected for the second round, all of them had available the full text.

After applying the second exclusion criteria and completion of the screening rounds, 34 articles (25 retrieved from PubMed and 9 retrieved from the browser search), 4 official recommendations

[1] http://www.ncbi.nlm.nih.gov/pubmed (Accessed: March 3, 2017).

[2] https://www.asf-stop.com/stsms/ (Accessed: December 14, 2017).

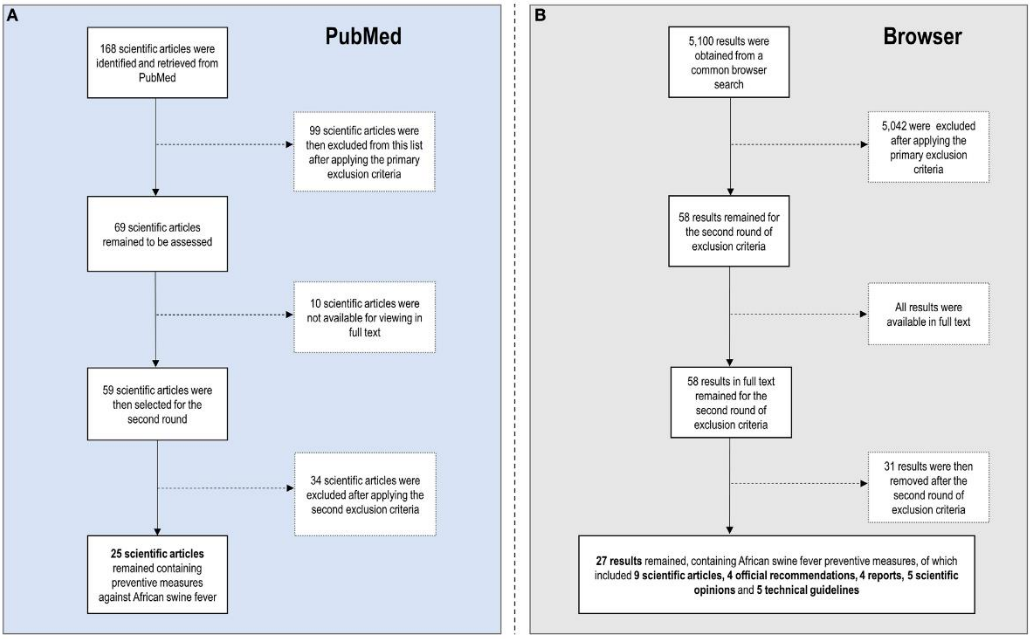

FIGURE 1 | Flowchart summarizing the literature selection process **(A)** on PubMed database and **(B)** on a common browser.

TABLE 1 | Pieces of literature included in the review process.

ID	Title	Search	Type	Reference
1	African and classical swine fever: similarities, differences and epidemiological consequences	PubMed	Article	(21)
2	Why is African swine fever still present in Sardinia?	PubMed	Article	(16)
3	African swine fever in eastern Europe: the risk to the UK	PubMed	Article	(22)
4	Understanding African swine fever infection dynamics in Sardinia using a spatially explicit transmission model in domestic pig farms	PubMed	Article	(23)
5	Control of African swine fever epidemics in industrialized swine populations	PubMed	Article	(24)
6	Preventive measures aimed at minimizing the risk of African swine fever virus spread in pig farming systems	PubMed	Article	(5)
7	Modelling African swine fever presence and reported abundance in the Russian Federation using national surveillance data from 2007 to 2014	PubMed	Article	(25)
8	English pig farmers' knowledge and behaviour towards African swine fever suspicion and reporting	PubMed	Article	(26)
9	Simulating the epidemiological and economic effects of an African swine fever epidemic in industrialized swine populations	PubMed	Article	(27)
10	A cartographic tool for managing African swine fever in Eurasia: mapping wild boar distribution based on the quality of available habitats	PubMed	Article	(28)
11	Transmission routes of African swine fever virus to domestic pigs: current knowledge and future research directions	PubMed	Article	(1)
12	Expert opinion on the perceived effectiveness and importance of on-farm biosecurity measures for cattle and swine farms in Switzerland	PubMed	Article	(29)
13	Spatiotemporal analysis of African swine fever in Sardinia (2012–2014): trends in domestic pigs and wild boar	PubMed	Article	(30)
14	Statistical exploration of local transmission routes for African swine fever in pigs in the Russian Federation, 2007–2014	PubMed	Article	(31)
15	Evaluation of the risk factors contributing to the African swine fever occurrence in Sardinia, Italy	PubMed	Article	(32)
16	Spatio-temporal modeling of the African swine fever epidemic in the Russian Federation, 2007–2012	PubMed	Article	(33)
17	Thirty-five-year presence of African swine fever in Sardinia: history, evolution and risk factors for disease maintenance	PubMed	Article	(6)
18	The medical and veterinary role of *Ornithodoros erraticus* complex ticks (Acari: Ixodida) on the Iberian Peninsula	PubMed	Article	(34)
19	Pig producers urged to review biosecurity as ASF and PED spread	PubMed	Article	(35)
20	African swine fever in the North Caucasus region and the Russian Federation in years 2007–2012	PubMed	Article	(36)
21	African swine fever (ASF): five years around Europe	PubMed	Article	(37)
22	African swine fever: an epidemiological update	PubMed	Article	(38)

(*Continued*)

TABLE 1 | Continued

ID	Title	Search	Type	Reference
23	Qualitative risk assessment in a data-scarce environment: a model to assess the impact of control measures on spread of African swine fever	PubMed	Article	(39)
24	Viruses in boar semen: detection and clinical as well as epidemiological consequences regarding disease transmission by artificial insemination	PubMed	Article	(40)
25	Temporal and spatial patterns of African swine fever in Sardinia	PubMed	Article	(41)
26	Do not bring African swine fever to Finland	Browser	Official recommendation	(42)
27	African swine fever facing Romania	Browser	Report	(43)
28	African swine fever	Browser	Official recommendation	(44)
29	African swine fever—Guidance	Browser	Official recommendation	(45)
30	Guidelines for the cost effective prevention and control of African swine fever	Browser	Report	(46)
31	African swine fever in Poland and Baltic countries	Browser	Report	(47)
32	Gaps in African swine fever: analysis and priorities	Browser	Article	(48)
33	African swine fever (ASF)	Browser	Article	(49)
34	African swine fever: new challenges and measures to prevent its spread	Browser	Article	(50)
35	African swine fever	Browser	Scientific opinion	(51)
36	Review of African swine fever: transmission, spread and control	Browser	Article	(52)
37	African swine fever: how can global spread be prevented?	Browser	Article	(4)
38	African swine fever	Browser	Scientific opinion	(53)
39	Epidemiological analyses of African swine fever in the Baltic States and Poland	Browser	Scientific opinion	(10)
40	Role of tick vectors in the epidemiology of Crimean-Congo hemorrhagic fever and African swine fever in Eurasia	Browser	Scientific opinion	(54)
41	African swine fever	Browser	Scientific opinion	(55)
42	Implementation of a regional training program on African swine fever as part of the cooperative biological engagement program across the Caucasus region	Browser	Article	(56)
43	African swine fever in the Caucasus	Browser	Report	(57)
44	African swine fever: detection and diagnosis. A manual for veterinarians	Browser	Technical guideline	(58)
45	African swine fever in wild boar in Europe: a notable challenge	Browser	Article	(59)
46	The costs of preventive activities for exotic contagious diseases-A Danish case study of foot and mouth disease and swine fever	Browser	Article	(60)
47	African swine fever strategy for Eastern part of the EU	Browser	Official recommendation	(15)
48	African swine fever in wild boar and African wild suids	Browser	Technical guideline	(61)
49	Transboundary and emerging viral infections of pigs in central and eastern Europe	Browser	Technical guideline	(62)
50	Guidelines on surveillance and control of African swine fever in feral pigs and preventive measures for pig holdings	Browser	Technical guideline	(63)
51	Good practices for biosecurity in the pig sector	Browser	Technical guideline	(18)
52	New insights into the role of ticks in African swine fever epidemiology	Browser	Article	(64)

(meaning information coming from governmental authorities), 4 reports, 5 scientific opinions, and 5 technical guidelines were included in the review. The rest of the documents including reports, recommendations, and guidelines were retrieved from the browser search.

Results From the Systematic Review

Preventive measures described hereinafter were obtained from the 52 pieces of literature selected during the systematic review. These measures were classified in four different groups: general prevented measures suggested for all types of farms (as some of them were common for commercial, non-commercial, and outdoor farms), and three groups of suggested measures for each of the identified types of farms.

General Preventive Measures

The risk of introduction and exposure to ASF depends on the epidemiological characteristics of the country, area, and type of farm (31, 70, 74–80). Pig production in Europe is highly heterogeneous with different biosecurity standards and productive levels (39, 81). Actions to prevent ASF introduction and spread should take into consideration the epidemiology of the disease, with especial focus on the virus resistance in the environment, routes of transmission, and excretion as well as the characteristics of the farming systems in place (5, 29, 38, 48, 58). As no vaccine for ASF is available, prevention of ASF relies upon implementing strict biosecurity measures to avoid potential contact between domestic pigs and ASF virus (35, 49, 58, 82). In the EU, movements of pigs or pig products coming from infected areas have been prohibited to prevent ASF spread (4, 19, 21). Moreover, the presence of infected wild boar in the area and its hunt constitutes an additional source of risk that cannot be discarded (82, 83). Minimum biosecurity requirements to apply during hunting in the affected territories have been proposed (5, 15, 61). First of all, hunters shall be authorized to hunt after receiving training on basic biosecurity practices. Hunted wild boar should be tested and only released after receiving negative results. Hunted animals should be moved to the dressing facilities in dedicated vehicles, private cars should be parked outside the hunting field. Dressing facilities would be used if they have tap water, electricity, freezers, and waste water collection. Evisceration should be performed with gloves at the dressing facilities and hands gently washed with soap and water. Offal should be stored in proper containers in the dressing area and then, cleaned and disinfected. Clothing, footwear, and hunting equipment should be cleaned and disinfect after each use (clothing washed at 60°C). Finally, contact with domestic pigs should be avoided, allowing a 48 h interval between hunting and being in contact with domestic pigs. All of above the needs to be implemented together with education and training campaigns to get hunters involved in control strategies as much as possible (5). Thoen et al. (84) and Sánchez-Vizcaíno et al. (50) also suggested that systems that wild boar can use as artificial feeding places (feeders, water holes, supplementary feeding of ungulates) should be avoided, as these systems can significantly increase wild boar abundance and spatial concentrations. However, it has been also suggested that this ban may be deemed effective only in regions where the habitat is unsuitable for wild boar and where feeding caused artificial population establishment (53).

The EU Commission has established minimum biosecurity requirements for commercial, non-commercial, and outdoor farms (63). Health status and free-ASF certificates have to be checked before acquiring new animals (15, 16, 18, 45, 58, 63, 64). On breeding farms, semen (21, 40, 45), embryos, or ova should come from free-ASFV certified farms (15, 19, 44, 57). Visits should be discouraged (44, 45), limiting access to the farm and animals, to workers and veterinarian services (5, 31, 52, 63). If visitors enter the farm, visits should be registered and visitors should follow strict biosecurity measures regarding footwear and clothing (45, 47, 52, 57, 58, 64). Farm staff should follow the same biosecurity procedures. Likewise, workers and owners should be aware and well trained with regard to ASF (22, 23, 37, 38, 47, 48, 52, 57, 63) as well as veterinarians and operators along the market chain (58). Moreover, farm staff must not have contact with animals from other pig premises nor own pigs (4, 5, 15, 16, 57). In addition to this, the Finnish Food Safety Authority recommends that farm staff should not directly enter the farm after visiting a farm abroad, they should wait at least for 48 h (42).

Regarding physical barriers on farms, animals should be kept in a way that ensures that no direct, nor indirect contact occurs with wild boar, feral pigs, or domestic pigs coming from other premises (15, 63). Additionally, perimeter fences should delimitate the commercial holding to prevent such contact (15). On outdoor farms, fences will be preferably doubled (63), at least 1 m apart (57), and proofed against wild boar and pigs (15, 16, 18, 45, 47, 65). Fences should be at least 2 m high of which 50 cm should be under the ground (66).

In addition, as part of good farming practices, carcasses, discarded parts from slaughtered pigs and food waste should be disposed in accordance with Regulation (EC) No. 1069/2009 (57, 58, 63, 67). Sharing equipment between holdings should be discouraged (45, 47, 52, 55, 57, 63), and footbaths should be used at the entrance of every unit where animals are held (5, 15, 52, 58, 63). Organic material should be removed from footwear prior to disinfecting (49). Animals must be checked at least once a day paying special attention to mortality rates and clinical signs compatible with ASF (45). Moreover, cleaning and disinfection protocols should be established and periodically performed on every farm facility, vehicle, and piece of equipment (15, 16, 18, 35, 42, 44, 55, 57, 63). Disinfectants effective against ASF virus include 2% caustic soda, 2% sodium hypochlorite, 0.3% formalin, 3% ortho-phenylphenol, and iodine compounds, among others (58, 85). Organic material (feces, feed, bedding materials) should be completely removed to maximize the efficacy of disinfection (49).

Moreover, regarding the location of pig farms, several scientific publications point out that farms should be located far from suitable wild boar areas and close to geographical barriers (such as mountains, rivers, etc.) (16, 28, 48, 53). Finally, Mellor et al. (68) observed experimental transmission of ASF through *Stomoxys calcitrans* flies. Therefore, given this potential role of stable flies as mechanical vectors, it has been suggested that sanitation, biological, and chemical controls should be applied to suppress stable flies. As an example, commercial and non-commercial farms could eliminate fly breeding sites in combination with placing insecticide-treated nets to reduce the potential risk posed by flies (45, 69).

Specific preventive measures based on biosecurity have been proposed depending on the type of farm: commercial, outdoor, or non-commercial (15).

Specific Measures Focusing on Commercial Farms

Commercial farms are significantly larger in size and number of animals (18) and so, the economic and animal health impact of ASF is greater than on outdoor and non-commercial farms (24, 29).

Key measures to prevent the introduction of ASF on commercial farms are to establish clear clean/dirty areas for personnel including changing rooms and shower (15, 18, 31, 49, 55) and to review logistical arrangement for entry of new animals. This measure will allow for the adequate identification of critical control points (15), which is particularly relevant since contaminated vehicles transporting pigs or carcasses are associated to a high risk of disease transmission (18, 70).

Several steps should be included when preparing a protocol for animal transport. First, farms should be designed to allow deliveries without entering the farm (5, 45, 63). If it is not possible, decontamination of vehicles is necessary before entering the farm (42, 58, 65). Employees involved in pig transport should not come in contact with farm workers nor with animals (5, 45). If other drivers (i.e., animal feed suppliers) need to enter the farm, footwear should be changed, cleaned, and disinfected when entering the farm and again before getting into the vehicle (45). Moreover, parking areas should be designed to avoid cross-contamination between workers and farm vehicles. In case vehicles have to enter into the farm, loading and unloading areas should be placed at least 20 m away from animal facilities within the perimeter of the farm (66). Vehicles transporting pigs and other vehicles must be cleaned and disinfected before and after each use (42, 45). Returning trucks should be cleaned and disinfected at the farm where pigs are unloaded (45). In addition to this, the Danish regulation applies a 48 h quarantine period before the next movement of animals (27). After that new animals should be kept in quarantine rooms (16, 35, 36, 55) between 14 and 30 days (5, 18, 45, 58, 64). Quarantine rooms should be located away from the main herd (45).

Furthermore, animals should be identified and all animal movements recorded (15, 23, 32, 45, 86); farm records should be ensured to easy track animals if an outbreak is reported; births and deaths, animal census, entry and exit of animals (live and dead), vehicles, visits, pest control, or cleaning and disinfection procedures should be properly registered in a farm record book (16, 55, 65). Moreover, internal audits or self-evaluation need to be periodically conducted to enforce biosecurity measures (15, 18). Furthermore, a set of rules on food for workers entering the farm should be clearly specify (31) and food should be restricted to eating rooms (15, 18) or not allowed (44, 52).

Finally, proper disposal of manure as well as dead animals and other removable material should be ensured (5, 58, 65). Containers and storage basins should accomplish with the minimum requirements for storage capacities recommended by the Best Available Techniques (71).

Specific Measures Focusing on Non-Commercial Farms

Backyard farms are characterized by limited farming management practices and nearly absent biosecurity levels (5, 6, 55). This type of farm is common in countries such as Romania (46), Bulgaria (80), Poland (87), or Sardinia (Italy) (6, 23), among others. Non-commercial farms are built for own consumption purposes, investment is minimum and animals could be fed on kitchen leftovers (88). Depending on the country and local practices, pigs are allowed to move freely (without physical restrictions) during the day or even scavenge for days or months (46, 74, 89). Pig slaughtering is usually carried out on the farm, although it may be restricted to proper slaughterhouses if there are local regulations on this issue (16, 46).

Specific measures focusing on these farms have been proposed, swill feeding practices are not allowed (15, 22, 43, 45, 47, 52, 57), as ASF can be transmitted through ingestion of contaminated raw pork or pork products (5, 15, 38, 64). Pigs should be kept in animal facilities ensuring no contact with domestic pigs from other non-commercial farms, feral pigs, wild boar nor their products (5, 15, 43, 55). If there were infected wild boar in the area, the owner or the person in charge of taking care of the pigs should allow a 48 h interval between hunting and being in contact with domestic pigs (15, 61) and should not use dogs during hunting (61). Any hunting equipment used as well as the dog's coat should be cleaned and disinfected (42). Effective disinfectants such as calcium hydrate (slaked lime), should be spread and renewed around the holding including its entrance (5). A veterinarian needs to supervise home slaughtering activities (15, 72). If a slaughterer comes to slaughter the animals, cleaned and disinfected clothing and footwear should be provided. Cleaning and disinfection protocols have to be applied after slaughtering on the facilities and to the slaughter tools (15, 16). The Directorate-General for Health and Food Safety and the Sardinian regulations agree that sows or boars cannot be held on non-commercial farms for matting purposes (15, 72) while Decision 830/2016 of the Romanian Government states that sows and boars might be present but they cannot be moved between holdings for matting purposes (43), movements from these farms are neither allowed in the Sardinian regulations (72). Furthermore, governments and institutions are encouraged to promote educational programs as well as improve access to health services on non-commercial farms (4, 15, 23). This measure is one of the novelties of the latest eradication program launched in Sardinia (20).

Moreover, the use of fresh fodder harvested in areas at risk for ASFV exposure should be avoided (15, 28, 53), as its consumption has been observed that could be related to ASF outbreaks in Eastern EU countries (53). If this is not possible, Directorate General for Health and Food Safety (15) recommends to perform treatments on grass or grains to inactivate ASFV or store them, out of reach of wild boar, for at least 30 days. In Estonia, according to the Regulation of the Minister of Agriculture No. 179, it is forbidden to bring green fodder to the farm (47). Likewise, Directorate General for Health and Food Safety (15) recommends to avoid using straw as bedding material unless treated to inactivate ASFV or stored for at least 90 days (15). Additionally, the Estonian Veterinary and Food Board established as compulsory biosecurity rule, no exchange feed and bedding material with other farms (47). Field experiences showed that no additional cases were reported when non-commercial farm had feed from reliable sources and contact with infectious free-ranging pigs was prevented (55).

Specific Measures Focusing on Outdoor Farms

The number of outdoor farms is increasing in Europe due to a growing interest in organic farming systems (90), particularly from pork consumers due to animal welfare concerns. Simultaneously, veterinarians and pig producers have been urging for improvements in biosecurity, so as to avoid health threats (91). Depending on the country and local practices, outdoor pig production may vary from outdoor farms that implement several biosecurity measures (92), to free-ranging herds where biosecurity is absent (6).

Spain is a good example of a country with strict biosecurity standards for outdoor pig production. Regulations regarding biosecurity on outdoor pig farms (73) are a result of the presence of ASF for more than 30 years in the Iberian Peninsula (65). Applied control and preventive measures allowed to eradicate ASF from outdoor pig production and avoided new introductions on outdoor farms, despite the constant threat posed by the presence of infected wild boar and infectious *Ornithodoros* ticks in the surroundings (4, 65). In other areas such as Sardinia in Italy, pigs are allowed to range free in public forests during the day, for days or even months under no biosecurity measures

(41). Free-range management practices in communal areas has been identified as a dangerous practice for the persistence and re-emergence of ASF in endemic areas like Sardinia (6, 16, 74). During the free-ranging period, pigs might be in contact with wild boar and pigs belonging to different herds (30, 32, 89). For this reason, free-range management practices in communal areas or public forest with no biosecurity measures nor veterinary control have been banned (5, 16, 20, 23), such as in Sardinia since 2012 (20).

Bearing in mind the current situation in Eastern Europe, the EU Commission has banned outdoor keeping of pigs as the main strategy to avoid ASF spread (15, 47). Although prevention becomes challenging in outdoor and semi-extensive pig production (74), several preventive measures can be implemented to ensure biosecurity levels. For instance, the territories/fields where animals are allowed to range free should be fenced (double fenced, if it is possible) to avoid the entrance and direct contact with wild boar, feral pigs, and other domestic pigs, as well as people and vehicles (5, 42, 49, 55). Sardinian regulations state farms should have perimeter barriers of at least 1.5 m high and wild boar proofed and fenced fields had a maximum extension of 3 ha (72). Outdoor farms should be separated from other outdoor farms to reduce the risk of ASF introduction through direct or indirect contact (73). This minimum distance between farms will vary depending on national and local regulations. If pigs were free to roam within no fenced fields, distance would become irrelevant (18).

So far, *Ornithodoros* ticks have not been implicated in the transmission of ASF in Eastern nor Central Europe (64). In Sardinia, ticks have also not been identified as a major transmission source (93). Several preventive measures were described in Portugal and Spain were *Ornithodoros erraticus* are present such as keeping traditional pig-housing facilities (typically, used in outdoor production), in good repair, otherwise it is recommended to fence them or destroy them if ticks are present (34, 64, 65). In case ticks are present, either chemical control with methylene bromide should be applied on the facilities, or treating pigs with an ivermectin treatment (34). If infected ticks were present in such constructions, it is not recommended to use the infested buildings (54) or keep these buildings empty for 6 years (19). Nevertheless, it should be considered that eradication of *O. erraticus* ticks is extremely difficult due to the long life of ticks, long survival without feeding, presence of accidental hosts, and possibility of penetrating into cracks and surfaces not accessible to acaricides (54).

Table 2 compiles the general preventive measures and specific preventive measures for commercial, non-commercial, and outdoor farms described in this review.

Assessment of the Importance of Described Preventive Measures

A total of 12 experts participated in the assessment of the importance of identified preventive measures. All of them completed the questionnaire and therefore, their responses were included in the analysis. Around 3% of assessed measures (2.85%) were categorized as "not applicable" preventive measure.

There was 100% agreement among experts (12 experts out of 12) that the identification of animals and farm records including animal movements; enforcement of the ban on swill feeding; and containment of pigs to not allow contact with pigs from other farms, feral pigs, or wild boar or their products, were important preventive measures for the three types of farms (commercial, non-commercial, and outdoor). Other important preventive measures identified for all farms were education of farmers, workers, and operators; no contact between farmers and farm staff and external pigs; appropriate removal of carcasses, slaughter residues and food waste; proper disposal of manure and dead animals; and a 48 h (minimum) interval between hunting and being in contact with domestic pigs for all farm staff, particularly those who work in an infected wild boar area.

Moreover, all experts identified as important preventive measures for non-commercial and outdoor farms, to improve access of those farms to veterinarians and health services. Between eight and nine of experts considered that logistical arrangement for the entry and exit of animals including protocols regarding entrance of vehicles, loading areas and role of pig transporters; quarantine period for purchased animals and quarantine rooms; and internal audits and evaluations to enforce biosecurity measures, were not important preventive measures for non-commercial farms. In addition, 10 experts concluded that control measures against flies were not an important preventive measure on outdoor farms.

Additional preventive measures were suggested by some experts such as the use of nets on animal facilities; establishment of pest control programs on farms; use of carbonic dioxide traps to check the presence of *Ornithodoros* ticks and change of boots before entering the farm and units. Furthermore, several respondents wanted to emphasize the importance of measures already included in the questionnaire. For instance, establishment of double fencing perimeter on outdoor farms; education of swine veterinarians and farmers paying especial attention to clinical signs and transmission routes; and discouragement of using the same injection syringes and instruments on different farms unless thoroughly disinfected sterilized.

Figure 2 and **Table 2** summarize the results obtained for preventive measures on commercial, non-commercial, and outdoor farms.

DISCUSSION

In the absence of an effective vaccine, prevention is the main tool to avoid further spread of ASF or an endemic situation. Both the systematic literature review as well as the expert opinion elicitation, highlighted three main areas where preventive measures would be very relevant to halt ASF spread in the domestic pig population: (1) control of entries into the farm, (2) control of pigs' feed, and (3) improvement of health services and education.

The first main area of prevention encompasses both the movements associated to production as well as the potential spill-over from infected wild boar in the surrounding areas. Both have been major drivers of spread in the current ASF epidemic in Eastern EU, where the majority of ASF notifications in domestic pigs have occurred in backyard or small commercial farms with limited biosecurity (11). The identification of animals and the containment of pigs were also identified by the experts

TABLE 2 | General measures to prevent African swine fever spread on domestic pig farms plus specific measures focused on commercial (CM), non-commercial (NCM), and outdoor holdings (OD).

ID	Preventive measures	Systematic literature review		Results of the assessment		
		Type	Reference	CM	NCM	OD
1	Check ASF-free certificates and health status before acquiring new animals as well as semen, ova or embryos on breeding farms	General (CM, NCM, OD)	(15, 16, 18, 19, 21, 40, 44, 45, 57, 58, 63, 64)	Yes: 100%	Yes: 83% No: 17%	Yes: 100%
2	Limited farm visitation with proper register and establishment of biosecurity measures regarding footwear and clothing	General (CM, NCM, OD)	(5, 31, 44, 45, 47, 52, 57, 58, 63, 64)	Yes: 100%	Yes: 83% No: 17%	Yes: 92% No: 8%
3	Farmers/workers and operators education	General (CM, NCM, OD)	(22, 23, 37, 38, 47, 48, 52, 57, 58, 63)	Yes: 100%	Yes: 92% No: 8%	Yes: 100%
4	Farmers/workers should not contact with external pigs	General (CM, NCM, OD)	(4, 5, 15, 16, 57)	Yes: 92% No: 8%	Yes: 92% No: 8%	Yes: 92% No: 8%
5	Perimeter fences to prevent contacts with external pigs and wild boar	General (CM, NCM, OD)	(15, 16, 18, 45, 47, 55, 57, 63, 65, 66)	Yes: 92% No: 8%	Yes: 67% No: 33%	Yes: 100%
6	Appropriate removal of carcasses, slaughter residues and food waste	General (CM, NCM, OD)	(57, 58, 63, 67)	Yes: 100%	Yes: 92% No: 8%	Yes: 100%
7	Discouragement of sharing used equipment between holdings and/or units	General (CM, NCM, OD)	(45, 47, 52, 55, 57, 63)	Yes: 100%	Yes: 83% No: 17%	Yes: 100%
8	Use of footbaths in entrance of units where animals are held	General (CM, NCM, OD)	(5, 15, 49, 52, 58, 63)	Yes: 75% No: 25%	Yes: 50% No: 50%	Yes: 33% No: 67%
9	Daily health checks for clinical signs and mortality rates	General (CM, NCM, OD)	(45)	Yes: 100%	Yes: 58% No: 42%	Yes: 92% No: 8%
10	Cleaning and disinfectant protocols for facilities, vehicles, and equipment	General (CM, NCM, OD)	(15, 16, 18, 35, 42, 44, 49, 55, 57, 63)	Yes: 100%	Yes: 50% No: 50%	Yes: 92% No: 8%
11	Farm location far from suitable wild boar areas and close to geographical barriers	General (CM, NCM, OD)	(16, 28, 48, 53)	Yes: 67% No: 33%	Yes: 58% No: 42%	Yes: 75% No: 25%
12	Control measures against flies	General (CM, NCM, OD)	(45, 68, 69)	Yes: 75% No: 25%	Yes: 42% No: 58%	Yes: 17% No: 83%
13	Establishing clean/dirty areas (including changing rooms and showers)	CM	(15, 18, 31, 49, 55)	Yes: 92% No: 8%	Yes: 58% No: 42%	Yes: 67% No: 33%
14	Logistical arrangement for the entry and exit of animals including protocols regarding entrance of vehicles, loading areas, role of pig transporters, etc.	CM	(5, 15, 18, 42, 45, 58, 65, 66, 70)	Yes: 100%	Yes: 33% No: 67%	Yes: 75% No: 25%
15	Cleaning and disinfection protocols for transport vehicles	CM	(27, 42, 45)	Yes: 100%	Yes: 58% No: 42%	Yes: 83% No: 17%

(Continued)

TABLE 2 | Continued

ID	Preventive measures	Systematic literature review		Results of the assessment		
		Type	Reference	CM	NCM	OD
16	Quarantine period for purchased animals and quarantine rooms	CM	(5, 16, 18, 24, 35, 45, 55, 58, 64)	Yes: 100%	Yes: 25% No: 75%	Yes: 67% No: 33%
17	Identification of animals and farm records including animal movements	CM	(15, 16, 23, 27, 32, 45, 55, 65)	Yes: 100%	Yes: 100%	Yes: 100%
18	Internal audits and evaluations to enforce biosecurity measures	CM	(15, 18)	Yes: 92% No: 8%	Yes: 25% No: 75%	Yes: 67% No: 33%
19	Rules for food staff entering the farm (i.e., restricted to eating rooms or not allowed)	CM	(15, 18, 44, 52)	Yes: 100%	Yes: 58% No: 42%	Yes: 75% No: 25%
20	Proper disposal of manure and dead animals	CM	(5, 58, 65, 71)	Yes: 100%	Yes: 92% No: 8%	Yes: 92% No: 8%
21	Strict enforcement of the ban on swill feeding	NCM	(5, 15, 22, 38, 43, 45, 47, 52, 57, 64)	Yes: 100%	Yes: 100%	Yes: 100%
22	Containment of pigs, do not allow contact with pigs from other farms, feral pigs, or wild boar or their products	NCM	(5, 15, 43, 55)	Yes: 100%	Yes: 100%	Yes: 100%
23	Farmers/farm staff should not have hunted, allowing a 48 h interval between hunting and being in contact with domestic pigs, if they work in an infected wild boar area	NCM	(15, 61)	Yes: 92% No: 8%	Yes: 92% No: 8%	Yes: 92% No: 8%
24	Effective disinfection and cleaning of the surrounding of the holding including its entrance	NCM	(5)	Yes: 58% No: 42%	Yes: 50% No: 50%	Yes: 42% No: 58%
25	Veterinary supervision prior and while home slaughtering	NCM	(15, 72)	Yes: 50% Na: 33% No: 17%	Yes: 83% No: 17%	Yes: 67% Na: 16.5% No: 16.5%
26	Cleaning and disinfection protocols before and after home slaughter (regarding slaughtering tools, facilities, clothing and footwear, etc.)	NCM	(15, 16)	Yes: 42% Na: 25% No: 33%	Yes: 83% No: 17%	Yes: 67% Na: 8% No: 25%
27	No sows or boars used for mating purposes held on non-commercial farm	NCM	(15, 72)	Yes: 58% Na: 42%	Yes: 67% Na: 8% No: 25%	Yes: 42% Na: 33% No: 25%
28	No movements between/from non-commercial farms	NCM	(43, 72)	Yes: 75% Na: 17% No: 8%	Yes: 92% No: 8%	Yes: 67% Na: 16.5% No: 16.5%
29	Avoid use of fresh fodder in areas at risk of exposure to ASF	NCM	(15, 22, 28, 47)	Yes: 67% No: 33%	Yes: 75% No: 25%	Yes: 75% No: 25%
30	Promote educational programs through governmental training programmes and improve access to health services	NCM	(4, 15, 20, 23)	Yes: 92% No: 8%	Yes: 100%	Yes: 100%
31	Treatment and storage (out of reach of wild boars) of grass or grains for at least 30 days or prohibit its use	NCM	(15, 47)	Yes: 75% No: 25%	Yes: 75% No: 25%	Yes: 67% No: 33%
32	Avoid the use of straw bedding unless treated to inactivate ASF and stored for at least 90 days	NCM	(15)	Yes: 83% No: 17%	Yes: 83% No: 17%	Yes: 83% No: 17%
33	No exchange of feed or bedding with other farms	NCM	(47)	Yes: 92% No: 8%	Yes: 92% No: 8%	Yes: 92% No: 8%
34	Banning of free-range management on communal areas or public forests with no biosecurity measure	OD	(5, 15, 16, 20, 23, 32, 47)	Yes: 67% Na: 16.5% No: 16.5%	Yes: 92% No: 8%	Yes: 92% No: 8%
35	Distance between outdoor farms (at least 1 km) to minimize the risk of ASF introduction through direct and indirect contact	OD	(73)	Yes: 16% Na: 42% No: 42%	Yes: 33% Na: 25% No: 42%	Yes: 67% No: 33%
36	If they were *Ornithodoros* ticks avoid using traditional pig-housing facilities (usually made of wood and stones were ticks can be hidden)	OD	(34, 54, 64, 65)	Yes: 84% Na: 8% No: 8%	Yes: 75% Na: 8% No: 17%	Yes: 84% Na: 8% No: 8%
37	Apply chemical control if ticks were present in traditional pig-housing facilities	OD	(34)	Yes: 84% Na: 8% No: 8%	Yes: 92% Na: 8%	Yes: 92% Na: 8%

Results of the assessment of identified preventive measures represented as percentage of yes, not applicable (Na) and no.

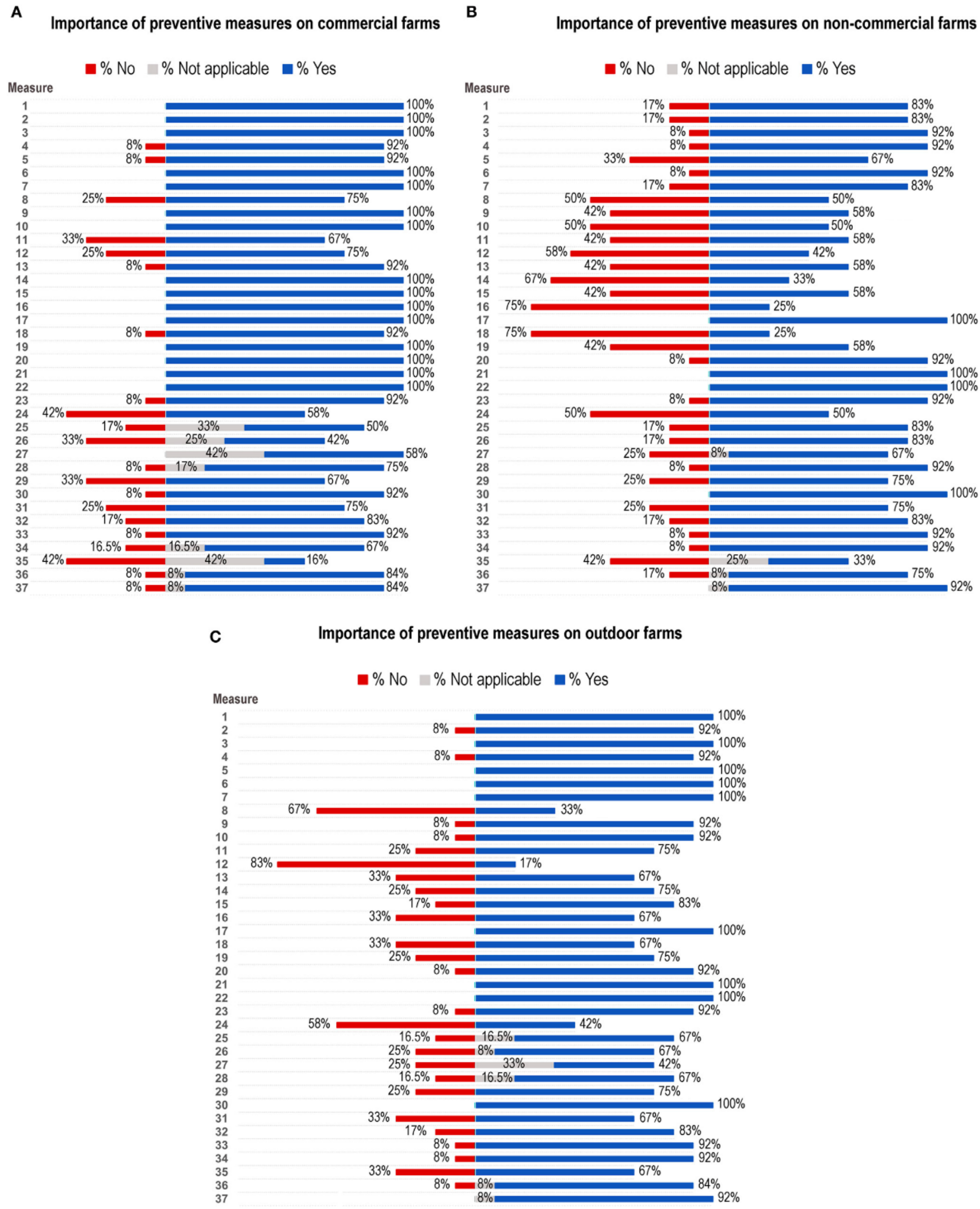

FIGURE 2 | Results of the assessment of identified preventive measures represented as percentage of yes (blue bars), no (red bars), and not applicable (gray bars) to **(A)** commercial farms, **(B)** non-commercial farms, and **(C)** outdoor farms. Listed preventive measures are described in **Table 2**.

as important preventive measures for all type of holdings. Quarantine period for purchased animals in quarantine rooms was identified as a relevant measure for commercial farms by 12 experts. In agreement with this result, experts in Switzerland perceived that purchasing from farms with known disease status and health certificates as 5/5 for importance and effectiveness as a biosecurity measure to prevent the introduction of ASF onto pig farms (29). Interestingly, 9 experts out of 12 did not consider this measure important on non-commercial farms, although the same number consider it important to check ASF-free certificates and health status before acquiring new animals. This may be explained because the feasibility of quarantine periods and establishment of quarantine rooms and procedure could be challenging on non-commercial farms where investment and facilities are minimum. This measure becomes particularly relevant when tackling the phenomenon of "emergency sale," in which farmers from non-commercial holdings attempt to sell infected pigs to minimize their economic losses (87, 94–96). The latest working document elaborated by the Directorate General for Health and Food Safety (15), which contains the majority of measures reviewed in the systematic literature review, aim at the improvement of biosecurity measures dealing with the replacement of animals, facilities design, and management practices, in particular in relation with cleaning and disinfection facilities, in such holdings. Very few outbreaks have led to secondary spread in the EU and there has been a significant progress in EU advice to improve preventive measures against ASF in non-commercial farms.

The Eastern EU scenario presents the additional challenge of spill-over from wild boar, where 95% of the ASF notifications have taken place (8) and which is playing a primary role in disease spread. However, additional measures were extracted during the review process (see **Table 1**). In Poland and Latvia, outbreak investigations carried out on several ASF positive farms determined that the most likely source of infection was wild boar (82, 97). Studies concluded that the poor biosecurity measures of affected holdings favored transmission between wild boar and domestic pigs (82, 97). Consequently, the EU elaborated a guidance where minimum biosecurity measures on farms were defined and biosecurity was enhance to minimize the risk of spread from wild boar (15, 63). One of the suggested measures found in the literature is "to locate farms far from suitable wild boar areas and close to physical barriers" (16, 48, 53, 83) since there is a disease interface where domestic pig and wild boar share location. Observations related to the wild boar–domestic pig interface indicated that all ASF notifications in domestic pig holdings were situated in areas with suitable wild boar habitat (53). Around 65% occurred in natural landscapes, the natural habitat for wild boar (28). The remaining 35% were located in mosaic agroforestry areas and buffer monoculture areas surrounding natural landscapes where agro-livestock activities are usually concentrated (28). In these areas, wild boars can receive, with minimal foraging, substantial amounts of protein from cultivated plants such as maize, wheat, barley, rapeseed, and sunflower seeds (98). Farm location far from suitable wild boar areas and close to geographical barriers was classified as important by more than half of experts. As expected, such measures were relevant to more experts on outdoor farms (9 experts), followed by commercial (8 experts), and non-commercial holdings (7 experts). This slight difference might be explained because the likelihood of wild boar being in contact with pigs would be higher on outdoor farms (where biosecurity is intrinsically lower) than on commercial or non-commercial farms. Experts who declined to consider it important, refereed that this measure is almost unfeasible considering the ecological characteristics of the European continent. Moreover, some of the experts who considered it important wanted to highlight that such a measure would only be applicable to new holdings.

Most experts (11 out of 12) recognized the importance of allowing a 48 h interval between hunting and being in contact with domestic pigs if farmers and farm staff worked in an infected wild boar area. Although it is not the scope of this article to cover the control measures in wild boar, the management of wild boar populations and hunting practices in affected areas has an undeniable effect over the prevention of ASF at the interface with domestic pigs located in the same area. Such measures have included the reduction of wild boar densities (53, 59) and the immediate removal of infectious carcases (5). However, wild boar cases have continued being notified in the area suggesting that there is still room for improving the strategy.

The second main area of prevention deals with avoiding ASF transmission through the ingestion of contaminated food. Even if swill feeding is banned in the EU, all experts agreed that it was an important measure to prevent ASF spread. Other measures identified in this sense are rules on food entry for farm workers in commercial farms; proper disposal of manure and dead animals; avoiding the use of fresh fodder from areas at risk of ASF unless a treatment to inactivate potential ASF virus, has been applied; or avoid sharing feed between farms. Long distance ASF transmission has been associated to the disposal of infected waste, meat or meat products in wild boar habitat, for example, in the Czech Republic, where the closest ASF cases were about 400–500 km away. Moreover, evidences of domestic pigs and/or pig sub-products as source of infection are scarce but they have been suspected in a few cases, like in Romania. On July 31, 2017, Romania's Veterinary Authority confirmed the first detection of ASF in a backyard herd of domestic pigs. Romania's Veterinary Authority suspects that contaminated Ukrainian products are the likely source of the Romanian detection (99). Human mistakes, lack of knowledge on ASF transmission, or insufficient enforcement are the most common reasons to fail to comply with these measures, particularly for non-commercial farms, and are directly related to the third main area of ASF prevention: improvement of health services and education.

Better access to veterinary health services and educational programmes, with specific training on ASF identification and biosecurity measures, are essential tools to improve human-mediated prevention measures. More than 11 experts agreed with this idea, considering both measures important for non-commercial farms but also, for commercial and outdoor facilities. In the end, effectiveness of prevention depends on awareness, compliance and diligence of people dealing with disease control and good timing of implemented measures

(100). The effectiveness of prevention is also influenced by socioeconomic, cultural, or traditional factors that will predispose the capability, attitudes, or willingness of people involved in disease control to implement preventive strategies. The understanding of such factors is particularly critical for backyards and small farmers, since economic and resources restraints can more easily limit the achievement of the preventive measure objective (16, 95). Generally, the effectiveness of preventive measures will be related to how farmers perceive the importance of each measures as well as what measures they are actually implementing (55). Farmers and workers are at the forefront of implementing biosecurity measures on the farms to prevent the spread of diseases. The application of these measures heavily depends upon the attitude and knowledge they have with regard to biosecurity measures (101). A study carried out in Great Britain showed that English pig farmers had poor knowledge about ASF as well as limited concern about it (26). Vergne et al. (102) also highlighted that the reasons for lack of immediate reporting in suspected ASF cases in Germany, the Russian Federation, and Bulgaria would be due to not knowing reporting procedures, fear that the report could have a negative impact on their reputation, and assuming they would be capable of handling the outbreak on their own. These studies (26, 102, 103) suggested that there is still room for improving farmers' knowledge to bridge the gap between authorities and farmers and consequently help prevent the spread of ASF (39). Similarly, to be able to effectively influence farm workers, veterinarians, and hunters' behavior, it is essential to analyze the "at-risk" practices that depended on human behavior which can perpetuate ASF spread and find out measures tailored to each specific situation.

From the research side, efforts have been made to fill in gaps that make disease control and eradication difficult. A recent publication identified current gaps in ASF and prioritized them into high importance, medium importance, and low importance (48). Highest importance was attributed to measures aimed at improving prevention and control of ASF, namely, (i) to raise awareness among hunters, farmers and veterinarians and (ii) to have adequate implementation of early warning systems, contingency plans, and control measures. Preventive measures of medium importance were (iii) to implement surveillance activities based on the risk of potential exposure, introduction and spread. Measures of low importance were (iv) to promote confinement of pigs in infected areas, and (v) to establish regulations to ensure farms are located far from areas suitable for wild boar. Finally, with regard to the importance of wild boar in ASF epidemiology, more research should be focused on (vi) increasing the availability of reliable population data, (vii) understanding role of this host in disease maintenance and spread, and (viii) developing non-invasive sampling methods (48, 50, 59). However, without an ASF vaccine, prevention of ASF becomes very challenging for the European pig sector. Despite advances, a safe and effective vaccine is still lacking. Thus, control and eradication of this disease still relies on rapid detection in field followed by the application of strict sanitary measures. Likewise, biosecurity is the only tool farms have to prevent the introduction of ASF. Therefore, joined efforts focusing on the domestic pig sector and wild boar need to be applied in parallel. This way, we will move forward to the final goal of eradicating ASF from the second largest world's pork producer, the EU.

CONCLUSION

African swine fever is currently one of the major threats to the pig production in the EU. As there is no a vaccine against ASF, biosecurity is key to prevent its spread between and within domestic pig farms. This study identified thirty-seven preventive measures aimed at reducing the spread of ASF among domestic pigs. These measures were also assessed by ASF experts within the framework of the EU scenario. According to this expert panel, the most important preventive measures for commercial, non-commercial, and outdoor farms were the identification of animals and farm records; enforcement of the ban on swill feeding; and containment of pigs to not allow contact with pigs from other farms, feral pigs, or wild boar or their products. In addition to this, other measures were considered relevant in preventing ASF introduction, namely education of farmers, workers, and operators; no contact between farmers, farm staff and external pigs; appropriate removal of carcasses, slaughter residues and food waste; proper disposal of manure and dead animals, and abstention from hunting activities for a period of 48 h prior to any contact with domestic pigs. Finally, all experts considered important to facilitate and promote the access of veterinarians and health services to non-commercial and outdoor farms. Adequate implementation of these measures can lead to significant advances in ASF prevention and control, and possibility contributing to the eradication of ASF from the EU pig sector.

AUTHOR CONTRIBUTIONS

All authors contributed to the literature review performed to build this review. CJ compiled the whole information and wrote the manuscript. CJ, JS-V, and SB designed the questionnaire for the assessment of preventive measures. CJ analyzed results from the expert opinion. CJ, SB, JS-V, MM-A, and AT participated in the creation of the argument line of this the text. All authors contributed to the critical review of the manuscript and approved the final version.

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Conflict of Interest Statement: HF was employed by company GD Animal Health Research Institute. All other authors declare no competing interests.

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CHAPTER 4

SUB-OBJECTIVE 4.1. To assess the risk of introduction of ASFV and CSFV into the United States via prohibited swine products carried by air passengers' luggage. The risk was characterised at time and space level (*i.e.* origin country, destination airport and connecting flight levels).

Main scientific publication of objective 4.1.

Jurado C, Paternoster G, Martinez-Lopez B, Burton K, Mur L. **Could African swine fever and classical swine fever viruses enter into the United States via swine products carried in air passengers' luggage?** Transbound Emerg Dis. 2018;66(1):166-180. DOI: 10.1111/tbed.12996.

SUB-OBJECTIVE 4.2. To reassess the risk of ASFV introduction into the United States considering that the disease distribution considerably changed with the notification of ASF in 2018 and 2019.

Main scientific publication of objective 4.2.

Jurado C, Mur L, Pérez Aguirreburualde MS, Cadenas-Fernandez E, Martinez-Lopez B, Sanchez-Vizcaino JM, et al. **Risk of African swine fever virus introduction into the United States through smuggling of pork in air passenger luggage.** *Accepted in Nature Scientific Reports on the 11th of September 2019.*

Related scientific contributions:

University congresses:

Jurado C, Paternoster G, Martinez-Lopez B, Burton K, Sanchez-Vizcaino JM, Mur L. **Could African swine fever and classical swine fever viruses enter into the United States via swine products carried in air passengers' luggage?** 4th VETINDOC, PhDay, UCM. Oral communication. 2018.

RESUMEN DE LOS RESULTADOS DEL CAPÍTULO 4

El valor de la producción del sector agrícola de EEUU se estimó en 407,8 mil millones de dólares americanos ($) en 2017, siendo 175,3 mil millones de $ atribuidos a productos de origen animal y animales vivos. La producción porcina aporta 20 mil millones de $ a la economía de los EEUU. Teniendo en cuenta que EEUU es el tercer productor de porcino del mundo (con más de 11 millones de toneladas de carne de cerdo producida durante 2016) y el segundo mayor exportador mundial de carne de cerdo (por un valor de 4,2 mil millones de $ en 2016), la introducción de enfermedades transfronterizas como la PPA causaría tremendas consecuencias económicas debido a las restricciones comerciales aplicadas después de la notificación de la enfermedad. Conscientes del riesgo al que se encuentran expuestos, las autoridades estadounidenses han establecido varios mecanismos para evitar la entrada de productos de origen animal a través de aeropuertos. La principal herramienta utilizada para mitigar este riesgo es la realización de controles por parte de los inspectores de aduanas.

Dentro de este capítulo, se evaluó el riesgo de introducción de los virus de la PPA (VPPA) y de la PPC (VPPC) a través del equipaje de pasajeros procedentes de vuelos internacionales. Para ello, se construyeron dos modelos estocásticos cuantitativos que permitieron caracterizar el riesgo de entrada a nivel espacial y temporal.

En el momento en el que se realizó el análisis (julio de 2016), los resultados mostraron que la probabilidad de introducción del VPPC era 7 veces mayor que la probabilidad de introducción del VPPA. Para ambas enfermedades, julio y mayo fueron los meses de mayor riesgo. Sin embargo, se observaron diferencias entre ambas enfermedades en términos de localización del riesgo. Para PPA, Gana, Cabo Verde, Etiopía y Rusia representaron más del 70% del riesgo total. Además, el riesgo de entrada se concentró en más de un 90%, en cinco aeropuertos de EEUU, en concreto, en los aeropuertos de Washington-Dulles (Virginia), John F. Kennedy-Queens (Nueva York), George Bush-Houston (Tejas), Warwick (Rhode Island) y San Juan (Puerto Rico).

Sin embargo, en 2018, la PPA se extendió a países occidentales de la UE como Bélgica y, por primera vez, a Asia. Un cambio tan dramático en las condiciones epidemiológicas globales de la PPA ha incrementado las preocupaciones sobre la posibilidad de que la enfermedad continúe propagándose a regiones libres, como EEUU Por ello, se consideró necesario revaluar el riesgo de introducción del VPPA a través de la vía anteriormente mencionada.

Los resultados mostraron que el riesgo medio de introducción se ha visto incrementado en un 183% en comparación con el riesgo estimado anteriormente. La probabilidad media de introducción se correspondería con al menos una introducción del virus cada 9 años (siendo el límite inferior del intervalo de confianza al 95%, una introducción cada 2 años). Tres países y una región (China, Hong Kong, Rusia y Polonia) representaron el 97% del riesgo. Por otro lado, más del 90% del riesgo se concentró en los aeropuertos de Newark-New Jersey (46,38%), George Bush-Houston-Tejas (32,71%), Los Ángeles-California (5,18%), John F. Kennedy-New York (5,04%) y San José-California (2,87%). Además, la fluctuación estacional del riesgo fue influenciada por la frecuencia de vuelos desde regiones afectadas, sin embargo el riesgo tendió a concentrarse en los meses de verano, y en especial, en julio.

Could African swine fever and classical swine fever viruses enter into the United States via swine products carried in air passengers' luggage?

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Summary

On average 8,000 pork derived products are annually confiscated by Customs and Border Protection at the United States (US) ports of entry such as international airports, harbours or mail offices. These swine products with unknown sanitary status could pose a risk for foreign animal diseases introduction into the US. This study aimed at analysing the risk of African swine fever virus (ASFV) and Classical swine fever virus (CSFV) being introduced into the US through prohibited swine products carried by air passengers (PSPAP) and identifying locations and time periods at higher risk where and when preventive and mitigation measures should be implemented. Our results estimated that the risk for CSFV entry was seven times higher and further spread between US airports than for ASFV. Specifically, the overall mean annual probability of ASFV entry was estimated as 0.061 at 95% confidence interval (CI) [0.007, 0.216] while the probability of CSFV entry was estimated as 0.414 (95% CI [0.074, 1]). For both diseases, July and May were the months at highest risk for entry. For ASFV, the origin countries of those PSPAP that represented the highest risk (above 70% of the total risk) were Ghana, Cape Verde, Ethiopia, and the Russian Federation, while for CSFV above 90% of the risk at origin was concentrated in the Dominican Republic and Cuba, followed by India, Colombia, Peru, Ecuador and China. These results could be used to implement and feed real time surveillance systems, which could potentially help customs to increase the detection rate of smuggled products, indicating when and where to look for them. Similarly, these systems could be adapted and implemented to other diseases improving the cost-effectiveness of the resources invested in preventing entrance of diseases via air passengers' luggage.

Keywords

Epidemiology, foreign animal diseases, international flights, quantitative risk assessment, smuggling products, targeted surveillance.

1. Introduction

The volume of smuggled and improperly imported agricultural products, including fruits, vegetables, plants, meat and animal products, arriving to the United States (US) increases every year (265). Data collected under the smuggling, interdiction and trade compliance program between 2005 and 2006 showed that approximately 30% of those smuggled agricultural products arriving to the US are animal products (266). Just considering pork products, an average of 8,000 units are annually confiscated by Customs and Border Protection (CBP) at the US ports of entry such as international airports, harbours or mail offices (Work Accomplishment Dataset from USDA and Animal and Plant Health Inspection Service, APHIS between January-2010 and December-2016). During the fiscal year 2016 (from October 1, 2015 to September 30, 2016), US agricultural statistics showed that more than 23 million passenger inspections were conducted versus almost 741,000 cargo inspections (267). Based on USDA-APHIS dataset, 45% of pork products confiscated at US ports of entry were intercepted at international airports carried by air passengers in their personal luggage. From 2010 to 2015, 81% of total confiscations occurred in airport and land border controls. Over this time period, the average monthly number of airport confiscations increased (from 292 to 306), whereas the number of interceptions in land borders controls decreased (from 252 to 215). Seasonality was observed, being July the month with the highest PSPAP confiscations along this time period.

These swine products with unknown sanitary status could be a potential source of infection of foreign animal diseases (FADs) such as African swine fever (ASF) (EFSA, 2014) or Classical swine fever (CSF) if they eventually contact US livestock populations (268, 269). The value of US agricultural sector production (mainly composed of crop and livestock) is expected to be $407.8 billion in 2017, being $175.3 billion attributed to total animal/animal product cash receipts (270). Focusing on US pig/pork production, this sector is expected to contribute around $20 billion to the US economy (270). Bearing in mind that the US is the world's third largest pig producer (more than 11 million tons of pork produced during 2016) and the world's second largest exporter of pork ($4.2 billion in 2016) (271), the introduction of those diseases would cause tremendous economic consequences due to trade restrictions applied after disease notification. As an example of this hypothetical situation, Rendleman *et al.* (1999) assessed the social costs and benefits of ASF prevention in the US. Their results showed that ASF occurrence would cost more than $4.25 billion while the cost benefit ratio of an ASF prevention program would be higher than 450. These costs should be summed to a decline in prices, decrease of consumers' confidence, direct and indirect costs of control and eradication and social consequences affecting farmers and thousands US workers directly and indirectly employed by livestock industries.

Aware of those risks, the US has several mechanisms in place to prevent the introduction of improperly imported animal derived products through numerous controls, including checking of air passenger's luggage. If any animal products were found in the incoming luggage, information on such findings would be recorded and those products would be subsequently confiscated and safely destroyed (272). However, the estimated detection rate of these products is relatively low, ranging from 10% to 50% depending on the source of information (273, 274). This means a large volume of prohibited animal products potentially contaminated with pathogens causing FADs or even zoonotic diseases, could be entering the US every day without being detected.

The possibility of animal derived products infected with pathogens being introduced into the US have been identified in previous analysis as one of the highest risks for the entry of both FADs into the US [*i.e.* for CSF in NABC (274)]. In addition, several authors have detected those risks from products intercepted in customs. Specifically, zoonotic viruses were detected in bush meat brought by air passengers into the US (275), foodborne pathogens were detected in 2.5% of food products confiscated in air passengers' luggage in Germany (276) and in up to 54.9% of meat samples tested in a Spanish airport (277).

Given the importance of the swine sector in the US and the current distribution of several swine diseases such as ASF and CSF, this study aimed at analysing the risk of ASFV and CSFV entry (formerly named release) into the US through prohibited swine products carried by air passengers (PSPAP), identifying locations and time periods at higher risk where and when preventive and mitigation measures could be more cost-effectively implemented to increase the number of interceptions and reduce the risk of FADs introduction into the US.

2. Materials and methods

2.1. Models design

Two quantitative stochastic models, one for ASF and another for CSF, were built to assess the monthly probabilities of ASF virus (ASFV) and CSF virus (CSFV) entry into the US through PSPAP. Country level for origin, and US airports for destination were selected as space units of analysis; and the seasonality of the risk was assessed by month. Risk models were developed in @RISK 7.5 (Palisade Corporation, Newfield, NY, USA) on Microsoft Excel 2007® and run 1,000 iterations using the Monte-Carlo sampling method.

Each quantitative model followed a binomial process according to the formula:

$$\mathrm{P}\ (\mathrm{x} \geq 1) = \sum 1 - (1 - \mathrm{Pi}_{\mathrm{vo}})^{N_{odm}}$$

where N_{odm} was the estimated volume (in Kilograms, kg) of PSPAP introduced into each US airport through each international flight, per month and origin country (common for both models/viruses); and Pi_{vo} was the estimated probability of at least one kg of PSPAP from each origin country to be contaminated with ASFV or CSFV (specific for each virus). The ∑ refers to the probability of entry considering all the different international flights.

Assuming that the probability of entry of both viruses is independent and non-mutually exclusive, the final probability of ASFV or CSFV being introduced into the US by PSPAP was calculated as $P_{ASFV} \cup P_{CSFV} = P_{ASFV} + P_{CSFV} - (P_{ASFV} * P_{CSFV})$

The specific details of each model component, the input parameters and data sources are explained in detail hereinafter. Specifically, we divided the explanation of the model into two modules: i) volume of PSPAP (N_{odm}) and ii) probability of PSPAP being contaminated with ASFV or CSFV (Pi_{vo}).

2.1.1. Estimation of PSPAP volume introduced into US airports per month and origin country (N_{odm})

The model structure and assumptions for the estimation of N_{odm} was heavily influenced by the quality and completeness of the data available. Two main datasets were essential for the estimation of the PSPAP arriving into the US. Firstly, the data on the number of PSPAP confiscated in US destination airports by CBP was obtained from USDA/APHIS. The Agricultural Quarantine Activity Work Accomplishment (WADS) database contains records of the agricultural products confiscated in US ports of entry. Specifically, we received access to the records of swine products intercepted from January-2010 to March-2016. Only the records of confiscations from air passengers at airport controls were used in the analysis, which represent 45% of the total pork products intercepted during those years in all of the US. Those records included detailed information on the port of entry (name of the airport) where they were confiscated, month, year, mode of introduction and quantity (however, based on personal communication with DHS personnel, this last field was not reliable due to differences between points of entry).

Secondly, the information on the number of air passengers arriving in the US via direct international commercial flights was obtained from the T-100 International Segment database (278). This database contained information from January-2010 until August-2016 on the international passenger flights arriving in the US including country of origin, arrival airport in the US, month and year of the travel. The number of destination airports considered in this study was 87, corresponding to all US airports receiving international flights. The number of origin countries was 128, for which international flights to the US were registered during the study period.

The biggest limitation of the WADs database is that no information was available for the origins of those confiscated PSPAP. Therefore, in order to estimate the origin of those confiscations, we assumed that the origin of the PSPAP confiscated in each destination airport per month was proportional to the volume of luggage arriving at that specific airport from each origin country.

Accordingly, we initially estimated the proportion of kg of air passengers' luggage arriving to each US airport from each origin country per month. For that purpose, we first parameterised the number of passengers arriving to the US via commercial flights per country of origin, destination airport, and month using a normal distribution considering data of the last 6 years to account for trends and seasonal variations over time (period 2010-2016). In parallel, as data on the actual weight of checked baggage per each flight was not available, the number of kg of checked luggage allowed per air passenger was estimated based on the free checked baggage allowance in the economy class of the three most important air carriers in the US (United Air Lines Inc., Delta Air Lines Inc., and American Airlines Inc.) which represented 31% of all international commercial flights arriving in the US from January 2010 to August 2016.

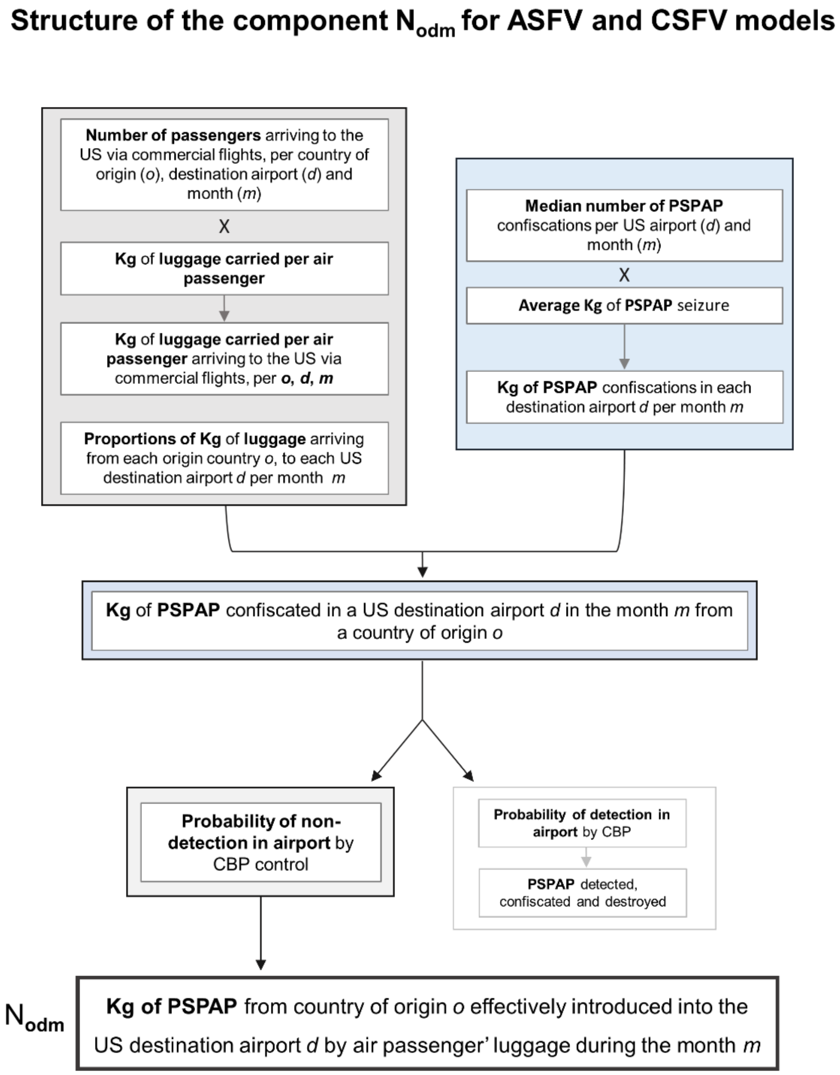
The variability between baggage allowance per airline and origin region was considered, as this input was parameterised as a pert distribution defined by the data collected. The minimum value was assumed to be 10 kg (hand baggage only), while the maximum value was assumed to be the sum of the hand baggage (10 kg) and the maximum free baggage allowance according to the origin region of the flight. The most probable value was the median of these values. The product of both parameters (passengers and the number of kg of luggage) resulted in the estimated number of kg of luggage per origin country, US airport and month. This information was used to estimate the proportion of kg of luggage arriving to each US airport from each origin country in month m. The results of this proportion were explored and fitted with a normal distribution for their use in following steps of the model.

In parallel, we estimated the volume (kg) of monthly confiscated PSPAP per US airport by multiplying the median number of PSPAP confiscations per month per airport (as this number is very stable for each airport according to WADS records) by the estimated weight of each individual PSPAP confiscation, based on previous data on US customs interceptions (273) and parameterised using a pert distribution. The volume (kg) of monthly confiscated PSPAP per US airport was multiplied by the proportion of kg of luggage from each origin country (*o*), arriving monthly (*m*) to each US airport (*d*) resulting in the estimated kg of PSPAP confiscated in a US destination airport *d* in the month *m* from a country of origin *o*.

However, we were not interested in the interceptions, but the products escaping from controls, and entering into the US. All PSPAP intercepted at US control borders are destroyed (272), thus assumed to not represent a hazard for ASFV or CFSV entry. However, between 50% and 90% of smuggled animal products carried in passengers' baggage escapes interception by CBP personnel (273, 274). These reference values (50% - 90% smuggled animals products are not detected) were modified assuming an improvement in the probability of detection since 1997, and modelled using a triangular distribution (as detailed in Table 1, shown at the end of the publication), to estimate the probability of non-detection of PSPAP at CBP controls. Then, this probability was used to estimate the kg of PSPAP that escape controls and are effectively introduced into the US by air passenger luggage per month and country of origin. Figure 1 depicts and summarises the structure of the component N_{odm} of the model, which is equal for ASFV and CSFV models.

In addition, Table 1 (shown at the end of the publication) describes in detail each model component, input parameters and data sources used to estimate the volume in kg of PSPAP getting into the US.

Figure 1: Model structure for the estimation of the volume (kg) of prohibited swine products carried in air passengers' luggage (PSPAP) introduced into the US. This component, named N_{odm}, was equal for African swine fever virus and classical swine fever virus models.



2.1.2. Probability of PSPAP being contaminated with ASFV or CSFV [Pi_{vo}]

The 128 countries of origin were classified in three categories based on the diseases status of ASF or CSF obtained from the OIE-WAHIS database (25). The three categories include: i) category "A" for countries where the disease is present and restricted to certain areas, and/or it is suspected in domestic pigs and/or wild boar; ii) category "B" for countries sharing geographical boundaries with countries belonging to category "A" or where disease outbreaks occurred in the near past (2015); and iii) category "C" for countries officially free from infection. The probability of ASFV or CSFV infection was modelled differently depending on the aforementioned categories (see Table 2).

For all the categories, it was assumed that the probability of one kg of PSPAP being contaminated with ASFV or CSFV was equivalent to the probability of at least one domestic pig being infected in the country of origin. This is a conservative approach, as infected pigs are often detected by farmers and/or veterinarians before arriving to the food chain. However, considering that this model includes products arriving from any country of the world (including ASF and CSF endemic countries), where the capacity of the veterinary services is extremely diverse, a conservative approach was assumed with the highest risk scenario. Similarly, no differences were considered between types of pork products. Virus survival and virus load in animal products vary depending on the piece of meat, time after the infection and processing with thermal/chemical treatments (260, 268).

Unfortunately, as no information on the type of products was recorded in the WADS dataset, according to the principle of maximum risk or worst case scenario, all PSPAP were considered as potentially contaminated with ASFV and/or CSFV.

For category "A" countries, the probability of ASFV or CSFV infection per origin country was estimated using a beta distribution taking into account the potential number of non-reported infected pigs and the number of pigs monthly slaughtered in the country. Specifically, the product of the apparent prevalence in the country by month, the duration of the infection (assuming that dead pigs do not go to slaughter) and the notification underreporting as previously estimated (279), resulted in the estimated number of non-reported infected pigs per month per country (Ni_{om}). In order to estimate the number of non-reported infected pigs slaughtered per month and country, we multiplied the number of non-reported infected pigs (Ni_{om}) by the modified proportion of pigs slaughtered per month (Mod Prop-S_m).

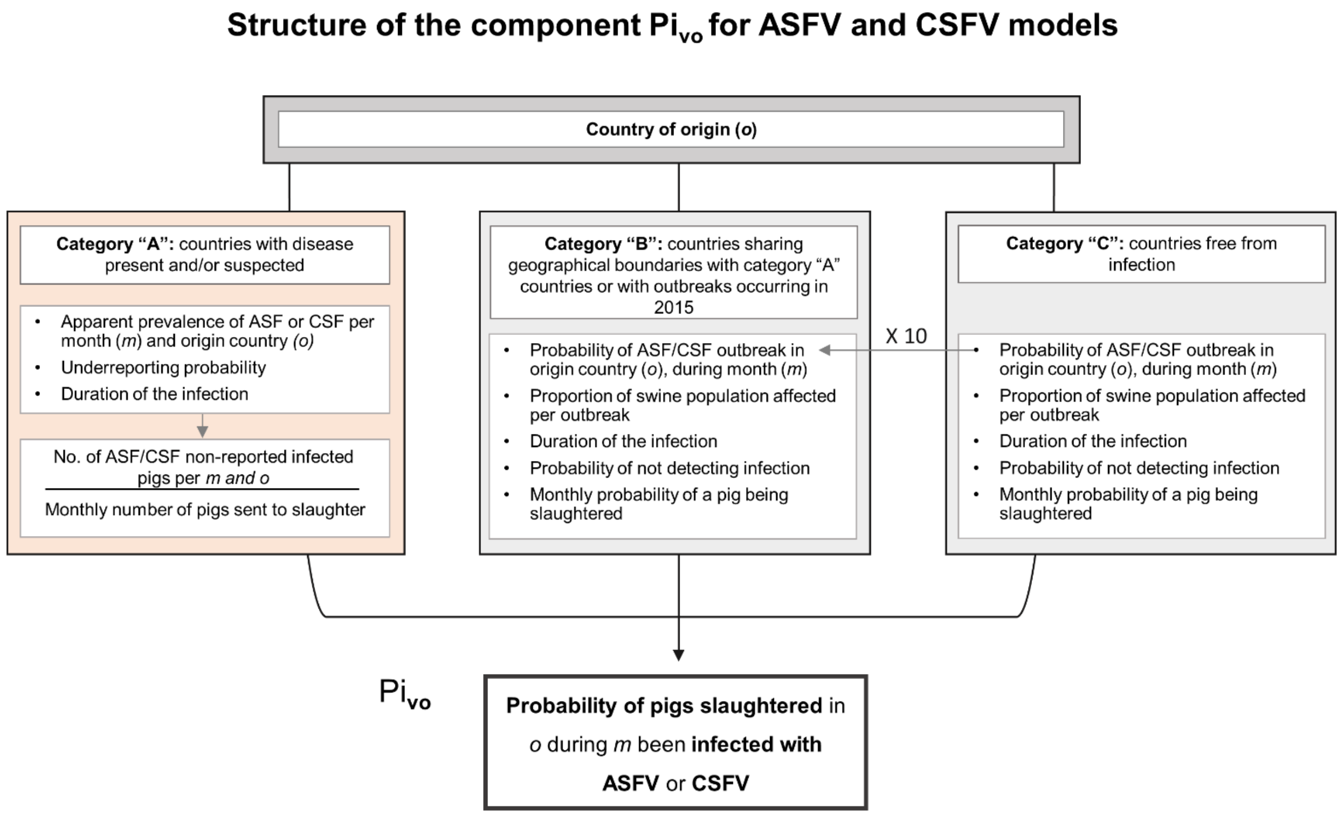
We took into account that the probability of slaughtering pigs can strongly vary depending on the approach of the farmers towards the disease, the clinical manifestations of the disease and other socio-economic factors (*i.e.* farmers tend to sell/slaughter the animals before they get sick). For that purpose, a conservative approach was used, as the Mod Prop-S_m was parametrised as a uniform distribution being the minimum, the mean value of the distribution Prop-S_m (normal situation), and the maximum, 100% (worst case scenario, where all pigs would be slaughtered if farmers suspected the disease). The number of pigs slaughtered per month (Prop-S_m) was estimated considering the annual pig census in each origin country by the proportion of pigs slaughtered per month.

In contrast, the probability of infection in free and neighbouring countries (categories “B” and “C”) was estimated following a similar approach used in Veterinary Laboratory Agency (279), by multiplying: the probability of an outbreak occurring in the country, the average size of the outbreak (number of affected pigs), the duration of the infection, the probability of outbreaks being undetected and the proportion of pigs going to the slaughterhouse that month. The only difference is that for countries located in regions considered at risk due to the proximity to affected countries (category “B”), the probability of outbreak occurrence was 10 times higher than for free countries.

Some assumptions were done for countries with no detailed information available.

For three African countries (*i.e.* Guinea, Liberia and Ethiopia) as data on ASF outbreaks and pig population were not available, the median value of the probability of infection of the other affected African countries was used. As no data on CSF outbreaks were found for Indonesia and Philippines, the apparent prevalence was estimated as the average apparent prevalence in two neighbouring countries with similar pig production characteristics (*i.e.* Thailand and Cambodia). Figure 2 shows the structure of the component Pi_{vo} of the model.

Figure 2: Model structure for the estimation of the probability of prohibited swine products carried in air passengers' luggage with ASFV or CSFV per origin country and month. This component, named Pi_{vo}, was specific for each model/virus.



2.2. Sensitivity analysis

Sensitivity analyses were independently performed for each model and/or virus in two steps. Firstly, the Spearman correlation coefficients (ρ_i) between each input and the annual probability of ASFV/CSFV entry into the US were calculated. Inputs with $\rho_i \geq 0.4$ and contribution to the variance of the output above 10% were identified as the most influential parameters for each model. Subsequently, these inputs were analysed in detail using the advanced sensitivity analysis tool of @RISK 7.5 running 1,000 iterations for each scenario. A total of 10 scenarios were assessed for each selected parameter, by changing the base values in ten consecutive steps, from a minimum of 50% reduction to a maximum of 50% increase.

2.3. Visualisation of results

Maps showing risks at origin country and destination airport and connecting flights were created in ArcMap 10.3 (ESRI®) using the "XY to line" tool. Maps showed the annual mean risk of ASFV or CSFV at three levels: i) origin country; ii) connecting flight; and iii) US destination airport, at 95% confidence interval (CI). Risks at origin country, flight and airport level were grouped into categories by using Jenks' natural break classification method (182). Specifically, destination airports and connecting flights were divided into 3 categories while 5 classes were established for the countries of origin. For visualising purposes, connecting flights representing 90% of the total risk were selected for final figures.

In addition, networks among origin countries and US airports per month and disease were drawn based on the obtained risk results by using visNetwork package (280) in R software (180). Connecting flights representing 90% of the total risk per month were selected to build final nets. Jenks's natural breaks were used to calculate cut-offs among categories.

3. Results

3.1. Risk of ASFV entry

The overall mean annual probability of ASFV entry into the US through the introduction of potentially contaminated PSPAP was estimated as 0.06 (95% CI [0.01, 0.21]), which approximately corresponds to an average of at least one introduction of PSPAP contaminated with ASFV into the US every sixteen years.

The risk of ASFV entry through PSPAP was highly concentrated (over 90% of the total risk) in five US airports, namely Washington-Dulles (Virginia), John F. Kennedy-Queens (New York), George Bush-Houston (Texas), Warwick (Rhode Island), and San Juan (Puerto Rico) (Figure 3B).

The origin countries of those PSPAP that represented the highest risk (above 70% of the total risk) to the US were Ghana, Cape Verde, Ethiopia, and the Russian Federation.

In terms of connecting flights, above 70% of the total annual risk was represented by flights between Cape Verde and Warwick airport, Ethiopia and Washington-Dulles airport, journeys from Ghana to the airports of San Juan, John F. Kennedy-Queens and Washington-Dulles; and flights from the Russian Federation arriving at George Bush-Houston airport (Figure 3A).

Interestingly, the risk of entry in each of those airports as well as in the countries of origin varied along the year, with July and May the months at the highest risk followed by August, June and September (Figure 3C). Additional networks are available representing the risk of ASFV entry per month (Figures S1-S3). For instance, during May the flights that pose the highest risk of ASFV for the US were the ones previously mentioned coming from Ghana. On the contrary, during July, the flights from Cape Verde to Warwick airport, from Azerbaijan and Ghana to John F. Kennedy-Queens airport and from the Russian Federation to George Bush-Houston airport posed the highest risk for the entry of ASFV (Figure S2).

Figure 3: Risk results of ASFV entry into the US through prohibited swine products carried in air passengers' luggage. (A) Risk map where the graduated colour, size and width in the map represent the annual average risk from the highest (darker/larger/thicker) to the lowest (lighter/smaller/narrower) per country, airport and connecting flight, respectively. (B) Zoomed map of the ASFV annual average risk at US international airports. (C) Temporal graph shows the average risk of ASFV entry in the US per month.

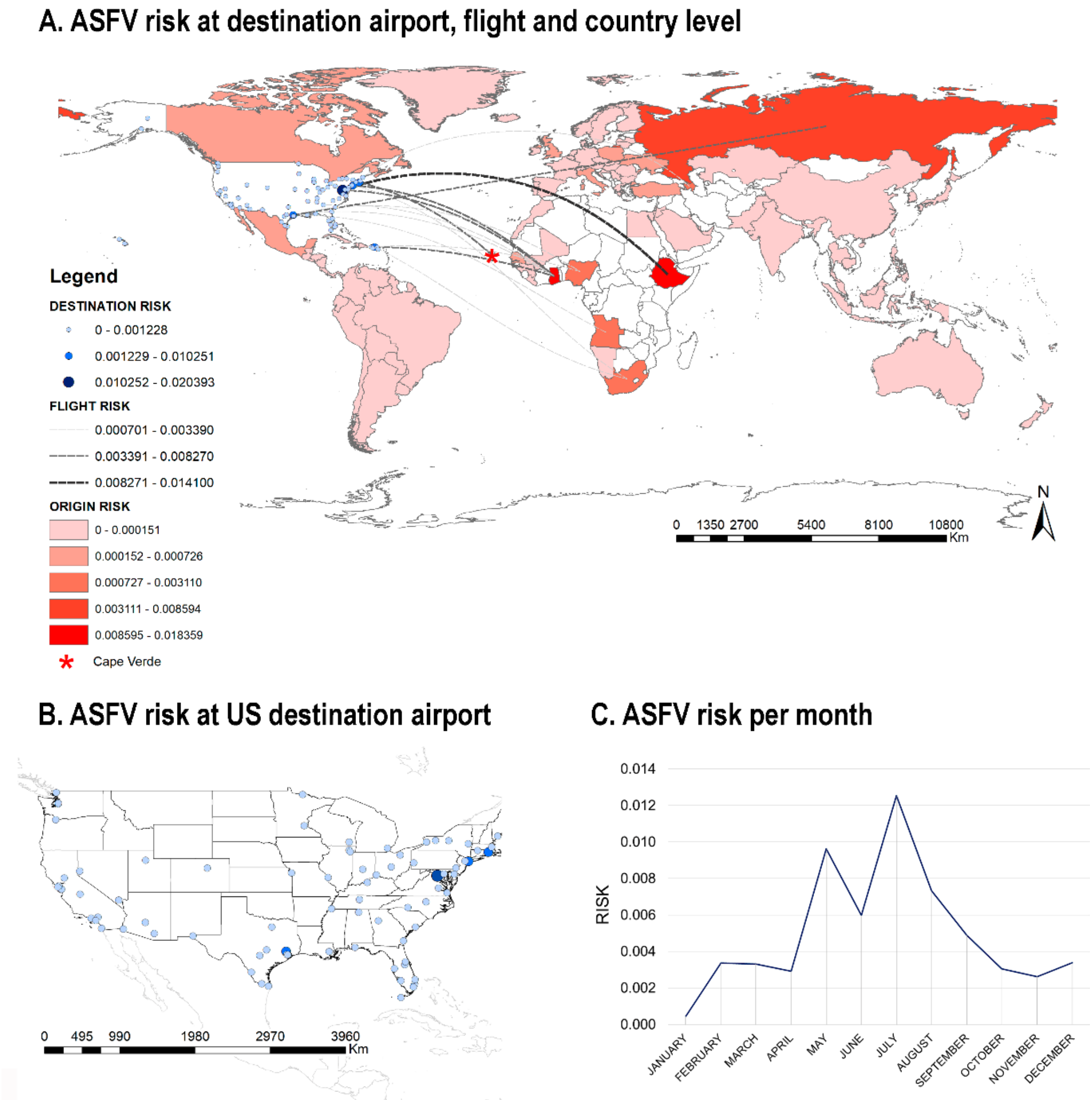


Figure S1: Visualisation of networks showing the risk of ASFV entry into the US through prohibited swine products carried in air passengers' luggage between origin countries and US destination airports in January, February, March and April. Networks characterised the risk of ASFV entry per origin country, destination airport and connections. Full name of airports are shown in Table S1.

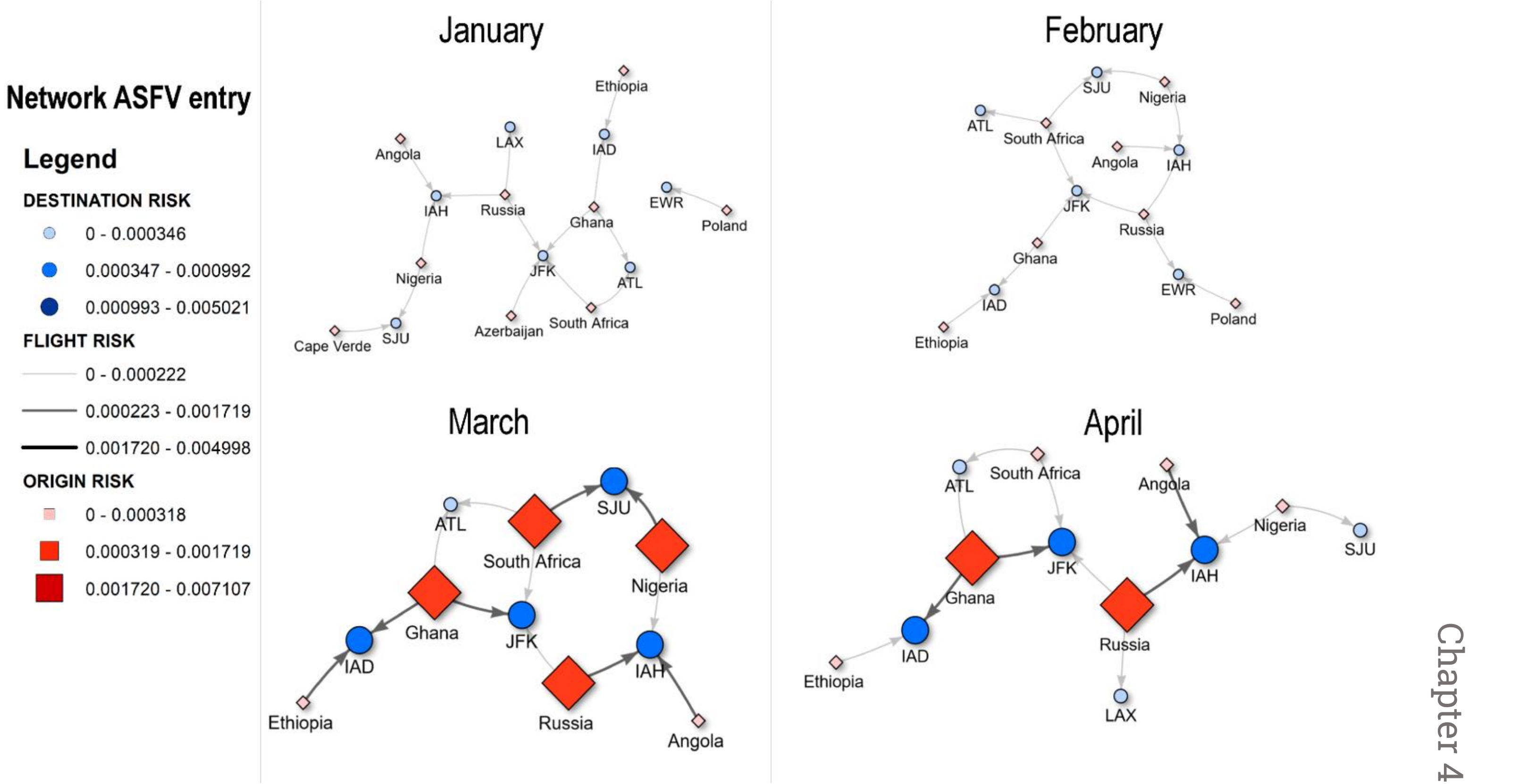


Figure S2: Visualisation of networks showing the risk of ASFV entry into the US through prohibited swine products carried in air passengers' luggage between origin countries and US destination airports in May, June, July and August. Networks characterised the risk of ASFV entry per origin country, destination airport and connections. Full name of airports are shown in Table S1.

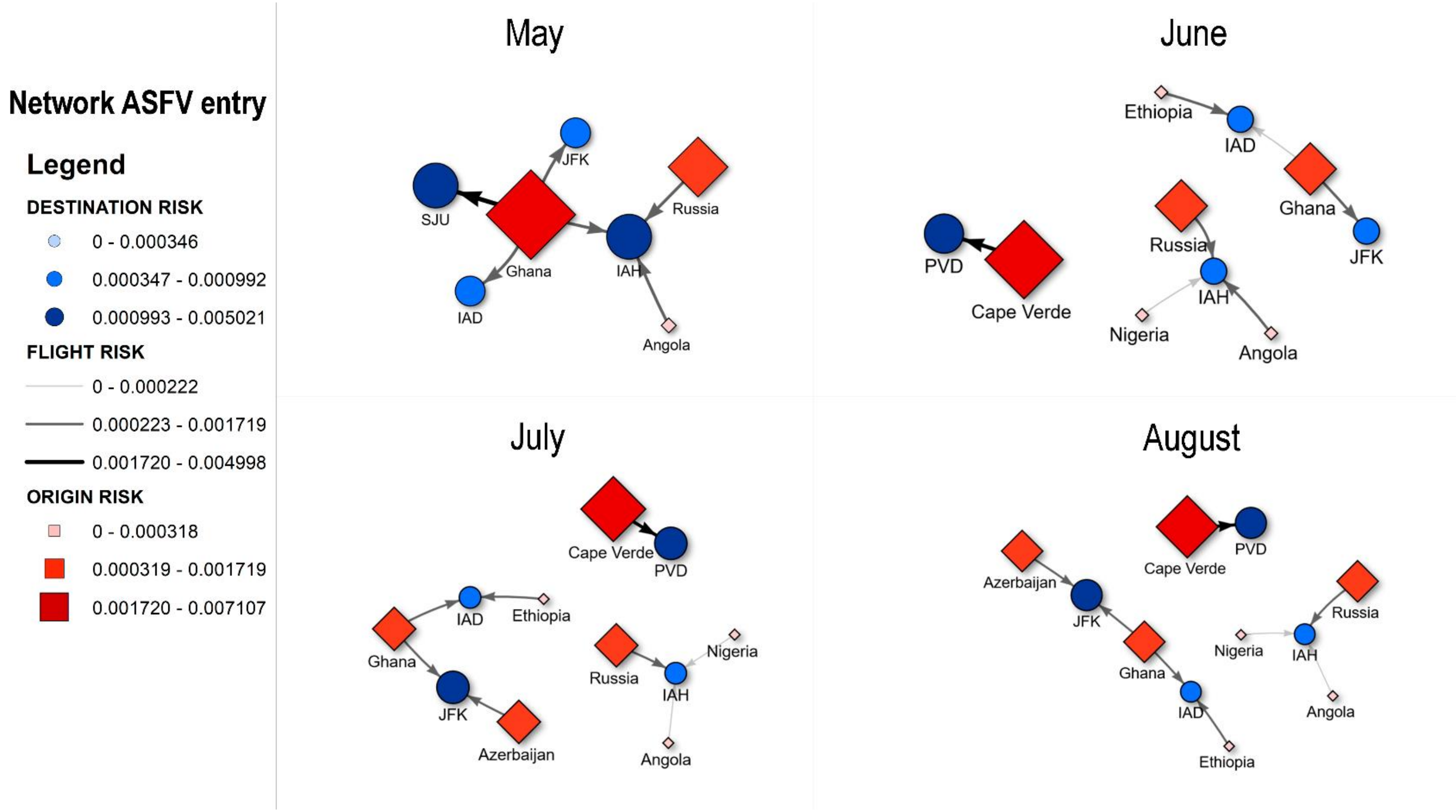
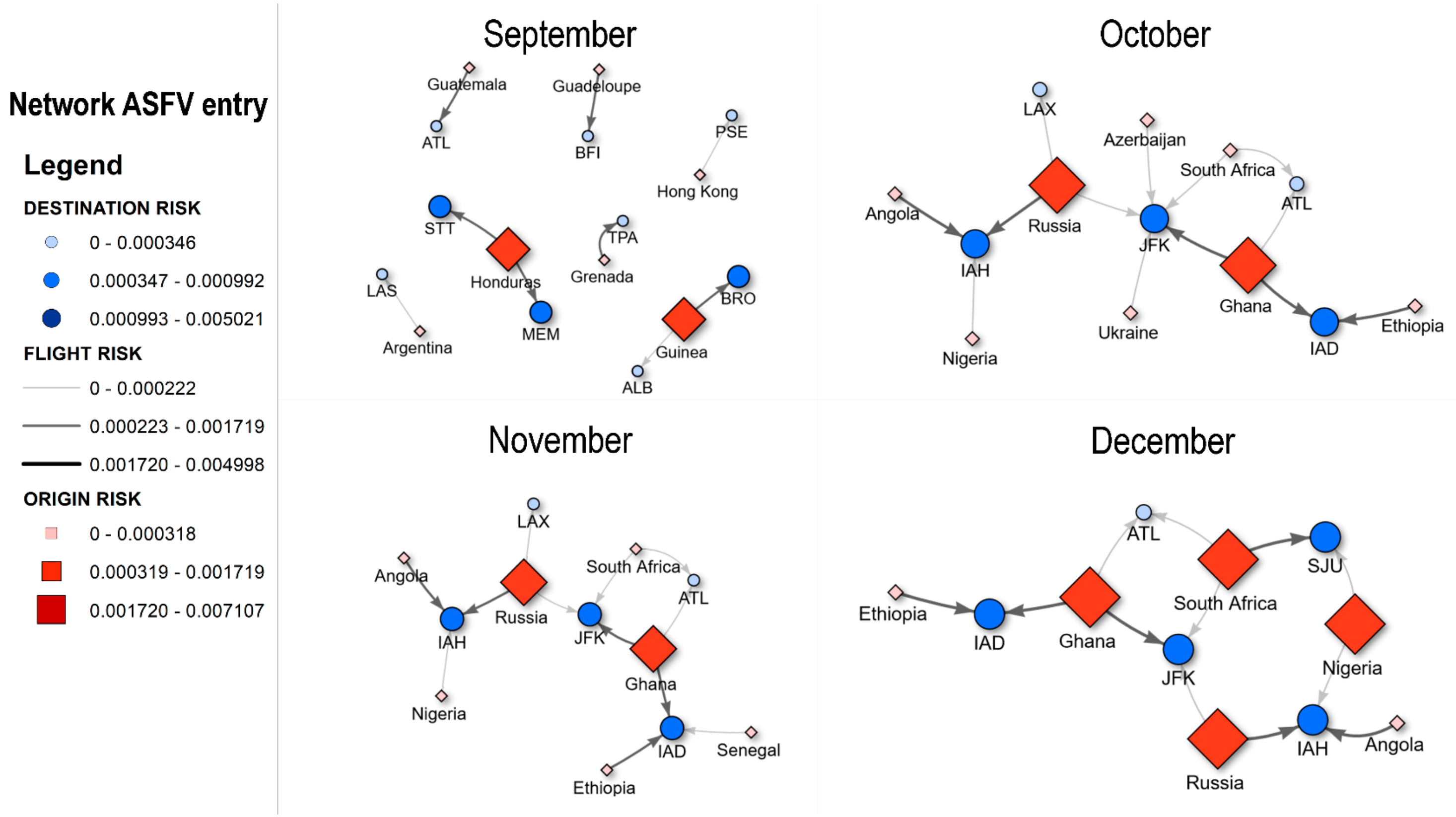


Figure S3: Visualisation of networks showing the risk of ASFV entry into the US through prohibited swine products carried in air passengers' luggage between origin countries and US destination airports in September, October, November and December. Networks characterised the risk of ASFV entry per origin country, destination airport and connections. Full name of airports are shown in Table S1.



3.2. Risk of CSFV entry

The overall mean annual probability of CSFV entry into the US through contaminated PSPAP carried by air passengers was estimated as 0.414 (95% CI [0.074, 1]) that approximately corresponds to one introduction of at least one PSPAP contaminated with CSFV every two years and a half. Therefore, the probability of CSFV entry is 7 times higher than the risk of ASFV by the same pathway.

The risk of CSFV entry through PSPAP was more widely distributed than the risk of ASFV, with 79% of the total annual risk concentrated in six US destination airports; namely San Juan (Puerto Rico), West Palm Beach (Florida), Charlotte (North Carolina), Fort Lauderdale (Florida), Newark (New Jersey), and Cleveland (Ohio). The countries of origin of those PSPAP that represented the highest risk (above 90%) to the US were Dominican Republic and Cuba, followed by India, Colombia, Peru, Ecuador and China, with May and July the months at the highest risk (Figure 4). The flight routes that pose the highest risk for CSFV entry were highly concentrated in the Caribbean region. Specifically, the Dominican Republic was the origin country that represented more than 80% of the total annual risk followed by Cuba (around 9% of the total risk).However, the risk at destination airports was widely spread since flights from the Dominican Republic arrived at up to 31 different airports along the year.

Networks representing the risk of CSFV entry per month are shown in Figures S4-S6. As it occurred with ASF, the risk of CSFV entry per connecting flights strongly varied per month. In May, flights coming from Cuba and arriving at West Palm Beach airport represented the highest risk for CSFV entry followed by connecting flights between the Dominican Republic and the airports of San Juan (Puerto Rico), Charlotte, Aguadilla (Puerto Rico) and Fort Lauderdale. However, in July the riskiest flights were the ones departing from the Dominican Republic and arriving at Cleveland, Charlotte, San Juan (Puerto Rico), Newark and Mercedita (Puerto Rico) airports (see Figure S5).

Figure 4: Risk results of CSFV entry into the US through prohibited swine products carried in air passengers' luggage. (A) Risk map where the graduated colour, size and width in the map represent the annual average risk from the highest (darker/larger/thicker) to the lowest (lighter/smaller/narrower) per country, airport and connecting flight, respectively. (B) Zoomed map of the CSFV annual average risk at US international airports. (C) Temporal graph shows the average risk of CSFV entry in the US per month.

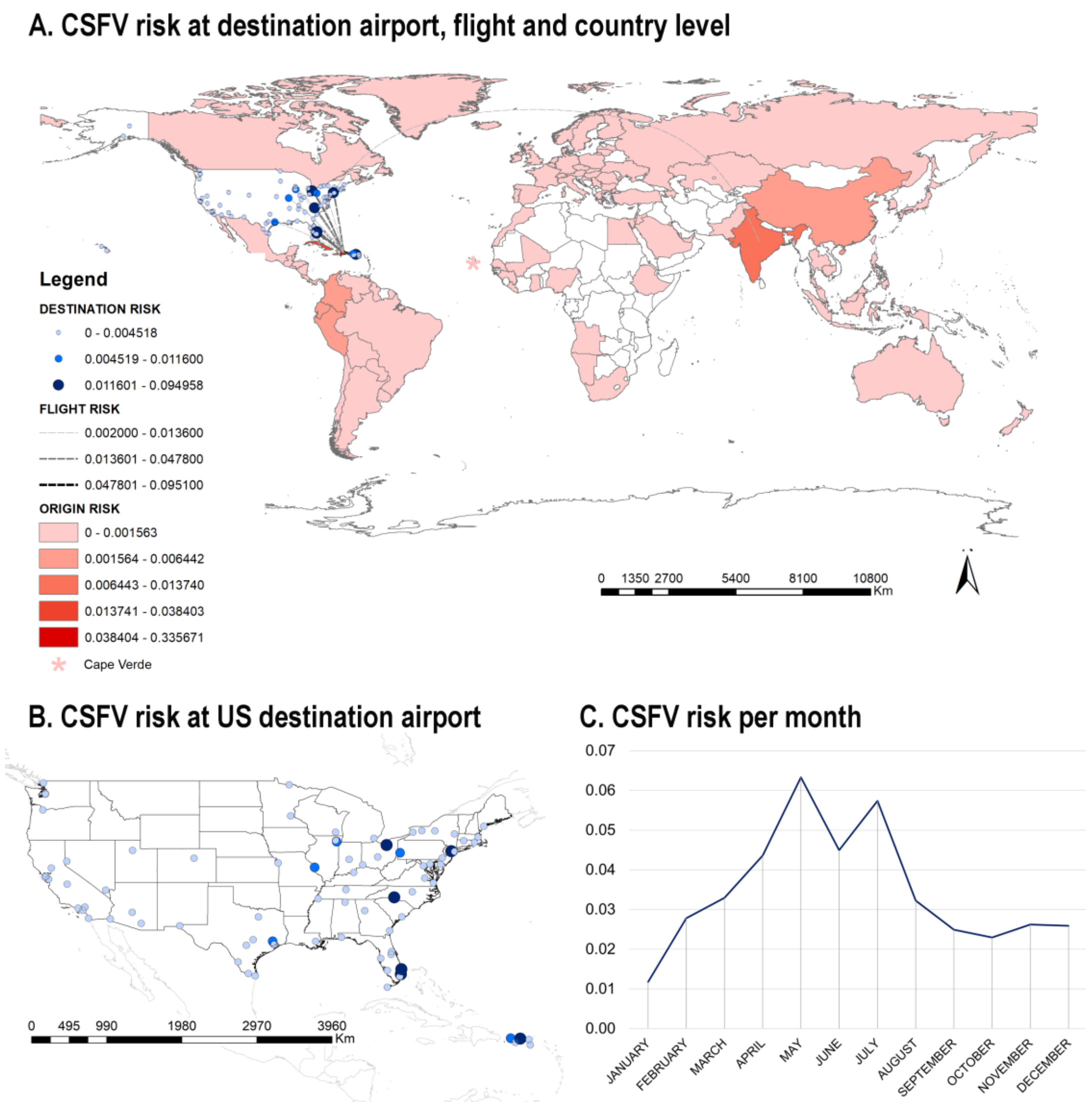


Figure S4: Visualisation of networks showing the risk of CSFV entry into the US through prohibited swine products carried in air passengers' luggage between origin countries and US destination airports in January, February, March and April. Networks characterised the risk of CSFV entry per origin country, destination airport and connections. Full name of airports are shown in Table S1.

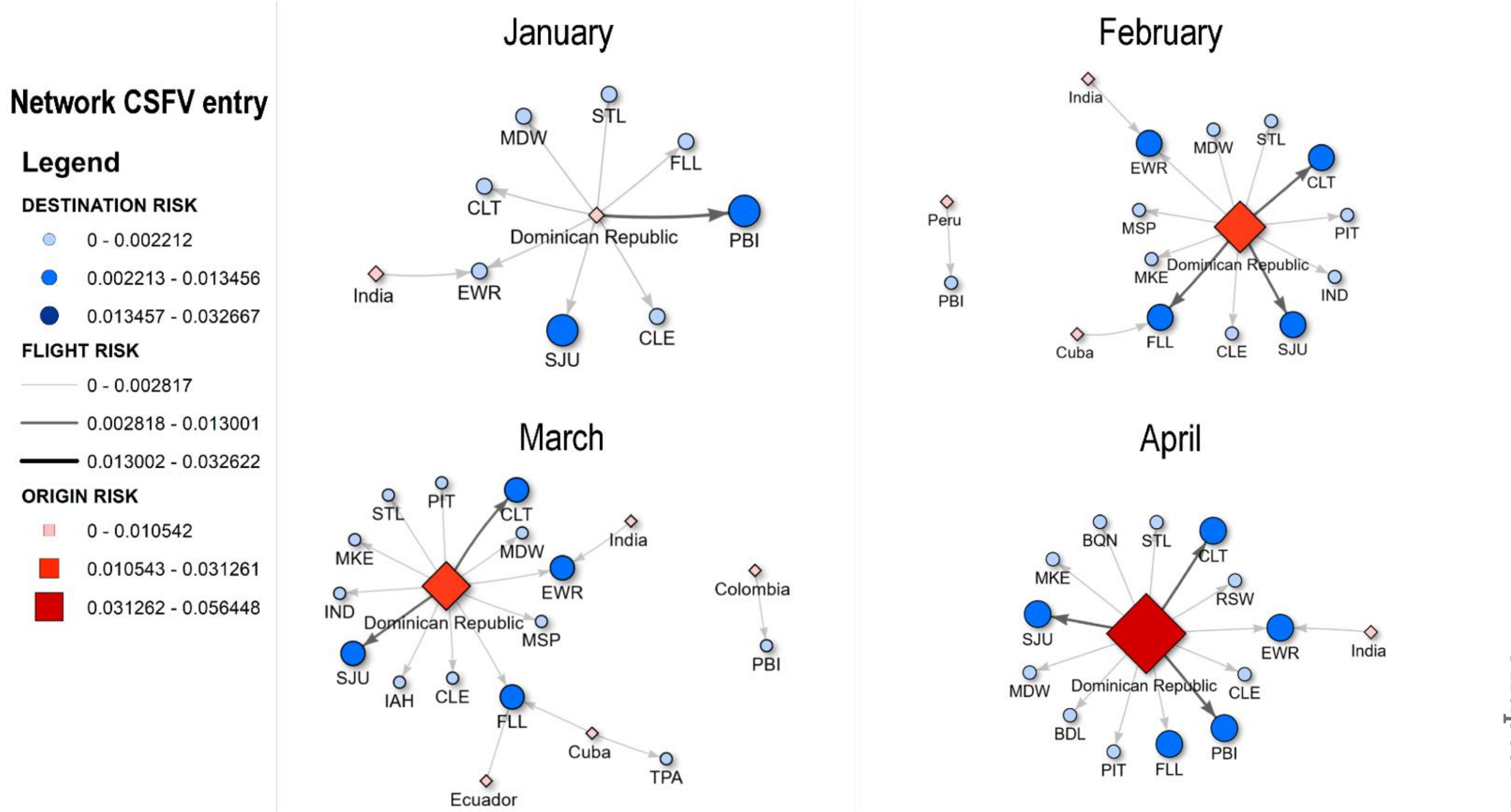


Figure S5: Visualisation of networks showing the risk of CSFV entry into the US through prohibited swine products carried in air passengers' luggage between origin countries and US destination airports in May, June, July and August. Networks characterised the risk of CSFV entry per origin country, destination airport and connections. Full name of airports are shown in Table S1.

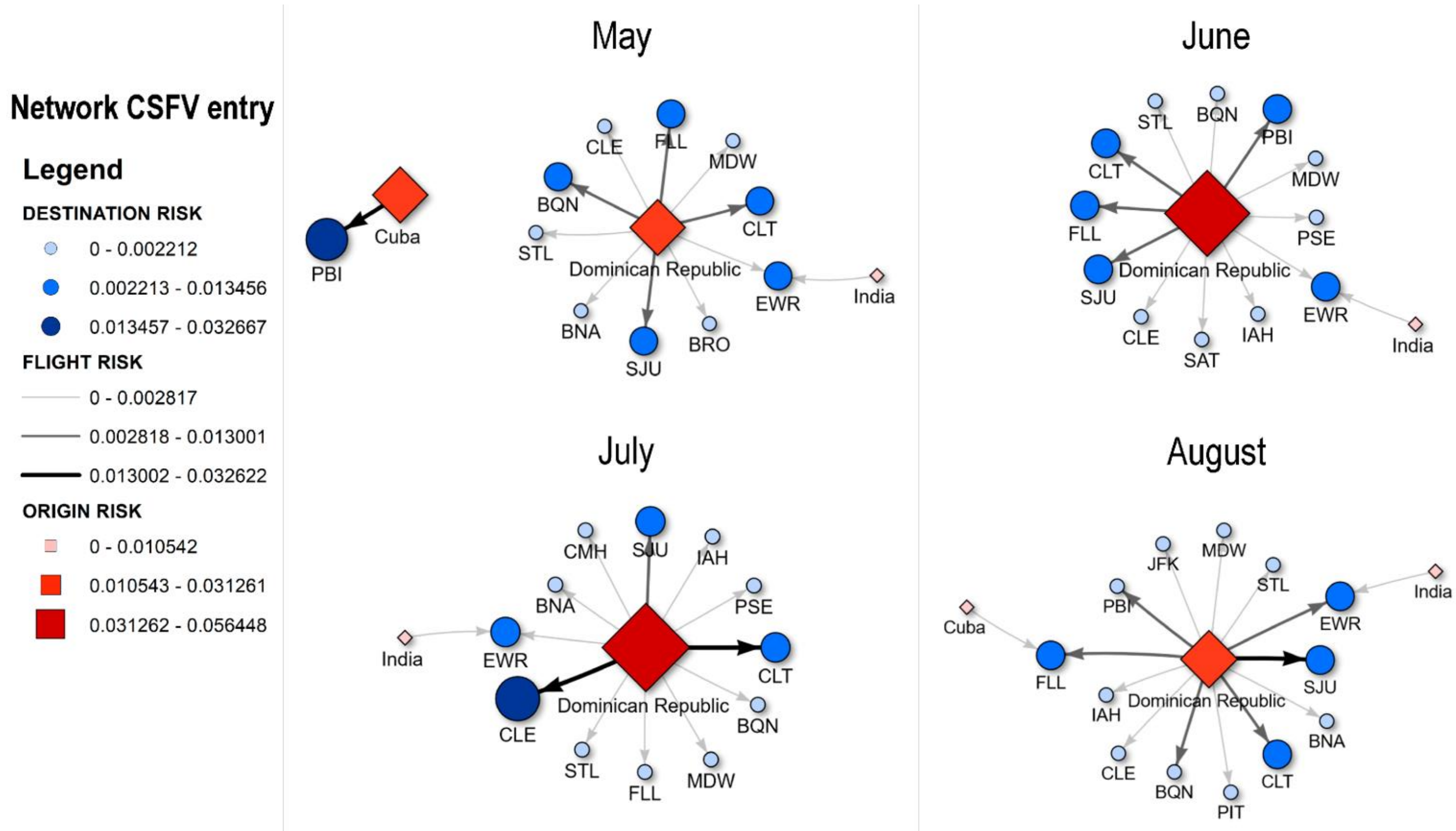
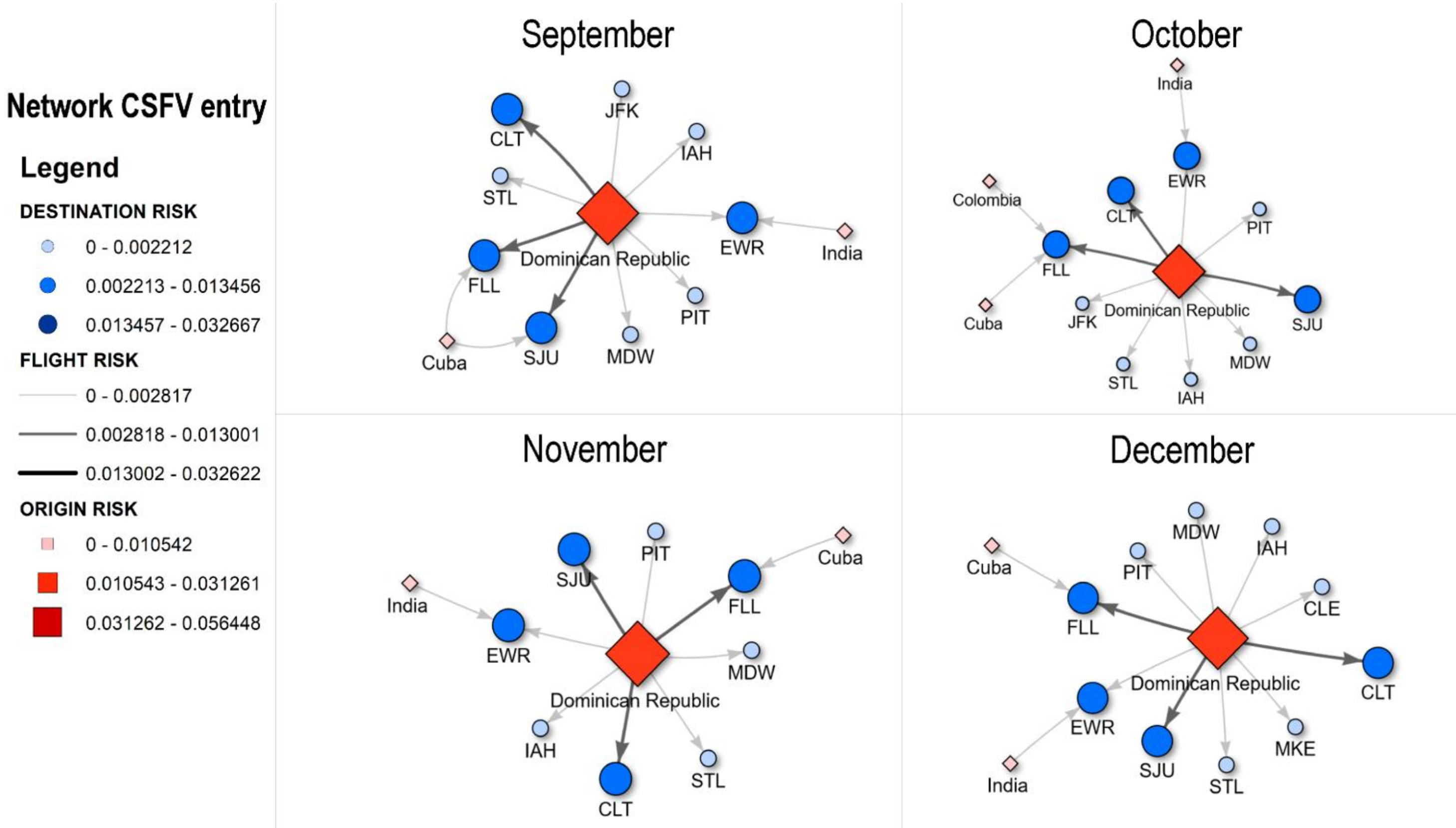


Figure S6: Visualisation of networks showing the risk of CSFV entry into the US through prohibited swine products carried in air passengers' luggage between origin countries and US destination airports in September, October, November and December. Networks characterised the risk of CSFV entry per origin country, destination airport and connections. Full name of airports are shown in Table S1.



3.3. Combined risk of ASFV and CSFV entry

The combined probability of the risk of ASFV and/or CSFV entry into the US by PSPAP was estimated as 0.45 (95% CI [0.08, 1]) which approximately corresponds to at least one introduction of PSPAP contaminated with ASFV or CSFV every 2.2 years.

3.4. Sensitivity analysis

Based on the Spearman correlation coefficients calculated, inputs strongly correlated with the final annual output ($\rho i \geq 0.4$) and contribution to the variance of the output above 10% were selected for advanced sensitivity analyses.

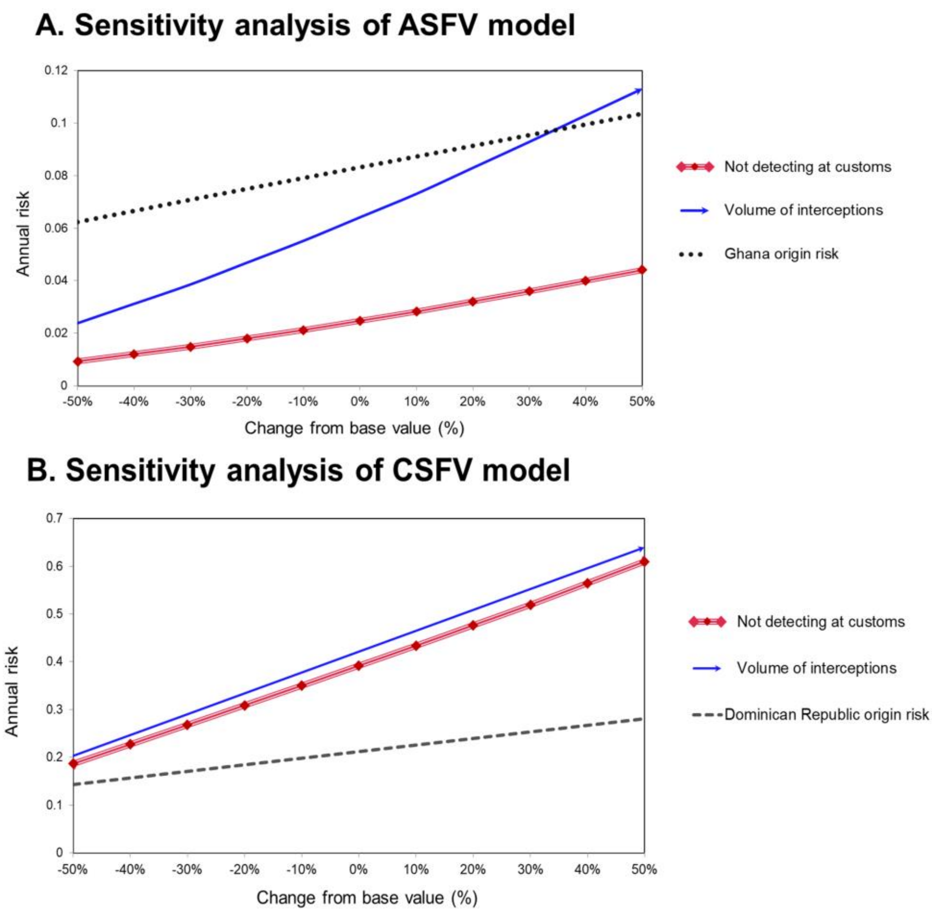
For ASFV model, the probability of not detecting PSPAP at customs, the individual volume (kg) of each PSPAP arriving in the US (V/PSPAP) and the probability of ASF infection in Ghana were the highest correlated input parameters of the model.

For CSFV model, the probability of CSF infection in the Dominican Republic, the probability of not detecting PSPAP at customs and the volume of interceptions were the inputs selected for advanced sensitivity analysis. All selected inputs were positively correlated with the response variable.

The advanced sensitivity analysis revealed that for both models the individual volume of each PSPAP arriving in the US was the most influential parameter followed by the probability of not detecting PSPAP at customs (second in CSFV model and third in ASFV model).

In addition, for the CSFV and ASFV models, the likelihood of CSF and ASF infection in the Dominican Republic and Ghana, respectively, also influenced the final results (Figure 5).

Figure 5: Advanced sensitivity analysis for the models of ASFV and CSFV risk of introduction through prohibited swine products carried in air passengers' luggage into the US. (A) Results for ASFV model. (B) Results for CSFV model. The spider graph plots the percentage change of selected input parameters against the annual output results.



4. Discussion

Control efforts made at US ports of entry to prevent the entrance of FADs and zoonotic diseases are essential to protect homeland security, international trade and US agriculture. This work analyses for the first time the risk of ASFV and CSFV entry through swine products illegally imported into the US by air passengers' luggage. Moreover, obtained results point out the countries of origin, US airports, flight routes and months where and when the introduction of these diseases would be more likely to occur and consequently, where and when preventive and control surveillance activities should be enhanced. Although international concerns are currently more aware of ASF risk due to its recent spread along Europe (27, 38, 281-283), our results suggest that the risk for CSFV entry into the US was 7 times higher and wider spread among US airports than for ASFV. Our results identified later spring and summer months as the highest risk period for the introduction of both analysed viruses into the US. However, while July was the month at the highest risk of ASFV entry, May was the one for CSFV.

The risk profile of US strongly varies between destination airports, as well as between months within the same airport. For instance, Washington-Dulles airport (Virginia) was identified as the airport at highest risk of ASFV entry in the global annual assessment, but San Juan airport (Puerto Rico) got the highest monthly risk of ASFV entry during May.

The obtained results demonstrate how dynamic risks are, changing drastically among months and airports, and the consequent need for a flexible and adaptable risk-based surveillance system in place. Unfortunately, there is no systematic method for anticipating imminent threats from passenger hand-carries and checked-in baggage. Moreover, passenger baggage inspection is constrained by limited manpower resources and short inspection time; and, as a consequence, not every single piece of luggage entering into any country can be inspected, opened or searched for prohibited agricultural quarantine materials. As expected, the risk of introduction was higher during the months when the air passenger traffic is the highest (peaks of summer and Christmas/winter). However, this result did not consider the potential variations in the control efforts effectuated at customs to adjust to the variations of the passengers' flow. In case that those controls would adapt proportionally to the flow of passengers, the risk would result more stable along the months. However, if those controls are constant throughout the year, effectively, during the periods of higher flow of passengers, the probability of detection would be lower, and consequently, as pointed here, the risk of introduction higher. These results can be therefore applied to distribute the control efforts proportionally to the risk, reducing these high risk period seasons. Thus, surveillance and control efforts could efficiently and effectively target scenarios at the highest risk, considering periods of the year, destination airports and origin countries, among others.

Outcomes obtained from risk assessment studies aim at identifying such sort of information. However, it is usually challenging for researchers to get access to or find the necessary data to perform analyses. As for any other kind of study, quality of data limits and interferes in the accuracy of obtained results. Information on the actual weight of checked baggage per passenger, as well as data on demographic characteristics of the passengers (*e.g.* age, gender, nationality) and the characteristics of their travel plans (*e.g.* duration and reasons for travel) were not available. Therefore, different behaviours (*i.e.* quantity and type of luggage, prohibited products carried) due to the passengers' and travel characteristics were not considered, resulting in possible over/underestimation issues. However, we conservatively estimated the quantity of luggage per passenger considering the variability between airlines and baggage allowance, including from the minimum (hand luggage only) to the maximum free baggage allowance (checked and carry-on luggage) in economy class of the three most important air carriers in the US. Although this approach is not perfect, at least it considered the variations due to the flight's origin, as the maximum baggage allowance per passenger varies according to the flight's region of origin (56 kg for passengers from Asia, South America and Australia; 48.3 kg for passengers from Africa and New Zealand; 33 kg for passengers from Europe and North America). For future studies, more detailed data on the factors influencing the illegal importation of meat products would be essential.

Fortunately, information on PSPAP confiscated in customs was made available for our study. In this study, we only used the confiscation data from air passengers. Analysing other routes of introduction of PSPAP such as maritime containers, land or mail shipments, would help obtaining a more complete estimation of the risk of introduction of ASFV and CSFV into the US. ASFV and CSFV survival in pork products mainly depends on the piece and processing with thermal/chemical treatments (*i.e.* dried, cured, salted or smoked) (260, 269). However, no detailed information about the type and characteristics of confiscated swine product was available in the provided records. Therefore, every PSPAP was considered presumptively contaminated with ASFV and/or CSFV according to the principle of maximum risk. This assumption could certainly have an impact in the outcomes and lead to an increase in the estimated risk probabilities.

Another important limitation of WADs database was the lack of information on the origin of confiscated PSPAP. In order to overcome this limitation, we assumed that the origin of confiscated PSPAP was proportional to the volume of luggage arriving at each airport from each origin country. However, some studies performed at airports in other countries such as Brazil, identified specific demographic characteristics of passengers and risk factors related with their travel plans associated with the presence of illegal animal products in their baggage (284). Therefore, additional research on this area, including sociological aspects of passengers carrying illegal products will be essential to improve the results of the analysis and better reflect the reality of the studied scenario.

In addition to this, no data on the true original departure country of each air passenger arriving into the US via international direct flights was available. In fact, on the same direct flight from an origin country to a US destination airport, there might be passengers with many different origins. Due to this lack of data on the entire passengers' journey (*e.g.* connecting flights), only direct international flights and their origin countries were considered in this study, and used to estimate the possible origin of the confiscated PSPAP in US airports, as discussed above. Consequently, a possible underestimation or overestimation of the risk of both viruses introduction was possible for origin countries having, respectively, few or many ports performing direct flights to the US. Data on the true original departure country of all passengers entering the US via international direct flights would be of utmost importance in order to better determine the countries that represented the highest risk for the introduction of PSPAP, although data on the origin of the intercepted PSPAP would represent the best information possible to estimate such risk.

Another assumption done in the study was that the likelihood of an ASF or CSF outbreak occurring in countries sharing geographical boundaries with infected areas was 10 times higher than in free countries. This assumption was established based on the epidemiological situation (*i.e.* ASF and/or CSF affecting domestic pigs and/or wild populations) and routes of transmission for both diseases. Currently, ASF and CSF are affecting wild populations such as wild boar in certain parts of Europe or wild African suids in Africa (25), with both types ranging freely across geographical boundaries. Wild suids and free ranging populations are able to spread diseases infecting other wild boar, feral pigs or even domestic pigs if biosecurity levels on farms are not properly in place (13, 152). This route of infection has been already proven in Eastern Europe where ASF spread from Belarus and the Russian Federation to the European Union (66). As an example of the importance of considering this assumption, the Czech Republic has recently notified several ASF cases in wild boar (July, 2017) even though the disease seemed to be far from its territory (the nearest cases were located close to the western Polish border at 400-500 km) (281). Moreover, the human action in spreading diseases should not be underestimated. After ASF or CSF are notified, pork prices dramatically drop which could encourage some people to make money through illegal trade of animals already infected. This situation was reported by Poland to the Standing Committee on Plants, Animals, Food and Feed celebrated in Brussels in August-2016 where the trade of infected animals gave rise to several outbreaks on pig farms (285). Therefore, it might seem valuable to consider the epidemiological situation of neighbouring countries when studying transboundary diseases.

The results obtained in our models showed two very different patterns of risk, at origin level, for each disease. Focusing on the ASFV model, Cape Verde and Ghana represented the biggest threat for US airports even though such countries do not have an important pig production. That might be explained by the fact that this study assesses the introduction of PSPAP, which represent any pig derived product homemade (highly appreciated by locals) or industrially produced. Considering the relative high prevalence of ASF in those countries (as OIE data suggested) and the small number of pigs produced in both territories, it is logic that the resulting probability of selecting an infected pig from those countries is much higher than in other regions with a lower prevalence of ASF. Transboundary movements of ASFV contaminated pig products were identified as the probably source of infection for Czech Republic. Contaminated food brought by Ukrainian workers was the most likely source of infection for wild boar in Zlin area (286). If such products had been introduced as air passengers' luggage still remains uncertain.

Moreover, Ethiopia arose as the third country in terms of risk to US airports. ASF disease status in Ethiopia is officially unknown. However, the presence of the disease was suspected between mid-2011 and 2012 (25). Thus, Ethiopia was considered an infected country which probability of infection was estimated from data on the rest of affected African countries (median value of the probability of infection). This assumption could have led to certain bias in obtained results, considering a worst case scenario where the final risk value for Ethiopia could have been overestimated. Some results showed by Achenbach *et al.* (9) reported the presence of ASFV in samples from domestic pigs collected between 2011 and 2014, but no information about the prevalence rate of the disease was provided in that study. Therefore, in the absence of official updated information, we cannot assume a high risk scenario, although this analysis could be updated when data is provided.

On the other hand, for CSFV the highest risk in origin was strongly clustered in the Caribbean. The biggest threat posed to US was the Dominican Republic (82% of the total risk in origin) followed by Cuba (10% of the total risk in origin). Controlling flights coming from these countries, but especially from the Dominican Republic, could considerably reduce the risk of CSFV introduction through PSPAP. However, flights from the Dominican Republic and Cuba landed at 36 and 7 different airports, respectively. Considering these numbers, controls in origin could be a possible solution to reduce costs and manpower needed.

When comparing both models, 94% of the total risk for ASFV introduction was concentrated in 5 US destination airports, while for CSFV, this level of risk was split in 14 different airports. Therefore, this implies that an effective control at borders of CSF would be much more challenging and pricey for US border control services than for ASF.

The final goal of this study, similar to others conducted during the last years (275-277, 284, 287, 288) is to prevent the entrance of FADs, zoonotic agents and/or human pathogens important to public health via air passengers' luggage. To do so, obtained results should be communicated to risk managers and agencies in charge of international borders to inform the implementation of controls and more cost-effective mitigation strategies. In addition, education and awareness are key to make passengers understand the risk that introducing agricultural products into foreign countries could pose as well as the socio-economic repercussions that it could have if they were contaminated with FADs and effectively contact susceptible livestock populations.

Taking into account the availability of advanced technologies existing nowadays, these results could potentially be used to implement and feed real-time surveillance systems helping customs to prevent the introduction of prohibited products, informing about when and where they should look for them. This improvement in surveillance systems is needed since as was previously referred, only between 10% and 50% of improperly imported products are intercepted by custom officers (273, 274). In fact, the sensitivity analysis revealed that the likelihood of detecting smuggled products at the border controls was high positively correlated with the final risk. Therefore, small improvements in the detection at borders will strongly help to reduce the risk of viral entry. Similarly, the volume of products intercepted was also identified as a critical parameter in the sensitivity analysis. This reflects the need for analysing and considering other routes of introduction of ASFV and CSFV such as maritime containers, land or mail shipments, where the volumes of smuggled products are more likely to be higher.

5. Conclusion

Two quantitative stochastic models were developed to assess the risk of entry of ASFV and CSFV into the US through PSPAP. Obtained results showed that the risk of CSFV entry was seven times higher than the risk of ASFV entry. Over 90% of the final ASFV risk was concentrated in five US airports (Washington-Dulles, George Bush-Houston, John F. Kennedy-Queens, Warwick and San Juan) while the risk of CSFV entry was somehow more distributed (79% of the total risk within San Juan, West Palm Beach, Charlotte, Fort Lauderdale, Newark and Cleveland airports). The origin of the flights posing a risk for ASFV and CSFV was completely different. Regarding ASFV, Cape Verde and Ghana represented the biggest threat for US airports. However, the Dominican Republic and Cuba were identified as the most likely route of entrance for CSFV. Interestingly, May and July were the months at the highest risk for both models. Information generated from this study could help to implement controls at customs as well as develop much more effective and cost-effective surveillance activities at borders to protect US livestock production.

Table 1: Description of input parameters, data sources and probabilities used for the estimation of the volume (kg) of prohibited swine products carried in air passengers' luggage introduced into airports of the US per month, origin country and destination airport.

Notation	Definition	Parametrisation	Values	Source/ Reference
PAS	Number of air passengers arriving in commercial flights in the US per origin (*o*), destination airport (*d*) and month (*m*)	Normal (μ, σ)	NA	(278)
VL/PAS_o	Volume in Kilograms (kg) of luggage per air passenger, per *o*	Pert (min, most likely, max)	NA	(289-291)
VL_{odm}	Total kg of luggage per *o, d* and *m*		PAS_{odm} * VL/PAS_o	
Prop-VL_{odm}	Proportion of kg of luggage from each *o* arriving at each *d*, per *m*	Normal (μ, σ)	$VL_{odm}/\sum_{d}^{i} VL_{odm}$	
$PSPAP_{dm}$	Median number of PSPAP confiscations per *d* and *m*	Median		WADS
VL/PSPAP	Individual volume (kg) of each PSPAP	Pert (min, most likely, max)	Pert (1.5, 3.25, 5)	Modified (273)
$\sum V_{PSPAP}$	Total volume (kg) of PSPAP confiscated per *d*, per *m*		$PSPAP_{dm}$ * VL/PSPAP	
CON-$PSPAP_{odm}$	kg of PSPAP confiscated at customs and border protection (CBP) control per *o, d* and *m*		Prop VL_{odm} *$\sum V_{PSPAP}$	
$P_{non\text{-}det}$	Probability of PSPAP non-detection at CBP controls	Triang (min, most likely, max)	Triang (0.2, 0.5, 0.9)	Modified (273, 274)
N_{odm}	kg of PSPAP escaping CBP controls, introduced into the US per *odm*		CON-$PSPAP_{odm}$ * $P_{non\text{-}det}$ / (1 - $P_{non\text{-}det}$)	

Table 2: Description of input parameters, data sources and probabilities used for the estimation of the probability of prohibited swine products carried in air passengers' luggage being contaminated with ASFV or CSFV per origin country and month.

Notation	Definition	Country category	Parametrisation	Values	Source/ Reference
N_o	Pig census in origin country (*o*)	A, B, C	NA	Data from year 2010	(25)
Prop-S_m	Proportion of annual pig census slaughtered per month (*m*)	A, B, C	Pert (min, most likely, max)	Pert (0.1, 0.18, 0.25)	(292)
NS_{om}	Number of pigs slaughtered for meat production in country *o* per month *m*	A, B, C	Pert (min, most likely, max)	N_o * Prop-S_m	
P_U	Probability of notification underreporting	A, B, C	Pert (min, most likely, max)	Pert (0.2, 0.4, 0.6)	(279)
d	Duration of ASF[1] and CSF[2] infection in months	A, B, C	Pert (min, most likely, max)	[1]Pert (3, 11, 30) [2]Pert (2, 9, 19)	[1, 2] Modified (279)
AP_{om}	Apparent prevalence of ASFV or CSFV in *o* and *m*	A		Number of infected pigs since disease introduction / time since disease presence in months	(25)
Ni_{om}	Number of ASFV or CSFV non-reported infected pigs in *o* and *m*	A		(AP_{om}* d) / P_U	

Mod Prop-S_m	Modified proportion of annual pig census slaughtered per month (m)	A, B, C	Uniform (min, max)	min = Prop-S_m max = 1	Modified (292)
Pi_A	Monthly probability of ASFV or CSFV infection in pigs slaughtered in *o*	A	Beta (α_1, α_2)	$\alpha_1 = Ni_{om}$ * Mod Prop-S_m + 1 $\alpha_2 = NS_{om}$ – (Ni_{om} * Mod Prop-S_m) + 1	
$Prop_{AF}$	Proportion of pig population affected by disease outbreak (ASF or CSF)	B, C	Pert (min, most likely, max)	Pert (0, 0.002, 0.02)	
Po_B	Probability of occurrence of an outbreak of ASF[1] or CSF[2] in countries belonging to category B	B	Pert (min, most likely, max)	[1]Pert (0, 0.0022, 0.022) [2]Pert (0, 0.005, 0.05)	[1]Modified (279) [2](279)
Pi_B	Probability of ASFV or CSFV infection in pigs slaughtered in *o* and *m*	B	Pert (min, most likely, max)	Po_B * $Prop_{AF}$ * d * P_U	
Po_C	Probability of occurrence of an outbreak of ASF[1] or CSF[2] in countries belonging to category C	C	Pert (min, most likely, max)	[1]Pert (0, 0.00022, 0.0022) [2]Pert (0, 0.0005, 0.005)	[1]Modified (279) [2](279)
Pi_C	Probability of ASFV or CSFV infection in pigs slaughtered in *o* and *m*	C	Pert (min, most likely, max)	Po_C * $Prop_{AF}$ * d * P_U	

Table S1: List of airports including airport code, name, city and state.

Airport IATA code	Airport name	City name	State
ALB	Albany International Airport	Albany	New York
ANC	Ted Stevens Anchorage International Airport	Anchorage	Alaska
ATL	Hartsfield–Jackson Atlanta International Airport	Atlanta	Georgia
AUS	Austin–Bergstrom International Airport	Austin	Texas
BDL	Bradley International Airport	Windsor Locks	Connecticut
BFI	Boeing Field	Seattle	Washington
BIF	Biggs Army Airfield	El Paso	Texas
BLI	Bellingham International Airport	Bellingham	Washington
BNA	Nashville International Airport	Nashville	Tennessee
BOS	Logan International Airport	Boston	Massachusetts
BQK	Brunswick Golden Isles Airport	Brunswick	Georgia
BQN	Rafael Hernández Airport	Aguadilla	Puerto Rico
BRO	Brownsville/South Padre Island International Airport	Brownsville	Texas
BUF	Buffalo Niagara International Airport	Buffalo	New York
BWI	Baltimore–Washington International Airport	Baltimore	Maryland

CHS	Charleston International Airport	Charleston	South Carolina
CLE	Cleveland Hopkins International Airport	Cleveland	Ohio
CLT	Charlotte Douglas International Airport	Charlotte	North Carolina
CMH	John Glenn Columbus International Airport	Columbus	Ohio
CVG	Cincinnati/Northern Kentucky International Airport	Hebron	Kentucky
DEN	Denver International Airport	Denver	Colorado
DFW	Dallas/Fort Worth International Airport	Dallas	Texas
DOV	Dover Air Force Base	Dover	Delaware
DTW	Detroit Metropolitan Airport	Detroit	Michigan
EFD	Ellington Airport	Houston	Texas
EWR	Newark Liberty International Airport	Newark	New Jersey
EYW	Key West International Airport	Key West	Florida
FAI	Fairbanks International Airport	Fairbanks	Alaska
FAT	Fresno Yosemite International Airport	Fresno	California
FLL	Fort Lauderdale–Hollywood International Airport	Fort Lauderdale	Florida
HNL	Daniel K. Inouye International Airport	Honolulu	Hawaii
HOU	William P. Hobby Airport	Houston	Texas
HSV	Huntsville International Airport	Huntsville	Alabama
IAD	Washington Dulles International Airport	Dulles	Virginia

IAH	George Bush Intercontinental Airport	Houston	Texas
IND	Indianapolis International Airport	Indianapolis	Indiana
INL	Falls International Airport	International Falls	Minnesota
JFK	John F. Kennedy International Airport	Queens	New York
KOA	Kona International Airport	Kailua-Kona	Hawaii
LAS	McCarran International Airport	Las Vegas	Nevada
LAX	Los Angeles International Airport	Los Angeles	California
LRD	Laredo International Airport	Laredo	Texas
MCI	Kansas City International Airport	Kansas City	Missouri
MCO	Orlando International Airport	Orlando	Florida
MDW	Chicago Midway International Airport	Chicago	Illinois
MEM	Memphis International Airport	Memphis	Tennessee
MFE	McAllen Miller International Airport	McAllen	Texas
MIA	Miami International Airport	Miami	Florida
MKE	General Mitchell International Airport	Milwaukee	Wisconsin
MSP	Minneapolis–Saint Paul International Airport	Minneapolis	Minnesota
MSY	Louis Armstrong New Orleans International Airport	Kenner	Louisiana
NGU	Norfolk Naval Station	Norfolk	Virginia
NYL	Marine Corps Air Station Yuma	Yuma	Arizona

OAK	Oakland International Airport	Oakland	California
ONT	Ontario International Airport	Ontario	California
ORD	O'Hare International Airport	Chicago	Illinois
PBI	Palm Beach International Airport	West Palm Beach	Florida
PDX	Portland International Airport	Portland	Oregon
PHL	Philadelphia International Airport	Philadelphia	Pennsylvania
PHX	Phoenix Sky Harbor International Airport	Phoenix	Arizona
PIT	Pittsburgh International Airport	Pittsburgh	Pennsylvania
PNS	Pensacola International Airport	Pensacola	Florida
PSE	Mercedita Airport	Ponce	Puerto Rico
PVD	T. F. Green Airport	Warwick	Rhode Island
PWM	Portland International Jetport	Portland	Maine
RDU	Raleigh–Durham International Airport	Raleigh	North Carolina
RIC	Richmond International Airport	Richmond	Virginia
RNO	Reno–Tahoe International Airport	Reno	Nevada
ROC	Greater Rochester International Airport	Rochester	New York
RSW	Southwest Florida International Airport	Fort Myers	Florida
SAN	San Diego International Airport	San Diego	California
SAT	San Antonio International Airport	San Antonio	Texas
SDF	Louisville International Airport	Louisville	Kentucky

SEA	Seattle–Tacoma International Airport	Seattle	Washington
SFB	Orlando Sanford International Airport	Sanford CBP	Florida
SFO	San Francisco International Airport	San Francisco	California
SJC	San Jose International Airport	San Jose	California
SJU	San Juan Luis Muñoz Marín Airport	San Juan	Puerto Rico
SLC	Salt Lake City International Airport	Salt Lake City	Utah
SMF	Sacramento International Airport	Sacramento	California
SNA	John Wayne Airport	Santa Ana	California
STL	St. Louis Lambert International Airport	Saint Louis	Missouri
STT	Cyril E. King Airport	Saint Thomas	United States Virgin Islands
STX	Henry E. Rohlsen Airport	Saint Croix	United States Virgin Islands
SYR	Syracuse Hancock International Airport	Syracuse	New York
TPA	Tampa International Airport	Tampa	Florida
TUS	Tucson International Airport	Tucson	Arizona

Risk of African swine fever virus introduction into the United States through smuggling of pork in air passenger luggage

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Summary

African swine fever causes substantial economic losses in the swine industry in affected countries. Traditionally confined to Africa with only occasional incursions into other regions, ASF began spreading into Caucasian countries and Eastern Europe in 2007, followed by Western Europe and Asia in 2018. Such a dramatic change in the global epidemiology of ASF has resulted in concerns that the disease may continue to spread into disease-free regions such as the US. In this study, we estimated the risk of introduction of ASF virus into the US through smuggling of pork in air passenger luggage. Results suggest that the mean risk of ASFV introduction into the US via this route has increased by 183.33% from the risk estimated before the disease had spread into Western Europe or Asia. Most of the risk (67.68%) was associated with flights originating from China and Hong Kong, followed by the Russian Federation (26.92%). Five US airports accounted for >90% of the risk. Results here will help to inform decisions related to the design of ASF virus surveillance strategies in the US.

1. Introduction

The US is the world's third largest pig producer, with over 11.5 million tons of pork produced per year, and the world's second largest pork exporter, with exports in 2017 valued at 4.6 billion USD (45). The introduction of a foreign animal disease (FAD) into the US may have far-reaching economic consequences for the country, due to the emergency response actions required to control the disease, such as herd depopulation and movement restrictions (293). Preparedness and prevention measures to avoid the introduction of FADs into the US include strict regulations on imports of live animals and animal products, checking of waste containing products that originated overseas (*e.g.* waste from international flights), application of thermal treatment to inactivate pathogenic microorganisms that may contaminate swill feed, restrictions on animal consumption of animal-derived by-products, and detection systems to facilitate rapid diagnosis of FAD through the country's national animal health laboratory network (294, 295).

African swine fever (ASF), caused by infection with the ASF virus (ASFV), is one of the most feared FAD in the US. ASF has traditionally been endemic to sub-Saharan Africa and the Italian island of Sardinia, with sporadic epidemics affecting a number of countries through the 20th century. However, in 2007, the ASFV spread into Georgia, Armenia, and Azerbaijan, and subsequently into the Russian Federation, Ukraine and Belarus. In 2014, four European Union countries became infected (Lithuania, Poland, Latvia, and Estonia). Despite prevention and control measures, ASF continued to spread and eight more European countries (Moldova, Czech Republic, Romania, Hungary, Bulgaria, Belgium, Serbia and Slovakia) reported the disease between 2014 and 2019 (25). In August 2018, China officially reported cases on a domestic pig farm (25), and as of August 2019, 32 Chinese provinces have been affected by the disease and more than 1,170,000 animals slaughtered (60). ASF has also been reported in Mongolia (296), Vietnam (297), Cambodia (298), North Korea (299), Laos (300) and Myanmar (301).

The recent ASF spread through Europe and Asia has raised concerns among US swine producers that ASFV-contaminated pork products may be illegally introduced into the country, which may infect susceptible animals, resulting in an epidemic in the country. According to the Agricultural Quarantine Activity Work Accomplishment database of the Animal and Plant Health Inspection Service of the US Department of Agriculture, screening activities conducted between 2010 and 2015 resulted in the confiscation of an average of 8,000 pork products per year. Nearly half (45%) of those prohibited pork products were intercepted at international airports inside air passengers' personal luggage. We refer henceforth to prohibited pork products carried in air passenger luggage as PSPAP.

It is unclear to what extent PSPAP pose a risk of bringing ASFV to US airports prior to customs inspection because seized PSPAP is not routinely diagnosed. The risk is likely greater than nil, given that ASFV has been detected in prohibited agricultural products seized at airports in South Korea, Japan, Taiwan, Thailand, Australia, the Philippines, and Northern Ireland (62, 302-307). In March-2019, the largest known illegal shipment of pork (1 million pounds) arrived from China at Newark, New Jersey port, one of the busiest entry ports into the US. Prior to the ASF spread in Western Europe and Asia, our analysis of data from July 2016 suggested that the annual average risk of ASFV introduction into the US via PSPAP was 0.06 (95% CI 0.01-0.21). In other words, ASFV could be expected to enter the US illegally in PSPAP once every 17 years on average (308).

It is unclear how much the risk has changed as a result of the spread of the disease through Europe and Asia in 2018 and 2019. The study here was aimed at i) quantifying the probability of arrival of ASFV-contaminated PSPAP at US airports (before customs inspection), ii) comparing the risk of ASFV introduction into the US via PSPAP (after customs inspection) before and after its spread into Western Europe and Asia, and iii) assessing how this risk varies with the US airport, country and month. These results may inform decisions related to the design of ASFV surveillance strategies in the US.

2. Material and methods

The probability of ASFV introduction into the US through PSPAP (defined as output) was assessed using a quantitative stochastic model. This probability (also named as risk) was estimated for each of 128 countries or regions of origin, for each of 87 US airports, and flights. The probability was also estimated across all US airports. The level of risk was assessed monthly and annually, using a model adapted from an earlier one (308). The risk model was developed in @RISK 7.6 (Palisade Corporation, Newfield, NY, USA) on Microsoft Excel 2007® and run for 10,000 iterations using a Monte-Carlo sampling method.

The primary output of the model was the probability that ASFV contaminated pork reaches the US (after customs inspection), based on data after the virus already spread through Europe and China in 2018 and 2019. This probability was compared to that obtained with data from before that expansion (308). As an additional output, the probability that ASFV-contaminated products arrive at US airports (before customs inspection) was estimated by computing the probability of ASFV introduction without considering the probability of non-detection at customs.

Briefly, the risk was calculated using two main input datasets (defined as inputs), namely, i) data on the number of PSPAPs confiscated at US airports by Customs and Border Protection from January 2010 to March 2016 at US airports, from the Animal and Plant Health Inspection Service of the US Department of Agriculture (obtained from USDA/APHIS dataset); and ii) information on the number of air passengers arriving in the US via international commercial flights from January 2010 through May 2018.

The probability of ASFV introduction via PSPAP was modelled as a binomial process of the form

$$\mathrm{P}\ (\mathrm{x} \geq 1) = \sum 1 - (1 - \mathrm{Pi}_{\mathrm{vo}})^{N_{odm}}$$

where Pi_{vo} is the estimated probability that at least 1 kg of PSPAP from each origin country or region is contaminated with ASFV. This probability was assumed to be equivalent to at least one domestic pig infected with ASFV. The probability of infection in each country or region of origin (n=128) was estimated based on disease information obtained from the OIE-WAHIS database (25) from the date when the disease was introduced through February 7, 2019. For the present study, countries or regions of origin were classified as i) “high risk” if ASF was present and/or suspected in domestic pig, ii) “medium risk” if ASF was present only in wild boar and/or the country or region bordered an infected country or region; or iii) “low risk” in all other cases.

The probability of ASFV infection in “high risk” countries or regions was estimated by taking into account the potential number of non-reported infected pigs and the number of pigs slaughtered monthly in the country or region, following an approach described elsewhere (308). This probability was estimated following a beta distribution with parameters α_1 and α_2, where $\alpha_1 = Ni_{om}$ x Mod Prop-S_m + 1 and $\alpha_2 = NS_{om} - Ni_{om}$ x Mod Prop-S_m + 1. Ni_{om} denotes the number of infected and non-reported domestic pigs in each origin country *o* per month *m*. The number of infected and non-reported pigs per *o* and *m* was estimated by multiplying the mean census on affected farms in *o*, the mean prevalence on affected farms, the mean number of outbreaks per month, and the duration of ASFV infection in months; this multiplicative product was divided by the probability of notification underreporting (274) and by the time in months since disease introduction in o. If the mean size of affected farms was unavailable, the mean pig farm size in o was obtained from the FAOSTAT database (45). The estimated number of slaughtered, infected, and non-reported pigs was calculated by multiplying the estimated number of infected and non-reported domestic pigs by the proportion of the annual pig census slaughtered per month in infected countries (Mod Prop-S_m). NS_{om}, which represents the number of pigs slaughtered for meat production per *o* and *m*, was calculated by multiplying the pig census per o by the proportion of annual pig census slaughtered per *m* (Prop-S_m). The probability of infection in countries belonging to “medium risk” and “low risk” countries or regions was calculated following the approach of Jurado *et al.* (309) where the probability of an outbreak was multiplied by the average size of an outbreak, the duration of the infection, the probability of an outbreak not being detected and the proportion of pigs slaughtered per month. The final probability of outbreak occurrence was considered to be 10 times higher for “medium risk” than “low risk” countries or regions.

Data on animal populations in countries or regions of origin for the present model came from 2016, whereas our model of risk from before ASFV spread to Europe and Asia relied on data from 2010. Since data on outbreaks and pig populations are unavailable for Guinea, Liberia and Ethiopia, we defined the probability of infection in these countries as the median of the probability for all other African countries. The probability of infection in Hong Kong was assumed to be the same as China, which supplies 94% of pork products consumed in Hong Kong (310).

N_{odm} is the estimated volume (kg) of PSPAP introduced into each US airport *d* from each *o* per *m*. Briefly, N_{odm} was estimated by multiplying the estimated number of kg of PSPAP confiscated from each *o* per *d* per *m* by the probability of PSPAP non-detection at customs (Supplementary Table S3). All PSPAP were assumed to be contaminated with ASFV based on the principle of maximum risk, especially since the database did not record whether the pork product was raw or treated chemically or thermally. The probability of non-detection at customs was assumed to be the same for all US airports and modelled following the approach of Jurado *et al.* (308), while taking into account improvements in detection estimated by previous studies (273, 274). Supplementary Table S3 lists formulas and sources of information used to estimate both parameters.

The input parameters of the model that most heavily influenced the output probability of ASFV entry into the US were identified by conducting two-step sensitivity analysis. First, inputs that had Spearman correlation coefficients (ρ_i) > 0.5 and that contributed >25% to the variance of the output were selected for analysis in detail involving 1,000 iterations for each of 16 scenarios generated by changing the base value of the parameter in consecutive steps, from a minimum reduction of -50% value to a maximum increase of 100%.

Flights accounting for 99% of the annual risk for US airports were selected to build networks per continent (*e.g.* flights representing <1% of the total risk were not displayed for clarity of the net), and depicted showing mean annual risk and the associated 95% confidence interval (CI) per country of origin, US destination airport, and flight level. Risks at the three levels were categorised using Jenks' natural break classification method (182). Nets were built using visNetwork package (280) implemented in the R software (180). Results were mapped using ArcMap 10.3 (ESRI®).

3. Results

The mean annual probability that ASFV-contaminated PSPAP arrives in a US airport prior to customs inspection) was estimated at 0.21 (95% CI 0.19 – 0.76). The mean annual probability that ASFV-contaminated PSPAP enters the US (after customs inspection) was estimated at 0.11 (95% CI 0.01 – 0.50), which is a 183.33% higher than the risk of introduction that we estimated prior to the spread of the disease through Europe and Asia in 2018 and 2019. Our latest estimate suggests that ASFV may evade customs controls and be introduced into the US at least once every 9 years on average, with the lower boundary of the 95% confidence interval corresponding to once every 2 years. China, Hong Kong, the Russian Federation, and Poland account for 97% of the risk, with all other countries contributing <1% of the risk (Table 1).

Five airports accounted for >90% of the risk: Newark-New Jersey (46.38%), George Bush-Houston-Texas (32.71%), Los Angeles-California (5.18%), John F. Kennedy-New York (5.04%) and San Jose-California (2.87%). Regardless of the country or region of origin, risk was higher during the summer, particularly July (Figure 1).

The primary output of the model (the probability that ASFV-contaminated PSPAP arrives in the US after customs inspection) was influenced most by the probability of infection in China ($\rho_i = 0.61$) and the probability of not detecting PSPAP at customs ($\rho_i = 0.72$). Consistent with these results, the probability of not detecting PSPAP at customs accounted for 38.17% of the variance in probability of PSPAP arrival in the US, while the probability of infection in China accounted for 19.53% of the variance. Sensitivity analysis showed that these two factors together doubled the annual risk of PSPAP arrival in the US, independently of other factors. Figure 2 illustrates the results of the advanced sensitivity analysis, showing how these two factors influenced the final annual risk from a reduction of 50% of their base values to an increase of 100%.

Table 1: Annual risk (probability) of African swine fever virus (ASFV) introduction into the US through prohibited swine products carried in air passenger luggage (PSPAP) per administrative unit and continent of origin.

Mean annual risk per continent	Administrative Unit	% Total risk	Probability
Asia [0.076]	China	38.35%	4.28×10^{-2}
	Hong Kong	29.33%	3.28×10^{-2}
	Others	0.36%	4×10^{-4}
Europe [0.034]	The Russian Federation	26.92%	3×10^{-2}
	Poland	2.43%	2.71×10^{-3}
	Others	1.17%	1.29×10^{-3}
Africa [0.002]	-	1.44%	1.6×10^{-3}

Figure 1: Mean annual and monthly risk (probability) of African Swine Fever Virus (ASFV) introduction per country of origin, destination airport in US and flight. A) Asia; B) Europe; and C) Africa. Airports are represented by their IATA codes; full names are shown in Supplementary Table S1.

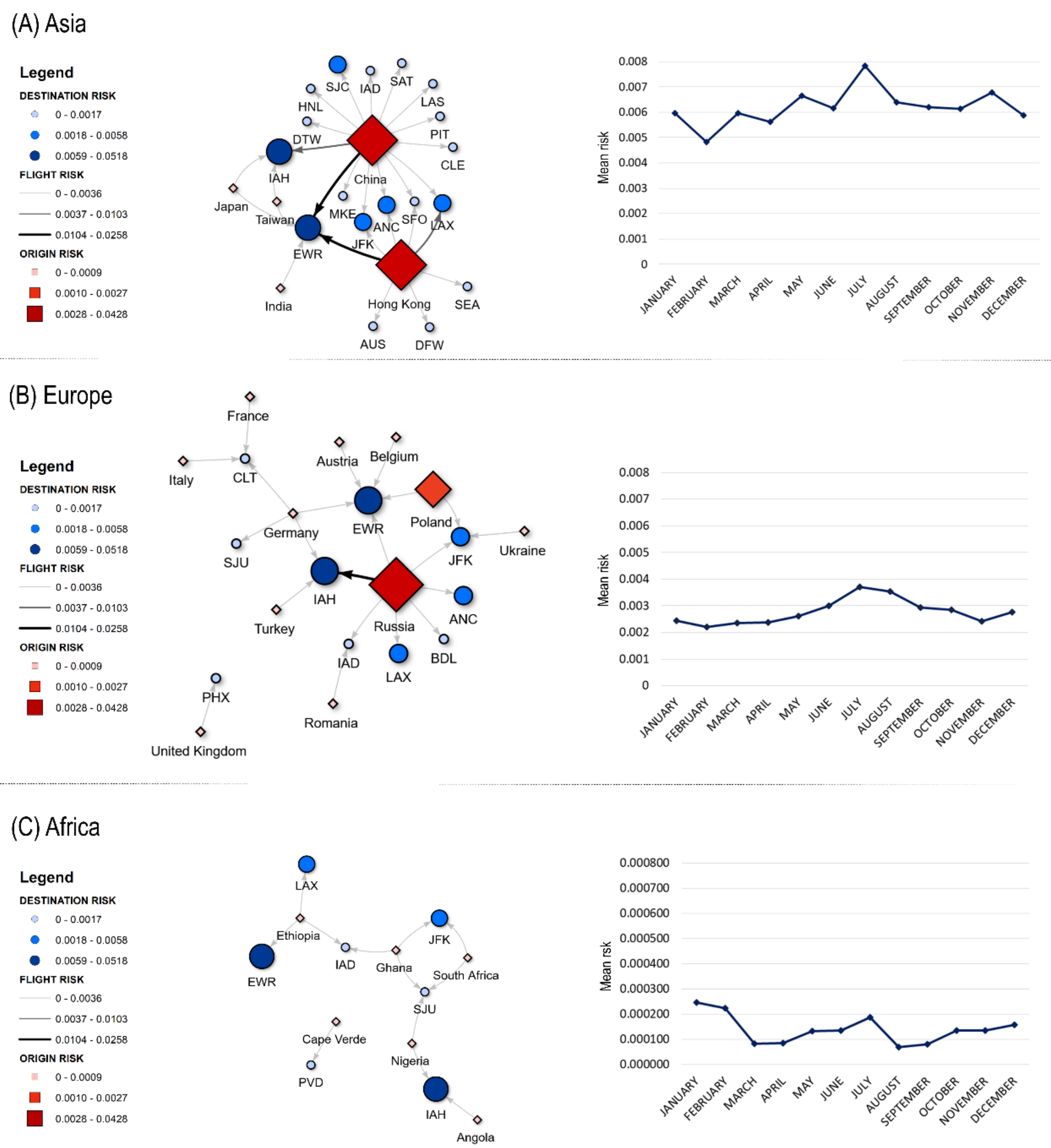
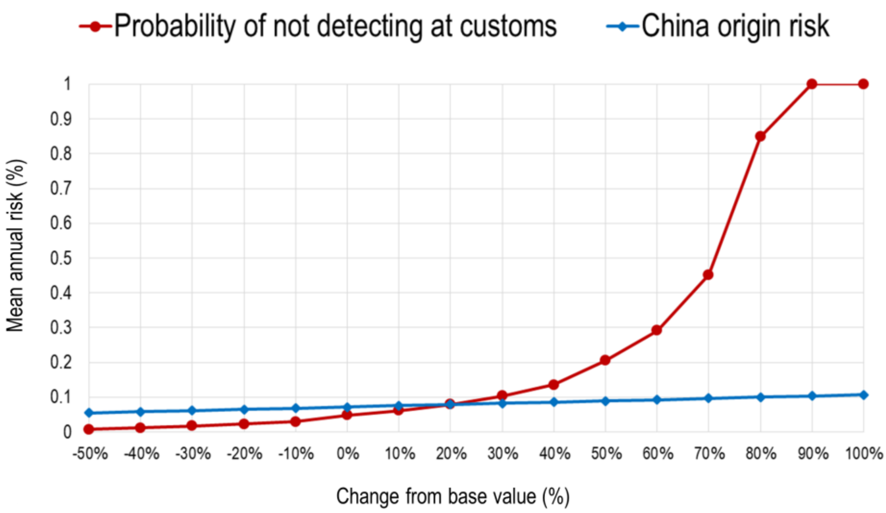


Figure 2: Advanced sensitivity analysis for the risk (probability) of African Swine Fever Virus (ASFV) introduction into the US through prohibited swine products carried in air passenger luggage (PSPAP). Graphs plot the percentage of change in the probability of non-detection of PSPAP at customs inspection (red line), the probability of infection in China (blue line) and the impact on the mean annual risk.



4. Discussion

In response to the spread of ASF in Western Europe and Asia in 2018 and 2019, disease-free countries have enforced strategies, control measures, and biosecurity protocols to protect their susceptible populations against the disease. Many believe that products illegally introduced through passenger luggage constitute a substantial source of risk for spread of FADs (275, 277, 284, 288, 311-313), such as ASF. For example, the UK Department of Environment, Food and Rural Affairs (DEFRA) has estimated that ASFV is likely (with moderate uncertainty) to be introduced into the UK from EU member states through contaminated products (314). Consequently, many countries have strengthened the control of air passenger luggage at points of entry (315-317). Perhaps in part as a result of these efforts, ASFV-contaminated pork products, such as sausages or dried pork, have been detected several times at ports and airports in Taiwan, South Korea, Japan, Thailand, Australia, Philippines and United Kingdom in 2018-2019 (Supplementary Table S2) (302-304, 306). The infectivity of these products is unknown, except for products confiscated in Japan, where some products were confirmed to contain infective virus (318). Most of the products confiscated at these airports originated from China, where meat and pork products have been found to contain ASFV (Table S2). Consequently, Chinese authorities have required companies to trace the source of contaminated raw materials and prevent contaminated pork raw materials from entering the food chain (60). The effectiveness of those preventive measures has yet to be measured.

The results here suggest a high risk that ASFV can reach US airports as PSPAP prior to customs control. In this study, all PSPAP were assumed to be contaminated with ASFV according to the principle of maximum risk, what might have led to overestimate the probability of introduction. Nevertheless, the viral genome has been detected in confiscated pork at many international airports, but whether the virus has entered US airports is unclear since pork seized in that country is not tested for ASFV. These results, along with the estimated 183.33% increase in risk of ASFV introduction into the country following spread of the disease in Asia and Europe, underscore the importance of prevention and detection measures at US airports. Most risk seems to concentrate on flights from China, Hong Kong, the Russian Federation and Poland, in contrast to earlier estimates that attributed most risk to flights from Africa (308). Indeed, the much higher cost of pork and much smaller pork production in the US than in China create an incentive for large-scale pork smuggling into the US. In March-2019, an illegal shipment of 1 million pounds of pork from China was seized at Newark airport (319).

Noteworthy, results were highly sensitive to the probability of non-detection of illegal products at airport. The probability of non-detection at customs was previously estimated by other authors (273, 274, 308). This probability was estimated as a unique value for the whole US. This assumption might have led to underestimate or overestimate the capacity of detection of some airports as the CBP resources and volume of passengers could considerably differ between airports. Because of the influence of that parameter in the model outputs, and because of the challenges associated with the accurate prediction of its true value, it is possible that the risk of ASFV introduction into the country would actually be lower or higher than the values estimated here. Therefore, it would be beneficial for the model to include actual information on the efficacy of control disaggregated by airport.

The US swine industry is one of the most industrialised in the world, and biosecurity measures are strictly enforced as the final line of defence to protect domestic pigs from ASFV incursions. Much of the concern related to the ASFV introduction through air passenger luggage is linked to the possibility that contaminated products may be disposed of outside the airport control zone, where it may infect feral pigs (314, 320). The population of feral pigs has steadily increased in the US, likely due to their flexibility in adapting to a variety of habitats as well as a lack of natural predators. Feral swine are present in at least 35 US states, giving rise to a population of >6 million animals (321). ASF spreads through populations of wild boar and feral pigs, so ASFV-contaminated products disposed of outside the airport may pose a risk to the domestic pig population in the US.

Summer months, and particularly July, accounted for most of the risk in our assessment. Summer is the time of the year when most tourists visit the US; in July 2017, for example, almost 23 million people flew into the US (278). The agricultural inspection process at US airports consists of primary activities (inspection of customs documents, interviewing of passengers, and searches by agriculture canine teams for agricultural products in the baggage area) and secondary activities (interviewing and luggage inspection). The large volume of passengers arriving every year into the US may compromise the efficacy of these activities. Targeted surveillance is a key strategy to increase effectiveness of prevention measures when resources are limited. Results here may be used to inform recommendations regarding how to strengthen surveillance activities in the US. Our results suggest that measures to detect ASFV early and prevent disease may be selectively targeted or prioritised to five airports, especially during the summer.

A limitation of the work here is that the probability of carrying pork products was assumed to be equal across countries and regions of origin. However, cultural and religious factors can influence passengers' behaviour (284, 313). Future studies should generate data on this point in order to incorporate it into the model. Another limitation of our study is that we had no information on connecting flights prior to arrival in the US, so we could not estimate risk from the point of departure. This means that our model may underestimate the risk of ASFV arrival due to the lack of information on the number of passengers from affected countries with no direct flights to the US. These passengers (i.e. passengers from Estonia, Belarus and Moldova, among others) would have layover at major hubs such as Amsterdam, London Heathrow, Frankfurt, or Paris-Charles de Gaulle. As a consequence of such absence of information, the risk estimated for countries with no direct flights into the US, and most importantly, the risk estimated for countries with major airport hubs, may have been underestimated here. Moreover, if data on connecting flights were available, the probability of detection of PSPAP at connecting hubs and data on confiscations at such customs control should be also taken into consideration to avoid underestimating or overestimating the final risk.

In conclusion, results suggest that the risk of ASFV introduction into the US through smuggling of pork through air passenger luggage has increased substantially since the disease spread into regions of Asia and Europe in 2018 and 2019. Most of the risk appears to come from China (38.35%), Hong Kong (29.32%), the Russian Federation (26.92%) and Poland (2.43%). The majority of risk concentrates in five US airports and is higher in the summer. These results will help to inform decisions related to design of ASFV surveillance strategies in the US.

Table S2: * Proportion of seized product detected positive. ** Apparent frequency (monthly based).

Country	Date published	Event	Prop*	Freq **	Source
South Korea	26/08/2018	ASF detected in dumplings and sausage declared by a South Korean tourist returning from Shenyang province	---	---	(302)
Japan	22/10/2018	Detection of ASF in packed pork sausage brought by a passenger from Beijing to Shin-Chitose airport in Hokkaido	---	---	(303)

Taiwan	31/10/2018	Since late August 2018, 928 products have been seized and test for ASF, 20 samples have been found to be positive	2.1%	2.86 events per month	(304)
Thailand	16/01/2019	African Swine Fever detected in pork products at Phuket Airport	---	---	(305)
Australia	17/01/2019	6 of 152 pork products seized over a period of two weeks were contaminated with ASF	3.2%	10 events per month	(306)
Philippines	14/06/2019	Canned pork products seized at the Clark International Airport in Pampanga brought in from Hong Kong in March resulted positive for ASFV by PCR			(307)
United Kingdom	11/07/2019	In July, airport authorities in Northern Ireland seized over 300 kg of illegal meat products. A sample of these seizures resulted positive for ASFV by PCR			(322)

DISCUSIÓN

Tras la erradicación de la PPA en Europa a finales del siglo pasado (a excepción de la isla de Cerdeña), el sector porcino de la UE ha sido capaz de prosperar, erigiéndose como la segunda potencia mundial en términos productivos y censales (45). La rentrada de la PPA en 2007 en Europa continental, a través de Georgia, impulsó el desarrollo de numerosos estudios epidemiológicos en aras de proteger este importante sector. Varios de estos estudios pretendieron identificar potenciales fuentes de riesgo que necesitaban ser mitigadas. En concreto, los estudios de análisis de riesgo elaborados para la UE, cuantificaron el riesgo de introducción en relación a la importación legal de animales vivos y productos de origen porcino, importación ilegal de carne de cerdo y productos de origen porcino o la entrada de vehículos y restos alimenticios contaminados procedentes de aviones y embarcaciones con rutas internacionales (212, 213, 323, 324). Del mismo modo, se caracterizó de forma semicuantitativa el riesgo de introducción de la PPA a través de movimientos naturales de jabalíes (67, 68).

Los resultados obtenidos en estos estudios reflejaron la importancia de controlar la entrada de vehículos potencialmente contaminados desde áreas infectadas, la necesidad de destruir adecuadamente restos alimenticios procedentes de rutas de transporte internacionales, así como el elevado riesgo al que se encontraban expuestos países como Letonia o Polonia en relación a la ruta mediada por jabalíes infectados. De acuerdo con los resultados obtenidos por Wieland *et al.* (217) en un estudio basado en la opinión de expertos de la Comisión Europea (CE) y grupos de trabajo de la EFSA, el riesgo de difusión desde la región del Cáucaso y Rusia hacia áreas próximas era muy alto. Así mismo, el riesgo de que la PPA permaneciese de forma endémica en dicha área era moderado.

En concordancia con los resultados de los estudios mencionados, el avance de la enfermedad a lo largo del continente europeo no tuvo freno y consiguió expandirse inexorablemente hacia áreas occidentales alcanzando en 2014, año de inicio de esta tesis doctoral, Lituania, Polonia, Letonia y Estonia. La nueva imagen epidemiológica y el contexto socioeconómico actual de la Unión, llevó al planteamiento del ***objetivo 1*** de esta tesis doctoral.

Este objetivo, se centró en la identificación y priorización en tres categorías (alta, media y baja) de las principales lagunas de conocimiento en relación a la PPA. Entre otras, el desarrollo de modelos epidemiológicos para la aplicación de medidas de control basadas en riesgo, así como la mejora de los sistemas de detección temprana, planes de contingencia y medidas de control fueron identificadas como prioridades de alta importancia. Del mismo modo, la necesidad de continuar los estudios con potenciales candidatos vacunales para hacer frente al VPPA, fue considerada por los expertos como una prioridad de gran relevancia. Otras prioridades relacionadas con la medicina preventiva fueron clasificadas en la categoría de relevancia media. Sin embargo, teniendo en cuenta las opciones disponibles por aquel entonces, la medicina preventiva se postulaba como la principal herramienta de lucha para la prevención y control de la PPA. En línea con la estrategia en sanidad animal 2007-2013: “más vale prevenir que curar”, para la UE, la realización de análisis de riesgo o la aplicación de medidas de vigilancia en zonas expuestas a un alto riesgo podría haber sido clasificadas como prioridades con importancia alta en lugar de media.

Del mismo modo en el estudio de Wieland *et al.* (217), se concluyó que si la PPA alcanzaba la UE, esta sería controlada de forma eficaz en sistemas productivos con bioseguridad alta o limitada. Sin embargo, en sistemas extensivos, el riesgo de endemicidad fue clasificado como medio, debido a posibles contactos con jabalíes, movimientos ilegales y dificultad de acceso a estos animales. Pese a las medidas preventivas y de control impuestas y la aplicación de estrategias de regionalización en áreas afectadas, la PPA se extendió por los países bálticos y regiones orientales de Polonia. Las notificaciones en estos países se atribuyeron en más de un 90% a casos en jabalíes (25). De hecho, las poblaciones silvestres de estos territorios ya son consideradas endémicas (26). En granjas de cerdo doméstico las notificaciones fueron inicialmente esporádicas (entre 2014-2016, 43 brotes/año de media), aunque con la llegada de la PPA a Rumanía la incidencia se ha visto incrementada a más de 1.000 brotes en 2018 (25, 26).

Las diferencias en los patrones epidemiológicos observados en los países afectados de la UE ha podido deberse a la heterogeneidad de los distintos sistemas productivos de porcino así como a las medidas de control establecidas en los países afectados (216). Dentro de la UE, países como Bélgica, España, Alemania, Países Bajos o Dinamarca, entre otros, representan el perfil de productores industrializados, donde el sector está integrado por granjas de gran tamaño con excelentes estándares de bioseguridad y sistemas de manejo altamente profesionalizado. En el otro extremo se encuentran países del este de la UE de reciente incorporación, como Bulgaria o Rumanía, donde la producción mayoritaria está representada por granjas de autoconsumo, con censos muy reducidos, manejo pobremente tecnificado y condiciones de bioseguridad muy limitadas o inexistentes (45, 216, 325). Por último, puede distinguirse una producción de tipo extensiva, que a su vez, presenta una gran variabilidad entre regiones. A modo de ilustración, las condiciones de bioseguridad distan considerablemente entre la producción de cerdo ibérico en la dehesa (246), las producciones extensivas de cerdo de capa blanca en granjas de ecológico (243), el mantenimiento de animales de "*brado"* en Cerdeña (48) o la producción de cerdo del este de los Balcanes en Bulgaria (208).

En Cerdeña, la PPA es una enfermedad endémica desde el año 1978 donde los programas de control habían conseguido limitar la incidencia de la enfermedad sin llegar a alcanzar la erradicación completa. Otra de las prioridades en investigación identificadas en el ***objetivo 1*** hacía referencia a la necesidad de emplear herramientas de modelización de enfermedades con el fin de emplear medidas de control basadas en riesgo. Además, se consideró una prioridad importante disponer de planes de contingencia, sistemas de detección temprana y medidas de control eficaces en la lucha frente a la PPA. Por ello, el ***objetivo 2*** se centró en la identificación de los factores de riesgo que estaban favoreciendo el endemismo de la PPA en la isla de Cerdeña, por aquel entonces único escenario endémico de la UE para esta enfermedad. La estrecha colaboración entre el grupo de investigación donde se ha desarrollado esta tesis doctoral y el Gobierno de la Región de Cerdeña, garantizó la utilización de datos epidemiológicos de gran valor, usados por primera vez para este estudio.

Los resultados obtenidos permitieron identificar como factores de riesgo con mayor relevancia, la alta densidad de granjas con censos entre 5-30 animales (variable altamente correlacionada con la alta densidad de granjas), la presencia de animales de "*brado*" y la combinación de altas densidades de jabalíes y altitudes por encima de la media de la zona. Este resultado refleja la naturaleza del sector porcino en la isla y es que una vasta mayoría de las granjas posee un tamaño reducido donde la bioseguridad es limitada. Desde la administración sarda se ha trabajado en mejorar esta situación, incrementando los controles en granja, certificándolas sanitariamente en función de su estado sanitario y nivel de bioseguridad (más de un 50% de las granjas ya se encuentran certificadas) y adecuando los sistemas de identificación individualizada de animales (52).

El rol de los animales de “*brado”* en la perpetuación de la infección de PPA había sido sugerido en repetidas ocasiones (23, 173), sin embargo hasta la publicación de estos resultados no existía ninguna evidencia científica que lo respaldase. Desde el año 2012, la práctica del “*brado”* en la isla había sido prohibida aunque seguía realizándose de manera indiscriminada en áreas de especial tradición como son las provincias de Nuoro y Ogliastra. Con la prohibición de esta práctica por parte de las autoridades sardas, comenzaron a existir registros de avistamiento de estos animales. No obstante, la realización de abatimientos no comenzó a realizarse con regularidad hasta 2015. La tardanza en la realización de estos sacrificios con fines sanitarios se debió en parte a la falta de comprensión de esta medida por parte de la población sarda.

Por ello, adquiere especial relevancia en cualquier plan de control y erradicación de enfermedades de declaración obligatoria, contar con la colaboración de ganaderos, veterinarios e incluso ciudadanos. Esta colaboración estrecha solo puede conseguirse siguiendo estrategias de comunicación adecuadas. De este modo, desde las administraciones locales sardas se comenzó a realizar en paralelo campañas de concienciación y formación, así como sacrificios controlados de estas poblaciones ilegales de cerdos. A modo de ilustración, entre diciembre de 2017 y junio de 2018, se realizó el sacrificio de más de 2.000 animales, de los que más de un 50% presentaron presencia de anticuerpos y alrededor de un 3% viremia positiva, todos ellos con ausencia de cualquier sintomatología compatible con PPA (36). De acuerdo con las estimaciones de las autoridades sardas, en la actualidad existen entre 500-600 cerdos de *“brado”* aún en libertad; estos se encuentran en zonas de difícil acceso y dispersos en grupos pequeños.

En dichas zonas de difícil acceso, podrían estar ocurriendo contactos entre animales de *“brado”* y jabalíes ya que el tercer factor de riesgo identificado representaba áreas donde se estimaba una elevada densidad de jabalíes y altitudes por encima de la media de la zona. De hecho, en un estudio reciente se ha podido caracterizar la interacción entre ambas poblaciones mediante la utilización de cámaras de foto trampeo (326). Los resultados de este estudio demuestran que las interacciones directas fueron más frecuentes entre animales juveniles (entre las 14-21 horas) mientras que en adultos contactaron con mayor asiduidad en zonas de agua. Sin embargo, las interacciones indirectas ocurrieron mayoritariamente entre individuos adultos. La transmisión del VPPA a través de estos contactos dependerá del estado sanitario de los animales (*i.e.* si existe excreción viral), así como de la supervivencia viral en el medio. Además, el tamaño de las poblaciones susceptibles también será un factor limitante en el ratio de transmisión. En este sentido, la población total de jabalíes de la isla se estima en 90.000 cabezas, con alrededor de 12.000 animales abatidos por temporada de caza (52). Esto se traduciría en una tasa de mortalidad del 13% atribuible a la actividad cinegética. Ciertos estudios estiman que las tasas de mortalidad efectivas deberían acercarse al 65% a fin de conseguir tendencias poblacionales estables (327). Por ello, un aspecto a tener en cuenta sería la adecuación de las bolsas de caza al tamaño de la población estimada a fin de evitar problemas de sobrepoblación y con ello, un posible recrudecimiento de la enfermedad en este hospedador.

En línea con las prioridades identificadas en el objetivo 1 y abordando el segundo escenario de estudio de esta tesis doctoral, el ***objetivo 3*** se basó en la realización de una revisión sistemática y posterior evaluación por expertos en PPA, de las medidas preventivas disponibles para su aplicación en granjas de cerdo doméstico en el contexto de la UE. Para ello, se tuvo en cuenta tres tipologías de granja de acuerdo conl documento de trabajo de la CE: comercial, no comercial y extensiva (206). Los resultados de este objetivo identificaron un total de 37 medidas preventivas aplicables. Esta diferenciación por tipo de granja se tuvo en cuenta dado que las características intrínsecas de cada modelo productivo condicionarán la susceptibilidad del sistema a la entrada del agente infeccioso. Por ejemplo, en granjas de tipo extensivo la probabilidad de contacto entre animales domésticos y poblaciones silvestres es mayor que en granjas industrializadas, donde los animales se encuentran estabulados de forma permanente. Por ello, medidas preventivas como es disponer de sistemas adecuados de vallado, se convierte en un aspecto crucial para evitar contactos de riesgo. Sin embargo, la agrupación de todos los sistemas extensivos en una misma categoría podría haber contribuido a una pérdida de granularidad en esta categoría.

De acuerdo con la opinión de los expertos, en el contexto de la UE, la identificación de animales y registro de actividades en granja, así como la prohibición de alimentar con restos alimenticios a los animales o estabular a los animales de forma permanente, son medidas preventivas relevantes para evitar la entrada y difusión de la PPA en cualquier tipo de granja. Estas medidas ayudarían a mitigar prácticas de riesgo llevadas a cabo en el escenario actual, en el que actividades humanas como la utilización de restos alimenticios contaminados para alimentar a los animales o la venta de emergencia de animales infectados, parecen haber favorecido la difusión de la enfermedad (328). En Rumanía por ejemplo, donde el 75% de las notificaciones se atribuyen a brotes en granjas de cerdo doméstico (25), la aplicación de estas medidas podría mitigar situaciones concretas identificadas por las autoridades veterinarias nacionales rumanas. El movimiento de animales sin certificaciones sanitarias y la falta de identificación adecuada de los animales fueron detectadas como prácticas ilegales realizadas en territorios infectados y por tanto, actividades de riesgo susceptibles de favorecer la difusión de la PPA en el territorio.

Del mismo modo, en China y Vietnam la PPA está afectando de forma mayoritaria al sector porcino con más de tres millones de animales sacrificados hasta la fecha (más de un millón en China y más de dos millones en Vietnam) (60). De acuerdo con Vergne *et al.* (329), algunos de los factores que favorecerían la entrada, difusión y persistencia de patógenos en la industria porcina china serían el alto porcentaje de granjas con bioseguridad limitada (40% de las granjas tienen un censo inferior a 30 animales), así como la falta de identificación de animales, acceso a servicios veterinarios y profesionalización del sector. Además, en las granjas industriales raramente se realizan cuarentenas, ni separación de animales por grupos de edad. En Vietnam, la PPA ha sido notificado en menos de 4 meses en 54 provincias diferentes (60). La entrada y difusión de la enfermedad podría haberse debido al movimiento ilegal de animales a través de la frontera con China y posteriormente, dentro del territorio vietnamita (330). Estos movimientos habrían sido propiciados por la falta de compensación a los ganaderos que notifican la enfermedad. Por ello, considerando la situación actual, sería necesario realizar una reevaluación para el escenario asiático, teniendo en cuenta sus sistemas productivos y las características culturales que pudieran estar afectando a la correcta aplicación de las medidas preventivas identificadas.

Una de las tareas fundamentales por parte de las administraciones y del propio sector es concienciar y educar a todos los miembros de la cadena productiva, desde ganaderos hasta el propio consumidor. La continua formación del personal veterinario también constituye una tarea fundamental, a fin de generar una sospecha y detección rápida antes de que se produzcan focos secundarios. Considerando la importancia de difundir estos resultados, en el marco de la acción COST 15116 “Understanding and combating African swine fever in Europe (ASF-STOP)”, se publicó el artículo científico recogido en el capítulo 3 de esta tesis doctoral. Además, estos resultados serán empleados para elaborar folletos informativos recogiendo las medidas preventivas más relevantes y serán distribuidos a productores de porcino de la UE. Del mismo modo, se han elaborado cursos de gestión en crisis y artículos de divulgación en revistas y páginas web del sector a fin de transferir los resultados obtenidos en esta tesis doctoral.

Por último, en la evaluación de las prioridades en investigación desarrolladas en el ***objetivo 1*** se identificó como prioritaria la necesidad de elaborar estudios de análisis de riesgo para identificar las rutas de introducción de PPA en áreas libres. Los estudios de análisis de riesgo constituyen una herramienta muy valiosa en materia de prevención ya que permite la toma de decisiones basada en riesgo. Los resultados de estos análisis permiten reforzar las tareas de vigilancia identificando puntos críticos que podrían ser mejorados a fin de salvaguardar el sector o área de interés. Por ello, de acuerdo con la situación epidemiológica descrita, se consideró una prioridad de gran importancia estimar el riesgo de entrada de PPA en un escenario libre, mediada por el comportamiento humano.

Así, el ***objetivo 4*** se centró en la evaluación del riesgo de entrada del VPPA en EEUU a través del movimiento ilegal de carne o productos de origen porcino transportados ocultos en el equipaje de pasajeros procedentes de vuelos internacionales. El escenario elegido fue Estados Unidos, tercera potencia mundial en producción porcina por detrás de China y la UE. Este estudio se inició en 2016, cuando la PPA limitaba su presencia al continente africano y europeo. De acuerdo con los resultados obtenidos, los países que representaron un mayor riesgo fueron Gana, Cabo Verde, Etiopía y Rusia.

Debido a la expansión de la PPA a China en verano de 2018, se consideró necesario actualizar el modelo desarrollado para reevaluar esta vía de entrada. Además, varios medios de comunicación informaron entre agosto de 2018 y enero de 2019, acerca de la detección de productos de contrabando de origen porcino contaminados con el VPPA, en controles realizados en aeropuertos de Japón, Corea del Sur, Taiwán, Tailandia, Australia, Filipinas y Reino Unido. En la mayoría de los casos se trataban de turistas procedentes de China, los cuales habían adquirido productos contaminados tales como salchichas, salami o empanadillas. En dichos productos se detectaron restos de genoma viral, sin embargo en la mayoría de países, a excepción de Japón donde se ha probado la infectividad de los productos, no se detallaba si dichas muestras presentaban virus infectivo.

Por tanto, considerando el cambio epidemiológico en relación a la distribución de la enfermedad así como la frecuencia con la que han sido detectados productos de contrabando procedente de los nuevos países infectados, se realizó una actualización del modelo utilizando los nuevos datos referentes a la situación epidemiológica de la enfermedad. Los resultados obtenidos reflejaron un incremento del riesgo anual de entrada del VPPA por esta vía del 183%. Además, se percibió un cambio en el perfil de riesgo en origen, aunando China, Hong Kong, Rusia y Polonia un 97% del riesgo total. Pese a que Hong Kong era una región libre en el momento en el que se realizó la estimación del riesgo, la probabilidad de infección en dicha región se consideró igual a la representada por China. Esta asunción se realizó en base a los estrechos lazos comerciales existentes entre China y Hong Kong ya que un 94% de la carne y productos de origen porcino consumidos en Hong Kong proceden de China (301). De hecho, un mes después se notificó oficialmente a la OIE la presencia de una canal positiva al

VPPA en un matadero de Hong Kong procedente de un cerdo importado de China (61, 62).

Del mismo modo, se observó un cambio en relación a los aeropuertos donde deben reforzarse las actividades de vigilancia. Mientras que en el modelo inicial cuatro de los cinco aeropuertos expuestos a un mayor riesgo se localizaron en la costa este, en los resultados del modelo actualizado, el riesgo se encuentra más disperso. En concreto, los aeropuertos de Newark-Nueva Jersey (costa este), George Bush-Houston-Tejas (costa sur), Los Ángeles-California (costa oeste), John F. Kennedy-Nueva York (costa este) y San José-California (costa oeste) recibirían de acuerdo con las estimaciones realizadas alrededor del 97% del riesgo anual total. Curiosamente, en el estudio inicial ningún aeropuerto de la costa oeste emergió como punto de entrada de interés en términos de riesgo. Este cambio se debió principalmente a los vuelos realizados desde China hacia los dos aeropuertos californianos.

Dado que los recursos humanos y logísticos en control de aduanas es finito, resultados de este tipo ayudan a caracterizar el riesgo al que se encuentran expuestos los aeropuertos estadounidenses y permiten invertir estos recursos de una forma más eficiente en base al riesgo estimado. Desde el sector porcino de Estados Unidos existe una preocupación real en cuanto a la posibilidad de expansión de la PPA. Prueba de ello es que en abril de 2019 se ha cancelado la “2019 World Pork Expo”, la cual es celebrada cada año en el estado de Iowa, atrayendo a más de 20.000 asistentes procedentes de 40 países diferentes, entre los cuales se encuentran áreas actualmente afectadas. Los organizadores han sustentado su decisión en la necesidad de extremar las precauciones en un intento de prevenir la entrada de la enfermedad en EEUU ya que en el contexto actual la única herramienta disponible sigue siendo la prevención.

Considerando que los países oficialmente afectados por PPA, poseen un 77% de la población porcina mundial en términos censales, de continuar esta situación, cabría esperar la aparición de problemas de desabastecimiento, especialmente en países como China donde la demanda interna obliga a importar carne de cerdo. En zonas infectadas de China, las medidas de control impuestas obligan al sacrificio de las granjas infectadas y la prohibición de repoblación de las granjas durante al menos 6 meses. Por ello, se espera un descenso considerable de la producción interna, a lo que se sumaría la aplicación de restricciones en el comercio. De hecho, las perturbaciones en el comercio se han traducido en un incremento del precio de la carne de cerdo en los mercados europeos y norteamericanos (331). Esta situación puede ser beneficiosa para países exportadores como Estados Unidos, España y Alemania, donde salvaguardar el estado sanitario de la cabaña ganadera porcina se ha convertido en una necesidad de primer orden. Para ello, se requiere una puesta a punto de los sistemas de detección temprana, revisión de los planes de contingencia y desarrollar tareas de concienciación e información a todos los niveles.

Desde los países la UE afectados, la erradicación la enfermedad pasa por el establecimiento de medidas de control eficaces en las poblaciones de jabalíes. Sin embargo, esto se encuentra dificultado por la falta de control sanitario de estos animales y su capacidad de libre movimiento. Algunas de las medidas puestas en marcha hasta la fecha han incluido la prohibición de alimentación suplementaria y actividades de caza a gran escala, sacrificio dirigido de animales a fin de reducir la abundancia poblacional y establecimiento de retribuciones económicas tras la retirada rápida y con garantías sanitarias de carcasas del medio ambiente. Muchas de estas medidas son controvertidas y su eficacia ha sido en ocasiones puesta en entredicho.

Sin embargo, la República Checa podría ser ejemplo de erradicación al haber sido reconocida como territorio libre por la UE, tras 10 meses sin ningún caso notificado (todas las notificaciones fueron atribuidas a casos en jabalí) (332). En las poblaciones de jabalíes se prohibió inicialmente la caza, se realizaron búsquedas activas de cadáveres con refuerzo de la vigilancia pasiva en jabalíes hallados muertos y prohibición de la entrada de personas al área infectada. Posteriormente se permitió la caza individualizada y uso de trampas por parte de cazadores entrenados, y se estableció un sistema de recompensa por cada animal encontrado muerto y/o cazado junto con un sistema de compensación para incentivar el manejo de las canales de jabalíes cazados en plantas de transformación. Además, las medidas empleadas para evitar la difusión de la enfermedad al sector porcino incluyeron la prohibición de granjas de traspatio y granjas no registradas, incrementar la vigilancia pasiva, la prohibición de movimientos sin autorización y alimentación con forrajes frescos o empleo de material de cama, realización de controles oficiales para reforzar la bioseguridad de las granjas y la realización de campañas de información (333).

Sin duda, los próximos años se prevén convulsos como consecuencia de la infección de importantes países productores y la aplicación de medidas de control, las cuales pueden afectar a los niveles de abastecimiento de países como China. Además, la aplicación de medidas de salvaguarda desde países libres podría dar inicio a posibles fricciones comerciales. Por ello, es necesario continuar con los esfuerzos en investigación a fin de reforzar las medidas preventivas y planes de vigilancia para evitar una mayor difusión de la enfermedad, así como avanzar en su control y conseguir al fin, la erradicación de la PPA en el mundo.

CONCLUSIONES

PRIMERA/FIRST

Se han identificado treinta y seis lagunas de conocimiento en relación a la peste porcina africana clasificadas en las categorías: virus, hospedadores naturales, formas clínicas, epidemiología, impacto socio-económico, respuesta inmune, prevención, detección y control, y diagnóstico y vacuna. Diecinueve de ellas fueron categorizadas como prioridades de relevancia mayor, once con importancia media y seis con importancia menor.

Thirty six gaps of knowledge regarding African swine fever were identified belonging to the following categories virus, natural hosts, clinical forms, epidemiology, socio-economic impact, immune response, prevention, detection and control, diagnosis and vaccine. Nineteen of them were categorised as high priority, eleven as medium priority and six as low priority.

SEGUNDA/SECOND

Nueve factores fueron asociados al riesgo de ocurrencia de peste porcina africana en Cerdeña, tres de los cuales habían sido previamente identificados (número de granjas medianas, presencia de animales de *"brado"* y la combinación de la densidad estimada de jabalíes y altitud) y otros seis identificados por primera vez (número de granjas familiares, numero de granjas que realizan movimientos de salida de animales, granjas que realizan matanzas domiciliarias, número de granjas no censadas, número de granjas de reproducción con ciclo abierto y número de granjas semiextensivas).

Nine factors were associated with risk of ASF occurrence in Sardinia, three of which were previously identified (number of medium-sized farms, presence of *“brado”* animals, and the combination of estimated wild boar density and altitude), and another six that are novel (number of family farms, number of farms reporting outgoing movements, number of farms reporting movements for self-consumption, number of non-censused farms, number of open-cycle breeding farms and number of semi-extensive farms).

TERCERA/THIRD

Considerando los factores de riesgo identificados en la isla de Cerdeña, el actual programa de control y erradicación podría ser implementado mediante la intensificación de la lucha contra los animales de “*brado”*, favoreciendo la profesionalización del sector e incrementando las medidas de bioseguridad en granja, especialmente en las de tipo semiextensivo.

Considering the identified risk factors in Sardinia, the current eradication programme could be implemented by intensifying the fight against “*brado”* animals, favouring the professionalisation of the pig sector and increasing biosecurity standards on farms, especially on semi-extensive farms.

CUARTA/FOURTH

Un total de treinta y siete actuaciones fueron identificadas como medidas preventivas disponibles para reducir la difusión de la peste porcina africana en el contexto de la Unión Europea. De acuerdo con el panel de expertos, las medidas preventivas más relevantes para granjas comerciales, no comerciales y extensivas fueron la identificación de animales y registro en granja, refuerzo en la prohibición de alimentar animales con restos procedentes de la alimentación humana y la estabulación permanente de animales a fin de evitar contacto con cerdos de otras granjas, cerdos asilvestrados, jabalíes y productos de origen porcino.

A total of thirty-seven measures were identified as preventive to reduce the spread of African swine fever among domestic pigs in the European Union context. According to the expert panel, the most important preventive measures for commercial, non-commercial and outdoor farms were the identification of animals and farm records, enforcement of the ban on swill feeding and containment of pigs to not allow contact with pigs from other farms, feral pigs or wild boar or their products.

QUINTA/FIFTH

El riesgo de entrada del virus de la peste porcina clásica, en Estados Unidos, a través de productos de origen porcino transportados ilegalmente en el equipaje de pasajeros procedentes de vuelos internacionales, fue siete veces más alto que el riesgo de entrada del virus de la peste porcina africana hasta agosto de 2018. El riesgo de entrada para peste porcina africana se concentró en más de un 90% en cinco aeropuertos americanos (Washington-Dulles, George Bush-Houston, John F. Kennedy-Queens, Warwick y San Juan). El origen de los vuelos que representaron mayor riesgo fue Cabo Verde y Gana. Mayo y julio fueron los meses expuestos a un mayor riesgo de entrada.

The risk of classical swine fever virus entry via pork prohibited swine products carried in air passengers' luggage was seven times higher than the risk of African swine fever virus entry up to August 2018. Over 90% of the final African swine fever virus risk was concentrated in five American airports (Washington-Dulles, George Bush-Houston, John F. Kennedy-Queens, Warwick and San Juan). The origin of the flights posing higher risk were Cape Verde and Ghana. May and July were the months at the highest risk.

SEXTA/SIXTH

La probabilidad media anual de entrada del virus de la peste porcina africana se ha visto incrementado un 183% comparado con el riesgo estimado antes de que la enfermedad se expandiese a China, este de Asia y oeste de Europa en 2018 y 2019. Tres países y una región (China, Hong Kong, Rusia y Polonia) aunaron un 97% del riesgo total. Cinco aeropuertos recibirían más del 90% del riesgo total, en concreto, Newark-Nueva Jersey, George Bush-Houston-Tejas, Los Ángeles-California, John F. Kennedy-Nueva York y San José-California. Julio continuó siendo uno de los meses expuesto a mayor riesgo.

The overall mean annual probability of African swine fever virus entry has increased 183% compared to the risk estimated before the disease spread into China, East Asia, and Western Europe in 2018 and 2019. Three countries and one region (China, Hong Kong, the Russian Federation, and Poland) accounted for 97% of the risk. Five airports accounted for more than 90% of the total risk, namely, Newark-New Jersey, George Bush-Houston-Texas, Los Angeles-California, John F. Kennedy-New York and San Jose-California. July was still the month at the highest risk.

OTRAS PUBLICACIONES CIENTÍFICAS

Other scientific publications not included in this doctoral thesis:

Bosch J, Rodríguez A, Iglesias I, Muñoz MJ, **Jurado C**, Sanchez-Vizcaino JM et al. **Update on the risk of introduction of African swine fever by wild boar into disease-free European Union countries.** Transbound Emerg Dis. 2017;64(5):1424-1432. DOI: 10.1111/tbed.12527.

Alkhamis MO, Gallardo C, **Jurado C**, Soler A, Arias M, Sanchez-Vizcaino JM. **Phylodynamics and evolutionary epidemiology of African swine fever p72-CVR genes in Eurasia and Africa.** PLoS One. 2018;13(2):e0192565. DOI: 10.1371/journal.pone.0192565.

Barasona JA, Gallardo C, Cadenas-Fernandez E, **Jurado C**, Rivera B, Rodríguez-Bertos A, et al. **First oral vaccination of Eurasian wild boar against African swine fever virus genotype II.** Front Vet Sci. 2019;6:137. DOI: 10.3389/fvets.2019.00137.

Ito S, **Jurado C**, Sanchez-Vizcaino JM, Isoda N. **Quantitative assessment of the risk of African swine fever virus introduction to Japan via pork products brought in air passengers' luggage.** *Submitted for publication to Transbound Emerg Dis.* 2019. *Under revision.*

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