Title
New tools for the risk assessment and modelling of African swine fever in backyard predominant settings

Author
O'Hara, Kathleen Clare

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New tools for the risk assessment and modelling of African swine fever in backyard predominant settings

By

KATHLEEN CLARE O'HARA
DISSERTATION

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Approved:

Beatriz Martínez López, Chair

Beate Crossley

Daniel Beltrán-Alcrudo

Committee in Charge

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Abstract:

The Food and Agricultural Organization of the United Nations (FAO) has classified African swine fever (ASF) as one of the most serious transboundary animal diseases (TADs) currently impacting the world due to its high lethality for pigs and crippling socio-economic consequences. ASF is a re-emergent viral hemorrhagic disease affecting domestic and wild suids. Until 2007, ASF was an exclusive problem of South Saharan African countries and the Italian island of Sardinia. However, since its introduction to Eastern Europe and the Caucasus region in 2007, ASF has continued a slow but steady spread north and westward, establishing itself in the wild boar populations of Europe and the Russian Federation, with numerous outbreaks in domestic pigs, primarily in backyard settings. In August 2018, ASF was introduced into China, and has since rapidly spread throughout Southeastern Asia, leaving economic devastation and millions of dead and/or culled pigs across more than 16 countries. In July of 2021, ASF was reported in the Western Hemisphere for the first time in 40 years, with outbreaks ongoing across the island of Hispaniola. The current spread of ASF is far from being controlled, and it is likely that new countries and regions will become infected during this ASF pandemic. Countries across the world are updating their surveillance programs and response planning based on the high risk of a potential ASF introduction. This work focused on North Macedonia, a country in the Balkan region of Europe, which is currently bordered to the north and east by countries with active ASF outbreaks. Working with FAO and the local Veterinary Authority, this work aimed to better understand the risk of ASF introduction and spread in North Macedonia's predominantly backyard pig sector. The first chapter of this work described the pork value chain, husbandry and biosecurity practices, and ASF awareness, in North Macedonia's swine industry as defined by a semi-structured questionnaire administered to more than 450 farmers. These data were used to calculate a biosecurity risk score for each farm, and multiple correspondence analysis (MCA) was used to evaluate patterns in farm

practices that may predict a farm's risk for disease introduction/spread. The eastern regions of the country were identified as being at highest risk for ASF introduction. Improvements in isolation of new pigs and basic sanitary practices, particularly among smallholder farms, was recommended. Chapter 2 described 2016-2020 swine census data and used 2017-2019 live pig movement data to conduct a network analysis to better understand pig movement patterns in the country. Census data revealed the improved identification and reporting of pig farms and numbers. Network analysis identified farms with consistently high rates of shipments and receipt of pigs for targeted intervention. Smallholder farms demonstrated a large amount of turnover, contributing to network instability in this subgroup. Live pig movement data showed that movements to slaughter predominated (85.6%), with movements between farms (5.4%) and movements to market (5.8%) playing a lesser role. Fragmentation of the 2019 network in comparison to previous years may aid in response planning. This information may be used to inform risk-based surveillance and interventions to prevent and better control disease spread, and to support business continuity in the country. Chapter 3 implemented an agent-based model to simulate the spread of ASF based on the live pig movement network and underlying farm, pig, and wild boar population densities. This model demonstrated that disease introduction into family and commercial farms resulted in larger and more widespread outbreaks. Outbreaks starting in backyard farms are expected to increase the probability of infection through the Eastern and Southeastern regions, while introductions into family and commercial farms result in more widespread outbreaks impacting the Vardar and Northeastern regions. Increasing surveillance was able to reduce the cumulative number of outbreaks over 18 months by up to 80%, while movement restrictions resulted in a 62% decrease. Overall, this work contributes to a better understanding of disease risk and transmission dynamics in backyard predominant settings and has provided practical information to the North Macedonian

Veterinary Authority on the current practices and biosecurity gaps in their swine industry. These results will inform targeted outreach and education campaigns, and risk-based mitigations to ideally prevent the introduction of ASF. These approaches can be broadly applied to countries working to improve their preparedness strategies for a variety of swine diseases and TADs.

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Introduction

Introduction:

African swine fever (ASF) is a World Organization for Animal Health (OIE) reportable, viral hemorrhagic disease of domestic and wild suids (World Organisation for Animal Health (OIE), 2021c, a). The Food and Agricultural Organization of the United Nations (FAO) has classified ASF as one of the most serious transboundary animal diseases (TADs) currently impacting the world, due to its high lethality for pigs and crippling socio-economic consequences. FAO defines transboundary animal diseases (TADs) as "those epidemic diseases which are highly contagious or transmissible, and have the potential for very rapid spread, irrespective of national borders, causing serious socio-economic and possibly public health consequences" (Food and Agriculture Organization of the United Nation and World Organization for Animal Health (FAO), 2021).

ASF was first diagnosed in 1909, and then reported 1921, after being observed in settlers' pigs in Kenya (Montgomery, 1921; Penrith, 2013). The disease has restricted the expansion of the pig production in Africa for decades, however it was not until it spread into the larger swine markets of Europe and Asia that it became well-known (Penrith, 2013). ASF was introduced to the Iberian Peninsula, Caribbean and Brazil in the 1950-1960's. After spreading through most of western Europe, ASF was eradicated, with the exception of Sardinia, in 1995 (Cwynar, 2019). In 2007, ASF re-emerged in Georgia; genome sequencing matched it to strains circulating in Mozambique, Madagascar and Zambia. Researchers have suggested the offloading of infected food materials from cruise ships and subsequent ingestion and exposure by feral swine as a possible route of introduction (Rowlands, 2008). Since then it has made a slow and steady march north and westward across Europe and into the Russian Federation where it has established itself in the wild boar population (World Organisation for Animal Health (OIE), 2021c). In August 2018, ASF was reported in China for the first time (World Organisation for Animal Health (OIE), 2021c). Viral sequencing matched the strain in China to one circulating in multiple European countries as well as Russia (Zhou, 2018). The route of introduction remains unclear, with many speculating the illegal movement of infected pork or pork products was to blame. From China, the virus rapidly spread throughout southeast Asia – fourteen countries in the region have now reported outbreaks (World Organisation for Animal Health (OIE), 2021c). During January 2021, 9 European counties (Germany, Hungary, Latvia, Moldova, Poland, Romania, Russia, Serbia and Ukraine), 12 Asian countries (China, India, Indonesia, Korea (Democratic People's Republic of), Korea (Republic of), Laos, Myanmar, Papua New Guinea, Philippines, Russia, Timor-Leste, and Vietnam), and 4 African countries (Namibia, Nigeria, South Africa, and Zambia) reported new or ongoing outbreaks (OIE, 2021d). In July 2021, ASF was detected in the Dominican Republic, marking the first time it has been in the western hemisphere in 40 years (World Organisation for Animal Health (OIE), 2021c). Haiti reported its first positive case in September 2021. The extent of this outbreak is still being determined.

The devastation caused by ASF's arrival in China has brought the disease into the limelight. China has the largest swine population in the world, and accounts for approximately half of all pork production and consumption globally (United States Department of Agriculture (USDA), 2021). Estimates suggest between 30-70% of China's pig population have been infected, and over 110 million pigs have been culled (Liu, 2020; Mason-D'Croz, 2020; Patton, 2020). Both large commercial and backyard production sites have been impacted, with primary transmission attributed to movement of domestic pigs and swill feeding (Liu, 2020). Asian Development Bank estimates the economic impact of ASF introduction to China is in the hundreds of billions. The introduction of ASF to a disease-free country can result in massive economic impacts via direct losses to the disease (i.e., mortality, stamping out, control measures etc.) and secondary losses associated with trade restrictions. In Europe, trade losses have greatly surpassed direct losses, and control measures have been associated with astronomical costs.

With the rapid spread of ASF through southeast Asia, recent arrival in the Caribbean, and the increasing outbreak frequency, and ongoing spread, in eastern Europe, countries worldwide are on high alert (World Organisation for Animal Health (OIE), 2021c). Risk assessments for multiple pathways of ASF introduction – wild boar, illegal importation of infected pork or pork products, vehicles - have been performed at the European Union and individual country level (Beltrán-Alcrudo, 2009; Costard, 2009; De la Torre et al., 2015; Bosch, 2016; Loi, 2019; Oleson, 2020; Bellini S, 2021). Vaccine development is underway at laboratories across the globe, but a commercial vaccine is not yet available (Borca, 2020; Teklue, 2020). Mitigation strategies are being evaluated and tools for the assessment of management practices and biosecurity level are being developed (Food and Agriculture Organization of the United Nations (FAO)/World Organisation for Animal Health(OIE)/World Bank(WB), 2010; Laanen, 2010; Filippitzi, 2017; Kukielka et al., 2017; Silva, 2018; da Costa, 2019; Zani, 2019; Dixon, 2020). Europe has been particularly interested in characterizing the practices of smallholder farms, leaning on knowledge from Africa, as recent outbreaks have been focused in this sector (Costard, 2009; Dione, 2017; Beltrán-Alcrudo, 2018; Fasina FO, 2020). Those few countries who have successfully eradicated ASF (Czech Republic, Belgium, Greece) are sharing their lessons learned, in hopes of slowing disease spread and helping others prevent introduction (Danzetta, 2020).

The Republic of North Macedonia, located on the Balkan Peninsula in southeast Europe, is bordered by Kosovo and Serbia to the north, Bulgaria to the east, Greece to the south, and Albania to the west. Bulgaria and Serbia are currently experiencing outbreaks of African swine fever (ASF) in both domestic pigs and wild boar, while Greece reported a single introduction in domestic pigs in 2020 (World Organisation for Animal Health (OIE), 2021c). The swine industry in North Macedonia is under imminent threat of disease incursion. The North Macedonian Food and Veterinary Agency (FVA) has been collaborating with FAO to better understand the swine industry in their country, to develop programs and policies to limit disease risk, and to prepare for an efficient and effective response if ASF should arrive. This work represents a large component of this effort and seeks to provide targeted guidance to reduce North Macedonia's risk of ASF introduction and spread.

ASF is caused by a large, double-stranded DNA virus, the only member of the *Asfarviridae* family (Dixon LK, 2005). It is the only known DNA arbovirus, infecting *Ornithodoros* ticks who can maintain the virus via sexual, transovarial and transstadial transmission (World Organisation for Animal Health (OIE), 2021a, b). Depending on the viral strain and host factors, ASF infection can present as acute, subacute, or chronic disease. Acute cases may be characterized by high fever, depression, anorexia, vomiting, diarrhea, abortion, hemorrhagic lesions and/or sudden death; while subacute or chronic cases may range from inapparent to having intermittent fevers, lethargy, weight loss, skin ulcers, arthritis and/or respiratory signs (Penrith, 2009; Blome, 2013). The non-specific nature of these clinical signs means that ASF can be confused with other diseases such as classical swine fever (CSF), porcine reproductive and respiratory syndrome virus (PRRS), erysipelas, and salmonellosis. Laboratory tests are required to differentiate these diseases. Whole blood, serum and tissue samples (spleen, lymph nodes, bone marrow, lung, tonsil and kidney) should be submitted from suspect cases (World Organisation for Animal Health (OIE), 2021b). Diagnostic tools for virus isolation, polymerase chain reaction (PCR), and serology are available (Oura, 2013; Gallardo, 2015; World Organisation for Animal Health (OIE), 2021b). The virus circulating in Europe (minus Sardinia) and Asia is Genotype II, and acute or peracute in its clinical presentation. When introduced to naïve populations, ASF can result in up to 100% lethality (Blome, 2013; Sánchez-Vizcaíno, 2015). Wild boar (*Sus scrofa ferus*) and domestic pigs (*Sus scrofa domesticus*) of all breeds and all ages are equally susceptible to the virus (World Organisation for Animal Health (OIE), 2021b). Animals who recover from infection can become persistently infected carriers. Wild boar are of concern due to their contribution to the maintenance and spread of this disease in Europe, while warthogs (*Phacocheoerus authiopicus*) and likely bushpigs (*Potamochoerus porcus*), while asymptomatic, contribute to the sylvactic cycle in Africa together with soft *Ornithodoros* ticks (Pérez, 1998; Beltrán-Alcrudo, 2009; Jori, 2009; Mur et al., 2012; Gallardo, 2014; De la Torre et al., 2015; Bosch, 2016; World Organisation for Animal Health (OIE), 2021b). ASF is not zoonotic. Disease transmission can occur via: direct contact with an infected animal, consumption of contaminated materials (e.g. swill feeding, discarded offal, scavenged carcasses or garbage), exposure to fomites, iatrogenically, or through the bite of infected *Ornithodoros* ticks (Beltrán-Alcrudo, 2009; Jori, 2009; Costard et al., 2013; Galindo-Cardiel, 2013; Gogin, 2013; Oura, 2013; Cwynar, 2019). No treatment and no vaccines currently exist for ASF. Current vaccine development is focused on subunit and live attenuated vaccines (Borca, 2020; Teklue, 2020).

Control of ASF is dependent on strict biosecurity, surveillance, rapid detection and stamping out (Penrith, 2013; Gallardo, 2015; Sánchez-Vizcaíno, 2015; Bosch, 2016). However, due to the absence of a vaccine, full eradication after introduction is uncommon, with few examples in recent years, namely the Czech Republic and Belgium (Danzetta, 2020). Biosecurity is a major component of disease control across industries, sectors and diseases. The primary elements of biosecurity include: segregation, cleaning and disinfection. These practices support external biosecurity, avoiding the entry of pathogens into a herd or farm, and internal biosecurity, preventing the spread of disease to uninfected animals within a herd or farm, and to other farms (Food and Agriculture Organization of the United Nations (FAO)/World Organisation for Animal Health(OIE)/World Bank(WB), 2010). This work aimed to assess these practices among swine producers in North Macedonia, and to better understand how they may inform risk of disease introduction across farm types and regions. Further, this work sought to evaluate the most likely pathways of disease spread to inform the most efficient and effective preparedness, response, and control strategies if ASF is introduced.

This dissertation represents the most complete evaluation of the swine industry in North Macedonia currently available, and provides critical information on backyard-predominant settings, which is urgently needed for ASF global prevention and control. Moreover, the methodologies presented here can be implemented in other countries to improve the understanding of risk associated with different husbandry practices and to provide the preparedness and response planning needed to prevent and mitigate the impacts ASF locally and globally.

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Chapter 1:

Descriptive and multivariate analysis of the pig sector in North Macedonia and its implications for African swine fever transmission

Title: Descriptive and multivariate analysis of the pig sector in North Macedonia and its implications for African swine fever transmission

Running Title: Descriptive Analysis North Macedonian Pigs

Authors: Kathleen C. O'Hara[1], Daniel Beltrán-Alcrudo[2], Mark Hovari[2], Blagojcho Tabakovski[3], Beatriz Martínez-López[1*]

Corresponding author*: Beatriz Martínez-López, beamartinezlopez@ucdavis.edu

Author Affiliations:

[1]Center for Animal Disease Modeling and Surveillance (CADMS), School of Veterinary Medicine, University of California, Davis, USA

[2]FAO Regional Office for Europe and Central Asia, Budapest, Hungary

[3]Food and Veterinary Agency, Republic of North Macedonia

Abstract:

North Macedonia, a country in the Balkan region of Europe, is currently bordered to the north and east by countries with active African swine fever (ASF) outbreaks. The predominantly traditional backyard pig farming sector in this country is under imminent threat of disease incursion. The characteristics and practices of such sectors have rarely been described, and thus the implications for these factors on disease introduction and spread are poorly understood. Using a semi-structured questionnaire, 457 pig producers were interviewed, providing information on 77.7% of the pig population in North Macedonia. In addition, a pilot study of 25 pig producers in Kosovo was performed. This study aimed to provide a detailed description of the North Macedonian pig sector, to make comparisons with nearby Kosovo, and to identify areas with high-risk practices for targeted mitigation. Descriptive data were summarized. Results of the questionnaire were used to identify farm-level risk factors for disease introduction. These factors were used in the calculation of a biosecurity risk score. Kernel density estimation methods were used to generate density maps highlighting areas where the risk of disease introduction was particularly concentrated. Multiple correspondence analysis with hierarchical clustering on principal components was used to explore patterns in farm practices. Results show that farms were predominantly small-scale with high rates of turnover. Pig movement is predominantly local. The highest biosecurity risk scores were localized in the eastern regions of North Macedonia, concerningly the same regions with the highest frequency of wild boar sightings. Veterinarians were highly regarded, regularly utilized, and trusted sources of information. Practices that should be targeted for improvement include isolation of new pigs, and consistent application of basic sanitary practices including washing hands, use of disinfection mats, and separation of clean and dirty areas. This study provides the most complete description of the North Macedonian pig sector currently available. It also identifies regions and practices that could be targeted to mitigate the risk of disease incursion and spread. These results represent the first steps to quantify biosecurity gaps and high-risk behaviours in North Macedonia, providing baseline information to design risk-based, more cost-effective, prevention, surveillance, and control strategies.

Keywords: African swine fever, biosecurity risk score, kernel density estimation, multiple correspondence analysis, North Macedonia, Kosovo

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Introduction:

The Republic of North Macedonia is located on the Balkan Peninsula in Southeast Europe. It is bordered by Kosovo* and Serbia to the north, Bulgaria to the east, Greece to the south, and Albania to the west. Bulgaria and Serbia are currently experiencing outbreaks of African swine fever (ASF) in both domestic pigs and wild boar, while Greece reported a single introduction in domestic pigs in 2020. African swine fever is a World Organisation for Animal Health (OIE) reportable, viral haemorrhagic disease of domestic and wild suids (World Organisation for Animal Health (OIE), 2021). Depending on the viral strain and host factors, ASF infection can present as peracute, acute, subacute, or chronic disease. The virus circulating in the Balkans (and the rest of Europe except for the Italian island of Sardinia, plus in Asia) is of genotype II and acute or peracute in its clinical presentation (among others, genotype II is also present in Africa) (Njau, 2021; World Organisation for Animal Health (OIE), 2021). Peracute cases are rapidly progressive, presenting with high fever, lethargy, anorexia and/or sudden death. Acute cases may be characterized by high fever, depression, anorexia, vomiting, diarrhoea, abortion, haemorrhagic lesions and/or sudden death; while subacute or chronic cases may range from inapparent to having intermittent fevers, lethargy, weight loss, skin ulcers, arthritis and/or respiratory signs (Penrith, 2009; Blome, 2013). When introduced to naïve populations, ASF can result in up to 100% lethality if no mitigation is enacted (Blome, 2013; Sánchez-Vizcaíno, 2015). Wild boar and domestic pigs are equally affected by the disease. Wild boar are of concern due to their contribution to the maintenance and spread of this disease in Europe; while warthogs and likely bushpigs are asymptomatic and contribute to the sylvatic cycle in Africa together with soft ticks of the genus *Ornithodoros* (Pérez, 1998; Beltrán-Alcrudo, 2009; Jori, 2009; Mur et al., 2012; Gallardo, 2014; De la Torre et al., 2015; Bosch, 2016). Disease transmission in both domestic and wild pigs can occur via direct contact with an infected

*All references to Kosovo should be understood to be in the context of United Nations Security Council resolution 1244 (1999).

animal, consumption of contaminated materials (e.g. swill feeding, discarded offal, scavenged carcasses or garbage), exposure to fomites, iatrogenically, or through the bite of infected *Ornithodoros* ticks if present in the area (Beltrán-Alcrudo, 2009; Jori, 2009; Costard et al., 2013; Galindo-Cardiel, 2013; Gogin, 2013; Oura, 2013; Cwynar, 2019). No treatment and no approved vaccines currently exist for ASF. Control is dependent on strict biosecurity, surveillance, rapid detection and stamping out with compensation (Galindo-Cardiel, 2013; Penrith, 2013; Gallardo, 2015; Sánchez-Vizcaíno, 2015; Bosch, 2016). The absence of a vaccine and the survival of the virus in ticks and the wild pig population, make full eradication after introduction challenging, with few examples in recent years, namely Belgium, Czech Republic, and Greece (Danzetta, 2020). The introduction of ASF into a disease-free country can result in massive economic impacts via direct losses to the disease (i.e., mortality, stamping out, control measures etc.) or secondary losses associated with trade restrictions (Berthe, 2020). In Europe, trade losses have greatly surpassed direct losses for countries exporting pigs and pork products. Control measures have been associated with high costs due to stamping out of infected farms. Within the Balkan region, ASF was first reported in Bulgaria in August 2018, in Serbia in August 2019, and in Greece in February 2020 (World Organisation for Animal Health (OIE), 2021). While Greece's only outbreak affected domestic pigs, Bulgaria and Serbia's outbreaks have impacted both domestic pig and wild boar populations (World Organisation for Animal Health (OIE), 2021). With this rapid timeline, the surrounding active outbreaks, and the mobility of infected wild boar, the pig industries in North Macedonia and Kosovo, while currently free of African swine fever, are under imminent threat of disease incursion.

Within North Macedonia, the Food and Veterinary Agency (FVA) developed programs and policies, and distributed educational materials, to aid in the prevention of ASF introduction into the country and improve early detection efforts. The FVA had a full ASF awareness campaign starting in 2018, which included billboards and leaflets, and media releases via radio and television. With the support of the Food and Agriculture Organization of the United Nations (FAO), the following awareness and training efforts were implemented: 1) the distribution to field veterinarians of several hundreds of the FAO manual on ASF detection and diagnosis in Macedonian; 2) ongoing distribution of editable ASF leaflets; 3) four veterinarians attended a training-of-trainers event in September 2019; 4) ten official veterinarians and 15 private veterinarians attended a biosecurity workshop in October 2019, 5) an ASF outbreak simulation exercise for official veterinarians was run in November 2019, and 6) a 4-week online certified training on ASF preparedness in Serbian. Additionally, FAO, in collaboration with the Veterinary Chamber of the Republic of North Macedonia (a non-profit organization of veterinarians and the veterinary statutory body for the country), undertook a survey of the pig industry to better characterize and define current husbandry practices, socioeconomic aspects, biosecurity capabilities, and disease awareness. FAO also administered this questionnaire to a small sample of pig farmers in Kosovo. This report will present the findings of this collaborative effort and provide some initial targets for ongoing mitigation efforts.

2. Materials and methods:

A questionnaire was designed and implemented by FAO to gather information about husbandry, veterinary care, socioeconomics, the pork value chain, biosecurity, and disease awareness throughout the pig sector in North Macedonia and Kosovo. The questionnaires were adapted from earlier work conducted by FAO in Georgia (Kukielka et al., 2017; Beltrán-Alcrudo, 2018). FAO followed the principles of the declaration of Helsinki and the Belmont report when designing and implementing the survey. The Institutional Review Board (IRB) of UC Davis Administration issued an exemption from the requirement for IRB review, the reasons being that the surveys would not elicit responses that would place the respondents at risk if obtained by individuals not associated with the research. The exemption criteria are available at 45 CFR 46.101(b)(2)–U.S. Code of Federal Regulation, Protection of human subjects. All the interviewed producers were informed of the study purpose, and of the facts that participation in the interviews was voluntary and they could drop from the study at any time.

2.1. Questionnaire

Semi-structured questionnaires were originally written in English and subsequently translated into Macedonian. In Kosovo, questionnaires were presented in English and translated into Serbian and Albanian by the surveyor as needed. Questionnaires included sections on: husbandry, veterinary care, socioeconomics, pork value chain, biosecurity including cleaning protocols, visitor access, exposure to other domestic and wild pigs, swill feeding practices and waste management and ASF awareness (Appendix 1). All questions referred to the 12 months prior to the date of interview. Questions related to slaughter focused on home-slaughter practices. North Macedonia has 14 commercial slaughterplants that process multiple species; however, these were not captured in the survey.

2.2. Sample selection

2.2.1. North Macedonia

Pig holdings, as identified by an annual census, were divided into three groups based on the number of pigs present: > 100 commercial, 11-100 family farm, and 0-10 backyard farm. Based on the

2019 pig census, the pig population of North Macedonia consists of around 125,230 pigs, distributed across 2315 farms with an average of 58 animals per farm. Under EU legislation, holdings with one pig for domestic purposes are not required to register, therefore these farms may be underrepresented in this count; illegal holdings are not thought to be an issue in North Macedonia. Five hundred farms were targeted, including all commercial farms (n=77), and a 2:1 split of family (n=282) and backyard (n=141) farms focusing on those farms with the most pigs. North Macedonia is divided into progressively smaller administrative levels: regions, municipalities, and town/villages, respectively. Family and backyard farms were proportionally divided between regions (but not municipalities). Within regions, and taking into account the availability of private veterinarians, farms were randomly selected for interviews. These farms were then visited to administer the questionnaires in person.

2.2.2. Kosovo

In Kosovo the major distinction was made between commercial (> 100 animals) and non-commercial farms (<=100 animals). The pig population of Kosovo consists of around 42,000 pigs distributed between one commercial farm and 3,948 non-commercial farms with an average of 11 animals per farm. Twenty-five farms were surveyed during a pilot study in August-September 2020. One survey was carried out in the one commercial farm in Kosovo located in Viti, while the remaining twenty-four samples were divided evenly into twelve surveys from the Serbian speaking community in the North and twelve samples from the Catholic Albanian community in the West. Farms were selected based on convenience and recommendations of the local veterinary offices.

2.3. Data collection

2.3.1. North Macedonia

In North Macedonia, questionnaires were conducted through the Veterinary Chamber of North Macedonia by private veterinarians selected based on the villages and municipalities they served. Prior to questionnaire implementation, training sessions were organized in each region for the interviewers, covering the survey goals, content, schedule, and basic interview techniques. Survey data was collected via the Epicollect5 mobile platform (Aanensen et al., 2009). Interviews were conducted between September 2019 and March 2020. A total of 457 questionnaires were implemented and are analyzed here. The semi-structured format of the survey allowed respondents to select multiple responses for some questions, therefore percentages discussed below represent the percent of respondents selecting a given answer - a given respondent may be counted across multiple answers if they selected more than one response.

2.3.2. Kosovo

In Kosovo one surveyor was hired and trained to fill in the twenty-five surveys in all of the locations. Data collection was also done via Epicollect5.

2.3.2. Data definitions

When collecting information on the types of pigs, sows were defined as females with litters in the last 12 months. The total number of pigs per farm was calculated as the sum of the reported boars, fattening pigs, piglets, and sows.

2.4. Data analysis

Descriptive statistics were computed from the questionnaire results from North Macedonia and Kosovo. Summary information on husbandry, veterinary care and practices, the pork value chain, biosecurity, and disease awareness, is presented as the proportion of respondents selecting or providing given answers (Appendix 2). Multiple choice questions allowed respondents to select multiple answers, meaning that one producer's response may contribute to the proportion of respondents for multiple answers. Data processing and analyses were performed in R Studio (v3.6.1) (RStudio Team, 2015). Spatial visualization and analyses were performed in ArcGIS Desktop v10.7. Mapping was conducting using the World Azimuthal Equidistant Projection.

2.4.1. Biosecurity risk scores

Biosecurity risk scores were calculated for farms in North Macedonia using a subset of responses from the questionnaire. Based on established literature and subject matter expertise, risk factors for disease introduction were identified and 28 questions that reflect those factors were selected: 21 questions that were answered by all farms, and an additional seven questions that were answered by family and commercial farms only. The answers to each of these questions were dichotomized, such that high risk answers/behaviors were assigned a score of one, and no/low risk answers/behaviors were assigned a score of zero (Supplemental Table 1). Missing values were scored as zero. A biosecurity risk score was calculated as a non-weighted linear combination of these values for each farm. The higher the biosecurity risk score, the worse the biosecurity practices were on that farm (maximum score for all farms: 21, maximum score for family and commercial farms: 28). Biosecurity risk scores were calculated for North Macedonia; due to limited data biosecurity risk scores were not calculated for Kosovo.

2.4.2. Generation of highest biosecurity risk maps using kernel density estimation

Kernel density estimation (KDE) is a non-parametric method to estimate the probability density function of a variable (Silverman, 1986). Using our biosecurity risk score, each farm serves as a point over which KDE fits a smooth curve with the true value at the exact location of the farm and diminishing values estimated with increasing distance from the farm/known biosecurity risk score. Using this method, we generated maps estimating the areas with highest biosecurity risk based on biosecurity risk scores from all farms. Additionally, we also generated risk maps using the biosecurity risk scores from family and commercial farms who answered both the initial 21 questions and the additional subset of seven biosecurity questions. KDE was used to generate risk maps for North Macedonia; risk maps were not generated for Kosovo due to the limited amount of data available. The kernel density function within ArcGIS was used, specifying a search radius of ten kilometers and an output cell size of one kilometer.

2.4.3. Generation of farm profiles using multiple correspondence analysis with hierarchical clustering on principal components

Multiple correspondence analysis (MCA) is an extension of simple correspondence analysis used for analyzing the association between two or more qualitative variables (Abdi, 2010; Husson, 2017; Kassambara, 2017). MCA is able to take the many variables generated by our survey responses and evaluate how they may be associated, e.g. if a respondent selected a specific answer to one question, is that associated with answering another question in a certain way? MCA further allows us to visualize the associations between variables by plotting them in space; variables near each other share a similar profile.

MCA was performed via forward stepwise selection selecting for the highest level of variance explained, resulting in the inclusion of nine categorical variables: household income from pigs, fate of meat and pork products produced, do you wash hands before going to pigs, do you use disinfection mat before going to pigs, which people are allowed access to your pigs, do you bring in external boar for mating purposes, biosecurity risk score, farm type and region. Farm type and region were used as supplemental variables, meaning they did not contribute to the calculation of the principle dimensions, but their coordinates were predicted to estimate how they might relate to those variables included in the analysis. Household income derived from pig production was divided into a categorical variable of less than or equal to 50%, or greater than 50%. Fate of products was divided into slaughtered for home consumption versus slaughtered for any other purpose. People pig access was divided into no access, veterinarians, and any other combination. External boar was divided into those farms that allowed their animals to interact with other pigs (their boar goes offsite, sows are crossed offsite, or external boar come to their farm), and those that allowed no interaction with other pigs. Biosecurity risk score was divided into low (0-2; lowest 50%), medium (3-5; middle 51-89%) or high (>=6; top 10%) risk.

After the MCA, we used hierarchical clustering on principle components (HCPC), which is a methodology that clusters individuals according to similar patterns of variable responses, e.g. two respondents who had similar answer profiles would be grouped together (Arguelles, 2014). HCPC grouped farms based on similar patterns in their survey responses. This allowed us to generate biosecurity farm profiles or groups of farms that share specific farm characteristics as defined by their questionnaire responses. MCA and HCPC were performed in R Studio using the FactoMineR (Le, 2008) and factoextra (Kassambra, 2017) packages. HCPC was performed using Ward's criteria. The number of clusters was determined using the 'elbow method', which entails plotting the explained variation as a function of the number of clusters and selecting the elbow of the curve as the best balance between number of clusters and variance explained (Kassambra, 2017).

Results:

A total of 457 surveys were completed in North Macedonia by March 29, 2020 (251 in 2019, 206 in 2020); 281 backyard (61.5% of respondents), 146 family (31.9% of respondents) and 30 commercial (6.6% of respondents) farms. The surveyed farms accounted for 77.7% of the pig population in North Macedonia. Additionally, a total of 25 questionnaires were administered during a pilot study in Kosovo, representing 24 non-commercial farms (<=100 pigs) and one commercial farm (>100 pigs). The breakdown of surveys by farm type and region/district are presented in Figure 1.

Husbandry

The number of sows, boars, fattening pigs, and piglets reported on North Macedonian farms was assessed by farm type (Table 1). Producers were asked about the current number of pigs, as well as the minimum and maximum numbers of each type of pig present on-site in the last 12 months (Table 1). Backyard and family farms tended to have more piglets than fattening pigs, in contrast to commercial farms in which fattening pigs predominate (Table 1). Overall, across pig and farm types, the number of pigs on any individual farm changed by about 30% over the course of a year. Commercial farms had more stable pig numbers, changing by 20-30%, compared to backyard or family farms whose pig numbers may change by up to 50-60%; fattening pigs and piglets had the highest turnover.

In North Macedonia, commercial breeds of pigs were the most common, with 96.7% of commercial farms, 65.8% of family farms, and 76.1% of backyard farms reporting only commercial breeds; the remainder reported local breeds only (commercial 0.0%, family 31.5%, backyard 22.4%), or a combination of local and commercial breeds (commercial 3.3%, family

2.7%, backyard 3.2%). In Kosovo, half of respondents reported only local breeds (48.0%), while the other half reported a combination of local and commercial breeds (48.0%); 4.0% reported commercial breeds only.

In North Macedonia, commercial operations used the highest proportion of hired workers to take care of their pigs (80.0%). Among backyard and family farms, husbands (83.8%) and wives (50.8%) were the most common pig caretakers, with children (21.5%), other family (15.9%), and rarely hired workers (2.8%) also contributing. More Kosovar respondents reported wives (80%) and kids (44%) caring for pigs, in addition to husbands (100%).

In North Macedonia, among backyard and family farms, the births of pig litters were seasonal; both farm types reported fewer litters over summer, with peaks in spring and winter (Figure 2A). Commercial farms reported litters being delivered throughout the year. The spring peak observed for backyard and family farms was variable by region, being most pronounced in Pelagonia, Northeastern, and Skopje (Figure 2B). Within Kosovo, births were concentrated in the spring, with the commercial farm reporting year-round litters.

North Macedonian pigs were predominately fed with grain (97.2%) and commercial feed (38.7%); commercial farms reported they only feed grain and commercial feed. About 15.1% of North Macedonian farms fed grass. Hay (7.2%) and agricultural by-products (6.6%) were each used to a lesser extent than other feed items. Butcher waste and food processing by-products were used by less than 1.0% of producers in North Macedonia. Food scraps were fed by 6.8% of farms in North Macedonia. Ninety-four percent of North Macedonian farms feeding food scraps reported the scraps they fed were from their own household. In North Macedonia, one backyard farm reported feeding scraps from a restaurant and one from a market. Of those North Macedonian farms feeding food scraps, 56.8% reported that they boil the scraps before feeding them to pigs. Only 3.5% of

North Macedonian respondents report that their pigs were allowed to scavenge (during the day, returning at night), with the remainder keeping their pigs enclosed year-round. Three of these farms explicitly report allowing scavenging outside of the household during September-November; these three farms were all located in the Eastern region.

All of the Kosovar respondents reported feeding grain, while 44% reported feeding commercial feed. The commercial farm in Kosovo reported they fed grain and commercial feed, as well as hay and agricultural by-products. Hay was fed by 84% of respondents in Kosovo. Feeding butcher waste and food processing by-products was reported by 56.0% of respondents in Kosovo. Food scraps were fed by 80.0% of respondents from Kosovo; 100% of respondents reported the scraps were from their own household. One farm in Kosovo fed scraps from their own as well as another household. Additionally, one family farm reported feeding food scraps from a market. No farms reported boiling food scraps before feeding them to their pigs in Kosovo. All Kosovar producers kept pigs enclosed year-round, with no scavenging reported.

Veterinary care

North Macedonian respondents reported an average of 14.6 contacts (including phone calls) with their veterinarian per year. Commercial farms consulted with veterinarians (mean number consults: 26.9, SD: 26.6) approximately twice as often as backyard (mean number consults: 12.1, SD: 17.0) and family farms (mean number consults: 16.9, SD: 18.6). Eighty-five percent of farms reported they consulted a veterinarian when they had a sick pig, with 43.9% also separating sick pigs and 8.6% disinfecting pens. Only 4.2% of North Macedonian respondents reported treating animals themselves. No farms reported selling off sick pigs or their meat, though two North Macedonian family farms reported sending remaining healthy pigs to slaughter if others became ill. Four percent of farms in North Macedonia reported killing and disposing of sick pigs. Kosovar responses to sick pigs were similar, with 84% reporting they consulted their veterinarian and 56% separated sick from healthy pigs. Cleaning and disinfecting of sick pig pens was reported by 24% of respondents. In Kosovo, 68% of respondents reported treating sick pigs themselves. No sick pigs were reported to be slaughtered or sold in Kosovo.

When asked what they do when an adult pig dies, across North Macedonian farm types, the most common responses were disposal via burial (47.3%) or pit disposal (26.6%), followed by contacting their veterinarians (19.7%) or the veterinary authorities (12.7%). No respondents reported selling the meat of pigs found dead or feeding carcasses to other pigs. In North Macedonia, 2.7% farms reported feeding meat of pigs found dead to dogs In Kosovo, adult pigs that died were thrown away (88.0%), disposed of in a pit (28.0%), or buried (8.0%). The commercial facility in Kosovo reported they contact their veterinarians. No respondents reported selling the meat of pigs found dead or feeding carcasses to other pigs. In Kosovo, 20.0% of farms reported feeding meat of pigs found dead to dogs.

The most common vaccine used in North Macedonia is that for classical swine fever (CSF), 87.7% of farms reported administration. In North Macedonia, erysipelas is the next most common at 32.8%, with Aujezsky's disease and Pasteurellosis rarely reported at 2.6% and 1.1%, respectively. Approximately 10.5% of North Macedonian farms (all backyard and family farms) use no vaccines at all. In Kosovo, 96.0% of Kosovar producers reported using CSF vaccines; however, only the commercial facility reported use of any additional vaccines beyond CSF. One non-commercial Kosovar farm reported using no vaccines.

Socioeconomics

In North Macedonia, the majority of farms reported pig rearing comprised only a proportion of the household income, with 29.1% of farms reporting all raised pigs were for home consumption only and only 11.6% of farms reporting pig rearing contributed more than 80.0% of the household income. Among backyard farms, 44.8% of pigs were reported to be raised for home consumption only, this number dropped to 2.7% for family farms. All of the producers interviewed in Kosovo reported household income from the pigs they raise (range: 2.0-80.0%). Removing the commercial farm, pig rearing contributed an average of 22.3% of household income on Kosovar farms.

About 19.5% of North Macedonian farms reported pig and/or piglet losses due to death on the farm or disappearance while free-ranging, with commercial farms having the highest proportion of respondents reporting such losses at 43.3%. In North Macedonia, results were similar for numbers of pigs reported lost to disease, with about 24.7% of farms reporting deaths due to disease. Approximately 66.7% of North Macedonian commercial farms report losses due to disease, versus 18.5% and 28.1% of backyard and family respondents, respectively. Only 1.5% of respondents reported having pigs disappear or not return while they were free-ranging. These losses were reported by three backyard and four family farms, including two backyard farms that had advised their pigs were enclosed year-round. In Kosovo, 16% of respondents reported pig or piglet deaths on the farm (Kosovo has no free-ranging pigs and thus reported no deaths or losses while free-ranging); 88.0% of respondents reported pigs died due to disease.

Pork value chain

The majority of North Macedonian respondents reported buying or sourcing their pigs from backyard farms (37.4%) or their own farms (42.2%) (Figure 3A). The majority of commercial farms reported sourcing only from other commercial farms or their own facilities; however, in

North Macedonia one commercial farm reported sourcing from backyard farms and one reported sourcing from a combination of family and commercial farms. In Kosovo, farms were more likely to source from non-commercial farms (64.0%), commercial farms (44.0%), and middlemen (28.0%), with only 12.0% sourcing from their own farms.

When buying in North Macedonia, the overall median number of pigs purchased was one. By farm type: backyard buyers bought a median of zero; family farms one; and commercial farms 21; with maximum purchases of 50, 200, and 25,000 for backyard, family, and commercial, respectively. Piglets for fattening (48.1%) and replacement sows (40.5%) were the most common types of pigs bought in North Macedonia (Figure 3B). Commercial farms buy throughout the year, while backyard and family farms tend to purchase early in the year (Figure 3C). In Kosovo, pigs for fattening (64.0%) and pigs fattened halfway (56.0%) were the predominate purchases, with replacement sows (28.0%) the next most common. Kosovar producers predominantly purchase their pigs at the beginning of the year: January (36%), February (52%), March (32%), April (12%).

The majority of backyard and family farms slaughtered their pigs at home, with 76.1% of North Macedonian farms reporting slaughter on-site by a family member (54.0%) or someone else (22.1%). North Macedonian farms slaughtering pigs at home overwhelming reported that they owned all the equipment used for slaughter or that the slaughterman brought everything needed. Only 2.1% of farms slaughtering pigs at home reported they borrowed all or only owned some equipment. Inedible materials from slaughter were primarily disposed of via offsite burial (33.6%) and pit disposal (26.1%) in North Macedonia. Sixteen percent of respondents in North Macedonia reported feeding inedible parts to dogs and cats. No respondents reported feeding parts to pigs. Fattened pigs were predominately slaughtered at the end of the year, with November the most common month across farm types, while the slaughtering of piglets had two peaks – April-May and November-January. Regarding the fate of pork products slaughtered at home, 90.2% of North Macedonian respondents reported the meat and products they produced were for home consumption, while most of the product from commercial farms ended up at butcher shops or with middlemen (Figure 4A). Backyard farms in North Macedonia reported they preserve (salt/smoke/dry) an average of 90.3% of meat slaughtered at home, with family farms reporting an average of 66.8%. This meat is then consumed over an average of 6.6 months for backyard farms and 4.5 months for family farms. Among those North Macedonian farms selling pigs, the majority reported selling to backyard farms (49.3%), markets (40.5%) and middlemen (33.4%) (Figure 4B). Almost all sales of meat and pork products were local. In North Macedonia this included sales within the same village (40.5%), same municipality (46.7%) or adjacent municipality (24.1%) (Figure 4C). One North Macedonian backyard farm located near the border reported sale of pork products in Bulgaria. In 19.5% of cases, North Macedonian sellers reported they were not aware of where their products ended up. In North Macedonia, fresh meat (87.9%) was the most common product sold or given away, followed by sausage (43.5%) and dried/smoked/salted meat (31.4%) (Figure 4D). Commercial farms sold consistently throughout the year, while backyard and family farms primarily sold at the end of the year (October-December).

About 64.8% of North Macedonian respondents answered questions regarding selling live pigs, suggesting there is a large segment of farms that do not sell pigs (this also corresponds with the numbers reporting production for home consumption only). The pigs sold in North Macedonia were primarily ready-to-slaughter pigs (50.9%) and piglets for fattening (69.4%). In a given year, North Macedonian commercial sites reported selling a median of 1,128 pigs (mean: 4570, SD: 7,561, range: 0-24,000), compared to backyard and family farms with medians of 1.0 (mean: 7.0, SD: 14.1, range: 0-80) and 27.5 (mean: 150.0, SD: 587, range: 0-6,404) pigs sold, respectively.

All responses from Kosovo reported slaughter on-site, with approximately half of slaughter performed by family (47.8%) and half by someone else (52.2%). Having all the equipment needed for slaughter was reported by 39.1% of respondents, while 47.8% borrowed or shared with neighbors. In Kosovo 95.7% of respondents reported inedible materials from slaughter were fed to dogs and cats, 43.5% disposed of via pit disposal and/or 39.1% thrown offsite. The commercial farm in Kosovo reported off-site burial or collection. No respondents reported feeding parts to pigs. Fattened pigs were reportedly slaughtered in October (25.0%), November (100.0%) and December (50.0%). Piglets were slaughtered in May (18.2%), June (77.3%) and July (45.5%). Pork products from home-slaughter were predominantly for home consumption (100.0%), or sold or given to relatives, friends and family (80.0%); however, pork products were also reportedly sold to middlemen (32.0%) and restaurants or bars (16.0%). Sale of pork products was primarily local, sold in the same village (100.0%), same municipality (95.4%), or adjacent municipality (52.4.0%). Pork products were also sold Skopje (28.6%). Fresh meat (100.0%), dried/smoked/salted meat (81.1%), fresh fat (38.1%), and sausage (14.3%) were the most commonly sold or gifted pork products.

About 44.0% of Kosovar respondents answered questions regarding selling live pigs. Among those selling pigs, 81.3% reported selling to backyard farms, followed by middlemen (54.5%) and family farms (27.3%). No respondents reported selling pigs to commercial farms or markets. The majority of pigs sold in Kosovo were ready-to-slaughter pigs (63.6%), piglets for fattening (54.5%) and pigs fattened halfway (45.5%). Pigs were primarily sold during October-November and April-June.

Biosecurity

Basic biosecurity

Producers were asked about a variety of biosecurity and sanitation practices on their farms. Over 90.6% of North Macedonian producers reported that their home or farm was fenced, with 98.2% reporting that their pig pens were fenced. Only 23.4% of North Macedonian producers reported isolating newly purchased pigs; of those who do isolate, the mean time was 24.9 days (SD: 12.2, range: 1-60). Even among commercial farms, the isolation of new pigs was not reported to be consistently practiced (46.7%). Equipment lending or borrowing between neighbours was reported by only 3.7% of respondents in North Macedonia, with commercial farms never lending or borrowing equipment. Changing shoes (94.1%) or clothes (92.8%) before going to the pigs was common in North Macedonia, with hand washing before going to the pigs being slightly less consistent (87.1%). Disinfection mats were used less reliably (68.5%). In general, commercial farms were the most consistent with their biosecurity practices, with all farms reporting fenced properties, fenced pig pens, and consistent practices of changing shoes and cloths, washing hands and using disinfection mats before going to pigs.

In Kosovo, 100% of respondents reported their farm/home was fenced; 92% reported their pigs were kept in a pen or fenced in. Among Kosovo respondents 40.0% reported isolating new pigs. Sharing of equipment was reported by 72.0% of respondents. In Kosovo, changing clothes (40.0%) and washing hands (28.0%) were performed less frequently than in North Macedonia; only the commercial farm used disinfection mats.

Visitors to farm

Next, producers were asked about the exposure of their pigs to people visiting the farm and pigs from other premises. Veterinarians were the most common persons allowed access to pigs at 86.7%

in North Macedonia. Twenty-three percent of North Macedonian farms had restricted access, with no one allowed near the pigs. Friends (9.0%), neighbours (8.5%), and buyers (8.1%) were each allowed in at a low rate. Slaughtermen had access at 4.2% of farms in North Macedonia. Only 1.8% of North Macedonian farms allowed fellow pig farmers access to their pigs. Commercial farms were generally the most restrictive, with 36.7% allowing no access and 56.7% only allowing access to veterinarians; one North Macedonian commercial farm reported allowing fellow pig farmers and one allowed buyers onsite. In Kosovo, veterinarians were allowed on 100.0% of farms. Among Kosovar respondents 28.0% allowed neighbors, 36.0% allowed buyers, and 28.0% allowed slaughtermen, to access their pigs. Fellow pig farmers were allowed access by 76.0% of Kosovar respondents.

Pigs from outside the premises

Bringing in external boar to cross with sows was reported by 8.6% of respondents in North Macedonia, including three commercial facilities. Most North Macedonian farms reported either using artificial insemination (35.9%) or owning their own boars (35.9%). Only 2.9% of farms, and only backyard and family farms, reported taking their sows offsite for breeding. Of the Kosovar farms assessed, 40.0% did not have breeding animals on-site; among those who did, 32.0% brought in an external boar, 12.0% sent their sows offsite, and 12.0% had their own boar. Artificial insemination was only reported by the commercial farm in Kosovo.

In North Macedonia, only 3.9% of farms reported having seen wild boar in the proximity of the farm in the last 12 months, with most sightings occurring late in the year. Wild boars were reported throughout the year in the Northeastern region, in November in the Eastern region, and in October and December in Vardar. Those farms who had seen wild boar were all in the eastern regions of the country. Among pig producers, 2.4% in North Macedonia reported hunting wild boar. Only one farm in Kosovo reported seeing wild boar. Hunting wild boar was reported by 8.0% of Kosovar respondents.

Waste disposal

Most farms in North Macedonia reported their household waste was collected by the municipality (77.2%). In North Macedonia, burning (9.2%) and throwing/dumping household waste off-site (8.1%) were the next most common disposal routes, with on-site burial of waste rarely reported (3.1%). All but one commercial farm report waste removal by the municipality. No farms reported burying off-site or discarding household waste on their premises. One third of North Macedonian farms reported that there was no disposal site available for household waste in their village. In North Macedonia, most village disposal sites were fenced sites (46.8%), with unfenced sites less common (11.1%). Burial (2.5%) or burning (5.8%) of household waste at village disposal sites was rare. In Kosovo, 68.0% of respondents reported household waste was collected by the municipality, with discarding household waste offsite the next most common form of disposal (36.0%). One farm reported burning some of their household waste. No disposal site available for household waste in the village was reported by 80.0% of Kosovar respondents; 12.0% reported a fenced disposal site, 4.0% a non-fenced disposal site, and 4.0% burial at the disposal site. No burning of waste at village disposal sites was reported.

Manure was most commonly disposed of in unfenced (49.2%) or fenced (27.8%) gardens or fields, or stored on-site (36.5%) in North Macedonia. Rarely manure was disposed of at a dumpsite (8.3%). It was very uncommon to sell or give away pig manure (1.3%) in North Macedonia. In

Kosovo, manure disposal was highly variable: 84.0% dump off-site, 36.0% spread in unfenced fields, 32.0% sell or give away, 20.0% store in a pit, and 8.0% spread in fenced fields.

Advanced biosecurity (only North Macedonian family and commercial farms)

A second series of biosecurity questions was targeted at family and commercial farm operations: 39.2% of farms reported having a double fence; 55.5% reported having separate clean and dirty areas for employees; and 42.1% reported restricting the kind of food products employees could bring on-site for their own consumption. No commercial farms allowed workers to keep their own pigs at home, with 86.8% of all respondents reporting workers could not keep pigs. Similarly, all but one commercial farm reported their workers were not allowed to hunt in their free time, with 91.1% of all respondents not allowing workers to hunt.

When asked about having detailed disinfection protocols, 55.3% reported protocols for vehicles, 68.8% for equipment, and 65.2% for people. Eighty-nine percent of commercial farms reported protocols in place for vehicles, equipment and people, compared to 41.2% of family farms.

About one third of farms report never re-assessing their biosecurity procedures. However, 27.1% were reassessing each month, with 18.6% doing so every three months, and 10.9% twice a year. Commercial farms were more likely to reassess more often.

Forty-three percent of farms reported never organizing events to educate workers about ASF; however, 14.6% did so each month, 12.3% every three months, 15.4% every six months, and 14.6% once a year. Commercial farms organized training more often.

ASF awareness

Producers were asked a series of questions regarding where they get information on animal diseases, their level of concern, and to test their knowledge of ASF. The most common sources of animal health information in North Macedonia were veterinarians (96.3%), television (75.6%), the internet (39.4%), and leaflets (29.8%). No one reported getting animal health information at church. These responses were consistent with responses about where producers heard about ASF. One percent of North Macedonian producers report not having heard of ASF – this represents three backyard farms, three family farms and one commercial farm. Reported sources of animal information were similar in Kosovo: veterinarians (96.0%), television (72.0%), local authorities (48.0%), newspapers (32.0%), leaflets/posters (20.0%). Among the Kosovo respondents, 32.0% reported not having heard of ASF.

Given a list of pig diseases – ASF, Aujezsky's disease, classical swine fever (CSF), foot-and-mouth disease (FMD), porcine reproductive and respiratory syndrome virus (PRRS), swine influenza, Senecavirus A (as a control; has not been reported in the region) - producers were asked to rank the top three diseases of most concern. African swine fever (85.6%), CSF (85.3%) and swine influenza (41.4%) were the predominant diseases of concern in North Macedonia. While ASF and CSF were consistently of concern, the remaining diseases showed some regional variation. In Kosovo, 68.0% of farms did not list ASF in their top three disease of concern, rather CSF (92.0%), swine influenza (92.0%), and FMD (68.0%) predominated.

In recognizing the signs of ASF, the most commonly reported signs from North Macedonian producers were: hemorrhages on the skin (60.6%), reduced appetite (60.0%), fever (60.0%) and sudden death (52.1%). Only 2.4% reported not knowing the signs of ASF, consistent with the previous numbers who had reported not hearing of ASF. Only 1.5% of producers thought ASF was zoonotic. The most common North Macedonian responses regarding the ways their pigs might contract ASF were: introduction or exposure to diseased animals (87.1%), fomites, e.g. infected boots or cloths (49.9%), and feeding infected pork products (39.2%). Twenty-four percent were concerned about transmission routes not relevant to ASF, such as 20.4% mosquitoes, 3.5% wind and 1.8% bad vaccines. In Kosovo, the most commonly reported clinical signs related to ASF were fever (68.0%), diarrhea (64.0%), reduced eating (44.0%), and sudden death (40.0%). Kosovar respondents reported diseased animals (76.0%), feeding infected pork products (28.0%), and fomites (20.0%) as paths of ASF transmission. Twenty percent of respondents did not know how ASF could infect their pigs.

When it comes to reporting suspect ASF cases, 76.4% of producers in North Macedonia reported they would quickly report ASF to veterinary authorities if they suspected it on their farms. Twenty-three percent in North Macedonia advised they would wait a few days to report due to concerns about it being a false report. In North Macedonia, only two farms would wait a few days to report to the veterinary authorities due to concern for financial losses. In Kosovo, 48.0% of respondents said they would quickly report suspect ASF, 12.0% would wait a few days due to concerns about a false report, and 40.0% would wait due to concern for financial losses.

Finally, when asked why an owner may not report ASF, producers in North Macedonia reported not knowing how to report (39.6%), being unclear about what might happen after reporting (31.1%), the culling of their pigs (27.8%), the subsequent restriction of sale of their pigs (24.3%), damaged reputation (15.1%), and no compensation (9.8%), as the top reasons. Only 2.4% said the owner would prefer to deal with the disease themselves. Reporting being too time consuming was only cited by 0.8% of respondents. In Kosovo, 60.0% reported not knowing how to report, 64.0% were concerned about post-reporting unknowns, 36.0% were concerned about banned sales, 28.0%

felt reporting was too time consuming, 20.0% were concerned about their reputations and 16.0% were concerned about their pigs being culled.

Biosecurity risk scores and high-risk areas for ASF introduction

A subset of survey questions was selected to reflect the biosecurity practices and associated risk level of each farm in North Macedonia. The responses to these questions were dichotomized into low/no risk or contributing risk based on whether a farm performs or does not perform certain activities, e.g. vaccinating versus not vaccinating pigs (Supplemental Table 1). The distribution of these answers is presented in Figure 5. The most common high-risk practices reported were allowing visitors (e.g. veterinarians, fellow pig farms, buyers, neighbors, friends) to access the farm, failure to isolate new pigs, and not using a disinfection mat. Among those questions targeted to family and commercial farms, more variability in answers was noted, with the most common high-risk practices including: not having a double fence, not regulating the food workers bring on the farm, not having separate clean and dirty areas, and not having events in which to educate and increase the awareness of employees about ASF.

Most farms have low biosecurity risk scores – indicating low risk of disease introduction and good biosecurity (Figure 6). When evaluating scores across all farm types, the highest biosecurity risk scores (those with the worst biosecurity) were generally observed among backyard and family farms. In both the all-farm and family and commercial focused assessments, commercial farms tended to score better (lower) than other types of farms (Figure 6).

Risk maps generated using the all-farm biosecurity risk scores, identified areas of high risk for ASF introduction in the Northeastern, Southwestern, and Southeastern regions of North

Macedonia (Figure 6C). When focusing on family and commercial farms, the Southeast region's focus is no longer highlighted and the Eastern region becomes lower in risk (Figure 6D); however, the high-risk areas in Northeastern and Southwestern regions remain. While the KDE maps identified high risk areas in the Northeastern and Southwestern regions, those individual farms with the highest biosecurity risk scores were located in the East, with the Southeastern region having the largest proportion of high-risk scoring farms (Figure 7A). Among the family and commercial farms subset, the highest individual scores were observed in the Northeastern and Eastern regions, with a high level of variability observed in the Southwestern region (Figure 7B). Among this subset, the Eastern and Southwestern regions have the highest proportions of high-risk biosecurity risk score farms.

Generation of farm profiles based on MCA and HCPC

MCA grouped not washing hands, allowing access to external boar, allowing access to people other than veterinarians, and not using a disinfection mat as variables highly correlated with dimension 2 and high-risk biosecurity risk scores (Figure 8A). Low and medium biosecurity risk scores were more difficult to delineate, as factors grouped around the X-Y axis did not strongly contribute to differentiating farms for these dimensions. Commercial farms grouped with pig rearing being more than 50% of household income, allowing no people to access pigs, and slaughtering for a purpose other than home consumption as variables highly correlated with dimension 1. Hierarchical clustering identified three separate groups of respondents with similar profiles, or pattens of responses to questions about their farm practices (Figure 8b).

Discussion:

This study provides the most complete profile of the pig industry in North Macedonia available, covering 77.7% of the pig population in the country, thanks to the large sample size and the comprehensive survey responses from pig producers on their husbandry practices, the pork value chain, biosecurity practices, and disease awareness. The recent ASF introductions into Bulgaria, Greece, and Serbia, highlight the need to better understand the pig sector in this region and to inform future targeted interventions. Like other countries in the Balkans, North Macedonia and Kosovo have numerous risk factors for ASF introduction including many low biosecurity small holder farms, free ranging pigs, farms practicing swill feeding, high wild boar suitability, and high connectivity to ASF positive countries through international travel (Jurado, 2018; EFSA Panel on Animal Health and Welfare (AHAW), 2019). This study has provided an in-depth description of the North Macedonian pig sector, contrasted these practices with those in Kosovo, and highlighted target areas for disease risk mitigation efforts.

North Macedonian farms had a high rate of turnover among their pigs; this is consistent with census data that shows a relatively large proportion of small farms do not maintain pigs year-to-year, making registration of, and outreach to, these small holder farms a challenge. The predominant use of commercial feed (97.2%) and grain (38.7%) suggests sites selling pig feed may provide good venues to access producers. The reports of feeding scraps and inedible parts to dogs and cats poses a zoonotic concern, not for ASF, but for other diseases such as pseudorabies or echinococcosis. Education on the risks of feeding food scraps to pets, and their role in the transmission of zoonoses, could be added to materials targeting swill feeding.

The North Macedonian pig sector seems to make good use of their veterinarians and to trust them as an information source (96.3%). However, only a third of producers called their veterinarians or the veterinary authority when they had pigs die. This should be highlighted as a major gap in current passive surveillance, a critical element for early detection and eradication. Burial and pit disposal predominated as methods of dead pig disposal; depending on the depth of burial, these methods should limit the access of wildlife to carcasses. The last outbreak of CSF in North Macedonia occurred in 2008 (World Organisation for Animal Health (OIE), 2021), yet vaccine compliance remains high. The vaccination campaign, financially sponsored by the state for farms with fewer than ten pigs, was suspended in October 2019 and North Macedonia is currently in the process of applying for CSF-free status. No other vaccines are compulsory. This history of vaccine compliance suggests that if an ASF vaccine were to become available, North Macedonia could expect high compliance from its producers, especially if financially backed. However, it should be mentioned, the initial phases after discontinuing a vaccine campaign are challenging, in that cases of ASF may be mis-diagnosed as re-emerging CSF. Diagnostic confirmation will be especially critical in differentiating the cause of illnesses among cases with similar clinical presentations.

A large number of households report raising pigs for home consumption and as a source of supplemental income. This reliance on pigs to feed families, as well as contribute to household income, highlights the extent to which an ASF introduction would impact the food and financial security of these producers. Adequate indemnity programs and education about these programs will be needed to support producers and get buy-in on timely disease reporting. Commercial farms reported higher rates of death and disease than backyard and family producers. These systems should be evaluated for potential husbandry, health (e.g. vaccination) and biosecurity interventions that may reduce these losses.

The pork value chain is predominantly localized, which may limit disease spread if ASF is introduced (Martínez-López, 2013). The sale and slaughter of pigs is also highly seasonal. Religion and cultural habits may influence these patterns as well as the probability of ASF introduction into domestic pigs. Serbians in the North, and Catholic Albanians in the West, keep pigs and may have different practices and seasonality in their pig rearing and trade. The large concentration of Muslims in Western North Macedonia likely contribute to the low density of pig farms in this area. Biosecurity is highest among commercial farms, but sanitary practices were in general fair to good. The primary areas that could consistently be improved upon would be the use of disinfection mats, the creation of separate clean and dirty areas, and the implementation of consistent disinfection protocols. The efficacy of disinfection mats and boot baths is dependent on removal of visible debris before use, and the use of appropriate disinfectants at adequate concentrations and for enough time (Amass, 2000, 2001). While effective when used properly (Amass, 2000; Dee, 2004), successful implementation of disinfection mats in small-holder settings may be a challenge due to lack of funds for disinfectants, rapid soiling, and improper protocols. Isolation of new pigs was reportedly uncommon – this may be associated with a lack of space, all-in all-out practices, or low perceived value. However, the overall percentage of producers reporting separating sick pigs was higher than that reporting isolating new pigs – suggesting that while areas for complete isolation may not exist, some level of separation may be possible. In general, most farms did not allow visitors near their pigs. Backyard and family farms were most likely to allow visitors to their premises to access their pigs. Training and future outreach should continue to highlight the risk of new pigs and visitors introducing disease. Visitors accessing pigs/farms was identified as a significant risk factor for disease introduction to backyard farms in Romania, and a case study of a backyard farm in Bulgaria cited visitors as the most likely route of ASF introduction (Zani, 2019; Boklund, 2020). Enclosure of pigs, and the removal and treatment of trash by the municipality, should help restrict wild-domestic pig interfaces contributing to disease exposure. While very few wild boar sightings were reported, ASF introduction via wild boar was listed as the highest risk pathway for Eastern Europe by recent studies (Taylor, 2020). Outbreaks in wild boar in Bulgaria and Serbia confirm this risk in the region. Additional data on wild boar populations in these countries is needed.

Addressing hurdles to timely reporting is critical to a country's disease detection. Kosovar producers reported a high level of concern about the financial implications of reporting, suggesting the need for clear messaging and planning around indemnity for animals culled to control disease. In both North Macedonia (39.6%) and Kosovo (60%), producers reported not knowing how to report suspect ASF, while about a third of respondents in each country were concerned about post-reporting unknowns, culling, and restricted sale of pigs. Concern about reputation or attempting to control disease oneself, was less commonly reported than previous studies in the region have shown (Vergne, 2016). These results indicate the need for transparency and communication about reporting. North Macedonia is in the process of improving their national surveillance programs. While they have ASF and CSF programs designed, they have not been widely implemented. The country currently relies heavily on passive surveillance, and the use of government authority to place quarantines during disease investigations. This heavy reliance on passive surveillance further emphasizes the need for education about diseases of concern, how to prevent disease introductions (e.g. biosecurity), what to look for, how to report, and what to expect during a disease investigation. Our biosecurity risk scores and KDE maps highlight specific areas for targeted intervention. On the KDE maps we observe diminishment of the foci in the Southeast and Eastern regions, while retaining the foci in the North and West, when focusing on family and commercial farms versus focusing on all farms, indicating that high biosecurity risk scores from family and commercial farms were contributing to high risk of ASF introduction in the North and Southwest, while backyard farms likely have a more important role for risk in the South and East. While the highest biosecurity risk scores were focused in the East, Southeast and West, our KDE maps register the highest risk areas in the West and North. This may be due to the small number of farms with high biosecurity risk scores and KDE being influenced by the number of farms in an area, particularly in the North; future work could consider standardizing biosecurity risk in a region by the number of farms in that region. Outreach for backyard farms at high risk of ASF introduction should be targeted in the East, particularly in Southeastern region. More general campaigns to reach all farm types are warranted in Southwestern, Northeastern and Eastern regions. Primary areas in which improvements could be made include: isolating/separating new pigs, using disinfection mats, and limiting access of visitors to pigs. Among family and commercial farms, investment in double fencing, separate clean and dirty areas, and educational training would improve current biosecurity risk scores.

MCA and HCPC divided farms into three groups – dimension 1 which captured commercial farms, dimension 2 which captured farms with high-risk practices, and a third group made up of the remaining farms. Our analysis suggests that farms with certain high-risk behaviours were likely to have profiles that demonstrated multiple risky behaviours resulting in an overall high biosecurity risk score profile. The specific behaviours that were highly correlated with dimension 2 – not washing hands, allowing visitors including friends, neighbors, buyers, and slaughtermen, and external pigs onto the farm, and not using a disinfection mat – were correlated with high-risk biosecurity risk scores. This grouping generated a profile of responses to this subset of questions. Farms with similar responses are expected to have poor biosecurity practices, and thus high biosecurity risk scores, and should be targeted for education and improved biosecurity, i.e. a farm that does not practice regular handwashing before working with their pigs likely has other poor biosecurity habits, will likely have a high biosecurity risk score, and should be targeted for intervention.

The Kosovo pilot study was intended to gain awareness of practices in their pig sector to support the expansion of FAO activities, including biosecurity training that is actively under development. The low sample size from the pilot study in Kosovo implies we should interpret these results with caution. However, a few marked contrasts between North Macedonia and Kosovo, that may impact the risk of ASF spread, should be noted. Kosovo has good, consistent practices around keeping pigs confined and not allowing scavenging. However, Kosovar pig producers reported a much higher rate of swill feeding, and not treating food scraps that were fed to pigs. These responses indicate that while swill feeding is banned in surrounding European Union countries, it is still widely practiced in this region and should be highlighted as a topic for education campaigns (Jurado, 2018). In general, losing pigs to illness was more widely reported in Kosovo than North Macedonia. The disposal of inedibles from slaughter and dead pig carcasses as thrown offsite and fed to dogs, could provide access from wildlife. More visitors and pigs from other farms were allowed on-site, and manure was moved offsite through sale and disposal methods, providing the means for disease introduction and spread. One third of respondents said they had not heard of ASF (compared to 1.5% in North Macedonia), and it was not reported as a top disease of concern from Kosovar producers. All of this suggests that education campaigns targeted at informing producers about ASF, its introduction pathways, clinical presentation, and how to report and seek aid, could improve early detection and reduce disease dissemination risk among these producers. The best means of reaching pig producers is through their veterinarians and television; North Macedonians also used the internet, while Kosovars preferred newspapers.

With data collected via a questionnaire, this study is subject to reporting bias by the respondents. In North Macedonia in particular, with questionnaires being administered by veterinarians, producers may have been more likely to report higher usage of veterinarians, higher levels of care, and stricter biosecurity practices. Additionally, outreach and educational campaigns targeting ASF awareness have been ongoing since 2018, which may have led producers to change or at least report higher quality practices. FAO training did occur in September, October, and November of 2019, while the initial phases of the survey were underway; however, these trainings were primarily targeted at veterinarians versus producers and are not thought to have had much impact on the respondents. Survey responses are being used to inform updates and development of training materials for producers in the region. In the calculation of the biosecurity risk scores, non-answers were assigned a value of zero. This practice may have resulted in an underestimation of the biosecurity risk scores for some farms.

Overall, this study has provided a thorough review of the practices of the pig sector in North Macedonia, highlighting some similarities and contrasts with neighbouring Kosovo, and discussing the potential strengths and vulnerabilities regarding the risk of ASF introduction and spread. We have highlighted some specific aspects (and regions) for improvement via additional and targeted educational campaigns and risk reduction interventions. This information will be of great value to inform risk assessments of ASF introduction/exposure, and modelling of ASF spread, if it is eventually introduced into the country. Ultimately, all of these tools will contribute to better prevention, early detection, and control efforts for ASF in North Macedonia and Kosovo.

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Contribution to the field statement (200 words):

This study provides the most extensive description of the North Macedonia pig sector available. As a country under imminent threat of ASF incursion, this work provides vital information to improve targeted, risk-based interventions and mitigation. Further, as a sector in which backyard farms predominate, this work provides insight into this under described community. Understanding the practices of these producers provides better insight into the decisions they are making, and what resources they may need to implement the changes that could reduce their risk for disease introduction, as well as the risk of disease spread between farms and across borders. Further, we describe the implementation of techniques to analyse this type of information, and highlight specific risk-based targets for intervention.

Conflict of Interest Statement:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Tables and Figures:

Tables:

Table 1. Number of pigs by farm type as reported by questionnaires administered to North Macedonian pig producers between September 2019-March 2020. The number of pigs currently on the farm were reported by type of pig. Producers also separately reported the maximum and minimum number of each type of pig that were on the farm in the last 12 months. Total pigs were calculated as the sum of the reported sows, boars, fattening pigs and piglets currently on-site. Percent change in average number of pigs was calculated as the difference between the average maximum and average minimum divided by the average maximum.

Farm Types		Sows	Boars	Fattening Pigs	Piglets	Total
All	Mean (SD)	7 (53)	1 (2)	121 (771)	84 (501)	213 (1304)
	Median	1	0	2	6	11
	Avg Minimum (SD)	8 (52)	1 (2)	97 (644)	66 (452)	
	Avg Maximum (SD)	11 (59)	1 (3)	145 (866)	91(555)	
	%Change AvgMax-AvgMin	0.3	0.3	0.3	0.3	
Backyard						
	Mean (SD)	1 (4)	0 (1)	3 (7)	7 (12)	11 (18)
	Median	1	0	1	2	6
	Avg Minimum (SD)	2 (2)	0 (1)	2 (3)	7 (12)	
	Avg Maximum (SD)	3 (4)	0 (1)	5 (13)	11 (18)	
	%Change AvgMax-AvgMin	0.4	0.2	0.6	0.4	
Family						
	Mean (SD)	3 (5)	1 (2)	30 (64)	35 (54)	69 (112)
	Median	2	1	3	20	29
	Avg Minimum (SD)	5 (7)	1 (1)	16 (37)	23 (46)	
	Avg Maximum (SD)	9 (8)	1 (4)	43 (77)	48 (86)	
	%Change AvgMax-AvgMin	0.5	0.4	0.6	0.5	
Commercial						
	Mean (SD)	82 (195)	5 (6)	1669 (2584)	1043 (1707)	2799 (4386)
	Median	15	2	460	335	737
	Avg Minimum (SD)	82 (189)	4 (5)	1371 (2171)	830 (1597)	
	Avg Maximum (SD)	103 (210)	6 (8)	1945 (2858)	1047 (1947)	
	%Change AvgMax-AvgMin	0.2	0.3	0.3	0.2	

SD: standard deviation, Avg: average, Avg Minimum: average of the minimum number of each type of pig reported; Avg Maximum: average of the maximum number of each type of pig reported; %Change: percent change

Figures:

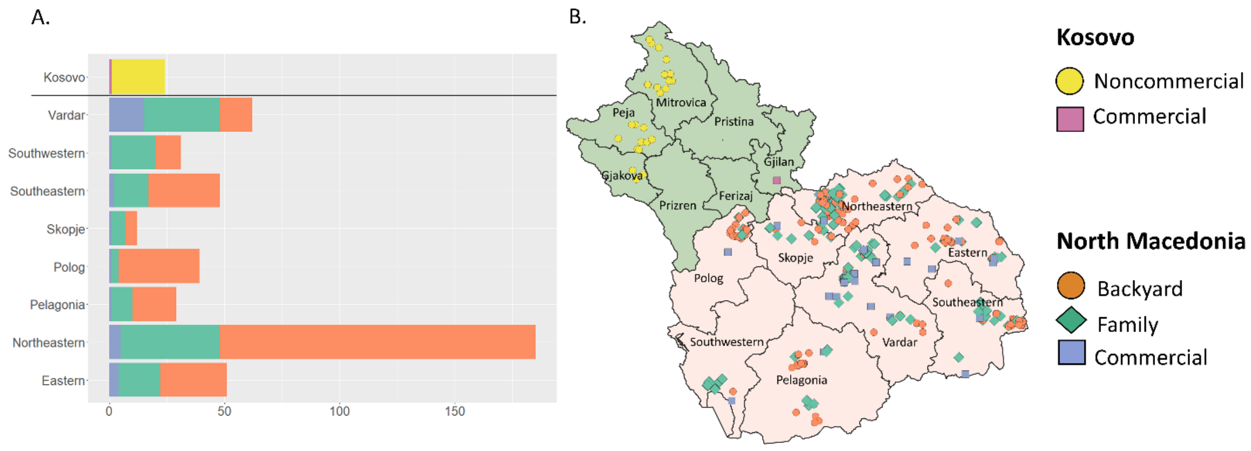


Figure 1. A) Number of questionnaires administered to North Macedonian pig producers by region and type of farm, and Kosovar producers characterized as commercial or non-commercial, during September 2019-March 2020. B) Map of questionnaire sites by farm type. Kosovo: green, North Macedonia: orange. Kosovo districts and North Macedonia Regions: black lines

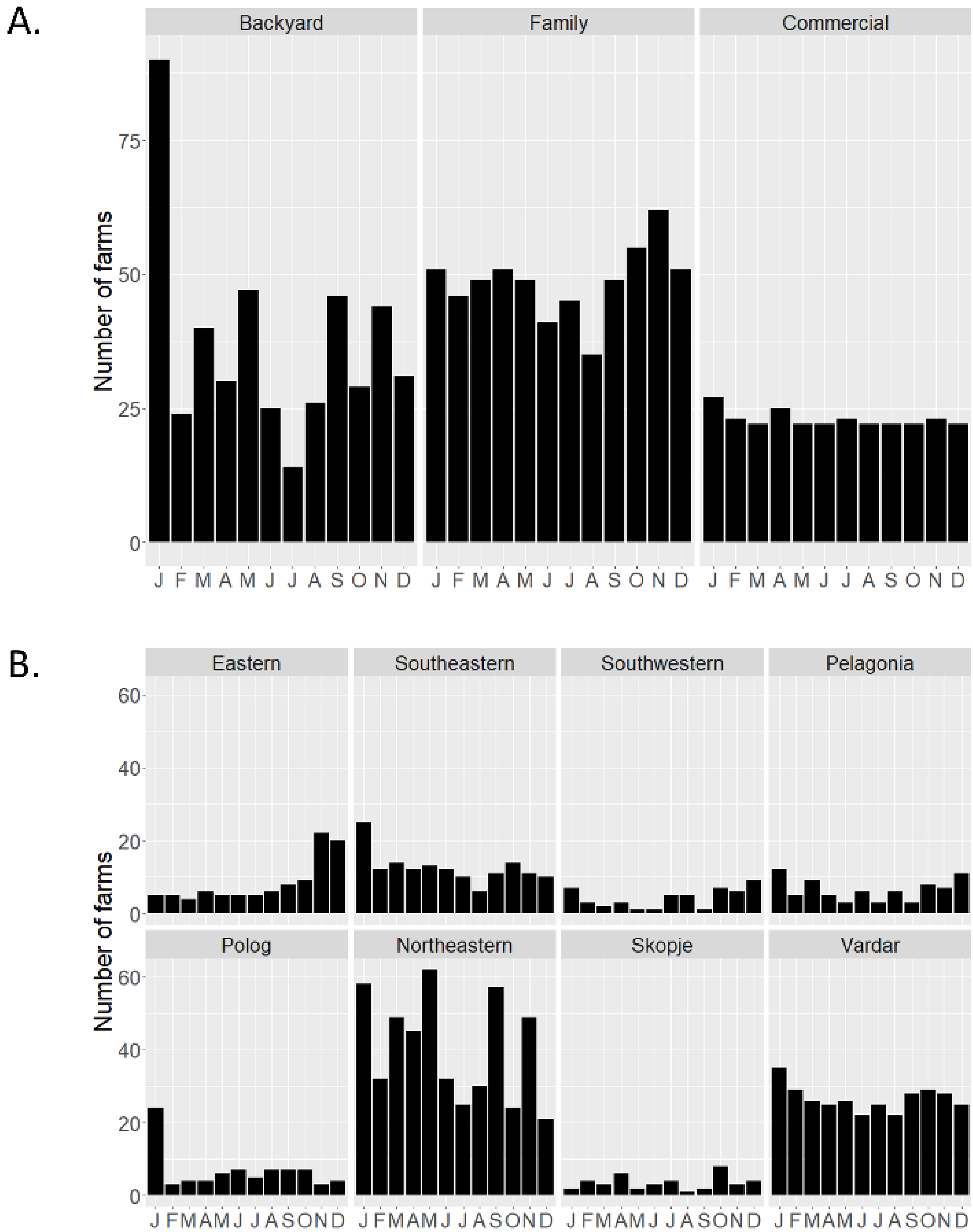


Figure 2. Number of North Macedonian pig farms reporting litters per month by A) farm type, and B) region, based on questionnaires administered between September 2019-March 2020.

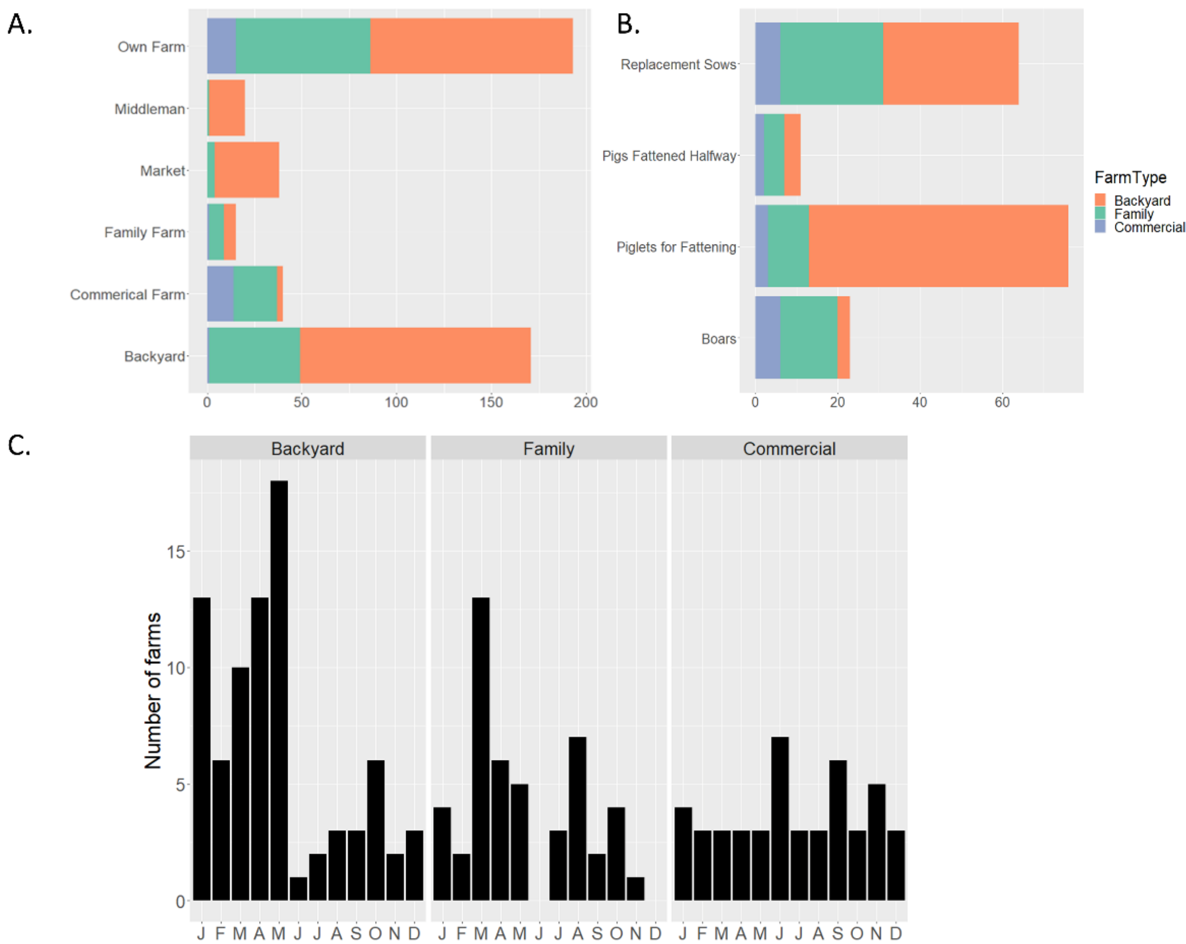


Figure 3. North Macedonian producer's pig buying practices by farm type by A) source of pigs, B) type of pig purchased, and C) when pigs were purchased by month, based on questionnaires administered between September 2019-March 2020 (281 backyard, 146 family, and 30 commercial farms). Types of pigs: replacement sows = intact female pig for breeding; pigs fattened halfway = pigs over 25 kg but under market weight; piglets for fattening: pigs from weaning to about 25 kg; boar: intact male pig for breeding.

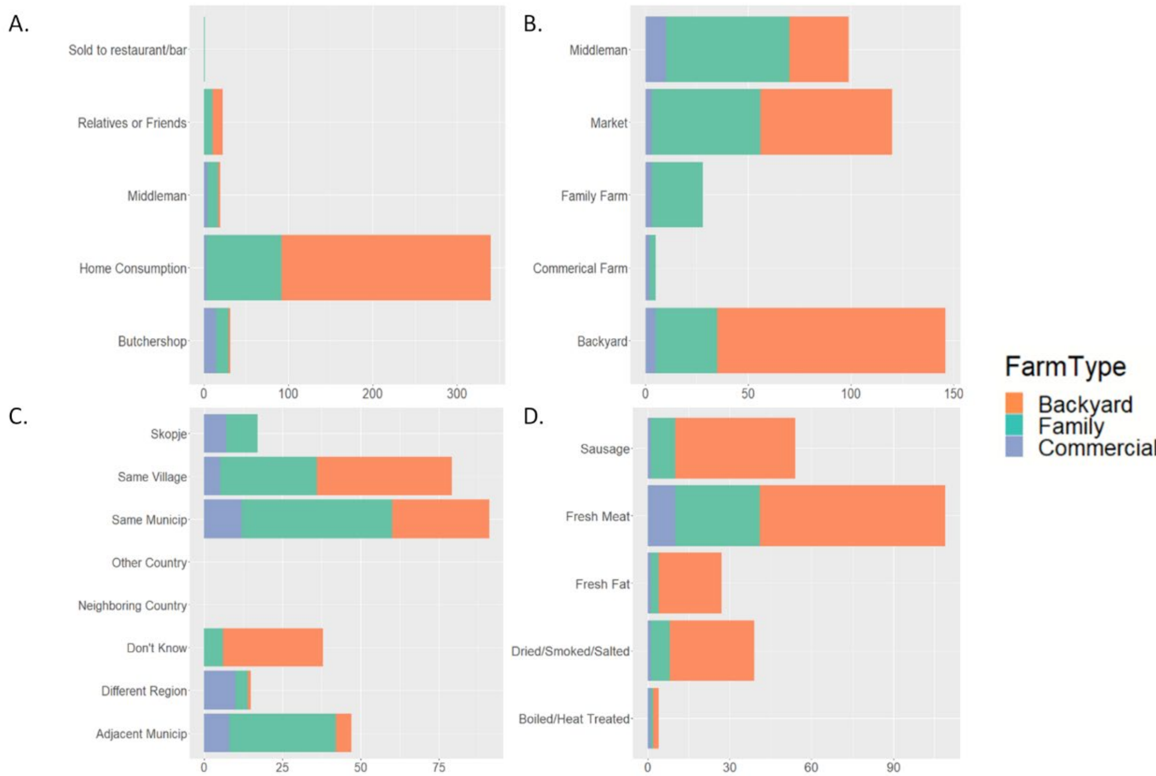
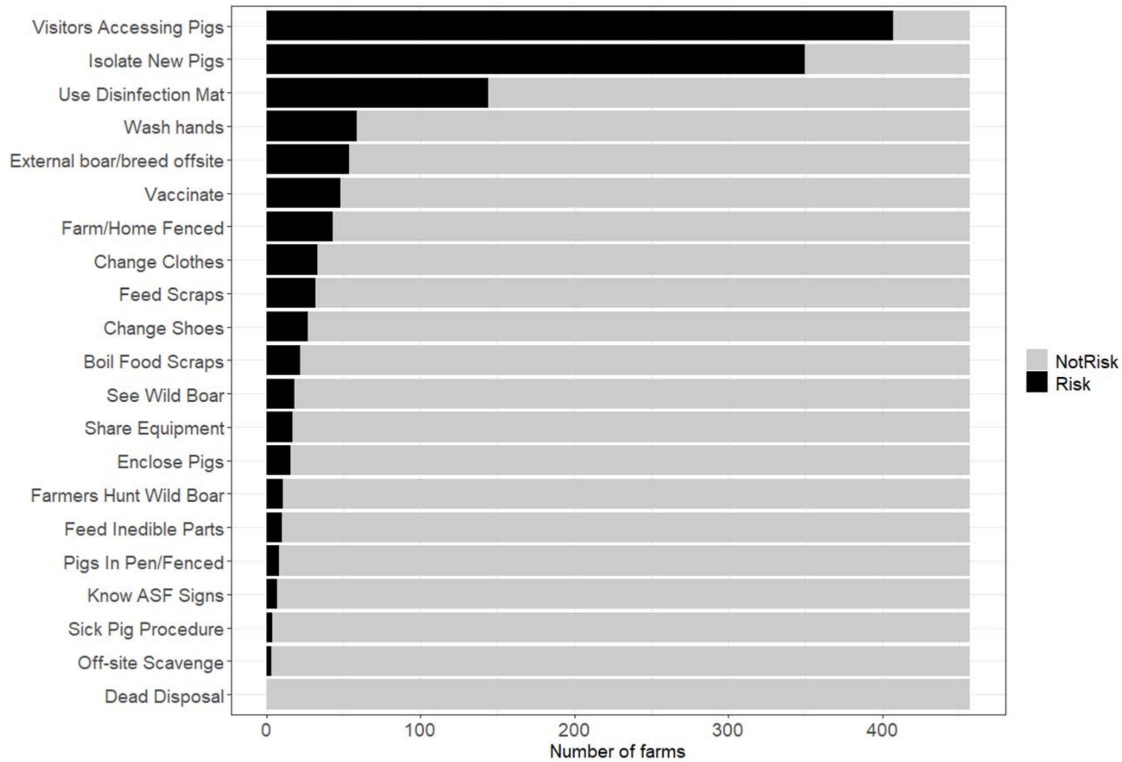


Figure 4. North Macedonian producer's pig selling practices by farm type by A) fate of products sold, B) who producers were selling to, C) location of buyers, and D) product type produced by farm, based on questionnaires administered between September 2019-March 2020 (281 backyard, 146 family, and 30 commercial farms). Notes: One producer did report selling to pork products to Bulgaria. Due to survey wording, sausages cannot be differentiated as fresh versus cooked or other.

A.


Visitors Accessing Pigs
Isolate New Pigs
Use Disinfection Mat
Wash hands
External boar/breed offsite
Vaccinate
Farm/Home Fenced
Change Clothes
Feed Scraps
Change Shoes
Boil Food Scraps
See Wild Boar
Share Equipment
Enclose Pigs
Farmers Hunt Wild Boar
Feed Inedible Parts
Pigs In Pen/Fenced
Know ASF Signs
Sick Pig Procedure
Off-site Scavenge
Dead Disposal
0
100
200
300
400
Number of farms
NotRisk
Risk

B.

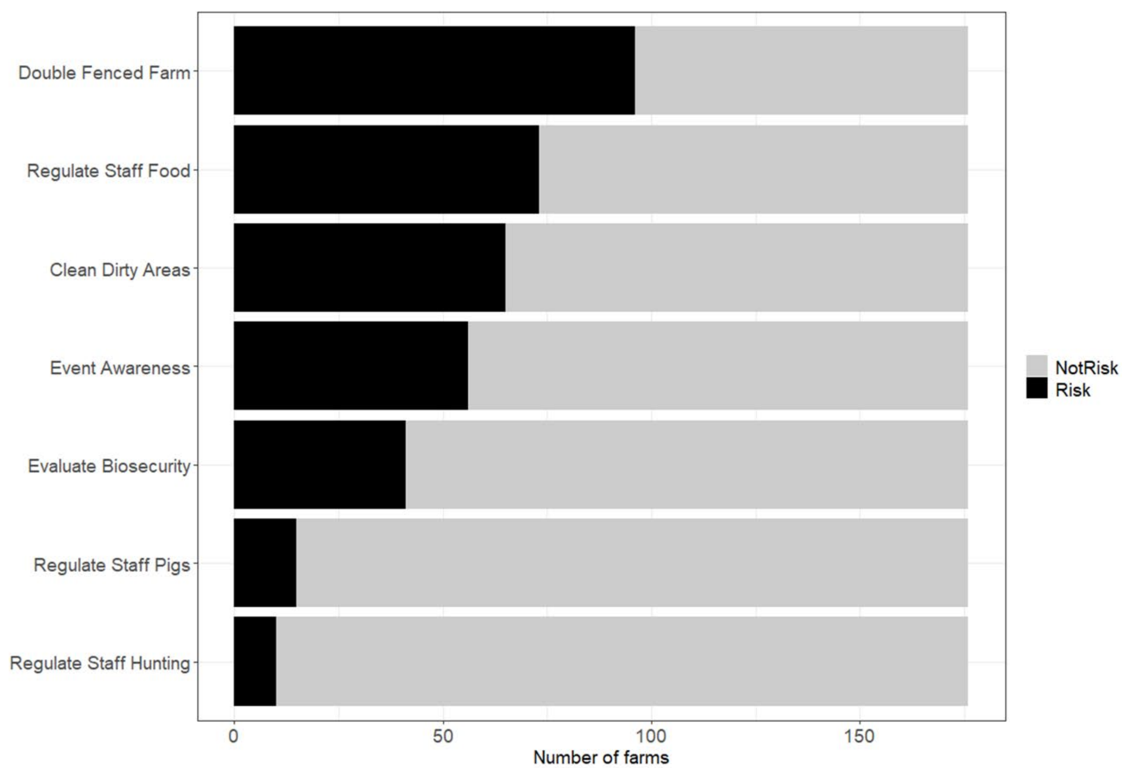

Double Fenced Farm
Regulate Staff Food
Clean Dirty Areas
Event Awareness
Evaluate Biosecurity
Regulate Staff Pigs
Regulate Staff Hunting
0
50
100
150
Number of farms
NotRisk
Risk

Figure 5. Dichotomous scoring responses for questions administered to North Macedonian pig producers between September 2019-March 2020, characterizing reported practices as no/low risk versus contributing risk for ASF introduction based on biosecurity characteristics for A) all farms, and B) family and commercial farms. Scores were used to calculate biosecurity risk scores. ‘Not risk’ answers were assigned a score of zero, ‘risk’ answers were assigned a score of one. Two separate sets of biosecurity risk scores were developed to account for additional information provided in a subset of biosecurity questions that was only answered by family and commercial farms.

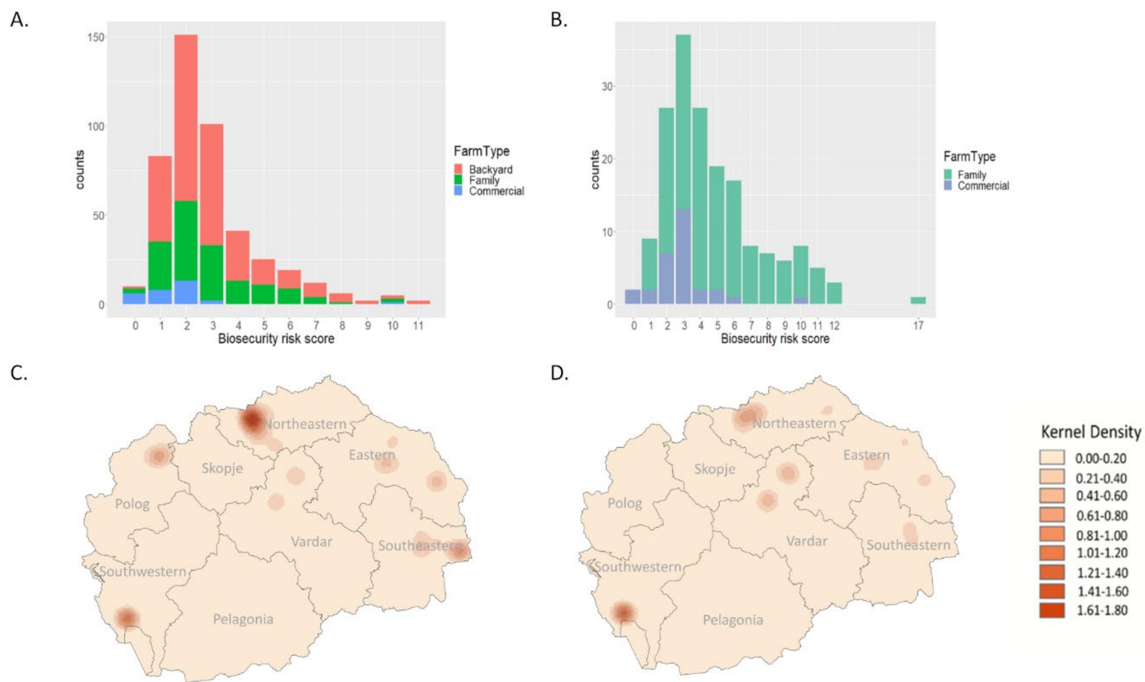
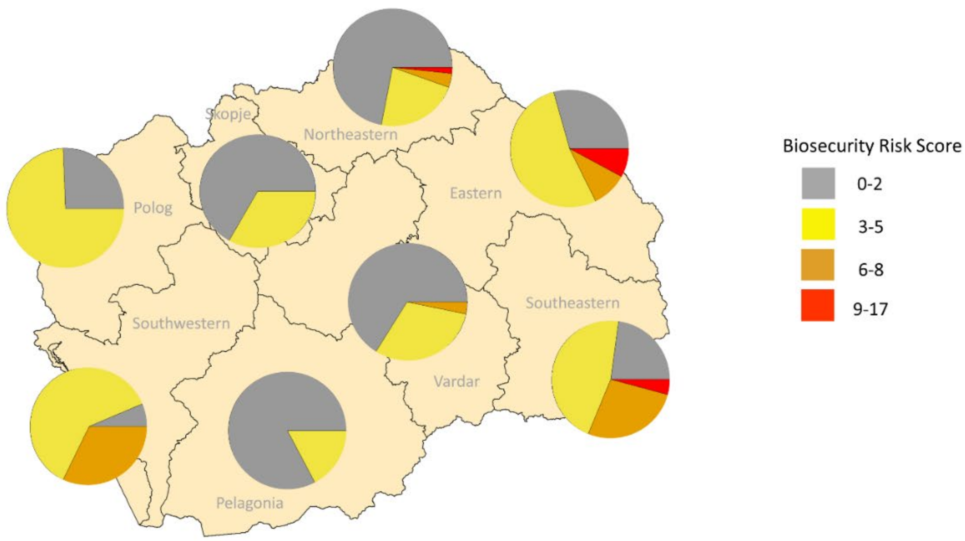


Figure 6. Biosecurity risk scores for A) all pig farms, and B) family and commercial pig farms, administered questionnaires in North Macedonia between September 2019-March 2020. Biosecurity risk scores represent a non-weighted linear combination of values assigned to dichotomized survey questions in which higher scores representing higher risk. Kernel density estimation (KDE) mapping of biosecurity risk scores for C) all pig farms, and d) family and commercial pig farms administered questionnaires in North Macedonia between September 2019-March 2020.

A.



B.

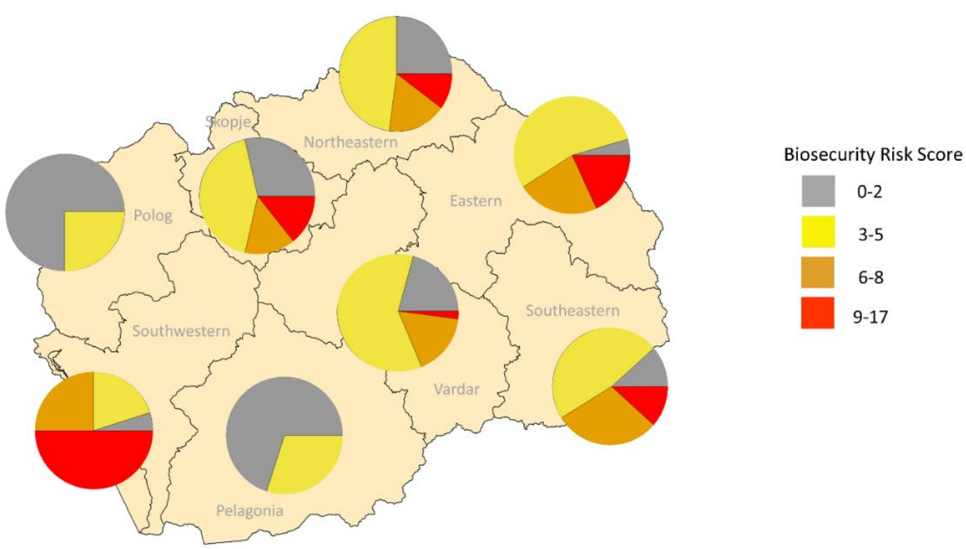
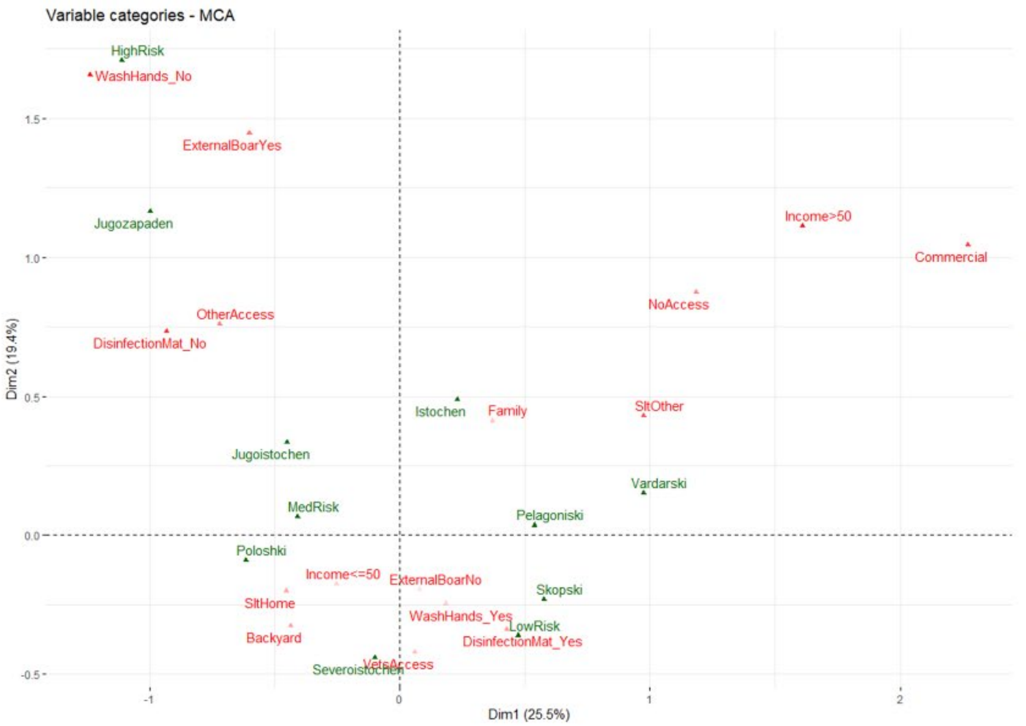


Figure 7. Mapping of biosecurity risk score by North Macedonian region. Pie charts represent the proportion of pig farms with the corresponding biosecurity risk scores in each region for A) all farms, and B) family and commercial farms. Biosecurity risk scores represent a non-weighted linear combination of values assigned to dichotomized survey questions collected between September 2019-March 2020 in which higher scores representing higher risk

A.


Variable categories - MCA
HighRisk
WashHands_No
ExternalBoarYes
Jugozapaden
OtherAccess
DisinfectionMat_No
Income>50
Commercial
NoAccess
Istochen
Family
SltOther
Jugoistochen
Vardarski
MedRisk
Pelagoniski
Poloshki
Income<=50
ExternalBoarNo
SltHome
Skopski
WashHands_Yes
LowRisk
Backyard
DisinfectionMat_Yes
Severoistochen
VetsAccess
Dim2 (19.4%)
Dim1 (25.5%)

B.

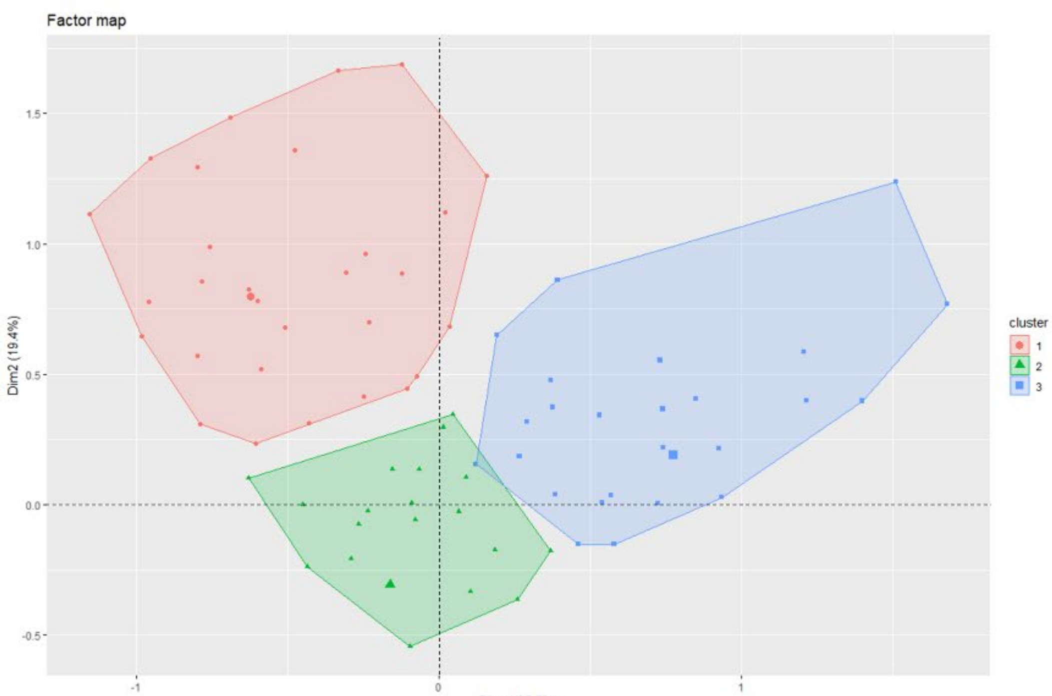

Factor map
Dim2 (19.4%)
Dim1 (25.5%)
cluster
1
2
3

Figure 8. Multiple correspondence analysis (MCA) and hierarchical clusters of principal components (HCPC) results for North Macedonian pig farms using farm characteristics and practices reported in questionnaires administered between September 2019-March 2020, with region and biosecurity risk score categories used as supplemental variables. A) Graph of the correlation of categorical variables by dimension. The distance between points gives a measure of their similarity; variables that group together have similar profiles. The distance from the axis represents the level of correlation that variable has with the given dimension; variables near the origin have low correlation with either dimension. Red: analyzed variables, Green: supplemental variables. Variables: WashHands_Yes/No: wash hands before going to pigs, External Boar_Yes/No: allow interaction with external pigs, DisinfectionMat_Yes/No: use disinfection mat, SltHome/SltOther: slaughtered for home consumption versus other, NoAccess/VetAccess/OtherAccess: allow no access to pigs, allow only veterinarians to access pigs, allow other people (neighbors, buyers, fellow pig farmers) to access pig, Income<=50/Income>50: household income from pig rearing less than or equal to 50% versus greater than 50%, Commercial/Family/Backyard: farm type. B) Plot of HCPC results. HCPC groups respondents into clusters based on their similar response profiles. Our analysis generated three clusters. The red cluster corresponds to high biosecurity risk farms, and groups respondents who reported not washing hands before going to pigs, allowing external pigs on the farm, allowing visitors other than veterinarians to access pigs and not using disinfection mats. The blue cluster groups respondents with profiles including commercial farms, household income from pigs >50%, not allowing visitors to access pigs, and slaughter done by someone outside the household. The green cluster groups the remaining respondents whose responses were not highly correlated with either dimension.

Appendices:

Appendix 1. African Swine Fever questionnaire administered via EpiCollect5 Sept 2019-Mar 2020.

Appendix 2. African Swine Fever questionnaire responses summarized for multiple choice questions by proportion of respondents for North Macedonia and Kosovo between Sept 2019-Mar 2020.

Supplemental:

Supplemental Table 1. Questions and attributed score by answer used to generate biosecurity risk scores for North Macedonian pig producers administered questionnaires between September 2019-March 2020.

All Farms	
Question	
Answer	**Attributed Score**
Are pigs enclosed all year round?	
Yes, the pigs are enclosed all year	0
No, the pigs are allowed to scavenge during the day, but return every night No, the pigs scavenge for several days or months	1
Against what diseases did you vaccinate your pigs, over the past 12 month?	
Classical Swine Fever Erysipelas Aujezsky's Pasteurellosis Other	0
I don't vaccinate	1
What did you do the last time a pig got sick?	
Separated the sick from the healthy ones Treated the animal/s yourself Consulted the veterinarian Slaughtered the sick pig for home consumption Slaughtered the sick pig and sold the meat Killed the sick pig and threw away the carcass Killed the sick pig and destroyed the carcass in my premises (by burial or burning) Killed the sick pig and destroyed the carcass outside my premises (by burial or burning) Slaughtered the remaining healthy pigs I cleaned and disinfected the pen(s)	0
Sold the sick pig to a slaughterhouse Sold the sick pig to someone Sold the remaining healthy pigs (before they became sick) to a slaughterhouse Sold the remaining healthy pigs (before they became sick) to someone Did nothing	1
What did you do with the last adult pig that died?	
Bury	0

Throw away Dispose of in a pit Burn Fed to the dogs Consumed the meat Collected from household by dedicated services Contacted your private veterinarian Contacted veterinary authorities	
Sold the meat Fed to the pigs	1
What did you do with the inedible parts of the pig after home-slaughter?	
Buried within premises Buried outside premises Burned within premises Burned outside premises Disposal in a pit Disposal as household waste Collected by others (companies, municipality, etc.) Fed to dogs/cats Other No nonedible parts left after slaughtering	0
Fed back to pigs Thrown away outside premises	1
Is your farm/home fenced?	
Yes	0
No	1
Are your pigs kept in a pen or fenced area within your home?	
Yes	0
No	1
Last time you bought pigs, how many days did you keep them isolated/separated from your pigs (quarantined)? Write 0 for "no quarantine". Question converted to quarantines new pigs yes/no. Answer 0=no, >0=yes	
Yes	0
No	1
Over the past 12 months, did you bring external boar into the farm to cross it with your sows?	
No, I have my own boar No, I perform artificial insemination No, there are no breeding animals (sows or boar) on the farm	0
Yes No, I have my own boar and also take him to other premises for breeding The sows get crossed while outside my premises	1
Do you (or your workers) lend or borrow equipment to/from your neighbors?	

No	0
Yes	1
Do you (or your workers) change shoes before going to the pigs?	
Yes	0
No	1
Do you (or your workers) change clothes before going to the pigs?	
Yes	0
No	1
Do you (or your workers) wash hands before going to the pigs?	
Yes	0
No	1
Do you (or your workers) use a disinfection mat before going to the pigs?	
Yes	0
No	1
Which persons are allowed to go to your pigs?	
Nobody, access is restricted	0
Friends Neighbors Buyers Slaughterman Fellow pig farmers Veterinarians	1
When did you see wild boar close your pigs over the past 12 months? Converted to yes/no: never=no, any other selection=yes	
Never	0
January February March April May June July August September October November December	1
Do you hunt wild boar?	
No	0
Yes	1
What do you feed your pigs?	
Grain/maize	0

Commercial feed Food processing-by-products (e.g. from cheese processing, bakery, etc.) Fresh grass Hay Agricultural by-products	
Kitchen waste/food scraps Slaughterhouse/Butcher leftovers	1
If you feed kitchen waste/food scraps, do you boil them first?	
Yes	0
No	1
What months do you allow pigs to scavenge outside the household? Converted to yes/no: never=no, any other answer=yes	
Never	0
January February March April May June July August September October November December	1
Name three clinical signs that you think are related to ASF (read the answers out loud and tick all the apply)	
Fever Coughing Diarrhea Vomiting Reduced eating Joint swelling Hemorrhages in the skin Bloody diarrhea Bloody urine Sudden death	0
I don't know	1

Family and Commercial Farms

Question	

Answer	Attributed Score
Is your farm double fenced?	
Yes	0
No	1
On your farm do you have established clean and dirty areas for your personnel?	
Yes	0
No	1
Do you regulate what kind of food workers can bring to the farm?	
Yes, certain products are not allowed	0
No, they can bring anything	1
Can workers on your farm keep pigs at home?	
No	0
Yes	1
Can your workers go hunting in their free time?	
No	0
Yes	1
How often do you organize events to raise the awareness and educate your workers / staff about ASF?	
Once a year Twice a year Every three months Every month	0
Never	1
How often do you evaluate the efficiency and enforcement of your biosecurity procedures?	
Once a year Twice a year Every three months Each month	0
Never	1

Chapter 2:

Network Analysis of Live Pig Movements in North Macedonia: Pathways for disease spread

Title: Network Analysis of Live Pig Movements in North Macedonia: Pathways for disease spread

Running Title: Network Analysis North Macedonian Pigs

Authors: Kathleen C. O'Hara[1], Daniel Beltrán-Alcrudo[2], Mark Hovari[2], Blagojcho Tabakovski[3], Beatriz Martínez-López[1*]

Corresponding author*: Beatriz Martínez-López, beamartinezlopez@ucdavis.edu

Author Affiliations:

[1]Center for Animal Disease Modeling and Surveillance (CADMS), School of Veterinary Medicine, University of California, Davis, USA

[2]Food and Agriculture Organization of the United Nations (FAO), Regional Office for Europe and Central Asia, Budapest, Hungary

[3]Food and Veterinary Agency, Republic of North Macedonia

Abstract:

The ongoing spread of African swine fever (ASF) beyond Africa and across Europe, Asia, and most recently into the Caribbean, highlights the need for countries to assess and enhance their surveillance and response plans. North Macedonia, bordered to the north and east by countries with ongoing ASF outbreaks, is under imminent threat of incursion. This study aimed to describe the distribution of pigs and pig farms in North Macedonia, and to characterize the live pig movement network. Census data for 2016-2020 documented an 31.6% increase in the number of reported farms and a 17.7% increase in reported pigs in North Macedonia, with consolidation of larger farms in the Polog region and increased numbers of smallholder farms in the Vardar and Eastern regions. Network analyses on movement data from 2017-2019 were performed for each

year separately, and consistently described weakly connected components, characterized by short diameters and path lengths. Fragmentation of the network was observed in 2019, with a marked decrease in betweenness, increase in community numbers, and loss of any giant strongly connected component. The majority of shipments occurred within 50 km, with movements less than 6 km being the most common (22.5%). Those nodes with the highest indegree and outdegree were consistent across years, despite a large turnover of unique identification numbers among smallholder farms. Movements to slaughter predominated (85.6%), with movements between farms (5.4%) and movements to market (5.8%) playing a lesser role. The scale of North Macedonia's movement network was significantly smaller than that described by other European nations, however movements and communities that spanned the country were observed. This work demonstrated North Macedonia's improvements in documenting and tracing pig farms, pig numbers, and live pig movements. Nodes that consistently contributed to high numbers of shipments and receipt of pigs were identified for enhanced surveillance and improved biosecurity. The description of North Macedonia's pig population and live pig movement network should enable implementation of more efficient and cost-effective mitigation efforts strategies, inform targeted educational outreach, and provide data for future disease modeling.

Keywords: African swine fever, social network analysis, North Macedonia

Introduction:

The ongoing globalization of agricultural trade has increased the international movement of animals, animal products, and disease (Marano, 2007; Manuja, 2014; Beltrán-Alcrudo D, 2019). African swine fever (ASF) provides a primary example of one such transboundary animal disease (TAD) (Food and Agriculture Organization of the United Nation and World Organization for Animal Health, 2004). This reportable, viral, hemorrhagic disease of pigs, re-emerged from Africa into Georgia in 2007 and has steadily spread westward through Europe, northeastward across Russia into China in 2018, and most recently to Hispaniola in the Caribbean in 2021 (World Organisation for Animal Health (OIE), 2021). The ongoing spread and associated risk of severe economic losses if ASF is introduced, have provided the impetus for many countries to develop and enhance their surveillance and response planning. North Macedonia, a country on the Balkan peninsula in Southeastern Europe, is currently facing, and working to prepare for, the threat of an ASF incursion.

North Macedonia is bordered by Kosovo and Serbia to the North, Bulgaria to the East, Greece to the South, and Albania to the West. Ongoing ASF outbreaks have been reported in both domestic pigs and wild boar in Bulgaria and Serbia, while Greece reported and was able to control a single introduction into domestic pigs in 2020 (World Organisation for Animal Health (OIE), 2021). Currently, North Macedonia is free from ASF, but the risk is imminent. This threat has increased the need for traceability and efficient data-driven methods to support disease surveillance, prevention, and outbreak response. The Food and Agriculture Organization of the United Nations (FAO) recently completed a survey in North Macedonia describing their pig sector (O'Hara, 2021), and continues to work toward enhancing and implementing targeted surveillance.

This work aims to describe North Macedonia's pig census and the live pig movement network to support ongoing planning efforts. Understanding the social network of this sector will support risk assessment of disease dissemination within the local industry (Thakur et al., 2016), as well as a faster response in case of an epidemic (Yang, 2019). ASF transmission in both domestic and wild pigs can occur via direct contact with an infected animal, through consumption of contaminated materials (e.g. swill feeding, discarded offal, scavenged carcasses or garbage), exposure to fomites, iatrogenically, or through the bite of infected *Ornithodoros* ticks if present in the area (Beltrán-Alcrudo, 2009; Jori, 2009; Costard et al., 2013; Galindo-Cardiel, 2013; Gogin, 2013; Oura, 2013; Cwynar, 2019). Therefore, movement of infected live pigs, pork products, or contaminated fomites, provides opportunities for disease introduction and spread. Understanding when, where and how frequently these contacts occur, and the network structure and vulnerabilities, may help to strategically allocate risk-based, more cost-effective, preventive and control measures.

Social network analysis (SNA) has been demonstrated to be a valuable tool to describe pig movement network structures and has been used with increasing frequency in the swine industry (Martínez-López, 2009; Martinez-Lopez et al., 2009; Nöremark, 2011; Ciccolini, 2012; Büttner, 2013; Smith, 2013; Büttner, 2015; Bütter, 2016; Guinat, 2016; Lentz, 2016; Relun, 2016; Schulz, 2017; Sterchi, 2019; Baron, 2020; Crescio, 2020; Rolesu, 2021). It has been used to evaluate the movement network dynamics and helps to quickly identify the individual farms, areas and time periods that may pose the highest risk for disease introduction to the system (Dubé, 2008; Dubé et al., 2009; Martínez-López et al., 2009; Dorjee et al., 2013). These insights allow for implementation of risk mitigation strategies at these spatial or temporal hotspots (Dorjee et al., 2013), as well as more realistic disease modeling.

Understanding the network of pig movements in North Macedonia is the first step toward risk analysis. Currently, there is very limited published information about the pig sector in North Macedonia and the Balkans. This lack of information is a critical gap in animal health and outbreak response planning. The predominance of small-scale subsistence farmers in North Macedonia, highlights the potential impact of a TAD of swine on food production and security in the country (O'Hara, 2021). SNA applied to the pig industry may also allow for the identification of potential super-spreaders (nodes likely to spread disease fastest or to the most additional nodes given their network contacts) or super-receivers (nodes at highest risk of disease exposure due to receipt of incoming movements from the most other nodes) of disease within North Macedonia’s pork industry chain, providing targeted locations for increased surveillance and risk mitigation (Dubé et al., 2009; O'Hara, 2020).

This study aimed to provide one of the first descriptions of North Macedonia’s pig population and the social network of its live pig trade. Our primary objectives were: to describe the distribution of pigs and pig farms; to describe the live pig network structure; to describe pig movement spatio-temporal dynamics; and to identify priority farms that may contribute to the risk of disease introduction and spread. An increased availability of pig demographic and movement data in North Macedonia and the Balkan region will help to better understand, and even predict, disease transmission patterns, supporting risk-based surveillance and control strategies for both endemic and emerging pig diseases such as ASF.

Materials and methods:

Data:

Annual pig census data for 2016-2020 was provided by the Veterinary Authority (Food and Veterinary Agency of North Macedonia). The 2016-2017 census data provided unique identification number (UIN), town/village, region, and number of animals for each farm. Census data for 2018-2020 included UIN, coordinates, town/village, region, total number of animals, number of piglets, number of fattening pigs, number of gilts, number of sows, and number of boars. North Macedonia is divided into increasingly smaller administrative levels from regions, to municipality, to towns/villages.

Records of permitted movements of live pigs for 2017-2019 were provided by the Veterinary Authority. Movement records for 2017-2018 provided data for the entire year, while 2019 data covered only Jan. 1-Nov 23, 2019. Records for 2017-2018 included movement type (completed movement, departed, departed with no document, movement off holding, movement without document), certificate number, date of departure, date of arrival, number of pigs departing, number of pigs arriving, origin UIN, destination UIN, and type of UIN for both origin and destination (farm, market, slaughterhouse, or unspecified). Records for 2019 included movement type, date of departure, date of arrival, for origin and destination: UIN, town/village, municipality, region, herd type, and coordinates were provided. Number of animals moved was not provided for 2019. A separate set of data on movements to slaughter was provided for 2019, which included date of departure, date of arrival, origin and destination: UIN, town/village, municipality, region, number of animals departed, number of animals arrived. Therefore, the number of animals moved is only available for movements to slaughter for 2019.

UIN types that were unspecified were assumed to be farms: 664 (3.0%) origin types and 304 (1.4%) destination types were reassigned from unspecified to farm. All of the 13 commercial slaughterhouses in North Macedonia were identified (no slaughterhouses were mis-identified as a farm or market). Five hundred fifty-nine (2.6%) movements did not have a destination recorded and were not considered in the network analysis. A total of 21,801 movements were included in this study.

Coordinate information for 2017-2018 movements were referenced from 2019 movement and census data by UIN. The remaining unassigned UIN's were assigned to the town/village centroid using the UIN coding system in which the first four digits reference a specific town/village. In 2017, this represented 53 (12.8%) UINs, associated with 127 (1.8%) of movements. In 2018, this represented 45 (10.9%) UINs, accounting for 110 (1.4%) of movements.

Data were collected, validated and cleaned in Microsoft Excel 2016 and R Studio (v.3.6.1)(R Core Team, 2017; RStudio Team, 2020).

Census:

Descriptive statistics were calculated for the census data in R Studio, excluding farms that reported zero total animals. Spatial visualization and analyses were performed in ArcGIS Desktop v10.7. Mapping was conducted using the World Azimuthal Equidistant Projection.

Network analysis:

The UIN's present in the census and reporting movements each year were highly variable, with only 23.2% being reported across all census years (Supplemental Figure 1) and only 163 (34.0%) present in the movement records across all years. In general, larger commercial farms were more stable year to year, while smaller backyard farms had a large amount of turnover. The movement networks were therefore analyzed separately for each year. Static networks for each year were defined using pig production sites as nodes or vertices, and shipments of live pigs as edges. The properties and characteristics of the networks were described using network parameters including number of nodes, number of edges, diameter, edge density, average path length, and transitivity. Centrality measures of in-degree and out-degree were calculated for each node. In-degree is defined as the number of incoming shipments to a production site, out-degree is the number of outgoing shipments from a production site (Wasserman, 1994; Lee, 2017; O'Hara, 2020). Betweenness is the number of shortest paths between any pair of nodes in the network that pass through an individual node (Freeman, 1977; Wasserman, 1994; Lee et al., 2017). Diameter is the longest of all the shortest path lengths between nodes in the network (Wasserman, 1994; Lee, 2017; O'Hara, 2020). Edge density is the ratio of the number of edges observed in the network to the number of possible edges (Wasserman, 1994; Lee, 2017; O'Hara, 2020). Average path length is the mean length of all the shortest paths between nodes in the network (Watts and Strogatz, 1998). Transitivity coefficient is the sum of the proportion of nodes that are connected to other nodes; this parameter is also known as the clustering coefficient (Watts and Strogatz, 1998; Lee, 2017). The igraph package (v 1.1.2; (Csardi, 2006)) in R Studio (v 3.6.1; (RStudio Team, 2020)) was used to generate and describe the static network and evaluate network parameters. Edge density, diameter, average path length and transitivity were calculated under the igraph package using functions: edge_density, diameter, mean_distance, and transitivity respectively. Type global was used for the transitivity function. Components are subregions within a network in which all nodes are directly or indirectly linked. For directed networks, components can be classified as strong or weak. Strong components are those in which every node can reach every other node by connected paths, while weak components area areas in which every node is connected when we ignore directionality (Robinson, 2007; Relun et al., 2016). The giant weak component (GWC) is the dominant large weak component, and the giant strong component (GSC) is the dominant strong component (Robinson, 2007). The Walktrap community finding algorithm was used to define communities within each year's network (Pons, 2005). Distance between movements was calculated based on Veness's equation for Excel derived from the spherical law of cosines (Vincenty, 1975; Veness, 2020).

Mapping movements

To address the large turnover in UINs each year, movements were also summarized at the municipality level. For each year, the number of movements into and out of each municipality was calculated and mapped for visual comparison. Summary data was mapped in ArcGIS for visual analysis.

Results:

Census

Census data were summarized in Table 1 and Supplemental Table 1. In general, the reported number of farms and number of pigs in North Macedonia have been increasing. The highest number of farms were reported in 2018 and 2020. The total number of farms increased by 31.6% between 2016-2020, while the number of pigs increased by 17.7%. Smallholder farms (≤10 pigs) saw the largest expansion. Consistent with this, the median and average number of pigs per farm decreased during this period. In the most recent data from 2020, there is a median of 3 pigs per farm, consistent with the predominance of backyard farms in North Macedonia (O'Hara, 2021). The Vardar (288.7%) and Eastern (260.9%) regions had the largest proportional increase in number of farms; the Northeastern (20.6%) and Polog (16.9%) regions had decreases in the number of farms. Skopje (46.6%) and the Southwestern (41.2%) regions had the largest proportional increase in number of pigs. Despite the decrease in number of farms in the Polog region, the number of pigs increased, suggesting consolidation of pigs into larger farms.

Based on the 2020 census, the highest densities of pigs are present in the Vardar, Polog and Eastern regions, while the highest densities of farms are in the Northeastern, Eastern and Southeastern regions (Figure 1). Vardar has the highest number of pigs per farm, consistent with the higher density of commercial farms in this region (O'Hara, 2021). Smallholder farms are distributed throughout the country.

Network Analysis

Network parameters for each year's static network are presented in Table 2. Even with incomplete data for 2019, the number of nodes increased by 23.4% between 2017 and 2019, and the number of edges or movements increased by 6.5%. The observed range of indegree was more stable than that of outdegree; the maximum outdegree increased by 32.4% between 2017-2018 and 11.3% between 2017-2019. All networks conform weakly-connected components, and are characterized by short diameters and path lengths, and low transitivity. A marked decrease in betweenness was observed in 2019, compared for 2017-2018. The GSC was composed of 31 nodes in 2017 and 34 in 2018, 10 of these nodes were common to each year. In 2019, the GSC was composed of only a single node, indicating the lack of a GSC that year. The GWC consistently made up the majority of the network, 98.5% in 2017, 97.7% in 2018, and 89.1% in 2019. Markets and slaughterhouses are observed to be aggregation points for incoming movements across years (Figure 2A-C). The 2019 network has more frequent occurrences of movements between two nodes that are otherwise independent of the rest of the network (Figure 2C). Community identification algorithms identified 17 communities in 2017 (nodes in community: mean: 23, median: 9, range 2-106), 33 in 2018 (nodes in community: mean: 12, median: 2, range: 2-100), and 85 in 2019 (nodes in community: mean: 6, median: 2, range 2-106: 1-75). This increase in community numbers in 2018-2019 reflects an increase in nodes that only contact one other node in the network (Figure 2D-F). When evaluated spatially, across all years, while some communities remain highly localized, there are communities that bridge regions and, in some cases, span the entire country. When evaluating a simplified network, removing repeated shipments, the median distance of a shipments across all years was 28.9 kilometers (average: 41.0, range: 0-187.5; note zero values reflect movements in which nodes were assigned to the same town centroid). The distribution of shipment distances (km) was stable between 2017 (median: 27.2, average: 38.2, range: 0-184.3) and 2018 (median: 25.7, average: 35.5, range: 0-176.8), with a moderate increase observed in 2019 (median: 41.8, average: 48.9, range: 0-187.5). When evaluating all shipments across all years, most shipments occur over distances less than 50 km; each year the largest number of shipments occurred within 0-6 kilometers (Supplemental Figure 2).

When the networks are visualized geospatially, the most frequent and stable movements are those to slaughter (Figure 3). Between 2017-2019, 85.6% of movements were to slaughter. A shift in slaughterhouse usage can be observed in Polog between 2017-2017 (Figure 3A,B), and in the Southeastern region between 2018 and 2019 (Figure 3B,C). When summarized at the municipality level, the receipt of pigs was spatially consistent across years, with the exception of a municipality in the Southeastern region with a slaughterhouse that received more shipments from 2018-2019 (Figure 3, D-F). Increases in small scale movements out of municipalities can be observed between 2017-2019, with increases in the Polog, Skopje and Pelagonia regions most evident (Figure 3, G-I). The network demonstrates seasonality, with peaks in the number of movements observed in April, July and November-December (Figure 4). Movements to markets follow this overall trend, with shipments occurring throughout the year, with March-April and

November-December peaks. In 2017, 25.3%, and in 2018, 27.5%, of movements to market occurred in November-December; this dropped to 3.9% in 2019.

Those nodes with the highest indegree and highest outdegree are consistent across the years, with 9 of the 10 nodes (all slaughterhouses) with the highest indegree consistent from 2017-2019 and likewise 9 of the 10 nodes (all farms) with the highest outdegree consistent from 2017-2019. When narrowed to those movements not to slaughter ('to live'), this consistency is largely retained. Among those nodes with the highest indegree in a 'to live' network, 7 of the top 10 nodes (4 markets, 3 farms) are consistent between 2017-2019. Among those nodes with the highest outdegree in a 'to live network' 8 of the top 10 (1 market, 7 farms) nodes are consistent between 2017-2018, but that drops to 6 of the top 10 (all farms) in 2019. Receiving and shipping at the highest volumes and throughout the year, these nodes were classified as presumptive super-receivers and super-spreaders using the live pig network as a proxy for disease spread. Summarizing the top ten nodes for each year together, within a 'to live' network, the average indegree per year is 60.5 (median:42, range:7-294), while the average outdegree per year is 40.2 (median:34, range:14-97).

When evaluating movements by type of origin and destination, the proportions of movements between farms, markets and slaughter are generally consistent between 2017-2019 (Table 3). Approximately 5-6% of movements are from one farm to another, about 6% from farm to market, and about 85% from farm to slaughter. Movements from markets compose less than 2% of movements, with no movements from markets recorded in 2019. The lack of information on destination improved across the years, decreasing from 5.1% to 0.0% of movements between 2017-2019 (Table 3).

Movements from one farm to another shipped the largest number of pigs, with an average number of pigs moved of 108.0 (median: 40; range: 1-700). Movements from farm to slaughter averaged 29.7 pigs moved (median: 20, range: 1-400), while movements from farm to market were smaller, with an average number of pigs moved of 8.2 (median: 7; range: 0-80). Movements from market to a farm averaged 8.0 pigs moved (median: 5; range: 1-30), while the 2 movements from market to slaughter had 4 pigs. The majority (83.0%) of movements from farm to market resulted in a record of zero pigs arriving. Excluding these zero arrival records, the average difference between number of pigs shipped and number of pigs arriving was <0.1 (median: 0; range: 0-40).

Discussion:

This study summarized North Macedonian pig population census data over the last 5 years, and provided one of the first descriptions of their live pig movement network. The number of reported pigs and pig farms has increased during 2016-2020. The network of movement of these pigs was weakly connected, with instability across years among those farms with infrequent movements. The top shippers and receivers of live pigs were more consistent. Movements to slaughter predominated the network. Most movements occurred within 50 km. These data are expected to provide key insights for targeted, risk-based, and economically efficient mitigations for disease spread. Further, these data allow us to make comparisons between North Macedonia and other countries in the region and throughout Europe, and support the development of regional training materials and intervention strategies.

While pig producing countries around the world are observing a consolidation of pig production into larger-scale, commercial farms (Key, 2007; Schulz, 2017; Crescio, 2020; O'Hara, 2020; Woonwong, 2020), the census data in North Macedonia reported smallholder farms taking up an increasingly larger proportion of the industry. This increase in smallholder farms may represent a true increase or reflect improved rates of discovery and inclusion in the national registry. The highest numbers of pigs and farms were reported in 2018 and 2020, both years in which there was a financial incentive provided for each farm reported. This suggests that rather than true growth in the number of smallholder farms, there is a proportion of these farms that has historically not been consistently captured in the pig census. Within the Polog region, a decrease in the number of farms and increase in the number of pigs was observed, suggesting that, at least in this region, North Macedonia's swine industry is following the trend toward consolidation. One of the benefits of consolidation into commercial production systems is the general increase in biosecurity standards of these farms. Within North Macedonia, the high density of smallholder farms in the Northeastern and Eastern regions, bordering ASF-positive Serbia and Bulgaria, is concerning. As an area with a high number of low biosecurity premises, consistent documentation of premises in these regions is critical to enabling risk-based awareness events and trainings and targeted disease surveillance and mitigation efforts.

North Macedonia closed animal markets in 2019 as part of their increased efforts to reduce the risk of ASF introduction. Though markets received about the same proportion of movements (even with partial 2019 data), no data on movements out of markets were reported during 2019. Markets have remained closed during the COVID pandemic. With the historically poor traceability of pigs arriving at and sold from markets, the ongoing closure of these sites is recommended until record-keeping can be improved. Evaluation of movements by origin and destination type (Table 3), did show a reduction in the number of movements with an unknown destination, suggesting North Macedonia is doing a better job with movement records. However, the lack of reporting on arrival numbers, dates and sales, indicate ongoing efforts to enhance reporting are warranted. Movement data for the remainder of 2019 and 2020 was not available at the time of this analysis, therefore it is unclear how the network may adapt to the removal of market sites. The increased use of the slaughterhouse in the Southeastern region may indicate a shift from markets to slaughter. This would be expected to reduce the risk of disease transmission via live pig movements, by increasing terminal movements. The closure of markets in 2019 and missing data during the peak months of November and December may explain the drop in betweenness observed in the 2019 network. Further investigation is needed to assess whether network connectivity dramatically increases with the surge of end of year movements, however the large proportion of movements to market that occur in November-December suggest this is the case. The drop in betweenness, increase in community numbers, and lack of a GSC in 2019, suggest the most recent network is significantly more fragmented than that of previous years. Future studies will need to determine if this pattern holds and how this may improve the ability to implement zoning if ASF was introduced. In general, shipments of live pigs remain localized, though a few network communities span the country. Again, this may aid in limiting disease spread to localized areas.

North Macedonia's live pig network demonstrated a seasonality that is consistent with other European countries, and that aligns with the Easter and Christmas holidays (Khomenko, 2013; Beltrán-Alcrudo, 2018; Crescio, 2020). Previously reported survey data was consistent with our network observations, identifying peak periods for slaughter of piglets in April to May and November to January, and for fattened pigs at the end of the year (O'Hara, 2021). Backyard and family farms also demonstrated a seasonal peak in the buying of new pigs, from March to May, contributing to additional movements during this time of year. While increased movements to slaughter are not expected to contribute to a large risk of disease spread (assuming good waste management practices and no access of free-ranging pigs or wild boar to infected offal), peaks in movements associated with purchasing of new animals may contribute to a higher risk during the spring season (Crescio, 2020). Implementation of pre-movement isolation periods, i.e. stopping movements into and out of a premises, and maintaining very high biosecurity, for a set period of time (e.g. one ASF incubation period) before shipping, during these seasonal peaks may increase the chance of observing a sick pig before shipment, and therefore decrease the risk of spreading disease.

As observed in many animal-production networks, large commercial farms and slaughterhouses acted as consistent shippers and receivers of live pigs in North Macedonia's network (Schulz, 2017; Sterchi, 2019; Crescio, 2020). Targeting those farms that ship most frequently, to the most other farms, and to non-slaughter destinations, for increased surveillance and training on the recognition of clinical signs and improvement of biosecurity, is expected to decrease the dissemination of disease in this network. While commercial and slaughter premises may provide consistency to the network, the high turnover of UINs by farms in North Macedonia suggests that smallholder farms may not maintain pigs year to year. The shift in municipalities shipping low numbers of pigs between 2017-2019 may reflect the instability in this group of producers, or inconsistent documentation of these farms and their movements. Improved implementation of UIN assignments, and maintaining consistency in these assignments, across years is expected to improve traceability and thus disease response efficiency.

North Macedonia's weakly connected network with low diameter and average path length, and right-skewed indegree and outdegree, is consistent with networks described for other backyard predominant countries including Georgia (Kukielka et al., 2017), Bulgaria, Extremadura (Spain) and Côtes-d'Armor (France) (Relun et al., 2016; Relun et al., 2017). EU member countries demonstrate much larger networks, with more community structure, and are additionally more likely to be impacted by international trade and movement of pigs (Lentz, 2016; EFSA Panel on Animal Health and Welfare (AHAW), 2019; Sterchi, 2019). North Macedonia's weak connectivity may provide an advantage in limiting disease spread if network vulnerabilities are appropriately targeted during a disease outbreak.

Consistent with previously reported trade information, none of the reported movement data indicated export or trade of live pigs with EU member states or other countries (EFSA Panel on Animal Health and Welfare (AHAW), 2019). Bosnia and Herzegovina, Kosovo, Montenegro and Serbia historically reported exporting no live pigs; only Serbia reported export of pig products (EFSA Panel on Animal Health and Welfare (AHAW), 2019). The disconnected nature of North Macedonia's live pig network, especially in more recent years, and the lack of international trade, data suggest the legal movement of live pigs is likely a low risk for disease spread in this region. This evaluation and the resultant risk-based recommendations for targeted interventions are limited to those supported by the live pig movement network. Additional information on the movement of pork products, vehicles, fomites, farm workers, veterinarians, and the illegal movement of live pigs and pork products, together with wild boar-related factors, is needed to make a better assessment of disease risk in the country. Indeed, these other factors are often seen as more important in the epidemiology of ASF than the movement of live animals.

This study has provided a foundation of information about the documentation and traceability of pigs in North Macedonia, and evidence to support ongoing improvement in this system. A better understanding of the live pig movement network has provided sites for targeted training and mitigation efforts, providing cost-effective, risk-based approaches to reduce the risk of disease introduction and spread. Future efforts will need to explore additional data sources, risk pathways, and modeling efforts to understand how this information may impact the spread of transboundary animal diseases, such as ASF, within North Macedonia's pig sector. The instability of North Macedonia's live pig movement network suggests that annual updates should be performed to analyses and resulting recommendations.

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Ethics Statement:

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

Conflict of Interest:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Tables and Figures:

Table 1. North Macedonia pig census data 2016-2020 summarized on the country and regional level.

Country		2016	2017	2018	2019	2020
Number of Farms	Total	1,976	2,344	3,096	2,175	2,889
	With <=10 pigs	1,262 (63.9%)	1,619 (69.1%)	2,589 (83.6%)	1,664 (76.5%)	2,339 (81.0%)
	With 11-100 pigs	638 (32.3%)	652 (27.8%)	435 (14.1%)	434 (20.0%)	465 (16.1%)
	With >100 pigs	76 (3.8%)	73 (3.1%)	72 (2.3%)	77 (3.5%)	85 (2.9%)
Number of Pigs	Total	109,845	110,058	128,983	125,230	133,448
	Avg Per Farm	55.6	47.0	41.7	57.6	46.2
	Median Per Farm	6	4	3	4	3
	Range (Min-Max)	1-18,576	1-19,837	1-21,747	1-22,459	1-21,159

Region		2016	2017	2018	2019	2020
Eastern	Number Farms	215	258	626	194	776
	Number Pigs	31,075	31,520	34,504	33,593	40,703
Northeastern	Number Farms	927	1205	918	874	736
	Number Pigs	12,592	11,977	10,602	10,519	10,454
Pelagonia	Number Farms	35	50	196	170	63
	Number Pigs	1,034	1,169	1,606	1,867	1,146
Polog	Number Farms	237	262	344	274	197
	Number Pigs	13,982	14,249	16,663	14,923	16,000
Skopje	Number Farms	15	14	19	19	28
	Number Pigs	1,653	1,951	1,760	1,751	2,424
Southeastern	Number Farms	289	242	371	165	480
	Number Pigs	8,177	7,034	8,275	8,149	7,568
Southwestern	Number Farms	196	230	418	344	368
	Number Pigs	1,652	1,813	2,720	1,959	2,333
Vardar	Number Farms	62	83	204	135	241
	Number Pigs	39,680	40,345	52,853	52,469	52,820

Table 2. North Macedonia live pig movement network parameters for 2017-2019.

	2017	2018	2019
Nodes	388	387	479
Edges	6,678	7,451	7,113
Indegree (Mean, (Min, Max))	17.21 (0, 1,469)	19.25 (0, 1,404)	14.85 (0, 1,461)
Outdegree (Mean, (Min, Max))	17.21 (0, 584)	19.25 (0, 773)	14.85 (0, 650)
Betweenness (Mean, (Min, Max))	26.01 (0, 5,922.1)	15.68 (0, 4,682.0)	0.21 (0, 15.0)
Edge Density	0.04	0.05	0.03
Diameter	5	5	4
Average Path Length	2.47	2.12	1.13
Transitivity	0.014	0.023	0.014
GSC size	31	34	1
GWC size	382	378	427
Communities	17	33	85

*GSC: giant strongly connected component, GWC: giant weakly connected component

Table 3 Movements of live pigs in North Macedonia from 2017-2020, summarized by type of premises moving from and to. Proportion is calculated as the proportion of total movements for a given year.

Year	From	To	Number	Proportion (%)
2017	Farm	Farm	283	4.0
		Market	384	5.5
		Slaughter	5949	85.6
		Unknown	352	5.0
	Market	Farm	60	0.9
		Market	0	0
		Slaughter	2	<0.1
		Unknown	5	<0.1
	Total		7035	
2018	Farm	Farm	375	4.9
		Market	473	6.2
		Slaughter	6526	85.3
		Unknown	184	2.4
	Market	Farm	77	1.0
		Market	0	0
		Slaughter	0	0
		Unknown	18	<0.1
	Total		7653	
2019	Farm	Farm	513	7.2
		Market	414	5.8
		Slaughter	6186	87.0
		Unknown	0	0
	Market	Farm	0	0
		Market	0	0
		Slaughter	0	0
		Unknown	0	0
	Total		7113	

Figures:

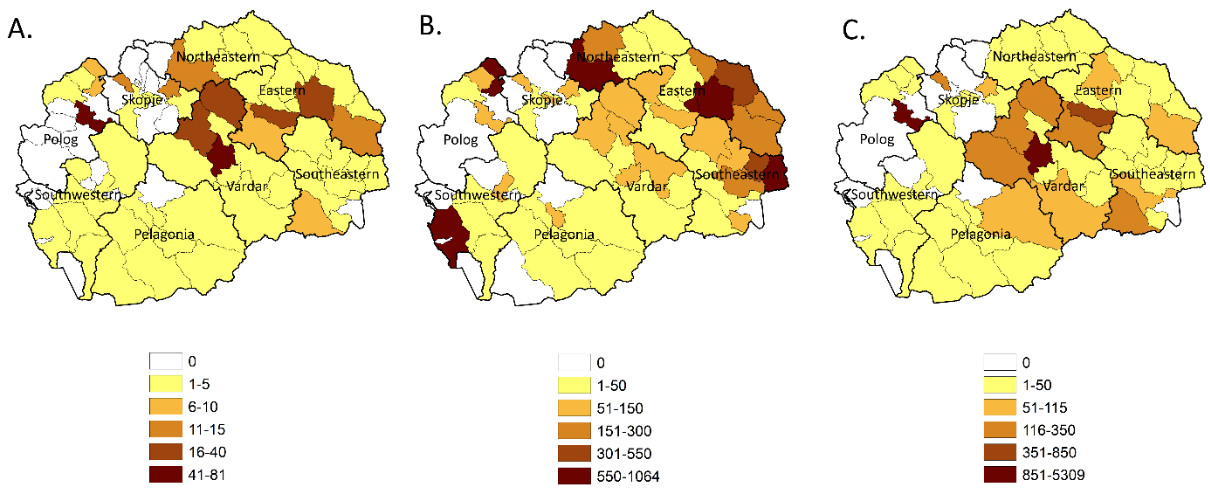


Figure 1. North Macedonian pig census 2020 summarized at the municipality level by, A) pigs per square kilometer, B), farms per 1000 square kilometer, and C) pigs by farm. Black lines outline regions; gray lines outline municipalities.

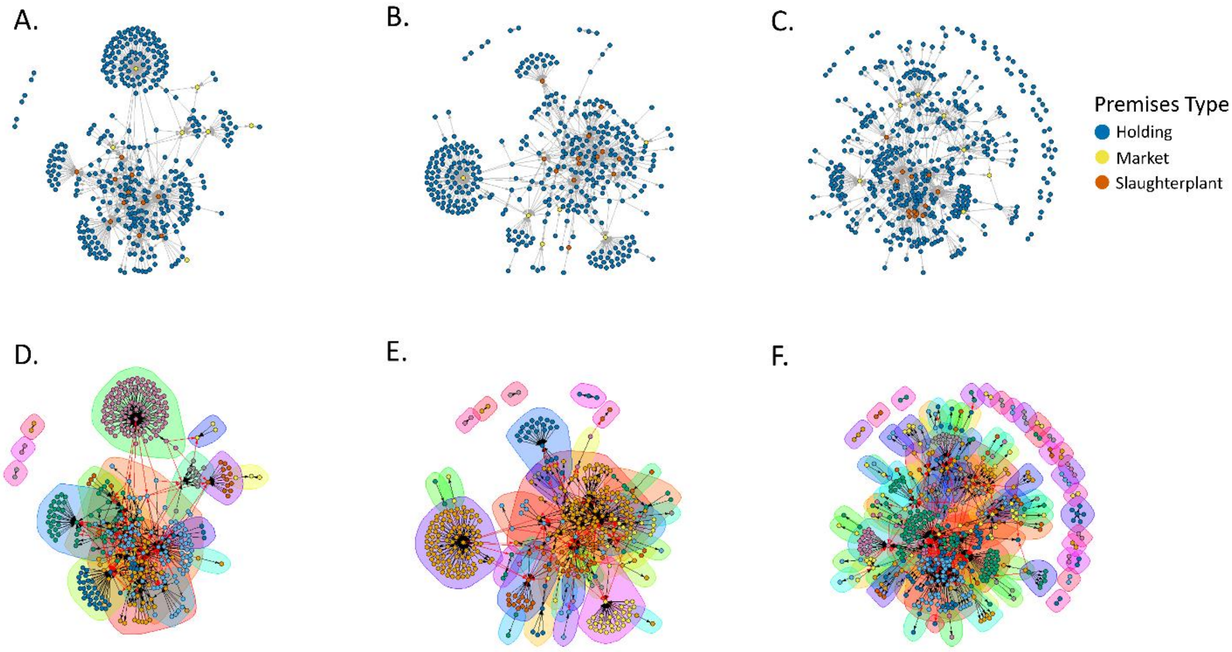


Figure 2. Non-spatially explicit simplified (repeated edges and loops removed) North Macedonian live pig movement networks for A) 2017, B) 2018, and C) 2019. Communities within the network were identified using the Walktrap community finding algorithm, with D) 17 communities identified in 2017, E), 33 communities identified in 2018, and F) 85 communities identified in 2019.

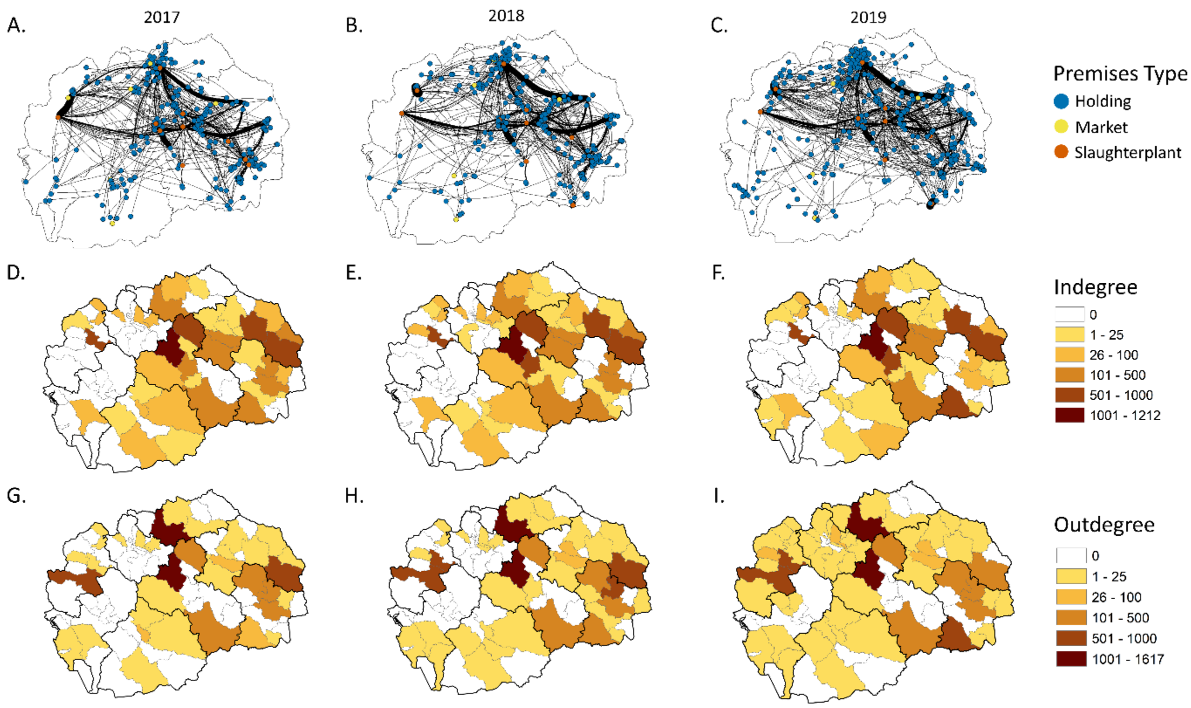


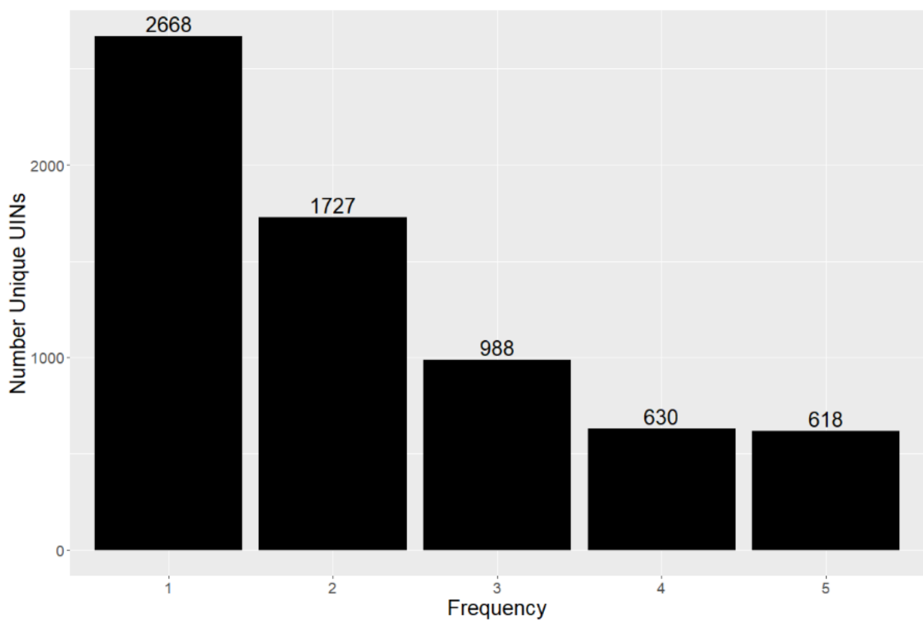
Figure 3. Network analysis of live pig movements in North Macedonia for 2017-2019. Simplified network with edge weight as the number of movements divided by 20 for A) 2017, B) 2018, C) 2019. Summary of the number of live pig movements into a municipality for D) 2017, E) 2018, F) 2019. Summary of the number of live pig movements out of a municipality for G) 2017, H) 2018, I) 2019.

Figure 4. Number of live pig movements within North Macedonia by shipment month for 2017-2019.

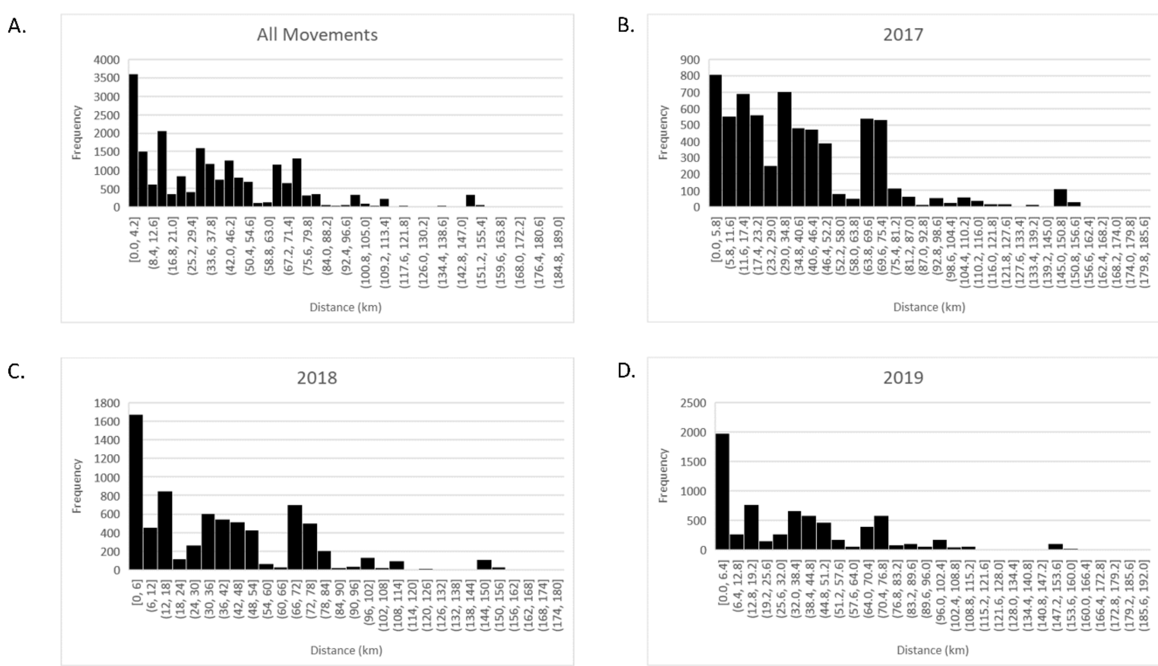
Supplemental Tables and Figures:

Supplemental Table 1. North Macedonia pig demographics based on the census data for 2016-2020. Summary data by total number of pigs, piglets, fattening pigs, gilts, sows and boar per farm. Farms reporting zero total animals were excluded.

		2016	2017	2018	2019	2020
Total	**Avg**	52	47	42	58	46
	Median	5	4	3	4	3
	Minimum	0	1	1	1	1
	Maximum	18576	19837	21747	22459	21159
Piglets	**Avg**			17	25	18
	Median			0	0	0
	Minimum			0	0	0
	Maximum			11058	10996	9955
Fattening	**Avg**			19	25	22
	Median			0	0	1
	Minimum			0	0	0
	Maximum			8488	9273	8930
Gilts	**Avg**			1	1	1
	Median			0	0	0
	Minimum			0	0	0
	Maximum			680	665	592
Sows	**Avg**			4	7	5
	Median			1	2	1
	Minimum			0	0	0
	Maximum			1498	1495	1657
Boars	**Avg**			0	0	0
	Median			0	0	0
	Minimum			0	0	0
	Maximum			28	30	25



Supplemental Figure 1. Frequency of occurrence of a given unique identification number (UIN) in the North Macedonia pig census between 2016-2020.



Supplemental Figure 2. Distance (km) of live swine shipments in North Macedonia for A) 2017-2019, B) 2017, C) 2018, and D) 2019.

Chapter 3:

Modelling the spread of African swine fever virus and targeted interventions in North Macedonian pigs

Title: Modelling the spread of African swine fever virus and targeted interventions in North Macedonian pigs

Running Title: Modelling ASF North Macedonian pigs

Authors: Kathleen C. O'Hara[1], Jose Pablo Gomez[1], Carlos Gonzalez Crespo[1], Daniel Beltrán-Alcrudo[2], Mark Hovari[2], Blagojcho Tabakovski[3], Beatriz Martínez-López[1*]

Corresponding author*: Beatriz Martínez-López, beamartinezlopez@ucdavis.edu

Author Affiliations:

[1]Center for Animal Disease Modelling and Surveillance (CADMS), School of Veterinary Medicine, University of California, Davis, USA

[2]FAO Regional Office for Europe and Central Asia, Budapest, Hungary

[3]Food and Veterinary Agency, Republic of North Macedonia

Abstract:

The global impact of African swine fever (ASF) on the pig industry continues to expand, most recently with its reintroduction into the Western Hemisphere. To best prevent introduction to free areas, countries need to understand their pig sector and identify gaps in existing surveillance, reporting and biosecurity practices. In addition to preventing disease introduction, countries need to have response plans in place, enabling rapid and efficient disease containment and eradication. North Macedonia, in the Balkan peninsula of southwest Europe, is currently free of ASF, but is bordered to the North and East by countries with ongoing outbreaks. In an effort to better understand how ASF might impact and spread through the pig sector in North Macedonia, this study aggregated domestic pig and wild boar population data, and considered movement data from 2017-2019 to inform a model of ASF spread. Using an agent-based approach ASF spread was simulated across a hexagonal grid of 10 km cells in North Macedonia. Each cell's disease transmission was modelled by a susceptible-infected-removed compartment approach. Random forest (RF) algorithms and classification regression trees (CART) were used to inform a global sensitivity analysis (GSA), assessing the influence and interactions of model parameters. Increased surveillance after the detection of infected pig herds, the index case region, and the global transmission rate had the greatest influence on the cumulative number of infected premises. Simulation results indicated that across an 18 month period between 42 to 612 premises, with a median of 128, would become infected. Outbreaks that begin in family or commercial farms, and those that begin in the Eastern regions, result in larger and more widespread outbreaks. Improving surveillance can decreased the number of infected premises by up to 80%, while movement restrictions dropped this number by 62%. This study simulated the spread of ASF based on pig density and live pig movements. Specific regions were identified as having a high probability of infection, and surveillance and movement restriction interventions were assessed. This information provides critical information to support risk-based, cost effective ASF prevention and response planning for the North Macedonian pig sector, and provides data on the pig sector in this region to inform future trainings, risk assessments, and further ASF modelling efforts.

Keywords: African swine fever, modelling, North Macedonia

Introduction:

African swine fever (ASF), a viral hemorrhagic disease affecting domestic pigs and wild boar (*Sus scrofa)*, continues to spread across Europe, Asia, and recently into the Caribbean (World Organisation for Animal Health (OIE), 2021). The immense direct and indirect economic losses associated with introduction into a free country (Berthe, 2020), have led the global swine community to seek better information about how local pork value chains interact and what strategies may be most effective in preventing disease introduction and reducing disease spread. Modelling approaches have been invaluable in exploring ASF disease transmission pathways, risk and assessing mitigations strategies.

ASF is a World Organisation for Animal Health (OIE) reportable disease of domestic and wild suids (World Organisation for Animal Health (OIE), 2021). When it is introduced to a naïve population, it can lead up to 100% mortality (Blome, 2013; Sánchez-Vizcaíno, 2015). Reintroduced to Europe via Georgia in 2007, it has since spread westward across Europe, north and east into Russia and Asia, and most recently to Hispaniola in the Caribbean (World Organisation for Animal Health (OIE), 2021). Disease transmission in both domestic and wild pigs can occur via direct contact with an infected animal, through consumption of contaminated materials (e.g. swill feeding, discarded offal, scavenged carcasses or garbage), exposure to fomites, iatrogenically, or through the bite of infected *Ornithodoros* ticks if present in the area (Beltrán-Alcrudo, 2009; Jori, 2009; Costard et al., 2013; Galindo-Cardiel, 2013; Gogin, 2013; Oura, 2013; Cwynar, 2019). No treatments or commercial vaccines currently exist. Control relies on strict biosecurity, surveillance, rapid detection and stamping out (Galindo-Cardiel, 2013; Penrith, 2013; Gallardo, 2015; Sánchez-Vizcaíno, 2015; Bosch, 2016).

North Macedonia, located in the Balkan peninsula of southeast Europe, is bordered by Kosovo and Serbia to the North, Bulgaria to the East, Greece to the South, and Albania to the West. Bulgaria and Serbia have ongoing ASF outbreaks in both domestic pigs and wild boar. Greece was able to control a single introduction among its domestic swine in 2020 (World Organisation for Animal Health (OIE), 2021). Currently, North Macedonia is free from ASF, but bordering these infected areas puts the country at high risk for disease introduction. In collaboration with the Food and Agriculture Organization of the United Nations (FAO), North Macedonia has been working to better characterize their pig sector (O'Hara, 2021a) and to use this information to inform data-driven approaches to support disease surveillance, prevention and response planning (O'Hara, 2021b).

Models have been used extensively to understand disease dynamics. Models may be used to aid in the interpretation of data, to identify primary drivers of disease or risk, or to better understand the epidemiology of a disease when data about specific populations is limited. A recent review of mechanistic models highlighted the growing literature base of ASF models (Hayes, 2021). Statistical and mechanistic models have been used extensively to simulate the spread of ASF and evaluate interventions in countries including China, Denmark, France, Italy, Latvia, Russia and Spain (Korennoy, 2014; Brarongo, 2016; Halasa, 2016a; Vergne, 2016; Mur et al., 2017; Relun et al., 2017; Halasa, 2018; Andraud, 2019; Halasa, 2019; Schulz, 2019; Gao, 2021; Taylor, 2021). Disease transmission in wild boar has been a particular focus of recent modelling, as researchers attempt to better understand ASF dynamics in these populations (Lange, 2017; Nurmoja, 2017; Halasa, 2019; Croft, 2020; O'Neill, 2020; Olševskis, 2020; Pautienius, 2020; Taylor, 2020). Agent- or individual-based models are used to understand the dynamics of individuals and their interactions with others, their environments and time (Macal, 2016). These approaches have been increasingly more common in the modelling of ASF and in the simulation of wild boar populations and their disease dynamics in particular (Thulke, 2017; Lange, 2018; Halasa, 2019; Croft, 2020; Yang, 2020; Gervasi, 2021).

This study aims to use an agent-based modelling approach to simulate the movement of ASF through North Macedonia, accounting for domestic pig and wild boar densities, and the live pig movement network. The objective is to understand how disease introduction in different populations or regions impacts the scale and distribution of the outbreak, and then to assess how different interventions can mitigate disease spread. The overarching goal is to improve North Macedonia's understanding of their risk for ASF introduction and spread, and to demonstrate the efficacy of strategies to improve early detection and limit disease dispersion.

Materials and methods:

1. Data:

Data were collected, validated and cleaned in Microsoft Excel 2016 and R Studio (v.3.6.1) (R Core Team, 2017; RStudio Team, 2020).

1.1. Census

Annual pig census data for 2020 was provided by the Veterinary Authority (Food and Veterinary Agency of North Macedonia). The data included a unique identification number (UIN), coordinates, town/village, region, total number of animals, number of piglets, number of fattening pigs, number of gilts, number of sows, and number of boars. Farms were defined as backyard (<=10 pigs), family (11-100 pigs), or commercial farms (>100 pigs) based on the total number of pigs on-site (O'Hara, 2021a).

1.2. Live pig movements

Records of permitted movements of live pigs for 2017-2019 were provided by the Veterinary Authority. Movement records for 2017-2018 provided data for the entire year, while 2019 data covered only Jan. 1-Nov 23, 2019 (321 days). Records for 2017-2018 included movement type (completed movement, departed, departed with no document, movement off holding, movement without document), certificate number, date of departure, date of arrival, number of pigs departing, number of pigs arriving, origin UIN, destination UIN, and type of UIN for both origin and destination (holding, market, slaughterhouse, or unspecified). Records for 2019 included movement type, date of departure, date of arrival, for origin and destination: UIN, town/village, municipality, region, herd type, and coordinates were provided. Number of animals moved was not provided for 2019. A separate set of data on movements to slaughter was provided for 2019, which included date of departure, date of arrival, origin and destination: UIN, town/village, municipality, region, number of animals departed, and number of animals arrived. Therefore, the number of animals moved is only available for movements to slaughter for 2019.

UIN types that were unspecified were assumed to be holdings: 664 (3.0%) origin types and 304 (1.4%) destination types were reassigned from unspecified to holding. All of the 13 commercial slaughterhouses in North Macedonia were identified (no slaughterhouses were mischaracterized). 559 (2.6%) movements did not have a destination recorded; these movements were not considered in the analysis. A total of 21,801 movements were included in this study.

Coordinate information was not provided in the 2017-2018 movement data, and were therefore referenced from 2019 movement and census data by UIN. The remaining unassigned UIN's were assigned to the town/village centroid using the UIN coding system in which the first four digits reference a specific town/village. This represented 53 (12.8%) UINs, associated with 127 (1.8%) of movements in 2017 and 45 (10.9%) UINs, accounting for 110 (1.4%) of movements in 2018.

1.3. Wild boar

The Veterinary Authority provided a pdf map of the hunting grounds in North Macedonia, along with a corresponding dataset that included the reported minimal number of parental stock maintained within each hunting ground, as well as an estimate of the number of wild boar within each hunting ground derived from this minimum. A shapefile was derived from the hunting ground pdf in ArcGIS Desktop v10.7. The estimated wild boar population density for the remaining geographic area was referenced from the ENETwild abundance model for wild boar (ENETWILD-consortium, 2020). The wild boar density map used in this study joined these two datasets, using the North Macedonia Veterinary Authority data as the primary layer and informing the remaining geographic area with the ENETwild modeled density.

2. Modelling:

2.1 General approach:

An agent-based model to simulate disease transmission dynamics on a local and national level was generated by aggregating demographic information from North Macedonia's domestic and wild pig populations. Data was aggregated using a hexagonal grid, where each cell is used as the base unit of analysis. Within each hexagon of 10 km diameter, the number of domestic pigs, number of wild boar and number of farms were summed. A probability of movement per day was calculated for each hexagon by dividing the number of movements reported by the total observation period in days. The spread of disease following an index case at one premises was iteratively simulated.

2.2. Local spread dynamics

A compartmental modelling approach was used to represent the transmission of ASF at the local level. The population of each cell (N_i) was subdivided into susceptible (S_i), infected (I_i) and removed (R_i) (Anderson, 1992). After introducing disease into a cell, the transmission between compartments was calculated using the following ordinary differential equations (ODE).

$$S_i = -\beta_i I_i S_i / N_i$$

$$I_i = \beta_i I_i S_i / N_i - \gamma_i I_i$$

$$R_i = \gamma_i I_i$$

The rate at which animals transition from the susceptible to infected compartment (β_i), for each cell is calculated as follows:

$$\beta_i = \beta \omega_1 \omega_2$$

Where β is the global transmission rate, and ω_1 and ω_2 are local weights for the influence of animal density and number of within cell movements obtained from census and movement records. It is assumed that cells with higher animal density and higher number of internal movements will have a higher transmission rate. The transition between infected to removed (γ_i) is represented as the inverse of the average number of days to detection and removal of infected farms; a baseline of 10 days is assumed. This information was based on data collected during the acute presentation of current genotype II strains of ASF currently circulating in Europe (Penrith, 2009; Blome, 2013; World Organisation for Animal Health (OIE), 2021).

Disease transmission between spatially adjacent cells was simulated to represent local between herds transmission. This probability of adjacent cell transmission was informed by the population within each cell and the proportion of infected animals. For the infected cells, every step of the simulation a Bernoulli distribution describes the probability of infecting one of the adjacent cells as follows:

$$P(y_{ij} = 1 | x_i = 1) = I_{ph,i}/N_{ph,i}$$

where $y_{ij} = 1$ indicates transmission between a pair of adjacent cells, $x_i = 1$ indicates that the current cell is infected, and $I_{ph,i}/N_{ph,i}$ is the proportion of infected farms in the cell.

Both domestic pig herds and wild boar populations are modeled. Disease transmission between domestic and wild populations is represented as:

$$P(y_{wb,ph} = 1 | x_{wb} = 1) = I_{wb}/N_{wb}$$

where $y_{wb,ph} = 1$ is wild boar to domestic transmission, $x_{wb} = 1$ is the current infection of wild boars in the cell, and I_{wb}/N_{wb} is the proportion of infected wild boars.

2.3. Long distance spread dynamics

To represent the transmission of disease across long distances and between different regions, an origin-destination probability matrix was calculated using the movement records. Each cell has a list of potential trade partners and probabilities associated for moving animals from one cell to another. The probability of exporting infected animals farm one farm to another is represented as a function of the proportion of animals infected at the origin.

2.4. Model implementation

Five hundred simulations of the model, each simulating a period of 18 months, were run using a controlled random seed. Model parameters were informed from the literature and expert opinion (Table 1). For each of the simulations, values for each parameter were sampled from a list representing low, moderate and high estimates, or yes/no, for the implementation of interventions (Table 1). Random forest (RF) algorithms were used to explore the influence of each parameter on the outcome of interest (disease spread), and classification regression trees (CART) were used to provide a graphical understanding of how the parameters interact to affect the outcomes selected, similar to the process described by (Harper, 2011) for global sensitivity analysis (GSA) of complex models.

Code for reproduction of these results is available at: https://github.com/jpablo91/ASF_Macedonia. The model was implemented in GAMA 1.8.1 (Taillandier, 2019) and the analysis of model outcomes performed in R Studio.

Results:

Pig population and movement data were aggregated to 5km^2 hexagons (Figure 1). The regions of North Macedonia and geographic overlay of the hexagons can be visualized in Figure 1A,B. The highest density of domestic swine lies in the center of the country, consistent with the high density of commercial production sites in the Vardar region (Figure 1A) (O'Hara, 2021b). Wild boar are reported or predicted to be present throughout the country, with the largest numbers in the Eastern, Southwestern and Vardar regions (Figure 1B). The highest numbers of farms are present in the Southwestern, Polog, and Northeastern regions (Figure 1C). When evaluating origin and destinations within the movement data, the highest densities of holdings are in the

Vardar and Southeastern regions (Figure 1D). Market density is highest in the Northeast and Southeast regions (Figure1E). Slaughterhouses are distributed through the Polog, Vardar and Eastern regions (Figure 1F). Movement records predict the highest probability of movements within the Polog, Vardar and Eastern regions.

Figure 2 presents the model framework, incorporating these density and movement probabilities. Estimates of number of infected premises were generated following single origin index cases. Across all simulations, the estimated final number of infected pig herds ranged from 40 to 612, with a median of 128 infected premises. RF and CART results show that the model predictions are sensitive to complex combinations of parameter estimate. The influence of the parameters and the complex interactions between them is presented in Figure 3. The classification and random trees generated explained 60% of the variance. The parameters with greatest influence in the cumulative number of premises infected included the effect of increased surveillance after the detection of infected pig herds, the index case region, and the global transmission rate (Figure 3B).

The highest number of infected premises resulted when outbreaks started in the Northeastern, Southeastern, and Eastern regions, when the effect of animal density (AnmlDnsB) and the global transmission rate (GBetaPh) were higher, and when the impact of local movements (loopsB) was low. An index case occurring in the Northeastern region resulted in an outbreak with the highest number of infected premises, with 192 infected premises on average. This can be compared to Pelagonia, which, as an origin, resulted in an average of 6 infected premises. More infected premises were predicted when the index case occurred in family or commercial farms than if the index case was in backyard farm. When surveillance was increased by 1.5 and 2.0 times (the rate of detection and removal was increased by 1.5 to 2 fold) after detection, the cumulative number of infected premises decreased by 46% and 80% respectively. Implementing movement restrictions after detection of a case decreased the number of infected premises by 62%.

Based on the number of times the outbreak affected each cell, the probability of infection for each of cell was calculated. This probability was used to generate risk maps, highlighting those regions that were predicted to be more affected once ASF had been introduced, depending on the index case type of farm (Figure 4). Following an introduction in a backyard farm, the Eastern and Southeastern regions were predicted to be the more highly affected (Figure 4A). While, introduction into either a family or commercial farm resulted in higher probabilities of infection in the Vardar and Northeastern regions, in addition to the Eastern and Southeastern regions (Figure 4B,C).

Discussion:

This study has aggregated pig population and live pig movement data to inform the risk of ASF spread through the North Macedonian pig sector. High risk regions in the east of the country have been highlighted for targeted mitigations efforts, and the value of improved surveillance and the implementation of movement restrictions has been shown.

In general, ASF introductions associated with long distance translocation are associated with human mediated activities, while cases introduced by wild boar often occur at border regions with infected countries (European Food Safety Authority (EFSA), 2018; Viltrop, 2021). Previous assessments have suggested that wild boar present the riskiest pathway for ASF transmission in Eastern Europe, while legal trade of pigs presents the highest risk in Western Europe (Taylor, 2020). Multiple incursions into the Balkan region, including into Romania, Hungary, Bulgaria, Poland and Greece, have been associated with human activities (Penrith, 2020; Viltrop, 2021).

These infections have often spilled over into wild boar, making disease eradication difficult (Penrith, 2020). While control within the domestic population has been achieved by some countries (Danzetta, 2020), Romania, with its large backyard sector, has struggled (Boklund, 2020). North Macedonia, with a similarly backyard predominant swine sector needs to understand how to best target risk-based mitigations and which interventions may be most effective in their pig sector.

Investigation of outbreaks among domestic pig farms in Romania indicated proximity to outbreaks (regardless of farm type), herd size, wild boar abundance, proximity of wild boar cases, and visits of professional working on farms, were significant risk factors for disease introduction (Boklund, 2020). While domestic pig and wild boar density are important factors in disease transmission, especially local disease transmission (Mur et al., 2017; European Food Safety Authority (EFSA), 2021). This study incorporated domestic pig and wild boar density data, with live pig movement data, to simulate how ASF might move through North Macedonia's pig populations. Similar to previous studies, areas with high pig and farm densities demonstrated higher risk of infection, and infection of larger farms was associated with in larger and more widespread outbreaks (Nigsch, 2013; Viltrop, 2021).

This study is focused on the movement of live animals as the route of disease transmission, and does not explicitly account for the movement of infected pork products or contaminated people, vehicles or other fomites. With family and commercial farms accounting for a higher proportion of, and longer distance, live pig movements, a model based on these movements may result in overemphasizing the role of these farms in disease spread. The impact of higher biosecurity practices is expected to both reduce the risk of disease introduction and disease spread from commercial farms in particular. However, studies from Estonia and other countries in the region do suggest that all farm sizes and types are at risk for infection (Nurmoja, 2020), and that index cases are large farms result in larger outbreaks (Nigsch, 2013). In general, the between farm movement of ASF is difficult to parameterize due to the many factors that may contribute, e.g. shared workers/load crews, shared vehicles/equipment, food sources, etc. Using a range of values for GBetaPh, AnimlDnsB and loopsB, and assessing the relevance of each parameter on predictions, attempts to account for some of this uncertainty in the true disease dynamics. More granular data is needed to better account for the critical role of human driven disease spread (Chenais, 2019). By accounting for a higher risk of disease spread between adjacent cells in the local spread component of the model, the authors attempted to acknowledge the increased likelihood of interactions between neighbors and family which may not otherwise be captured.

Risk maps generated from the simulations in this study were consistent with those previously developed based on biosecurity practices and farm type for North Macedonia (O'Hara, 2021a). By biosecurity risk score, the Southeastern and Eastern regions were of consistent concern when assessing all farm types, but when focusing on family and commercial farms, the Northeastern and Southwestern regions carried higher risk for introduction. The regional pattern based on index farm type described here demonstrates a similar pattern. This study suggests that introduction to family or commercial farms results in larger outbreaks, therefore the Northeastern and Southwestern regions, where the probability of infection of those farms is highest, should be targeted for increased training and funding support improvements in surveillance, reporting and biosecurity.

Those interventions assessed here, increased surveillance and movement restrictions represent some baseline strategies to improve disease detection and reduce spread. The SurvGamma parameter, provides a baseline level of surveillance and the ability to incorporate increases in early detection, awareness and reporting. The MovRestrictions parameters captures interventions occurring once the disease has been introduced. The scale of the impact of rapid implementation of movement restrictions observed here, 62% reduction, is consistent with that of other ASF models (Lee, 2021).

The authors recognize there are limitations to the current work, and are actively expanding and updating the model to address these. While the wild boar population is accounted for in the model, the impact of disease spread within and movement of this population could be improved. Particularly, accounting for the impact of infected carcasses on the landscape as ongoing sources of infection, will be critical to capture the disease dynamics in this population (Lange, 2017; Halasa, 2019). The maintenance of ASF within the wild boar population has contributed to the spread of the disease across Europe and the difficulties some countries have had in eradicating the disease (Nurmoja, 2017; Olševskis, 2020; Pautienius, 2020). Therefore, a better understanding of these dynamics in North Macedonia is warranted. Additionally, the assessment of further control measures is of interest. Other countries in the region have successfully implemented, and models have supported the efficacy of, culling of infected farms, fencing to control wild boar movement in infected areas, increased hunting pressure, and active searches for and removal of wild boar carcasses (Lange, 2015; Halasa, 2016b).

This study has provided some foundational information about the role live pig movements could play in dispersing ASF throughout North Macedonia following a disease introduction. The Veterinary Authority can use these results to inform risk-based, efficient and cost-effective educational campaigns and preparedness planning. Future efforts will continue to revise these recommendations and provide further evaluation of the most effective interventions to be implemented if an outbreak were to occur. Further, these data provide additional information on the factors driving movement of disease in this region, providing targets for regional training and exercises.

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Tables and Figures:

Table 1. Parameters explored for global sensitivity analysis of modelling ASF spread among North Macedonian pigs

Parameter	Definition	Sample Space
GBetaPh (β)	Global transmission rate for pig herds	(0.2, 0.25, 0.3, 0.35)
AnmlDnsB	Effect of the animal density on the local transmission rate	(0.1, 0.2, 0.3)
loopsB	Effect of the number of internal movements on the local transmission rate	(0.1, 0.2, 0.3)
IndexCaseRegion	Region where the index case was simulated	(regions 1 to 9)
IndexCaseType	Type of farm where the index case was simulated	(backyard, family, commercial)
SurvGamma	Effect of increased surveillance after the detection of an infected pig herd	(1.0, 1.5, 2.0)
Movement Restrictions	Whether or not movement restrictions are implemented after the dection of a case	(yes, no)

Figures:

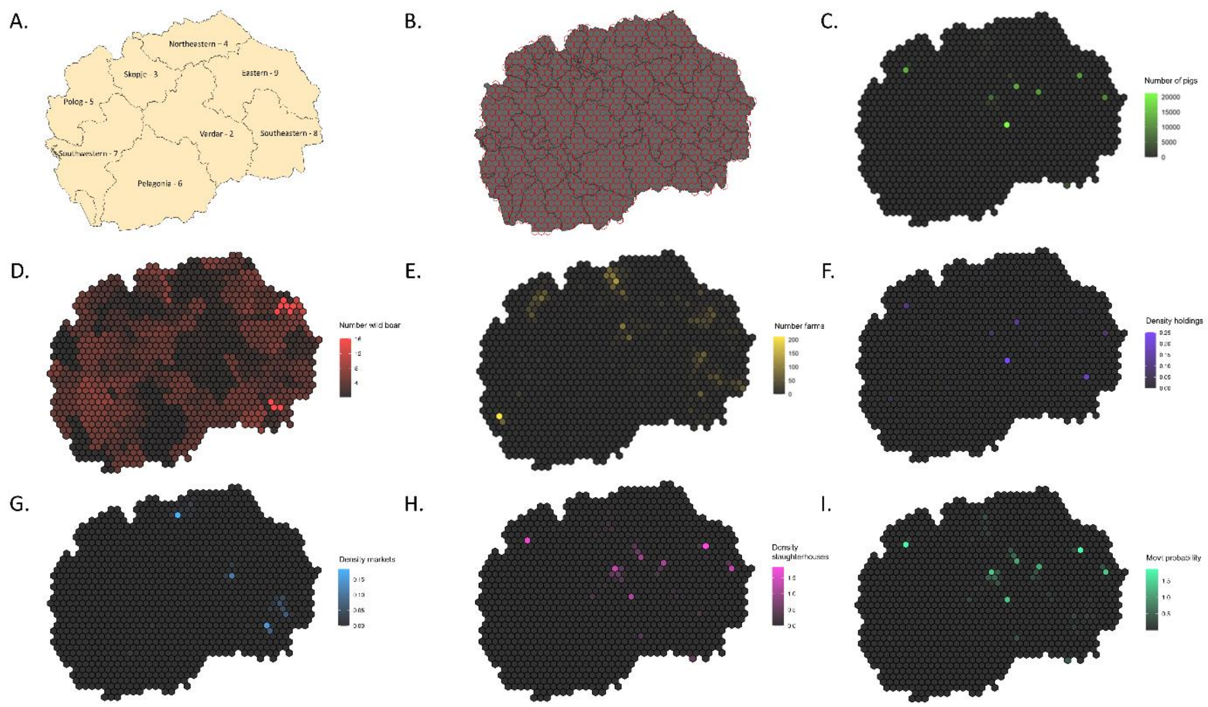

Figure 1. Aggregation of North Macedonia pig population and movement data to hexagons of diameter 10 km. A) Regions of North Macedonia, B) hexagon division of North Macedonia, C) domestic pig population, D) wild boar population, E) number of farms, F) holding density (pig production premises as defined in the movement data; non-market or slaughter premises), G) market density, H) slaughterhouse density, I) movement density.

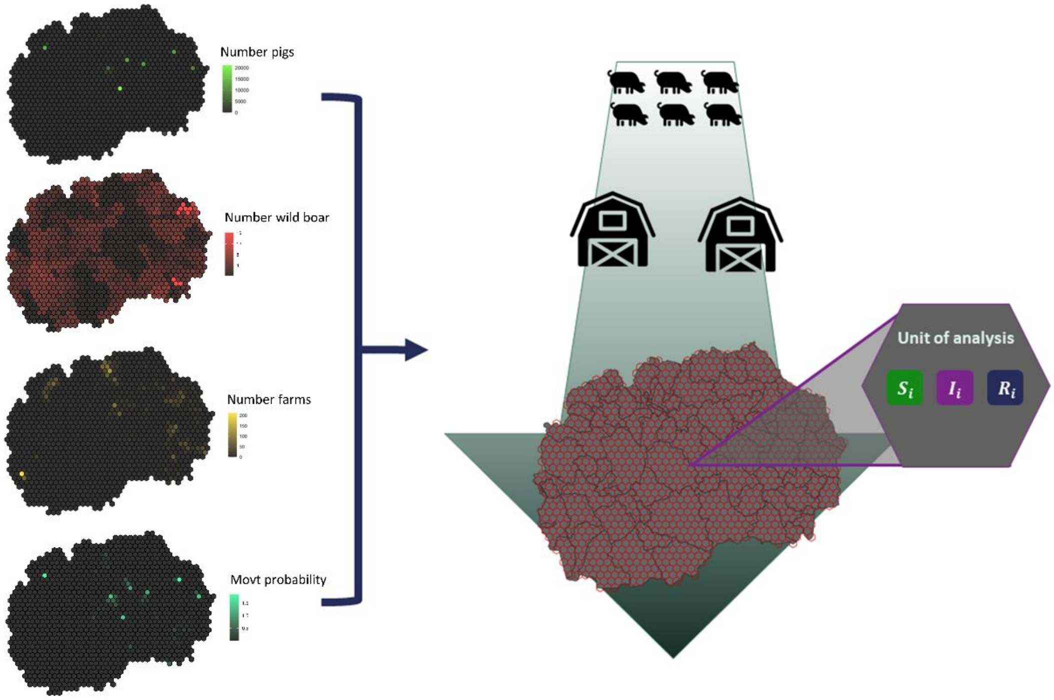

Figure 2. Framework for modelling ASF spread in North Macedonian swine accounting for domestic pig density, wild boar population, farm density, and movement probability. Populations were aggregated for a 5km^2 hexagonal grid. Within each grid, disease spread was based on a Susceptible-Infected-Removed (SIR) compartment model).

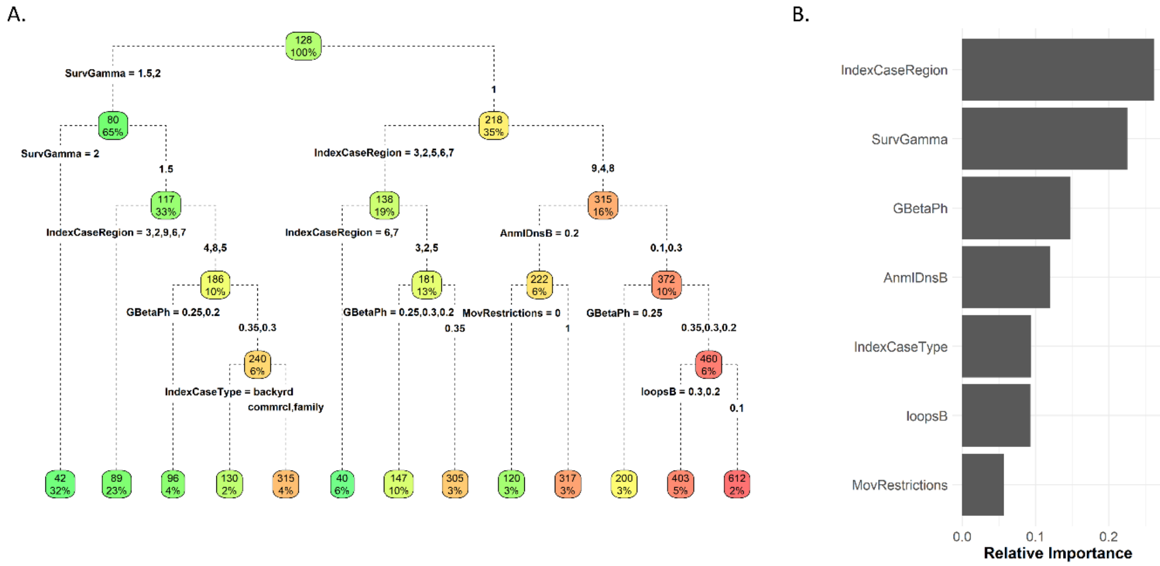

Figure 3. Global sensitivity analysis (GSA) for ASF spread in North Macedonia pigs. A) Classification trees show the interactions between parameters that were explored using GSA for each of the outcomes analyzed. The number in each box represented the number of infected premises. Each horizontal row represents the different parameters considered, with the branches reflecting the impact of different values for each parameter. B) Normalized relative importance of the parameters used for the GSA. Descriptions of the parameters can be found in Table 1. Regions numbers correspond to those in Figure 1A.

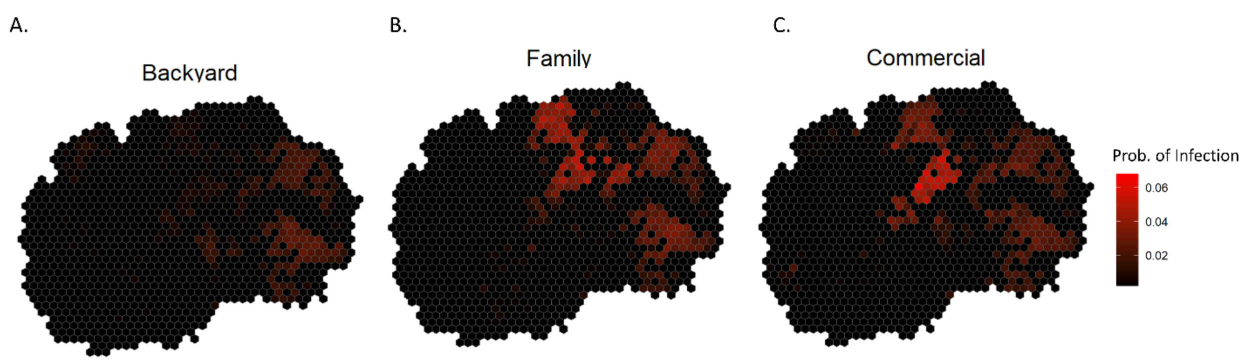

Figure 4. Risk maps for the probability of infection with ASF in North Macedonian pigs based on the type of farm at which the index case began.

Discussion

Discussion:

This work represents the most extensive description of the North Macedonian pig sector currently available. Detailed producer information collected via surveys was combined with government information on domestic pigs, wild boar, and live pig trade, to provide a global understanding of the industry in the country. A range of descriptive, statistical, and mechanistic approaches were used to estimate the risks of disease introduction and spread across space, time, and population. The tools used in this work can be applied in different countries and for a variety of diseases. This work provides a framework for a ground up evaluation of a country's swine sector, and detailed approaches for the application of this information for preparedness and response planning, particularly in backyard predominant settings.

Describing the husbandry practices, veterinary care, pork value chain, biosecurity, and disease awareness within North Macedonia's pig sector, provided vital information to improve targeted, risk-based interventions and mitigation within the country, but also information to improve disease control and outreach in the region. As the rapid disease spread and ongoing outbreaks indicate, the Balkans is a high-risk area for ASF spread. As a sector in which backyard farms predominate, North Macedonia provides insight into this under-described community. An improved understanding of the practices of smallholder predominant sectors will help to further define the implications of these practices on risk for disease introduction and spread. Additionally, by defining the extent of high-risk practices, such as swill-feeding, and identifying gaps in current biosecurity practices, training for producers and veterinarians in the region can be tailored to address these vulnerabilities.

While the European Food Safety Authority (EFSA) has performed an ASF risk assessment in the region (EFSA Panel on Animal Health and Welfare (AHAW), 2019), some of the data collected in the producer survey described here revealed the extent to which some restrictions are or are not complied with. For example, swill feeding and scavenging are banned in North Macedonia, yet these practices were still reported by a small proportion of backyard producers. Again, this information can help direct the need for training and discussions on the risks of certain practices, despite the expectation that they are not being used. Further, the fact that high-risk practices continue in the face of regulations, suggests that additional discussions are warranted on what resources producers need to move away from these behaviors.

In countries with limited resources to focus on ASF prevention and control, a rationale to prioritize interventions is critical to optimally balance economic efficiency and the efficacy of control strategies. Chapter 1 used descriptive statistics to develop farm level biosecurity risk scores, defining which farms are at highest risk for disease introduction. Chapter 2 used network analysis to identify those premises with the highest rates of outgoing and incoming movements; using animal movement as a proxy for disease spread, these are that sites that should be targeted for improved biosecurity and increased surveillance respectively. Chapter 3 used agent-based modelling to highlight areas with the highest probability of ASF infection. Further, Chapter 3 evaluated the efficacy of intervention strategies, enabling the impact of these measures to be weighed against the costs. These approaches can be generalized to other countries to inform risk-based, economically efficient deployment of resources to best prevent and mitigate the impacts of disease.

Producer reported information was combined with live pig movement records to help contrast the localized trade reported by smallholders with the more frequent and long-range movements of larger production sites. Movement of live animals can be a good proxy for movement of infectious diseases. Using network analysis and modelling approaches to describe movement patterns, can help inform the ability to use zoning and compartmentalization strategies for disease control. By understanding the connectivity of the pig network, officials can more easily identify specific premises or trade patterns that could be interrupted to prevent disease spread. North Macedonia is already taking steps to achieve this with the closure of markets. The full impact of that change will require an updated analysis with more current data, however the data indicated that 2019's pig network was significantly more fragmented than that of previous years. North Macedonia's pork value chain provides insights into the network structures of other backyard-predominant swine sectors. In those regions with high proportions of backyard farms, trade is localized, and disease spread is expected to be limited. Larger range disease translocations will likely be coming from larger producers during the seasonal peaks in movement and through anthropogenic factors not captured in this study. North Macedonia's movement data provided another important insight. While no export of live pigs or pork products is officially reported (EFSA Panel on Animal Health and Welfare (AHAW), 2019), at least one instance in which pork products were moved across the border to Bulgaria was reported. This highlights the knowledge gap around illegal movement of pigs and pork products, known high risk activities for ASF spread.

The importance of addressing barriers to reporting is another critical message. Enhanced surveillance, and thus improved early detection, was able to reduce outbreak size by up to 80%. The value of early detection on limiting disease spread, and thus reducing economic losses, cannot be overemphasized. The role of producers and hunters in early reporting is critical. Over one-third of North Macedonian producers reported that "not knowing how" was a reason pig owners may not report ASF. This hurdle should be surmountable with improved communication and development of accessible resources. Not knowing how to report is an issue cited by producers in many countries. The recurrence of this barrier suggests the need for development of an easy-to-use reporting interface, that could be adapted across countries. Other reporting barriers included being uncertain about what happens after reporting, and resistance to the culling of pigs or restriction of the sale of pigs. Transparency about the critical role of reporting, and the expectations and support to be provided in the event of an outbreak, are critical to attaining buy-in and bolstering early detection capacity. Inclusion of these topics in any training should therefore by prioritized.

In collaboration with the Food and Agriculture Organization of the United Nations (FAO), North Macedonia has been making a large effort to improve their readiness for an ASF challenge. This study was able to report high levels of ASF awareness and trust in the veterinary community, suggesting that ongoing education campaigns and outreach activities have been successful. Evaluation of census records demonstrated improved identification and reporting of farms, which will improve the ability to reach target groups for interventions and enhance disease traceability in the face of an outbreak. These advancements should provide validation to the North Macedonian government of the impact of their efforts to date. However, this work has also highlighted gaps and provide risk-based guidance to target additional resources.

Each chapter of this dissertation identified the eastern regions of North Macedonia as being at high-risk for ASF introduction. This was a product of large populations of backyard farms, higher incidence of poor biosecurity practices, scavenging practices, and live pig movements in these regions. With ASF positive Serbia and Bulgaria to the north and west, these areas must be targeted for enhanced surveillance and fortification of biosecurity practices. Positive wild boar populations are also present in these bordering countries, suggesting this as a likely pathway of introduction. Enhancement of carcass reporting through organized searches, and the improvement of relations with hunters and other people who regularly access the landscape, are highly suggested.

Overall, this work has provided an in-depth description of the North Macedonian pig sector and highlighted farms, practices, and regions at high risk for ASF introduction. This information will be vital to informing risk-based, cost-effective prevention, surveillance, and control strategies in the country and region. More broadly, this work outlined a framework from which to approach describing a population of animals and the tools that can be applied in assessing the drivers of risk for disease introduction and spread in new countries and for additional diseases, particularly in backyard predominant settings.

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