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RISK ASSESSMENT OF THE INTRODUCTION OF AFRICAN SWINE FEVER FROM HUNGARY TO AUSTRIA VIA WILD BOAR

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Abbreviations

ASF – African Swine Fever

ASFV – African Swine Fever Virus

CLC – Corine Land Cover

DNA – Deoxyribonucleic acid

EFSA – European Food Safety Authority

EU – European Union

FASFC – The Federal Agency for the Safety of the Food Chain

GF-TADs – Global Framework for the Progressive Control of Transboundary Animal Diseases

LAU – Local Administrative Unit

OIE – The World Health Organisation for Animal Health (Office International des Epizooties)

PCR – Polymerase Chain Reaction

WAHIS – World Animal Health Information System

Introduction

African Swine Fever (ASF) is a viral disease of domestic swine caused by the African Swine Fever Virus (ASFV). The disease is characterised by haemorrhagic fever with high mortality in affected domestic and feral pig populations (Galindo and Alonso, 2017). A mortality of up to 100% can be observed in peracute and acute forms of the disease, whereas the chronic and subclinical form shows decreased mortality, while being much rarer (Sánchez-Vizcaíno *et al.*, 2015). ASF is a highly contagious disease, mainly transmitted via direct contact, contaminated feed as well as ticks of the *Ornithodoros* genus acting as a virus reservoir (Beltrán-Alcrudo *et al.*, 2009; EFSA, 2015). The virus is spread to different populations within and across borders by transportation of infected animals, animal products or fodder (Cwynar *et al.*, 2019).

The disease first appeared in Kenya in 1909 where the term "East African Swine Fever" was later coined. Already back then a potential link between wild living suids, warthogs (*phacochoerinae*) and appearing infections was drawn as a high density of warthogs was found close to the spread out farms (Eustace Montgomery, 1921). In 1957 ASFV arrived in Europe the first country being Portugal but the virus quickly spread further east to other European countries such as Spain where the virus persisted in backyard farms due to suboptimal biosafety measures, an uncontrolled wild boar population and *Ornithodoros* ticks acting as reservoirs. Spain was only declared free off the disease in 1995. The disease spread further to France, Italy and Malta where it was kept in check with the utilisation of measures such as wild boar hunting and stamping out of infected domestic pig herds.

The exception was the island of Sardinia where ASF remains endemic to this day. This may be due to the widespread prevalence of extensive keeping systems combined with close contact to wild boars (Firinu and Scarano, 1988; Cwynar *et al.*, 2019).

The second spread of ASFV in Europe was first noticed in Georgia in 2007. Upon the confirmation that domestic pigs were indeed infected with the ASFV and not with a respiratory circovirus as was previously believed, authorities immediately implemented disease-control methods. ASFV likely travelled to Europe via contaminated animal products (Cwynar *et al.*, 2019).

From there the virus quickly spread to the Russian federation. Many rivers flowing from the affected area, which are often where free roaming boars are to be found, were a contributing geographical factor in the movement of the disease (Gogin *et al.*, 2013). The first infected domestic pig populations were reported in North Ossetia in 2008. Within two years 177 outbreaks were reported. As outbreaks have been reported consistently for over three years the area is now deemed as being endemically infected with ASF (Oganesyan *et al.*, 2013).

ASFV further spread to the Ukraine, Belarus, Lithuania and Latvia as well as Estonia before arriving in Hungary (Cwynar *et al.*, 2019). The first case of Hungary was found in the form of a dead wild boar on the 19th of April 2018 in Heves county. People travelling to the country from the Ukraine to work were likely responsible for the spread. In the following months ASF was detected close to the Ukranian border in Szabolcs-Szatmár-Bereg county and the neighbouring county Borsod-Abaúj-Zemplén (Cwynar *et al.*, 2019). As of 2019, according to the WAHIS-Interface of the OIE, ASF has spread as far west as the Pest county, with many outbreaks still unresolved. Every ASF outbreak reported in Hungary up to this day has been in a wild boar population (OIE, 2020b).

The fact that the disease is limited to wild suids in Hungary proposes certain epidemiological dynamics. This means that wide jumps of the disease where the virus suddenly appears across a large distance as it happened in Sardinia in 1978 and Georgia 2007 are less likely. Wild boars are to a large part responsible for the current situation in Hungary and several Caucasus nations (Boklund *et al.*, 2018; Sánchez-Cordón *et al.*, 2019).

In the year 2019 there were almost 2,7 million pigs being kept in Austria (Neumann, 2019). Currently there has never been a report of a case of ASF in Austria, "Pure Luck" according to Thomas Mettenleiter, President of the Friedrich-Loeffler-Institute. Knowing that the current epidemic that started in Georgia in 2007 spread to neighbouring country via wild boar, it is paramount to stop the spread of the disease, the stamping out of many herds of swine and the devastating blow to the economy by trying to use the knowledge that was gained over the previous decade. To use the measures available effectively the most likely location of occurrence should be predicted. If the spread to Austria is inevitable like many experts claim, where will we be able to find the virus if it is carried by wild boar and in which regions should Austrian swine farmers be especially cautious?

Literature Review

The Disease

Viral characteristics

ASF is a double stranded DNA virus and the only member of the "Asfarviridae" virus family (Sánchez-Vizcaíno *et al.*, 2015). The virus causes chronic, inapparent infection in African warthogs and other wild pigs as well as ticks occurring in Africa, allowing these species to act as reservoirs for the virus. The sylvatic cycle between infected ticks and wild or domestic pigs is the reason the disease has been endemic in several African countries since it was first described (Galindo-Cardiel *et al.*, 2013).

Clinical picture

Unlike their African counterpart, the European wild suids such as "Sus scrofa" as well as domestic pigs, often exhibit very severe haemorrhagic clinical signs. After an infection has taken place the virus replicates in phagocytic cells, cells lining the endothelium and others.

Galindo-Cardiel et al. explain how the infected cells lead to the appearance of symptoms:

"Infected macrophages can release inflammatory mediators like TNF-alpha, IL-1alpha and IL-6, which in turn cause severe injury to endothelial cells. This damage may provoke an increase in vascular permeability and haemostatic alterations, leading to disseminated intravascular coagulation (DIC), coagulation factors consumption, fibrinolysis activation and, subsequently, extensive haemorrhage."(Galindo-Cardiel *et al.*, 2013)

Of course, the clinical presentation of an infection will take different forms in different individuals depending on their condition and immune status as well as the infective dose and route of infection (Sánchez-Vizcaíno *et al.*, 2015). Apart from that different ASFV strains are also classified by virulence. Highly virulent strains are associated with per-acute and acute courses of the disease meaning infected individuals die with one to eight days post infection and the clinical signs develop within 7 days in the case of an acute infection. Moderately virulent strains are involved in the acute and sub-acute form of the disease which develops over 10-20 days. Lower virulence strains of ASFV, inducing a chronic form of ASF, have only been described in areas where the disease had been endemic for some time (Mebus and Dardiri, 1979).

Peracute course of ASF is very quick with affected pigs usually perishing suddenly after 1-4 days after symptoms appear. These include high fever, inappetence, reluctance to move, hyperpnoea and reddening of the skin. Typical post-mortem lesions are frequently absent (Sánchez-Vizcaíno *et al.*, 2015).

The most common form that occurs in naïve populations of domestic pigs is the acute one. The symptoms seen in these animals are high fever with body temperatures of 40-42°C, depression, inappetence. Many animals will develop a change in colour of the skin on several body regions such as: the ears, snout, abdomen, limbs and tail. This discolouration is due to cyanosis as well as potential skin lesions combined with petechial bleedings and/or ecchymosis. Pulmonary oedema form resulting in respiratory distress and nasal discharge (Salguero *et al.*, 2002). Pigs may also suffer from vomiting and diarrhoea, the latter potentially mixed with blood in the faeces. Pregnant sows are prone to abort if infected. As mentioned above the mortality rate may reach 100% (Salguero, 2020).

Congruent with the clinical symptoms of subcutaneous haemorrhagic disorders, the post-mortem lesions, that are the most characteristic for the disease, are the bleeding related signs. Haemorrhagic splenomegaly with a spleen that is larger than normal as well as darker in colour is often cited. Followed by multifocal haemorrhagic lymphadenitis. The lymph nodes take on a marbled appearance. Haemorrhages may be found on several organs such as lymph nodes, small and large intestine, the epicardium, the bladder and in form of petechial bleeding on the surface of the kidney (Salguero, 2020).

The subacute form of the disease has a very similar course as the acute form but the clinical signs don't appear to be as severe (Sánchez-Vizcaíno *et al.*, 2015). Mortality rates reach from 30 to 70% over 7 to 20 days post infection. In these cases less the haemorrhagic but the oedematous changes are prevalent with lesions such as ascites, multifocal oedema and hydropericardium. The typical splenomegaly may still be present however often times the spleen is only partially enlarged while leaving other areas of the organ unaffected. In general the post mortem lesions are largely similar (Salguero, 2020).

Due to being infected by the rare low virulence strains of ASFV, pigs may suffer from a chronic version of ASF. Surprisingly even though ASFV has been endemic in several regions only the Iberian Peninsula is home to this form of the disease (Giammarioli *et al.*, 2011; Sánchez-Vizcaíno *et al.*, 2015). Skin necrosis as well as arthritis characterizes chronic ASF. Dyspnoea and abortion are still possible signs, however no vascular changes are observed and most lesions inspected after the animal has passed are mostly due to secondary bacterial infection (Salguero, 2020).

Route of infection

If infection occurs the susceptible pigs usually become infectious after three to five days after inoculation (Guinat *et al.*, 2016).

Concerning the route of infection, it was found that, among domestic pigs, direct contact was the most important factor in spreading the disease fast. If housed together with infected individuals, susceptible pigs also became infected within one to nine days post-exposure. Even if solid separators were used to keep the animals apart infection usually occurred between six to 15 days post-exposure (Guinat *et al.*, 2016). However, a study from the year 2019 showed, that not only direct contact was responsible for the fast spread of the virus within a herd, but that feed and drink which had not been contaminated with infected pork -meat or -product was also, quite easily, able to infect the animals if inoculated with the *Georgia 2007* strain (Niederwerder *et al.*, 2019). While the route of direct transmission may be important for the fast spread within a herd, what is more worrying on an international level is that the disease can travel vast distances if carried within contaminated pork products. The virus can survive for months in the meat, fat and other tissues of slaughtered pork. It even survives the processing into for example sausage. Domestic pigs being fed swill, a common practice in many backyard farms across the world, and wild boars which come in contact with trash or disposed foods that contain infected products, infect themselves with the virus that was carried over hundreds of kilometres and bring about a new cluster of ASF cases (Gogin *et al.*, 2013; Guinat *et al.*, 2016).

Treatment

Presently there exists no treatment for an infection with ASF. Preventive measures are limited to enforcing hygiene rules on farms, movement restrictions and sanitary handling of pork products as a vaccine against ASF is currently not available. Diagnosis of the disease is made on the basis of clinical signs and detection of specific antibodies indicating that an infection had already taken place. Clinical signs are not pathognomonic and often similar to other diseases such as classical swine fever, making it more difficult to accurately detect ASF in affected livestock. Eradication of the disease is based on the removal of seropositive herds. A successful eradication was accomplished in the Iberian Peninsula in 1990 where quick serodiagnosis and culling of infected animals proved to be essential in getting rid of the disease (Cubillos *et al.*, 2013).

The wild Boar

The boar in Europe

The wild boar (*Sus scrofa*), an even toed ungulate belonging to the family of suidae, is one of the most wide-ranging mammals on earth and the ancestor of the domestic pig (Chen *et al.*, 2007). They can live and thrive in a large variety of habitats ranging from deserts to forests, taigas and alpine regions. 16 different subspecies have been derived from *Sus scrofa* each of them occupying according distinct geographical areas. Likely originating from South-East-Asia, the wild boar is now found in virtually all corners of the earth from western Europe to Siberia and Japan (Chen *et al.*, 2007; Massei *et al.*, 2015). In the last few years wild boar activity has sprung up again in countries such as Sweden, Finland, Estonia, Norway and many others. During the past few decades, the number of wild boars has increased steadily. (Fig.1) This is due to *Sus scrofa* having a very high reproductive potential with their annual population growth rate sometimes exceeding 2.0. Natural causes of death include starvation and predation, however these causes of mortality seem to not be as significant anymore if compared to traffic accidents but mostly hunting as the predominant controlling factor of boar populations nowadays (Bieber and Ruf, 2005). In several European countries the number of active hunters is decreasing, potentially leading to a larger survival rate of wild boar. (Fig.2)

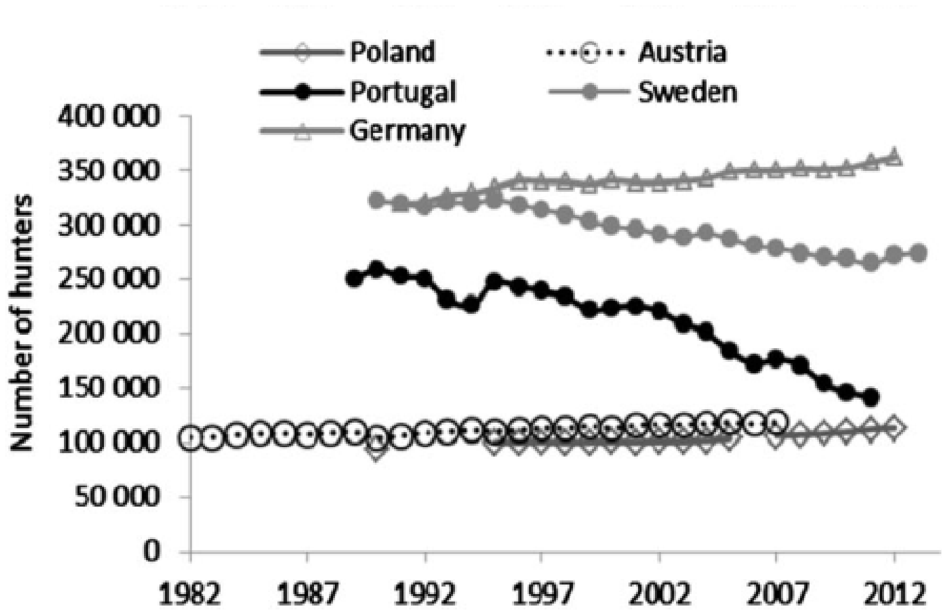

Figure 1 Number of hunters in selected European countries

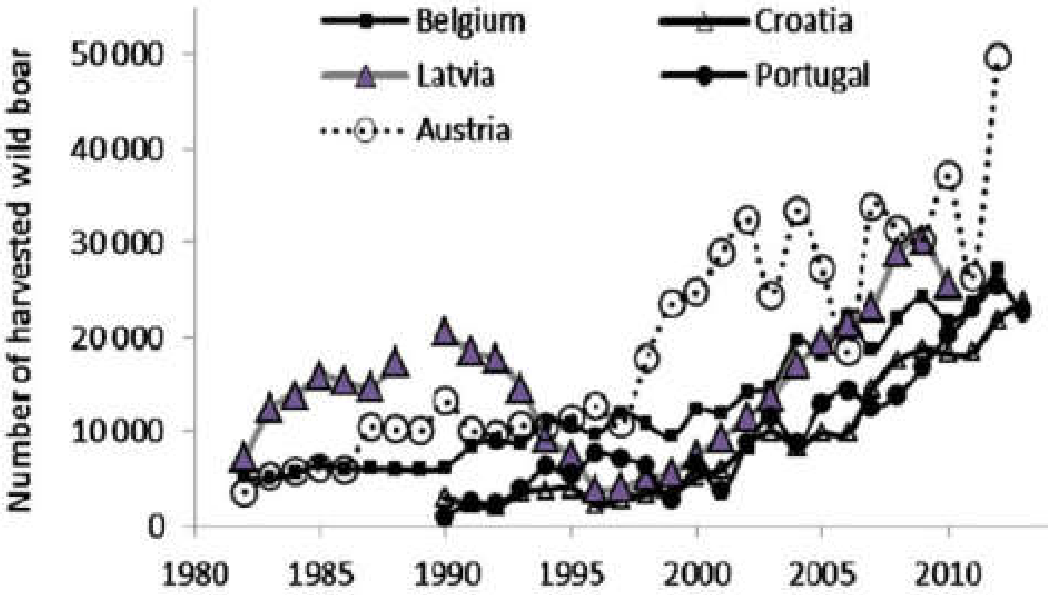

Figure 2 Trends in the number of harvested wild boar in selected European countries

For Austria specifically the number of boars has increased sharply while the number of hunters remained about the same, making the question of how dangerous wild boars are for the introduction of ASF into local pig farms very important.

ASF in wild boar

Concerning the clinical picture and pathology of ASF in wild boar, it can be said, that due to the taxonomic kinship the clinical picture is similar to that of the domestic pig described above. The risk of death if infected is similarly at 90-100%. Neither in the acute nor subacute course of the disease was there enough of a difference in the clinical presentations to warrant a distinction between domestic and wild suid. In regards to pathology, wild boars also display hemorrhagic splenomegaly, lymphadenitis and multifocal petechial bleedings (Salguero, 2020). Some studies describe these lesions as even more severe than domestic pigs (Sánchez-Cordón *et al.*, 2019).

The danger lies in wild boar infected with a low virulent isolate surviving the infection and acting as a carrier and potential source of infection for months.

The spread in 2007 across Europe

Georgia

As touched upon in the introduction the ongoing epidemic of ASFV sweeping through several continents in the previous years got its' start in Georgia in 2007. Despite the Georgian authorities responding quickly to the newly confirmed occurrence of the disease, over 60000 pigs were infected in the first two months of its emergence and the infected herds were stamped out (Cwynar *et al.*, 2019). The specific origin point of the Georgian 2007 strain of ASFV is unknown, although some research points to the origin being on the African continent like Rebecca J. Rowlands et al. found in their study

" ...DNA fragments amplified from the genome of the isolates from domestic pigs in Georgia in 2007 were sequenced and compared with other ASF virus (ASFV) isolates … the Georgia 2007 isolate is closely related to isolates belonging to genotype II, which is circulating in Mozambique, Madagascar, and Zambia." (Rowlands *et al.*, 2008)

ASFV is still considered endemic in Georgia as the authorities did not manage to eradicate the disease (Cwynar *et al.*, 2019).

The Russian Federation

In November of 2007, The Russian Federation identified its' first case of ASFV in the 21st century, when five deceased wild boars were found along the Argoun and the Shatoy-Argoun rivers, bordering Georgia. Even though the origin of the outbreak could not be determined, it is likely that the appearance of the virus in Russia is linked to the outbreak in Georgia. In June 2008 the disease was first diagnosed in domestic pigs. In 2009, domestic, backyard pigs and wild boars were diagnosed with the disease hailing from Stavropol and Krasnodar Krays before an outbreak occurred in Mga Village in October of 2009. This village was located 200km to the north from where the previously affected areas had been (Beltrán-Alcrudo *et al.*, 2009). Until February 2009 52 ASF outbreaks were recorded (Cwynar *et al.*, 2019). In 2011 the disease reached St. Petersburg, Murmansk and Archangel.

20 outbreaks have been reported from these areas since (Lange *et al.*, 2014). In the following years, ASF travelled to the southern Russian region of Krasnodarskiy and the western regions Smolensk and Pskov close to the Belarusian border.

Until 2016 110 outbreaks of ASF had been reported to the OIE by the Russian Federation (Gulenkin *et al.*, 2011).

Ukraine

In the Ukraine, the death of three domestic backyard farm pigs, on July 30. 2012, in the Zaporozhye region, was the first ASF outbreak reported to the OIE (Cwynar *et al.*, 2019).

After the disease was also detected in wild boar close to the Russian border, measures were taken to stop the spread. Within a radius of 10 kilometres all backyard pigs were slaughtered and three villages were placed under quarantine (EFSA, 2014).

The last outbreak in the Ukraine was reported on a backyard farm in 2016. The disease is currently limited to one or more zones according to the WAHIS interface of the OIE (OIE, 2020b).

Belarus

The first ASF case in Belarus was reported on June 21. 2013 in domestic pigs being kept in a backyard farm in Grodno region (EFSA, 2014). Only several days later but 400 kilometres away from the first outbreak, in the Vitebsk region, the second case was reported. The disease is currently absent in the country (OIE, 2020b).

Lithuania

In January 2014 Lithuania's State Food and Veterinary Service confirmed that an ASF outbreak had taken place in the Varena district, close to the Belarusian border. The isolate sampled at the Lithuanian outbreaks was proven by the European Union Reference Laboratory for African Swine Fever to be 100% homologous with the ASFV form implicated in the 2013 outbreak in Grodno, Belarus.

A large commercial pig farm, home to over 19 thousand pigs, reported an outbreak of ASF. Despite the high biosecurity level of the farm, 18 pigs perished in the weaner unit, and the farmer reported suspicion of a contagious disease. ASFV was likely introduced onto the farm through human involvement (EFSA, 2015). In total 821 outbreaks in Lithuania have been reported to the OIE. The disease is currently present in wild as well as domestic pigs. The situation is described as "sufficiently stable" by the WAHIS Interface of the OIE (OIE, 2017).

Poland

Just a month after the first outbreak in Lithuania, in February 2014, Poland reported the country's first occurrence of ASFV as the virus was identified in a DNA sample of a wild boar carcass. The boar had been found just under a kilometre from the Belarusian border. After the confirmation of another ASF positive dead wild boar, 623 blood samples from domestic pigs on 118 farms were examined. All samples turned out negative. To limit the spread of the disease, the Polish government implemented an ASF monitoring program with special attention on the buffer zones close to the Belarusian and Lithuanian borders. On top of that large awareness campaigns were put in place (Ja *et al.*, 2014).

Despite the effort of the authorities the disease continued to spread and on 23 July 2014 the first ASF outbreak in domestic pigs was reported. ASF cases became a common occurrence in 2014 as the virus seemed to spread fast among the wild boar population. The largest part of cases was found in Podlaskie Voivodeship, this region has since been continuously monitored to prevent the spread to regions with an even higher wild boar density such as Lubelskie and Warminsko-Mazurskie. But again, efforts proved to be ineffective as ASF outbreaks were reported in neighbouring voivodeships such as Lubelskie and Mazowieckie (Cwynar *et al.*, 2019).

In total there have been 613 confirmed outbreaks of ASF in Poland. The disease continues to spread as many outbreaks have not been resolved yet (Fig.3). For now, ASFV seems to be confined to the eastern part of Poland.

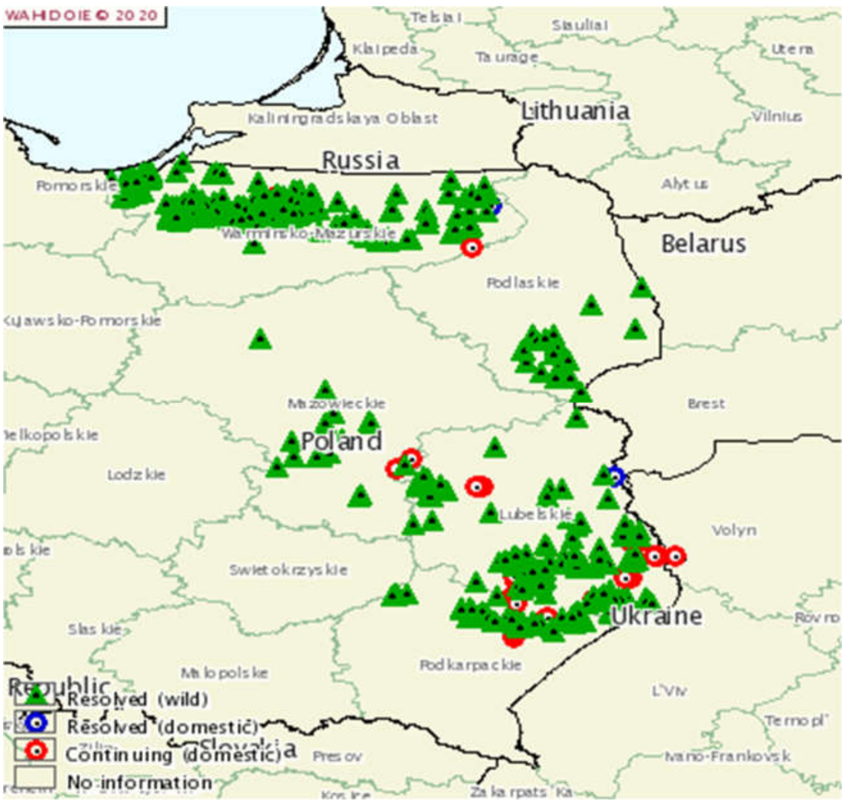

Figure 3 ASF Situation in Poland 2020 (WAHIS Interface, OIE)

Latvia

Starting on 25 June 2014 Latvia experienced a rapid spread of ASF when three dead wild boars close to the Belarusian border were confirmed to having been infected. The following day, Latvias' National Reference Laboratory was able to confirm three cases of ASF in backyard farm domestic pigs. In July the disease had already made its way to Madona region, in the central part of Latvia (Cwynar *et al.*, 2019).

Almost all outbreaks were reported by the owners of the affected animals at an early stage of infection. This may have been helpful in preventing an even larger spread within the domestic pig populations. The largest infected farm housed 196 pigs. ASFV spread faster in Latvia when compared to the spread in other countries. Possible reasons include Latvia's location, as it borders countries that had also been affected by the disease. Also using kitchen waste (swill feeding) as a source of food for the pigs and uncontrolled movement of people having backyard farms were listed as contributing factors (EFSA, 2015).

The disease is currently present in wild boar and domestic pigs (OIE, 2020b).

Estonia

In Estonia, the spread of ASF appeared more sporadic and at locations not as close to each other. The first report of ASFV came on 2 September 2014 in Vlaga region 6 kilometres from the Latvian border. The second and third case, both in wild boar, were located 30 and 180 kilometres respectively from the location of the first case (EFSA, 2015).

The disease is absent from domestic pig populations as of 2018 and is currently present in wild boar (OIE, 2020b).

The Czech Republic

In the Czech Republic, two dead wild boars infected with ASFV were found in June 2017. The location was Zlín City in the Zlín district.

The question of how the virus was introduced is quickly posed as the distance to the closest outbreak at the time was over 450 kilometres away in Poland. A potential introduction might have come in the form of a container transport depot under four kilometres from where the carcass was found.

The Czech Republic had been testing any wild boar found dead since 2011. In total 230 cases of ASF were reported from the primary outbreak to the last. All of the cases reported in the Czech Republic have been confined to the Zlín district and to wild boars. There was no infection of domestic pigs. With the help of measures being outlined in a later chapter, the country managed to eradicate the disease and is free of ASFV as of March 2019. This has been confirmed by the European Commission (Miteva *et al.*, 2020) .

Romania

Romania came in contact with the virus in July 2017, when two outbreaks were reported in domestic pigs in Satu Mare County. Interestingly, the disease spread from and to different directions with contrasting dynamics. While the south-eastern areas suffered through a rapid spread with a large number of affected farms, the north-western regions where the first case had been reported did not see such an intense spread.

In Romania, contrary to other countries the worse affected population was that of domestic pigs instead of wild boar. By October 2018, 155 cases of ASF in wild boar had been confirmed while the number of outbreaks in domestic pigs was much higher at 1073 (Cwynar *et al.*, 2019). In 2019 ASFV had been spreading fast close to the Danube and the cases in wild boar increased. According to the EFSA report however, humans still seem to be the largest threat of spread:

"Human-mediated spread, however, is still considered as the main risk factor. The low level of biosecurity in backyard farms and the traditional particularities of pig keeping in Romania have facilitated the introduction of ASF in many backyard farms over a short period of time. It is assumed that due to the high infection pressure of the environment, combined with possible breaches in biosecurity, ASF was also introduced in commercial farms" (Miteva *et al.*, 2020).

Bulgaria

In Bulgaria, the first ASF outbreak happened in a small pig farm in August of 2018 in the Varna Region. Throughout 2018 the number of cases kept relatively low. However, in 2019 cases started to appear all over the country. Eight industrial pig farms, three smaller farms, 25 backyard farms and five East Balkan pig farms suffered from infected individuals. 1,420,000 pigs were stamped out in response to the outbreaks. An epidemiological inquiry was conducted on the backyard farms were the primary outbreak had taken place.

According to the EFSA, again, human involvement seems to be the most likely way of spread in this particular case as the holding owner had travelled to affected countries and had visits of a hunter and a veterinary assistant as well as contaminated vehicles (Miteva *et al.*, 2020). In total 222 outbreaks have been recorded all over Bulgaria (OIE, 2019).

Moldova

Moldova experienced its' primary outbreak of ASF in form of two affected backyard farms in Donduseni, the northern part of the country, in July of 2018.

Though all of the active cases were resolved quickly, the disease remains in the country, in wild boar as well as domestic pigs, although limited to one or more zones (Cwynar *et al.*, 2019).

Belgium

Belgium is located hundreds of kilometres away from the closest ASF affected country. Nevertheless, in 2018, when a huntress discovered remains of a dead wild boar and a forester went to examine the area where the bones had been found, instead found a dead adult female boar, as well as a weak, clearly ill young wild boar, which was subsequently euthanized and both of the carcasses were examined, they tested positive for ASFV. After the notification of the cases on 13. of September, the samples were also sent to the European Reference Laboratory for gene typing.

"On 14 September, the EURL confirmed the ASF cases and identified the strain based on the sequence of the p72 protein as p72 genotype ll that had been circulating in eastern European countries since the first introduction in Georgia in 2007. Further subtyping throughout the analysis of three independent ASFV genome regions, clustered the Belgium wild boar viruses within the CVR-I, IGR-2 and MGF1 variants. From the data available at the EURL, these are the variants mostly circulating within the EU countries as well as described in Moldova, Ukraine, Belarus, and in certain areas of the Russia Federation." (Boklund *et al.*, 2018)

The EFSA ASF report of 2020 speculates that, since the disease jumped over 1000 kilometres, human activity was the cause for the introduction of ASFV. The origin of the outbreak is still unknown.

Fortunately, the course of the disease in Belgium has so far been constricted to wild boar, domestic pigs have not yet been affected. To keep it that way, preventive measures have been put into place by the federal authorities. Domestic pigs and captive wild pigs have been stamped out preventively in the "infected zone" spanning over 630 km^2. 5222 pigs were eliminated in this action according to the FASFC. The preliminary "infected zone" was replaced by zone I and zone II, the former being the area around zone II where no cases have been reported and the latter defining the area where ASF had been detected in wild boar only. Regional operational zoning was further implemented to allow the authorities to act on control methods quicker. Carcass searching and fencing as well as depopulation of wild boar via hunting and trapping were also utilised methods to keep the disease in check.

The measures seem to show some effect as all of the 827 ASFV positive wild boars have been found in the infected zone. Different surveillance methods, such as active analysis of hunted boars as well as passive carcass removal and subsequent analysis, coupled with stringent biosecurity rules in place, are being maintained (Miteva *et al.*, 2020).

Germany

At the time of writing, it has only been several days since Germany reported the first occurrence of ASFV in the countries' history. On 10. September 2020, the infection of the remains of a wild boar located in Brandenburg in the Spree-Neiße-Kreis close to the border with Poland, was reported. Since the carcass of the boar was very decomposed the animal may have lain there for quite some time (REUTERS, 2020b).

Five more cases of ASF in wild boar were reported on 15. September in Brandenburg, close to the primary finding place. Among other countries China, the largest importer of German pork products, has declared an embargo on said wares (REUTERS, 2020a).

Hungary

Hungary confirmed the first case of ASFV on April 21. 2018 via a PCR test done by the National Reference Laboratory of the sample of a wild boar in Heves county. May and October of 2018 the disease was then found in Szabolcs-Szatmár-Bereg county as well as Borsod-Abaúj-Zemplén and Nógrád county, respectively. The wild boar found in Szabolcs-Szatmár-Bereg county was only one kilometre from the border to Ukraine. Sequencing the strain revealed that it shared 99-100% identity with the 2007 Georgia strain. In 2019 ASFV spread further to Hajdú-Bihar county, while numerous cases of dead wild boars infected with the virus were reported in the already affected counties (Boklund *et al.*, 2018; Cwynar *et al.*, 2019; Miteva *et al.*, 2020) (Fig.4).

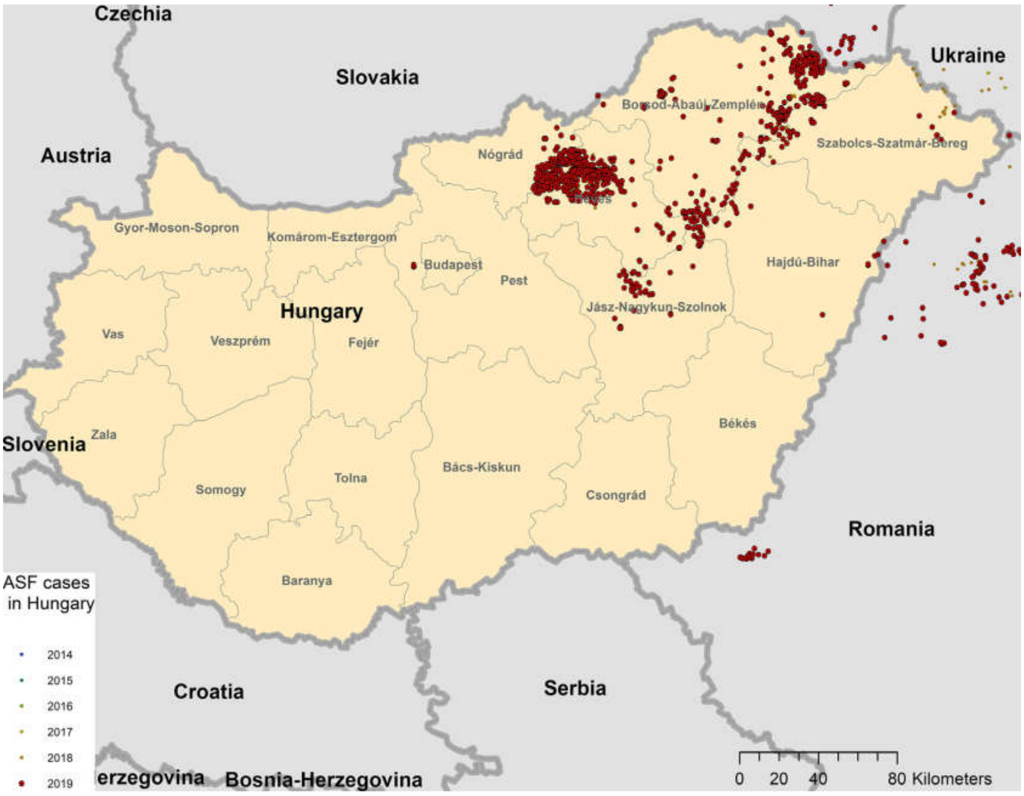

Figure 4 ASF cases in wild boar reported to the ADNS in Hungary (31 July 2019 to 31 October 2019)

Asia

As the aim of this thesis is to better understand the epidemiological situation of ASF in Europe, an overview of the status of the epidemic in Asia should still be given.

Except for the Russian Federation, no countries in Asia had come in contact with ASFV prior to August 2018, when the People's Republic of China reported its first ASF case. The disease quickly spread to all regions of Mainland China. In 2019 ASFV continued its spread to several other Asian countries. In January, Mongolia announced its primary outbreak, Vietnam in February, Cambodia in March, North Korea in May, Laos in June, the Philippines in July, Myanmar in August, South Korea and Timor Leste in September and finally, Indonesia in December (Fig.5) (Lu *et al.*, 2020; OIE, 2020a).

In India ASF hit in 2020 in the North Eastern Part of the country, the state Assam. The disease killed 14000 pigs across 306 different villages. It is believed that the virus was brought into the country by carcasses floating down the Brahmaputra river from China. Despite the common reactionary measure in other countries primary outbreaks having been the culling of domestic pigs in the affected area, Animal Husbandry Minister: Atul Bora said:

"We have discussed with experts if we can save the pigs without culling them. The death percentage of the pigs affected by the disease is almost 100 per cent. So we have made some strategies to save the pigs, which are not affected by the virus,"(Bhattacharyya, 2020; The Economic Times, 2020).

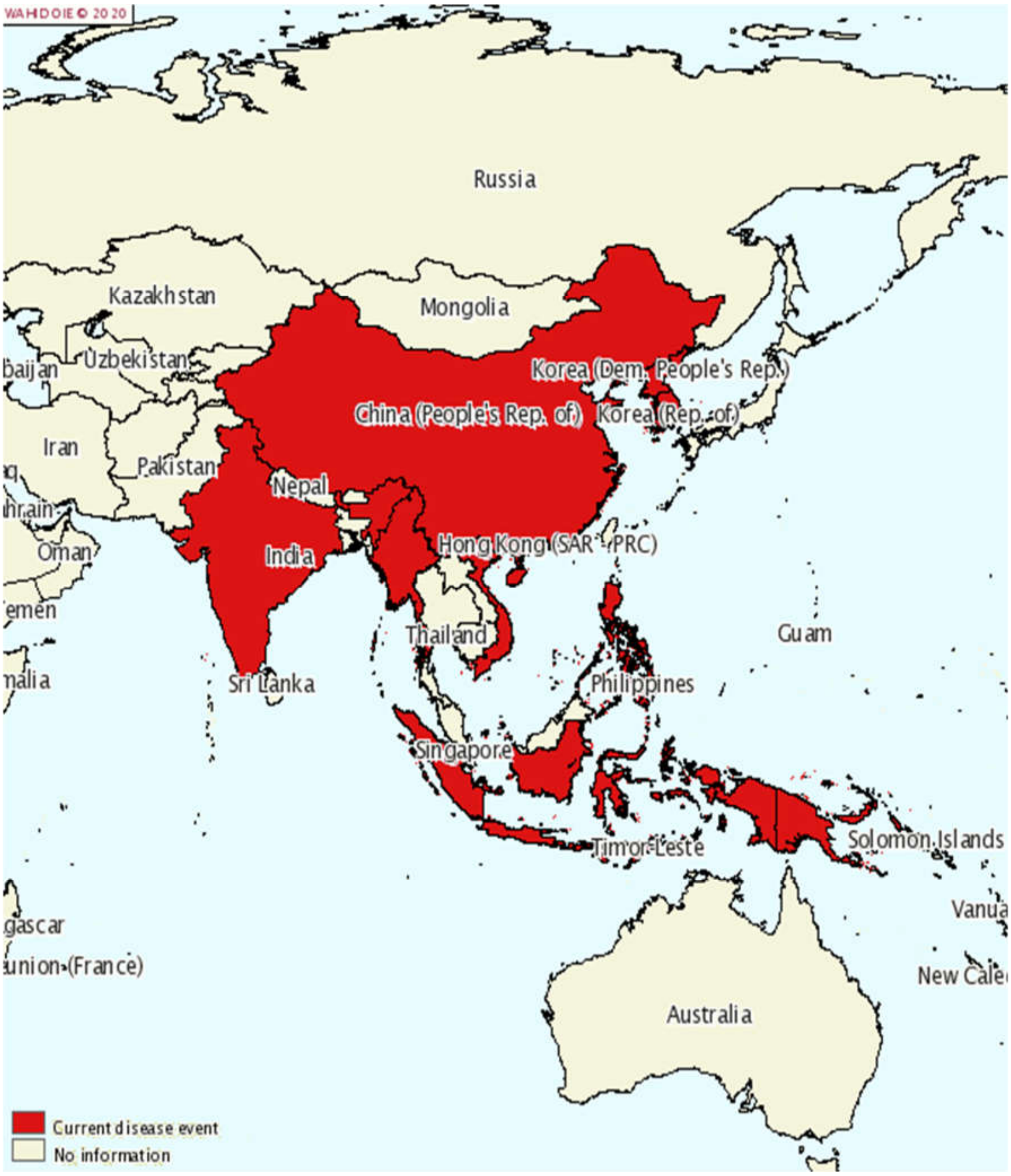

Figure 5 Spread of ASF in Asia from Jan.-Jun. 2020

Measures

ASFV is a great threat to the global pork productions at large as well as the lives of billions of pigs, be they in small scale or industrial farms. To minimise the spread and reduce harm to the economy different control measures have been implemented by countries facing the disease.

Measures in Domestic Pigs

National health authorities are usually tasked with creating a plan to help combat the spread of the disease in their respective nation. But, of course, an issue this large is multifaceted and several stakeholders in the pork industry will be interested in the strategies applied. These often feature biosecurity safety regulations, surveillance protocols as well as plans of action in case of a new outbreak.

Socio-economic factors and cultural factors are often not fully appreciated when it comes to implementation of measures which might lead to decreased effectiveness and the further spread within the country (DIxon *et al.*, 2020). The spread of ASF in Romania could be an example of this, where potential biosecurity breaches and traditional particularities of pork production combined with a high number of backyard farms was a bad environment for control measures (Boklund *et al.*, 2018; Miteva *et al.*, 2020). Also the fact that "emergency sales" often happen when farmers feel the need to minimise their losses when part of their livestock becomes infected with the disease (Costard *et al.*, 2015).

As implemented measures are often tied to renumeration of farmers, wealthier countries are often in a better position to tackle the problem of the pandemic (DIxon *et al.*, 2020). This is further shown in the fact that during the spread of ASFV in Eastern Europe, spill-over from wild boar played a primary role in disease spread. Meaning a severe lack in biosecurity must be present in a large percentage of pig farms in order for wild boars to have contact with domestic pigs enough to transmit the virus (FAO, 2009; Reyna-Hurtado *et al.*, 2014; Jurado *et al.*, 2018).

Of course, a large emphasis should be put onto preventing the disease from entering a country in the first place. Bio-security guidelines must be in place for all kinds of pig farms. A guideline for minimum biosecurity measures exists, brought upon by the EU. Jurado et al. conducted an extensive systematic literature review on the topic and highlights three forms of measure that prove particularly important in trying to stop ASF.

The first one being the control of entries into the farm, making sure that pigs coming into the farm are in a healthy state as well as keeping of records, and identification papers. Pigs also should be contained in quarantine rooms for a set amount of time after arriving at the farm. These principles are of high importance in commercial, non-commercial and backyard farms (Jurado *et al.*, 2018).

Second, the review mentions the feed of the pigs as a possible source of infection. Swill feeding is banned in the EU since 2002 as a response to the Foot and Mouth Disease epidemic in the United Kingdom. Experts agree that this measure remains an important one in the fight against the spread of ASF. Furthermore, regulations about food for workers in commercial farms should be in place, manure as well as dead pigs should be disposed of properly and fresh fodder should not be given to pigs if the area is at risk of ASF (Jurado *et al.*, 2018).

A failure to comply with the measures mentioned above is often attributable to a lack in understanding of the transmission mechanics of ASFV and/or a not stringent enough enforcement of the rules imposed by national authorities. As such, a core tenet of the measures, reviewed by experts, is the improvement of health services and educational campaigns with a focus on ASF. Of course, socioeconomic, cultural and traditional factors will influence how proposed measures will be implemented and how well actors in the pig industry will respond to them. Farmers and workers are most affected in their way of working by biosecurity measures. The success of the measures will depend on their willingness to adapt them. It was shown that during outbreaks in Asia and Europe farmers would often only report the disease after two weeks had passed since the first case due to not recognising the symptoms of ASF in single animals (DIxon *et al.*, 2020). Awareness must be raised and farmers, hunters and veterinarians should be familiarised with the protocol of early warning and surveillance systems (Jurado *et al.*, 2018).

To combat an outbreak as effectively as possible, an early detection surveillance system must be implemented. As the virus can be maintained in the local wild boar population as well as in processed pork products, early detection as possible is paramount for the chance of eradication.

Passive surveillance is carried out through parties in the pork industry such as farmers or veterinarians performing pre-slaughter inspections, who can observe the pigs and report potential infection.

Active surveillance comes in the form of diagnostic testing of newly acquired livestock or dead pigs. In the Republic of China routine testing for the virus is already in place in slaughterhouses, where a selection from every slaughter batch is being examined, as well as in large commercial pig farms. Unfortunately no data is available on the utility of these approaches yet (DIxon *et al.*, 2020).

Should an outbreak occur despite early surveillance systems, a fast response is vital. Forward and backward tracing is important to make out the origin of the primary infection. However, in a highly complex system, such as the pork industry, this tracing is often very difficult to perform. In recent years, countries which suffered through outbreaks of ASF have implemented protection and surveillance zones around the occurring disease events. These along with the active search for, for example, wild boar carcasses are important first responses to an outbreak of ASF. The decision of whether to stamp out pig herds pre-emptively is often very hard to make. Again, Eco-social considerations must be made in order to not worsen the problem via, for example, farmers moving their animals before they can be culled, spreading the disease further (DIxon *et al.*, 2020).

Measures in Wild Boar

In the European Union, most outbreaks since 2007 have occurred via introduction of the virus through wild boar. As such measures to halt the epidemic must not be restricted on the domestic pig sector but expanded to the wild boar population of the respective nation.

Examples can be seen in the outbreak events of the Czech Republic and Belgium both of which could be contained and never crossed over to the domestic pig. In both cases the outbreaks were brought under control relatively quick. This was achieved through several strategies. Zones were set up categorising the affected area in infected, control and buffer zones. In the Czech Republic at-risk areas were surrounded with fences. Different measures were applied in the corresponding zones, such as a ban on hunting and feeding. Depopulation of the wild boar population was performed to decrease the risk of natural spread within the virus reservoir (DIxon *et al.*, 2020).

Of course, the group that naturally comes in contact with wild boar the most is that of the hunters. Control measures should also be extended to their field and minimum biosecurity guidelines should be followed in order to ensure the proper response to a potentially infected found wild boar and to not spread the virus further through improper handling of infected carcasses or body tissues. In recent outbreaks that involved wild boar it was shown, that carcass detection is the most important method of detecting geographical spread of ASF in wild boar. If an infected carcass is found it must be transported, sampled and subsequently destroyed in a save manner.

The "Handbook on African Swine Fever in wild boar and biosecurity during hunting" by the GF-TAD proposes a model, in where, within the core zone, hunting is not permitted but carcasses are routinely removed. The core zone would be surrounded by an area where hunting is reserved for hunters trained in biosecurity measures (Guberti *et al.*, 2018; Chenais *et al.*, 2019).

Silvia Bellini et. al. outlines minimum requirements for hunters to follow (Bellini *et al.*, 2016) (Table 1).

Table 1 Minimum Biosecurity Guideline for Hunters (Bellini *et al.*, 2016)

- Hunters shall be authorised to hunt in the area only after a specific training on basic hygiene and biosecurity practices;
- Hunted wild boar should never leave the hunting area unless checked and tested and the carcasses released only when resulted negative to ASFV.
- Transport of hunted animals to the dressing facility is carried out using dedicated vehicles. Private cars shall be parked outside the hunting house, possibly on the main road.
- The use of the dressing facilities should be authorised only in case it is available: tap water, electricity, waste water collection and freezers.
- Animal dressing should be performed using appropriate aprons which must remain in the facility. Working tools cannot be transported to other places.
- Hunting suits, including boots/shoes should be kept in specific bags. Boots are worn in the dressing room before hunting and re-placed in the same bag after hunting.
- Boots and apron shall be cleaned and disinfected after each use.
- Dressing rooms are to be equipped with effective disinfectants.
- Offals should be stored in proper containers inside the dressing areas and before storing, containers shall be cleaned and sprayed with effective disinfectants.
- Ground pits for offal disposal should be at least 1.5 m deep, fenced and closed with a locked closure. Pits should be located in close proximity to the dressing room.
- Wild boar carcasses shall be individually identified before storing. In case of ASFV positive outcome all stored carcasses have to be disposed under veterinary supervision and the whole dressing room cleaned and disinfected.

Materials and Methods

The goal of our study was to predict the probability of the occurrence of ASF in Austrian regions bordering regions in Hungary in the state of being suspected of being infected. Unfortunately, several sensitive data from both countries needed for a more accurate prediction were not available to us, as such, we focused on the forest coverage of each local administrative unit as a predictor.

Data

Maps were retrieved on the local administrative units' level 2 (LAU 2), from the site https://gadm.org/ and https://data2.openstreetmap.hu/hatarok/index.php?admin=8 for Austria and Hungary, respectively. To obtain data on the forest coverage of LAUs the Corine Land Cover database version 2018 (CLC) (Feranec *et al.*, 2016) was used.

The data of Hungarian ASF outbreaks was collected for the period between 2018-04-20 and 2020-09-30 using the R-package OIEparser. The World Organisation for Animal Health (OIE) oversees a list of 117 notifiable terrestrial and aquatic diseases. Outbreaks of these diseases must be reported due to specific hazards or importance in international trade. The reported events are accessible to the public in the form of individual pdf documents at the OIE's World Animal Health Information System (WAHIS). While these reports carry important disease information, WAHIS could not be searched as a database to give researchers easy access to disease statistics over a set time period or regarding one specific disease. OIEparser allows researchers to navigate the vast WAHIS database with ease and use all available wildlife and domestic animal disease data in a structured way. Knowledge of incidence rates and appearances have tremendous application possibilities. Data analysed this way can show trends in disease spread over time and geographical locations, can be incorporated into disease risk analysis, and facilitate preventive measures (Horváth *et al.*, 2018).

Methods

The CLC categories ranging from 22 to 29 were handled as forest. For each LAU2s the proportion of these categories were calculated. The ASF outbreak case numbers were aggregated for all Hungarian LAU2s. For each Hungarian LAU2 we counted the number of neighbouring LAU2s with an ASF disease event in the study period. Settlements within one kilometre in distance from each other were considered as neighbours. The neighbour affected counts were converted to be binary, if a settlement was neighbour to a settlement which suffered through an outbreak in the study period, the value 1 was assigned to it, did no neighbouring settlements report cases, the value 0 was assigned to it.

Based on this dichotomic variable as dependent and the forest coverage variable as independent, logistic regression was applied to estimate their relationship (Gelman and Hill, 2012). Using the fitted model, we made predictions for the likelihood of introduction of ASFV into each Austrian LAU2 which was neighbour to a Hungarian LAU2.

All data processing, statistical analysis and mapping was performed in R-environment (R Core Team, 2020)

Results

In the OIE reporting repository 1283 distinct outbreaks were available for Hungary, for the period of 2018-04-20 to 2020-09-30 (Figure 1). Figure 2 shows the forest coverage of the Hungarian settlements. By logistic regression we calculated that the forest coverage of local administrative units has a statistically significant ($p<0.0001$) relationship with the infection status of neighbouring settlements. One percent increase in forest coverage of a LAU increases odds of infected status of neighbouring LAUs by 1.11 (OR: 1.11, 95%CI: 1.08-1.15).

Based on this fitted model we performed predictions for the Austrian settlements neighbouring with Hungarian LAUs. The results are presented in Table 2 and Figure 3.

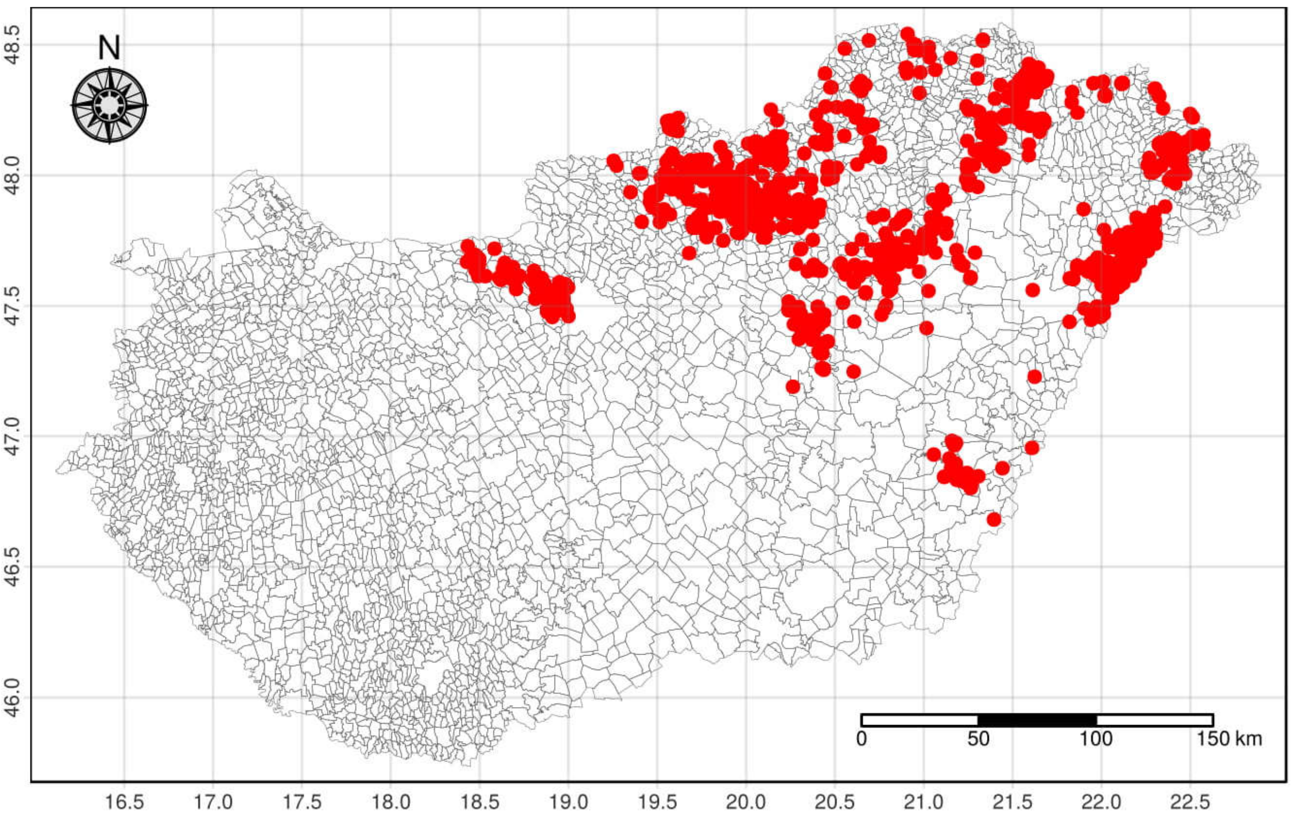

Figure 7 The map of the distinct reported ASF outbreaks in Hungary, between 2018-04-20 and 2020-09-30 obtained from OIE reports

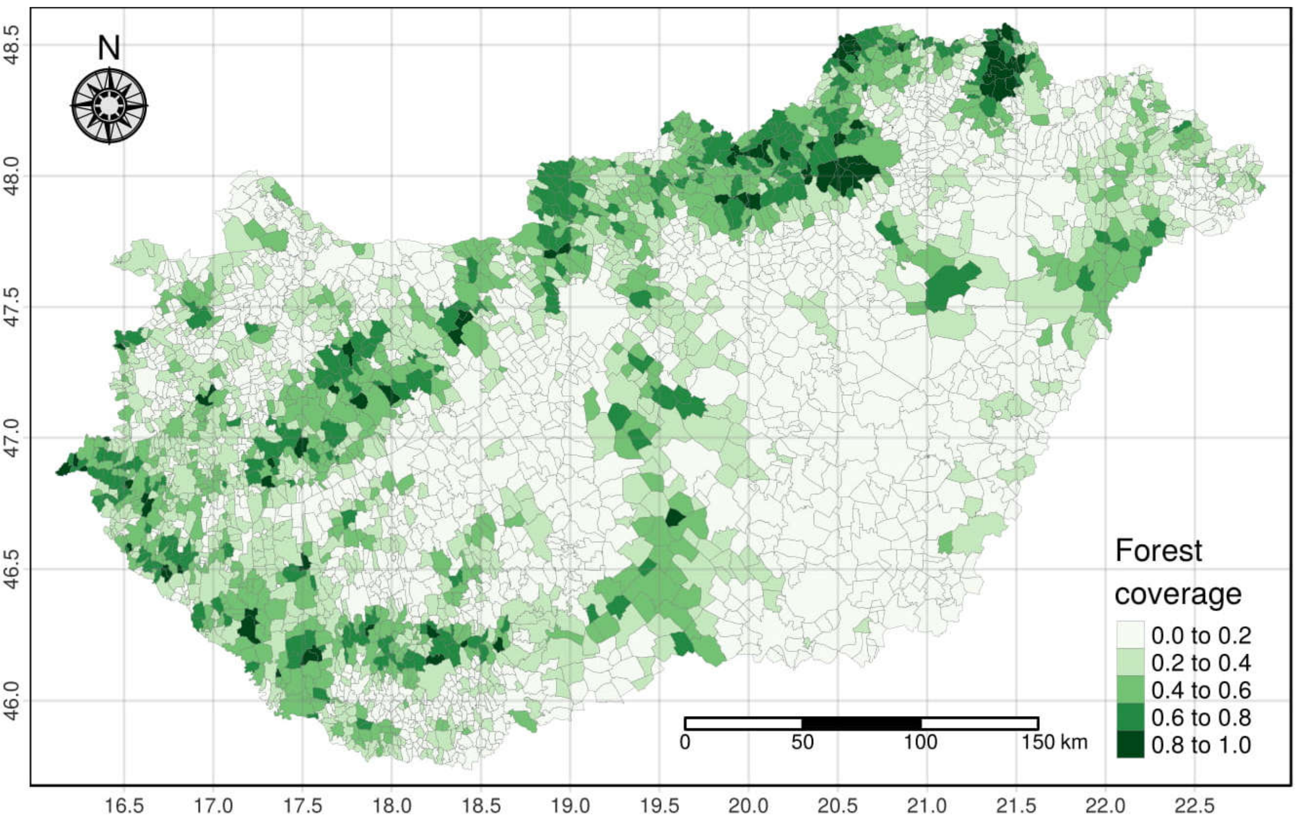

Figure 6 Forest coverage of Hungarian local administrative units based on Corine Land Cover database

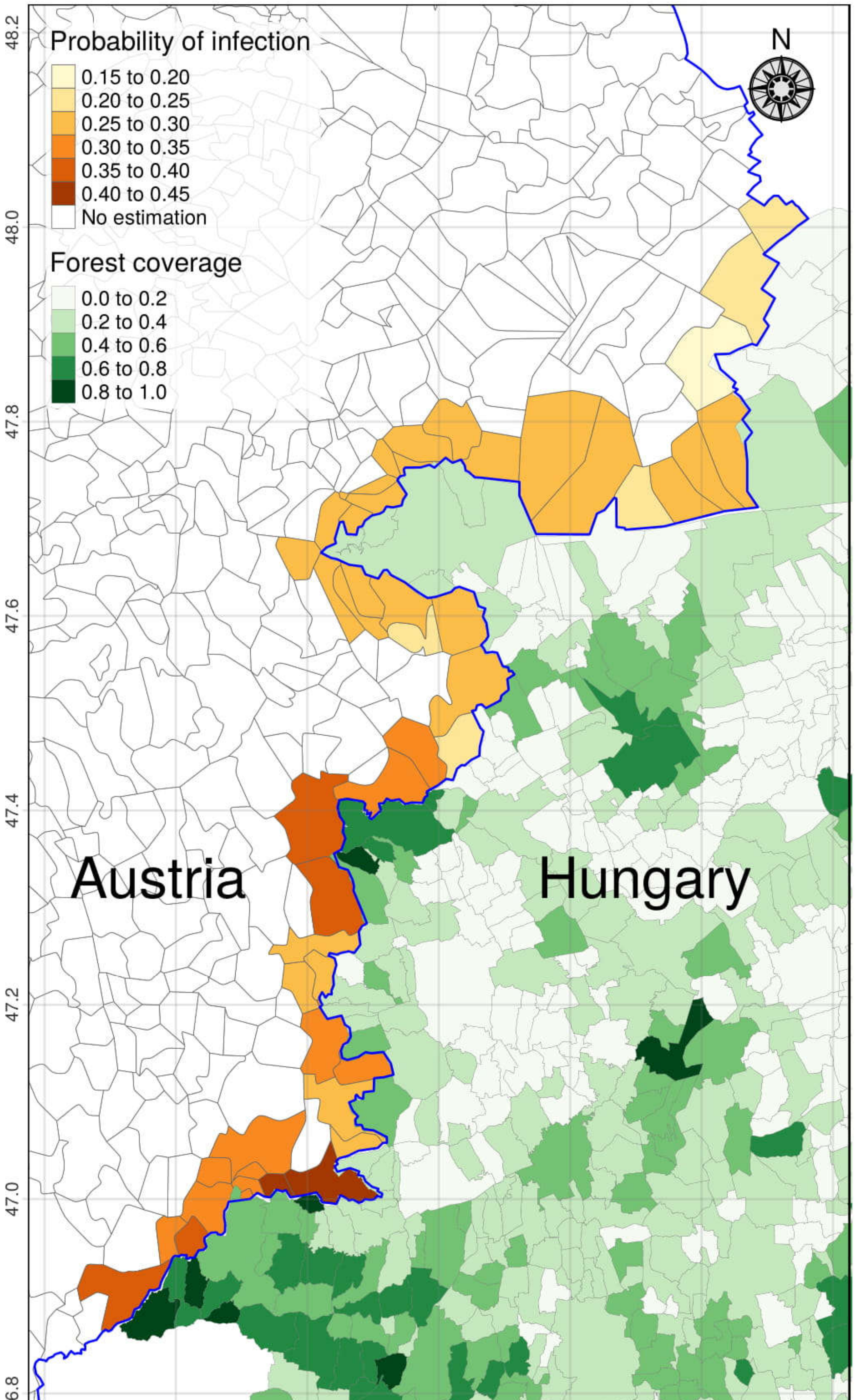

Figure 8 Estimated probability becoming the LAU infected based on the forest coverage of Hungarian neighbouring settlements

LAU	Probability of being infected
Andau	0.26
Apetlon	0.27
Baumgarten	0.27
Bildein	0.31
Deutsch Jahrndorf	0.23
Deutsch Schützen-Eisenberg	0.31
Deutschkreutz	0.27
Eberau	0.30
Frankenau-Unterpullendrof	0.33
Großmürbisch	0.41
Güssing	0.31
Halbturn	0.20
Hannersdorf	0.27
Heiligenbrunn	0.41
Heiligenkreuz im Lafnitztal	0.31
Horitschon	0.24
Illmitz	0.27
Inzenhof	0.31
Kleinmürbisch	0.31
Klingenbach	0.27
Lackenbach	0.27
Lackendorf	0.27
Lockenhaus	0.38
Loipersbach im Burgenland	0.27
Lutzmannsburg	0.23
Mannersdorf an der Rabnitz	0.33
Mogersdorf	0.37
Mörbisch am See	0.27
Moschendorf	0.30
Neckenmarkt	0.27
Neustift bei Güssing	0.31
Nickelsdorf	0.23
Nikitsch	0.29
Pamhagen	0.22
Rechnitz	0.38
Ritzing	0.27
Rohrbach bei Mattersburg	0.27
Sankt Margarethen im Burgenland	0.27
Sankt Martin an der Raab	0.37
Schachendorf	0.25
Schandorf	0.27
Schattendorf	0.27
Siegendorf	0.27
Sieggraben	0.27
Tadten	0.26
Tschanigraben	0.31
Wallern im Burgenland	0.26
Weichselbaum	0.34
Zagersdorf	0.27

Table 2 Estimated probability becoming the LAU infected based on the forest coverage Hungarian neighbouring settlements

Discussion

Limitations

This work was severely limited by the fact, that we did get granted access to vital datasets regarding the wild boar population from Hungary as well as Austria. The wild boar reproductive behaviour is very varied and as such very difficult to predict, making estimations or counts of populations very difficult. We tried to combat this, by making the approximation that the wild boar is most likely found in uninhabited forest areas.

The nature of this work, being international, brings with it its’ own sets of problems, mainly the fact of the language barrier, which may lead to information getting lost in translation or not being accessible to all parties.

ASF is a multifaceted disease, in this work, several ways of transmission are described. To perform a risk assessment the factors have to be somewhat predictable. This is impossible when it comes to the spread of ASFV by man. In several countries the disease seems to have jumped thousands of kilometres. We assume this is due to contaminated pork products or the like. Large jumps of the disease are impossible to predict as too many factors play into them. As such this work could only focus on a part of the transmission of ASFV, namely the assumed spread via wild boar.

This work was in such a way limited as it was the authors first array into scientific writing. As students of veterinary medicine only bring a surface level understanding of working in a scientific context, this work did not benefit from the experience of someone having already worked with the subject at hand.

The border between Hungary and Austria was the only thing considered in this risk assessment. Of course, the introduction of ASFV into Austria is also possible from other countries dealing with the epidemic.

Risk of infection along the border

The data analysed gives us a better picture of how to perceive the risk of ASF introduction into Austria. As Hungary is fighting the epidemic since 2018 the spread has not been haltable and the Austrian authorities might need to consider the possibility of wild boar being the carrier. 49 local administrative units were involved in the calculation.

The probability of being infected ranged from 0,20 in the unit Halbturn to 0,41 in Großmürbisch and Heiligenbrunn. The median of probability of infection is 0,29 for all the LAUs at the border. At the northern part of the border, probability of infection is generally low. Deutschkreutz, Nikitsch and Lutzmannsburg with a chance of 0,27, 0,29 and 0,23 respectively, seem to pose a lower threat than their southern counterparts.

A statistically very homogenous part can be seen in the middle of the border with relatively low forest coverage and infection risk. Near the more southern parts of the border the forest coverage seems to increase and with it the risk of infection. The neighbouring LAUs Lockenhaus and Rechnitz show a stark rise in risk with 0,38 each. This is due to the high forest coverage of their Hungarian LAU counterparts on the other side of the border.

After a relatively low stretch of forest coverage Heiligenbrunn and Großmürbisch sit at the upper end of infection risk with 0,41, being the most likely to introduce the virus from all the LAUs discussed in this work. The risk of infection remains relatively high at the southern part of the border due to the forest coverage on the Hungarian side.

Taking this information into consideration, the results seem congruent with the way ASF travelled across Europe and crossed borders into several nations, whereas in heavily forested areas wild boar carcasses where found containing the virus.

Implications for Austrian health authorities should be to recognise where ASF might cross to border from Hungary and take necessary precautions in advance. Austrian measures indicate that if the virus should enter the country, stamping out would be the appropriate response to an occurrence on a farm. Meaning severe economic losses not only for the affected farm, but the entire region.

The information of where along the border diseased wild boar might appear could be used by national authorities to be passed along to hunters, forest workers and veterinarians to assess the risk of encountering a case of ASF. As it is already mandatory to report any findings of dead wild boar in Austria, having additional information to judge the chance of facing a potential contagious carcass, might lead hunters and others to treat the situation with more care. Population control of wild boar, especially along the border and in the Burgenland-region, might help to keep the disease at bay.

Knowing where the risk is the highest also might make pig farmers reconsider their stance on any outdoor cages for their livestock or, if they find themselves in a possibly dangerous area, tighten up on their biosecurity measures and align themselves with rules and guidelines published by the Austrian Government.

Future research into more accurate tracking of the movement of wild boar as well as a better understanding of the population dynamics and migratory behaviour especially in western Europe could lead to better results in preventing outbreaks of the virus. Since there is no causative treatment against the virus, vaccine development remains one of the top priorities in the fight against African Swine Fever.

Conclusions

ASF is a very intimidating disease that has spread all over the eastern parts of Europe and Asia and is now spreading even further west, with countries like Germany having to deal with the epidemic. The economic implications for any country that reports cases of ASF are very significant. So, it should be in every nations interest to keep themselves free from it. In recent years it has been shown that the disease travels in a variety of ways.

Wild boars are one of the ways the disease gains entrance to a country, shown by how many of primary infections within a country happen via the contact of wild boar carcasses. Making the spread via wild boar a promising subject for further research and a tool for potentially estimating the risk of introduction into a previously unaffected country such as Austria.

Hungary has been affected by ASF since 2018 and has, as of yet, been unable to control it. As there is currently no vaccine to effectively combat the virus, for the potential measures to work effectively, similar to how they did in Belgium and the Czech Republic, they must be implemented by national veterinary authorities very early. Making the early detection of the disease a very important goal.

49 Austrian Local Administrative Units border Hungary. We established that along this border the forest coverage in the LAUs plays a significant role in predicting the risk of infection in each unit. The southern parts of the Austrian-Hungarian-border are more prone to introduction of ASF than some of the LAUs in the northern part.

Should ASF arrive in Austria, the national authorities must make use of active and passive surveillance and catch the disease as early as possible. Preventive measures might help fight the spread. Hunters should be informed about the transmission mechanics and dangers of ASFV. Active and passive surveillance should be implemented, and protocols should be in place in case of introduction of the disease. The question of stamping out arises and how the economic consequences can be kept to a minimum.

Summary

African Swine Fever is a devastating disease of domestic and wild suids which has been spreading across the globe. It is a very lethal disease with infected pigs showing haemorrhagic symptoms. Mortality rates reach up to 100% in peracute and acute cases in domestic pig. The virus is mostly spread via direct contact but is also able to be transmitted in processed pork products and remain infective for months, allowing the disease to traverse large distances and cause disease in another country. ASFV may also infect wild boar and cause the wild boar population to act as a reservoir for the disease. The wild boar also played a vital role in the recent spread of ASF in Europe and beyond. In 2007 the current epidemic started in Georgia and has since made its' way across most of Eastern Europe, where several countries have been fighting the disease. Countries like Belgium, The Czech Republic and recently Germany have been affected. Causing Western Europe to look toward preventive measures like newer biosecurity and surveillance measures to catch the disease as soon as it appears. Hungary reported its' first case of ASF in 2018. Since then the disease has spread further west within the country, as more wild boar carcasses, who were found to have suffered from the disease were identified. Trying to assess the risk that comes from the introduction of ASFV into Austria via wild boar over the Hungarian border, this work takes a look at the local administrative units (LAUs) at the border which show a dense forest coverage. An increase in forest coverage was found to significantly increase the risk of a neighbouring LAU of becoming infected. We found that the southern part of the border suffers from a higher risk of becoming infected than the northern part. Especially Heiligenbrunn and Großmürbisch, the two LAUs with the highest probability.

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