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A Study on the Risk of African Swine Fever Entering in Kosovo

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Review paper

SUMMARY

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- African Swine Fever (ASF), active in Europe in domestic pigs and wild boars, has extended into the Balkans. While does not affect human health, high morbidity and mortality, coupled with costs of control and eradication, result in large national and individual costs of the disease. While ASF transmits from infected to susceptible porcines by direct contacts, it can also be spread by infected pork products and on other fomites such as contaminated transport.
- ASF can be maintained and spread by soft ticks. Where spread is through fomites, outbreaks can occur a long distance from origin of the virus.

- When infecting wild boars, spread is more local but maintenance and spread of disease in wild boars has been supported by increasing wild boar populations in Europe.

- As the disease spreads into the Balkans, risks of spread to Kosovo and steps which can be taken to stop its entry and, if unsuccessful, mitigate effects of the disease and control it, are studied.

- Legislation to control ASF in the EU is reviewed and compared with relevant legislation in place in Kosovo. Control of ASF with consideration of surveillance, movement control, carcass disposal, fomite control, biosecurity, public awareness and wild boar management.

- A risk assessment of ASF spreading to Kosovo considers: numbers, distribution and movements of domestic pigs in Kosovo and the wild boar population and its ability to cross Kosovo's borders with neighbouring countries.

- Risk of entry and spread is considered to lack of public awareness of the disease and its consequences.

- **Key words:** African Swine Fever; risk analysis; surveillance; disease control; Balkans; Kosovo

INTRODUCTION

- African Swine Fever (ASF) is a viral disease which causes a haemorrhagic fever with high mortality rates in domestic pigs, but persistently infects its African natural hosts, warthogs, giant forest hogs and bush pigs, with no clinical signs of disease, in Africa where it originates.

- The disease is highly transmittable and there is currently no treatment or vaccine available to control it.

- The virus causing ASF (ASFV) is a large,

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double-stranded DNA virus which replicates in the cytoplasm of infected cells. ASFV infects domestic pigs and wild pigs in Africa, as well as soft ticks (*Ornithodoros spp.*), which can act as intermediate hosts.

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- The disease originates from, and was once confined to, Africa where it existed as unapparent infections of wild porcine species. But when domestic pigs were introduced they suffered severe clinical disease and high mortality.
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- Control of disease in them relied on their separation from the indigenous porcine species, for example, by double fencing pig paddocks. ASFV is a very resistant virus, resilient to external factors. For instance, it can survive in the external environment for at least 150 days at 4°C; it can live up to 140 days in dry salted ham; in frozen products it can survive for several years. Although ASFV only infects porcine species and is not a zoonosis, economic consequences of infection are serious and immediate. Herds of infected domestic pigs are culled. A single case of ASF in a country can lead to bans on export of that country's pork products. In Estonia, when ASF entered in 2015, 22,000 pigs were slaughtered, pork prices collapsed, and more than a third of pig farms went out of business.
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Epidemiology: Means of transmission include:

- Direct contact between porcine species where one is infected;
- Contact of susceptible porcines with infected faeces and urine and discharges from infected porcine species;
- Contact of susceptible porcines with infected meat or processed products from infected wild boar or domestic pigs;
- Transmission via an intermediate arthropod host.
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- Due to the resistance of ASFV and its ability to survive outside the host, transmission via fomites has become the most important method of long distance spread
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and, particularly in an age of long distance travel by air and sea, movements of infected pork products have been responsible for long distance spread of ASFV.

Role of wild boars: are a key factor in maintenance and spread of ASF in Europe. Wild boar populations have undergone an increase, in both size and distribution, across most parts of Europe over the past 30 years (Tack, 2018).

30 (Tack, 2018).

This factor, together with the difficulty of controlling ASF in wild boars, can explain the increasing importance of wild boars in maintenance of the disease. The increase in numbers of wild boars has been explained by a combination of factors: very high reproduction rates; dispersal potential; lack of large predators; reforestation; deliberate release and conservation for sport hunting; habitat alterations due to humans, for example, increase in availability of palatable and nutritive farm crops such as maize, potatoes, beans and sugar beets; mild winters resulting from climate change.

Countries in Europe showing increasing wild boar populations measured as numbers of harvested wild boar are: Austria, Belgium, Croatia, Czech Republic, France, Germany, Hungary, Italy, Latvia, Luxembourg Poland, Portugal, Slovenia, Spain and Switzerland.

Wild boars have been identified as an important source of spread of ASF in the Baltics and Eastern Europe where passive and active surveillance of wild boar populations and wild boar carcass removal are described as important control activities. However, it is difficult to eliminate ASF from wild boar populations once it has become endemic (Gavier-Widen et al., 2015). A review of the scientific literature on hunting and trapping of wild boars revealed that hunting and trapping has never achieved

(Gavier-Widen et al., 2015).

a drastic reduction in a wild boar population in Europe. Depopulation efforts can lead to perturbation and adaptive behaviour of the hunted wild boars, compensatory growth of the population and the influx of wild boars from adjacent areas. Wild boar density thresholds for introduction, spread and persistence of ASFV in wild boar populations have not been established. If depopulation attempts are undertaken, these can even increase transmission and facilitate progressive geographical spread of ASFV as intensive hunting pressure on wild boar populations may lead to dispersal of groups and individuals.

Fencing can restrict wild boar movement but the feasibility and effectiveness of implementing (emergency) fencing is not clear. Better knowledge on the ASF epidemiological situation and spatial distribution of the wild boars is required to identify the areas where fencing could be used as one element of a control programme and to assess the feasibility of its implementation (Anon., 2014).

The European Food Safety Authority (EFSA), using a simulation model, concluded that early detection, coupled with the application of measures such as quick removal of carcasses and intensive hunting in the specially designated hunting areas, increases the probability of eradication.

They also observed seasonal peaks in the numbers of animals that tested positive and were found dead – summer and winter for wild boars and summer for domestic pigs (Anon., 2018a).

Role of soft ticks: With regard to transmission via an intermediate arthropod host, Argasid (soft) ticks of the genus *Ornithodoros* can host the virus and are capable of transmission of the virus to porcine hosts they parasitize.

This is of importance in Africa as the

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burrows of wart hogs become infested with soft ticks and these are then important in maintenance of the infection. A large selection of arthropods has been investigated as potential vectors of ASFV. *Ornithodoros spp.* ticks are competent biological vectors of ASFV and need not necessarily have evolved with it, since *Ornithodoros erraticus* proved to be competent in transmission as well as in maintenance of the virus over long periods (at least 5 years) when ASF reached Portugal and Spain, and several species of *Ornithodoros* in the USA and Caribbean and *O.savignyi* in southern Africa have been shown experimentally to have the same competence, although transovarial transmission as occurs in the natural host in southern and East Africa (*Ornithodoros moubata* complex), was not demonstrated in all species.

The only other arthropod demonstrated experimentally to be able to maintain the virus for up to 48 hours and transmit it mechanically is the stable fly, *Stomoxys calcitrans*.

First spread of ASF from Africa:

During the 20th century, due to movement of fomites over long distance by sea, via illegal imports of pork products by tourists or for commercial purposes, or via the illegal disposal of waste from ships or planes originating from ASF-affected areas, ASF outbreaks started to occur outside Africa.

The first known outbreak of ASF outside of Africa was in 1957 in Portugal, when pigs near Lisbon were fed with food waste from an airplane. Though this outbreak was controlled, there was a new outbreak in the same region in 1960 and, until the mid 1990s, ASF was a constant presence on the Iberian Peninsula.

Between 1964 and 1986, ASF also surfaced in some other European countries, namely France, Italy, Sardinia, Malta, Belgium and the Netherlands, but

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was stamped out in all except Sardinia where, due to the presence of uncontrolled wild boar and extensively managed domestic pigs, it became endemic, though the virus did not spread from Sardinia to the mainland of Europe. Epidemics of ASF occurred in Cuba in 1971 and 1980 but the disease was rapidly eradicated through strenuous control activities and the fact that the disease affected only domestic pigs: wild animals (feral pigs) and soft ticks did not play a role in the epidemiology and transmission of the disease.

Curent epidemic situation: More recently, ASFV started an ongoing spread in continental Europe from 2007, initially in Georgia from where it spread eastward and subsequently north-westward into Russia where it persists throughout the western parts. It has also been reported in this period in Armenia (2007 to 2011) and in Azerbaijan (2008) in domestic pigs.

It entered Russia in 2009, infecting both domestic pigs and wild boar and, subsequently, it entered Ukraine (2012 to 2014) also affecting both domestic pigs and wild boar. From this point on, wild boar appear to have become important in proliferation as it spread to Ukraine (2014), Belarus (2015), and entered the EU: Lithuania (2014), Latvia (2014), Poland (2014) and Estonia (2014).

In all of these countries, it entered the wild boar population as well as infecting domestic pigs. In 2015, outbreaks of ASF continued in pigs and wild boar in Russia, Ukraine, Poland and the three Baltic countries (Lithuania, Latvia and Estonia) and in 2016, Russia, Ukraine, Poland, Estonia and Moldova. In 2017, while the same countries were affected, importantly, ASF cases in wild boar were diagnosed in Czech Republic and in domestic pigs in Romania, indicating a spread westwards and southwards into the Balkans.

	<ul style="list-style-type: none"> - In 2018 was a very important year with regard to spread of ASF. While outbreaks continued in Russia, Ukraine, Poland, Latvia, Moldova, the Czech Republic and Romania, it made its first appearance in wild boars in Hungary and also in domestic pigs in Bulgaria close to the Romanian border, this despite a fence being built along Bulgaria's land border with Romania (most of the border follows the River Danube) in an attempt to prevent the crossing of wild boar that could spread the disease into its territory. Also, of huge importance, as it contains half of the world's pig population, the disease spread into domestic pigs in China (Anon. 2018e), genotyping indicating that the virus emanated from Eastern Europe (Anon. 2018f) but a long way from the nearest border (Russian). It was reported on 22 Mar 2019 that since the China Ministry of Agriculture and Rural Affairs confirmed its first ASF outbreak in Liaoning Province on 3 Aug 2018, a total of 114 ASF outbreaks have been detected in 28 municipalities. More than 950,000 pigs have been culled in an effort to halt further spread (Anon. 2019a). Outbreaks in China have so far been confined to the Eastern half of the country where they are distributed from the north to the south. All except two outbreaks in the north-east in wild boars, have been in domestic pigs. In 2019 China's neighbours have become infected: Mongolia (Anon. 2019b), Vietnam (Anon. 2019c), Cambodia (Anon. 2019d), North Korea (Anon. 2019e), Myanmar (2019f), Laos (2019g) and in Eastern Russia close to its border with China (2019h).
<p>2018e), (Anon, 2018f), (). 22</p>	<ul style="list-style-type: none"> - - -
<p>2018 ., 28 114 950 000 (Anon, 2019a)</p>	<ul style="list-style-type: none"> - - - - -
<p>2019 . (Anon, 2019b), 2019c), (Anon, 2019d), (Anon, 2019e), 2019f), (Anon, 2019g) (Anon, 2019h).</p>	<ul style="list-style-type: none"> - - - - -
<p>e</p>	<ul style="list-style-type: none"> - An extreme example was the spread of FMD virus from south east Asia to a rural part of northern England in 2001. With

<p>2001</p>	<p>regard to ASF, a similar unpredictable, and so far unexplainable, spread of the virus to Belgium occurred.</p>
<p>2018</p>	<p>In September 2018, ASF in wild boars was detected in Belgium. Phylogenetic analysis of the virus revealed that the causative strain belongs to genotype II, and its closest relatives are viruses previously isolated in Ukraine, Belarus, Estonia, and European Russia (Anon. 2018b).</p>
<p>2018b). ASFV</p>	<p>To date, the manner by which ASFV was able to spread to wild boar in Belgium is unknown, bearing in mind that the whole of Germany separates Belgium from other infected areas, namely Poland and the Czech Republic.</p> <p>However, it appears that wild boar meat from Poland has been sold widely in Belgium and this could have been the route of transmission. A map showing spread of ASF in Europe is in Figure 1.</p>
<p>1.</p> <p>:</p> <p>2002/60/</p> <p>(Anon, 2002).</p>	<p>Legislation to control ASF in the EU: The EU has laid down prevention and control measures to be applied where ASF is suspected or confirmed either in holdings or in wild boars. The overarching piece of legislation providing the tool for the control of ASF in the EU is Council Directive 2002/60/EC which lays down minimum measures to be applied within the Union for the control of ASF, including establishment of an infected area following the confirmation of one or more cases of ASF (Anon. 2002). In this framework, new specific regionalisation measures have been taken with respect to evolution of the ASF situation in the EU:</p>
<p>27 2014 . (2014/178/)</p> <p>(Anon, 2014b).</p>	<p>Commission Implementing Decision of 27 Mar 2014 (2014/178/EU) concerning animal health control measures relating to ASF in certain Member States (Anon. 2014b).</p>

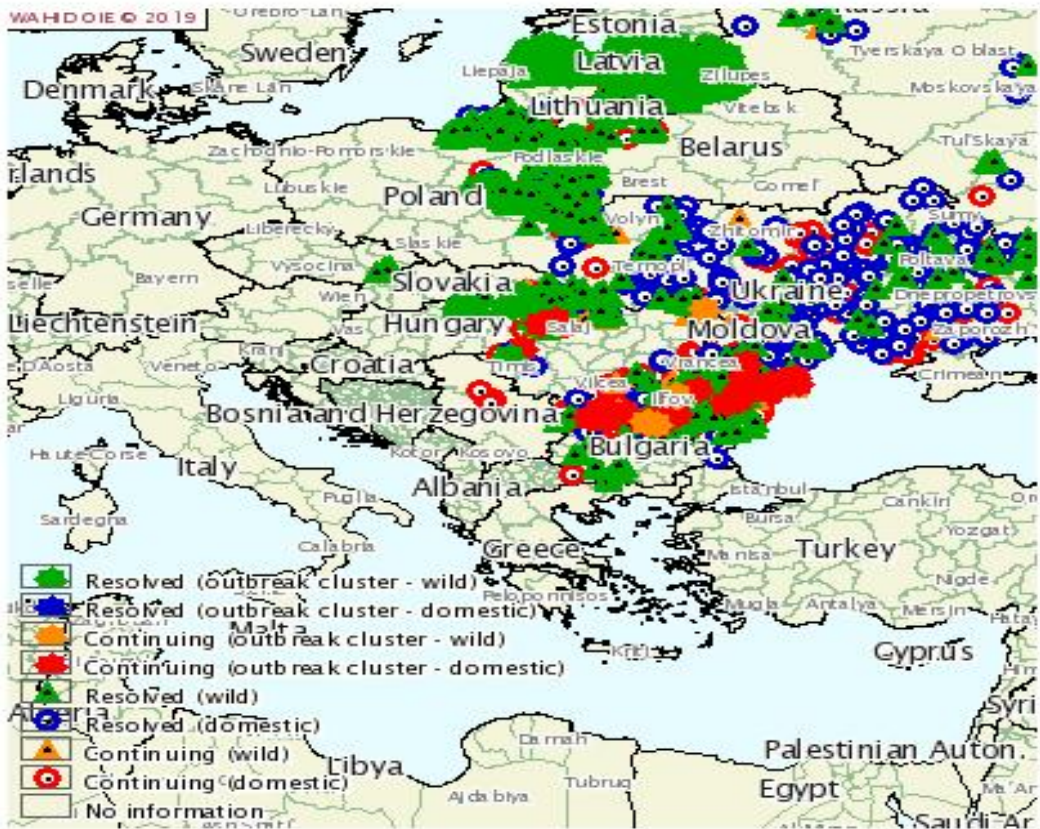


Fig. 1. Map of spread of ASF in Europe (taken from OIE website)

<p>SANCO/7138/2013 (Anon, 2013)</p> <p>2002/60/</p> <p>15 16; - IV ()</p> <p>2003/422/</p> <p>EFSA</p> <p>AHAW []</p> <p>EFSA</p>	<p>Document SANCO/7138/2013 (Anon. 2013) contains guidelines on surveillance and control of ASF in feral pigs and preventive measures for pig holdings. The aim of this document is to provide Member States guidance for controlling ASF when the disease is suspected or confirmed in feral pigs.</p> <p>Guidelines are based on: -the provisions of Council Directive 2002/60/EC, and in particular of Articles 15 and 16; -chapter IV(H) of the Annex to Commission Decision 2003/422/EC; -the EFSA Scientific Opinion of the Panel on AHAW [Animal Health and Welfare] on the control and eradication of classic swine fever in wild boar; -the EFSA Scientific Opinion of the Panel on AHAW on ASF.</p>
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The EU legislation referenced above is fully in line with OIE international standards.

Nevertheless, in order to ensure a higher level of animal health protection, the EU goes beyond the OIE requirements and applies stricter standards. In the current application of regionalisation in Lithuania and Poland, for example, no pigs, their semen, embryos, or ova are allowed to be moved from the infected area.

The European Commission published on 28 Nov 2018 in the Official Journal of the EU, L 302, the Implementing Decision (EU) 2018/1856 of 27 Nov 2018, which amends the annex to "Implementing Decision 2014/709/EU concerning animal health control measures relating to African swine fever in certain Member States (Anon. 2018c). In an amended annex, Belgium's ASF-related zones are described in detail and the ASF-related zones in the other infected member countries (Bulgaria, the Czech Republic, Estonia, Latvia, Lithuania, Poland and Romania) are updated (Anon. 2018d).

MATERIAL AND METHODS

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 OIE
 ASFV,
 et al., 2019).
 (CVET)

Control: Vaccination against ASF would be an obvious control method and lack of a vaccine is of major concern in the fight against ASF. OIE has accelerated laboratory research, with much finance poured into the effort, but with no success so far because "of ASFV genetic complexity, gaps in knowledge concerning ASFV infection and immunity, lack of development of neutralizing antibodies, and technical difficulties such as the lack of stable cell lines" (Barasona *et al.* 2019). In fact, vaccine development has been identified as a major gap in ASF control and eradication. In the absence of a vaccine, the EU Community Veterinary Emergency Team (CVET) focuses on the following points for control:

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- Surveillance in wild boar and domestic pigs;
- Standstill and movement control;
- Carcass disposal;
- Swill feeding;
- Biosecurity;
- Awareness campaign;
- Hunting practices. (Anon. 2017).

- Surveillance in wild boar and domestic pigs: is crucial as quick detection of ASF decreases the period of silent spread which is the period after incursion during which the virus spreads unchecked without control because of lack of knowledge of its presence.

- Early detection through good surveillance, before the disease has had a chance to spread very far, decreases the size of the infected area and numbers of affected animals, thus decreasing costs of control activities and increasing the odds of stamping out viral incursions.

- Stand still and movement control: In the EU countries, controlled movement of pigs and disinfection procedures of vehicles used for their transport helps limit the spread of ASF from an outbreak even before the outbreak has been diagnosed.

- Generally in the EU, the veterinary authorities have the powers and resources to implement standstills in restricted areas around diagnosed outbreaks of pigs, their products and other fomites, thus mitigating the risks of further spread.

- While this can happen with pigs, where wild boars are involved these measures become problematic.

- Carcass disposal: As there is high mortality in outbreaks of ASF and as all parts and discharges from carcasses affected by ASF are highly infectious, disposal of carcasses in a secure manner is very important.

- Swill feeding: Kitchen waste and

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food leftovers can be fed as swill to domestic pigs. As well as containing domestic pig products which could be contaminated with ASFV, if pig keepers are hunters or are associated with them, there is the possibility that domestic pigs might come into contact with discarded food of wild boar origin which could also be contaminated with ASFV.

- Biosecurity: Relevant biosecurity measures can be most important for control of ASF entry and spread. Measures to be considered are:

- Domestic pigs for breeding and fattening sourced from home bred animals, or if it is necessary to introduce pigs from outside, selection from neighbouring breeders whose freedom from disease can be ascertained. Avoiding introduction of pigs from unknown sources and through markets, particularly pigs which have been imported;

- Secure housing and paddocks which do not allow kept pigs to escape and roam and prevent other pigs/wild boar from coming into direct or indirect contact with faeces, urine and discharges;

- Ban on feeding swill and kitchen waste, especially fresh or processed meat from either domestic pigs or wild boar

(If pig keepers are also hunters they must be particularly careful not to allow contacts between their domestic pigs and any parts or fluids from wild boar they may have hunted);

- Barrier husbandry: not allowing outsiders to enter pig housing or have contact with pigs; disinfectant footbaths at entrances to holdings and to pig housing.

Where essential visits are required (e.g. for veterinary treatment), the visitor should be provided with disposable overshoes and disposable, biosecure, coveralls;

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(Mur et al., 2014)

- Soft tick control: if soft ticks are present in pig housing they should be controlled by filling cracks and crevices in walls and by spraying interiors with residual acaricides.

Awareness campaign: Awareness campaigns can be powerful tools to inform pig keepers of the dangers they are facing, the consequences for them if they face an ASF outbreak and the steps they can take to mitigate the risk of this happening.

Risk analysis: This risk analysis, performed according to the methodology described by OIE, has been conducted to identify and assess the likelihood of ASF being introduced and spreading or becoming established in Kosovo together with the likelihood of and the likely magnitude of any consequences for animal health. As the disease does not affect man consequences for human health can be ruled out. The risk analysis considers four components: Hazard identification, Risk assessment, Risk management and Risk communication. The hazard in this case is ASFV and its potential to spread and become endemic in the domestic pig and wild boar populations in Kosovo causing actual and economic damage.

The risk assessment identified factors which affected the likelihood of the hazard to occur. The following factors were identified in Kosovo:

- high proportion of backyard pig farms (large commercial pig farms generally maintain better levels of biosecurity);
- wild boar contact,
- suitable vector (argasid tick) populations,
- swill feeding,
- existence of pathways of introduction

One study (Mur et al, 2014) aimed

to integrate five of these pathways into an overall assessment of risk:

- legal imports of pigs (in Kosovo, illegal imports of pigs also occur),
- legal imports of products,
- illegal imports of products,
- fomites associated with transport and
- wild boar movements.

Results of the study indicated that 48 percent of EU countries are at relatively high risk (risk score 4 or 5 out of 5) for ASFV entry for at least one analyzed pathway.

Four of these countries had the maximum risk score for one pathway: Bulgaria for legally imported pig products during the high risk period (HRP); Finland for wild boar; Slovenia and Sweden for legally imported pigs during the HRP.

Distribution of risk considerably differed from one pathway to another; for some pathways, the risk was concentrated in a few countries (e.g., transport fomites), whereas other pathways incurred a high risk for 4 or 5 countries (legal pig imports, illegal imports, and wild boar).

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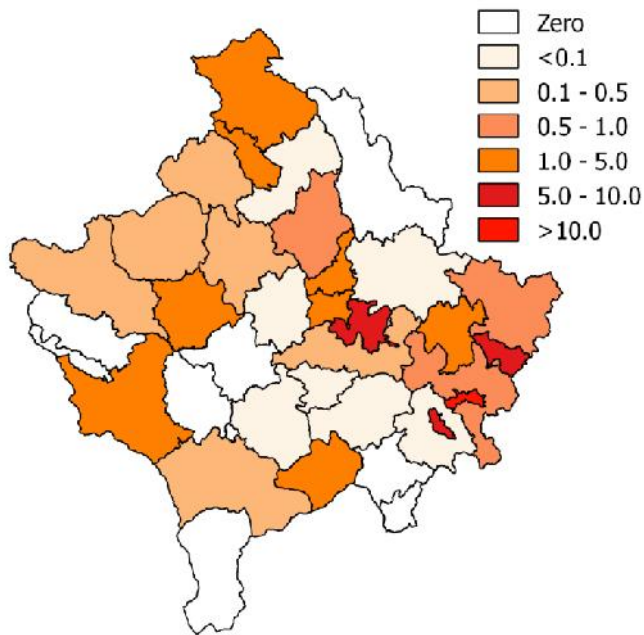
RESULTS AND DISCUSSION

Assessment of risk of ASF entering and spreading in Kosovo:

Domestic pigs: Being a predominantly Muslim country, 95.7% of the population are Muslims, most do not keep pigs. However, there are Serbian (Orthodox Christian) and Kosovo Albanian (Roman Catholic) minorities who breed, rear and trade pigs and domestic pigs (approximately 40,000) are distributed quite widely in Kosovo as can be seen in Figure 2. Pig keeping is almost exclusively small scale 'back yard' production. There is considerable local movement of pigs through sale of pigs from breeders, either directly or through markets, to those pig keepers who rear small numbers of pigs through the

- summer and slaughter them in their back
- yards in the autumn for home processing
- and consumption. The situation is
- different in the four northern municipalities
- where the ethnicity is predominantly
- Serbian. Here there is at least one large
- pig farm and a pig abattoir at Leshak in
- the most northerly municipality,
- Leposaviq. Rather than breeding all their
- pigs, many rearers are imported for
- fattening from Serbia (estimated at as
- much as 10,000 pigs per year which could
- emanate from any part of Serbia).

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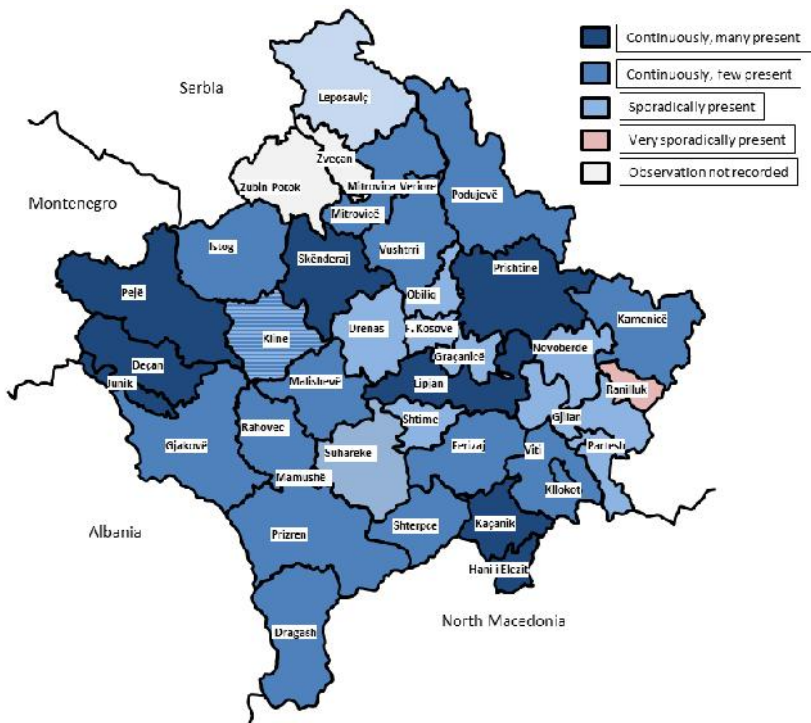
Fig. 2. Distribution of pig keeping in Kosovo (Based on the data in the identification and registration (I&R) database and a survey of a sample of pig farms from eight municipalities, the figure shows the density of premises with registered pigs. Municipalities that were surveyed are labelled)

- While most imported pigs are kept in the
- four northern municipalities for fattening,
- some of them may travel further through
- internal markets to reach other pig
- keepers for fattening in any of the other
- regions of Kosovo.
- Both imports and local movements are

- 'informal' in that they are not subjected to any customs or health checks, traders of imported pigs being able to bypass border inspection posts by crossing at other points in the north which are currently difficult to control. Major risk: introduction of ASF by informal importation and spread via the movement and marketing of infected animals.

Wild boars: Distributions and estimates of numbers of wild boars in Kosovo have been investigated through interviews with hunting associations and by camera trapping in different municipalities. Results indicate that wild boar are common in all municipalities except in Suhareka, Ranilluc, Gllogoc, Leposaviq, Fushe Kosova/Obilic, Gjilani, and Novo Brdo where they are reported to be sporadic or very sporadic. Wild boars were confirmed by camera trapping in Dragash, Podujevo, Junik, Mitrovica, Kamenica, Skenderaj, and Shtime.

- Population numbers were estimated on the assumption that the average group size is 2.1 individuals and the average daily range (distance of movement) is 5 km. The estimated population in the observed areas was between 0.1 individuals/100 ha in Mitrovica and 2.2 individuals in Junik. The wild boar population seems to be not as big as in Middle Europe where an average population density of 8 individuals per 100 ha has been estimated. Distribution of wild boars in Kosovo is presented in Figure 3. Some risk of introduction of ASF via cross border movement of infected wild boar to introduce ASF into Kosovo's wildlife population.



3. **Fig. 3. Distribution of wild boar in Kosovo according to hunters' reports**

: ASFV

Cross border spread of ASF virus into Kosovo: ASFV has been able to spread in Europe by a variety of pathways which are dependent on its highly infectious nature, capability to survive for long periods in fomites and ability to infect both domestic and wildlife porcine hosts. In its continuing spread it has now reached the Balkans, being currently active in Hungary, Romania, Bulgaria and, recently, Serbia (domestic pigs).

Considering that Romania and Bulgaria have ASF in wild boars and that they have borders with Serbia which are mainly rural, agricultural and forested, there is a major risk that ASF will spread across these borders to wild boars in Serbia.

ASFV

- There is a high risk of ASF entering Kosovo from Serbia because of the long border it has with Serbia which is largely rural and, in most places, passes through very suitable habitats for wild boar, and is also suitable for informal movement of pigs from Serbia into Kosovo along unguarded roads and tracks. Mitigating the risk of ASFV entering and becoming established in Kosovo is that a large part of the population of Kosovo does not keep pigs. Exacerbating the risk is that a large number of pigs come into Kosovo each year from across the border with Serbia into the northern municipality of Leposavic. While most pigs imported in that manner stay in the northern municipalities of Leposavic, Zubin Potok, Zvecan and Northern Mitrovica, others may be taken direct to other Serbian communities in the country or maybe sold in markets and get further dispersed, possibly to the Roman Catholic communities keeping pigs, mostly in Dukagjini, western Kosovo.

ASFV

- There is a high risk that ASFV will enter Kosovo from Serbia via informal imports of pigs for fattening, particularly at the border with Serbia in Leposavic. There is some risk that ASF will enter from Serbia into Kosovo via movement of wild boars. This will become a major risk if ASF establishes in the wild boar population of Serbia.

- **Potential direct contacts between domestic pigs and wild boar:** The possibility of contacts between the domestic pig and wild boar populations has been investigated in Kosovo. With regard to free-range pig keeping, the investigators could only find evidence of holdings in the municipality of Novo Brdo where pigs have been allowed free range into meadows. In this municipality, wild

² Novo Brdo municipality was selected for study as a report indicated that in that municipality, domestic pigs were allowed to forage in forests neighbouring their farms, thus risking direct/indirect contact with wild boars.

boars have only been reported sporadically. Farmers report that while free range pig husbandry had been practiced in previous years, it has been discontinued because pigs have been repeatedly attacked by wolves. The risk of direct contacts between domestic pigs and wild boars in the region of Novo Brdo and other parts of Kosovo seems to be low. However, there is a need for caution with regard to Mangalica pigs.

These hardy pigs are widely kept in Serbia in extensive systems where they can scavenge in pastures and forests. Under these extensive husbandry systems there are increased possibilities for them to come into direct and indirect contact with wild boar and thus a risk of transmitting ASF between the two populations.

To date, there is only one Mangalica herd in Kosovo, in Zubin Potok. Generally, the risk of transmission of CSFV by direct contacts between domestic pigs and wild boar in Kosovo appears to be low.

CSFV

Potential indirect contacts between domestic pigs and wild boar:

Results of a study of pig keeping in Novo Brdo Municipality² showed that most of the pigs are consumed in the household of the owners while some are sold to a small abattoir in Gracanica.

The whole carcass is consumed. Bones and skin are fed to the dogs. No remains are discarded in the forest. But as there is, as yet, no rendering system in Kosovo, the carcasses of pigs which might die on the farm may be disposed of in surrounding woodlands where parts could be scavenged by wild boar. Generally, the risk of indirect transmission of ASFV between domestic pigs and wild boar in Kosovo appears to be low.

ASFV

Risk of ASF entering in Kosovo on fomites: There are many unrestricted movements of people moving to and fro

<p>ASFV</p>	<p>across the border between the northern municipalities of Kosovo and Serbia.</p>
<p>ASFV</p>	<p>ASFV is capable of being transmitted by contaminated vehicles and footwear. Also infected pork products can be brought in and discarded riskily.</p>
<p>ASFV</p>	<p>Considering the many unrestricted movements of people and their vehicles across the border between Serbia and Kosovo, particularly in the north, the risk of transmission of ASFV by fomites such as contaminated vehicles or in infected pork products can be regarded as high.</p>
<p>ASF</p>	<p>Risk of ASF entering in Kosovo via infected wild boar: If ASFV gets into the wild boar population of Serbia, given the rural nature of the border between Kosovo and Serbia, the risk of infected wild boar carrying ASFV across from Serbia into Kosovo is high.</p>
<p>ASF</p>	<p>Mitigating the risk is that the wild boar population in Kosovo appears to be at a low density compared to other parts of Europe. If ASF becomes endemic in wild boar in Serbia, the risk of it spreading to wild boar in Kosovo is high but, unless it spreads from them into domestic pigs, ASFV might not maintain itself in wild boar in Kosovo due to their relatively low numbers.</p>
<p>ASF</p>	<p>Risk of ASF spreading of wild boar to domestic pigs by direct contact: The husbandry system for keeping pigs in Kosovo relies on keeping them intensively in small houses and enclosures and not allowing them to go to free range. This means that the risk of wild boar spreading disease to domestic pigs, and indeed the risk of domestic pigs spreading ASFV to wild boar by direct contact is small.</p>
<p>ASF</p>	<p>Risk of ASFV spreading to domestic pigs from wild boar by direct contact in Kosovo is low.</p>

ASF

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Risk of ASF spreading of wild boar to domestic pigs by indirect contact:

Indirect contact through fomites (contaminated vehicles, footwear or discarded pig products) is considered to be unlikely.

However, if an epidemic of ASF occurs in domestic pigs in Kosovo, there is considerable likelihood that pigs that die from the disease are dumped in forested land near the holding. If this occurs there is risk of wild boar becoming infected by contact with ASFV in contaminated carcasses and their discharges.

Risk of ASFV spreading to wild boar from infected domestic pigs in Kosovo if dead carcasses are dumped in the forest. It is noteworthy that a rendering plant has been established in Kosovo. When this becomes operational it is hoped that disposal of carcasses in the forest will occur less frequently

Risk of ASF spreading from wild boar to domestic pigs by indirect contact:

There could be a risk of pig farmers, who are also hunters, introducing the disease to their pigs through contact with infected wild boars and also allowing their pigs' access to scraps of infected wild boar meat from animals they have hunted.

Risk of spread of ASF from wild boars to domestic pigs by hunters who are also pig keepers bringing fomites to their farms

Risk of ASF spreading to domestic pigs through swill feeding:

While swill feeding on a large scale is not a usual procedure in Kosovo, kitchen waste and food leftovers are often fed to domestic pigs.

As well as containing pig products which could be contaminated with ASFV, if pig keepers are hunters or are associated with them, there is the possibility that domestic pigs might come into contact

ASFV.

ASF

- with discarded food of wild boar origin, which could be contaminated with ASFV.

Risk of ASF spreading to domestic pigs through feeding domestic food waste

Risk of ASF spreading to domestic pigs through lack of biosecurity:

- Pig keepers in Kosovo are almost all back yard farmers who are not aware of the need for biosecurity to prevent disease in their holdings.
-
-

There is a high risk of ASF entering and spreading in Kosovo because of a lack of biosecurity

Risk of ASF spreading to domestic pigs through lack of awareness:

- Public awareness campaigns have been undertaken in Kosovo for disease control, particularly as a tool for control of rabies during national rabies control campaigns targeting foxes, but as yet, no such awareness campaigns have been used to target ASF.
-
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Lack of government and public awareness of the threats of entry and spread of ASFV create a high risk of ASFV entering and spreading in Kosovo.

ASFV

Current situation in Kosovo:

- Under the Veterinary Law no.2004/21, ASF is listed as a scheduled dangerous disease requiring particular veterinary control measures, including with regard to domestic animals (in this case pigs): obligation to report; restrictions on movement; isolation; slaughter; disposal.
-

With regard to wild animals (in this case wild boar) particular veterinary control measures are: treatment; vaccination and/or reduction in number of affected wild animals or otherwise limiting their movement.

	<p>There is also regulation regarding</p> <ul style="list-style-type: none"> - establishment of a laboratory for - diagnosis of animal diseases and - establishment of contingency plans - involving the law enforcement bodies, - municipal authorities and civil protection - bodies to implement the above provisions.
<p>(MAFRD)</p>	<p>There are provisions for gathering</p> <ul style="list-style-type: none"> - information for the analysis of the - epizootic and enzootic situations in and - out of Kosovo, risk assessment, monitor- - ing and formulating plans for the - prevention, control and, where feasible, - eradication. There is also a provision on - co-operation with the Forestry Department - of the Ministry of Agriculture, Forestry and - Rural Development (MAFRD), to formulate - and implement prevention, control and - eradication programmes for infectious - diseases affecting both wild and domestic - animals.
<p>(IEMS)</p>	<p>As required in its Veterinary Law, Kosovo</p> <ul style="list-style-type: none"> - has a general contingency plan for the - management of actual and suspected - outbreaks of notifiable diseases of - animals in Kosovo. While the response to - animal diseases emergencies will always - be dealt with by the Kosovo Food and - Veterinary Agency (KFVA), there are - linkages to the 'Integrated Emergency - Management System' (IEMS) under the - Ministry of Internal Affairs and the - expected emergency response to an - animal disease emergency is integrated - with the IEMS.
<p>(JFC).</p> <p>, IEMS</p>	<p>Kosovo's planned response to</p> <ul style="list-style-type: none"> - emergencies of all types is embodied in - "The National Response Plan" (NRP). The - NRP is a flexible plan that can be - implemented partially or completely. Key - elements of the NRP are: - <ul style="list-style-type: none"> • When needed, Joint Field Centres (JFC) are established in the locality of the incident. • Particularly relevant to animal disease contingency planning, the IEMS defines and establishes the resource

KFVA.

1)

2)

ASFV

management process. This process should be followed in case of animal disease emergencies when resources beyond the immediate capacity of KFVA are required.

In addition to the general contingency plan, a specific contingency plan has been developed to cover ASF and CSF.

While the epidemiology of the two diseases is similar in most respects, two important differences should be taken into consideration with regard to ASF:

- 1) unlike CSF, there is no vaccine available for ASF;
- 2) soft ticks can act as intermediate hosts for ASFV and maintain the virus in the absence of pigs, for example after culling has taken place.

CONCLUSIONS

Surveillance in wild boar and domestic pigs.

Domestic pigs: Passive surveillance in Kosovo is mostly provided by a network of private veterinarians who, through providing support to their pig keeping clients, are the most likely to be alerted to sudden outbreaks of acute disease with high mortality in pigs which they are obliged to report to KFVA.

Many of the private veterinarians have contracts with the KFVA to provide government funded services. In the case of pigs, this includes visiting their pig keeping clients for annual vaccination of pigs against CSF.

This provides a good opportunity for private veterinarians to establish a close working relationship with pig keepers and enables them to keep up to date with on farm pig disease situations.

Wild boars: In a virgin epidemic of ASF in wild boar mortality is high and the most obvious finding is likely to be dead

(Sharri Bjeshket e Nemuna),
MAFRD,

wild boars.

As they are mostly confined to the forests, the most probable finders are forest rangers from the Forestry Department of MAFRD, staff of National Parks (Sharri and Bjeshket e Nemuna) belonging to Ministry of Environment and Spatial Planning and hunters who, if legal, are members of hunting associations.

Standstill and movement control:

The problem with movement control, particularly with regard to movement between Serbia and Kosovo is that, especially in the north, there is a border between the two countries with unguarded space between border inspection posts and ample opportunity for informal movement of pigs in pickups and trucks from one country to the other.

Coupled with this, traditionally, weaner pigs are imported from Serbia into the four northern municipalities, finding their way to owners of back yard holdings who do not breed their own pigs but rear weaners for slaughter in the autumn for immediate consumption and preservation of meat by smoking and salting for consumption during the winter. Some of these imported weaners are moved further south, directly to holdings, or through markets, to Serbian and Roman Catholic pig keepers in Dukagjini in the west and to the Serbian enclaves in the east and south of Kosovo.

In the absence of a diagnosed outbreak of ASF, movement control of pigs is currently not strictly enforced, both within the country and across its borders.

This particularly applies to the four northern municipalities where much of the pig population is kept by the Serbian minority.

KFVA,

This situation is because, as yet, there are not enough KFVA inspectors to enforce movement control and pig keepers do not

appreciate the need for it. If an outbreak is diagnosed, it is probable that, due to evidence of the damage the disease is causing, standstill and move-ment control will be easier to enforce.

Carcass disposal: A rendering system is just now being introduced in Kosovo. While this is a major step forward with regard to carcass disposal generally, it may have little effect with regard to pigs.

Pig keeping is very small scale in Kosovo and, while there is a pig abattoir at Leshak in northern Kosovo and two small abattoirs at Gracanice, near Pristina, most pig slaughtering is done in the back yard.

In the latter case, it is likely that waste products from slaughter not consumed by dogs will be disposed of in the neighbouring common land (usually forest).

In the event of a disease outbreak, pigs that die are likely to be disposed of in the same manner.

Swill feeding: Though not banned, swill feeding is not done on a commercial basis as almost all of pig production is 'back yard'.

Pigs are, however, fed left over food and kitchen waste which could include pig products, some of which could be imported. It could also include waste from hunted wild boars.

Biosecurity: Currently, there is little awareness by pig keepers of security measures they could take to protect their pigs from infections such as ASF although pig keepers and private veterinarians observe that there is more disease problems in pigs imported from Serbia than there is in locally bred animals.

Awareness: Currently, there are no

particular awareness campaigns regarding the danger of ASF entering the pig population in Kosovo directed at pig keepers or private and public veterinarians.

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Horizon

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particular awareness campaigns regarding the danger of ASF entering the pig population in Kosovo directed at pig keepers or private and public veterinarians.

Hunting practices: Hunting of wild boars is restricted to members of Hunting Associations licensed by MAFRD. Hunting associations are widely distributed throughout Kosovo.

Wild boars are a protected species and limitations on hunting them are imposed by MAFRD. However, there are also illegal hunting activities which are uncontrolled.

Recommendations for risk control and mitigation

It is appropriate that a risk assessment of ASFV entering Kosovo is undertaken so that appropriate risk mitigations can be created and put in place to decrease the risk of entry and to generate preparedness to quickly control and minimise the effects of ASF on pig production and welfare and associated economic and socio economic effects caused by an outbreak at local and national levels.

There is a generic contingency plan for entry of exotic diseases into livestock in Kosovo and also a specific contingency plan for ASF/CSF.

However, to be useful, these plans need to be reviewed and circulated to key stakeholders and procedures need to be tested by simulation exercises, all to provide emergency preparedness.

Surveillance

Horison scanning: Staff of the Animal Health and Welfare Directorate, MAFRD, should on a daily basis monitor OIE reports on ASF outbreaks, noting their location, whether they are in

	<p>domestic pigs, wild boars, or both, and report on the risks they pose with regard to ASFV entering Kosovo.</p>
<p>ASFV</p>	<p>Where appropriate and dependent on the outcomes of passive surveillance, active surveillance operations can be undertaken in areas where there are particular risks of ASFV incursions.</p>
<p>ASFV.</p>	<ul style="list-style-type: none"> - <u>Domestic pigs:</u> Passive surveillance for ASF can be improved through a stakeholder awareness programme to pass on information on signs of ASF and reporting obligations to pig keepers, as well as to public and private veterinarians.
<p>ASFV</p>	<p><u>Wild boars:</u> If ASFV enters the wild boar population in Kosovo, there will be, at least initially, high mortality in infected wild boars.</p>
<p>()</p>	<p>Hunters, forest rangers, as well as the general public, should be made aware of this and be encouraged to report presence of dead (or sick) wild boars immediately to veterinarians, police, or other responsible persons such as forest rangers, without moving, or interfering with, the carcass.</p>
<p>KFVA KVL</p>	<ul style="list-style-type: none"> - Field staff from the KFVA and laboratory staff from the KVL should, as part of the contingency plan and emergency preparedness, be ready to collect the carcass in a biosecure manner to take to the laboratory for laboratory diagnosis and to decontaminate the area where the carcass was found.
	<p>Standstill and movement control: The problem with movement control, particularly with regard to movement between Serbia and Kosovo is that, especially in the north, there is a border between the two countries with unguarded space between border inspection posts and ample opportunity for informal movement of pigs in pickups and trucks from one country to the other. Coupled</p>

with this, traditionally, weaner pigs are imported from Serbia into the four northern municipalities, finding their way to owners of back yard holdings who do not breed their own pigs but rear weaners for slaughter in the autumn for immediate consumption and preservation of meat by smoking and salting for consumption during the winter.

Some of these imported weaners are moved further south, directly to holdings, or through markets, to Serbian and Roman Catholic pig keepers in Dukagjini in the west and to the Serbian enclaves in the east and south of Kosovo.

As yet, the movements described above are not monitored or fully controlled by police or government inspectors.

While every effort should be made to increase control of unauthorised movements of pigs by inspections at borders and markets, and along trade routes, pig keepers should be made aware of the usefulness of controls of movement and standstills to protect their pigs from ASF so that they respect the restrictions placed on unauthorised movements of their pigs.

Carcasses: While it is important to note that a rendering service is being introduced in Kosovo, it is unlikely, in the short term at least, to be dealing with carcasses from deaths in the smallholder pig keeping sector which may be due to ASF when the owner may not want to report and will find it easier to dispose of a carcass by dumping it on neighbouring common land which is usually forest. An information campaign which warns of the danger of this may improve the situation. Where the rendering service will become most useful is if an outbreak of ASF does occur, after slaughter of infected herds, disposal of carcasses by rendering provides a better alternative for disposal of carcasses rather than burial or incineration on site.

Swill feeding: While large scale

- feeding of swill is not practiced in Kosovo,
- there is a need to make pig keepers aware of the potential dangers of feeding food waste to pigs which contains discarded remnants of preserved and fresh pork from domestic pigs and wild boars.

- **Biosecurity:** Pig keepers in Kosovo have not yet learnt to observe biosecurity practices which can protect their pigs from infectious diseases, including ASF.

- There is great need for bringing good biosecurity practices to the attention of pig keepers and promoting their use.

- **Avareness:** Awareness campaigns can be powerful tools to inform the backyard pig keepers in the pig keeping areas of the dangers they are facing, the consequences for them if they face an ASF outbreak and the steps they can take to mitigate the risk of this.

KFVA

- There is an annual campaign mounted by the KFVA through contracted private veterinarians to vaccinate pigs against CSF. This opportunity to contact pig keepers is an ideal time to distribute information on the risk and consequences of ASF outbreaks. Increasing pig keepers' awareness of ASF and providing them with the information they need to avoid incursions of ASFV and, at least, reduce the size of outbreaks, appears to be the first important step towards avoiding ASF, or at least limiting its impact.

- It is proposed that a handout for pig farmers is prepared in the relevant languages which simply describes the disease and its effect on farmers' livelihoods, as well as showing the clinical signs likely to be seen if an outbreak occurs.

- It should also describe importance of reporting suspected outbreaks and the

biosecurity measures which farmers should adopt in general, including secure pig housing in which the pigs are kept at all times, avoidance of feeding food waste which may contain pig or wild boar products and purchase of pigs from a known source where there is no evidence of disease and, if an outbreak of ASF occurs, standstill on movement, banning of visitors to pig pens and barrier husbandry with disinfectant footbaths and protective clothing).

This handout to be delivered primarily during the visit by contracted veterinarians each year to each pig premises when they vaccinate the pigs against CSF and also when other opportunities arise.

It is proposed that veterinarians' awareness with regard to ASF is updated by sending all veterinary practices a handout in appropriate languages detailing, with pictures, the clinical signs of ASF in domestic pigs and wild boars and reminding veterinarians of their reporting obligations of this notifiable disease.

The handout should be accompanied by a wall poster for display in veterinary surgeries to be a constant reminder of the likelihood of incursions of this disease. Increasing awareness of hunters, forestry and park rangers and others working in the forests of Kosovo is also important.

A handout should be prepared for them on what they should do if they find sick or dead wild boars, emphasising their reporting obligations.

Also, hunters should be warned about their contacts with domestic pigs after hunting wild boars and feeding waste from wild boar carcasses. Similarly, a poster on these subjects should be prepared for display in forestry and park offices.

The general public should also be made

MAFRD

ASFV

aware of ASF through the media with regular updates, reassuring them that there is no public health concern associated with the disease but informing them of the potential serious impact on the pig industry in Kosovo.

Hunting practices: Hunting of wild boars as an ASF control activity is a complex issue. Eliminating wild boars would remove a wildlife host capable of maintaining ASF but this would be difficult to achieve and, as there is a good environment for wild boars in Kosovo, there is the likelihood that the wild boar population would be replenished with wild boars from neighbouring countries. Also, hunting of wild boars can cause their perturbation which can cause them to disperse, carrying infection with them if they are infected with ASF.

On the other hand, hunting wild boars may keep the population to a level at which ASF cannot be maintained in wild boar populations unless reintroduced by domestic pigs or wild boars from neighbouring countries. Officers in MAFRD need to be informed on current knowledge of best practice regarding hunting wild boars when making decisions on hunting policy for wild boars in Kosovo.

Soft ticks: Studies have not been undertaken on the prevalence and distribution of soft ticks in Kosovo, particularly on their presence in pig housing. Because of their potential to act as intermediate hosts for ASFV and maintain the virus in the absence of pigs, for example after culling has taken place, it is recommended that they should be studied and, if found necessary, provisions for their control be included in contingency planning.

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(*Cyprinus carpio*)

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Histopathological Changes of Liver and Intestine of Common Carp (*Cyprinus carpio*) after Long-Term Starvation

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SUMMARY

Starvation is defined as deprivation of food for a prolonged period of time, which can cause permanent internal organs damage. In order to assess the effect of starvation, especially histological changes on some of the digestive organs like liver and intestine of common carp (*Cyprinus carpio*), the present study was conducted among 20 fish were starved for 150 days.

Samples for analyses were collected and fixed in Davidson's solution at room temperature for 24 h, dehydrated in 70%

20

150

70% (24), (4-5 μm) (H&E).

(Atanasoff et al., 2017).

(Atanasoff et al., 2018; Zapryanova et al., 2016), (Koynarski et al., 2018), (Jain et al., 2011)

ethanol, treated with xylene and embedded in paraffin wax. Paraffin blocks were sectioned (4-5 μm thickness) on a microtome dewaxed and stained with haematoxylin and eosin (H&E) reagents.

The microscopic findings indicate hepatic steatosis and hyperemic liver blood vessels. Numerous small vacuoles were found in the cells' cytoplasm. Most of these were fat droplets extracted during treatment with the preparation. A small part of the cells' nuclei was smaller (pyknosis) and intensively stained which indicates karyopnicosis. In others, chromatin was collected in small clumps along the nuclear membrane (hyperchromatosis), while others dystrophic processes were necrobiotic. Part of the epithelial cells of the mucosa were separated from the basement membrane and dropped into the intestinal lumen (enteritis).

Key words: common carp, histopathological changes, intestine, liver, starvation

INTRODUCTION

Under natural or controlled conditions (aquaculture), some fish species face the risk of limited food intake, that could lead to prolonged period of starvation and degradation of important internal organs (Atanasoff et al., 2017).

Negative effects of starvation could be detected in hematological and biochemical indicators (Atanasoff et al., 2018; Zapryanova et al., 2016), non-specific immune parameters (Koynarski et al., 2018) as well as acute phase proteins (Jain et al., 2011) and structural changes in fish digestive organs.

A structural change in the hepatocyte nuclear height preceded by reduction in the intestinal epithelial cell height and connective tissues has been observed in this species common carp (*Cyprinus*

2012). (Cyprinus carpio) (Seong et al.,

carpio) also (Seong et al., 2012).

(Raškovi et al., 2011).

Conventional histopathological analysis of the digestive tract, especially intestine and liver serves as major indicator for the aquatic animals' nutritional status. In fact, all existing methods for histochemical, immunohistochemical and stereological examination of fish do not depart substantially from those used in other animals (Raškovi et al., 2011).

The present investigation aimed to study the effect of prolonged starvation on histological and morphological structure of liver and small intestine of common carp.

MATERIAL AND METHODS

(*Cyprinus carpio*) 20

The experimental 20 common carp (*Cyprinus carpio*) were obtained from the carp farming soil ponds, near the city Nikolaevo, Bulgaria. Fishes starved for a period of 5 months during the winter period when weather conditions did not allow access to the farm. Six fish obtained from fish farm and fed with commercial feed was used as control.

After transportation fishes were randomly assigned in the aquaculture experimental base at Trakia University.

(2010/63/)

Fish were sacrificed by decapitation according to the Guidelines of the European Union (2010/63/UE) and Animal Welfare Act and approved by the Committee on Animal Experimentation at the Trakia University, Stara Zagora, Bulgaria.

(462,04 ± 2,13 g).

The liver and small intestine were collected and prepared for histopathological analysis. Body weight was measured using electronic weighing machine (462.04±2.13 g). Materials for histopathology examination were fixed in Davidson's solution at room temperature for 24 h, dehydrated in 70% ethanol, treated with xylene and embedded in paraffin wax. Paraffin blocks were

24

70%

sectioned (4-5 μm) on a microtome Leica RM 2125 (Leica Microsystems GmbH, Austria), dewaxed and stained with haematoxylin (Sigma-Aldrich-HHS16), and eosin (Merck 109844, Merck KGaA, Germany), according to the method described by Culling (1963).

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RESULTS AND DISCUSSION

The starved fish did not show any alteration throughout the period of experiment in health status and behavior. No mortality and morbidity was observed during this study.

Histopathological changes observed in the liver, and intestine of the exposed fish to prolonged starvation and control fish are illustrated in Figures 1-4.

The liver of the food deprived fish group were characterized hepatic steatosis. Numerous small vacuoles were found in the cytoplasm of hepatocytes (Figure 1) compare with control fish (Figure 2).

The described structures were fat droplets extracted during treatment with organic and alcohol reagents (Figure 1). Typically, changes in response to starvation include the disappearance of lipid droplets. The turnover time for lipid in digestive tract is a few hours, hence starved fish devoid lipid droplets for a period of few weeks.

In food deprived cyprinid fish (from few hours to a period of 10 weeks) the observations showed progressive reduction of glycogen and lipid vacuoles in cytoplasm (Ba-Omar and Victor, 2000; Al-Nieem et al., 2010).

Prolonged starvation of carp has been reported to decrease cytoplasm of hepatocytes (Al-Niaeem et al., 2010).

During starvation, the contents of the hepatocyte nucleus are converted to non-chromosomal protein, resulting in changed

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(Storch and Juario, 1983).
 (Liza abu) 13-
 (Jasim, 2006). 30-
Misgurnus anguillicaudatus
 (Park, 2018).

shape. Therefore, the observation on hepatocyte nuclear size is useful to determine nutritional status of the cell (Storch and Juario, 1983). Some of the observed nuclei were smaller and intensively stained (karyopicnosis). In other, chromatin was collected in small clumps along the nuclear membrane (hyperchromatosis). The aforementioned histopathological findings are essentially the same as reported in mullet (Liza abu) in a 13-week study (Jasim, 2006). A 30-days starvation study with *Misgurnus anguillicaudatus* detected decreased hepatocyte nuclear areas without karyolysis (Park, 2018). Obvious histological changes were not found in liver of control fish. The liver had normal structure, hexagonal shaped hepatocytes had round nuclei and a uniform cytoplasm (Figure 2).

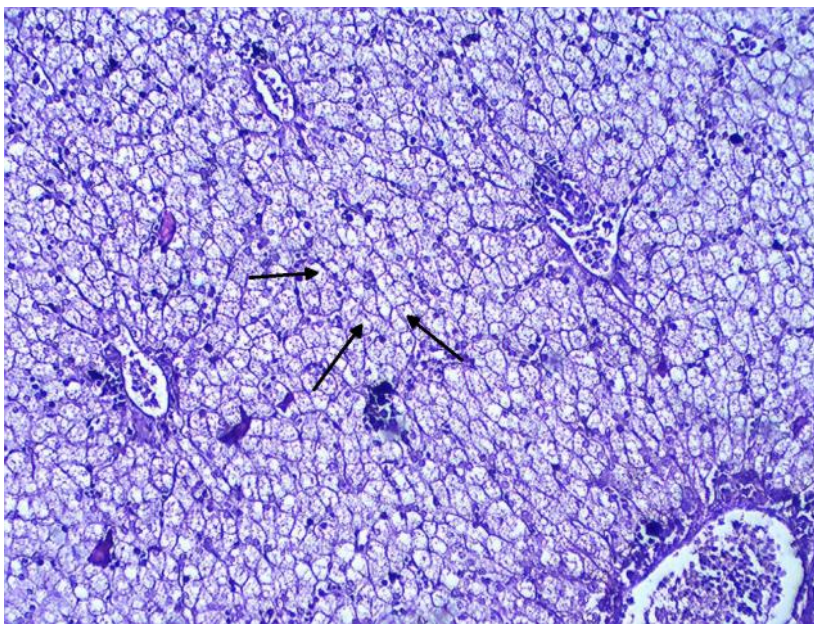


Fig. 1. Liver histopathology of starved fish. Small bright vacuoles (black arrows) observed in the cytoplasm of the liver cells, which are fat droplets extracted during preparation. (H&E stain; original magnification 10x)

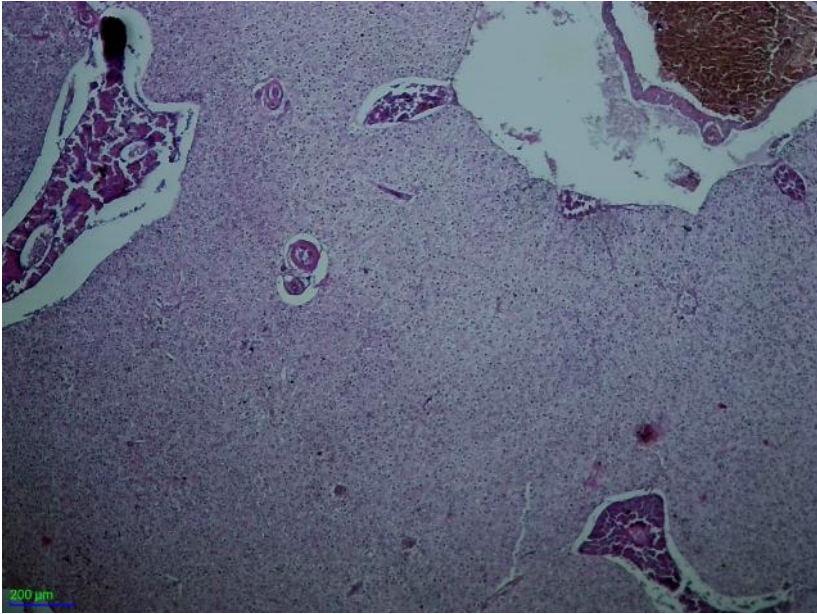


Fig. 2. Light micrograph of normal liver structure (H&E stain; original magnification 4x)

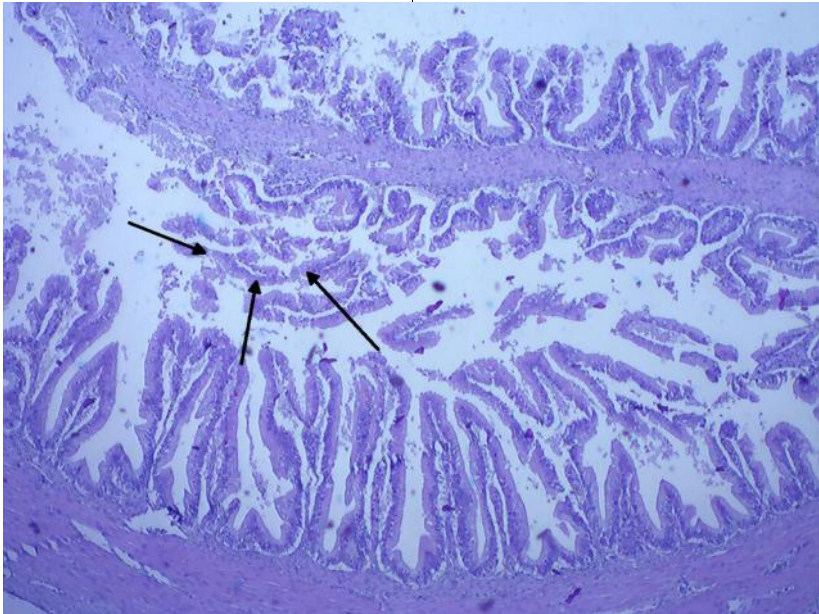
In present study, intestinal mucosal epithelium of starved fish consisted of enterocytes showing basically located large oval nuclei. Connections between the epithelial cells of the mucosa and basement membrane were not well preserved, resulting in partial dropped off into the intestinal lumen, which is a sign for enteritis. The goblet cells of common carp, when starved, showed changes in their number, shape and location (Figure 3).

Some cells were found close to the submucosa, away from their usual location in the apical region of the mucosa. There was a considerable reduction in the number of cells which were more rounded shape compared with control fish (Figure 4).

The intestinal histological changes of the long-term starved carp contradict findings from previous study conducted by

Al-Nieem et al. (2010),

- Al-Nieem et al. (2010), where after longitudinal section of intestinal epithelium a desquamation of the apical portion of the mucous folds was detected.
- In control fish, the intestine was normal.
- All intestinal villus (transverse folds on the walls) in fed fish were poorly developed.
- In contrast, the mucosa showed large number of well-defined longitudinal folds extending along the whole intestinal.
- Goblet cells are the dominant mucus cell type in fish intestine epithelium.
- Furthermore, in common carp their number is highest in the crypts. However, in the fed carps the intestinal goblet cells were clearly visible and differentiated but the number was reduced.

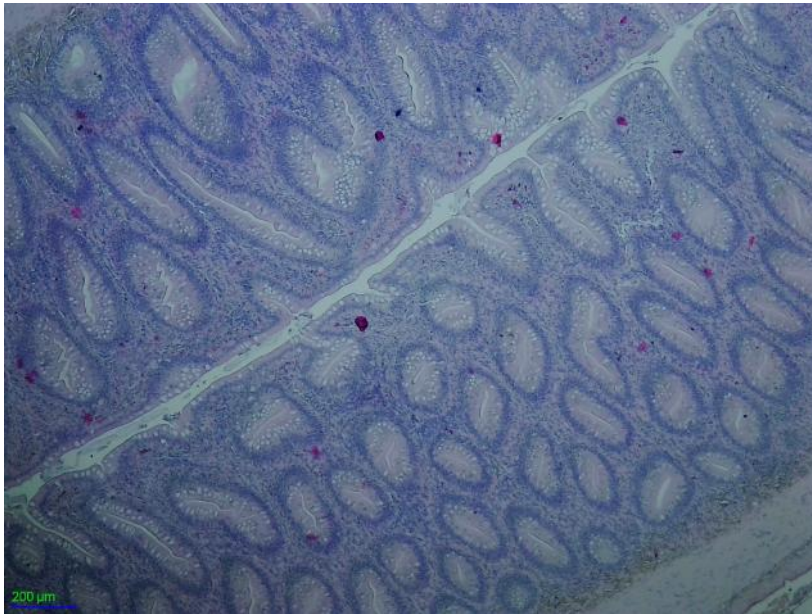


3.

(H&E

40 ×)

Fig. 3. Intestine histopathology of starved fish. Part of the epithelial cells of the mucosa disconnected from the basement membrane and slough in the intestinal lumen showing with black arrows (H&E stain; original magnification 40×)



4 ; 4x) (H&E
 Fig. 4. Light micrograph of normal intestine structure (H&E stain; original magnification 4x)

CONCLUSIONS

- Our results provide an accurate indication of the histological changes identified in liver and intestine in 5 months starved common carp. This study indicates that common carp have the ability to withstand a relative long-time starvation.
- The results obtained in the end of study had yielded some data on the effect of pro-longed starvation such as hepatic steatosis in the liver, thickening of muscularis mucosa decrease the number of the goblet cells in the intestine.
- This data would be important in determining the optimal feed and feeding regime of common carp reared of earthen ponds through the different seasons.

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