SCIENTIFIC OPINION

Scientific Opinion on foot-and-mouth disease in Thrace

EFSA Panel on Animal Health and Welfare

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT:

Introduction of FMDV into Thrace by wildlife is less likely than introduction due to movement of domestic animals or animal products. Based on a systematic literature review, currently available data of surveillance in wildlife and the epidemiological model, FMD will not be sustainable in the wildlife population in Thrace although limited spread of FMDV in time and space may occur. There are several potential risk factors associated with both introduction and spread of the FMDV infection in the region. The most important of these are biosecurity, movement of live animals and animal products, swill feeding and access to landfill waste. The absence of significant clinical signs in sheep in particular, and the increased levels of livestock movements associated with particular festivals in this region, give rise to specific concerns. Active surveillance for early detection of FMDV infection in wildlife could be a useful addition to an effective passive surveillance system in domestic animals. The EFSAwbFMD model indicated that when the sampling strategy in wildlife was based on hunting alone, the time needed to detect at least one seropositive animal for an FMDV incursion in January and July would be 39 and 13 weeks after incursion of the virus into the population respectively, whilst, when regular sampling was implemented over the whole year, about one month is needed. The precise pathway for the introduction of FMDV into Bulgaria for the 2010/2011 outbreak and its subsequent spread is not known. One possible explanation based on the genetic relationships between viruses in Bulgaria is a single introduction of virus into the country from Anatolian Turkey but it is also possible that the common ancestor was introduced into Turkish Thrace and quickly moved to Bulgaria either through a single introduction or through several introductions from the same source within a relatively short time span.

KEY WORDS

Foot-and-mouth disease, wildlife, epidemiology, Thrace, surveillance, early detection, genetic characteristics

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SUMMARY

Following a request from the European Commission and the Bulgarian Food Safety Risk Assessment Agency–Risk Assessment Centre, the Panel on Animal health and Welfare was asked to deliver a scientific opinion on foot-and-mouth disease (FMD) in Thrace. The scientific opinion addresses the following three terms of reference (TOR):

TOR 1. The relative significance of and the role played by wild and feral bi-ungulates, notably wild boar and deer species in the epidemiology of foot-and-mouth disease (FMD) in Thrace (Bulgaria, Turkey and Greece), taking into account the different FMD virus (FMDV) strains circulating in the region.

Introduction of FMDV into Thrace by wildlife is less likely than introduction due to movement of domestic animals or animal products.

Based on a systematic literature review focusing on experimental FMDV infections in wild boar and deer, it was concluded that transmission from wild boar or deer to domestic animals et vice versa can occur so wildlife can play a role in the spread of FMD. The evidence however did not prove that such cross-transmission of the virus will occur in a natural setting when proximity of infected animals may be much lower than under experimental conditions.

Historical observations of FMD in wildlife populations and a systematic literature review of observational studies in wildlife revealed no evidence for maintenance of infection within wildlife in Europe. The important issues were considered to be whether these wildlife animals are active players in the spread of the disease and can transmit the virus to domestic animals or whether they only become infected through contact (direct or indirect) with FMDV infected domestic animals but do not pose a threat to domestic animals.

In the recent outbreak in Bulgaria, 2011, no virus was isolated from wildlife except for the index case. Results of the sero-surveillance of wildlife in Bulgaria and Turkey (February 2011 to January 2012) suggested that circulation of FMDV in wildlife was spatially related to the outbreaks in livestock. There was no evidence of disease occurrence in wildlife anywhere else in Turkish Thrace or further north in Bulgaria. The relatively low sero-prevalence and clustered spatial distribution of positive wild boar and deer indicated that the wildlife FMD event in the Bulgarian-Turkish cross-border area apparently failed to develop into a large scale epidemic wave. It remains questionable, if and for how long after the last series of outbreaks, the virus could have been present in the population of wild boar, and to which extent the FMD affected domestic livestock contributed to the disease spread in wildlife in April 2011.

The epidemiological model to simulate spread and maintenance of FMD in the wild boar and deer populations also indicated that FMD will not be sustainable within a wild boar and deer host system alone but limited spread of FMDV in time and space may occur. Naturally, if there is a continued cross-over of FMDV between domestic and wildlife population then circulation may be prolonged. Moreover, the epidemiological model supported that decisive spatial differences in host density and hot summer temperatures may have shaped the limited size of the observed outbreak region in Thracian wildlife.

Epidemiological observations, published literature and modelling support the conclusion that the wildlife population is not able to maintain FMD in the absence of FMDV infection in the domestic host population. All of the FMD viruses sampled in Bulgarian Thrace were of serotype O PanAsiaII-Ant10 lineage. There is no evidence that any of the circulating strains of FMDV (serotypes O, A and Asia 1) in the wider region would cause a different outcome.

TOR 2. The risk factors and other relevant epidemiological features, in particular for the different FMD virus strains circulating in Thrace (Bulgaria, Turkey and Greece) which must be
taken into account for the design of surveillance systems (including estimation of advantages and disadvantages), that could be implemented for the early detection of any FMD virus incursion in the territory of Thrace (Bulgaria, Turkey and Greece).

There are several potential risk factors associated with both introduction and spread of the FMDV infection in the region. The majority of these factors are related to biosecurity, movement of live animals and animal products (either legally or not), swill feeding or access to landfill waste, animal density, and the implementation and efficacy of vaccination programmes. The interaction between all risk factors also needs to be considered.

In addition to the above, more general risk factors for introduction of FMDV, the absence of significant clinical signs in sheep in particular, and the increased levels of livestock movements associated with particular festivals in this region, give rise to specific concerns.

An active but spatially restricted/targeted surveillance system for early detection of FMDV infection in wildlife could be a useful addition to an effective early passive detection system in domestic animals. The EFSAwbFMD model indicated that when the sampling strategy is based on hunting alone, the time needed to detect at least one seropositive animal for an FMDV incursion in January and July into a wild boar population, would be 39 and 13 weeks after incursion of the virus into the population respectively. The implementation of a surveillance system throughout the year required about a month to detect the first seropositive animal after incursion of the virus into the population. Disadvantages related to a surveillance system that could be implemented for early detection in wildlife based on hunting alone could be associated with one or more of the following adverse factors: collection of samples us limited to the hunting season (e.g. 4 months during winter); low public awareness of FMD and its association with hunted animals and poor sample quality for laboratory testing.

TOR 3. The relevance and significance of epidemiological data and genetic characteristics for the different FMD strains recently isolated in Bulgaria and Turkey, with regards to the hypothesis of single versus multiple introductions into Bulgaria.

Each of the Bulgarian outbreak viruses appear to share a single recent ancestor which is most closely related to viruses detected previously circulating in Anatolian Turkey. The precise pathway for the introduction of FMDV into Bulgaria and its subsequent spread is not known. One possible explanation based on the genetic relationships between viruses in Bulgaria is a single introduction of virus into the country from Anatolian Turkey, but it is also possible that the common ancestor was introduced into Turkish Thrace and quickly moved to Bulgaria either through a single introduction or through several introductions from the same source within a relatively short time span. Significant gaps in the genetic relationships between the sampled viruses from Bulgaria point to the potential presence of unreported (and hence unsampled) infection in domestic animals (consistent with the identification of seropositive domestic animals) and/or to spread in wildlife reservoirs (consistent with the detection of seropositive wildlife). Epidemiological data and genetic characteristics of the different FMDVs detected in Bulgarian Thrace indicate that FMD did spread through wildlife population but it also involved human transportation.
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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION AND THE BULGARIAN FOOD SAFETY AUTHORITY- RISK ASSESSMENT CENTRE

Foot-and-mouth disease (FMD) is a viral disease primarily of cloven-hoofed animals that can profoundly affect animal husbandry by evolving into severe epidemics that reduce the productivity of susceptible livestock. FMD is certainly the most significant animal disease in terms of international trade. The potential impact on the EU is evident from the 2001 FMD epidemic that affected four Member States.

EFSA has previously delivered scientific opinions on FMD\(^4\). In particular EFSA has assessed the risks of re-introduction of FMD into Europe, identified the likely pathways by which this could occur, and assessed the potential of possible intervention measures in infected non-EU countries to reduce the likelihood of FMD virus introduction.

On 5\(^{th}\) January 2011, Bulgaria notified a case of FMD in a wild boar shot in the municipality of Tsarevo in the Region of Burgas. Outbreaks of FMD were detected in domestic animals on 9\(^{th}\) January following laboratory testing of samples taken in the restricted area. This was the first outbreak of FMD in Bulgaria since 1996. Until 7\(^{th}\) April, the date of the last outbreak, 11 outbreaks were confirmed in livestock kept in that mountainous forest area which stretches into Turkish Thrace and is inhabited by a rich susceptible wild and feral fauna.


The causative FMD virus of serotype O belongs to the PanAsia-2-lineage, which is widespread in the Anatolian Part of Turkey and other countries in western Eurasia, according to genotyping performed at the EU Reference Laboratory for FMD. The genotype of the FMD virus isolated from the wild boar is almost identical to an FMD virus isolate collected near the eastern Black Sea coast of Turkey in July 2010, showing only one nucleotide change in VP1 sequencing. However, the FMD virus from the initial cluster of cases in livestock in Kosti and the virus of the second outbreak in Rezovo and moreover the viruses isolated from the second wave of outbreaks in the Sredets municipality are, following full-genome sequencing, genetically distinct, suggesting no direct connection between any of these outbreak clusters. The virus may have evolved from a putative common ancestor in late 2010 that is not less than 31 nucleotide changes different in the whole genome from the aforementioned Bursa isolate. This suggests that wildlife might have been affected and a series of transmissions might have occurred leading to a genetically distinct virus affecting the domestic animals in the first and second waves of outbreaks in livestock.

Despite epidemiological investigations, the primary source of the FMD outbreaks in Bulgaria remains unknown. One hypothesis is that the FMD virus may have entered Bulgaria through infected wild boar from Turkish Thrace, although it has been recognised by the World Organisation for Animal Health (OIE) since 2010 as free of FMD with vaccination. In addition, surveillance carried out by the Turkish authorities in January and February 2011 did not reveal clinical infection in livestock and no clustering was found for the limited number of livestock that had antibodies to non-structural proteins of the FMD virus. An alternative hypothesis suggested that wild boar, extensively kept domestic pigs or feral pigs could have had access to waste food or other fomites contaminated with the virus.

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\(^4\) Opinion of the Scientific Panel on Animal Health and Welfare (AHAW) ON "Assessing the risk of Foot-and-mouth Disease introduction into the EU from developing countries, assessing the reduction of this risk through interventions in developing countries / regions aiming at controlling / eradicating the disease, and Tools for the control of a Foot-and-mouth Disease outbreak: update on diagnostics and vaccines", adopted on 5 February 2006 (HTTP://WWW.EFSA.EUROPA.EU/EN/EFSAJOURNAL/PUB/3 13.HTM)


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However, it appears that with the exception of the index case, no FMD virus-positive wild boar or other wildlife have been found in the Bulgarian part of this particular biotope which is shared between Bulgaria and Turkey. Investigations carried out on that biotope in Thrace revealed wild boar which tested positive for antibodies to non-structural proteins of the FMD virus. In addition, it remains unclear whether the maintenance of endemic infection in a wild boar population is likely to occur, or whether the duration of an epidemic depends on the size of the wild boar population, contact with non-immune domestic animals or other characteristics of the ecosystem.

The extent of infection in the wild boar or other wild populations of FMD susceptible animals and the duration and epidemiological characteristics of a sylvatic FMD epidemic in a European context are unknown. A possible mechanism for the introduction of FMD into the EU might therefore be an undetected epidemic in wild boar in countries adjacent to Member States. Further transmission within the EU could also be related to susceptible wildlife, notably wild boar.

Hunting activities are valuable for surveillance in hunting areas, especially at the forest edges, and are a possible option for surveillance particularly in summer. Surveillance in the Thrace region should therefore be designed and implemented taking into account the expected duration of an epidemic and the possible repeated rounds of sampling to provide further information for risk assessment and risk management.

The presence of FMD in the EU neighbourhood represents a challenge for all risk managers; the Commission is therefore in need of scientific advice to update and strengthen risk management measures on FMD. In addition, several scientific questions have been raised following the recent epidemiological situation. In particular, these relate to the role of wildlife in the epidemiology of FMD under European conditions, the risks of transmitting the disease and maintaining a persisting FMD infection in wild and feral animals of susceptible species, the surveillance systems and to what extent phylogenetic data can be linked to geospatial data for virus tracking purposes.

**TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION AND THE BULGARIAN FOOD SAFETY AUTHORITY-RISK ASSESSMENT CENTRE**

1. The relative significance of and the role played by wild and feral bi-ungulates, notably wild boar and deer species in the epidemiology of foot-and-mouth disease (FMD) in Thrace (Bulgaria, Turkey and Greece), taking into account the different FMD virus strains circulating in the region.

2. The risk factors and other relevant epidemiological features, in particular for the different FMD virus strains circulating in Thrace (Bulgaria, Turkey and Greece) which must be taken into account for the design of surveillance systems (including estimation of advantages and disadvantages), that could be implemented for the early detection of any FMD virus incursion in the territory of Thrace (Bulgaria, Turkey and Greece).

3. The relevance and significance of epidemiological data and genetic characteristics for the different FMD strains recently isolated in Bulgaria and Turkey, with regards to the hypothesis of single versus multiple introductions to Bulgaria.
ASSESSMENT

1. Structure of the report

To answer the three ToR’s described above, the following approach was used:

For the first ToR, relating to the relative significance and the role played by wild ungulates in the epidemiology of FMD in Thrace, the report describes first the necessary background information to understand the epidemiology of FMD in Thrace.

It starts by giving a short description of the geographical boundaries of Thrace (Section 2). This is followed by a summary of FMD occurrence in domestic and wild animals in Thrace in recent decades (Section 3). More detailed information of the FMD outbreaks and surveillance activities in livestock is given in Appendix A and more information on the livestock density and husbandry systems is provided in Appendix B. A detailed description of the recent surveillance activities in the wildlife populations in Thrace is also provided in Section 3. All details related to the ecological, behavioural and population characteristics relevant to the epidemiology of foot-and-mouth disease in susceptible wild ungulates of Thrace are given in Appendix C. The report then continues by summarising the risk factors that are important for introduction and spread of FMD in the region (Section 4).

After presenting the background information on the occurrence of FMD in Thrace, and the risk factors for introduction of the disease, the report provides a systematic literature review of FMD observations in wild boar and deer, both at a population level (Section 5) and at an individual animal level (Section 7), the latter being a systematic review of experimental infections in wild boar and deer that also describes the infection dynamics. Furthermore, Section 6 provides the necessary background information about FMD virus (FMDV) characteristics.

Some of the background information given in Sections 2-7 is then used to fit into an epidemiological model, addressing the possible maintenance of an FMDV incursion into an area populated by wildlife (i.e. wild boar and/or deer) (Section 8). The answer to ToR 1 (Section 9) is based on a review of the background information, especially the systematic literature review of FMD in wild boar and deer, the known risk factors for introduction of FMDV into Thrace, the surveillance results of the recent surveillance programme in wildlife in Thrace, and the outcomes of the simulation model.

The second ToR, concerning the risk factors that need to be taken into account for designing surveillance systems, is answered firstly by listing the most important risk factors for introduction of FMD in Thrace (Section 4). During the course of preparing this Scientific Opinion, it was considered by both the requestors of the Scientific Opinion and the AHAW Panel that guidelines for passive and active surveillance for early detection of FMD in domestic animals were already sufficiently developed.

However, targeted surveillance systems in wildlife for early detection of new FMDV incursions do not exist. It is up to the competent authority of the affected Member State to develop and present plans for surveillance, control and eradication of FMD in wild animals in an affected area. A model was developed to assess the suitability of different surveillance systems for early detection of FMD incursions in wildlife. The advantages and disadvantages of the different surveillance systems were provided.

The third ToR, regarding the significance of epidemiological data and genetic characteristics of the different FMD strains recently isolated in Bulgaria and Turkey, and the hypothesis of single versus multiple introductions into Bulgaria, is answered by a narrative discussion. First, the heterogeneity and the genetic characterisation of the FMDV’s involved in Thrace are discussed, and, secondly, the genetic and epidemiological information concerning the spread of the virus in Thrace was combined in order to discuss the likelihood of single versus multiple introductions of the virus in Bulgaria.
2. Geographical description of Thrace

Thrace is a geographical area in south-east Europe, including the territories of south-eastern Bulgaria, north-eastern Greece and the European part of Turkey. The area is bounded by the Balkan Mountains in the north, the Black Sea in the east, the Aegean and Marmara Sea in the south, and the Rhodope Mountains in the west (Figure 1 and 2).

The portion of Thrace that is part of Greece is bounded by the Néstos River to the west (Encyclopædia Britannica, online), the Bulgarian border to the north, and the Turkish border to the east. It comprises the areas of Drama, Xanthi, Rhodope Prefectures and Evros. The Bulgarian part of Thrace includes the regions of Smolyan, Pazardjik, Plovdiv, Kardzhali, Haskovo, Stara Zagora, Sliven, Yambol and Burgas. On the west, it is bounded by the Rhodope Mountains and to the north by the Balkan Mountains. European Turkey, including the Gallipoli Peninsula, constitutes the remainder of the geographical region of Thrace (Encyclopædia Britannica, online).

Figure 1: Thrace and present-day state borderlines

Figure 2: Regions or prefectures in Thrace
3. FMD occurrence in Thrace

Detailed epidemiological investigations of the FMD outbreaks that have occurred in 2011 in Bulgaria have been conducted by national authorities and international institutions (i.e. EUFMD, 2011; EU Veterinary Expert Team Mission, 2011; FAO/OIE/EC, 2011a, b; SCoFCAH, 2011; BFSA, 2012). To determine the “relative significance of wildlife in the epidemiology of FMD in the region” (ToR 1) and to determine the risk factors that need to be taken into account for designing surveillance systems for early detection of FMD (ToR 2), the most important observations concerning FMD occurrence in Thrace during the last two decades were considered. More detailed descriptions of the earlier FMD history in Thrace can be found in Valarcher et al. (2008), and the recent FMD surveillance activities in domestic animals and a detailed description of the latest Bulgarian FMD outbreaks can be found in Appendix A.

3.1. FMD outbreaks during the last decade in Thrace

In the last two decades, outbreaks have occurred in Bulgarian Thrace in 1991, 1993, 1996 and 2011 (Figure 3). In Greece, major outbreaks occurred in 1961, 1994 and 1996 and the last outbreak occurred in 2000 (Valarcher et al., 2008).

Figure 3: Outbreaks of FMD in Bulgaria during the period 1991-2011

All the outbreaks in Bulgaria (Figure 3) and Greece (with the exception of the outbreak in 1994) were located close to the Turkish border. Illegal movements of susceptible domestic animals were indicated as the probable source of infection in these outbreaks. The outbreaks were all stamped out quickly and both Greece and Bulgaria returned to the FMD-free status without vaccination (see Appendix A).

On the 5th of January 2011 Bulgaria notified a case of FMD in a wild boar which was hunted about 2 km north of the Turkish border. After this index case, 11 outbreaks in susceptible domestic animals were detected in southeast Bulgaria from January to April (Table 1 and Figure 4). Two series of outbreaks were observed. The first series of outbreaks (outbreak 1 to 3) occurred in January 2011 at the southeast of the Burgas region in the municipalities Tsarevo and Malko Tarnovo. The second series of outbreaks (outbreak 4-11) occurred more in the southwest of the Burgas region in the Malko Tarnovo and the Sredets municipalities in March and April 2011.
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Figure 4: Locations of the place where the FMDV-positive wild boar (wb) was shot and the 11 outbreaks/IP codes declared by Bulgaria (1-11)

A detailed description of the outbreaks in Bulgaria in 2011 can be found in Appendix A. The chronology of laboratory confirmation of infection or previous infection of premises does not necessarily represent the actual chronology of the cases with virus infection, for example the infection of the seropositive sheep of IP 9-11 may have occurred in time before the virus positive cattle in IP 8.

Table 1: Outbreaks in Bulgaria in 2011

<table>
<thead>
<tr>
<th>Outbreak</th>
<th>Date of suspicion</th>
<th>Date of confirmation</th>
<th>Age lesions (days)</th>
<th>Large ruminants</th>
<th>Serology/antibodies detected (a)</th>
<th>Virology/virus detected (b)</th>
<th>Small ruminants</th>
<th>Serology/antibodies detected (a)</th>
<th>Virology/virus detected (b)</th>
<th>Pigs</th>
<th>Serology/antibodies detected (a)</th>
<th>Virology/virus detected (b)</th>
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<tr>
<td>Wild boar Kosti</td>
<td>30/12/2010</td>
<td>05/01/2011</td>
<td>&gt;10</td>
<td></td>
<td>NA</td>
<td>NA</td>
<td></td>
<td>NA</td>
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</tr>
<tr>
<td>IP1 Kosti</td>
<td>07/01/2011</td>
<td>09/01/2011</td>
<td>2-5 days old lesions</td>
<td>1</td>
<td>1</td>
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<td>-</td>
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<td>17/01/2011</td>
<td>4-5</td>
<td>12</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td></td>
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<td>0</td>
<td>23</td>
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<td>-</td>
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<td>19/03/2011</td>
<td>4-5</td>
<td>7</td>
<td>12</td>
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<td>9</td>
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<td>0</td>
<td>6</td>
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<td>3-5</td>
<td>0</td>
<td>6</td>
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<tr>
<td>IP9 Bliznak</td>
<td>30/03/2011</td>
<td>03/04/2011</td>
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<td>8</td>
<td>-</td>
<td>78</td>
<td>-</td>
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</tbody>
</table>

*Positive for FMDV, serotype O on Ag ELISA, real time RT-PCR and LFD (SVANOVIR, SVANOVA)
Foot-and-mouth disease in Thrace

(a) Serological tests used for surveillance in infected premises (IP) were FMD ELISA type O and/or FMD NSP ELISA
(b) Virological tests used for FMD detection and surveillance in IP were RT PCR and/or Ag ELISA
(c) 21 of the 77 seropositive large ruminants were buffalos. All the other large ruminants tested were cattle.
NA: not applicable, IP: Infected premise
Source: Animal Health and Welfare Directorate and National Reference Laboratory for FMD at the Bulgarian Food Safety Agency

In Turkish Thrace, the last four FMD outbreaks were observed in 2007. Illegal movement of animals, or contact with infected grazing animals were indicated as the source of infection.

In March 2010 (OIE, 2011b), Turkish Thrace was recognised officially as an FMD-free zone in Turkey, where vaccination is practised. Cattle in Turkish Thrace are vaccinated twice annually against FMD, while sheep are vaccinated once. Vaccination, however, will never be 100% efficient and there may be periods (prior to vaccination) when susceptible animals are present. Furthermore, vaccination alone does not completely prevent infection (i.e. it does not provide sterile immunity). Nevertheless, vaccination prevents clinical disease and drastically reduces the possibility of virus spread from one infected animal to another. Therefore, if virus is introduced, only low virus circulation should occur and no clinical disease would be observed within the vaccinated domestic animals in Turkish Thrace. However, in some individual animals limited virus replication and excretion at a low level might occur.

Since September 2011, the FMD-free status with vaccination was suspended in Turkish Thrace, due to the detection of seropositive wild boar in the same region.

3.2. Surveillance of domestic animals in 2011 in Thrace
The details of the surveillance activities in domestic animals are described in Appendix A.

In Bulgarian Thrace, passive and active surveillance activities for domestic animals were implemented in an area in south-east Bulgaria defined as the “cordon sanitaire” (Figure 5) and they were conducted by a plan approved by the European Commission (Decision 2011/493/EU).

![Figure 5: Divisions of three sampling areas within the cordon sanitaire.](image)

Since August 2001 pursuant to the one year plan for the control of foot-and-mouth disease in susceptible wildlife in south-east Bulgaria, all 106 villages in the cordon sanitaire have been visited every 21 days by a team of veterinary experts and all animals of susceptible species were clinically examined. In addition to clinical surveillance, routine blood sampling for serological tests was conducted every third month. A total of 10,498 samples were taken in August and November 2011 and

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they had negative results using Type O ELISA. In addition, no FMD clinical signs were observed during the 5th round of clinical examinations in December 2011.

In Greece, all the 4946 samples taken in 2011 tested negative by anti-NSP ELISA.

In Turkish Thrace, intensive surveillance activities from autumn 2010 until autumn 2011 did not reveal clinical signs of infection in livestock. This may be due to the fact that these animals were vaccinated (see table 12 in Appendix A). A total of 34,609 blood samples from large ruminants and 28,187 from small ruminants were tested with NSP-ELISA with a Se/Sp 90%, 99.1%, respectively. The number of positive animals was less than 1%, and there were no strong positives after retesting. No virus was detected by RT-PCR in probang samples collected from seropositive animals.

3.3. **Surveillance in wildlife 2011 in Thrace**

![Figure 6: Locations of the three sampling areas within the cordon sanitaire in Bulgaria: infected areas A B and C. In Turkey there were eight sampling units, of which units 1 and 2 were given highest priority (from: Khomenko et al, 2011)](image)
Foot-and-mouth disease in Thrace

Figure 7: Sample collection locations (n = 1,077) with results of serological tests conducted from February 2011 to January 2012

Bulgaria and Turkey adopted statistically similar approaches to surveillance in wild boar (Khomenko et al., 2011). The sample size of 59 head per sampling unit (Figure 7) was designed to achieve 95% confidence for detection of FMD antibodies, with an expected FMD antibody (Ab) prevalence of 5%. Other FMD-susceptible species (red deer, roe deer, unowned stray livestock) were targeted, with a sample size of 35 head (10% sero-prevalence at 95% confidence).

In Bulgaria, a defined infected area (area A) has been established, based on the results of epidemiological considerations and the geographical distribution of the disease in January and April 2011. This 20 km-wide region along the Turkish border in south-east Bulgaria covers about 1,240 km². Two risk areas (areas B and C), adjacent to area A, cover about 2,160 km² in the north and west, along the Turkish border (~ 240 km). Together these form a cordon sanitaire to control the possible spread of FMD. The estimated wild boar population was about 1,500 animals in area A and about 3,000 in the risk areas B and C. In autumn 2011, Turkish veterinary authorities extended the original design of the wildlife surveillance scheme (Khomenko et al., 2011), initially focused on the Strandzha Mountains, (Figure 1) to also include parts of Edirne, Tekirdag and Istanbul Provinces (Figure 2).

The sampling framework developed for serological and virological monitoring generally followed the European Food Safety Authority (EFSA) recommendations for classical swine fever surveillance, but with some adaptations. The animals were either hunted (in Bulgaria and Turkey) or trapped (only in Bulgaria). Blood samples and tissue samples (pharyngeal area, skin with lesions, lymph nodes and vesicular fluids, where available) were collected from each animal for serological and virological tests. In Turkey, the NSP ELISA kit PrioCHECK FMD NS (Prionics Lelystad B.V.) was used for initial testing, and positive samples were retested with another commercially available NSP ELISA kit.
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(Svonovir FMD 3ABC-Ab Ruminant, Svanova Biotech AB). In Bulgaria, Type O ELISA was used for serosurveillance in wildlife.

According to the information provided by the respective country veterinary authorities (as of 1st February 2012) in the whole of the Thrace region between February 2011 and January 2012, a total of 1,077 individuals from four susceptible wild species were tested serologically and virologically for FMD (Table 2). No virus was detected, and seropositive animals were found only amongst wild boar and roe deer. Sample sizes for other species (red deer and mouflon (*Ovis musimon*)) were too small (n = 7 and 2, respectively) to draw any conclusions. For 11 samples (wild boar, n = 6; roe deer, n = 4; and one red deer), no laboratory results were available at the time of finalising this report.

Table 2: Numbers of wild animals tested monthly in the course of surveillance campaign in Thrace during the period from February 2011 to January 2012 by species and age groups.

<table>
<thead>
<tr>
<th>Species</th>
<th>Age group</th>
<th>Months, 2011</th>
<th>2011 Total</th>
<th>2012</th>
<th>Grand Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 3 4 5 6 7 8 9 10 11 12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild boar</td>
<td>Ad.</td>
<td>5 11 22 23 19 193 166 160</td>
<td>599</td>
<td>29</td>
<td>628</td>
</tr>
<tr>
<td></td>
<td>Juv.</td>
<td>1 2 30 39 24 96 109 50 351</td>
<td>7</td>
<td>358</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>17 1 18 18 18 18 18 18</td>
<td>968</td>
<td>36</td>
<td>1004</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>5 12 2 0 52 62 43 306 276 210</td>
<td>968</td>
<td>36</td>
<td>1004</td>
</tr>
<tr>
<td>Roe deer</td>
<td>Ad.</td>
<td>1 2 17 12 3 17 9 61</td>
<td>61</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Juv.</td>
<td>1 1 2 2 2 2 2 2</td>
<td>64</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>1 1 1 1 1 1 1 1</td>
<td>64</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1 2 0 17 12 5 18 9 64</td>
<td>64</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Red deer</td>
<td>Ad.</td>
<td>2</td>
<td>1 3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>3 1 4 4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>2 3 1 1 1 7</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Mouflon</td>
<td>NA</td>
<td>0 2 2 2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>All 4 species</td>
<td>Total</td>
<td>6 14 2 0 71 62 55 3 314 294 220</td>
<td>1041</td>
<td>36</td>
<td>1077</td>
</tr>
</tbody>
</table>

Note: Ad. – adults; juv. – juveniles; NA – not available

An average seroprevalence of 7.8 % was found in all the wild boars sampled (Table 8). Adult animals (n = 628) had a seroprevalence of 9.5 % compared to 5.6 % in juveniles (born in 2011, n = 358). This difference was not quite statistically significant according to Fisher’s exact test (two-tailed P value = 0.0634). Among animals of undefined age (n = 18) there was just one positive sample. In roe deer (n = 64, of which 61 were adult animals), seroprevalence was 4.7 % (95 % CI: 0.1 - 13.1 %).

The difference in seroprevalence between all wild boar and all roe deer was not found to be statistically significant (Fisher’s exact test; two-tailed P value = 0.7938). Importantly, the overall seroprevalence in all four species did seem to decline with time, since the percentage of Ab+ animals decreased from 12.7 (95 % CI: 8.5 - 17.9) in February-September 2011 (before the start of the hunting season) to 6.4 (95 % CI : 4.8 - 8.3) during the hunting season (October 2011 to the end of January 2012). This difference was statistically significant (Fisher’s exact test; two-tailed P value = 0.0036).
Specifically in wild boar, seroprevalence calculated by the three month periods starting from February 2011 (including 11 wild boar shot in Turkey in February 2011, of which 5 were seropositive; Naci Bulut, pers. comm.) appeared to be clearly declining with time (Figure 8).

On average, seroprevalence in all wild boar in Turkey (11.3 %) was significantly higher (Fisher’s exact test; two-tailed P value = 0.0498) than in Bulgaria (6.9 %). The difference in prevalence between countries was not significant for adults but, on the contrary, it was extremely significant for juveniles (Fisher’s exact test; two-tailed P values were 0.5510 and >0.0001, respectively; see Table 3). These differences, however, might be strongly influenced by spatial and temporal biases in sample distribution (Figures 7 and 9, and Table 3).

Table 3: Results of the serological surveillance for FMD in wild boar in Turkey and Bulgaria by age groups (February 2011 – January 2012).

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Total sampled (n)</th>
<th>Antibody positive (n)</th>
<th>Prevalence (95% CI) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BG</td>
<td>TR</td>
<td>ALL</td>
</tr>
<tr>
<td>Ad.</td>
<td>538</td>
<td>90</td>
<td>628</td>
</tr>
<tr>
<td>Juv.</td>
<td>257</td>
<td>101</td>
<td>358</td>
</tr>
<tr>
<td>?</td>
<td>17</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>812</td>
<td>192</td>
<td>1004</td>
</tr>
</tbody>
</table>

Note: BG - Bulgaria; TR - Turkey; Ad. - adults; Juv. - juveniles; ? - age unknown.

In fact, closer examination revealed that most positive detections were clustered in the cross-border area near FMD outbreaks in livestock, although some were found further from the border in Turkish Thrace (Figures 7 and 9). Calculation of prevalence in all age groups of wild boar by the 6 buffer zones around outbreaks (defined as direct distances from outbreaks without taking into account differences in height; Table 4 and Figure 9) shows that the highest one (17.9 %) was found at a distance of 6 to 10 km from the outbreak in livestock. No positive animals were reported from the areas further than the 50 km zone around FMD outbreaks (Figures 7 and 9).
Seropositive juveniles (n=20) were found only to the south of FMD outbreak locations with the majority of them being detected in Turkey (n=16), while positive adult wild boar were clearly concentrated at a distance of ~15 km. A few positive animals outside this zone with known sex appeared to be adult males shot during the rutting period between November 2011 and January 2012 (e.g. when they could already move quite long distances away from where they contracted FMDV).

It was not possible to determine the antibody status of the mothers of the juvenile seropositive wild boar (n = 20, Figure 9) during pregnancy, which made it impossible to identify the likely timing of the infection based on their life spans. At the time of testing, some of these positive piglets (n = 8) could still have had maternal antibodies (e.g. piglet Nr. 11-14, 15-18, 20; Figure 9). Otherwise, if their mothers were not immune, one can not exclude that the piglets contracted the virus at any time during their life from April to August 2011, based on their estimated months of birth (4 piglets in April, 3 in May and 1 in June) and dates of killing. The juveniles that lived longer than 3 months (n = 12; e.g. ## 9, 10, 15, 19; Figure 9) were unlikely to have maternal antibodies at the time of testing. However, based on the estimated month of their birth (2 piglets in January, 2 in February, 6 in March, 1 in April and 1 in May), it was again impossible to draw any definite conclusion on the timing of the infection. If these piglets did have maternal antibodies, the earliest they could have been challenged with the FMDV is April and the latest is November 2011. Otherwise, if they were not immune at birth, the period would theoretically extend from January to November 2011.

The fact that the seropositive adult animals, which were killed late in 2011 further than 10 km from outbreaks in livestock, were only males (generally more mobile) also suggested that movements of single positive animals some time after encountering the infection rather than its active spread in an epidemic wave had occurred outside the main infected zone (< 10 km from outbreaks).

The observed significant decline of seroprevalence in all species sampled during 2011 implied that the extent of FMD spread in the wildlife population was not enough to sustain a long-lasting epidemic wave that would produce a constant or increasing seroprevalence over time, and the disease most likely disappeared shortly after the second wave of outbreaks in livestock in April.

**Table 4.** Sero-prevalence in all age groups of wild boar calculated according to the direct distance (not accounting for differences in height) from FMD outbreaks by zones

<table>
<thead>
<tr>
<th>Zone</th>
<th>Negative (n)</th>
<th>Positive (n)</th>
<th>Total (n)</th>
<th>Prevalence (95 % CI) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 5 km</td>
<td>151</td>
<td>17</td>
<td>168</td>
<td>10.1 (6.0 - 15.7)</td>
</tr>
<tr>
<td>6 – 10 km</td>
<td>147</td>
<td>32</td>
<td>179</td>
<td>17.9 (12.6 - 24.3)</td>
</tr>
<tr>
<td>11 – 20 km</td>
<td>204</td>
<td>19</td>
<td>223</td>
<td>8.5 (5.2 - 13.0)</td>
</tr>
<tr>
<td>21 – 30 km</td>
<td>113</td>
<td>-</td>
<td>113</td>
<td>-</td>
</tr>
<tr>
<td>31 – 50 km</td>
<td>176</td>
<td>10</td>
<td>186</td>
<td>5.4 (2.6 - 9.7)</td>
</tr>
<tr>
<td>&gt;50 km</td>
<td>134</td>
<td>-</td>
<td>134</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 9: Locations of sample collection from wild boar with results of serological tests (positive detections shown separately for adult and juvenile animals) and 5 to 50 km buffer zones around outbreaks in livestock.

Figure 10. Estimated life spans of 20 seropositive piglets tested in Bulgaria and Turkey in the course of a surveillance campaign from February 2011 to January 2012.
4. Risk pathways for introduction and spread of FMD in Thrace

![Diagram showing different pathways for introduction and spread of FMDV into Thrace]

**Figure 11:** Different possible pathways for introduction and spread of FMDV into Thrace

There are several potential risk factors that are associated with both introduction and spread of the FMDV infection in the region (McLaws and Ribble 2007; Valarcher et al. 2008). The majority of these factors are related to biosecurity, movement of live animals and animal products (either legally or illegally), swill feeding or access to landfill waste, animal (both domestic and wild life) density, and the implementation and efficacy of vaccination programmes. The interaction between all risk factors also needs to be considered.

In addition to the above, more general risk factors for introduction of FMDV, the absence of significant clinical signs in sheep, in particular, (Uppal 2004) and the increased levels of livestock movements associated with particular festivals in this region, give rise to specific concerns.

Before the outbreak of FMD detected in Bulgaria in early 2011, the previous outbreak in Bulgaria had been in 1996, while no outbreaks have been detected in the Thrace part of Turkey since 2007, and Greece had its last outbreak in 2000 (see section 2). The new incursion into the region could have occurred by a variety of routes. The general risk factors for the introduction of the disease (see Alexandersen et al., 2003) include the movement of infected livestock or products from such animals, or the introduction of virus contaminated materials (e.g. straw) or even by airborne spread from infected animals, although this is normally only applicable over relatively short distances and most likely occurs when a herd of infected pigs (which exhale large amounts of virus) is upwind of cattle (which are highly susceptible to aerosol infection). The virus, once introduced, could be taken up by and infect either domestic livestock (e.g. cattle, sheep and pigs) or susceptible wildlife (e.g. wild boar or deer) and then be spread to other animals by direct contact or indirectly through contamination of the environment.

5. Historical observations of FMD in wild boar, roe and red deer populations

5.1. Wild boar

Wild boar (*Sus scrofa*), the ancestor of the domestic pig, is fully susceptible to all diseases of swine, including foot-and-mouth disease (FMD). The question whether FMD virus can be maintained in a wild boar population and which conditions may facilitate the development of an endemic situation in such a population is of epidemiological importance.
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Literature describing FMD in wild boar populations goes as far back as the beginning of the 20th century (Hutyra and Marek, 1906; cited in Sludskiy, 1956). In the countries of the ex-USSR, clinical disease in wild boar was most often observed in the Caucasus (1902-1925), but also occasionally in southern Kazakhstan (1927-1941) and in 1953 in Kyrgyzstan (Sludskiy, 1956; Danilkin, 2002), until FMD was finally eradicated from the USSR in the 1980s due to the country-wide vaccination of livestock and other control efforts. Donaldson et al. (1988) speculated that two independent FMDV introductions to Israel in 1985 might have been due to airborne spread of the virus emitted by infected wild boar from across the border in Jordan. Following these incidents, a total of 740 boar sera were sampled in Israel between 1987 and 1999, of which 108 (14.6 %) were found positive in serum neutralisation (SN) tests (ProMED-mail, 2007; # 20070517.1571). Additionally, virus was found in 2 out of 73 (2.8 %) of these animals in 1992. In addition, it was reported that as many as 85.7 % (18 out of 21 sampled) wild boars from three locations along Israel’s northern and north-eastern frontiers were positive in the non-structural protein (NSP) ELISA for FMD (ProMED-mail, 2007; # 20070517.1571). In July 2011, wild boar were again implicated as “potential virus disseminators” in northern Israel (ProMED-mail, 2011; # 20110713.2120).

In July 2011, wild boar were again implicated as “potential virus disseminators” in northern Israel (ProMED-mail, 2011; # 20110713.2120).

Figure 12: Anecdotal and documented historical observations of FMD in different regions of the wild boar historical occurrence range (Oliver et al, 1993: background map http://www.ultimateungulate.com/artiodactyla/sus_scrofa.html)

5.2. Roe deer and red deer

European (Capreolus capreolus) and Siberian (C. pygargus) roe deer are also historically known to become infected with FMD virus (FMDV) and develop clinical disease both in the wild, as well as by experimental infection (Thomson et al., 2003). The significance and implication of deer species in the spread of FMDV in case of an FMD epidemic is always an important question as to the role these species may play in maintaining the disease or introducing it into neighbouring unaffected areas. FMD in Siberian roe deer as a large-scale epidemic with high mortality rates was reported in the 1950’s from the Altay region of the former USSR (Sobianskiy, 1992) and occasionally in Kazakhstan (Goreglyad, 1972). Epizooties of FMD in red deer (Cervus elaphus) were observed in the Caucasus in 1908, 1911 and 1925 (Geptner et al., 1961).
5.3. Systematic literature review reviewing observational studies investigating FMD occurrence in wild boar and deer populations.

A systematic literature review was carried out from October 2011 to January 2012, reviewing observational studies investigating FMD occurrence in wild boar and deer populations (see Appendix D for the detailed protocol and the results). The papers that were included after relevance and eligibility screening indicated that FMDV has never been an important pathogen of wild ruminants in Europe. During the 2001 FMD epidemic in Europe, the roe deer tested in the vicinity of outbreaks were found to be serologically negative in Germany, the Netherlands and the UK, which has been explained by relatively rare contacts between cervids and livestock in these countries (Elbers et al., 2001, 2003; Mouchantat, 2005; Mouchantat et al., 2003, 2005; Frolich et al., 2006). At the time of the FMD epidemic in 2001, no exposure of wild boar to FMDV was found in the Netherlands (Elbers et al., 2001, 2003).

This is in line with the anecdotal and review papers where observations of seropositive wildlife were made in areas with concurrent FMD outbreaks in livestock. They were usually considered to be the result of transmission from domestic animals rather than stand-alone epidemics in wild boar or deer species (Sludskiy, 1956; Goreglyad, 1977; Danilkin, 2002), which seems so far to be the case in the absolute majority of FMD detections in wildlife, with the exception of the African buffalo (Thomson, et al., 2003).

6. Foot-and-mouth disease virus (FMDV)

FMDV is the prototype member of the Aphthovirus genus within the picornavirus family. Seven distinct serotypes of FMDV are known (O, A, C, SAT1, SAT2, SAT3 and Asia 1). Serotypes O and A are widely distributed around the globe, whereas the SAT (Southern African Territories) serotypes and the Asia 1 serotype are usually quite restricted in their location. Serotype O is the most common. There is limited or no cross-protection following either infection or vaccination between each serotype and the virus sequences are quite diverged. Within each serotype there are also many strains. At one time different strains were assigned to different subtypes on a serological basis but now different viruses are frequently classified into lineages and sub-lineages based on nucleotide sequence analysis of one of the viral proteins (called VP1). The complete virus genome (RNA) is about 8400 nucleotides (nt) in length while the VP1 coding sequence is only about 640 nt long and thus represents less than 10 % of the genome.

FMDV can infect a diverse range of cloven-hoofed animal hosts (about 70 different species), including cattle, pigs and sheep together with wildlife species such as buffalo, deer and wild boar. In general, the different serotypes and strains of FMDV each cause the same clinical disease within a single species of animal (thus, if FMDV infection is observed in pigs then this could result from infection by any of the serotypes). Furthermore, it is believed that most strains of the virus can affect each of the major susceptible domestic species, although there are a few notable exceptions. For example, in Taiwan in 1997, a strain of serotype O FMDV caused clinical disease in swine but not in cattle. Such strains are described as porcinephilic. There is no evidence that any of the serotypes/strains currently circulating in Eurasia have these atypical properties.

The virus was essentially eradicated from West Europe by the 1970’s using a combination of control measures, including stamping out and the use of chemically inactivated vaccines in conjunction with restriction on the importations of live animals and animal products from endemic countries. Currently, no vaccination against FMDV is permitted in Europe, except in the face of an outbreak, thus the domestic and wildlife animal population is highly susceptible to infection. Sporadic outbreaks still occur within Europe, notably in the UK in 2001, which resulted in infection of over 2,000 premises and resulted in the slaughter of several million animals.
7. Foot-and-mouth disease infection dynamics at the individual animal level in wild boar and deer

A systematic literature review was carried out to provide an overview of existing evidence pertinent to the maintenance of foot-and-mouth disease (FMD) in wild boar and deer, in order to use this evidence to assess the significance and role of wild boar and deer species in the epidemiology of foot-and-mouth disease (FMD) in Thrace, as specified in the terms of reference (ToR 1). The detailed protocol and results are given in Appendix D.

7.1. Wild boar

FMD in wild boar has, to date, not been studied in depth, since this species has so far not played a major role in the spread of the disease. In particular, questions concerning the clinical course, viraemia, virus shedding, incubation period and pre-infection period were and are still uncertain. So far, only two well documented infection experiments exist in wild boar, one conducted in the USA with feral swine of predominantly Eurasian wild boar heritage (Mohamed et al., 2011) and one conducted in Germany with wild boar (Breithaupt et al., in press), while some other reports only provide sparse details in terms of the role of wild boar in the epidemiology of FMD (Ercegovac et al., 1968; Yadin and Chai, 1994).

The wild boar experiment conducted by Breithaupt et al (in press) was performed with the 2011 Bulgarian FMDV type O isolate while the feral swine experiment described by Mohamed et al (2011) was performed with the FMDV A 24 Cruzeiro strain.

In the wild boar experiment by Breithaupt et al. (in press), two animals (four months old) were experimentally inoculated in the bulb of the heel with FMDV. One day later, two wild boar of the same age and two domestic pigs (8 weeks old) were placed as contact animals in the same containment room. Clinical signs appeared after 2 days for domestic pigs and 4 days for wild boar. The most striking result of the experiment was the discrepancy in the clinical course of FMD between wild boar and domestic pigs. While the domestic pigs had to be euthanized due to severe clinical FMD, the general condition of wild boar was less affected. Although wild boar displayed severe foot lesions, the animals’ mobility did not appear to be impaired. These findings suggested that infected wild boar may survive the disease, stay mobile and excrete FMD virus over a longer period, up to 9 days after contact infection.

In the feral swine experiment of Mohamed et al. (2011), 15 feral swine were used of which 2 animals were kept as controls, 4 were inoculated with FMDV and the rest were placed in contact either with inoculated feral or domestic pigs. It was noted that feral swine exhibited a higher tolerance for FMD compared to domestic pigs. The authors observed not only transient fever and vesicular lesions but also lameness in feral swine. The incubation period in feral swine was only 2 days.

There are several factors that have to be considered when evaluating susceptibility of swine to FMDV infections. Certainly, age and size of the experimental animals can influence the clinical course of disease, in particular when the feet are affected. Also the different viruses (A 24 Cruzeiro vs. O Bulgaria 2010) used in the experiments may have had an effect on the clinical signs. A striking example for the importance of the virus strain was seen in the FMDV type O epidemic in Taiwan 1997 when only pigs but no cattle showed clinical signs. In another study, a strong relationship could be demonstrated between virus dose and length of the incubation period (Alexandersen et al., 2003). Furthermore, the genetic background of the wild boar and feral swine used may have played a role.

In the wild boar study, animals already started to shed virus during the incubation period one day after infection or contact. Thus, it could be demonstrated that transmission can occur before the onset of clinical signs. The virus excretion via nasal discharge and saliva lasted for up to 9 days.

Also in respect to the detection of virus specific RNA, there were differences between the wild boar study with O Bulgaria 2010 and the feral swine study with A24 Cruzeiro. Whereas in the A24 Cruzeiro experiments, FMDV specific RNA was detectable only intermittently in the oral swabs of
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feral swine between 1 and 8 DPI/DPE and not beyond 14 DPI/12 DPE, in the wild boar study FMDV specific RNA was found constantly until 13 DPI/DPE and intermittently until 23 DPE and 24 DPI in the oral and nasal swabs. Again, the age of the animals as well as the viral strain and dose may have played a role, but also the sensitivity of the assays and RNA isolation protocols may have been different.

It is generally believed that pigs do not become virus carriers (Alexandersen et al, 2003). However, in the A24 Cruzeiro experiment, FMDV specific RNA persisted in the tonsils up to 33–36 DPI/DPE, whereas virus isolation was negative.

The available data on FMD in wild boar can be summarised as follows. After an incubation period of only a few days (2 to 4 days) first clinical signs are noticed. The severity of the clinical course is rather mild, compared with disease in domestic pigs. Although severe foot lesions are seen, the animals’ mobility does not seem to be impaired substantially. Viraemia starts very early, already two days after exposure and lasts for up to one week while the virus shedding, which starts also during the incubation period, may last up to 9 days. Viral RNA can be detected in tissue samples and oropharyngeal fluids constantly or intermittently for much longer, over two weeks (up to 14 days). Antibodies can be already detected after the first week of infection and have been shown to remain for at least 28 days, when the experiment was terminated (see Appendix D).

Since the mobility of wild boar may not be substantially impaired by the disease, as shown in the wild boar experiment and that these animals show an early onset of viral excretion, there is a potential role for wild boar to spread FMDV and to maintain an epidemic, particularly in areas with a high wild boar density. However, based on these findings and the lack of evidence for a carrier state in domestic pigs, it can be concluded that wild boar probably do not play a crucial role as long term virus carriers.

From a diagnostic point of view the data presented above data might help to determine the timing of infection (Figure 13). Long-term studies to determine the entire duration of anti-FMDV antibodies in wildlife species are needed.

![Figure 13: Suggested FMD infection dynamics in wild boar based on the experimental data of Breithaupt et al. (in press) (n=4).](image)

7.2. Deer

Experimental studies conducted with species of deer found in Europe indicate that the pathogenesis and clinical course is similar to that seen in domestic ruminants. Fallow deer can become persistently infected and virus has been recovered from the pharynx up to 63 days post infection. Experimentally,
red deer only occasionally became carriers, while in roe deer persistence could not be proven (Forman et al., 1974; Forman and Gibbs, 1974; Gibbs et al., 1975). Furthermore, there is no experimental evidence for transmission of the disease from carrier animals to other domestic animals.

From data available from the systematic literature review (see Appendix D), it can be concluded that FMDV has never been an important pathogen of deer in Europe and that they do not play a significant role in the spread of FMDV to domestic ruminants or pigs.

8. Modelling spread and maintenance of FMD in the wildlife populations

8.1. Objectives and description of the epidemiological model.

FMDV once introduced into wild animal populations may be hypothesised to spread well within a population based on wildlife hosts (i.e. epidemic) and eventually also switch to continued perpetuation (i.e. endemicity). The actual situation is influenced by a complex set of factors (i.e. susceptibility, between-host contact structures, intensity of indirect transmission between adjacent host groups, survival of the virus outside the host, seasonality, reproduction dynamics). Several of these factors have been investigated experimentally for domestic animals, whereas others such as ecological characteristics are available without the context of a contact infection. Therefore, the possible maintenance of an FMDV incursion into an area populated by wildlife (i.e. wild boar and/or deer) was addressed with a dynamic, spatially explicit, individual-based eco-epidemiological model. The host ecology (i.e. density per unit area and reproduction season) was adjusted to the data presented in Appendix C while using a hypothetical population of wild boar and deer. The model mechanisms represent the available knowledge, as well as mimicking individual host animals of both species and simulating the potential host-to-host spread of the infection given its introduction.

Figure 14: Annual reproduction dynamics following the data provided in Appendix C. The two distributions are applied to determine reproduction in the model.

With regard to the objectives, the simulation study specifically addressed the assumption of whether the infection can be maintained by wildlife alone. Therefore, the model excluded human interactions (i.e. excluding domestic farm units or human activities that may transport the virus over non-biological distances). Also, stray cattle were not considered in the model scenarios except in as much as they could be thought of as represented by a “deer flock” (i.e. by one of the moving ruminant groups included in the model).

The model is described in Lange (2012) in full formal detail following the standard protocol for documentation of complex individual-based models (i.e. the ODD protocol; Grimm et al., 2006, 2011). Moreover, the part representing wild boar ecology was applied in previous reports regarding the spread of classical swine fever virus (CSFV) (Alban et al., 2005; Fernandez et al., 2006; Kramer-Schadt et al., 2009; Lange, 2012). Next, the conceptual overview is summarised.
The ecological part of the model represents a fictitious geographical area of about 100km x 50km, which is monitored for population dynamics and the presence of infection once every week. The area is assumed to be connected wild boar habitat (e.g. comprising an amount of forested structures that allow reproduction; Cargnelutti et al., 1990; Gerard et al., 1991). Wild boar live in social groups established by sows and each group recognises a core home range of 4-20 km² (Leaper et al. 1999). Thus, the model landscape is subdivided into small spatial patches each of which holds one wild boar group and proportional males. Group size depends on local habitat quality that governs reproductive success (Jedrzejewska et al., 1994) and the stochastically varying annual living conditions that govern natural mortality (e.g. mast years, cold winters, etc.). Litter size and the natal dispersal distance distribution follow the literature (see Fernandez et al., 2006 and references therein). Each habitat patch is additionally assigned a deer flock. Deer temporal ecology follows that of wild boars with adapted density regulation and reproduction season. Every individual’s life history is followed in weekly time-steps until death. Total population size in the model is emergent from the sum of all local groups and matches the highly dynamic year-to-year variation known from natural populations (Figure 15).

![Simulated population dynamics of the wildlife population.](image)

**Figure 15:** Simulated population dynamics of the wildlife population. Top: Long-term simulation of the ecological model for two extreme density scenarios found for Bulgarian Trace (see Figure 40 in Appendix C). Bottom: Boar and deer population in detail for standard simulation runs reflecting age structure, population fluctuation and seasonal cycling due to turn over. In the simulated outbreaks the virus would be introduced into the region at the year time point marked by “0”.

For infection by FMDV in a model each animal is represented by the successive states S-E-I-R (Susceptible-Exposed-Infectious-Removed). Here ‘Removed’ either means “dead” or “immune”, depending on the individual outcome of the infection in the particular host animal. The epidemiological part of the model now calculates thousands of times how an animal will be exposed, to the virus whether it gets infected, and how the disease course proceeds for that infected animal.
A host animal can be exposed either to infectious social group mates (direct transmission, for example, within social groups or flocks) or to an environment contaminated by infectious material (indirect transmission, for example, at water sources, grazing places or in aerosols). Both approaches follow the idea that the more infectious hosts present in a place, the higher the resulting exposure will be for other animals in the same place at that point in time.

An exposed animal is infected stochastically where the probability is evaluated each time from: (i) the number of infectious group mates, plus (ii) the contact with infectious material in the environment the animal reaches (i.e. home habitat patch and, with much lower weight, the adjacent habitat patches). The latter was used to represent the roaming of animals with resulting spatial overlap between their foraging areas, although core home ranges are assumed distinct in both species.

An infected animal passes its own – stochastically assigned – incubation and infectious period and concludes its disease course by recovery. During the infectious period the animal is shedding infectious material to the environment (i.e. to its home patch and at a lower level to the adjacent ones). The infectious material is accumulated for all shedding animals and ages by a given rate of decay before forming the next generation of environmental exposure to close the transmission cycle.

In principle, modelling the outcome of a single infection is comparable to challenge experiments, from which the reported distributions of, for example, lethality, duration of infectiousness, re-emerge when data of all infected animals recorded during one model run are jointly evaluated.

8.2. Simulation experiments and Analysis

The investigated scenarios were constructed to demonstrate under which assumptions transmissibility of FMDV infection will establish in wildlife, spread through the population, and will finally be maintained by the available host populations. Additionally, three hypothetic situations were combined with each scenario, in order to test the importance of the presence of deer in the simulation area:

(i) Situation B involving only wild boar i.e. deer virtually sheltered from infection;

(ii) Situation B+D involving wild boar and deer;

(iii) Situation D involving only deer i.e. wild boar virtually sheltered from infection.

Simulated spread of FMDV infection in wildlife was structured in three ways in order to address the establishment, spread, and maintenance of the infection. First, for each simulation run, the duration of virus presence was recorded, which, for all repetitions, may be summarised by a survival curve (Figure 16). From the resulting graph, it is possible to read out immediately what proportion of simulation runs became extinct quickly after virus introduction (i.e. the infection does not establish in the wildlife population, as shown by the end of year one in (Figure 16). Moreover, the graph also reveals whether an epidemic that affected the whole landscape already becomes prone to frequent extinction. The steeper the drop in the curve, the lower the potential for the simulated population to maintain the infection beyond the first wave, in other words the lower the potential to create an endemic infection.
Figure 16: Survival curve of 120 simulations by time of simulation. Each drop in the curve reflects the end of one simulation by final fade out of the infection from the total population.

Geographical spread was monitored in the simulations by measuring the probability of each home-range patch of the landscape being involved in simulated outbreaks. The representations reveal if established outbreaks more often ended while spreading through the population or after affecting the whole population.

The temporal dynamics of the spreading FMDV front were observed along the longitudinal extent of the landscape. Therefore, selected subpopulations were focused during each simulation run (i.e. size 6km x 50km perpendicular to spread of the epidemic). At the beginning of either season (1st week of April, July, October, January) those subpopulation were selected for evaluation that were not yet affected by the disease but closest to the spreading epidemic (i.e. right in front of the epidemic wave; Lange, 2012).

For each of the observed subpopulations, weekly virus- (left) and sero-prevalence (right) were recorded for both species (see example for wild boars in Figure 17).

Figure 17: Local virus- and sero-prevalence dynamics after FMDV incursion through wildlife depending on season of introduction into an area of 6 x 50 km. Left panel: Virus prevalence in the wild boar population. Right panel: Sero-prevalence in the wild boar population.

The mean environmental pathogen load of each observation zone was recorded. Observations of a particular box were averaged over all simulation runs of a scenario. Averaging was performed after temporal alignment by setting t=0 when first infection was observed in either particular run.

8.3. Assumptions and uncertainties

The conceptual model only takes natural mortality and intensive hunting into account as the causes of removal of animals from the susceptible population (see Figure 15). The reproduction and life expectation data applied to calibrate the model, therefore, come from intensively hunted European
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There is lack of quantitative evidence about how infectious diseases and FMD in particular are transmitted between host groups of animals that have no direct contact due to lack of social relationship. Thus, usually, a non-specific transmission probability is applied between separated host groups. This is probably meant to represent the result of a plausible mechanism that may bring infectious material from an infected host in one group into contact with susceptible animals from other groups (male boar multi-paternity, aerosol spread, environmental contamination, shared pathways and drinking places, etc.).

The method of modelling between group / between species transmission proposed here (Lange, 2012), resolves the transmission processes into more detail by separating release of infectious material to the environment (i.e. virus excretion) from the total amount accumulated therein (i.e. exposure to infection).

Evidence exists on the (range of) amount of infectious material shed by domestic pigs and cattle, as well as the species specific dose-response curve for a successful infection. Excretion is expected to be much higher in pigs compared to ruminants (see, for example, Sørensen et al., 2000; Alexandersen et al., 2003; Kitching et al., 2005; Garner et al., 2006). On the other hand, pigs are reported to be less susceptible to FMD than domestic ruminants. In the absence of data for the modelled species, excretion and uptake rates reported for domestic pigs were applied to wild boar. Characteristics of ruminants were assumed to apply for deer. Although, according to Alexandersen et al. (2003), susceptibility was greater for swine than for cattle through the oral route, and the necessary dose by the oral route was higher by two to five orders of magnitude compared to infection by inhalation. Thus, the critical value is the relative susceptibility by inhalation, and for the susceptibility parameter (i.e. infection probability per dose) a relation of 1:10 was applied as a combination of both routes (Table 5).

However, the dose a susceptible animal receives from the contaminated environment when sharing an area with acutely infected animals is not addressed. Therefore, the major unknown in the proposed modelling is the (range of the plausible) proportion of infectious material (in TCID50 units) a susceptible animal will receive from the environment. Once the infectious material is shed into the environment inactivation of virus is well accepted. The presence of infectious virus in the environment (in terms of accumulated TCID50 doses) is modelled by the standard time of inactivation. The parameterisation was agreed to be based on data from FMDV survival curves in pig and cattle slurry (Bøtner and Belsham, 2011). According to this published study, experimental data provided the basis to regress half-life of FMDV in pig slurry onto the temperature between 5°C and 45°C ($R^2 =0.996$ See Lange, 2012, for details). In particular the strong temperature dependence was respected by seasonally varying the time for inactivation (i.e. half-life). Figure 18 depicts the half-life of infectious material in the environment as applied with the model (black line), calculated from the regression using mean monthly temperatures from the Thrace region (red line).
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**Figure 18:** Seasonal dynamics of the half-life, as applied to model virus survival in the environment. The red line represents mean monthly temperature for Edirne (Turkish State Meteorological Service, 2012), whereas the black line represents the seasonal half-life of FMDV in pig slurry resulting from temperature dependent experimental data provided by Bøtner and Belsham (2011). In particular, temperatures above 20 °C critically reduce FMDV survival outside the host.

Table 5 summarises the standard parameter values for the transmission models by considering parameters between socially related hosts (i.e. beta within group) and between unrelated hosts through the environment (i.e. excretion dose per day; relative efficiency of uptake from environment contaminated by 1,000,000 infectious doses; infection probability per dose; neighbourhood forage).

**Table 5:** Standard transmission parameters of the simulation model for direct animal-to-animal transmission within social groups and transmission mechanism via contamination of the environment (spread of infectious material by shedding hosts).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Boar</th>
<th>Deer</th>
<th>Motivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta within group</td>
<td></td>
<td>0.25</td>
<td>0.25</td>
<td>Pinf ~1 with more than 2 animals in the group. See transmission experiments (Mohamed et al., 2011 and Breithaupt et al., in press)</td>
</tr>
<tr>
<td>Excretion dose per day</td>
<td>Environmental contamination outside the host</td>
<td>1,000,000</td>
<td>10,000</td>
<td>100 fold higher for pigs compared to ruminants</td>
</tr>
<tr>
<td>Relative efficiency of uptake from environment contaminated by 1,000,000 infectious doses</td>
<td>Contact with environmental contamination as accumulated and decayed outside the host</td>
<td>1</td>
<td>1</td>
<td>Uncertain Results provided for [0.1; 0.5; 1.0; 2.0] doses</td>
</tr>
<tr>
<td>Infection probability per dose</td>
<td>Dose-response relation for FMDV</td>
<td>0.003</td>
<td>0.03</td>
<td>Ten-fold less for pigs compared to cattle</td>
</tr>
<tr>
<td>Neighbourhood forage</td>
<td>Spatial overlap of movement area of two unrelated groups</td>
<td>0.1</td>
<td>0.1</td>
<td>Plausible but uncertain</td>
</tr>
</tbody>
</table>

**8.4. Results of the model**

If not stated differently, the following model output was recorded with standard parameterisations according to Table 5 for transmission mechanisms, and table 4 in for the ecological and disease parameterisation in the scientific report delivered to EFSA (Lange, 2012).
Figure 19: Model output of survival times for FMD virus in the total wildlife population from 120 simulations, following introduction of 10 infected animals at one edge of the area (i.e. entry point), assuming standard transmission parameters (see Table 5) were applied. The graphs differentiate the survival curve for the three scenarios that assume “B+D” are wild boar and deer; “B” is only wild boar; and “D” is only deer susceptible to FMDV.

Figure 19 reveals that deer play a marginal role in spreading and maintaining an FMDV incursion through the model population. The dotted line represents virus circulation if wild boars are hypothetically excluded as susceptible to FMDV. The drastic effect is reasonable considering the relationship between pig and ruminant hosts regarding shedding and uptake. The ruminant host – although more susceptible per dose taken – shed too little infectious material (i.e. TCID\textsubscript{50} doses) into the model environment to allow for a sustained transmission chain. On the contrary, the survival curve for FMDV outbreaks, assuming only wild boar as susceptible, did not differ from that in the real two-species population.

Standard transmission parameters (see table 5) were applied but assuming either constant or seasonal half-life values for survival of infectious material in the environment (Figure 20). The temperature dependence of virus survival in pig slurry indicated an increased chance of fade-out of FMDV circulation in wildlife during hot summer months. To assess the impact of variations in half-life of infectious material on the maintenance of virus circulation both scenarios were simulated. The model results underpinned the assumption that seasonally varying temperatures will dramatically increase the chance of virus fade-out from the affected populations always in the summer months (Figure 20). The outcome is consistent with the survey data from Bulgaria where spatial spread was demonstrated (see Figures 7 and 9) by serology in spite of not being able to detect the virus in wildlife. Indeed, assuming seasonally varying temperatures, the chance of virus fade-out from the affected populations would have been increased during the summer months (Figure 20).
Figure 20: Virus survival curves for virus circulation (left) and prevalence in terms of infectious wild boar and deer hosts out of the total wildlife population (right) for different modelling of the half-life of infectious material in the environment. Thin line: assuming constant inactivation over time in the environment; thick line: assuming seasonal inactivation over time of infectious material outside the host animal.

Figure 21: Average and 5%+95% percentile of the spatial forward spread of FMDV outbreak in a wildlife population.

Figure 21 shows how the spatial spread will cover the whole population if FMDV parameterisation allows for a sufficient number of animals that are simultaneously infected in a locality and hence sufficiently large dose on FMDV accumulated in the environment. Consequently, the main issues for the spread are the local number of hosts available (to allow shedding into the environment) and sufficiently high uptake from the environment (to link neighbouring host groups for transmission of infection). These two issues are explored in greater detail (see Figure 22)

The population density was a variable ecological feature in the Thrace region where the FMDV positive wildlife was found. Therefore, the simulations were performed for two different population densities (Figure 22 left vs right column) adapting the ecological data from the surveillance cordon (see Figure 9). The obvious outcome (see each row in Figure 22) that FMD circulated only for the high density scenario (i.e. the eco-region which corresponds to the area were most seropositive wildlife was detected – comparing figure 41 in Appendix C the map of sero-survey and wildlife population estimates).

Moreover, the less certain parameter (the uptake dose from 1,000,000 TCID$_{50}$ accumulated in an environment) was additionally subjected to sensitivity consideration regarding circulation and spread. Figure 22 shows that in the high density scenario (right column) only the very high uptake (first row) was sufficient to guarantee the full range of spread through the simulation area for most simulations (nearly all places are coloured in red, meaning approximately 100 % of simulations reached this point). Already with halved uptake the spatial spread is not assured anymore. Any further lowering of the uptake would not correspond to the spatial spread observed in the sero-survey region.

Most interesting, however, was that, for each of the uptake dosages, the low population density areas were unable to allow an epidemic to become established (corresponding to green coloured patches in Appendix C, Figure 40). That might be a plausible reason why the sero-survey revealed such a distinct spatial cluster of seropositive wildlife in the surveillance cordon. The cluster coincides roughly with the high density patches as shown in Figure 40 (Appendix C). In addition to the high summer temperature (i.e. low virus survival in the environment), the particular shaped areas of high vs. low population density may also have supported both the spatial spread, as seen in the sero-survey result,
and the fade-out of the infection north- or westwards of the seropositive cluster in the surveillance cordon.

![Image: The average spatial spread achieved by different dosages acquired from each 1,000,000 TCID50 of FMDV accumulated within the environment at the different population densities as shown from the study region (Appendix C, Figure 40.). The rectangle represents the simulation area in x-y-coordinates with an extent of 100 x 50km. Each point/pixel is coloured according to the percentage of simulated epidemics that affected the location (100% means that all 120 simulation runs affected the animals at the x-y-location, and dark blue indicates no epidemic simulations covered the locality). Low density corresponds to green areas in Figure 40 while High density corresponds to the red/orange areas where the seropositive animals have been detected later.]

**Figure 22:**

9. The relative significance of -and the role played by wild and feral bi-ungulates, notably wild boar and deer species, in the epidemiology of foot-and-mouth disease (FMD) (TOR 1)

The observations in Sections 2-7 have led to the following conclusions concerning the relative significance of wild boar and deer species, in the epidemiology of foot-and-mouth disease TOR 1:
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The role of wildlife regarding introduction and spread of FMDV in Thrace can be considered minor relative to the more important risk factors such as poor biosecurity, movement of live animals and animal products (either legally or illegally), swill feeding or access to landfill waste, animal density, and the availability and efficacy of vaccination programs (Sections 2 and 3 and Appendix A).

Based on a systematic literature review focusing on experimental FMDV infections in wildlife (Section 6 and Appendix D), it was concluded that transmission from wildlife to domestic animals can occur so wildlife can play a role in the spread of FMD.

In particular, studies performed at the Friedrich-Loeffler-Institut (FLI), Germany (Breithaupt et al., in press) have shown that the Bulgarian strain of FMDV can infect wild boar under experimental conditions and transmission of the virus to other in-contact porcine animals was observed. Similar studies using a different strain (serotype A) of virus in feral pigs have been performed in the USA and they also showed transmission from domestic pigs to feral pigs and vice versa (Mohamed et al., 2011). This evidence however does not prove the potential for transmission of the virus in a natural setting when the proximity of infected animals may be much lower.

Historical observations of FMD in wildlife populations and a systematic literature review of observational studies in wildlife (Section 3.3 and Appendix D) revealed no evidence for maintenance of infection within wildlife in Europe either from within the U.K. (in 2001 when over 2000 premises were identified as infected and deer were no doubt exposed to the virus) or earlier in the 1960’s within mainland Europe when FMDV outbreaks were common and were controlled using movement controls, slaughter and vaccination of domestic animals alone (not applied to wildlife that included wild boar and deer).

FMD was present in domestic animals (and FMDV was isolated in some cases) from December 2010 to January 2011 and from March 2011 to April 2011. However, no cases were reported between these periods (and hence no virus samples were collected) but some undetected infection in domestic animals did become apparent through serosurveillance. Undoubtedly, some wildlife also became infected during these periods, as indicated by the serosurveillance of wildlife (February 2011 to January 2012) and the serological results. The relatively low seroprevalence and clustered spatial distribution of positive wild boar and deer indicated that the wildlife FMD event in the Bulgarian-Turkish cross-border area apparently failed to develop into a large scale epidemic wave. It remains questionable if, and for how long after the last series of outbreaks, the virus could have been present in the population of wild boar and to which extent the FMD affected domestic livestock contributed to the disease spread in wildlife in April. It is still uncertain whether wildlife animals were active players in the spread of the disease and could transmit the virus to domestic animals or whether they became infected through contact (direct or indirect) with FMDV infected domestic animals but did not pose a threat to domestic animals. No virus was isolated from wildlife except for the initial case. The model to simulate spread and maintenance of FMD in the wild boar and deer populations (Section 8 and Lange, 2012) indicated that FMD will not be sustainable within a wild boar + deer host system alone but limited spread of FMDV in time and space may occur.

Naturally, if there is a continued cross-over of FMDV between domestic and wildlife population then circulation may be prolonged.

There is no evidence that any of the circulating strains (serotypes O, A, Asia 1) in the wider region would cause a different outcome (Section 5).

10. Surveillance systems for early detection of new FMDV incursions in Thrace (TOR 2)

For domestic animals, a passive surveillance system for early detection of FMD is based on the appearance of pronounced clinical signs in cattle and swine. In sheep, clinical signs are less pronounced and in some cases subclinical infections occur but, as contact between sheep and other domestic animals commonly occur, then passive surveillance has been found to be sufficient.
Furthermore, as soon as a Member State confirms a primary case of FMD in wild animals, it has to apply the measures provided for in Council Directive 2003/85/EC, Part A of Annex XVIII in order to reduce the spread of disease and to draw up an eradication plan in accordance with Part B of that Annex. According to the eradication plan the infected area has to be defined and the measures have to be applied to the holdings in that area. In Annex III of the above-mentioned directive the procedures for sampling and clinical examinations are laid down.

10.1. Targeted surveillance systems for early detection of new FMDV incursions in Thrace in wildlife

Targeted surveillance systems in wildlife for early detection of new FMDV incursions do not exist. It is up to the competent authority of the affected Member State to develop and present plans for surveillance, control and eradication of FMD in wild animals in the affected area. The model described below was developed to assess the suitability of different surveillance systems for early detection of FMD incursions in wildlife.

10.2. Objectives of the assessment

The objective of the assessment was to contribute to answer ToR 2. This assessment provides information on the performance, in terms of early detection, of different monitoring and sampling schemes in case of an incursion of FMD virus in a free wild boar population area. It should be noted that detection has to be based on serology rather than FMD virus isolation, since the duration of viraemia in any individual animal is very short (a few days). In particular, the impact of different types of surveillance strategies on the weekly probability of detection over time was evaluated. The assessment took into account different epidemiological features, such as the period of the year at which the virus incursion occurs and the sensitivity and the specificity of the surveillance system.

The following main output for the comparison between the surveillance strategies under investigation was provided by the assessment:

*Number of weeks needed to first detection:* defined as the minimum number of weeks needed to detect at least one seropositive animal calculated via simulation over one year.

10.3. Outline of the approach

The assessment consisted of two major steps:

The first step of the assessment estimated the sample size needed to detect at least one positive animal for a given prevalence of sero-converted individuals, with a given level of confidence, using the conventional formula of Cannon (2001). Various prevalence values and different testing system sensitivities were considered, including the parameters used in Council Directive 2003/85/EC, i.e. 5% prevalence to be detected with a 95% Confidence Level.

The second step consisted of the development of a simulation model, called the “EFSAwbFMD model”, which was able to generate the true sero-status of the population. From that population, it was possible to implement virtually different sampling strategies and calculate the probability of detecting at least one seropositive animal for a given time period, after assumed exposure to infection. Once the week of first detection was recorded from the simulation, it was possible to calculate the number of weeks needed to detect the first case as the difference between the week of first detection itself and the week of incursion. In addition, the model consisted of the following more detailed sub-steps:

- *Individual probability of testing positive (APs).* From the true individual probability of being seropositive (Ps) (simulated by epidemiological model described in section 8.1), the *apparent probability* was calculated using the Rogan and Gladen formula solved for the apparent prevalence (i.e. the characteristics of a non-perfect test/system are taken into account).
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- **Generation of the weekly individual sero-status.** It was then possible to generate the weekly population sero-status as the outcome of a random sample from a Bernoulli distribution where the population size was the Population Trend Overtime (N) and the probability was either the true individual probability of being seropositive overtime (Ps – see Equation 1 below) or one of the apparent probabilities overtime calculated in the previous step (APs – see Equation 2 below). In the latter case, the outcome is the apparent serostatus of the population or, from another point of view, the individual probability of testing positive.

\[ (1) \]

- **Virtual implementation of a sampling strategy.** Once the population was generated, the sample of the desired size was collected virtually according to the chosen sampling strategy and sampling rate.
- **Calculation of the probability of detection overtime.** For each week, the probability of detection was calculated as the ratio between the number of times each week recorded a number of positive results greater that zero (number of times the cases>0) and the number of simulations run.
- **Weeks needed to first detection.** Based on the same set of simulated data (collected sample from the whole population), the minimum number of weeks needed to first detection was also reported and central tendency measurements were provided.

Two sampling strategies were explored. The first being based on samples taken from hunted animals during the hunting season only; and the second being based on regular sampling over the whole year (i.e. fixed sample size every week of the year or a fixed proportion taken every week of the year). The outputs from the simulation allowed the effectiveness of the different surveillance strategies to be compared for early detection.

10.4. **Model input**

- **Population trend overtime (N)**

The wild boar population size overtime used in the EFSAwbFMD model was, for consistency, the same one used in the epidemiological model. This hypothetical population was estimated taking into account the available information on the distribution of the wild-boar population in the relevant geographical areas as well as the ecological and behavioural characteristics of this animal specie. Wild boar population dynamics emerge from individual behaviour, defined by age-dependent seasonal reproduction and mortality probabilities and age- and density-dependent dispersal behaviour, all including stochasticity (Lange, 2012).

- **Individual probability of being seropositive (Ps)**

The true individual probability of being seropositive overtime was one of the outputs of the epidemiological model and was obtained via simulation. The epidemic course emerged from within the groups representing virus transmission, wild boar dispersal, individual stochastic disease courses and infectious periods for infected wildlife hosts, and survival dynamics of infectious virus outside the host and uptake of a wild boar of another group.

10.4.1. **Assumptions underpinning the EFSA model**

- The population in the area under investigation was free of anti-FMDV antibodies before the virus incursion;
- The number of animals hunted (sample size) was estimated by the experts to be approximately 25 % of the population in February;
- The only reason for the reduction of the population size during the hunting season was the hunting exercise itself;
In terms of the individual probability of being seropositive, the age of the animal did not have any impact on the parameter (i.e. the age did not represent a risk factor or, in other words, the Relative Risk (RR) between age groups was 1;

- The area under investigation was considered homogeneous: the individual probability of being seropositive was the same over the whole area (i.e. the geographical situation did not represent a risk factor);
- The wild animal population was homogenously distributed in the area of concern.

10.4.2. Scenario’s considered in the EFSAwbFMD simulation model

- Scenario’s were built for virus incursion in January and in July. These choices were made to evaluate the possible impact on the individual probability of being seropositive (Ps) due to the different climate conditions and their impact on the behaviour of the wild boar, and to evaluate the impact of time of infection on number of weeks to first detection.

- Two scenarios were considered in the model for the potential different capacities of FMDV for spreading in a wild boar population. These were referred to as “high dose uptake” and “low dose uptake”, respectively;

- Two different sampling strategies were considered: (i) a sampling exercise mimicking hunting and (ii) a theoretical, but realistic, sampling exercise. In detail:
  - The hunting was simulated by mimicking a realistic scenario where the samples were collected and tested from the 1st of October until the 31st of January. The number of animals hunted is not equally distributed over the hunting season, (i.e. a different number of individuals is hunted every week). A random sample from a multinomial distribution was used for this purpose (R function: “rmultinom”) and the resulting sample size, for the 18 hunting weeks, was the following: 74, 97,161, 244, 40, 259, 285, 194, 179, 20, 58, 45, 194, 108, 199, 155, 205 and 268 (EFSA, 2012).
  - The theoretical sampling exercise was performed in two different ways: (i) considering a weekly constant sampling proportion over the year (see Equation 3) obtaining a total number of sampled animals equivalent to the hunting bag; (ii) considering a fixed number of animals to be sampled each week. In particular, from the formula of Cannon (2001), the sample size needed to detect a prevalence from 5% to 1% (CL 95%) was used.

- As the model aimed to investigate a realistic implementation of a surveillance system based on hunting, the whole process leading to a laboratory test result needed to be taken into account. However, this flow has some critical points which create an important amount of bias in the analytical results. Some examples include the biological quality of the collected samples, the way the samples are kept and preserved, etc. However, all samples that test positive were submitted to further laboratory analysis (e.g. alternative ELISAs or VNT), which minimises the number of false positives. For these reasons, it was agreed to include the sensitivity and the specificity of the whole process (system) in the model rather than the values referring to the single laboratory test. The following five system scenarios were explored: i) Se=85 % and Sp=100 %; ii) Se=90 % and Sp=100 %; iii) Se=95 % and Sp=100 %; iv) Se=99 % and Sp=100 %. An additional scenario was also considered, as an ideal setting, where the system was perfect (Se=100 % and Sp=100 %).

Where:
- Hunting Bag = estimated by the experts to be around 25 % of the population in February;
- Begin = First week of the hunting season;
- End = Last week of the hunting season;
- N = population size;
10.5. Results of the assessment

This opinion, reports only the results on the sample size needed to detect a disease and the results on the time (weeks) needed to detect the first seropositive animal. For more details on these results and for the results on the probability of detection over time, see EFSA (2012).

10.5.1. Sample size calculation to detect seropositive animals

The formula of Cannon (Cannon, 2001 – see EFSA, 2012) was used to calculate the sample size needed to detect a seropositive animal when the number of seropositive individuals reaches a given proportion. The population size was calculated as the average over one year from the four inputs (N) provided by the epidemiological model. The results are shown in table 6. They indicate that to detect a seropositive animal when the prevalence of seropositive individuals in the population is 5%, with a 95% confidence level, the sample size needs to be between 59 (if the sensitivity of the testing system is perfect) and 69 (if there is a lack of sensitivity in the testing system, i.e. Se = 0.85). Specificity is assumed to be perfect as the system does not allow for false positive test results, since all positive test results undergo further analytical tests to confirm the outcome.

Table 6: Sampling to detect disease

<table>
<thead>
<tr>
<th>CL=95%</th>
<th>System Sensitivity</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sp = 1</td>
<td>0.85</td>
<td>347 328 310 298 295</td>
</tr>
<tr>
<td>N=11524</td>
<td>0.9</td>
<td>174 164 156 149 148</td>
</tr>
<tr>
<td></td>
<td>0.95</td>
<td>116 109 104 99 98</td>
</tr>
<tr>
<td></td>
<td>0.99</td>
<td>87 82 78 74 74</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>69 65 62 59 59</td>
</tr>
</tbody>
</table>

(a) Desired prevalence value at which seroprevalence needs to be detected

10.5.2. Effectiveness of the different surveillance strategies for early detection (based on the EFSAwbFMD simulation model)

10.5.2.1. Surveillance strategy based on hunting

Figure 23 shows the number of weeks needed to detect at least one seropositive animal for an incursion in January and July when the samples come only from the hunting bag, for a high and low dose uptake and perfect test conditions (Se =1 and Sp = 1). There is no information available before the hunting season begins. When the first samples are collected (i.e. the first animals are hunted) the probability that at least one animal is seropositive is 100% (if the incursion is assumed to be in January) or occurs between 58% and 76% (if the incursion is assumed to be in July and according to the probability of spreading).

In the scientific report (EFSA, 2012), graphs for tests with lower sensitivity were also shown, however, the mean and median number of weeks needed coincide (39 weeks for incursion in January, and 13 weeks for incursion in July). This highlights that the test/system sensitivity within the range examined has a negligible impact on the number of weeks to first detection. Furthermore, the results do not change according to the probability of spreading, since no differences were observed between a high and a low dose uptake.

<table>
<thead>
<tr>
<th>INCURSION IN JANUARY</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIGH DOSE UPTAKE</td>
</tr>
</tbody>
</table>
The model indicates that a sampling strategy based on hunting needs a long time before the first seropositive animal is detected. If the incursion is far from the hunting season (e.g. when the incursion is in January), almost 10 months are needed to detect the first case and the proportion of seropositive animals at that time will be quite high (17.5 % if the probability of spreading is low and 24.5 % if the probability of spreading is high).

10.5.2.2. Surveillance strategy based on regular sampling over the year

Figure 24 shows the number of weeks needed to detect at least one seropositive animal for an incursion in January and July for a high and low dose uptake for perfect test conditions (Se =1 and Sp = 1). In the scientific report of EFSA (EFSA, 2012), graphs for a test with lower sensitivity were also shown, however, the median number of weeks needed was always a minimum of five, except for the sample strategy with a fixed sample size, when assuming high dose uptake and incursion in January. In this case, one week less is needed to detect at least one seropositive animal. This observation shows that the test/system sensitivity has a negligible impact on the number of weeks to first detection.

When a regular sampling overtime is performed, the first sample will test positive on average after 5 weeks (see Figure 24), when the prevalence in the population would lay between 0.8% and 1.3%, according to the time of incursion and the probability of spreading. When looking at the performance of a sampling scheme based on a weekly fixed number of samples over time, it is interesting to notice that in order to have 95% probability of detecting at least one seropositive animal when the prevalence in the population is equal to 5%, a number of weeks between 6 and 9 are needed (depending on the assumed probability of spreading and the time of incursion (Figure 24). Actually, the system is on average able to detect the disease when the prevalence is lower than 5%, but in this case, obviously, the probability of detection will be lower than 95%.
Foot-and-mouth disease in Thrace

INCURSION IN JANUARY

<table>
<thead>
<tr>
<th>HIGH DOSE UPTAKE</th>
<th>LOW DOSE UPTAKE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed Sample Size</td>
<td>Fixed Sample Size</td>
</tr>
<tr>
<td><img src="image1" alt="Graph" /></td>
<td><img src="image2" alt="Graph" /></td>
</tr>
</tbody>
</table>

INCURSION IN JULY

<table>
<thead>
<tr>
<th>HIGH DOSE UPTAKE</th>
<th>LOW DOSE UPTAKE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed Sample Size</td>
<td>Fixed Sample Size</td>
</tr>
<tr>
<td><img src="image3" alt="Graph" /></td>
<td><img src="image4" alt="Graph" /></td>
</tr>
</tbody>
</table>

Examples of outputs of EFSA (for Fixed Proportion) or 95% percentile (for Fixed Sample Size)

**Figure 24:** Frequency plot of the number of weeks needed to detect at least one seropositive animal. Incursion is assumed to be in January and July for low and high probability of spreading. The sampling strategy is based on regular sampling over the year.

A sampling strategy based on regular sampling over time obviously performs much better, since, independently from the time of the incursion and the probability of spreading, the maximum number of weeks needed to detect the first seropositive animal is five. From this point of view, the implementation of a surveillance system based on a constant fixed sample size over time or on a constant proportion of samples leads to the same result.
However, it is important to note that a sampling system based on a constant fixed sample size over time seems to allow for early detection particularly when the probability of spreading is high. This is an important feature that must be taken into account because the sooner the detection, the lower will be the proportion of seropositive animals at that time. Moreover, a system based on a constant fixed sample size allows for a stable probability of detection of 100% when the proportion of seropositive animals in the population is lower (from 12.7% to 14.6%) if compared to a system based on a constant proportion (from 14.3% to 16.1%).

The good performance of a system based on a constant fixed sample size is probably due to the formula used to calculate the number of samples needed to detect a disease. In fact, the formula of Cannon takes into account a possible lack of sensitivity. Therefore, if the estimate of the sensitivity of the test/system is available, the best option to detect a disease as early as possible is to calculate the sample size needed using this formula. Table 7 summarises the results from the simulation exercise.

### Table 7: Summary of the assessment outcome

<table>
<thead>
<tr>
<th>INCURSION</th>
<th>January</th>
<th></th>
<th>July</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High Dose</td>
<td>Low Dose</td>
<td>High Dose</td>
<td>Low Dose</td>
</tr>
<tr>
<td>Hunting</td>
<td>Uptake</td>
<td>Uptake</td>
<td>Uptake</td>
<td>Uptake</td>
</tr>
<tr>
<td>Weeks to first detection</td>
<td>39</td>
<td>39</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Ps at first detection</td>
<td>24.5 %</td>
<td>17.5 %</td>
<td>1.6 %</td>
<td>1.2 %</td>
</tr>
<tr>
<td>Ps when Pdet=1</td>
<td>24.5 %</td>
<td>17.5 %</td>
<td>10.2 %</td>
<td>5.7 %</td>
</tr>
<tr>
<td>Fixed Sample Size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weeks to first detection</td>
<td>4 (6)(^{(a)})</td>
<td>5 (7)(^{(a)})</td>
<td>5 (9)(^{(a)})</td>
<td>5(9)(^{(a)})</td>
</tr>
<tr>
<td>Ps at first detection</td>
<td>0.9 %</td>
<td>1.3 %</td>
<td>0.8 %</td>
<td>0.8 %</td>
</tr>
<tr>
<td>Ps when Pdet=1</td>
<td>(3.5 %)(^{(b)})</td>
<td>(3.2 %)(^{(b)})</td>
<td>(1.2 %)(^{(b)})</td>
<td>(1 %)(^{(b)})</td>
</tr>
<tr>
<td>Fixed Proportion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weeks to first detection</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Ps at first detection</td>
<td>2 %</td>
<td>1.3 %</td>
<td>0.8 %</td>
<td>0.8 %</td>
</tr>
<tr>
<td>Ps when Pdet=1</td>
<td>16.1 %</td>
<td>14.3 %</td>
<td>14.6 %</td>
<td>14.9 %</td>
</tr>
</tbody>
</table>

\(^{(a)}\) In brackets: values at 95 % CL. Not in brackets: values at 50 % CL.
\(^{(b)}\) In brackets: Ps at first detection with 95 % CL. Not in brackets: Ps at first detection with 50 % CL.

11. The relevance and significance of epidemiological data and genetic characteristics for the different FMD strains recently isolated in Bulgaria and Turkey, with regards to the hypothesis of single versus multiple introduction into Bulgaria (TOR 3).

11.1. FMDV heterogeneity

The nucleotide sequences of FMDVs continually evolve, and there are several factors that contribute to the rate of change of the sequence over time that is observed when isolates are obtained directly from an outbreak. When the virus replicates within a host animal, new mutations are always introduced into the virus genome, and so the virus always exists as part of a population of closely related viruses. The consensus (most dominant) sequence of this population can be observed to change gradually over time (Figure 25) but will always contain a large pool of variants.
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Figure 25: Representation of progressive accumulation of nt substitutions (indicated by letters) within the FMDV RNA genome. Virus strains numbered 1 to 10 are all very similar, differing at most by 6 nt from the shared ancestral sequence (1) of approximately 8300 nt. However, it can be deduced that viruses in sample 10 evolved from viruses 8 and 9 but not from strain 7. Strain 4 is the last shared ancestor of all subsequent strains depicted here. Potentially viruses actually sampled during the surveillance period may only include viruses 1, 7, 8 and 10 (marked by red ovals) but the existence of the intermediate virus sequences can be inferred (although the order in which the separate mutations were generated cannot be deduced).

Both within endemic settings and also during outbreaks within areas that are normally free from FMDV infection, the average rate of change within the virus population corresponds to approximately $5 \times 10^{-3}$ substitutions per nucleotide per year (e.g. Cottam et al., 2006). For the FMDV genome with a total of 8400 nt, this corresponds to an average of 40-80 nt-changes in the whole genome/year or an average of 1 nt-change/week. This rate of change can vary on a short-term basis depending on several other factors related to the host and environment but it serves as a useful overall guide (see Cottam et al., 2006; Cottam et al., 2008). If virus is shed into the environment from a host then it can remain infectious for several weeks under cool conditions, providing there are other appropriate required environmental factors, such as sufficient humidity (Bøtner and Belsham, 2011), but it will not change. Similarly if the virus is present within boned-meat from a killed infected animal and then preserved in some form (e.g. frozen), then the virus can survive but will not evolve until it is again introduced back into a susceptible host and can initiate an infectious cycle again. If such breaks in the spread of the virus occur, then this will have the effect of slowing the apparent rate of genetic change of the virus. On the other hand, selection pressure (e.g. from vaccination or from changing host species, such as cattle to pigs) can, in principle, increase the apparent rate of sequence change as a result of the selective amplification of a small proportion of the virus population. Thus, the 40-80 nt sequence changes in the genome/year is a long term average and cannot be applied too precisely within a short time scale.

11.2. Genetic characterisation of FMDV’s involved in Thrace

Complete genetic characterisation (i.e. full genome sequences; FGS) of virus samples obtained from seven isolates within Bulgaria in 2011 has been performed (Valdazo-González et al., 2011), but no virus isolates were obtained from the other outbreaks in domestic animals which were only identified from serology (see Table 1). With the exception of the initial case detected in wild boar, all the other samples, which were sequenced, were obtained from cattle.

Samples from the wild boar that was shot near Kosti on December 30th, 2010 were supplied to the EU Reference Laboratory at the Institute for Animal Health, Pirbright, U.K. Attempts to isolate infectious virus were unsuccessful but isolation was achieved from samples submitted to FLI, Germany. Nevertheless, the Pirbright laboratory was able to detect virus antigen (by ELISA) and viral RNA (by real time RT-PCR), which confirmed the presence of FMDV. Initially, the coding sequence for just one of the capsid proteins, VP1, was amplified by RT-PCR and then sequenced. Comparison with
other known FMDV sequences from VP1 indicated a close genetic relationship (>99% identity) to recent (2010) serotype O viruses (lineage PanAsiaII-Ant10) which have been characterised from Anatolia in Turkey and Iran. Indeed, the sequences differed by just a single nt within this portion of 639nts of the viral genome (encoding VP1) (see Valdazo- González et al., 2011). Subsequently the full genome sequence (FGS) of the wild boar sample was determined, as was the complete sequence of virus samples obtained from six other outbreaks in domestic animals during 2011.

Sequence comparisons suggested that all seven FGSs from Bulgaria had a putative common ancestor that was closely related to the virus present in the initial case in the wild boar near Kosti village (in December 2010). The FGS of the seven Bulgarian isolates, collected over the period from December 2010 to April 2011, varied by up to 28 nt substitutions (out of 7346 nt compared) from the common ancestor. For comparison, the FGSs have been determined for ten different strains (all O-Ant10 lineage) obtained from Anatolian Turkey during 2010 and 2011 (Valdazo-González et al., 2011). The Bulgarian viruses varied by 37 to 63 nt changes from the closest relative identified (isolate TUR/926/2010) which was collected from Bursa in Anatolian Turkey, but this still represented >99% identity. It is noteworthy that the Bursa sample (collected in July 2010) was 31nt distinct from the predicted common ancestor shared by all the Bulgarian isolates from 2011 (see the TCS tree, Figure 26). It is important to stress that sequence comparisons to determine relationships between different isolates rely not only on the number of sequence changes but also on their particular locations within the genome (at the individual nt level). Thus as the virus evolves from a common ancestor it gradually acquires changes, which build on those previously gained (6). The virus genomes sequenced from the first series of outbreaks in Kosti (IP1) (collected on 7th January 2011) and Rezovo (IP2) (collected on 15th January 2011) differed by 8nt and 14 nt (out of 7087nt from within the genome that has been compared) respectively from the putative common ancestor which must have existed at some date prior to the initial case detected in the wild boar when it was killed in December 2010. The sequences obtained from the second series of outbreaks (IPs 4-7) in March and April 2011 in Bulgaria, still shared the same initial common ancestor but also shared a second common ancestor (6) or sub-ancestor. Each of the viruses from the second series of cases differed by 17–28nt from the initial common ancestor and by 4-15 nt from the second common ancestor. The genetic evidence indicates that the viruses isolated from the diseased cattle in these later outbreaks were not derived directly from the viruses involved in the initial outbreaks in Kosti and Rezovo (since they acquired a distinct pattern of nt-changes) but they did share the same initial common ancestor (Figure 276). In addition, the accumulation of the additional genetic changes indicates that virus replication had been occurring during the time between the first and second series of outbreaks in 2011.
Putative common ancestor of all the Bulgarian isolates in both the first and second series of outbreaks
Putative common ancestor (sub-ancestor) for the second series of outbreaks
Putative un-sampled viruses (equivalent to the number of nt-changes)

**Figure 26:** Genetic relationships determined from full genome sequencing of the Bulgarian isolates

In contrast to previous studies on the FMD outbreaks in the U.K. in 2001 and 2007 (Cottam et al., 2006, 2008), there appeared to be many unidentified intermediate viruses among the sampled Bulgarian viruses since there were some significant breaks (up to 14 nt substitutions) in the genetic relationships between the viruses obtained from the different Bulgarian outbreaks in 2011. Since evolution of the virus sequence requires active replication within a host animal then it is apparent that unrecognised cases of infection occurred in between those that were detected. This infection could have occurred in either wildlife (e.g. wild boar) or within domestic animals. The identification of seropositive domestic animals in Bulgaria without observed clinical signs is shown in Table 1 and seropositive wildlife in both Bulgaria and in Turkish Thrace is consistent with both possibilities, which are also not mutually exclusive. If spread of the disease occurred within wildlife then this would also require that wildlife could spread the infection back to domestic animals in the natural environment (rather than just under experimental conditions; see section 4) since each of the virus samples obtained (other than from the initial wild boar sample) were collected from domestic animals.

11.3. **Combining genetic and epidemiological information on the spread of FMD in Thrace**

ToR3 addresses the issue of whether epidemiological evidence in conjunction with the genetic evidence can differentiate between single or multiple introductions of FMDV into Bulgaria. The TCS tree (in Figure 26) does not link the FGS findings to geographic positions or to the time of the collection of the samples. Normally this limitation is reasonable with outbreaks resulting from spread between animal premises that are usually close to each other, however activities related to human intervention (e.g. by transportation of animals) can result in a great deal of long-distance spread as was demonstrated during the FMD outbreaks during 2001 in the U.K. The long-distance transportation of the virus, therefore, will washout spatio-temporal relationships between successively acquired changes in virus sequence. However, for a virus spread only by wildlife, all changes in the virus genome are subjected to biological mechanisms and would, therefore, associate reasonably well with the time between detection or distance between the reporting places of FGS if the number of nt-changes was equated with the speed by which the virus generations were transmitted from wildlife host to neighbouring wildlife hosts (this “speed per nt-change” is symbolised by particular lengths of the arrows in Figure 27 below). The spread of infection within Bulgaria has been considered using the genetic linkages derived from the FGS findings (see Figure 26) in relation to both the spatial proximity and temporal aspects of the outbreaks (Figure 27).
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**Figure 27:** Relation of the FMDV isolates by genetic relatedness to distance and time of outbreaks

The black and green arrows fit with the known information about the common ancestors in the first series of outbreaks. However, it is not possible to generate a satisfactory prediction for the location of the second common ancestor (green square) in relation to both the first and second series of outbreaks. This indicates that spread is likely to have involved human intervention procedures such as transportation of virus. The other possibility is the potential for wind-borne spread but it is unlikely to occur in this situation (Figure 27).

In respect to the first series of cases (WB, IP1-3), if it is assumed that spread of the virus occurred only within wildlife, then the required changes leading to the recorded series of nt substitutions within the viruses must have happened along the paths of advancing outbreak waves produced by successive spread from one wildlife group to the next. Therefore in Figure 27 the length of arrows indicate the hypothetical spatial spread consistent with the corresponding amount of nt-changes (“speed”). In Figure 27, the first three FGS (i.e. WB, IP1 and IP2) are easily associated to the unknown first common ancestor (black square). Arrows representing relative distances congruent with 4, 8 and 14 nt-changes, respectively, connect the common ancestor with the locations of the WB, IP1 and IP2 (left to right). Geometrically, the location of the common ancestor would need to be placed close to the border between Bulgaria and Turkish Thrace. This might also account for the seropositive animals detected in Turkish Thrace.

Without also considering the possibility of human transportation of infectious material, there is minimal flexibility to move the common ancestor (i.e. black square). Nevertheless, the precision of the
exploited methods (e.g. nt-changes per time; spatial speed of spread) will not allow the black square to be positioned on either side of the border between Bulgaria and Turkish Thrace.

With respect to the second series of outbreaks, the second common ancestor (or sub-ancestor) -also unknown- was considered and is represented by the green circle in the TCS tree. To link the single common ancestor (black square) to the sub-ancestor (green square) in Figure 27, arrows of the same length were drawn as the one already connecting IP2 with the common ancestor (13 nt- compared to 14 nt-changes). Its location, however, could have been at any of the green squares marked in Figure 27 or even in the full circle.

Assuming that FGS cases were observed and reported as they occurred, the timeline between the main ancestor and the first observed FGS after the sub-ancestor (>80 days) is sufficient to allow for the multiple nt-changes (13 nt with about 1 change per week). The length of this arrow between the black and any of the green squares, however, does not permit the connection between the initial infected area A (right yellow ellipse) to the second series of cases in area B (left yellow circle). Consequently, instead of continuous forward spread in wildlife, a human intervention procedure (such as transportation of domestic or wild animals or animal products) is speculated to have been involved in the spread of the disease from area A to B.

If this is the case, the green square could be placed anywhere (as it cannot be decided whether the transportation happened before or after the nt-changes or even if it occurred several times). If it is assumed that the sub-ancestor is associated with affected area A, then one hypothetical scenario would be that wildlife-mediated spread towards the south-west (into the region where seropositive wildlife was detected), before introduction of the virus into area B, occurred as a result of human transportation of infectious material. Therefore, this would not involve another introduction into Bulgaria. On the other hand, the green square may also be placed to match up with the enlargement of the affected area towards the north-east (again into a region were seropositive wildlife was detected, see red dots in Figure 27.

The nature of the relationship between the isolate from Fakia (IP7) and that of Golyamo Bukovo (IP6) is unclear. To maintain the pathway of sub-ancestor (green square) --> Fakia (IP5+IP7) --> Golyamo Bukovo (IP6) as indicated in the TCS tree would appear to require violating the standard assumptions about the rate of change (i.e. 11 nt-changes with only 1 day gap between sample collections). It should be noted, however, that there can be significant uncertainty about the timing of virus entry into an infected premises. The detection of disease can take some time and the same amount of virus replication within a domestic holding does not cause the spatial spread which is necessarily associated with virus spread in the wildlife.

In addition, the significant “gaps” shown in the TCS tree leave uncertainty about other unsampled outbreaks, which may have modified the predicted pathway and the statistical parsimony of the particular branching of the TCS that was based on a relatively small number of samples. Human interventions (i.e. through transportation) could bias the spatial conclusiveness of Figure 27. In addition, variations in the rate of sequence change (e.g. due to bottlenecks in the spread of infection or change between wildlife or domestic hosts) may bias the findings, on a short-term basis, for the average number of nt-changes per unit of time in circulation. On the other hand, the concentration of contact animals in pig holdings might speed up the amount of virus replication per time unit compared to spread through wildlife populations, which could shorten the length of the arrows from/to pig holdings, although airborne spread from such holdings could dramatically enhance the spatial spread of the virus.

Neither the TCS tree, nor the additionally considered epidemiological relationships between the premises from which the seven FGS were collected, are sufficient to support or fully exclude the possibility that multiple introductions occurred. In particular, the unknown link between infected areas A and B prevents an understanding of how often the virus crossed the border between Turkey and
Bulgaria and in which direction. Thus, further information is needed before this genetic relationship can be explained.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

ToR 1. The relative significance of - and the role played by- wild and feral bi-ungulates, notably wild boar and deer species in the epidemiology of foot-and-mouth disease (FMD) in Thrace (Bulgaria, Turkey and Greece), taking into account the different FMD virus strains circulating in the region.

- Introduction of FMDV into Thrace by wildlife is less likely than introduction due to movement of domestic animals or animal products.

- Recent experimental studies have shown that FMD can be transmitted from wild boar to domestic pigs and earlier studies have shown transmission from deer to cattle so wildlife can play a role in the spread of FMD.

- No direct evidence for the spread of FMDV from wildlife to domestic animals in Thrace has been demonstrated but it cannot be excluded given the limited observations from the area

- Epidemiological observations, published literature and modelling support the fact that the wildlife population is not able to maintain FMD in the absence of FMDV infection in the domestic host population.

- Results of the sero-surveillance of wildlife in Bulgaria and Turkey suggest that circulation of FMDV in wildlife was spatially related to the outbreaks in livestock.

- In the absence of virus isolates and information on the antibody status of the respective mothers of the seropositive piglets, the timeframe of the FMD event in wild boar in relation to outbreaks in livestock can not be identified. It remains questionable if and for how long after the last wave of outbreaks the virus could have been present in the population of wild boar and to which extent the FMD-affected domestic livestock contributed to the disease spread in wildlife in April.

- Most likely the disease died out in mid-summer 2011, and the areas subject to intensive surveillance both in Bulgaria and Turkey are now free from FMD in wildlife (and domestic animals). This is based on the observations with epidemiological considerations (a hot summer in 2011, which was detrimental for environmental survival of FMDV, a relatively low density of wild boar of ~2-3 heads km, absence of clinical signs or virus detections in a considerably large proportion of animals inspected and tested in Bulgaria in October-December 2011 exactly in the former area of the infection, as well as further away in Turkey).

- The epidemiological model indicates that the presence of deer in the populated area does not alter the spatio-temporal dynamics of the infection in the model and that deer alone are not able to facilitate spread of the infection through the whole landscape.

- The epidemiological model indicates that the strong temperature dependence of FMDV survival in the environment explains the seasonal increased chance of virus fade-out.

- The epidemiological model indicates that continued maintenance (e.g. with moderately virulent CSFV in wild boar) cannot be expected from a wild boar + deer host system alone for FMDV. There is need for cross-transmission between wildlife sub-populations due to human movement or cross-transmission to the domestic sector for virus circulation to be maintained.
The duration of infection in the wild boar population will depend on the availability of continuous wild boar habitat harbouring susceptible wild boar.

As host ecology (density and reproduction) and seasonal environmental drivers are critical for FMD circulation in wildlife, outbreak dynamics in Central Europe might be different than those observed in Thrace.

All the FMD viruses sampled in Bulgarian Thrace were the serotype O PanAsiaII-Ant10 lineage. There is no evidence that the circulating strains in the wider region (O, A, Asia 1) would cause a different outcome.

ToR 2. The risk factors and other relevant epidemiological features, in particular for the different FMD virus strains circulating in Thrace (Bulgaria, Turkey and Greece) which must be taken into account for the design of surveillance systems (including estimation of advantages and disadvantages), that could be implemented for the early detection of any FMD virus incursion in the territory of Thrace (Bulgaria, Turkey and Greece).

- No new risk factors were identified to be associated with the introduction and the spread of FMDV in the region.

- There are several potential risk factors that are associated with both introduction and spread of the FMDV infection in the region. The majority of these factors are related to biosecurity, movement of live animals and animal products (either legally or illegally), swill feeding or access to landfill waste, animal density, and the implementation and efficacy of vaccination programmes. The interaction between all risk factors also needs to be considered.

- An active but spatially restricted/targeted surveillance system for early detection of FMDV infection in wildlife could be a useful addition to an effective passive detection system in domestic animals.

- The EFSAwbFMD model indicated that when the sampling strategy is based on hunting alone, the time needed to detect at least one seropositive animal for an FMDV incursion in January and July into a wild boar population is 39 and 13 weeks after incursion of the virus into a population with similar characteristics, as in the cordon sanitaire in Bulgarian Thrace.

- Disadvantages related to the surveillance system that could be implemented for early detection in wildlife, based on hunting alone, could be associated with one or more of the following adverse factors:

  - collection of samples are limited to the hunting season (e.g. 4 months during winter);

  - low public awareness of FMD and its association with hunted animals;

  - poor sample quality for laboratory testing;

  - inadequate hunting bag; caused by irregular frequency of hunting or unregulated hunting strategies.

- To detect a prevalence of 5% each week, with a CL of 95 %, the ideal surveillance system would require a sample size of between 59 (if the system is perfect) and 69 animals (if the sensitivity of the system is 85%) for a population of greater than 10,000 units and with homogeneous disease distribution.

- The specificity of the surveillance system should be 1 to avoid false positive results. This is achieved when a combination of tests is used.
Foot-and-mouth disease in Thrace

- A critical factor of surveillance systems for early detection of FMDV in wildlife is the availability of samples. If early detection of a new FMDV incursion in a wildlife population is required in a region at risk, then sampling outside the hunting season is necessary.

- The EFSAwbFMD model indicated that the implementation of a surveillance system throughout the year would allow shortening the period for the detection of the first seropositive animals to a median of about a month after incursion of the virus into the population.

TOR 3. The relevance and significance of epidemiological data and genetic characteristics for the different FMD strains recently isolated in Bulgaria and Turkey, with regards to the hypothesis of single versus multiple introductions into Bulgaria.

- The precise pathway for the introduction of FMDV into Bulgaria and its subsequent spread is not known. One possible explanation for the observed genetic relationships between viruses in Bulgaria is a single introduction of virus into the country from Anatolian Turkey but it is also possible that the common ancestor was introduced into Turkish Thrace and quickly moved to Bulgaria either through a single introduction or through several introductions from the same source within a relatively short time span.

- Each of the Bulgarian outbreak viruses appear to share a single recent ancestor which is most closely related to viruses detected previously circulating in Anatolian Turkey.

- Significant gaps in the genetic relationships between the sampled viruses from Bulgaria point to the potential presence of unreported (and hence unsampled) infection in domestic animals (consistent with the identification of seropositive domestic animals) and/or to spread in wildlife reservoirs (consistent with the detection of seropositive wildlife).

- Epidemiological and genetic data indicate that the spread of FMDV in Thrace involved wildlife but must also have involved human transport of animals or animal products.

RECOMMENDATIONS

- A comprehensive surveillance system for the entire region is required with the aim of early detection of the FMDV infection in both domestic and wild susceptible animals. The surveillance system should consider the region as a whole with the ability to trace infected animals to their sources of exposure to the virus. The surveillance system should also consider the vaccination history of the animals in the region.

- Genetic analysis for the FMDV isolates from the region should be linked to epidemiological and other related data with the aim of deriving reliable conclusions about the source of infection.

- To obtain optimal information from genetic analyses it is important to collect samples for virus isolation from all infected premises and from wildlife. Therefore, when serological detection is achieved following the end of clinical signs then probang samples should be collected.

- To trace the incursion of viruses into new areas it is important to collect and sequence viruses circulating in endemic areas.
REFERENCES


BFSA (Bulgarian Food Safety Agency), 2012. Plan for the control of foot and mouth disease (FMD) in susceptible wildlife in the southeast of Bulgaria. 50 pp.


EFSA (European Food Safety Authority), 2012. Simulation-based approach for the assessment of different monitoring strategies for early detection of FMD incursion in a free wild boar population.


Foot-and-mouth disease in Thrace


BULGARIAN THRACE

- History of FMD outbreaks

In the 1990’s Bulgaria experienced three FMD type O outbreaks, all in the Thrace region (Figure 28).

The first outbreak (O/BUL/1/91) was reported in July 1991. During that period Bulgaria had already stopped prophylactic vaccination against FMD in the established 30 km buffer zone bordering Turkey. The outbreak was restricted to Stefan Karadjovo village (1), Yambol region. Stamping out and ring vaccination was successfully applied as the control policy.

In May 1993, a second FMD outbreak was notified. The disease was detected in a dairy cattle holding near the town of Simeonograd (2), Haskovo region, located very close to an international transit road.
For the second time, stamping out with ring vaccination was successfully applied. In total, 304 infected and contact cattle were killed and buried.

The third outbreak was notified in October 1996 in the village of Malko Sharkovo (3), Yambol region, and stamping out was the control policy used to eradicate the outbreak. No vaccination policy was applied. The hypothesis for the origin of the disease was that the virus was introduced with illegally moved susceptible animal(s) from Turkey.

**Figure 28:** Location of FMD outbreaks in Bulgaria in the 90-ies

- **Description of FMD outbreaks in Bulgaria in 2011**

On the 5th of January 2011, Bulgaria notified a case of foot-and-mouth disease (FMD) in a wild boar, which had been shot about 2 km north of the Turkish border. After this finding, 11 outbreaks in susceptible domestic animals were detected in south-east Bulgaria from January to April (Figure 4). Stamping out and burial of animals on the spot was applied as the control policy.

On 30th December 2010, three wild boars were shot during a regular hunting event on Strandzha Mountain in the vicinity of Kosti village, Tsarevo municipality, Burgas region. Lesions on the four feet suggested FMD was observed in one of the animals. Tissue samples from all three wild boars were collected and sent to the National Reference Laboratory (NRL) for FMD in Sofia for laboratory examination. In the evening of 4th January 2011, the samples from only the wild boar with lesions were confirmed positive for FMDV, serotype O using Ag ELISA, real time RT-PCR and LFD (SVANOVIR, SVANOVA). On 5th January, the first case of FMD in wild boar in Bulgaria was declared. Serology was not performed and, consequently, the antibody status of the animals was not determined.

The veterinary service immediately started clinical and serological surveillance in FMD susceptible domestic livestock in all settlements and their vicinities in Tsarevo, Malko Tarnovo and Sredets municipalities of the Burgas region.

On 9th January 2011, within the framework of serological surveillance, 1 bovine, 14 sheep, 12 goats and 8 pigs grazing in the grounds of Kosti village (IP-1) (sampled the previous two days) were confirmed positive using NSP ELISA and Priocheck FMDV type O ELISA. Most of the animals were
Foot-and-mouth disease in Thrace

grazing outdoors on common village grounds throughout the year without stables. No clinical signs had been reported by the local people. FMD clinical signs were also observed during killing of a fenced Hereford cattle herd on 14th January. The disease was confirmed by rRT-PCR and Ag ELISA in tissue samples from one adult bovine from this herd. Blood samples collected from these cattle on 7-8th January had been tested already but with negative results. All susceptible animals grazing in the village and its grounds were killed and buried on the spot.

On 15th January, FMD clinical signs were observed in cattle by veterinary teams in the village of Rezovo (IP-2), Tsarevo municipality, located south-east of Kosti close to the border with Turkey. The disease was confirmed by the NRL on 17th January, and 8 cattle were found to be positive by rRT-PCR and Ag ELISA, whereas 12 cattle and 3 pigs were positive for NS antibodies (Priocheck FMDV NS ELISA). In this area, all cattle had been grazing together on common pasture and were stabled in the vicinity (<200 m) of the pig sties at the entrance of the village. During killing of animals, clinical signs were observed in one sheep as well. The last blood sampling in Rezovo was carried out on 8-9th January as part of serological surveillance before clinical signs appeared in the herd. All samples were negative for FMDV and antibodies against FMDV. Samples for genome sequencing were taken from cattle with lesions on 15th January.

On 31st January, a third outbreak was detected in Gramatikovo village (IP-3), Malko Tarnovo municipality, 10 km west of IP-1, during the second round of the serological survey in the 10 km surveillance zone around Kosti. A total of 1 bovine, 13 sheep and 10 goats were confirmed positive by Priocheck FMDV NS ELISA. All FMD susceptible animals in the village were killed and buried on the spot.

During February and the beginning of March, regular clinical examinations and serological surveillance of susceptible livestock had been carried out in all villages in the “at risk” municipalities of the Burgas region without any evidence or suspicion of FMD virus infection.

However, on 18th March, clinical signs were reported by farmers and confirmed by a veterinary team in a herd of 143 cattle grazing in a fenced forest area (IP-4) located 3 km south of Kirovo village, Sredets municipality, 7 km north of the border with Turkey and 42 km west from IP-3. About 28 of 60 inspected cattle showed salivation and 4-5 days old mouth lesions. The NRL confirmed 12 cattle were positive by rRT PCR the next day.

During January and February, the infected herd had been clinically examined and blood sampled five times without any suspicion or evidence of the disease. The last blood sampling for serological surveillance was carried out on 24-28th February.

On 23rd March, FMD clinical signs were detected by veterinary teams in cattle that were previously grazing in Granichar village (IP-5), Sredets municipality, located in the 10 km surveillance zone around IP-4 and 3 km north of the border with Turkey. In the village, there was only one herd of 133 cattle. No other susceptible animal species were kept there. On 24th March, the NRL confirmed the diagnosis of 11 cattle positive by rRT PCR. Since January 2011, the bovine herd had been clinically examined and blood sampled in five rounds was examined with negative results by serological testing (08 Jan, 28 Jan, 07 Feb, 16 Feb and 25 Feb).

On 24th March, three new suspect cases with clinical signs were noticed by the veterinary teams in the Sredets municipality. On 25th March, the NRL confirmed the three new outbreaks by rRT-PCR and FMD Ag ELISA: a mixed cattle and pig farm (IP-6) located near Goliamo Bukovo village and a cattle farm (IP-7) in the vicinity of Fakia village. Both outbreaks located in the 10 km surveillance zone around IP-4, as well as in a cattle farm (IP-8) near the village of Momina Tsarkva located west of IP-7 and 6 km north of the Turkish border.
During January and February, these three farms were sampled five times. All serological tests were negative. The last blood sampling before detection of the outbreaks was carried out during 24-28th February 2011.

On 3rd April, two new outbreaks were detected within the framework of serological surveillance in the 10 km surveillance zones around IP-5 and IP-6. Clinical signs had been missed mainly due to the difficulties related to the mountain landscape of the area and the limited number of veterinary personnel dealing at the same time with management of the already confirmed outbreaks.

Eight cattle, 42 sheep and 36 goats from the village of Bliznak (IP-9), Malko Tarnovo municipality were declared positive using Priocheck FMDV type O ELISA. During January and February, the animals from this village were serologically tested four times with negative results.

The same day the disease was confirmed by the NRL, using Priocheck FMDV type O ELISA, in a mixed buffalo, sheep and goat herd grazing 2 km south of IP-9 and 5 km north of the border with Turkey (IP-10). The animals were confined during the time of the outbreak.

The last outbreak was notified on 7th April in a herd of cattle, sheep and goats in Dolno Yabalkovo village (IP-11), Sredets municipality. The outbreak was detected during serological surveillance, using Priocheck FMDV type O ELISA, in the 3 km protection zones around IP-4. Since January 2011, the herd had been clinically examined and blood sampled six times with no evidence of sero-conversion.
### Table 8: Chronology of the FMD outbreaks in Bulgaria, including detailed epidemiological information

| Outbreak | Date of suspicion/Date of first sample collection for detection of outbreak | Date of notification/Laboratory confirmation date | Number of animals with clinical signs | Age of lesions in days | Number of animals | Serological tests(a) | Number tested | Number positive | Virological tests(b) | Number tested | Number positive |
|----------|--------------------------------------------------------------------------------|--------------------------------------------------|-------------------------------------|-----------------------|-------------------|-------------------|---------------|----------------|-------------------|---------------|----------------|-----------------|
| wild boar | 30/12/2010 05/01/2011                                                        |                                                   | 1                                   | > 10                  | NA                | NA                | NA            | NA            | NA                | NA            | NA            |
| IP 1 Kosti | 07/01/2011 09/01/2011                                                        |                                                   | 196                                 | 132                   | 1                 | 0                 | 0             | 0             | 0                 | 326           | 266           |
| IP 2 Resovo | 15/01/2011 17/01/2011                                                        | 18/03/2011 19/03/2011                             | 87                                  | 31                    | 12                | 26                | 8             | 0             | 0                 | 76            | 0             | 0              | 0             | 29            | 6              | 3              | 0              | 0            |
| IP 3 Gramatikovo | 30/01/2011 31/01/2011                                                        |                                                   | 1                                   | 1                     | 1                 | 1                 | 0             | 0             | 0                 | 303           | 303           |
| IP 4 Cattle herd south of Kirovo Granicha | 18/03/2011 19/03/2011                                                        |                                                   | 13                                   | 4                     | 28                | 143              | 28            | 7             | 15                | 12            | 0             | 0              | 0             | 0             | 0             | 0             | 0             | 0            |
| IP 5 Granicha | 23/03/2011 24/03/2011                                                        |                                                   | 133                                 | 15                    | 9                 | 11                | 11            | 0             | 0                 | 0             | 0             | 0              | 0             | 0             | 0             | 0             | 0             | 0            |
| IP 6 Cattle & pig farm near Goliamo Bukovo | 24/03/2011 25/03/2011                                                        |                                                   | 49                                  | 8                     | 4                 | 15                | 11            | 0             | 0                 | 0             | 0             | 0              | 0             | 0             | 0             | 0             | 0             | 0            |
| IP 7 Cattle farm near Fakia | 24/03/2011 25/03/2011                                                        |                                                   | 81                                  | 81                    | 0                 | 6                 | 6             | 0             | 0                 | 6             | 6             | 0              | 0             | 0             | 0             | 0             | 0             | 0            |
### Foot-and-mouth disease in Thrace

<table>
<thead>
<tr>
<th>IP</th>
<th>Location Description</th>
<th>Start Date</th>
<th>End Date</th>
<th>Cattle</th>
<th>Sheep</th>
<th>Goat</th>
<th>Serology</th>
<th>Virology</th>
<th>Total</th>
<th>Other Animal</th>
<th>Serology</th>
<th>Other Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Cattle farm near Momina Tsarkva</td>
<td>24/03/2011</td>
<td>25/03/2011</td>
<td>2</td>
<td>3-5</td>
<td>209</td>
<td>11</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>Bliznak</td>
<td>30/03/2011</td>
<td>03/04/2011</td>
<td>0</td>
<td>NA</td>
<td>21</td>
<td>21</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Mixed Buffalo, sheep and goat herd</td>
<td>30/03/2011</td>
<td>03/04/2011</td>
<td>0</td>
<td>NA</td>
<td>72</td>
<td>57</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>72</td>
<td>57</td>
</tr>
<tr>
<td>11</td>
<td>Dolno Yabalkovo</td>
<td>04/04/2011</td>
<td>07/04/2011</td>
<td>0</td>
<td>NA</td>
<td>45</td>
<td>35</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

(a) Serological tests used for surveillance in IP are FMD ELISA Type O and/or FMD NSP ELISA
(b) Virological tests used for FMD detection and surveillance in IP are RT PCR and/or Ag ELISA
NA: not applicable

Source: Animal Health and Welfare Directorate & National reference laboratory for FMD at the Bulgarian Food Safety Agency
On 18-19th April, clinical examinations and blood sampling of susceptible animals had taken place in the villages in the risk municipalities of Burgas and Yambol regions, as well as in other randomly selected villages in the Burgas, Varna, Shumen, Sliven, Yambol and Haskovo regions, in order to prove the absence of FMD virus infection in domestic livestock in the areas north and west of the detected outbreaks.

Clinical examinations and blood sampling had been repeated again with negative results on 28-29th April in the risk regions of Burgas and Yambol municipalities.

The next round of clinical examination was carried out on 18-19th May in the border municipalities of Burgas and Yambol and in the additional randomly selected villages of Burgas, Varna, Shumen, Sliven, Yambol and Haskovo bordering the risk area. No suspicion or evidence for FMDV were detected.

On 4th July, the protection and surveillance zones imposed were removed from all outbreak areas.

- **Surveillance in domestic animals**

During January – May 2011, repeated clinical inspections and blood sampling for serological assays took place in the course of the FMD epidemics in designated areas overlapping the protection and surveillance zones established around the detected outbreaks in the villages of Tsarevo, Malko Tarnovo, Sredets, Primorsko and Sozopol municipalities of the Burgas region and villages of the Yambol region bordering Turkey.

It was proposed that all susceptible animals in the villages along the Turkish border and in the villages within the protection and surveillance zones around the outbreaks should be bled, while in all other villages considered “at risk”, samples should be taken for detection of at least one seropositive animal if the seroprevalence was 5 % with 95 % confidence per epidemiological unit. The Commission implementing Decision 2011/493/EU of 5th August 2011 approved this plan, which was submitted by Bulgaria on 4th April 2011 for the eradication of foot-and-mouth disease in wild animals in south-east Bulgaria. The plan had to be implemented within a one year period following submission to the Commission, and included certain passive and active surveillance activities in wildlife and domestic livestock in an area in south-east Bulgaria defined as the “Cordon Sanitaire”.

**Figure 29:** Cordon sanitaire – FMD surveillance and control area in South-East Bulgaria

The “Cordon Sanitaire” was formed by a defined infected area and neighbouring risk areas to the north and west based on the results of epidemiological considerations, the geographical distribution of the disease and the FMD situation in wildlife in Turkish Thrace.

The defined infected area (Area “A”) was a 20 km-wide region in the south-east of Bulgaria along the Turkish border with a size of about 1,240 km². In this area, the outbreaks in domestic livestock
Foot-and-mouth disease in Thrace occurred in January and March-April 2011. The area comprised part of Tsarevo, Malko Tarnovo and the southern part of the Sredets municipalities (Burgas Region). A total of 32 villages were located within the defined infected area. The risk area of approximately 2,160 km² was divided into two parts (Areas “B” and “C”) and neighboured the infected area in the north and west along the Turkish border. These areas were defined taking into consideration the generally unclear epidemiological situation of FMD.

The risk areas comprised the following municipalities or parts of them: North Tsarevo, Primorsko, Sozopol and North Sredets (Burgas Region), which formed Area B, as well as Svilengrad, Topolovgrad (Haskovo Region), Elhovo and Boliarovo (Yambol Region), which formed Area C. A total of 74 villages were located within the defined risk area.

Since August 2011, pursuant to the one year plan for the control of foot-and-mouth disease in susceptible wildlife in south-east Bulgaria, all 106 villages in the “Cordon Sanitaire” have been visited every 21 days by a team of veterinary experts, and all susceptible animals have been clinically examined in accordance with Annex III of Directive 2003/85/EC. In suspect cases, samples for laboratory testing (blood and tissue samples) have to be taken immediately and the measures laid down in Directive 2003/85/EC, Articles 4 and 5 applied.

In addition to clinical surveillance, routine blood sampling for serological tests was conducted every third month. The minimum number of animals to be sampled in an epidemiological unit allowed detection of at least one seropositive animal if the seroprevalence is 5 % with 95 % confidence. The epidemiological unit was the village. In case of a pure sheep/goat population in the epidemiological unit, blood sampling was carried out as specified by the clinical surveillance plan. Blood sampling of animals for slaughter was also carried out before they left the Cordon Sanitaire if the animals had not been tested within the previous 21 day period. At the slaughterhouse, a thorough ante- and post-mortem inspection was performed for detecting FMD at this stage.

The first active surveillance activities within the framework of the plan were carried out during 16-25th August 2011. All 106 villages were checked and all results were negative for FMD clinical signs. A total of 5,203 blood samples from susceptible livestock tested negative using Type O ELISA. The next two rounds of clinical examinations were carried out according to plan in September and October 2011 and they were all negative for FMD clinical signs.

During 21-25th November, the next blood sampling for serological surveillance was successfully conducted in the “Cordon Sanitaire”. A total of 5,295 blood samples to detect one seropositive animal if the seroprevalence is 5 % with 95 % confidence tested negative using Type O ELISA. Moreover, no FMD clinical signs had been observed either during the 5th round of clinical examinations in December 2011.

Table 9: Serological surveillance in the “Cordon Sanitaire” in November 2011

<table>
<thead>
<tr>
<th>Sampling period</th>
<th>No of villages</th>
<th>No of animal holdings</th>
<th>No of animals</th>
<th>No of blood samples taken (a)</th>
<th>Laboratory results (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21st-25th Nov</td>
<td>106</td>
<td>3,701</td>
<td>10,834</td>
<td>44,172</td>
<td>17,059</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>176</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3,277</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5,295</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
</tr>
</tbody>
</table>

(a) Number of blood samples taken to detect 5 % prevalence with 95 % confidence within a village
(b) Type O ELISA

TURKISH TRACE

- History of FMD outbreaks
Along the natural and political borders of Turkish Thrace, a buffer zone was established in 1962-1965 to protect Greece and Bulgaria from SAT-1 and A22 pandemics. Since that time, Turkey’s FMD control policy has become more committed and has included a vaccination programme targeting circulating serotypes and subtypes. In conjunction with the programme, the EC and EUFMD specifically started to support Turkey’s efforts. Vaccines needed for Thrace have been mostly supplied by the EU, EuFMD and UN institutions since the 1960s to the present day. The current FMD control measures in Turkish Thrace are outlined below:

1. Vaccination policy and coverage: All the bovine population in Turkish Thrace is vaccinated twice a year, while the ovine population is vaccinated once in spring. The levels of vaccine coverage are given below in Table 10.

**Table 10:** FMD vaccination coverage in Turkish Thrace in 2010 and 2011

<table>
<thead>
<tr>
<th>Vaccination coverage (%)</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR (spring)</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>LR (spring and autumn)</td>
<td>92 and 94</td>
<td>100 and 100</td>
</tr>
</tbody>
</table>

2. Vaccine: EU supplied purified vaccines (free of non-structural proteins) produced in Europe are used against serotypes A, O and Asia 1.

3. Animal movements to Thrace: Regulated according to Article 8.5.14 of Chapter 8.5 of the OIE Terrestrial Animal Health Code (OIE, 2011a) considering “importation from FMD infected countries or zones” (Anonym, 2010b). However, probang samples are not always tested as required.

4. Control points established between Thrace and Anatolia to prevent illegal movements (Anonym, 2010b).

5. When there is an outbreak in Thrace, legislation fully compliant with Directive 2003/85/EC has been implemented since 4th February 2011 (Anonym, 2011b).

6. Movement of large ruminants or small ruminants from Thrace (Edirne, Kırklareli, Tekirdağ, Çanakkale) to Istanbul is only permissible in one direction, in order to prevent possible contact with infected animals and potential future carriage of the disease, which is of particular importance for markets that are established to provide animals for sacrifice during festivals (Anonym, 2012).

7. When smuggled animals are detected in Thrace, all of them, including contacts, are destroyed regardless of their infection status and this is followed by local serosurveillance (Anonym, 2012).

The last series of FMD outbreaks occurred in 2007, and were due to two different serotypes, type A and type O. Details are given in the Figure 30 and Table 10.
Foot-and-mouth disease in Thrace

Since 2007, FMD has not been seen in Thrace. As a result Turkish Thrace was recognised as an official FMD-free zone, where vaccination is practised, in March 2010 (OIE, 2011b). Since September 2011, however, this free status with vaccination was suspended, due to the detection of seropositive wild boars in the region.

Table 11: Last reported FMD outbreaks in Turkish Thrace (Anonym, 2009)

<table>
<thead>
<tr>
<th>Location (Province, District, village)</th>
<th>Date of the outbreak</th>
<th>Virus serotype</th>
<th>Number of diseased animals</th>
<th>Source of the outbreak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edirne, Havsa, Oğülpása</td>
<td>4.01.2007</td>
<td>A</td>
<td>2 cattle</td>
<td>Animal Market</td>
</tr>
<tr>
<td>Kirklareli, Merkez, Armağan</td>
<td>22.02.2007</td>
<td>O</td>
<td>1 cow</td>
<td>Illegal animal movements</td>
</tr>
<tr>
<td>Çanakkale, Gelibolu, Evreşe</td>
<td>30.03.2007</td>
<td>O</td>
<td>22 cattle</td>
<td>Illegal animal movements</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Contact with infected animal(s) at grazing/watering</td>
</tr>
<tr>
<td>Kirklareli, Merkez, Çukurpinar</td>
<td>12.09.2007</td>
<td>O</td>
<td>83 cattle</td>
<td>Introduction of live animals</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Illegal animal movements</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Contact with infected animal(s) at grazing/watering</td>
</tr>
</tbody>
</table>

- **Surveillance in domestic animals**

FMD surveillance in Turkey mainly consists of clinical, virological and serological components besides characterisation of circulating FMDV strains in the field. As specified in Section 11.2, the virus involved in the 2011 Bulgarian outbreak, O-ANT-10, is a pandemic strain and was first collected in south-east Turkey, in a village of the Gaziantep province (20 km north of the Syrian border) on 19/03/2010. It became the dominant subtype of serotype O and caused approximately 1,000 outbreaks in Anatolia until it became extinct in July 2011. Coinciding with this period, six serosurveillance
Foot-and-mouth disease in Thrace

studies were carried out in domestic animals of Turkish Thrace, and their short descriptions are as follows:

1. 2010 autumn serosurveillance was regular serosurveillance in the whole of Turkish Thrace (TT) (Figure 32A).
2. 2011 spring serosurveillance (forests) was designed to target wild and domestic animals in forested areas of TT, where possible contacts could occur between them and permit virus circulation that could contribute to part of the Bulgarian outbreak (Figure 33A). Results for wild animals are given in Section 2.5.
3. 2011 summer serosurveillance (near the border) was designed to target small ruminants (SR) and large ruminants (LR) in locations close to the high risk area in Bulgaria (Figure 34).
4. 2011 summer serosurveillance was regular serosurveillance and was carried out in the whole of Turkish Thrace (Figure 35A).
5. 2011 summer water buffalo serosurveillance was species targeted and sampling locations are shown in Figure 35A.
6. 2011 autumn serosurveillance (sub-zonal TT) excluded borderline districts and villages in Kirklareli province confined by the red line and Bulgarian border shown in Figure 36.

Serosurveillance designs, laboratory tests and interpretation of results were compliant with the current OIE FMD Terrestrial Animal Health Code (OIE TAHC) (Figure 31 and risk factors due to contamination of wildlife were also managed (OIE, 2011a). Details are given in Table 12 regarding designs, tests, interpretation, and follow ups.

Figure 31: Algorithm and flow of laboratory tests for determining evidence of FMDV infection through or following serological surveys (cited from OIE-TAHC) (OIE, 2011a)
### Table 12: Summary of all sero-surveillance activities in TT during FMDV serotype O ANT-10 outbreak (Bulut, 2011)

<table>
<thead>
<tr>
<th></th>
<th>1-2010 autumn (whole TT)</th>
<th>2-2011 spring (forests)</th>
<th>3-2011 summer (borderline)</th>
<th>4-2011 summer (Whole TT)</th>
<th>5-2011 summer (Water buffalo)</th>
<th>6-2011 autumn (sub-zonal TT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SR 88/46/86/13/NA</td>
<td>1197/80-70/19/NA</td>
<td>1178/11/15</td>
<td>9108/66/13/NA</td>
<td>240/All</td>
<td>896/864/140/NA</td>
</tr>
<tr>
<td>Age targeting</td>
<td>LR/NS 4-18</td>
<td>All ages 4-24</td>
<td>All ages</td>
<td>All ages</td>
<td>All ages</td>
<td>All ages</td>
</tr>
<tr>
<td></td>
<td>LR/NS 95/95</td>
<td>95</td>
<td>97.59/7.5 NA</td>
<td>95/95</td>
<td>95/95</td>
<td>95/95</td>
</tr>
<tr>
<td>CI%</td>
<td>LR/NS 5/5</td>
<td>NA/2</td>
<td>NA/5</td>
<td>NA/2</td>
<td>NA/5</td>
<td>NA/NA</td>
</tr>
<tr>
<td>Minimum expected prevalence % (primary/secondary)</td>
<td>LR 2/5</td>
<td>NA/2</td>
<td>NA/5</td>
<td>NA/2</td>
<td>NA/5</td>
<td>NA/2</td>
</tr>
<tr>
<td></td>
<td>SR 2/5</td>
<td>NA/2</td>
<td>NA/5</td>
<td>NA/2</td>
<td>NA/5</td>
<td>NA/2</td>
</tr>
<tr>
<td>Mass screening results (positive %)</td>
<td>LR 0.49 (18 w+ve)</td>
<td>0.91 (SLR and 13 SR)</td>
<td>0.26 (215R, 28 w+ve, 14 ve)</td>
<td>0.34 (1LR, 8SR)</td>
<td>0.18 (2 w+ve)</td>
<td>0.16 (2 w+ve)</td>
</tr>
<tr>
<td></td>
<td>SR 0.32 (27 w+ve, 5 ve)</td>
<td>0.045 (5 w+ve)</td>
<td>0.08 (1 w+ve)</td>
<td>0.18 (11 w+ve)</td>
<td>0.98 (SLR, 5 SR)</td>
<td>0.16 (2 w+ve)</td>
</tr>
<tr>
<td>Retesting (positive %)</td>
<td>LR 0.02 (1 w+ve)</td>
<td>NA/0.045 (5 w+ve)</td>
<td>NA/0.08 (1 w+ve)</td>
<td>NA/0.18 (2 w+ve)</td>
<td>NA/0.035 (w+ve)</td>
<td>NA/0.00 (w+ve)</td>
</tr>
<tr>
<td></td>
<td>SR 0.04 (3 w+ve, 1 ve)</td>
<td>NA/0.00</td>
<td>NA/0.00</td>
<td>NA/0.00</td>
<td>NA/0.00</td>
<td>NA/0.00</td>
</tr>
<tr>
<td>LPB-ELISA (confirmatory for +ve)</td>
<td>LR +ve/ +ve</td>
<td>+ve/ +ve</td>
<td>+ve/ +ve</td>
<td>+ve/ +ve</td>
<td>+ve/ +ve</td>
<td>+ve/ +ve</td>
</tr>
<tr>
<td></td>
<td>SR +ve/ +ve</td>
<td>+ve/ +ve</td>
<td>+ve/ +ve</td>
<td>+ve/ +ve</td>
<td>+ve/ +ve</td>
<td>+ve/ +ve</td>
</tr>
<tr>
<td>Western blotting (confirmatory for +ve)</td>
<td>LR -ve/ -ve</td>
<td>-ve/ -ve</td>
<td>-ve/ -ve</td>
<td>-ve/ -ve</td>
<td>-ve/ -ve</td>
<td>-ve/ -ve</td>
</tr>
<tr>
<td>Probang (virological)</td>
<td>LR NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td></td>
<td>SR NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
<td>NA/NA</td>
</tr>
<tr>
<td>Clustering of positive animals</td>
<td>LR -ve/ -ve</td>
<td>-ve/ -ve</td>
<td>-ve/ -ve</td>
<td>-ve/ -ve</td>
<td>-ve/ -ve</td>
<td>-ve/ -ve</td>
</tr>
</tbody>
</table>
Foot-and-mouth disease in Thrace

1 Total number of animals bled from the target area/number of sampled animals from each village/number of villages sampled/number of animals sampled for probang

2 None sampled in the first step

3 As close as possible to a balanced sample of 40SR/40LR from each village

4 Biased design in favour of borderline locations (Figure 33A) such as Kırklareli, Demirköy close to high risk areas shown in Figure 31

5 Vaccinated at least three times

6 Survey design targeted borderline villages only shown in Figure 34

7 Sub-zonal TT: corresponds to the whole of TT, excluding villages in Demirköy, Kofçaz and central Kırklareli shown in Figure 36

8 Primary: first stage sampling level (= village); secondary: second stage sampling level (= individual animals)

Mass screening assay: Prionics NSP-ELISA with a Se/Sp 90%, 99.1%, respectively.

Retesting: Second testing of +ves with Prionics NSP-ELISA

Confirmatory assays: Only positive animals were tested with LBP-ELISA and Western blotting

Probang testing: Only positive animals were re-sampled for their oesophago-pharyngeal fluids in the follow up step and tested with real-time- and/or agarose-based RT-PCRs having universal primers and probes.

NA: Not applicable or not available

LR: Large ruminants

SR: Small ruminants

w+ve: weak positives in ELISA (for NSP between 50-70 % inhibition value, or for LPB-ELISA <1/96 titre)

s+ve: strong positives in ELISA (for NSP ≥ 70% inhibition value, or for LPB-ELISA > 1/96 titre)

TT: Turkish Thrace

CI: Confidence interval
Figure 32: Map indicating the distribution of the sampled epidemiological units (A), and the distribution of positives (B) in the autumn 2010 (Whole TT). (📍: LR;📍: SR; positive LR📍; positive SR📍) (Bulut, 2011).

![Map indicating the distribution of the sampled epidemiological units (A), and the distribution of positives (B) in the autumn 2010 (Whole TT). (📍: LR;📍: SR; positive LR📍; positive SR📍) (Bulut, 2011).](image)

Figure 33: Map indicating the distribution of the sampled epidemiological units (A) (📍: Çanakkale;📍: İstanbul;📍: Tekirdağ;📍: Kırklareli;📍: Edirne), and the distribution of positives (📍: LR;📍: SR) (B) in spring 2011 (forests) (Bulut, 2011).

![Map indicating the distribution of the sampled epidemiological units (A) (📍: Çanakkale;📍: İstanbul;📍: Tekirdağ;📍: Kırklareli;📍: Edirne), and the distribution of positives (📍: LR;📍: SR) (B) in spring 2011 (forests) (Bulut, 2011).](image)

Figure 34: Map indicating the distribution of the sampled epidemiological units in summer 2011 (borderline) (Bulut, 2011).

![Map indicating the distribution of the sampled epidemiological units in summer 2011 (borderline) (Bulut, 2011).](image)
Figure 35: Map indicating the distribution of the sampled epidemiological units (A), and the distribution of positives (B) in summer 2011 (Whole TT) (▼: Cattle; ◆: SR; and ◇: water buffalo; ◇+: ve water buffalo; ▼+: ve cattle; ◆+: ve SR) (Bulut, 2011).

Figure 36: Map indicating the distribution of the sampled epidemiological units in autumn 2011 (sub-zonal Thrace, the area confined by the red line and the Bulgarian border is excluded) (▼: Cattle; ◆: SR) (Bulut, 2011).

According to mass serosurveillance results, the percentage of positive animals was not higher than the false positive limits (1%) of the NSP ELISA in all cases (Figures 32-36), and there were no strong positives after retesting. Confirmatory test results were also not suggestive for the presence of antibody to live FMDV. In order not to miss any hidden virus circulation risk and to obtain stronger confidence, epidemiological inquiry, assessment and re-sampling (follow up step) were conducted in all villages (regardless of the age) where positive sera were detected without proving virus circulation in the first step. Similar to the first step, interpretation of the results from the follow up data also did not reveal any proof of live virus circulation in TT.

GREEK THRACE

- History of FMD outbreaks

Greece has a disease free status for FMD without vaccination. Major outbreaks occurred in 1994 and 1996, and the last outbreak occurred in 2000 (Valarcher et al., 2008). The last outbreak of FMD in Greece was at the prefecture of Evros in the Delta of Evros. Serotype Asia 1 was isolated, which had originated from Turkey. There were three primary outbreaks in bovines; one occurred in the Evros Delta, one in the village of Peplos and one in the village of Mandra. There were nine secondary outbreaks: four occurred in the Evros Delta in bovines, three in the Ferres village, of which two were
Foot-and-mouth disease in Thrace

in bovines and one in sheep, one outbreak occurred in the village of Selino in bovines and one outbreak occurred in the village of Mandra in the prefecture of Xanthi, also in bovines.

- **Surveillance in domestic animals**

All the results of the serological surveillance for FMD carried out in 2011 were negative by anti-NSP ELISA (PRIONICS).

**Table 13: Serological surveillance for FMD carried out in 2011**

<table>
<thead>
<tr>
<th>prefecture</th>
<th>bovine</th>
<th>small ruminants</th>
<th>swine</th>
<th>wild boar holdings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evros village</td>
<td>133</td>
<td>850</td>
<td>39</td>
<td>77</td>
</tr>
<tr>
<td>Orestiada</td>
<td>186</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soufi</td>
<td>138</td>
<td>291</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alex/polis</td>
<td>164</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xanthhi</td>
<td>1883</td>
<td>509</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rodopi</td>
<td>339</td>
<td>337</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(FMD institute, Ministry of Rural Development and Food, unpublished)

**APPENDIX B. LIVESTOCK POPULATION DENSITY AND HUSBANDRY SYSTEMS IN THRACE**

**POPULATION SIZE AND GEOGRAPHIC DISTRIBUTION OF SUSCEPTIBLE LIVESTOCK**

The population size and geographic distribution of cattle and small ruminants in Thrace are presented in Figure 37 and 38.

**Figure 37: Density of Cattle in Thrace**
Figure 38: Density of small ruminants in Thrace

In January 2012, the domestic susceptible livestock population was comprised of 297,934 large ruminants, 841,037 small ruminants and 197,197 pigs in the Bulgarian part of Thrace.

HUSBANDRY SYSTEMS

• Greek Thrace

In the south-east of Evros Prefecture, there are 48 bovine dairy production farms, where the animals stay in permanent facilities, and 521 extensive farms with beef cattle, which consist of 5 to 10 free ranging animals. These are mostly in the Delta of Evros near the Soufli village mountain area where they stay for 10 months outside and for the remaining 2 months they are transferred to the Agriculture Park of Ferres.

In the same farms, the ovine and caprine animals are kept under extensive farming. In the north-east of Evros Prefecture, small ruminants are kept in permanent stable facilities during the night, and during the day time they are free under extensive farming. Large ruminants for dairy production are kept within facilities but beef cattle are free ranging. There are two wild boar farms in the village of Pedalofo in Orestiada near the border with Bulgaria.

• Bulgarian Thrace

High biosecurity animal holdings (see European Commission, DG (SANCO) 2010, for definition of high biosecurity holdings) are located mainly in the central and eastern territories of Bulgarian Thrace in the regions of Pazardjik, Plovdiv, Stara Zagora, Sliven, Yambol and Burgas.

Sheep, goat and cattle herds are grazed on common open pastures during most periods of the year. This practice is mainly observed in the semi-mountain and mountain areas of Rhodopes, the and southern outskirts of the Balkan Mountains.

Family pig farming is well developed in the whole of Bulgarian Thrace. Backyard pig production (up to 5 pigs for own consumption) is widespread in the smaller villages. A unique free range Bulgarian breed of East-Balkan pigs is found only in defined areas of the Burgas region covering small territories.
of the Balkan and Strandzha Mountains. East-Balkan pigs are free ranged outdoors throughout the year, after which they are usually slaughtered for the owner’s consumption and are generally not marketed.

Strandzha Mountain is a unique ecosystem allowing outdoor free ranging of cattle, sheep, goats and pigs throughout the whole year, mainly for local needs.

The holdings in south-east Bulgaria, along the border with Turkey, are of a family and back yard type, although some herds are larger in size. The villages belong to a remote region with a relatively low animal density, which are generally not involved in industrialised animal production. Most of the animals graze on common village grounds throughout the year and are kept for own consumption or for the local/regional market, generally for animals or animal products.

- **Turkish Thrace**

In spite of more intensive agriculture, the husbandry system in Turkish Thrace has a similar structure to that in Anatolia. Villages and holdings are under veterinary supervision and owners in Thrace are more conscious in terms of disease hazards. Moreover, cattle husbandry is more organised in this region for marketing and breeding. The FMD susceptible species present include cattle (504,256), sheep (643,764), goats (163,000) and buffalo (~7,000). The average cattle herd and sheep flock sizes in Thrace are 502 and 900 per village, respectively. Cattle and sheep husbandry practices are relatively smaller in size compared to Anatolia, and the animals are kept for personal meat and milk consumption, as well as for commercial use. On the other hand, acquisition of the FMD vaccinated free status in Thrace in 2010 has been seen as an investment opportunity by the larger sectorial actors, which is supported by the Ministerial statement that Thrace would “turn into such a hub” and operate under OIE rules, which largely limit animal movements into Turkish Thrace (Anonym, 2010a, 2010b; Aslankılıç, 2010). Moreover, one of the largest cities in the world, the European part of Istanbul, is now largely dependent on the beef produced within Thrace. These factors will expand the marketing opportunities for animal husbandry in Thrace. On the other hand, rapid urbanisation and industrialisation resulting in limitations on pasture and feedstuff resources have been threatening sheep husbandry and pushing cattle husbandry from outdoors to higher cost indoor practices (Anonym, 2004a, 2004b, 2004c; Aslan et al., 2004; Sarı, 2005).

Villages are commonly accepted as the epidemiologic unit, which consists of small-scale large ruminant (e.g. cattle) and small ruminant (e.g. sheep and goat) holdings with the houses owned by different farmers. Large and small ruminants are generally in close contact with each other and share the same grazing and water sources, which might be shared with neighbouring villages as well. Biosecurity practices in these holdings are generally low. Owners of different holdings in a village traditionally visit each other and share their farm equipment, reducing biosecurity at the village level. On the other hand, there are some larger-scale holdings outside the villages that are accepted as independent epidemiologic units (Anonym, 2009).

During the 2011 Bulgarian FMD outbreak, which included infection of wildlife, local authorities in Kırklareli ordered certain precautionary measures on 13th January 2011 in order to prevent contact between wildlife and farm animals. The second article of this decision ordered that in the villages under FMD risk near the Bulgarian border, all large and small ruminants had to be kept indoors and not allowed to graze until after a second announcement that was made in March 2011 (Anonym, 2011a).
APPENDIX C. ECOLOGICAL, BEHAVIOURAL AND POPULATION CHARACTERISTICS RELEVANT TO THE EPIDEMIOLOGY OF FOOT-AND-MOUTH DISEASE IN SUSCEPTIBLE WILD UNGULATES OF THRACE (WILD BOAR, ROE AND RED DEER)

POPULATION ESTIMATES

The current distribution of roe deer, red deer and wild boar (Figure 39) is primarily associated with areas occupied by broadleaved forests. The majority of the species concerned occur in the FMD-free countries where they are distributed nearly continuously, though with varying density.

Figure 39: Distribution of wild boar, roe deer and red deer according to IUCN (Lovari et al., 2008; Oliver and Leus, 2008).

The estimated post-harvest number of the European population of wild boar, red and roe deer altogether reaches a minimum of 14.2 million individuals (Burbaitė and Csányi, 2009; Burbaitė and Csányi, 2010; Putman et al., 2011), which can increase annually to over 22.4 million following the spring reproduction season, with maximum population recruitment rates for wild boar of 100 %, for roe deer of 50 % and for red deer of 25 % (Danilkin, 1999, 2002). Their actual population size may be significantly higher; however, population estimates of these wild ungulates on such a large spatial scale based on low quality census data tend to be lower than in reality. According to the 2011 census data, in the whole of Bulgaria there were 76,402 wild boar, 86,648 roe deer and 21,140 red deer. For the Burgas region these figures were 6,958, 4,467 and 1,905 heads, respectively (data: Bulgarian MoF).

During winter, mortality rates in the populations of wild ungulates generally increase due to predation, hunting, disease, starvation and other reasons (Geptner et al., 1961; Danilkin, 2002). Therefore, their lowest numbers are observed before the start of the reproduction season, and this is when population censuses normally take place. In wild boar, reproduction begins in February and continues until June, although sometimes it finishes even later in the season. Most wild boar piglets are normally born in March-April. Specifically in south-east Bulgaria, as shown by the age distribution of piglets shot in the Strandzha Mountains in 2011 (n=257), 73.5 % of them were born in the period from February to April (18.7 % in February, 31.9 % in March and 23.0 % in April). Reproduction in both deer species takes place from mid April to mid June, with a peak of the calving period occurring in May (Danilkin, 1999). Importantly, unlike in most Western European countries, in south-east Bulgaria, populations of wild ungulates are subject to high predation pressure from the grey wolf Canis lupus and the golden jackal Canis aureus. According to 2011 census data, there were 106 wolves and 4,401 jackals in the Burgas region.

In Thrace, data on populations of wild ungulates are available only for the Cordon Sanitare, as identified by the Bulgarian Veterinary Authority (Figure 40). According to the spring 2011 population census, wild boar is the most abundant species (5,059 heads, average density 2.1; 0.43–2.140 heads per square km), followed by roe deer (3,931 heads, 1.5; 0.57–1.48 heads per square km) and red deer (1,657 heads, 0.7; 0.24–0.7 heads per square km). Overall, in spring, there are around 11,000 FMD susceptible wild animals found most abundantly in the districts of Malko Tarnovo (Burgas region) and Bolyarovo (Yambol region). From May to June, ungulate populations receive a significant influx of
juvenile individuals and reach the peak of their annual density. Due to the lack of field data on the age population structure of the species concerned at this time of the year, we can consider using the maximum known recruitment rates for these animals (although they might underestimate predation pressure on juveniles). These are a conservative 100 % for wild boar, 50 % for roe deer and 25 % for red deer. This brings the total population estimate for the Cordon Sanitaire from 10,600 in February to 18,100 (more likely to be approximately 15,000) in June for all FMD susceptible individuals. Their overall density increases respectively from 4 to 6-7 individuals per square km.

Figure 40: Densities of wild boar (top left), roe deer (top right), red deer (bottom left) and species composition (bottom right) within the Cordon Sanitaire in Bulgaria at the municipal level (data: spring 2011).

No ungulate population estimates are available for the Turkish part of Thrace. Calculations made on the basis of the forest cover area (only in the north-eastern forested half of Turkish Thrace), provide average home ranges and herd sizes of wild boar that give a post-harvest total of approximately 3,500 individuals (Khomenko et al., 2011), which is likely to be an underestimate as wild boar distribution here is not limited to forest covered areas only and the species penetrates far inland into the agricultural landscapes through the bush overgrown river valleys and gullies (N. Bulut, pers. comm.). Both roe and red deer are reportedly found in very low numbers.

In Greek Thrace, an estimated total of 915 animals were found in 2004, in densities ranging from 0.13 to 1.1 (average 0.89) heads per square km (Tsachalidis and Hadjisterkotis, 2009). This is the lowest density in Greece. Also, according to Tsachalidis and Hadjisterkotis (2009), the wild boar occurrence range in Greek Thrace is primarily associated with the forests of the Rhodope Mountains, while on the border between Evros and Edirne in Turkey they do not occur at all. The current population size of wild boar in this area might have changed by now, although, until 2004, the whole Greek population did not show any apparent increase (Tsachalidis and Hadjisterkotis, 2009). The population of red deer in Greece is apparently very small and, in 2001, it was estimated at 130 individuals (Burbaitė and
Csányi, 2010). It is not clear how many red deer are found in Greek Thrace, but it is probably just a few tens of individuals. No data is available for the roe deer population (Burbaitė and Csányi, 2009).

**SOCIAL ORGANIZATION**

The three species normally occur in more or less territorial (and in this case usually family) groups of just a few individuals, most often females with their off-springs.

![Figure 41: Annual variation in herd size of wild boar, roe deer and red deer (data from: Danilkin, 1999, 2002).](image)

Herds of wild boar are typically comprised of 4-5 individuals (Figure 41), although much larger groups reaching a size of 100 individuals may be occasionally observed (Danilkin, 2002). In the Strandzha Mountains, a group of about 70 wild boar was seen in 2011 (Tsviatko Alexandrov, pers. comm.). Such large groups are usually temporary aggregations of animals that occur in response to the availability of food, weather conditions, disturbance, etc., and they never last for a long time. There is relatively little seasonal variation in the average herd size of wild boar (Figure 41). It increases slightly in May-June following the reproduction season, due to the presence in the population of many piglets accompanying sows, but normally stays at approximately 3.8 individuals throughout the year (Danilkin, 2002). Nonetheless, social organisation of wild boar is highly flexible and labile. It depends on local conditions and the phase of their life cycle. Average herd size may increase with population density, but it also does so at the times and places with abundant food resources (such as acorns in mast years), as well as during the rut period. In different populations, and dependent on the season, from 14 to 60 % of the observed animals may remain solitary. Groups of 2-5 individuals of different age and sex comprise from 4 to 26 % of all observations. Such small family groups may join together in summer or in the beginning of autumn in the habitats where food is found abundantly.

An average roe deer herd size throughout the year normally stays in the range of 2.1-2.4 individuals, increasing to 3.9 in winter and declining to 1.1 individuals in summer months. The maximum known herd size of European roe deer is 27 individuals (Danilkin, 1999). Most of observations in summer are those of single individuals (60-90 %). Towards autumn, groups of 3-4 animals are observed more frequently (up to 50 % of cases). In winter, the majority of animals stay in herds of three and more individuals. Social organisation of red deer is similar to that of roe deer. Average yearly herd size is 3.1 (max. 65) individuals, which increases in winter and declines in summer (Figure 41). Larger herds comprised of a stag and several hinds with offsprings can be seen during the rut period (September). Animals of different sex and age group join together mainly in winter and disaggregate by the calving period.

**TERRITORIALITY AND USE OF SPACE**

As in most of Europe, in the Strandzha Mountains, the three ungulate species are sedentary. They occupy relatively stable home ranges of seasonally variable size that strongly overlap in space at the inter- and intra-species level, thus providing opportunity for their direct or indirect contact and potential spread of pathogens such as FMDV across the populated landscape.

Typically, the average annual home range of a matriarchal family group of wild boar is around 4 sq. km, however, it is highly variable for individual animals and depends on sex, age and season (Massei...
et al., 1997), as well as on the population density (Danilkin, 2002). Weather (particularly snow), disturbance, hunting and predation can strongly affect space use patterns of wild boar (Danilkin, 2002; Sodeikat and Pohlmeyer, 2002; Keuling et al., 2008). In general, sows with piglets and juvenile females have smaller home ranges and are more site faithful compared to boars and juvenile males, which may wander for tens and, sometimes, hundreds of kilometres (Danilkin, 2002). Home range dynamics in wild boar in Thrace have never been studied and no data are available on the subject. During summer, particularly during the reproduction period and rut, the roe deer population is highly territorial (Figure 42), but switches to a loose aggregation type distribution pattern during the cold period of the year. According to Danilkin (1996, 1999), territories of adult males in summer range from 2 to 200 hectares. Their size is inversely correlated with population density. Daily home ranges of males average around 49 hectares (from 4 to 97), which is 40 % of the territory protected during the summer period (122 hectares). During calving, the daily home range of adult females declines to its minimum. It is limited to the most favourable areas and increases during the lactation period to an average of 24 hectares (from 7 to 34). Their seasonal summer home range averages approximately 73 hectares (39-107). Immature males that have to emigrate from their native locations have summer home ranges up to 1,000-2,000 hectares. Immature females usually occupy much smaller summer ranges that are most often found close to their mother’s territories.

**Figure 42**: Territorial organization of roe deer population in summer (“a complex family”, after: Danilkin, 1999)

1. Territories of adult males;
2. Home ranges of adult females with calving sites;
3. Home ranges of one year old females;
4. Emigration of one year old individuals both males and females;
5. Home ranges of one year old males, which may or may not overlap with animals of other sex or age as shown in the picture

The home ranges of roe deer from October to April may increase compared to their home range in summer, depending on the availability of food and the weather conditions. They often overlap and change their boundaries. At this time of the year, there is no difference in the size of the home range between sexes or age groups (Danilkin, 1996).

Annual home ranges of red deer vary from a few hundred to a few thousand hectares (Danilkin, 1999), but their daily movements normally rarely exceed 1-4 km. Daily home ranges vary from a few to a few tens of hectares and may strongly overlap in the case of small herds of hinds with young. Stags live singly or form all male herds in summer. They establish harems in the rut season where females are found and are more territorial during the rut. According to Kamler et al. (2008), in Poland, the mean annual home-range size of adult males (36.0 km$^2$) was significantly larger than that of adult females (8.4 km$^2$). Seasonal home-range sizes differed significantly between seasons for both males and females, with the largest home ranges being in autumn for males (23.0 km$^2$), and winter for females (7.1 km$^2$). Both sexes were shown to exhibit strong range fidelity. However, home range dynamics in cervids at different spatial and temporal scales, and in different populations, is fairly variable and complex (Danilkin, 1996). It is influenced by various factors, including season, sex, age, landscape characteristics, weather, hunting and predation pressure, hunting management, etc. (Tufto et al., 1996; Bevanda et al., 2010; Rivrud et al., 2010). To date, there have been no special studies into spatial population structure or general ecology of both roe and red deer in the Thrace region.

Movements of ungulates in the Strandzha Mountains involve several patterns, which may have implications for geographic (including transboundary) spread of the disease:
Foot-and-mouth disease in Thrace

a) Dispersion/emigration of juvenile animals, which peaks twice during the year: at the time of the reproduction season (March to May) and the rut period (August to December). One year old animals, particularly males, wander across larger distances as compared to other animals (wild boar, red and roe deer);

b) Snow cover reportedly influences distribution of wild boar, that tend to move away from snow covered tops or slopes of the mountains to the areas with less snow. Thus, during particularly heavy snowfalls, animals usually move from the Demirkoy District in Turkey, where mountains are higher, to the neighbouring districts in Bulgaria (Malko Tarnovo, local hunters);

c) In winter, distribution and predation pressure from the grey wolf Canis lupus reportedly drives wild boar to escape from wolf packs to safer areas (Malko Tarnovo, local hunters);

d) According to information from local villagers (both in Turkey and Bulgaria), availability of food, such as acorns in mast years or maize in the agricultural fields, both of which change seasonally, influences distribution of wild boar and may cause their movements from Bulgaria to Turkey and vice versa. There are more crops available in summer in Turkey, while in certain years, in the beginning of autumn, oak forests in Bulgaria may become more attractive feeding grounds for wild boar;

e) Strong hunting pressure (particularly driven hunts) comes from organised legal hunting activities, and poaching may also drive wild boar away from their normal home ranges. Both in Bulgaria and Turkey, these animals are subject to intensive hunting three times a week from October to the end of February.

This interplay of the natural dynamics of ungulate populations, effect of various ecological factors, variability in space use patterns, as well as uncertainty for the predominant FMDV transmission mechanisms in different species of wildlife (aerosol, direct contacts, virus contamination of the environment, scavenging on carcasses) is further complicated by the hunting management practices widely applied in Bulgarian Strandzha. These involve fencing, provision of supplementary food (most intensive in winter) in order to increase the survival rates and prevent animals from dispersion (wild boar, red and roe deer), and attraction with salt (deer). In winter, feeding locations are normally attended by several groups of animals, thus increasing the chances of pathogen spread in the population. Locations with salt are most frequently attended by deer in the beginning of summer. Additionally, hunters themselves can spread pathogens from one feeding location to another (as well as to the livestock) even without shooting and skinning of infected wild animals.

Wild boar is the most abundant and social ungulate in the Thrace region, which sometimes extensively congregates in the feeding habitats (crop fields, oak forests), and attends supplementary feeding locations or mud baths, which are typically shared by several groups of animals. This might have played a role in the wildlife FMD epidemic in 2010/2011, possibly originally serving as an entry point for the virus through scavenging on contaminated meat products or carcasses. In terms of FMDV transmission risk, the rut period (November-December) is probably most important as it involves intensive social contacts, movements of adult boars searching for females, as well as emigration of young males. This would also be in agreement with the timing of the first and the only detection of FMDV in a wild boar shot in Bulgarian Strandzha in December 2010. FMDV circulation in the populations of both deer species is probably more likely to be better sustained in the period starting with the rut (August in roe deer and September in red deer), and throughout the cold part of the year when animals are more gregarious and access to food is limited (September - April). During the cold part of the year, higher virus survival rates in the environment should increase the basic reproduction rates of the infection and facilitate its maintenance in the multi-species communities of wild ungulates.
APPENDIX D. SYSTEMATIC LITERATURE REVIEW ON FOOT-AND-MOUTH DISEASE IN WILD AND FERAL BOAR AND DEER

1. BACKGROUND AND OBJECTIVES

Due to its methodological rigor and its transparent nature, systematic literature reviews can provide additional value for answering well-formulated questions generated by risk assessment processes. Detailed guidance and examples for the conduct of key steps in the systematic review process are described in the EFSA guidance for systematic reviews (EFSA, 2010). The steps proposed for the systematic review of foot-and-mouth disease in wildlife are described in Figure 43.

**Figure 43:** Seven core steps for performing a systematic literature review (adapted from Cochrane handbook for Systematic Literature Reviews of Interventions; Higgins and Green, 2009).

**Objectives:**

To provide a systematic overview of existing evidence pertinent to the maintenance of foot-and-mouth disease (FMD) in wild boar and deer, in order to use this evidence to assess the significance and role of wild boar and deer species in the epidemiology of foot-and-mouth disease (FMD) in Thrace, as specified in ToR 1 of the mandate for a Scientific Opinion on FMD (see below). Using pre-specified and standardised methods to identify and appraise evidence, documenting the review process, the criteria used in selection of papers, and the interpretation of the results, allowed the review to be critically assessed and, where necessary, repeated or updated.
The terms of reference of the mandate for a Scientific Opinion on FMD, as agreed upon by the European Commission and the Bulgarian Food Safety Authority-Risk Assessment Centre were:

1. The relative significance of and the role played by wild and feral bi-ungulates, notably wild boar and deer species, in the epidemiology of foot-and-mouth disease (FMD) in Thrace (Bulgaria, Turkey and Greece), taking into account the different FMD virus strains circulating in the region.

2. The risk factors and other relevant epidemiological features, in particular for the different FMD virus strains circulating in Thrace (Bulgaria, Turkey and Greece) which must be taken into account for the design of surveillance systems (including estimation of advantages and disadvantages), that could be implemented for the early detection of any FMD virus incursion in the territory of Thrace (Bulgaria, Turkey and Greece).

3. The relevance and significance of epidemiological data and genetic characteristics for the different FMD strains recently isolated in Bulgaria and Turkey, with regards to the hypothesis of single versus multiple introductions into Bulgaria.

**Study question:**

To assess the significance and role of wild and feral boar and deer in the epidemiology of FMD, as specified in ToR 1, the following review question was defined for the systematic review:

“What is the maintenance of foot-and-mouth disease in wild and feral boar and deer populations?”

Sub-questions:

- “What is the duration of the infectious period of FMD in deer/wild or feral boar?”
- “What is the duration of FMDV antibody detection in deer/wild or feral boar?”
- “What is the transmissibility of FMDV between deer/wild or feral boar?”
- “What is the occurrence of FMDV in deer/wild or feral boar?”
- “What is the mortality and morbidity of FMD in deer/wild or feral boar?”

**2. SEARCHING FOR RESEARCH STUDIES AND INFORMATION SOURCES**

**Electronic databases:** CAB Abstracts, PubMed, Web of Science (WoS)

Remark: the search equations differed according to the electronic database (DB) searched. For the search in PubMed and CAB Abstracts, search equations rely on terms extracted from the thesaurus. The search in the WoS also relies on these terms and on additional synonymous terms. No attention was paid to capital letters as the same results were obtained by doing a test search in the three databases with or without capitals.

Additional information sources were screened, such as HAL, Agricola, Agris (FAO), Belgian Veterinary and Agrochemical Research Centre, DEFRA, NAHIS, OIE, USDA-APHIS, DART-Europe E-theses Portal, Index to Theses in France (Fichier Central des Thèses), Index to Theses in Germany, Index to Theses in Great Britain and Ireland.

Unpublished data: unpublished data was provided by the experts of the working group and the sources of these data were documented. The working group experts also provided papers that were not found via the electronic search or screening the websites.
Keywords:
- FMDV, FMD, foot and mouth disease,
- Excretion, shedding, infectious virus,
- Carriers, clinical signs, persistence,
- Transmission, transmissibility,
- Wild boars, feral swine, sus scrofa scrofa, feral pig, deer, capreolus, cervus.
Foot-and-mouth disease in Thrace

Table 14: Search strings:

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</tr>
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<td>Total number of records retrieved: 0 records (1 non-relevant record)</td>
<td>Total number of records retrieved: 50 records (11 reviews excluded from the 61 records initially retrieved)</td>
<td>Total number of records retrieved: 45 records (8 reviews excluded from the 53 records initially retrieved)</td>
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</table>

Total number of summary records retrieved: 293 after automatic suppression of duplicates

3. SELECTING RELEVANT RESEARCH STUDIES AND INFORMATION SOURCES

The records obtained from the literature search, applying the search criteria outlined above, were screened for relevance to the review question. There were two steps, first the titles and abstracts were screened for relevance, excluding records which were obviously irrelevant. Records which appeared relevant or were of unclear relevance, passed to step two, where the abstract was assessed for relevance. Studies not excluded in step two were classified as relevant or of unclear relevance.

The screening for relevance of the records was carried out by two reviewers (KW and SD) in the Endnote reference manager and Distiller. Records that fulfilled one of the two relevance criteria below proceeded to the eligibility screening. Any disagreement between the reviewers was solved through discussion and, if necessary, by a third reviewer (WG member). All the experts of the working group had full access to the review process.

- **Language**: Only records with a title and abstract in English were considered for the relevance screening

**Relevance criteria:**

- Is the paper reporting a primary research study OR surveillance outcomes? (not a review paper)
- Does the paper report a study assessing one of the topics listed below?
  - Infectious period of FMD in wild or feral boar or deer,
  - transmissibility of FMD in wild or feral boar or deer,
  - FMD occurrence in wild or feral boar or deer populations,
  - clinical manifestations of FMD in wild or feral boar or deer,
  - duration of detectable antibodies to FMDV.

4. ASSESSING ELIGIBILITY AND METHODOLOGICAL QUALITY OF RELEVANT STUDIES

The relevant papers were then scrutinised by two reviewers (KW and SD) for their eligibility. The methodological quality and reliability of the included studies needed to be assessed in order to avoid bias.

**Observational studies investigating FMD occurrence in wildlife populations:**

Answering “NO” to one of the first two questions (underlined) led to exclusion of the publication for data extraction. The other questions helped in assessing the quality of the paper (i.e. when a posteriori the results were doubtful).

1. **Is the diagnostic method used in the study (antibody detection or virus detection) mentioned in the paper?**
2. **Is the number of sero- OR RNA- OR virus-positive animals mentioned in the paper?**
3. **Is the sampling procedure applied in the study (active versus passive surveillance, random versus targeted) described in the paper?**
4. **Is the number of animals sampled mentioned in the paper?**
5. **Is the population size mentioned in the paper?**
6. **Is the country, in which the study was carried out, mentioned in the study? For studies which did not cover the entire country: are the country AND the region mentioned in the paper?**
7. **Is the year in which the study was carried out mentioned in the paper?**
8. **Is the studied FMD serotype mentioned in the paper?**
9. **Is it a peer reviewed paper?**
Experimental infection studies to study clinical manifestations, duration of the infectious period, duration of virus shedding or transmissibility of FMD in wild or feral boar or deer:

Answering “NO” to one of the first two questions (underlined) led to exclusion of the publication for data extraction. The other questions helped in the methodological quality assessment of the paper (i.e. when a posteriori the results were doubtful).

1. Was either PCR/virus isolation to detect virus/viral nucleic acid performed OR serological testing to detect antibody carried out?
2. Is the number of positive animals mentioned in the paper?
3. Is the duration of the experiment mentioned in the paper?
4. Is the FMDV serotype that was used for inoculation mentioned in the paper?
5. Are results for the individual animals described?
6. Is it a peer reviewed paper?

5. DATA EXTRACTION FROM INCLUDED STUDIES

Parameters to be extracted from observational studies:

1. What was the type of survey undertaken?
2. Specify the country and region where the samples were taken
3. What animal species was/were involved in the study?
4. What was the population size of the wildlife species under consideration in the region?
5. What FMDV serotype was tested for?
6. Have concurrent FMDV infections been reported in domestic livestock in the region OR when was FMDV infection last reported in domestic livestock in the region?
7. What was the age of the animals?
8. What was the sample size?
9. Which diagnostic test was used? (i.e. serology, PCR, antigen ELISA, virus isolation)?
10. What was the number of positive samples for test 1? (\(\rightarrow\) morbidity)
11. How many of the positive samples were taken from animals with clinical signs for test 1? (\(\rightarrow\) morbidity)

Parameters extracted from experimental studies:

1. Which animal species was/were used in the experiment?
2. What was the FMD serotype/strain inoculated?
3. What was the route of infection?
4. What was the dose used for inoculation?
5. What was the total duration of the experiment (in days)?
6. What was the diagnostic method used? (i.e. serology, PCR, antigen ELISA, virus isolation)
7. How many animals were inoculated?
8. What was the sample site (i.e. blood, oral swab, nasal swab, lymph node, probing sample, air filter)
9. How many days post inoculation was the first positive sample detected?
10. How many days post inoculation was the last positive sample detected?
11. How many infected animals showed clinical signs? (\(\rightarrow\) morbidity)
12. How many animals died following infection? (\(\rightarrow\) mortality)
13. What was the number of in-contact/exposed animals in the experiment (not inoculated)?
14. What animal species was/were exposed in the experiment (not inoculated)?
15. How were the animals exposed in the experiment (if not inoculated)?
16. What is the number of infected in-contact/exposed animals?

6. COLLECTING DATA FROM THE STUDIES INCLUDED AND PRESENTING THE RESULTS
The search in the databases delivered a total of 354 publications, of which 294 publications remained after removal of electronic duplicates, and 270 publications remained after manual removal of duplicates. From these 269 publications, 44 papers were considered relevant (37 observational studies, and 8 experimental studies). From these papers, 5 were considered eligible as experimental studies, and 11 were considered eligible as observational studies. The data extracted from these publications is listed below.

### 6.1 Experimental infections

**Capreolus capreolus**

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**Cervus spp. (Cervus elaphus, Cervus elaphus nelsoni, Cervus nippon)**

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Route of infection: ID=intradermal inoculation, DC=direct contact, IC=indirect contact
Clinical signs: none; mild=fewer or/and minor lesions (i.e. on the inoculation site only); severe=fewer and lesions on sites other than inoculation sites and/or lameness
ns=not specified

Terminated: experiment terminated (last detection is the minimum duration of antibody or virus detection)
Route of infection: ID=intradermal inoculation, DC=direct contact.
Clinical signs: none; mild=fever or/and minor lesions (i.e. on the inoculation site only); Severe=fever and lesions on sites other than inoculation sites and/or lameness
No= not specified

### Dama dama (fallow deer)

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</table>
### Foot-and-mouth disease in Thrace

| Route of infection: ID= intra-dermal inoculation, DC = direct contact, IC = indirect contact |
| Clinical signs: none; mild = fever or and minor lesions (i.e. on the inoculation site only); severe = fever and lesions on sites other than inoculation sites and/or lameness |
| ns = not specified |

| Virus isolation | Blood | O : 1 BFS | ID | 1 | 1 | No | None | Gibbs, 1974 |
| Virus isolation | Blood | O : 1 BFS | ID | 1 | 1 | No | None | Forman et al., 1974; Forman and Gibbs, 1974 |
| Virus isolation | Blood | O : 1 BFS | ID | 1 | 1 | No | None | Forman et al., 1974; Forman and Gibbs, 1974 |
| Virus isolation | Blood | O : 1 BFS | ID | 1 | 1 | No | None | Forman et al., 1974; Forman and Gibbs, 1974 |
| Virus isolation | Blood | O : 1 BFS | ID | 1 | 1 | No | None | Forman et al., 1974; Forman and Gibbs, 1974 |
| Virus isolation | Blood | C : Noville | ID | 2 | 3 | No | Severe | Forman et al., 1974; Forman and Gibbs, 1974 |
| Virus isolation | Blood | C : Noville | ID | 2 | 3 | No | Mild | Forman et al., 1974; Forman and Gibbs, 1974 |
| Virus isolation | Blood | C : Noville | ID | 1 | 3 | No | Severe | Forman et al., 1974; Forman and Gibbs, 1974 |
| Virus isolation | Blood | C : Noville | IC | 1 | 4 | No | ns | Forman et al., 1974; Forman and Gibbs, 1974 |
| Virus isolation | Blood | C : Noville | DC: deer | 5 | 5 | No | None | Forman et al., 1974; Forman and Gibbs, 1974 |
| Virus isolation | Blood | C : Noville | DC: deer | 3 | 7 | No | Mild | Forman et al., 1974; Forman and Gibbs, 1974 |
| Antibody | ELISA AB | Blood | C : Noville | ID | 4 | 21 | Yes | ns | Forman et al., 1974; Forman and Gibbs, 1974 |
| Antibody | ELISA AB | Blood | C : Noville | DC | 7 | 21 | Yes | ns | Forman et al., 1974; Forman and Gibbs, 1974 |
Other deer (*Odocoileus virginianus* and *Muntiacus muntjac*)

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Route of infection: ID=intradermal inoculation, DC=direct contact

Clinical signs: mild=fever or/and minor lesions (i.e. on the inoculation site only); severe=fever and lesions on sites other than inoculation sites and/or lameness

ns= not specified
## Sus scrofa scrofa

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<td>DC: domestic swine</td>
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<td>6</td>
<td>No</td>
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<td>Mohamed et al., 2011</td>
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<td>No</td>
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<td>Mohamed et al., 2011</td>
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<td>No</td>
<td>Severe</td>
<td>Mohamed et al., 2011</td>
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<td>Mohamed et al., 2011</td>
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<td>Antibodies</td>
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<td>28</td>
<td>Yes</td>
<td>Mild</td>
<td>Breithaupt et al., in press</td>
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Route of infection: ID=intradermal inoculation, DC=direct contact, IC=indirect contact
Clinical signs: none; mild=fever or/and minor lesions (i.e. on the inoculation site only); severe=fever and lesions on sites other than inoculation sites and/or lameness
ns=not specified

*Mohamed et al., 2011: feral swine used for the experiment were predominantly of the *Sus scrofa scrofa* heritage
### 6.2 Observational studies

<table>
<thead>
<tr>
<th>References</th>
<th>Period</th>
<th>Country</th>
<th>FMD occurrence in area</th>
<th>Species</th>
<th>Serotype tested</th>
<th>Sample site</th>
<th>Diagnostic test</th>
<th>Detection</th>
<th>Sensitivity-Specificity test</th>
<th>Number samples</th>
<th>Positive samples</th>
<th>Conclusions in publication</th>
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<tr>
<td>Araujo et al., 2010</td>
<td>1998</td>
<td>Brazil</td>
<td>Outbreaks occurred in the area during the time of sampling</td>
<td>Blastocerus dichotomus (marsh deer)</td>
<td>A, O and C</td>
<td>Blood</td>
<td>ELISA AB</td>
<td>Antibody</td>
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<td>108</td>
<td>19 (low log*10 titres in the range of 1.0-1.5 for A 24 Cruziero)</td>
<td>Marsh deer are unlikely to carry FMD</td>
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<td>Araujo et al., 2010</td>
<td>1998</td>
<td>Brazil</td>
<td>Outbreaks occurred in the area during the time of sampling</td>
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<td>A : 24 Cuzeiro</td>
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<td>Frolich et al., 2006</td>
<td>2000-2002</td>
<td>Germany</td>
<td>21 March-22 April 2001 in The Netherlands</td>
<td>Capreolus capreolus (roe deer), Cervus elaphus (red deer), Dama dama (fallow deer)</td>
<td>Capreolus capreolus (roe deer)</td>
<td>A</td>
<td>Blood</td>
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<td>Mouchantat, 2005 ; Mouchantat et al., 2003, 2005 ; Mouchantat, 2001-2002</td>
<td>2001-2002</td>
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<td>Capreolus capreolus (roe deer)</td>
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<td>Blood</td>
<td>Liquid phase blocking Elisa + virus neutralisation</td>
<td>Antibody</td>
<td>SP 95.6 for LBPA, and 88.3 for SPCE</td>
<td>43</td>
<td>2 positive for LPBE, but negative after VNT confirmation</td>
<td>The results suggest that FMDV was not transmitted to free-ranging roe deer in the investigation area</td>
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<td>Deem et al., 2004</td>
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<td>Bolivia</td>
<td>Unknown exposure of population to FMDV</td>
<td>Mazama gouazoubira (brocket deer)</td>
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<td>Blood</td>
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<td>17</td>
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<td>Larger sample size needed</td>
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<td>Uhart et al., 1995-1998</td>
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<td>Elbers et al., 2001</td>
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