

## ORIGINAL ARTICLE

# Assessing the potential spread and maintenance of foot-and-mouth disease virus infection in wild ungulates: general principles and application to a specific scenario in Thrace

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## Keywords:

foot-and-mouth disease; wild boar; systematic literature review; epidemiological model

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## Summary

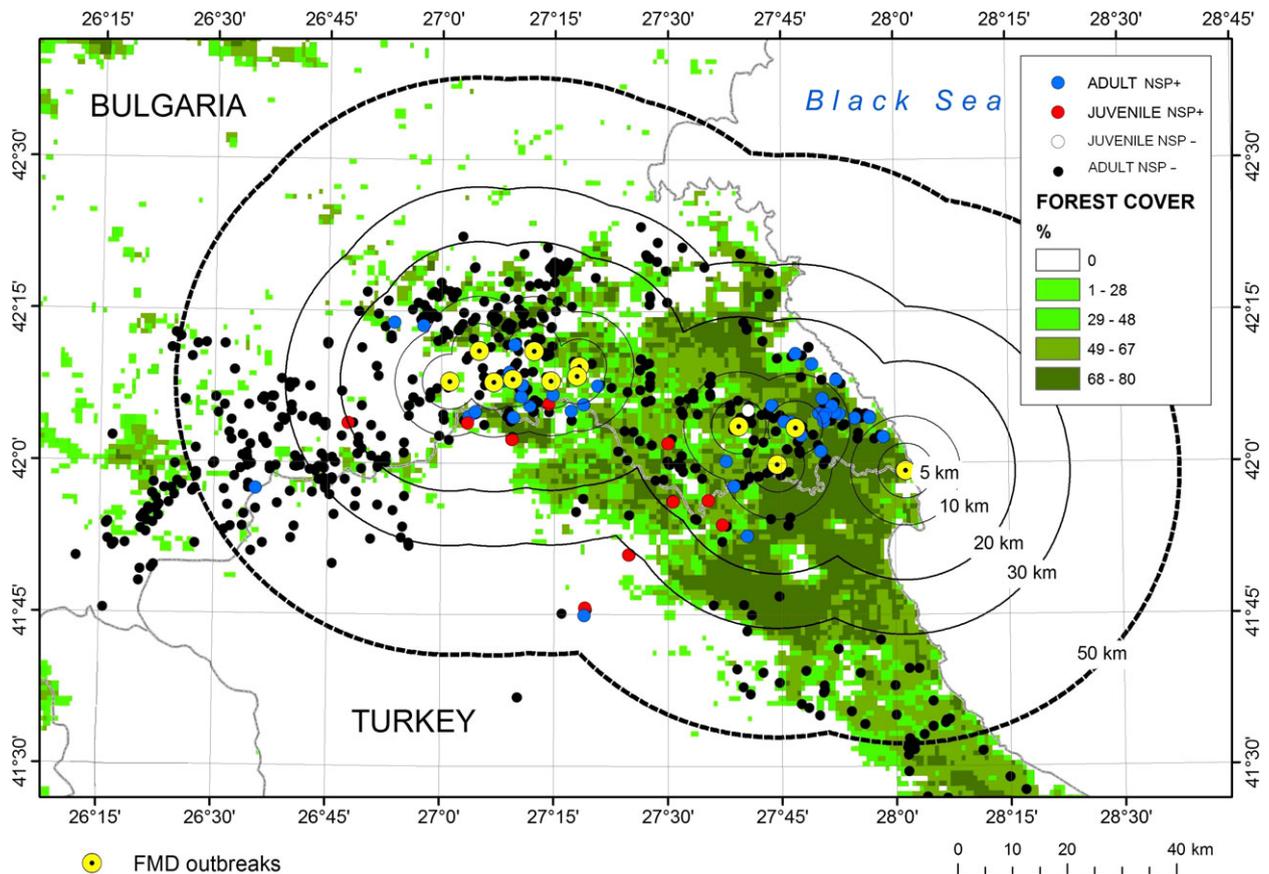
Foot-and-mouth disease (FMD), due to infection with serotype O virus, occurred in wild boar and within eleven outbreaks in domestic livestock in the south-east of Bulgaria, Thrace region, in 2011. Hence, the issue of the potential for the spread and maintenance of FMD virus (FMDV) infection in a population of wild ungulates became important. This assessment focused on the spread and maintenance of FMDV infection within a hypothetical wild boar and deer population in an environment, which is characterized by a climate transitional between Mediterranean and continental and variable wildlife population densities. The assessment was based on three aspects: (i) a systematic review of the literature focusing on experimental infection studies to identify the parameters describing the duration of FMDV infection in deer and wild boar, as well as observational studies assessing the occurrence of FMDV infection in wild deer and wild boar populations, (ii) prevalence survey data of wild boar and deer in Bulgaria and Turkey and (iii) an epidemiological model, simulating the host-to-host spread of FMDV infections. It is concluded, based on all three aspects, that the wildlife population in Thrace, and so wildlife populations in similar ecological settings, are probably not able to maintain FMD in the long term in the absence of FMDV infection in the domestic host population. However, limited spread of FMDV infection in time and space in the wildlife populations can occur. If there is a continued cross-over of FMDV between domestic and wildlife populations or a higher population density, virus circulation may be prolonged.

## Introduction

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 surrounded 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.

In January 2011, Bulgaria notified OIE of a case of foot-and-mouth disease (FMD), due to serotype O virus, in a wild boar in the region of Burgas (Fig. 1). This first reported case was followed by 11 outbreaks of FMD in



**Fig. 1.** Locations of follow-up surveillance samples of wild boar, collected between February 2011 and January 2012, and results of serological tests within the 50 km buffer zones around outbreaks in livestock (yellow circles).

domestic animals kept in that mountainous forest area of the Strandzha Mountains, which stretches into Turkish Thrace and is populated by susceptible wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*).

Two series of outbreaks were observed in domestic animals (Fig. 1). The first series of outbreaks (three in total) occurred in January 2011 in the south-east of the Burgas region in the municipalities of Tsarevo and Malko Tarnovo. The second series of outbreaks (eight in total) occurred in the south-west of the Burgas region in the Malko Tarnovo and the Sredets municipalities in March and April 2011. Detailed epidemiological investigations of the FMD outbreaks that occurred in 2011 in Bulgaria have been conducted by national authorities and international institutions (EUFMD, 2011; EU Veterinary Expert Team Mission, 2011; FAO/OIE/EC, 2011a,b; SCoFCAH, 2011).

In the Strandzha Mountains, roe deer, red deer and wild boar are largely sedentary. They occupy relatively stable home ranges of seasonally variable size (Danilkin, 1999, 2002) that strongly overlap in space at the inter- and intraspecies level, providing the chance for direct or indi-

rect contact and the potential spread of pathogens, such as FMD virus (FMDV) (Cowled and Garner, 2008), particularly where supplementary feeding is provided by hunters (common in Bulgaria). Population data for wild ungulates were only available for the Bulgarian part of the Strandzha Mountains (Bulgarian Food Safety Agency, unpublished data). According to the spring 2011 population survey in the 10 concerned municipalities, wild boar is the most abundant species (population estimate 5059 heads, with the average density varying between 0.43 and 2.14 heads per km<sup>2</sup> per municipality) followed by roe deer (population estimate 3931 heads and average density varying between 0.57 and 1.48 heads per km<sup>2</sup> per municipality) and red deer (population estimate 1657 heads and average density varying from 0.24 to 0.70 heads per km<sup>2</sup> per municipality).

The epidemiological characteristics of sylvatic FMDV infections in a European context have not been described in detail previously. Therefore, a suspected wildlife FMD epidemic in the south-east of Bulgaria was of theoretical and practical interest for disease managers, particularly for the affected and neighbouring regions and countries. The maintenance of FMD by continued circulation in wildlife,

that is, without spillover, back and forth, from infected livestock, depends on a variety of factors such as the size of the population and the local density of susceptible animals, demographics and social structure, the proportion of individuals that recover from infection and the potential occurrence of carrier animals (Kramer-Schadt et al., 2007; Ruiz-Fons et al., 2008). In this context, very little data regarding FMD outbreaks in wild boar exist, which could be used as guidance. However, a number of publications deal with theoretical considerations regarding the propagation, persistence and control of an outbreak of FMD in feral pig (wild boar) populations of Australia or Texas (Pech and Hone, 1988; Pech and McIlroy, 1990; Pech et al., 1992; Dexter, 2003; Doran and Laffan, 2005; Ward et al., 2007, 2009). European (*Capreolus capreolus*) and Siberian (*C. pygargus*) roe deer are also historically known to become infected with FMDV and develop clinical disease both in the wild, as well as by experimental infection (Thomson et al., 2003).

Circulation of FMDV in wildlife may pose a threat for introduction of the virus into domestic animal populations in the region. It is therefore important for risk managers to know the role of wildlife in the epidemiology of FMD and the risk of wildlife populations transmitting the virus to domestic livestock and/or maintaining FMDV infection. When FMD was detected in Thrace, it was unclear whether spread and maintenance of the FMDV infection in the wildlife population was likely to occur. The findings from a meta-study combining a literature review, data provided through a prevalence survey and epidemiological modelling are presented regarding the likelihood of spread and maintenance of the FMDV infection in a population of wild ungulates in Thrace.

## Methods and Materials

The assessment of the potential spread and maintenance of FMDV infection in the wild boar and deer population was based on three aspects: (i) a systematic literature review (SR), (ii) the results of wildlife prevalence surveys carried out in Thrace between February 2011 and January 2012 and (iii) an epidemiological model.

### Systematic review of literature

Systematic reviews (SRs) use an evidence synthesis approach that provides robust and transparent answers to clearly formulated questions. Originally developed for use in clinical practice, SRs have wider applicability, including food and feed safety risk assessment (O'Connor et al., 2012). EFSA has developed a framework for identifying the different types of question suitable for SRs within broad risk assessments (EFSA, 2010) and also implemented their

use in animal health risk assessments. The objective of this SR was to provide a comprehensive overview of existing evidence pertinent to FMDV infections in wild boar and deer under both field and experimental conditions. The detailed review protocol can be found in EFSA, (2012). In summary, the procedure involved screening of publications in the electronic databases of CAB Abstracts, PubMed and Web of Science. The search equations differed according to the electronic databases (Table 1). Information sources, such as HAL, Agricola, Agris (FAO), 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, were manually screened. The search took place in October 2011 and retrieved 293 publications. Exclusions of publications were based on the absence of the description of the diagnostic tests carried out to detect FMDV or the absence of the description of the number of positive animals for both the experimental infection studies and the observational studies. In total, data were extracted from seven experimental studies and 12 observational studies of FMD in wild boar and deer species.

### Prevalence survey

Data collected by two serosurveys across the Bulgarian–Turkish border in Thrace have been combined (Khomenko et al., 2011; EFSA, 2012; Alexandrov et al., 2013). Between February 2011 and January 2012, blood samples and tissue samples (pharyngeal area, skin with lesions, lymph nodes and vesicular fluids, where available) were collected from wild ungulates for serological and/or virological examination (PCR and /or virus isolation). In total, 1077 samples from wild boar, roe deer and red deer were tested for current or earlier infection by FMDV. The serosurvey was designed to detect 5% and 10% seroprevalence in wild boar and deer populations respectively with 95% confidence (Alexandrov et al., 2013). The sampled animals were either hunted (Bulgaria and Turkey) or trapped (only Bulgaria) and the sex, age and geographical coordinates of the sample collection site recorded (Khomenko et al., 2011; Alexandrov et al., 2013).

### Epidemiological model

An ecological disease spread model was constructed to estimate the likelihood of maintenance of FMDV infection in a simulation area which was assumed to be populated by wildlife only (Lange, 2012). The likelihood of maintenance was estimated by the survival time of FMDV introductions in the model, that is, at each time step the cumulated proportion of simulation runs with circulating FMDV infection was recorded. The modelling was intentionally

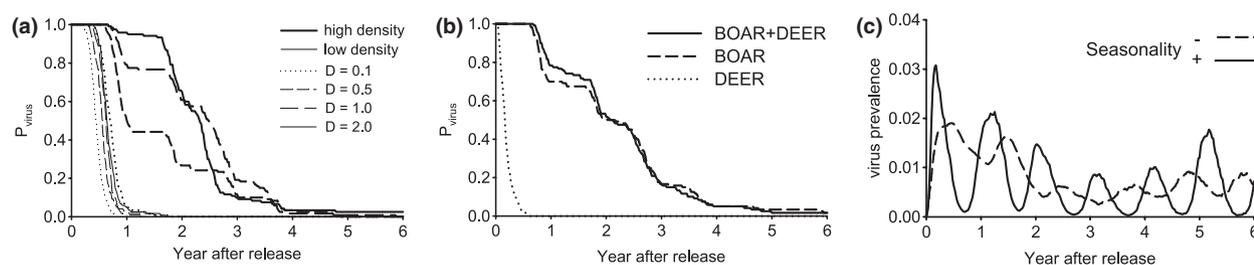
**Table 1.** Search strings for systematic literature review

Database 1: CAB (WoK)	Database 2: CCC (WoK)	Database 3: FSTA (WoK)	Database 4: PubMed (NLM)	Database 5: Web of Science (WoK)
<p>Search strategy</p> <p>Topic=('Foot-and-mouth disease' OR 'Foot and mouth disease' OR 'FMD' OR 'Foot-and-mouth disease virus' OR 'Foot and mouth disease virus' OR 'FMD virus' OR 'FMDV') AND Topic=((Boar<i>ORgilt</i> OR hog<i>ORpig</i> OR piglet<i>ORporcineORSow</i> OR swine<i>ORdeer</i> OR 'sus scrofa' OR cervus OR capreolus) AND (wild OR feral)) NOT Topic=(guinea-pig\$)</p> <p>Timespan=All Years.</p> <p>Databases=ABES, CM, LS.</p> <p>Lemmatization=On</p>	<p>Search strategy</p> <p>Topic=('Foot-and-mouth disease' OR 'Foot and mouth disease' OR 'FMD' OR 'Foot-and-mouth disease virus' OR 'Foot and mouth disease virus' OR 'FMD virus' OR 'FMDV') AND Topic=((Boar<i>ORgilt</i> OR hog<i>ORpig</i> OR piglet<i>ORporcineORSow</i> OR swine<i>ORdeer</i> OR 'sus scrofa' OR cervus OR capreolus) AND (wild OR feral)) NOT Topic=(guinea-pig\$)</p> <p>Timespan=All Years.</p> <p>Databases=ABES, CM, LS.</p> <p>Lemmatization=On</p>	<p>Search strategy</p> <p>Topic=('Foot-and-mouth disease' OR 'Foot and mouth disease' OR 'FMD' OR 'Foot-and-mouth disease virus' OR 'Foot and mouth disease virus' OR 'FMD virus' OR 'FMDV') AND Topic=((Boar<i>ORgilt</i> OR hog<i>ORpig</i> OR piglet<i>ORporcineORSow</i> OR swine<i>ORdeer</i> OR 'sus scrofa' OR cervus OR capreolus) AND (wild OR feral)) NOT Topic=(guinea-pig\$)</p> <p>Timespan=All Years.</p> <p>Databases=ABES, CM, LS.</p> <p>Lemmatization=On</p>	<p>Search strategy</p> <p>('Foot-and-Mouth Disease'[MeSH Terms] OR 'Foot-and-Mouth Disease virus'[MeSH Terms] OR 'Foot-and-Mouth Disease'[All Fields] OR 'Foot-and-Mouth Disease virus'[All Fields]) AND ('swine'[MeSH Terms] OR 'swine'[All Fields] OR 'sus scrofa'[MeSH Terms] OR 'sus scrofa'[All Fields] OR 'deer'[MeSH Terms] OR ('deer'[MeSH Terms] OR 'deer'[All Fields])) AND ('feral'[Supplementary Concept] OR 'feral'[All Fields] OR wild[All Fields])</p>	<p>Search strategy</p> <p>Topic=('Foot-and-mouth disease' OR 'Foot and mouth disease' OR 'FMD' OR 'Foot-and-mouth disease virus' OR 'Foot and mouth disease virus' OR 'FMD virus' OR 'FMDV') AND Topic=((Boar<i>ORgilt</i> OR hog<i>ORpig</i> OR piglet<i>ORporcineORSow</i> OR swine<i>ORdeer</i> OR 'sus scrofa' OR cervus OR capreolus) AND (wild OR feral)) NOT Topic=(guinea-pig\$)</p> <p>Timespan=All Years.</p> <p>Databases=ABES, CM, LS.</p> <p>Lemmatization=On</p>
<p>Total number of records retrieved: 230 records (no 'review' tagged by CAB)</p>	<p>Total number of records retrieved: 48 records (7 reviews and 1 editorial material excluded from the 56 records initially retrieved)</p>	<p>Total number of records retrieved: 0 (1 non-relevant record)</p>	<p>Total number of records retrieved: 50 (11 reviews excluded from the 61 records initially retrieved)</p>	<p>Total number of records retrieved: 45 records (8 reviews excluded from the 53 records initially retrieved)</p>
<p>Limits: no limit applied for this search</p> <p>Date of the search: 4 October 2011</p> <p>Date span of the search: 1 day</p>		<p>Justification for choosing the sources: major sources in this field</p> <p>Total number of summary records retrieved: 293 after automatic suppression of duplicates</p>		

performed in an environment assumed devoid of livestock. The model is documented in detail with reasoned biological assumptions, in Appendix S1. The simulation area of the model was populated by individuals according to the range of densities of deer and wild boar observed in Thrace. The model represents direct within-group contacts, indirect between-group and between-species contacts as well as demography based on ecological literature (for references, see Appendix S1). Foot-and-mouth disease virus infections were assumed to be spread through direct host-to-host transmission, as well as indirect transmission via the environment. Both transmission mechanisms are modelled stochastically (Appendix S1, section 1.3.3.2). Direct transmission reflects the chance to become infected from the number of infectious hosts accessible from the neighbourhood. Indirect transmission reflects the chance to become infected from exposure to FMDV in the environment. The mechanism was motivated by the reported long-term survival of the virus in the environment as plausible link between the different species. Reported survival outside the host ranges from hours to months (see, e.g. Cottral, 1969; Ferguson et al., 2001), depending on the medium in

which it is imbedded and other environmental factors such as temperature, humidity and pH (Cottral, 1969; Alexandersen et al., 2003). The exposure rate  $D$  is a combined measure of virus excretion and uptake from the environment.  $D$  is the dose, a host is assumed to ingest if the virus load of its home range cell would equal the amount excreted by one infectious boar per day. The exposure of susceptible animals results from all infectious boars excreting to the local neighbourhood and subsequent decay of infectious material over time in the environment. The exposure is the input to a dose-response relationship (Appendix S1, Fig. 2) providing the infection probability for susceptible hosts becoming infected during the time-step at their location. Host demography was based on the reproduction, mortality and dispersal behaviour on the individual level. The host ecology, its population density and its reproductive season were calibrated to the wild boar and deer population characteristics reported in Thrace.

The details and parameter logic concerning the wild boar ecology were described previously (Alban et al., 2005; Fernandez et al., 2006; Kramer-Schadt et al., 2009; Lange et al., 2012a,b). The details and parameters concerning the



**Fig. 2.** Output of the epidemiological model. (a) Survival probability of the virus in the model population depending on time since first infection for the two animal density scenarios and the range of exposure rates to virus in the environment; (b) Survival probability of the virus in the model assuming both species (BOAR+DEER), boar only (BOAR) and deer only (DEER) as competent hosts at high host density and exposure rate  $D = 1.0$ ; (c) Virus prevalence in wild boar for different modelling of inactivation of virus in the environment. Dashed line: constant inactivation over time assuming annual mean temperature; continuous line: temperature-dependent (seasonal) inactivation over time using the scenario of wild boar only, high host density and exposure rate  $D = 1.0$ .

deer ecology were based on the literature of territoriality and use of space of this host species complex.

## Results

### Systematic review of literature

#### Observational studies

The detailed results of the SR of observational studies investigating FMDV occurrence in wild boar and deer populations can be found in Appendix S2. The publications indicated that FMDV was previously not observed to be an important pathogen of wild ungulates. During and after the 2001 FMD epidemic in Europe, the roe deer tested in the vicinity of outbreaks were found to be seronegative, which has been explained by relatively rare contacts between cervids and livestock in the affected areas (Elbers et al., 2001, 2003; Mouchantat et al., 2003, 2005; Mouchantat, 2005; Frölich et al., 2006).

#### Experimental infections

Only two relevant and well-documented FMDV infection experiments of 'wild boar' were identified through the SR: one conducted in the USA with feral swine of apparently predominantly Eurasian wild boar heritage (Mohamed et al., 2011) and one conducted in Germany with wild boar (Breithaupt et al., 2012). Other reports provided sparse details in terms of the role of wild boar in the epidemiology of FMD (e.g. Ercegovac et al., 1968).

The available experimental data on FMDV infections in wild and feral swine are summarized in Appendix S3. The FMDV infection experiment in wild boar (Breithaupt et al., 2012) reported that after an incubation period of 2–4 days, first clinical signs were noticed. The severity of the clinical course was rather mild, compared with disease in domestic pigs. Although severe foot lesions were seen, the mobility of the animals did not seem to be impaired substantially. Viraemia started by 2 days after exposure and lasted up to

1 week. Virus shedding also began during the incubation period and lasted up to 9 days. Viral RNA could be detected in tissue samples and oro-pharyngeal fluids constantly or intermittently for rather longer, lasting for a period of 2 weeks. Antibodies could be detected after the first week of infection and were shown to remain for at least 28 days, when the experiment was terminated (Breithaupt et al., 2012).

Experimental studies conducted with different species of deer indicated that the pathogenesis and clinical course is similar to that seen in domestic ruminants. Most fallow and red deer showed less severe clinical signs compared with roe deer and other deer species (see Appendix S3). 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, that is, virus positive for more than 28 days, while in roe deer persistence could not be proven (Forman and Gibbs, 1974; Forman et al., 1974; Gibbs et al., 1975).

### Prevalence survey

There was no FMDV detected in the samples obtained from the prevalence survey in wildlife, and seropositive animals were found only amongst wild boar (7.8%,  $n = 1004$ , 95% CI: 6.2–9.6%) and roe deer (4.4%,  $n = 68$ , 95% CI: 0.9–12.4%). This difference between species was not significant (Fisher's exact,  $P = 0.79$ ). Most positive detections were found in the cross-border area near to the FMD outbreaks in livestock, although some were found further from the border in Turkish Thrace (Fig. 1). Calculation of prevalence in wild boar by distance from livestock outbreaks showed that the highest prevalence (17.9%) was found at a distance of 6–10 km. All wild boar further than the 50 km from FMD outbreaks tested negative (Fig. 1). Adult wild boar had a significantly higher seroprevalence (9.1%,  $n = 628$ , CI: 6.9–11.6%) than juveniles (5.6%,  $n = 358$ ,

95% CI: 3.4–8.5%). No significant difference in seroprevalence between sexes was found either in adults or in juveniles ( $n = 820$ ).

The overall seroprevalence for all species sampled 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;  $P = 0.004$ ).

#### *Epidemiological model*

In simulations applying the highest wild ungulate density estimate together with standard parameterization of exposure to virus in the environment, the spatial spread of FMDV infection covered the whole model population (Fig. 2a; exposure rate  $D = 1.0$ , that is, susceptible hosts ingest one unit, if the virus load of its home range cell was equal to the amount excreted by one infectious boar per day, that is,  $10^6$  TCID<sub>50</sub>). However, the time of continued circulation was sensitive to the level of exposure to virus present in the environment. Just by halving the parameter (Fig. 2a; exposure rate  $D = 0.5$ ), in every second simulation, the infection circulated for less than 1 year and covered only a limited part of the population. Simulations assuming one-tenth of the standard exposure rate did not spread at all (Fig. 2a, exposure rate  $D = 0.1$ ) and would not be consistent with the spatial distribution observed in the serosurvey region. Simulations of the low density estimate did not allow establishment of spatial spread and infection faded out within 1 year of simulation, independent of the assumed exposure rate to virus in the environment (even if the parameter was doubled, Fig. 2a; exposure rate  $D = 2.0$ ).

In the output of the simulation model, deer played only a marginal role in maintaining FMDV infection following incursion (Figure 2b). This seemed reasonable, because ruminant hosts in the model were considered to shed much less infectious material into the model environment than wild boar [i.e. wild boar shed  $10^6$  TCID<sub>50</sub> per day, compared with a daily excretion of deer of  $10^4$  TCID<sub>50</sub> (Sørensen et al. 2000; Garner et al. 2006; Hess et al. 2008)].

In comparison with the simulations assuming average temperature, that is, constant inactivation of virus in the environment, the temperature-dependent model of inactivation resulted in a substantial increase in virus fade-out in the summer months due to a reduced number of new infections occurring by indirect transmission (Fig. 2c).

#### **Discussion**

From the literature data, it can be concluded that transmission from wild boar or deer to domestic animals, and vice versa, can occur under experimental conditions; however, the incidence of this process under natural conditions

remains unknown. As the mobility of wild boar may not be substantially impaired by the disease, as shown in the wild boar experiment, and because these animals show an early onset of viral excretion, there is a potential role for wild boar in the spread of FMDV infection, particularly in areas with a high wild boar density and during the cold season when environmental survival of the virus is higher. Some deer species, such as fallow deer, may become virus 'carriers' (defined as virus positive for more than 28 days). However, there is no experimental evidence for transmission of the virus from carrier animals to other susceptible animals (except for African buffalo; Alexandersen et al., 2003). Thus, carriers are not expected to have any major role in spread and maintenance of the infection in wildlife.

Recently, Moniwa et al. (2012) studied the clinical signs and the level and duration of virus replication in white-tailed deer (*Odocoileus virginianus*) inoculated with FMDV O UKG. The study was published after this SR was finalized, and thus, the outcomes were not included in the summary tables in Appendix S3. The results of the study, however, were consistent with the reports that were retrieved through the SR (Forman et al., 1974; Forman and Gibbs, 1974; Gibbs et al., 1975) studying experimental FMDV infections in deer species. The white-tailed deer were severely affected by the virus. The deer inoculated experimentally in this study did not appear to become carriers, because virus was not detected beyond 28 dpi.

Observational studies in wildlife populations did not reveal evidence for spread or maintenance of infection within wildlife in Europe during earlier FMD outbreaks. Seropositive wildlife was observed in spatial or temporal connection with concurrent FMD outbreaks in livestock. This is considered to be the result of transmission from domestic animals (Sludskiy, 1956; Goreglyad, 1972; Danilkin, 2002). However, there have been no reports of observational studies that investigated wild ungulates independently of FMD outbreaks in livestock. Therefore, data from the systematic review were inconclusive to strictly exclude maintenance of FMDV infection in wild ungulate populations.

A prevalence survey in the wild deer and boar populations in Thrace carried out until 1 year after the first detection of FMDV infection in Bulgaria in 2011 also suggested that circulation of FMDV infection in wildlife was spatially correlated with the outbreaks in livestock. There was no evidence of disease occurrence in wildlife anywhere else in Turkish Thrace or further north in Bulgaria. Nonetheless, in the absence of precise information on spatio-temporal association between seropositive samples taken from wildlife and outbreaks in domestic animals, it will be difficult to identify whether the seropositive wildlife occurred only as occasional disease spillover events from the associated outbreaks in livestock or the other way round. However, the

decreasing seroprevalence during the period of the prevalence survey together with the clustered spatial distribution of positive wild boar and deer, as well as the lack of further outbreaks in domestic animals, indicated that the wildlife population was most likely not able to sustain the virus circulation. Based on the higher seroprevalence found in adult wild boar, it seems plausible that the FMD event in wildlife started before the farrowing period (February–March) and died out shortly after (April–May) without involvement of the new generation of piglets (Alexandrov et al., 2013).

The prevalence survey retrospectively indicated that the spread of FMDV infections in Thracian wildlife was spatially restricted. However, the geographical distribution of the data from the serosurvey in Thrace demonstrated that, once introduced, FMDV infection can, to a limited extent, develop into an epidemic wave within susceptible wild ungulates.

In accordance with these observations, the epidemiological model indicated that although limited spread of FMDV infection in time and space may occur, FMD is unlikely to be maintained within the wild boar and/or deer host population in Thrace alone. The model assumptions that were most influential on the extent and duration of spatial spread and thus prevented the long-term maintenance of FMDV infection in the wildlife population were the high summer temperature (i.e. with low virus survival in the environment) and the limited population density (i.e. with low chance of spread into or within low density areas), both of these features applied to Thrace. For the ecological conditions in eastern Australia, the model of Pech and Hone (1988) concluded that, assuming homogeneous distribution, a density of more than two wild boar per km<sup>2</sup> would be required to allow an FMD epidemic to establish in the wild boar population, which was reached only in the high-density part of the survey region in Thrace (EFSA, 2012).

Ward et al. (2007, 2009) investigated how the size and distribution of wild deer and feral pig herds at the location of FMDV incursions might affect the initial size of an outbreak, using spatially referenced data from southern Texas, USA. Their model's objective was thus different from the one reported here. They concluded that lack of continuity within the feral pig herd distribution across the landscape makes predicting disease spread more difficult than for deer, which are more homogeneously distributed. When assessing the potential of wild and feral animal species at a locality to act as maintenance hosts of FMDV, estimates of the population size and distribution might serve as a useful indicator of potential outbreaks in some circumstances. In this study, the latter has been adjusted to the characteristics of the population studied.

Additionally, hunting management interventions (such as supplementary feeding, hunting pressure) or ecological factors (snow, predator pressure) may also have supported

both the limited spatial spread, as seen in the serosurvey, and the fade-out of the infection in the survey area. Unlike in most of Western Europe, predators such as wolves (*Canis lupus*) and golden jackals (*Canis aureus*) occur commonly all over the Thrace region.

In a wildlife population different from that of Thrace, where the animal density is much higher, ecological settings are different (e.g. significant predation is absent) and the climatic conditions are suitable for the virus survival in the environment, FMD might be maintained for a longer time, as indicated by the model. Depending on the spatial extent of the high-density wildlife population, transient FMDV circulation may last for several years (e.g. up to three in the high-density scenarios simulated for the 100 km region in the model). The presence of FMDV infection for three or more years in wildlife might be considered as a huge threat for the domestic ungulates, even if the disease eventually will fade out from the wildlife population.

The outcome of the model is also influenced by the input parameters concerning the properties of the virus and these do vary, to some extent, between different strains; for example, some strains of FMDV replicate exclusively within pigs (so-called porcophilic strains) and not in cattle. As FMDV rapidly evolves, essentially all outbreaks are caused by different virus variants and thus the particular features of each strain are necessarily unpredictable in terms of its virulence within the particular host species present within a region and its ability to be transmitted from one host to another. Hence, the output of the model should be considered as a useful guide and cannot be a statement of fact.

The applied epidemiological modelling was explicitly addressing whether wildlife alone can maintain the infection. Therefore, any interference of wildlife with livestock or virus transported by humans was excluded, which rarely might be the case in reality. Obviously, if there is a continued cross-over of FMDV between domestic and wildlife population, then circulation may be prolonged. Human actions, such as hunting of infected wild animals (as we observed with the first livestock outbreak in south-east Bulgaria) or attending wildlife feeding locations by hunters involved in livestock breeding, have the potential to facilitate this cross-over even in the absence of an apparent way for wild and domestic animals to interact.

## Conclusions

Epidemiological observations, published literature and epidemiological modelling support the conclusion that the wildlife population in Thrace, and so wildlife populations in similar ecological settings, are not able to maintain FMD in the long term. However, based on our study and currently very limited knowledge of the epidemiology of FMD in wildlife, the potential threat of longer and more wide-

spread FMD epidemics involving the most abundant European wild ungulates cannot be ruled out for other parts of the continent. This might be the case in some Western European countries that have large continuous populations and high densities of wild boar, roe and red deer (Putman et al., 2011) in combination with a climate with lower temperatures favouring FMDV survival in the environment and hence transmission.

In the model discussed here, the domestic species were explicitly excluded because it was considered that when outbreaks of disease occur within domestic animals that there will be human intervention, for example the culling of the animals on the affected premises. Such an event is most likely going to increase infection within the wildlife population rather than suppressing it. The model here has focused on how FMD can be maintained in a region without the involvement of deliberate human intervention, that is, when it is essentially an unnoticed event. Under such circumstances, if the wildlife is able to maintain and spread the virus over large geographical areas, then potentially 'spill-over' events into the domestic animals could occur at widely dispersed sites over a significant period of time. However, the outcome of the model, for the environment considered, is that the wildlife in Thrace is only able to maintain the virus and disperse it for a limited period of time.

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### Conflict of interest

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### References

Alban, L., M. M. Andersen, T. Asferg, A. Boklund, N. Fernández, S. G. Goldbach, M. Greiner, A. Højgaard, S. Kramer-Schadt, A. Stockmarr, H.-H. Thulke, Å. Uttenthal, and B. Ydesen,

- 2005: Classical Swine Fever and Wild Boar in Denmark: A Risk Analysis. Project report, DFVF, 118.
- Alexandersen, S., Z. Zhang, A. I. Donaldson, and A. J. Garland, 2003: The pathogenesis and diagnosis of foot-and-mouth disease. *J. Comp. Pathol.* 129, 1–36.
- Alexandrov, T., D. Stefanov, P. Kamenov, A. Miteva, S. Khomenko, K. Sumption, H. Meyer-Gerbautet, and K. Depner, 2013: Surveillance of foot-and-mouth disease (FMD) in susceptible wildlife and domestic ungulates in Southeast of Bulgaria following a FMD case in wild boar. *Vet. Microbiol.* 166, 84–90.
- Breithaupt, A., K. Depner, B. Haas, T. Axandrov, L. Polihronova, G. Georgiev, H. Meyer-Gerbautet, and M. Beer, 2012: Experimental infection of wild boar and domestic pigs with a Foot-and-mouth disease virus strain detected in the south-east of Bulgaria at the end of 2010. *Vet. Microbiol.* 159, 33–39.
- Cottral, G. E., 1969: Persistence of Foot-and-Mouth Disease Virus in Animals, their Products and the Environment. *Bull. Off. Int. Epizoot.* 71, 549–568.
- Cowled, B., and G. Garner, 2008: A review of geospatial and ecological factors affecting disease spread in wild pigs: considerations for models of foot-and-mouth disease spread. *Prev. Vet. Med.*, 87, 197–212.
- Danilkin, A. A., 1999: Deer (*Cervidae*). Mammals of Russia and Adjacent Areas, 552 pp. GEOS, Moscow. (in Russian).
- Danilkin, A. A., 2002: Suids (*Suidae*). Mammals of Russia and Adjacent Areas, pp. 309. GEOS, Moscow. (in Russian).
- Dexter, N., 2003: Stochastic models of foot and mouth disease in feral pigs in the Australian semi-arid rangelands. *J. Appl. Ecol.* 40, 293–306.
- Doran, R. J., and S. W. Laffan, 2005: Simulation of the spatial dynamics of foot and mouth disease outbreaks in feral pigs and livestock in Queensland, Australia, using a susceptible-infected-recovered cellular automata model. *Prev. Vet. Med.* 70, 133–152.
- EFSA, 2010: Application of Systematic Review Methodology to Food and Feed Safety Assessments to Support Decision Making. *EFSA J.*, 8, 1–90. Available at [www.efsa.europa.eu/efsajournal.htm](http://www.efsa.europa.eu/efsajournal.htm) (accessed May 8, 2014).
- EFSA, Panel on Animal Health and Welfare (AHAW), 2012: Scientific opinion on foot-and-mouth disease in Thrace. *EFSA J.*, 10, 2635. 91 pp. doi: 10.2903/j.efsa.2012.2635.
- Elbers, A. R. W., L. J. M. Dekkers, G. J. Spek, L. J. M. Steinbusch, and A. C. van Exsel, 2001: Sero-monitoring of notifiable diseases in wild boar in the Netherlands 1999-2001. *Tijdschr. Diergeneesk.* 126, 779–781.
- Elbers, A. R. W., A. Dekker, and L. J. M. Dekkers, 2003: Serosurveillance of wild deer and wild boar after the epidemic of foot-and-mouth disease in the Netherlands in 2001. *Vet. Rec.* 153, 678–681.
- Ercegovic, D., R. Golosin, D. Panjevic, M. Borojevic, and Z. Calic, 1968: Potential role of game animals in the epidemiology of foot and mouth disease. *Acta Vet. (Beogr)* 18, 119–126.

- EU Veterinary Expert Team Mission, 2011: FMD in Bulgaria. Preliminary report as presented 21st January, 1000 hrs to National Veterinary Service of Bulgaria, pp. 21.
- EU FMD (European Commission for the Control of Foot-and-Mouth), 2011: 81st Executive Committee of the EuFMD, Budapest, Hungary, 2 February 2011, pp. 22.
- FAO/OIE/EC, 2011a: EuFMD Research group/FAO EMPRES Wildlife Unit Joint Meeting Berlin, 11–12 April 2011. On the risks of persistence of FMD circulation in wild boar/wildlife in southeast Bulgaria and Turkish Thrace, pp. 6. Available at: [http://www.fao.org/ag/againfo/commissions/docs/Workshop/Workshop\\_berlin\\_2011/report.pdf](http://www.fao.org/ag/againfo/commissions/docs/Workshop/Workshop_berlin_2011/report.pdf), accessed on May 7, 2014.
- FAO/OIE/EC, 2011b: Tripartite meeting on control of foot-and-mouth disease and other exotic diseases in the Southern Balkans, 25th March 2011, pp.3. Available at: <http://www.oie.int/doc/ged/D11554.PDF>, accessed on May 7, 2014.
- Ferguson, N. M., C. A. Donnelly, and R. M. Anderson, 2001: The Foot-and-Mouth Epidemic in Great Britain: Pattern of Spread and Impact of Interventions. *Science* 292, 1155–1160.
- Fernandez, N., S. Kramer-Schadt, and H. H. Thulke, 2006: Viability and risk assessment in species restoration: planning reintroductions for the wild boar, a potential disease reservoir. *Ecology and Society* 11, 6.
- Forman, A. J., and E. P. J. Gibbs, 1974: Studies with foot-and-mouth disease virus in British deer (red, fallow and roe). I. Clinical disease. *J. Comp. Pathol.* 84, 215–220.
- Forman, A. J., E. P. J. Gibbs, D. J. Baber, K. A. J. Herniman, and I. T. Barnett, 1974: Studies with foot-and-mouth disease virus in British deer (red, fallow and roe). II. Recovery of virus and serological response. *J. Comp. Pathol.* 84, 221–229.
- Frölich, K., C. Hamblin, S. Parida, E. Tuppurainen, and E. Schettler, 2006: Serological survey for potential disease agents of free-ranging cervids in six selected national parks from Germany. *J. Wildl. Dis.* 42, 836–843.
- Garner, M.G., G.D. Hess, and X. Yang, 2006: An integrated modelling approach to assess the risk of wind-borne spread of foot-and-mouth disease virus from infected premises. *Environ. Model. Assess.* 11, 195–207.
- Gibbs, E. P., K. A. Herniman, and M. J. Lawman, 1975: Studies with foot-and-mouth disease virus in British deer (muntjac and sika). Clinical disease, recovery of virus and serological response. *J. Comp. Pathol.* 85, 361–366.
- Goregryad, K. S., 1972: Diseases of wild animals. Minsk, Former USSR, Nauka i Tekhnika, pp. 520 (In Russian).
- Hess, G.D., M. G. Garner, and X. Yang, 2008: A sensitivity analysis of an integrated modelling approach to assess the risk of wind-borne spread of foot-and-mouth disease virus from infected premises. *Environ. Model. Assess.* 13, 209–220.
- Khomenko, S., T. Alexandrov, K. Sumption, N. Bulut, and S. Aktas, 2011: Role of wildlife in foot-and-mouth disease dynamics in Thrace Region in 2011 and beyond. *EMPRES Transbound. Animal Dis. Bull.* 39, 55–63.
- Kramer-Schadt, S., N. Fernández, and H.-H. Thulke, 2007: Potential ecological and epidemiological factors affecting the persistence of classical swine fever in wild boar *Sus scrofa* populations. *Mammal Rev.* 37, 1–20.
- Kramer-Schadt, S., N. Fernández, D. Eisinger, V. Grimm, and H.-H. Thulke, 2009: Individual variations in infectiousness explain long-term disease persistence in wildlife populations. *Oikos* 118, 199–208.
- Lange, M., 2012: Scientific report submitted to EFSA. Spatial spread and maintenance of foot-and-mouth disease virus infections in wildlife populations of Thrace region applying epidemiological modelling. Available at [www.efsa.europa.eu/efsajournal](http://www.efsa.europa.eu/efsajournal) (accessed May 8, 2014), pp. 29.
- Lange, M., S. Kramer-Schadt, B. Blome, M. Beer, and H.-H. Thulke, 2012a: Disease severity declines over time after a wild boar population has been affected by classical swine fever - Legend or actual epidemiological process? *Prev. Vet. Med.* 106, 185–195.
- Lange, M., S. Kramer-Schadt, and H.-H. Thulke, 2012b: Efficiency of spatio-temporal vaccination regimes in wildlife populations under different viral constraints. *Vet. Res.* 43, 37.
- Mohamed, F., S. Swafford, H. Petrowski, A. Bracht, B. Schmit, A. Fabian, J. M. Pacheco, E. Hartwig, M. Berninger, C. Carrillo, G. Mayr, K. Moran, D. Kavanaugh, H. Leibrecht, W. White, and S. Metwally, 2011: Foot-and-mouth disease in feral swine: susceptibility and transmission. *Transbound. Emerg. Dis.* 58, 358–371.
- Moniwa, M., C. Embury-Hyatt, Z. Zhang, K. Hole, A. Clavijo, J. Copps, and S. Alexandersen, 2012: Experimental Foot-and-Mouth Disease Virus Infection in White Tailed Deer. *J. Comp. Pathol.* 2012, 330–342.
- Mouchantat, S., 2005: Serologische Untersuchungen zum Vorkommen von Maul- und Klauenseuche (MKS) bei Rehen (*Capreolus capreolus*) in ausgewählten Gebieten in Deutschland – Untersuchungen im Rahmen des Europäischen Seuchenzuges im Jahr 2001. Freie Universität Berlin, Berlin, Germany 133.
- Mouchantat, S., B. Haas, W. Lutz, K. Pohlmeier, and K. Frölich, 2003: A serological survey for antibodies against foot-and-mouth disease virus (FMDV) in free-ranging roe deer (*Capreolus capreolus*) from selected areas of Germany. *Erkrankungen der Zootiere: Verhandlungsbericht des 41. Internationalen Symposiums über die Erkrankungen der Zoo- und Wildtiere*, Rome, Italy, 28 May–1 June, 2003, 141–144.
- Mouchantat, S., B. Haas, W. Lutz, K. Pohlmeier, and K. Frölich, 2005: Absence of antibodies to foot-and-mouth disease virus in free-ranging roe deer from selected areas of Germany (2001–2002). *J. Wildl. Dis.* 41, 599–605.
- O'Connor, A. M., G. L. Lovei, J. Eales, G. K. Frampton, J. Glanville, A. S. Pullin, and J. Sargeant, 2012: Implementation of systematic reviews in EFSA scientific outputs workflow. Supporting Publications: EN-367. 36 pp. Available at [www.efsa.europa.eu/publications](http://www.efsa.europa.eu/publications) (accessed May 8, 2014)
- Pech, R. P., and J. Hone, 1988: A model of the dynamics and control of an outbreak of Foot and Mouth Disease in Feral pigs in Australia. *J. Appl. Ecol.* 25, 63–77.

- Pech, R. P., and J. C. McIlroy, 1990: A model of the velocity of advance of Foot and Mouth Disease in Feral pigs. *J. Appl. Ecol.* 27, 635–650.
- Pech, R. P., J. C. McIlroy, M. F. Clough, and D. G. Green, 1992: A Microcomputer model for predicting the spread and control of Foot and Mouth disease in feral pigs. The proc. 15-th vertebrate pest Conference., 360–364. Available at <http://digitalcommons.unl.edu/vpc15/60> (accessed May 8, 2014).
- Putman, R., M. Apollonio, and R. Andersen, 2011: Ungulate Management in Europe: Problems and Practices. Cambridge University Press, New York, 408. Available at <http://books.google.bg/books?id=02zA9-jJT8wC&hl=cs&sitesec=reviews> (accessed May 8, 2014).
- Ruiz-Fons, F., J. Segalés, and C. Gortázar, 2008: A review of viral diseases of the European wild boar: Effects of population dynamics and reservoir role. *Vet. J.* 176, 158–169.
- SCoFAH (Standing Committee on the Food Chain and Animal Health), 2011: Implementation of the plan for the control of FMD in susceptible wildlife in the southeast of Bulgaria. Available at [http://ec.europa.eu/food/committees/regulatory/scfcah/animal\\_health/presentations/0708092011\\_fmd\\_bulgaria.pdf](http://ec.europa.eu/food/committees/regulatory/scfcah/animal_health/presentations/0708092011_fmd_bulgaria.pdf) (accessed May 8, 2014).
- Sludskiy, A. A., 1956: Wild Boar (Morphology, Ecology, Economic and Epizootological Role, Harvesting). Former USSR, Izdatelstvo Akademii Nauk Kazhskoi SSR, Alma-Ata pp. 220. (In Russian).
- Sørensen, J.H., D. K. J. Mackay, C. Ø. Jensen, and A. I. Donaldson, 2000: An Integrated Model to Predict the Atmospheric Spread of Foot-and-Mouth Disease Virus. *Epidemiol. and Infect.* 124, 577–590.
- Thomson, G., W. Vosloo, and A. D. Bastos, 2003: Foot and mouth disease in wildlife. *Virus Res.* 91, 145–161.
- Ward, M. P., S. W. Laffan, and L. D. Highfield, 2007: The potential role of wild and feral animals as reservoirs of foot-and-mouth disease. *Prev. Vet. Med.* 80, 9–23.
- Ward, M. P., S. W. Laffan, and L. D. Highfield, 2009: Modelling spread of foot-and-mouth disease in wild white-tailed deer and feral pig populations using a geographic-automata model and animal distributions. *Prev. Vet. Med.* 91, 55–63.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Model documentation.

**Appendix S2.** Systematic review of observational studies studying FMD in wild boar and deer populations. Data extracted from papers which were included in the final steps of the systematic literature review.

**Appendix S3.** Experimental infection of wild boar and deer with FMDV. Data extracted from papers which were included in the final steps of the systematic literature review.