

who are now in the field conducting targeted FMD vaccination campaigns. The training also included details of the FMD progressive control pathway (PCP) and evaluated where Mongolia should focus its efforts to move through PCP stages towards FMD freedom.

Further TCP activities will include gazelle surveillance, such as capture and sampling, to measure the extent of exposure to FMD and to determine whether there could be an FMD reservoir. Historical analysis will be undertaken to evaluate where gazelle and livestock ranges overlap and the potential for FMD transmission between these species. When combined with FMD outbreak maps, this analysis will help to clarify the role of gazelles in the epidemiology of FMD in Mongolia, as well as helping to identify management steps that can be taken to minimize disease impacts and spread.

FAO and the EMPRES Wildlife Health and Ecology Unit anticipate that this TCP support will ensure that the Mongolian Government is better prepared to address outbreaks of FMD or other livestock and wildlife diseases that may occur in the future.

Contributors: Tracy McCracken (FAO), Scott Newman (FAO)

Role of wildlife in foot-and-mouth disease dynamics in Thrace Region in 2011 and beyond

Historical notes and background

Wild boar (*Sus scrofa*), ancestor of the domestic pig, is fully susceptible to all diseases of swine, including foot-and-mouth disease (FMD). Specifically clinical FMD (or less frequently laboratory-confirmed disease) has been reported from a number of locations across the historical range of the species (Figure 1). There is little doubt that these occasionally observed (and reported) cases represent just a tiny proportion of such events on the global scale. Marek and Hutýra (1931, cited in Sludskiy, 1956) mention a widespread epidemic in *S. scrofa* in a European country at the beginning of the twentieth century. In the countries of the Former USSR, clinical disease in wild boar was most often observed in the Caucasus (1902 to 1925), but also occasionally in southern Kazakhstan (1927 to 1941) and, in 1953, in Kyrgyzstan (Sludskiy, 1956; Danilkin, 2002), until the country-wide vaccination of livestock and control efforts finally eradicated FMD from the Former USSR in the 1980s.

Donaldson and Shimshony (1988) speculated that two independent FMD virus (FMDV) introductions into Israel in 1985 might have been due to air-borne spread of the virus emitted by infected wild boars 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 percent) were found positive in serum neutralization (SN) tests (ProMED-mail, 2007). Virus was also found in two out of 73 animals (2.8 percent) in 1992. It was reported that as many as 85.7 percent of wild boars (18 out of 21 sampled) from three locations along Israel's northern and northeastern frontiers were positive in the non-structural protein (NSP) enzyme linked immunosorbent assay (ELISA) for FMD (ProMED-mail, 2007). In July 2011, wild boars were again implicated as potential virus

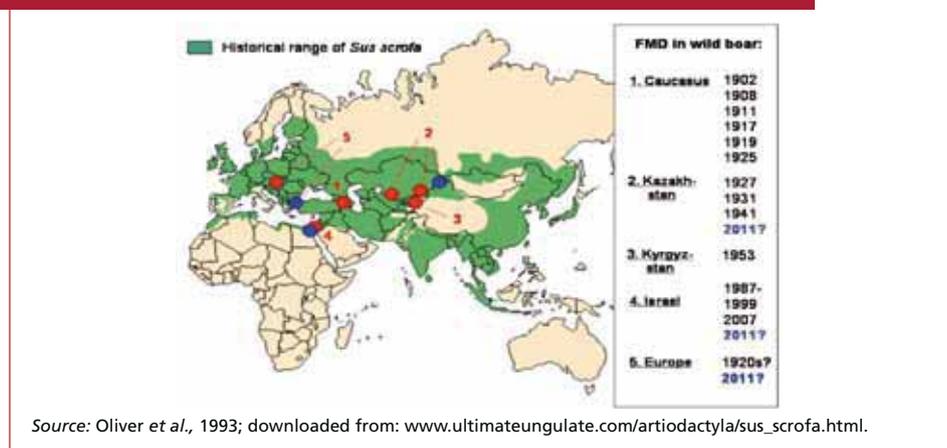
FMD lesions in cattle in Kost, Bulgaria



© FAO/ALEXANDROV TSVIATKO (AGAH)



Figure 1: Anecdotal and documented historical observations of FMD in different parts of the wild boar historical occurrence range



disseminators in northern Israel (ProMED-mail, 2011). Similarly, following unexplained mortality in wild boars, an FMD type O outbreak in eastern Kazakhstan in September 2011 was attributed to possible migration of the species from China (A. Tanraev, personal communication). All these anecdotal or surveillance observations were made in areas with concurrent FMD outbreaks in livestock (often involving domestic pigs) and were usually considered to be the result of transmission from domestic animals rather than stand-alone epidemics in wild boars (Sludskiy, 1956; Goreglyad, 1971; Danilkin, 2002), which is the case in a large majority of FMD detections in wildlife in general (Thomson, Vosloo and Bastos, 2003).

Late in 2010, type O FMD virus was detected in a wild boar shot in southeast Bulgaria (Figure 3). The VP1 genotyping of the FMD virus isolate performed at the European Union (EU) Reference Laboratory for FMD, Institute for Animal Health Pirbright (United Kingdom of Great Britain and Northern Ireland) confirmed its close genetic relationship with recent isolates from the Asian part of Turkey (Valdazo-Gonzales et al., 2011). On 5 January 2011, Bulgaria notified this case. Prior to 2011, the last outbreak of FMD in Bulgaria was in 1996, and since then the country had been free of the disease. Further investigation of the epidemiological situation in this area – where 14 FMD outbreaks in livestock (Figure 3) were reported to the World Organisation for Animal Health (OIE) in 2011 – from January to April 2011, coupled with molecular data on Bulgarian isolates, showed that the virus was possibly amplified in an unidentified reservoir (supposedly wild boars), from which it was independently introduced into livestock in different locations of the area at least four times (based on the available epidemiological and genetic evidence). Simultaneous intensive surveillance in livestock on the Turkish side of the border did not reveal circulation of FMD virus (Khomeenko and Honhold, 2010) and found only limited and localized occurrence in Bulgaria. The Bulgarian Ministry of Agriculture, the Turkish veterinary authorities and international organizations therefore raised concerns regarding:

- the possibility that transboundary spread of FMDV from Turkish Thrace, which has disease-free status with vaccination, was facilitated by infected wild boars and/or other FMD-susceptible wildlife species;
- the suspicion that local wild boar or other wild ungulate populations may represent a silent epidemiological reservoir of FMDV that is separate from livestock (which has 100 percent cover from vaccination in the Turkish part of Thrace);
- the actual and future risk of FMDV introduction into EU Member States, and the chances of persistence in European populations of wild ungulates if this occurs.

For these reasons, serological surveillance in wild boars and other ungulate species, such as roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*), was initiated to find out what actually happened in the population of wildlife inhabiting the Turkish-Bulgarian cross-border area and what the immediate and long-term implications of this unique epidemiological situation were likely to be. Short- and long-term surveillance plans were developed by both Bulgaria and Turkey, the latter with assistance from the FAO Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases (EMPRES) Wildlife Health and Ecology Unit (see next section).

FMD surveillance in susceptible wildlife in Southeast Bulgaria and Turkish Thrace.

Both Bulgaria and Turkey adopted statistically similar approaches to surveillance. The sample size of 59 head per sampling unit (see following) was set to achieve 95 percent confidence for detection of FMD antibodies, with an expected FMD antibody (Ab) prevalence of 5 percent. Another assumption was that as a highly contagious disease, once FMDV is introduced to a wild boar herd (family group) it is likely eventually to infect all herd members. Assuming that hunters can kill all the animals in a group (which is not usually the case), with this sample size a minimum of 12 average-sized herds of wild boars (e.g., quasi-epidemiological units) would be sampled. Thus, the infection would be detectable if it had affected about a quarter of the herds in a sampling unit (with herd sero-prevalence of approximately 25 percent at 95 percent confidence). Other FMD-susceptible species (red deer, roe deer, unowned stray livestock) were targeted, with a sample size of 35 head (10 percent sero-prevalence at 95 percent 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 2011 and in March 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) cover about 2 160 km² adjacent to area A 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. The sampling frame developed for serological and virological monitoring generally followed European Food Safety Agency (EFSA) recommendations for classical swine



© FAO/SERGEI KHOMENKO

Interview with a local veterinarian, Azdavay, Turkey



fever surveillance, with some adaptations. The animals were either hunted (in Bulgaria and Turkey) or trapped (only in Bulgaria). From each animal, blood samples for serological and virological tests, and tissue samples (pharyngeal area, skin with lesions, lymph nodes and vesicular fluids where available) were collected. In Turkey the NSP ELISA kit PrioCHECK FMD NS (Prionics Lelystad B.V.) was used for initial testing; positive samples were re-tested with another commercially available NSP ELISA kit (Svonovir FMD 3ABC-Ab Ruminant, Svanova Biotech AB).

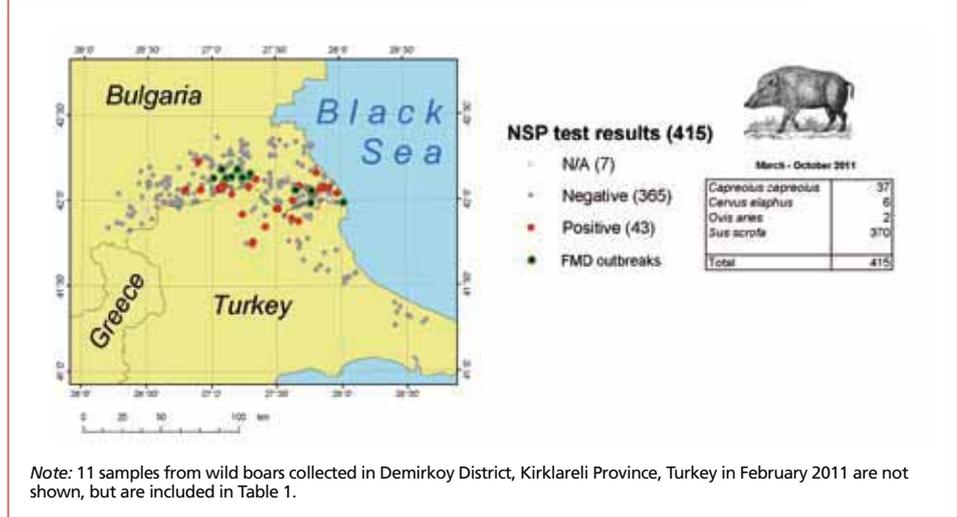
In Turkey the whole forested area extending from the border with Bulgaria to Istanbul was defined as the area at risk of FMD persistence in wildlife and was divided into patches based on the map of catchment areas (originally produced with ArcInfo 9.3 based on 90-m resolution digital elevation model [DEM]), which were grouped following the relief into eight larger sampling units averaging 342 km² of forested area each (and ranging from 232 to 438 km²). Catchment areas were selected because they were easier for hunters to locate on the ground and because particularly high ridges are likely to serve as ecological barriers for the movement of wild boars. In the absence of official wild boar population estimates, numbers were calculated based on the total forested area, the average home range for a wild boar group (4 km²) and the average group size (five head) (Danilkin, 2002). This gave a total of 3 500 to 7 000 head (depending on the season), with between a few hundred and 1 000 wild boars estimated to occur in each sampling unit.

Owing to the foreseen logistical difficulties for hunting wild boars in July and August 2011, sampling units 1 and 2, which are in immediate proximity to FMD-affected locations in Bulgaria, were given priority. Several hunting teams led by professional veterinarians were established to hunt wild boars in July and August. A specially

Figure 2: Locations of the three sampling areas within the cordon sanitaire in Bulgaria



Note: Infected area A – South Tsarevo, Malko Tarnovo and South Sredets Municipalities; risk area B – North Tsarevo, Primorsko, Sozopol and Central Sredets Municipalities; and risk area C parts of Bolyarovo, Elhovo, Topolovgrad and Svilengrad Municipalities. In Turkey there were eight sampling units, of which units 1 and 2 were given highest priority.

Figure 3: Sample collection locations and results of serological tests


designed sample collection protocol was followed in the field. Geographical coordinates, a full-size photograph of each animal killed and close-up photographs of its feet, snout and tongue were taken, and its age, sex and any other relevant information (e.g., size of its group) were recorded. In addition to standard blood and tissue samples, pharyngeal lymph nodes were also collected to attempt virus isolation. In sampling units with lower priority (units 3 to 8) in Turkey, wild boar populations were to be tentatively surveyed with a maximum target sample size of ten.

Between February and 20 October 2011, a total of 426 individuals from four susceptible wild species were tested serologically and virologically for FMD in Bulgaria (total $n = 321$, wild boars = 280) and Turkey ($n = 98$, all wild boars). No virus was detected. Average sero-prevalence of 11.6 percent was found in all the wild boars sampled. Adult and juvenile (born in 2011) animals ($n = 361$) had the same sero-prevalence (12.2 percent), while among animals of undefined age ($n = 28$) there was one positive sample. In adult roe deer ($n = 33$) sero-prevalence was 9.1 percent. Sample sizes for other species (red deer and mouflon [*Ovis musimon*]) were too small ($n = 6$ and 2 , respectively) to draw any conclusions. On average, sero-prevalence in wild boar in Turkey (27.6 percent) was significantly higher (four times) than in Bulgaria (6.5 percent), including when the prevalences in adults and juveniles were compared separately (2.6 and 14.8 times higher in Turkey, respectively, Table 1). Most positive detections seem to be clustered in the cross-border area near FMD outbreaks in livestock, although some were found further from the border in Turkish Thrace (Figure 3). This should be taken into account, as differences between countries might be influenced by spatial bias in sample distribution. No positive animals were reported from sampling units other than 1 and 2 (Figures 2 and 3), but sample sizes in the districts of Vize, Saray, Catalca and Gaziosmanpas (east of $27^{\circ} 30' E$) were too small to exclude the possibility of failure to detect past or current infection.



The age distribution of sero-positive juvenile animals suggests that although some of them might have had maternal antibodies at the time of testing, others could have been challenged with FMDV either during the period of their maternal immunity (probably similar in length to the three months of domestic piglets) or after. This seems particularly likely for several juveniles aged seven to nine months shot in Bulgaria in October. Surveillance efforts are continuing in the area, and further testing of juvenile animals and analysis based on a more comprehensive data set will help improve understanding of the timeframe and spatial extent of this FMD epidemic in wildlife. However, it is already clear that FMDV introduced into wild ungulate populations somewhere in Thrace Region, probably as early as the Kurban Bayram period (16 November in 2010), resulted in a fairly extensive and long-lasting (at least six to eight months) epidemic involving at least two abundant species (wild boar and roe deer), but probably also some unowned stray domestic animals in the Strandzha area of Bulgaria. These preliminary results should be treated with caution and need to be properly evaluated to avoid possible biases and artefacts of sample distribution and size.

EU-FMD/FAO wild boar surveillance project in Anatolian Turkey

In addition to this study, the EU-FMD Secretariat and the EMPRES Wildlife Health and Ecology Unit have developed a study project proposal and submitted it to the European Commission for funding. The proposed project aims at exploring further the epidemiological role of wild boars in Anatolian Turkey, where FMD is endemic in some areas. Prior to developing the project proposal, an EU-FMD/FAO mission visited Anatolia in July and August 2011 to evaluate the feasibility of such a study. The mission visited the Turkish Ministry of Environment and Forestry and the General Directorate for Protection and Control (GDPC) in Ankara, and the Provincial Directorates of Environment and Forestry (EFD) and Agricultural (AD) in Gumushane, Kastamonu, Samsun

Table 1: Results of serological surveillance for FMD in wildlife in Turkey and Bulgaria, by species, February to 20 October 2011

Country	Species	Age group	No. sampled	Number NSP-positive	Prevalence (%)	95% confidence interval +/-
Turkey	<i>Sus scrofa</i>	Adult	46	11	23.9	12.3
		Juvenile	52	16	30.8	12.5
		All	98	27	27.6	8.8
Bulgaria	<i>S. scrofa</i>	Adult	167	15	9	4.3
		Juvenile	96	2	2.1	2.9
		?	17	1		
		All	280	18	6.4	2.9
	<i>Capreolus capreolus</i>	Adult	33	3	9.1	9.8
	<i>Cervus elaphus</i>	Adult	2	0		
	<i>Ovis musimon</i>	Adult	2	0		

and Rize. These provinces were selected based on the occurrence and frequency of FMD outbreaks, livestock husbandry systems and the availability of wild boar habitats (broad-leaved or mixed forests, Figure 4). Modalities, logistics, legal aspects and practical options for project implementation on the ground were discussed with EFD and AD staff. The project's aims, objectives and details were explained to district-level government and private veterinarians, who were invited to participate in the project.

Leaders of local hunting communities were interviewed regarding wild boar population trends, wild boars' movement/seasonal migration patterns, crop damage, estimated annual hunting bags, methods and timing of hunts, historically known cases of mortality and disease, number of licensed hunters available for organized hunts, normal frequency of hunts, etc. Most respondents agreed that wild boar numbers had been increasing recently, reportedly because of warmer winters, a general decline in grey wolf (*Canis lupus*) numbers (related to an overall decline in sheep breeding) and a decrease in hunting pressure due to hunting restrictions. A respondent in Azdavay (Kastamonu Province) reported regular migration of local wild boars to the Black Sea coast (70 to 80 km away) in August and September, to feed on chestnuts. No historical accounts of mortality or disease were reported. Crop damage (on both private garden plots and larger croplands) by wild boars was commonly reported everywhere in late summer. At the moment, driven wild boar hunts are forbidden (permits have to be requested from EFD). Individual chase hunting with dogs takes place from October to January (when snow cover facilitates this type of hunting). Both registered hunters and EFD authorities admitted that up to 80 to 90 percent of the people who hunt wild boars are poachers. The enforcement of legislation in this part of Turkey is weak to non-existent, particularly in remote mountain villages.

In summary, the mission drew the following conclusions:

Figure 4: Provinces and districts visited/selected for project implementation





- A sample collection strategy can be based only on animals harvested under licensed hunts, of which commercial hunts organized by safari companies are the best option.
- A sample size of 60 animals would be feasible in three of the four provinces visited (not in Rize). The total target sample size was set at 210 samples.
- Collaboration between local hunting clubs and veterinarians for the collection of samples would be feasible with proper organization, particularly with official endorsement and support from the local AD and EFD.

References

- Chung, W.-B., Sorensen, K.J., Liao, P.-C., Yang, P.-C. & Jong, M.-H.** 2002. Differentiation of foot-and-mouth disease virus-infected from vaccinated pigs by enzyme-linked immunosorbent assay using nonstructural protein 3AB as the antigen and application to an eradication program. *J. Clin. Microbiol.*, 40: 2843–2848.
- Danilkin, A.A.** 2002. [Suids (*Suidae*). In *Mammals of Russia and adjacent areas.*] Moscow, GEOS. 309 pp. (in Russian)
- Donaldson, A.L., Lee, M. & Shimshony, A.** 1988. A possible airborne transmission of foot and mouth disease from Jordan to Israel – a simulated computer analysis. *Israel Journal of Veterinary Medicine*, 44: 92–96.
- Goreglyad Kh.S.** 1972. [*Diseases of wild animals.*] Minsk, Former USSR, Nauka i Tekhnika. 520 pp. (in Russian)
- Khomenko, S. & Honhold, N.** 2010. FAO/European Commission for the Control of Foot-and-Mouth Disease mission to Turkey concerning foot-and-mouth disease outbreaks in Bulgaria. *EMPRES Transboundary Animal Diseases Bulletin*, 37: 14–18.
- Oliver, W.L.R., Brisbin, I.L. Jr. & Takahashi, J.** 1993. The Eurasian wild pig (*Sus scrofa*). In W.L.R. Oliver, ed. *Pigs, peccaries and hippos: status survey and action plan*, pp. 112–121. Gland, Switzerland, IUCN.
- Panel on Animal Health and Welfare.** 2009. Scientific opinion on a request from Commission on “Control and eradication of classic swine fever in wild boar”. *EFSA Journal*, 932: 1–18.
- ProMED-mail.** 2007. *Foot-and-mouth disease, wild boar – Israel (north and northeast)*. ProMED-mail No. 20070517.1571. www.promedmail.org. (accessed 10 November 2011)
- ProMED-mail.** 2011. *Foot-and-mouth disease – Israel (13): (Hazafon), request for information*. ProMED-mail No. 20110713.2120. www.promedmail.org. (accessed 10 November 2011)
- Sludskiy A.A.** 1956. [*Wild boar (morphology, ecology, economic and epizootological role, harvesting)*]. Alma-Ata, Former USSR, Izdatelstvo Akademii Nauk Kazhskoi SSR. 220 pp. (in Russian)
- Thomson, G.R., Vosloo, W. & Bastos, A.D.** 2003. Foot and mouth disease in wildlife. *Virus Research*, 91(1): 145–161.
- Valdazo-González, B., Knowles, B.N. J., Wadsworth, J., King, D.P., Hammond, J.M., Özyörük, F., Firat-Saraç, M., Parlak, Ü., Polyhronova, L. & Georgiev, G.K.** 2011. Foot-and-mouth disease in Bulgaria. *Veterinary Record*, 168: 247 doi:10.1136/vr.d1352.

Contributors: Sergei Khomenko (FAO), Tsviatko Alexandrov (FAO), Keith Sumption (FAO), Naci Bulut (SAP Institute, Turkey), Sinan Aktas (FAO)