

## **Meeting report**

### **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 South-East Bulgaria and Turkish Thrace

#### **Follow-up Actions :**

1. Finalize the report of the meeting (KS);
2. Ryan Waters (IAH) to send sampling protocols for tonsils/lymph nodes to BG and TUR participants;
3. Refinement of wildlife surveillance plan (BFSA/Bulgaria)
4. Further develop surveillance protocol for FMD in wildlife, for summer period surveillance in Thrace region of Turkey and autumn/winter surveillance project proposal for Anatolia (EuFMD/FAO: S.Khomenko);
5. Present results of the report of the meeting at the EuFMD General Session (S.Khomenko and Keith Sumption);
6. Subject to EuFMD/EC agreement, issue call for Concept Notes for studies recommended [see Recommendations 2 and 4].

#### **Background to meeting:**

The meeting was organised by the EuFMD Commission, in collaboration with FAO and with the support of the EC (via MTF/INT/003/EEC) in response to the unprecedented situation in south-east Europe, where wild boar (WB) had been confirmed positive in January for FMDV, and were considered to be a plausible source of infection for several of the following outbreaks in Bulgaria.

**The purpose of the meeting** was to identify gaps in knowledge, and priorities for follow-up actions, in order to improve guidance to countries on surveillance for infection (or its absence).

It was also a valuable opportunity to review the findings of FMD surveillance in domestic and wildlife species in the two countries, to review findings from FMD experiment studies in wild boar, and to identify the likelihood of persistence of virus circulation in the wildlife in this region.

#### **Agenda and Participation**

The Agenda of the Meeting, the List of Participants, and the List of Presentations are given in Appendices 1-3

The nineteen participants were from the two current affected countries, with experts from Germany, UK, Belgium, FAO, and EC.

Expertise included, from Turkey (on FMD and on wild boar ecology), Bulgaria (FMD Laboratory experts, and disease surveillance in wildlife (Bulgarian Food Safety Agency), the EU-CRL for FMD (Pirbright) and FLI (FMD and wildboar disease pathogenesis and ecology) , and experts on CSF epidemiology and dynamics in wildboar (3 –from Germany and Belgium). Dr Bellini represented DG\_SANCO, and Drs Khomenko, Guberti and Newman represented the FAO input on wildlife/wildboar disease ecology.

#### **Structure**

Day 1 involved presentations and discussions in three sections:

Part I. Background (*presentations: S Khomenko, FAO, and K Depner. FLI*)

Part II. Current situation - surveillance findings ( *Tsviatko Alexandrov, BFSA Bulgaria, G Georgiev, Bulgaria, and Naci Bulut, SAP Institute Turkey*).

Part III. Conditions for persistence *Presentations: V Guberti, FAO; H Thulke and S Kramer-Schadt, Germany)*

Part IV. Infection dynamics and transmission in wildboar/wildlife; in vivo findings *Presentations: A Breithaupt, FLI, and R Waters, IAH Pirbright)*

Day 2: Working Groups on knowledge gaps, follow-up actions and studies required

### **Part I: Background**

In the recent Tripartite meeting (Greece, Bulgaria, Turkey) in Bulgaria, the Turkish participants, following-up from the EuFMD mission in January, had reported the results of surveillance in wild boar and revealed that five out of twenty-six animals were positive for FMD antibody. The meeting was called with the purpose of identifying gaps in knowledge on the transmission of FMD by wild boar and wildlife that would affect the estimation of the epidemic duration in wildlife in this region, or indeed in other parts of Europe.

The meeting opened with a review of the literature on FMD in wild boar in Eurasia, given by S Khomenko (FAO); of note is that there are many reference n old publications (mainly pre-WWII and in Russian ) but few from western Europe in same period, and of recent only those recent publications from Israel. since FMD in western Europe became rare after 1970, as a result of mass vaccination, and wildboar populations have increased in the period, the risks associated with wild boar may need to be revised in light of recent findings and population increase/expansion. He placed the recent findings from Bulgaria and Turkey in the context of known and estimated wildboar populations in the Strandzha ecosystem, a forested region straddling the Turkish/Bulgarian border; this region is a extensive area with limited domestic animals within the forest; recent cases in Bulgaria are on the more western edges, where forest gives way to more populated valleys with denser and more continuous domestic animals. The virological (full genome sequence) findings do suggest entry of infection into animals in this region, expansion of the infection and spillover as separate secondary infections into domestic animals. The serological evidence from wildboar in Turkey supports the view that part of this reservoir has been in wildlife. The duration infection has been present in this ecosystem must be at least 4 months; the likely continuation is the subject of the meeting.

Dr Depner (FLI) then summarised issues where knowledge is needed to better assess the risk of FMD spread within wildboar populations and their consequence for domestic animals.

### **Part II Current situation: surveillance findings**

The talks from Bulgaria reviewed the epidemiological findings, and the laboratory assessment including sequencing studies conducted to shed light on transmission events and connection between outbreaks. With the exception of the first, index case (confirmed 4<sup>th</sup> January), no FMD antibody or virus positive wild boar or WL had been found . FMDV from the initial index case in wild boar, plus the domestic animal cases in Kostinbrod and Resovo villages (1<sup>st</sup> wave, in the east) and Kirovo (2<sup>nd</sup> wave in the west) were genetically distinct, suggesting no direct connection between any of these cases, instead FMDV had evolved, from a common ancestor, presumably within unknown (wild?) animals in this area. Since the area is highly forested and all domestic animals subject to serological surveillance (within protection zones, and surveyed in surveillance and at risk zones) , then in this reservoir is likely to be wildlife, which almost equal domestic animals in this area.

The talk from Turkey focussed on surveillance in wild boar, and the evidence for absence of circulation in domestic animals in Thrace. Hunting was banned after the index case in Bulgaria, except for surveillance; a plan had been developed by the EuFMD mission team working with Turkish authorities, and 26 animals had been shot for surveillance from 3 priority forested areas of Thrace.

Five of eleven animals from the Strandzha ecosystem had tested NSP positive, and had type O antibody reactions, confirming past infection; all were virus negative. However, animal ages and exact location data were missing from most of these; nevertheless the locations were sufficiently far apart to say that these were not from the same groups, and infection therefore had been successful in reaching a significant fraction over a dispersed area close to Bulgarian border.

No substantially new results were presented in comparison to those reported at the meeting in Plovdiv (Bulgaria) two weeks previously. This was, however, a very good opportunity to share knowledge with experts from Germany, Italy, Belgium and the UK, all of whom were new to the situation.

### **Part III Conditions for persistence**

Subsequent presentations were made on the potential for persistence for FMD in wild boar wildlife by Vittorio Guberti (VG) (FAO), Stephanie Kramer-Schadt (SKS) and Hans Thulke (HT) from Germany. The latter two presented some preliminary work on modelling, adapting the models for CSF to FMD in which they looked at parameters that might result in an extended epidemic, as well as the findings for the conventional model. The results of the modelling suggested an epidemic would last between one to two years with little likelihood of persistence beyond two years according to their model assumptions. This model was based largely on a block of country which would approximate to the Strandzha eco-system. The implication that an epidemic could persist for many months, possibly to 2 years, is new and of high importance; the current evidence, although limited, does suggest the epidemic has been successful in wild boar in Turkey (the locations of positive boar were distant) and suggests that they could be right. They could find only very rare circumstances for an extended epidemic; the duration depends largely on the size of the population and other parameters such as the presence or absence of maternal immunity, the latter affecting the role of piglets in amplification of infection in the population. This model did not take into account possible antigenic drift, which is a very big difference between CSF and FMD.

### **Part IV Infection dynamics and transmission in wildboar/wildlife; in vivo findings**

Angele Breithaupt, of the **Friedrich Loeffler** Institute (FLI), presented the findings of an experiment with the Bulgarian strain in wild boar. This showed there was a longer incubation period and also a higher level of virus excretion by a wild boar than domestic pigs. Subsequently a presentation was given by Ryan Waters (RW) from Pirbright (Institute for Animal Health – IAH) on the possible role of infection in pregnancy and neonatal animals in which the potential exists for abortion and delayed expulsion of infected material into the environment, and also death in neonates; these factors have not yet been taken into consideration in the modelling study, but could extend the period of infectiousness of an animal group or their environment. The role of other wildlife was briefly discussed as well, but it was thought that if the model of Hans Thulke was correct, then the role of short-lived infections in other wildlife might be not really any different from wild boar, but given their lower reproductive (breeding) rate and this might limit their role in the epidemic.

**Relating to field sampling of wild animals**, FLI requested to receive serum samples from wild boar to compare for levels to those in experimental infection; IAH (Pirbright) to receive tissue samples for virus propagation, full genome sequencing [subject to funds].

Regarding other species, experimental infections would be difficult and need greater evidence of an issue (e.g. findings of exposure in the field) and possibly implication of importance from modelling studies (the multi-species models) before experiments justified.

The implications of these findings were then worked upon by four groups, and their conclusions and recommendations were presented on the second day.

### **Day 2 FMD in wildboar/wildlife: Issues and Outcome:**

The **main conclusions and recommendations** were that

- more work is needed to establish the models and to test the reality of an extended epidemic occurring in wild boar populations and this model development would not only take into account those factors which have been mentioned, but would also need to have their assumptions checked by a wider group of experts.
- the opportunity to validate these models with in depth field studies on exposure of different species to FMD must be taken
- immediate (summer period) surveillance in wildlife in the Strandzha ecosystem is needed in both Bulgaria and in neighbouring parts of Turkey to give a rapid assessment (snapshot) of the current exposure/distribution of infection in areas posing a threat for domestic animal exposure, and
- surveillance plans for the autumn/winter period must be developed, considering the probable duration of the epidemic
- further experimental studies (domestic or wild boar )should be designed and costed, focussing on the transmission parameters that most affect estimated duration of an epidemic

Of immediate importance would be the planned surveillance in Bulgaria and their representatives agreed that they would review their surveillance plan, in light of the new findings. On the Turkish side, SK would work with NB and would develop a plan for 'emergency surveillance' during the summer months; involving hunting wild boar on the edges of the forests where they invade the crop land. This would therefore also inform on the risk from wild animal to domestic at the interface and could provide some valuable information on the extent to which the epidemic has spread to the south. Since the epidemic was expected to last in the ecosystem for longer than six months, then studies in the autumn during the normal hunting season would be planned in both countries and autumn would be the time for the other extended study to take place in Turkey.

The other follow-up include experiments to be conducted; perhaps the principal ones of concern would be how long the immunity is in wild boar, because if it were short they would re-enter the epidemiological cycle, and also on the possibility of using lymph nodes as an "archive material" possibly indicating the timing of the initial infection of the animals, which could be very valuable in trying to establish the sequence of events and whether infection in a population is old or recent from hunted samples. This would require an experimental study over some months and there is a possibility of the FLI, IAH and SAP Institute (Turkey) collaborating to make a cost-effective study. These reports and proposals will be costed and funding will be sought, possibly from the EU after the General Session of the EuFMD Commission in April 2011.

### **Conclusions**

1. The lack of reports on the role of wildboar/wildlife in maintaining FMD in western Europe in the past should not be considered conclusive on their future role, given the number of historic reports from Eastern Europe and the increase in population densities and distributions in Europe in the past 10 years;
2. The data from Bulgaria and adjacent part so Turkey is currently insufficient to draw conclusions on the relative role of wildlife and domestic animal infections in maintaining the epidemic since its introduction in late 2010;

3. The genome sequencing , which suggests amplification and evolution of the strain from a common ancestor in late 2010, and the finding of sero-positive wildboar in Demirkoy district of Thrace, together with the index case in a wildboar in Bulgaria, suggest wildlife have been affected and may have been a significant part of the series of transmissions that led to genetically distinct FMDV affecting the domestic animals in the first and second waves of domestic animal outbreaks in Bulgaria;
4. Since all wild and domesticated ungulate species may play some role in transmission, wild ungulates (principally three deer species in the area) should also be sampled, and included in modeling;
5. Given the short period of excretion by ungulates, and low reproduction rate, deer species are unlikely to change the duration of persistence in a population;
6. Based on the assumptions used, including the results of the transmission experiment with the Bulgarian isolate, endemic infection in a wildboar population would be unlikely to occur; however, as the duration of an epidemic depends on size of the population, duration of the endemic in a population and areas similar to the Strandzha ecosystem could last between 1 and 2 years;
7. A longer duration would require the regular introduction of new susceptible animals, which may occur through a high breeding rate, contact with non-immune domestic stock, declining immunity after recovery, and evolution of antigenic variants;
8. Key parameters that need experimental evidence or expert opinion are the duration of maternal immunity (since it affects the role of piglets in amplification cycles) , rate of antigen drift and its impact on transmission by partially immune animals; and the role of infection in pregnancy or neonatal period upon mortality and virus survival in discharges and carcasses (which could be an a form of environmental persistence )
9. Sero-surveillance , in all relevant species, should be adequate for demonstrating presence of infection, and for data needed for model development and validation;
10. Give the above, FMDV infection can be expected to persist through 2011 in the ecosystem, with risk to domestic animals principally at the forest edges and within the forest;
11. Hunting for surveillance at the forest edges is a possible option in Turkey for summer period surveillance; the results, and risk features at the forest/domestic interface, may be useful to design local control measures to protect the status of domestic animals in Thrace region;
12. Surveillance in Bulgaria should be designed and implemented to provide results within a useful period for risk assessment; given the expected duration of the epidemic, repeated rounds of surveillance should be anticipated;

### **Recommendations**

1. Sampling wildboar for FMD exposure; serum, tonsil, lymph nodes draining sites of replication (submandibular, pre-scapular....) should be taken from all sampled animals;; if NSP positive go back to these samples for virus/genome detection/sequence analysis. [ Action: Ryan Waters to send LN collection protocol sent to BG and TUR - to be used in studies in both countries]
2. Further development of the models to include parameters such as antigenic drift, multi-species and domestic/wildlife infection exchange is needed [Action: EuFMD -to Invite Concept Notes from interested parties? ]
3. That current opportunity to validate models with field data from Strandzha and from Anatolia should be taken, with well structured surveillance designs ; these studies would be likely to be

possible autumn/winter hunting season 2011 [Action: FAO/EuFMD in collaboration with Turkey, BG in relation to the statutory surveillance plan]

4. Animal expts are needed to optimize information from the sampling and diagnostic protocols; a longitudinal study of Infection in domestic and wild boar with weekly sampling over a year to determine antibody decay and presence of viral RNA in lymph nodes (needle biopsy method) ;  
*[Concept Note needed? FLI will propose, working with SAP Institute and IAH ]*
5. Maternal antibodies and protection; review of the literature needed [Action: Bernd Haas – EuFMD research group]

#### **Appendix 1: Agenda**

#### **Appendix 2: Participants:**

Angele Breithaupt (FLI)

Sandra Blome (FLI)

Bernd Haas (FLI)

Klaus Depner (FLI)

Hans-Herman Thulke (UFZ)

Stephanie Kramer (IZW)

Ryan Waters (IAH, UK)

Frank Koenen (Coda Cerva VAR, Brussels)

Georgi Georgiev (Bulgaria)

Hinrich Meyer-Gerbaulet (Bulgaria)

Liliana Polychronova (Bulgaria)

Tsviatko Alexandrov (Bulgaria)

Naci Bulut (SAP Institute, Turkey)

V. Beskardes (Turkey)

Keith Sumption (EUFMD)

Vittorio Guberti (FAO)

Scott Newman (FAO)

Serghei Khomenko (FAO)

Silvia Bellini (DG-SANCO)