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datasets provide important information on the molecular epidemiology of FMDV during outbreaks and will be applied to further outbreak tracing studies to identify and determine patterns of viral transmission and mechanisms of evolution.

#### References

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### Classical Swine fever in wild boar: Surveillance strategies under the microscope

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**Objective:** Classical Swine Fever (CSF) is a highly contagious viral disease, which affects all suid species. CSF infection in wild boar can play an important role in disease introduction to commercial pig holdings. Due to its high economic impact, efficient but also cost-effective surveillance strategies have to be implemented not only in commercial pig holdings but also in wild boar.

In Germany, the last CSF outbreak occurred in wild boar in 2009. Consequently, vaccination had been implemented until 2012. Since June 2012, Germany is considered to be free from CSF. Following this, active surveillance in wild boar has been carried out according to the directives of the European Union (Council directive 2001/89/EC). Germany's implementation of these regulations plan to take 59 samples per district per year to be capable to demonstrate freedom from disease on district level (conventional method).

Compared to conventional surveillance, risk-based surveillance approaches may achieve similar performance at lower cost or better performance at the same cost. Within the framework of the European project RISKSUR (<http://www.fp7-risksur.eu/>) we conducted a simulation study to compare the performances of different surveillance approaches for CSF in an unvaccinated wild boar population in an area free from the disease.

The aim of the study was to find out whether surveillance with the objective of demonstrating freedom from disease in wild boar could be designed more effectively using risk-based or alternative methods compared to using conventional methods.

**Methods:** For the simulation model, R ([www.r-project.org](http://www.r-project.org)) was used for statistical computing and displaying graphs. A virtual wild boar population was generated and an infection initiated within this population. As study area we used the federal state of Rhineland Palatinate. The population size estimates for the considered region, determined using fecal DNA samples, were used to calculate the total number of wild boar within the simulation study. To assure a general pattern of the population structure we chose districts from three areas with different epidemiological situations. The simulated population was structured containing information on age, gender and the type of carcass (shot healthy, shot sick, injured through road traffic accident and found dead). The setup of the infection was done on the basis of data from Mecklenburg-Western Pomerania to estimate the increase of the seroprevalence at the beginning of an infection.

The risk factor analysis used to define the risk-based surveillance approaches was done through literature search, expert opinion and bivariate analysis of infection data. Due to a lack of appropriate information in Rhineland Palatinate, infection data of the federal state of Mecklenburg-Western Pomerania were used as data basis as well.

The simulation was conducted on the basis of real hunting data of Rhineland Palatinate and the surveillance approach, performed following the regulations of the European Union was simulated.

With these simulations, the probability of case detection and the time until the first case detection were determined and used as reference values. These values were then compared to the values resulting from the simulation of different alternative and risk-based surveillance methods. Alternative surveillance methods were increased sampling in the age class at higher risk of infection or in the season with an assumed higher detection probability. Furthermore we simulated the sampling in dependence of the population density in the different districts in two different ways. One approach was to sample only in districts where the population density was above a predefined threshold, whereas the other approach was to determine the sample size in the individual districts in dependence of the population density.

**Results:** The results of the fecal DNA analyses showed an estimated mean of 2,593 wild boar per district with a maximum of 8,524 and a minimum of 30 wild boar (median: 2,163). The real hunting data collected in specified districts (27 districts) of Rhineland Palatinate between 2003 and 2014 constituted the basis for the age and sex structure of the simulated population. In total we had 105,439 records. In the analyzed data the sex proportion of shot male and female animals was almost equal (male: 52.4%; female: 47.6%). The age distribution showed that the number of shot animals aged less than one year were highest (55.1%) and the number of samples coming from animals over 2 years was lowest (10.8%). Almost all samples (99.6%) were collected from hunted healthy animals, i.e. from active surveillance. Most samples were taken in the months of November (15.92%), December (13.68%) and January (12.57%). For the simulation of hunting, averaged values of the hunting bag data from 2003-2011 were used. In average 1,340 wild boar/district/year were hunted (min: 0; max: 4,573; median: 1,113).

For the setup of the infection into the simulated population, data from Mecklenburg-Western Pomerania were used. They consisted of 17,492 data sets resulting from the years 1993 and 1994, which were collected in six non-vaccination districts. Within this dataset, 2,652 samples had tested seropositive for CSF.

Due to the low number of positive cases identified through serological and virological testing in the data set of Rhineland-Palatinate, surveillance data from non-vaccination districts within Mecklenburg-Western Pomerania were used for risk analysis. The data consisted of 85,105 data sets from 1994-2000. The sex, age and carcass distribution showed a similar pattern as the data from Rhineland-Palatinate. Bivariate analysis showed that age plays a role in the probability of being serologically or virologically positive. These findings were supported by the findings of the literature search as well as by expert opinion. In the literature, it was found that the probability of samples gathered through passive surveillance being positive is assumed to be higher. However, the low number of available passive surveillance data made it impossible to support this assumption through statistical analysis. Additionally, experts suggest that seasonality of the hunting and infection as well as population density may be considered as risk factors for infection. The following initial results refer to simulations in a defined area of Rhineland Palatinate consisting of three neighboring districts. From 1000 simulation repetitions of sampling 59 samples per district over the year, by serological examination the infection was detected 1000 times per district (reference value for detection probability). On average 331 cases were detected in the first month of infection (reference value for time until first case is detected). By serological investigations of 59 animals aged less than 1 year infection was not detected at all in 11 simulation runs. On average 162 cases were detected within the first month. However, investigating 59 animals aged over 2 years serologically, the infection was detected 1000 times and 688 times already in the first month of infection.

**Conclusion:** The first results of the simulation model indicate that alternative surveillance strategies show a similar probability of detection. However, serological surveillance of animals aged over two years reduced the time until the first case was detected significantly.

Additionally, it might be assumed that through changes in sampling strategies e.g. based on population density thresholds, a reduction of costs is possible. The resulting financial resources could be used to increase sampling following the risk based approaches, therefore increasing the detection probability. They could also be used for campaigns supporting for example passive surveillance. However, the effectiveness of the single surveillance approaches should not only be measured by the outputs of the model, but also through a comprehensive evaluation of the whole surveillance system, including acceptability and practicability of the system. Moreover, the surveillance could be designed taking different risk factors into account and could therefore have the potential to result in a better performance.

If higher numbers of animals found dead were investigated, passive surveillance would be an alternative. However, also in the future it will be difficult to reach higher numbers of samples from passive surveillance.

Finally it has to be mentioned that due to the limitation of getting accurate population abundance data in wild life, designing surveillance schemes and the establishment of effective surveillance strategies will always constitute a certain challenge.

## Quantification of African swine fever virus transmission parameters in carriers and the possible role of indirect virus transmission

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**Objective:** In 2007 African swine fever (ASF) virus (ASFV) was introduced in Georgia, and through Russia it reached the EU (Poland and the Baltic states) in 2014. So far the disease seems to be a typical disease of backyard holdings, with virus spread mainly through direct contact and swill feeding, with only occasional introductions in industrial herds. Wild boar are heavily involved in many areas, and while no one doubts they play a role in the spread of the virus, the extent of their role is unclear and under discussion. The Eastern European ASFV strain seems almost 100% lethal, at least under experimental circumstances. Infections with less virulent strains, however, result in some pigs that survive the infection and remain persistently infected for several months (1), while showing no clinical signs. These carriers could potentially play a major role in the virus being sustained in wild boar population. This will, however, also depend highly on the infectiousness of these carriers, and it is still unclear how that compares to the infectiousness of pigs in the acute phase of the infection.

Besides through direct contact or swill feeding, spread of virus may occur through indirect routes as a result of contamination of the environment or fomites. A high stability of the virus in the environment will facilitate the virus to become and remain endemic in a population. Furthermore, this would, especially for industrial herds, facilitate the spread of the virus to other farms. Indirect transmission routes, which are difficult to identify and control, are for instance a major problem during outbreaks of classical swine fever (CSF). Both the excretion patterns from infected pigs and the infectious dose of ASFV suggest that indirect transmission may be of limited importance for ASFV as compared to CSF virus (CSFV). This hypothesis has, however, never been tested under controlled circumstances.

The objective for the current study was to estimate quantitative parameters for ASFV transmission by carriers and to elucidate the possible role of indirect virus transmission.

**Methods:** Twenty pigs, housed in four pens, were successfully inoculated with ASFV through the intranasal route with the moderately virulent strain Netherlands '86. In previous experiments with this strain, roughly half of the inoculated pigs tend to survive and become a carrier. Excess faeces were removed on a daily basis from the pens with a shovel, but no further cleaning and disinfection was carried out.

**Contact infections by carriers:** Six pigs, divided over three pens, survived the acute phase of the infection and became carriers. Four weeks after the inoculation, these six carriers were moved to new, clean pens, one pig per pen. In each pen, one contact animal was added for a maximum of 13 days, or until the contact animal became infected. After 13 days, all remaining contact pigs were removed and 6 new contact pigs were added 24 hours later for another 13 days (or until they became infected). Contact animals were tested frequently by PCR on oropharyngeal swabs and blood for evidence of an infection. The six carriers were regularly tested for viremia and virus excretion by PCR and virus isolation. Transmission rate parameters ( $\beta$ ) were estimated for each period separately and for both periods together.

**Infections from the environment:** The four pens were repopulated with two sentinel piglets each, 24 hours after the inoculated pigs were removed, for a period of 14 days. The sentinel pigs were frequently tested by PCR on oropharyngeal swabs and blood for evidence of an infection. Environmental samples were collected from the pens on a regular basis and tested for virus by PCR and virus isolation.

**Results:** *Contact infections by carriers:* In the first group of contact pigs, who were in contact with the carriers for 13 days between day 28 and 41 after the inoculation, none of the contacts became infected with ASFV. In the second group of contact pigs, who were in contact with the carriers for 13 days between day 42 and 55 after