

7. Summary

Characterization of virulence of CSFV isolates originating from wild boar

The purpose of this study was to characterize the virulence of 3 CSFV field isolates obtained from wild boar in the 1990's using *in vivo* and *in vitro* methods. The isolates belong to genotypes 2.3 Rostock (2 isolates from Mecklenburg-Western Pomerania) and 2.3 Guestrow (1 isolate from Brandenburg). A special focus of this work was to validate *in vitro* methods for their suitability to characterize the virulence of different CSFV strains.

Infection experiments with each CSFV isolate were carried out using both, domestic pigs and wild boars, to elucidate the influence of host factors and species on clinical outcome and etiopathology. Clinical picture, course of body temperature, duration of virus shedding, and production of antibodies against CSFV were used as parameters for the evaluation of clinical course and severity of the virus infection. A virulence score for the respective CSFV isolate was then calculated for every single animal as well as for the particular species of swine.

Independently of the applied CSFV isolate all infected domestic pigs showed a subacute etiopathology and a lethal outcome of the disease. After an initial onset of fever the animals developed typical clinical symptoms of CSF in the second to third week *p.i.* and died or were euthanized in a moribund stage until the end of week four *p.i.* Viraemia and virus shedding prolonged until the death of the infected animal. No neutralizing antibodies were detected in any serum sample of the domestic pigs. All 3 tested CSFV field isolates proved to be highly virulent for the infected domestic pigs.

In contrast wild boars partially showed a less homogenous clinical outcome. As in domestic pigs, infection of wild boars with one of the virus isolates of genotype 2.3 Rostock caused a subacute course and all infected animals died. Others, infected with the second isolate of type 2.3 Rostock developed a chronic form of the disease and survived until the end of the experiment at day 61 *p.i.* One of these wild boars showed viraemia and virus shedding during the entire experiment. Unlike the other two infected animals this wild boar did not produce any neutralising antibodies during the observed period. Infection of wild boars with the CSFV isolate of genotype 2.3 Guestrow resulted in an even more variable clinical outcome. While 3 out of 5 animals died of subacute CSF the remaining 2 showed only a transient form of the disease. Due to the different etiopathology the examined CSFV isolates were classified as moderately virulent for wild boars. Potential reasons for the differences in virulence scores of the tested virus isolates observed between individual wild boars and in comparison with

domestic pigs are elucidated. Furthermore, the relevance of chronic CSFV infections in wild boars for the epidemiology of CSF is discussed. Taken together the results emphasize the impact of host factors for the outcome of a CSFV infection.

Experiments for the *in vitro* characterization of CSFV additionally included several older isolates of genotype 1.1 representing different virulence scores as well as another field isolate from wild boar belonging to genotype 2.3. The latter proved to be low virulent in experimentally infected wild boars.

One-step growth curves showed significant differences in mean titer ratios of cell-bound and secreted virus between CSFV of genetic groups 1.1 and 2.3. On the other hand no correlation between these ratios and virulence of the respective viruses could be shown. Nevertheless, ratios of cell-bound and secreted virus titres at single time-points as well as the growth rate of cell-bound virus per hour support a correlation between *in vitro* growth and genotype of the tested CSFV isolates.

Furthermore, glycoprotein E2 expression was detectable at an earlier time-point in growth kinetics of CSFV strains belonging to group 1.1 than in those of group 2.3 field isolates. Luminescence counts detected in E2 kinetics of group 1.1 strains reached a multiple of those measured for group 2.3 isolates. Thus, expression of gpE2 also depends on the genotype of the respective CSFV, but not on its virulence.

Determination of plaque sizes after blocking of infected cell monolayers with hyperimmune serum showed no differences between the CSFV isolates tested. Plaque diameters varied over a relatively wide range. Infections of cells with and without the addition of hyperimmune serum resulted in distinct differences in the distribution of infected cells when performed with a low virulent CSFV, but not when a highly virulent virus was used. However, since CSFV of different genetic groups were used for those experiments no final conclusion can be drawn with regard to the role of genetic differences and virulence.

In vitro methods used in this study did not allow a classification of the CSFV strains and isolates examined. Nonetheless similar growth properties were shown for genetically more closely related viruses but not for CSFV of the same virulence score. Consequently, in our investigations animal experiments proved to be the most useful tool for characterization of the CSFV virulence.