7. Summary

Efficacy of a new bait form for oral immunization of wild boar against classical swine fever virus

A comparative study of vaccination with lyophilised C-strain and chimeric pestiviruses

This thesis deals with the advancement of oral immunization of wild boar, focussing on the creation of a new bait formulation. The basic objective of oral immunization is to enhance herd immunity and to eliminate the CSF pathogen within a short time frame. Higher bait uptake rates in gruntlings would on one hand lead to an increase of the seroprevalence rate in the wild boar population and on the other hand to a reduction of the number of susceptible animals. This would permit an even more effective control of CSF in the wild boar population.

The uptake studies performed with domestic pigs and wild boar described in this exposition revealed, that gruntlings below four months of age only played with the baits and showed an incomplete uptake. By four months of age, the animals began to develop an active bait uptake initially showing a preference for the small baits (1.8 cm diameter). By the age of five to six months, the animals began to pick up the spheroidal 3 cm bait.
The 3 cm bait was taken as a model for the creation of a new box vaccine container. In order to incorporate a sufficient amount of virus antigen into the bait, the vaccine ("C"-strain/CP7_E2alf) was lyophilised and filled into hard gelatine capsules which were then covered with the maize bait masse. Following investigations on the bait uptake, the lyophilised vaccine (we used the conventional "C"-strain and the chimeric pestivirus CP7_E2alf) was examined for its stability in comparison to the liquid formulation under different environmental conditions. It could be shown, that both vaccines are much more stable in the lyophilised than in the liquid form ($p < 0,05$). Due to the higher stability of lyophilised vaccine at temperatures over 20°C, the new formulation represents an improvement and is especially suitable for immunization campaigns carried out at higher environmental temperatures (in summer or in warmer regions).
In the first part of the animal experiments carried out for the present thesis, the efficacy of the bait formulation containing lyophilised "C"-strain virus was studied. After administration of

the spheroidal vaccine baits to wild boar and domestic pigs followed by an infection with a low dose of CSFV strain “Alfort 187”, all vaccinated domestic pigs and wild boar were protected from a CSF infection and we were neither able to isolate challenge virus from BC nor from nasal mucous, eye fluid, saliva or faeces.
In a second confirmatory trial, pigs were immunised with lyophilised “C”-strain and the spheroidal 3 cm bait and were subsequently subjected to a challenge infection with 1,000 $TCID_{50}$/ml.
Again, the animals, which had picked up the bait completely, were protected from a lethal CSFV infection. Neither viremia nor virus shedding could be detected. The pigs developed high neutralising antibody titres.
To conclude, the new bait-formulation consisting of:

a) a smaller bait (d = 3 cm)
b) a hard gelatine capsule with snap-lock and
c) lyophilised “C”-virus

is able to protect pigs against a severe CSF infection and simultaneously prevent viremia and virus transmission.

As in the future DIVA vaccines might have a positive impact on the control strategies against CSF in domestic pigs and wild boar, the development of new marker vaccine candidates also was one of the aims of an EU-project.
Therefore, the last two animal experiments described in this thesis were performed in line with this project, using the chimeric marker vaccine CP7_E2alf in a lyophilised formulation with the bigger spheroidal bait and analogously to the experiments carried out with “C”-virus. In contrast to the “C”-strain, the freeze-dried chimera was not able to protect the infected pigs from a lethal infection. All vaccinees (wild boar and domestic pigs) became ill showing CSF typical symptoms. In the virological examination, virus was found in the blood as well as in all body fluids examined (nasal mucous, eye fluid, saliva and faeces), so that the animals died or had to be killed in a moribund state.
We could not clarify conclusively whether the negative result of this animal experiment was due to low virus antigen content or rather the consequence of a non-optimal E1E2-heterodimer-complex in the chimera.
Based on these findings, another animal trial was performed with an improved CP7_E2alf chimera consisting of the E2-gene of CSF-strain “Alfort 187” and the E1-gene of this CSFV strain instead of the E1-gene of BVDV (CP7_E1E2alf). Besides this newly created virus the

previous chimera CP7_E2alf and the “C”-virus were used for parenteral vaccination. In animal experiments with domestic pigs, a complete protection from clinical signs and virus excretion could be observed with both chimeric pestiviruses and in the “C”-virus group after i.m. immunization. After vaccination, the weaner pigs developed neutralising antibodies, whereas at the time point of challenge the animals of the CP7_E1E2alf group reached the highest antibody titres in the virus neutralisation test, followed by the “C”-virus vaccinated and the CP7_E2alf immunised pigs. Because of the higher antibody titres in the CP7_E1E2alf group, it could be assumed that this construct with its CSF-E1E2-dimer is more effective with regard to the production of neutralising antibodies.
Further studies on the efficacy of this new chimera after oral administration as well as on its stability and safety are not part of this thesis, but are regarded as important exploratory focuses for future research.

With the new bait formulation it should be possible to reach a larger number of gruntlings by oral immunization. As a result, more animals will be protected against CSFV infection and in the consequence will not be able to serve as virus carriers spreading and shedding the CSF pathogen. Due to the improved stability of the lyophilised vaccine in comparison to the liquid suspension, the virus antigen content should not fall below the MID, even at high environmental temperatures and if the baits are not taken up for several days after distribution. This should lead to an effective immunization of wild boar over a long time period.

If further scientific studies prove that the lyophilised CP7_E1E2alf chimera is efficient after oral administration, this vaccine can be considered as a classical DIVA vaccine candidate which allows to discriminate field-virus infected animals from vaccinated animals also in wild boar.