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Comparative evaluation of live marker vaccine candidates "CP7_E2alf" and "flc11" along with C-strain "Riems" after oral vaccination

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ABSTRACT

Due to the tremendous socio-economic impact of classical swine fever (CSF) outbreaks, emergency vaccination scenarios are continuously under discussion. Unfortunately, all currently available vaccines show restrictions either in terms of marker capacities or immunogenicity. Recent research efforts were therefore directed at the design of new modified live marker vaccines. Among the most promising candidates the chimeric pestiviruses "CP7_E2alf" and "flc11" were identified. Within an international research project, these candidates were comparatively tested in challenge experiments after a single oral vaccination. Challenge infection was carried out with highly virulent CSF virus strain "Koslov", 14 or 21 days post vaccination (dpv), respectively. Safety, efficacy, and marker potential were addressed. All assessments were done in comparison with the conventional "gold standard" C-strain "Riems" vaccine. In addition to the challenge trials, multiple vaccinations with both candidates were performed to further assess their marker vaccine potential.

All vaccines were safe and yielded full protection upon challenge 21 days post vaccination. Neither serological nor virological investigations showed major differences among the three vaccines. Whereas CP7_E2alf also provided clinical protection upon challenge at 14 days post vaccination, only 50% of animals vaccinated with flc11, and 83% vaccinated with C-strain "Riems" survived challenge at this time point. No marked differences were seen in protected animals. Despite the fact that all multiple-vaccinated animals stayed sero-negative in the accompanying marker test, the discriminatory assay remains a weak point due to delayed or inexistent detection of some of the vaccinated and subsequently infected animals. Nevertheless, the potential as live marker vaccines could be confirmed for both vaccine candidates. Future efforts will therefore be directed at the licensing of "Cp7_E2alf" as the first live marker vaccine for CSF.

1. Introduction

Classical swine fever (CSF) is considered one of the most important infectious diseases of pigs and is notifiable to the World Organization for Animal Health (*Office International des Epizooties*, OIE). The multisystemic disease with a wide range of clinical pictures has tremendous socio-economic consequences (Edwards et al., 2000). The

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causative agent, classical swine fever virus (CSFV), is a small enveloped RNA virus belonging to the genus *Pestivirus* within the family *Flaviviridae* (Fauquet and Fargette, 2005). In several countries worldwide, CSF has been eradicated from the domestic pig population. To achieve this, mandatory vaccination with modified live vaccines in combination with sanitary measures was often used as a tool to control the disease (Van Oirschot, 2003). Yet, despite intensive efforts on the national and international level, complete eradication from the European Union (EU) has proven to be elusive, especially since the wild boar populations in several European countries are endemically infected. The current control strategy within the EU is a strict stamping out strategy without prophylactic vaccination. Nevertheless, community legislation also foresees the possible use of emergency vaccination, both in domestic pigs and wild boar (Anonymous, 2001). Legislation does not limit emergency vaccination to a certain type of vaccine, but only vaccines that allow differentiation of infected from vaccinated animals (DIVA) are discussible in terms of trade impact.

In general, two types of vaccines are available on the European market: (a) several modified live vaccines that have proven to be both efficacious and safe, and (b) E2 subunit vaccines that allow differentiation of infected from vaccinated animals (DIVA) but lack some properties that live vaccines exhibit, e.g. fast and sound protection after single application, complete block of transplacental infection or the oral application route. Thus, the current options are potent live vaccines that are accompanied by severe trade restrictions and subunit vaccines that are barely suitable for emergency situations. Due to these drawbacks, it is not surprising that within the EU emergency vaccination of domestic pigs has only been implemented once so far in Romania.

However, due to the vast economic consequences of CSF outbreaks, emergency vaccination plans are now again under discussion in EU Member States. To ease the trade restrictions, potent marker vaccines are required that allow reliable differentiation of infected from vaccinated animals. As wild boar populations will require oral vaccination, only live vaccines are suitable (Van Oirschot, 2003).

Despite the fact that several research projects dealt with the design and characterization of new marker vaccines, none of these candidates is close to market authorization. To meet the above mentioned demand, it is therefore of utmost importance to choose candidates and bring them to the level of licensing.

At the moment, vaccines based on viral vectors or chimeric pestiviruses seem to be among the most promising candidates (Beer et al., 2007). Among the chimeric pestiviruses two have been previously described, namely "CP7_E2alf" (Reimann et al., 2004) and "flc11" (van Gennip et al., 2000).

Within the EU funded project "Improve tools and strategies for the prevention and control of classical swine fever" (CSFV_goDIVA, KBBE-227003), the above mentioned marker vaccine candidates were assessed in several vaccination/challenge experiments in different facilities and compared to the "gold standard" C-strain "Riems" vaccine in order to choose a final candidate that should be brought to registration and licensing. In this context, we report on comparative vaccination trials conducted at the Friedrich-Loeffler-Institute (FLI), Isle of Riems, Germany, and in parallel at the Institute of Virology and Immunoprophylaxis (IVI), Mittelhäusern, Switzerland. The aims were to evaluate both vaccine candidates independently in two different laboratories by performing challenge experiments after one-shot oral immunization, and to assess DIVA capacities after multiple vaccinations.

2. Materials and methods

2.1. Vaccines and challenge virus

Two chimeric pestiviruses were assessed as marker vaccines in the present study: The previously characterized "CP7_E2alf" is a chimeric pestivirus based on the cytopathogenic bovine viral diarrhea virus (BVDV) strain "CP7" expressing the E2 glycoprotein of CSFV strain "Alfort/187" (Reimann et al., 2004). Differentiation of CSFV field strain infection from CP7_E2alf vaccination is possible by a commercially available CSFV-specific E^{RNS} ELISA. CP7_E2alf vaccine virus stock was produced under 'Good Manufacturing Practice (GMP)' conditions in swine kidney cells (SK-6) grown in bioreactors and provided by Fort Dodge Veterinaria S.A./Pfizer (Spain). The titer of the GMP-produced CP7_E2alf was $10^{6.4}$ tissue culture infectious dose 50% ($TCID_{50}$) per ml. The liquid vaccine was used as formulated, one dose consisting of 2 ml without further dilution.

The second vaccine candidate termed "flc11" is based on a CSFV C-strain backbone in which the E^{RNS} gene (amino acids 268–494) was replaced by the corresponding region of BVDV II strain "5250" (van Gennip et al., 2000). Again, differentiation of CSFV field strain infection from flc11 vaccination is possible by the same CSFV-specific E^{RNS} ELISA mentioned above. Vaccine virus stock of flc11 was obtained from the Central Veterinary Institute (CVI), Leylstad, the Netherlands, and diluted tenfold in phosphate-buffered saline (PBS) for vaccination. One vaccine dose was 2 ml of diluted flc11 per animal.

Commercially available and licensed C-strain "Riems" vaccine (RIEMSER® Schweinepestoralvakzine) was used according to the manufacturer's recommendations (Riemser Arzneimittel AG, Greifswald – Insel Riems, Germany). The content of one vaccine blister (1.6 ml C-strain "Riems" vaccine with a minimum titer of 10^4 $TCID_{50}$ per vaccine dose) was administered to the animals individually by the oral route.

Challenge infections were carried out using the highly virulent CSFV strain "Koslov" derived from an animal experiment at the FLI where whole blood was collected, defibrinated, and prepared as challenge material. The mean titer after three titrations was $10^{7.5}$ $TCID_{50}$/ml blood. For challenge infection, the blood was diluted 1:120 in PBS to obtain a titer of $1 \times 10^{6.0}$ $TCID_{50}$/ml, and a dose of 4 ml was applied to the animals by oronasal inoculation.

Both, vaccines and challenge virus were back-titrated after administration.