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Pre-registration efficacy study of a novel marker vaccine against classical swine fever on maternally derived antibody positive (MDA+) target animals

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ABSTRACT

Maternally Derived Antibodies (MDA) can have a negative effect on the efficacy of live attenuated vaccines against classical swine fever (CSF). For this reason, a marker vaccine candidate CP7_E2alf was tested for its efficacy in the presence of MDA. Pregnant sows were vaccinated four weeks before farrowing with CSF virus (CSFV) strain "Thiverval". A total of 40 piglets with MDAs were included in this study. At six weeks of age the piglets were allocated into three treatment groups using generalized randomized block design (GRBD) blocking on serological status and pen location.

Of the 40 piglets with MDAs, 30 piglets were vaccinated either orally (n = 15) or intramuscularly (n = 15) with a single dose of vaccine candidate produced under Good Laboratory Practice (GLP) conditions. The ten remaining piglets were allocated into the untreated control group. All 40 piglets were oronasally challenged with 2 ml of the highly virulent CSFV strain "Koslov" 14 days after vaccination.

It was revealed that presence of MDAs negatively influences the efficacy of the live marker vaccine candidate, however, the extent of this negative impact depends on the route of vaccine administration. Based on our observations, intramuscular vaccination is recommended during CSF control programs in order to develop superior immune protection.

1. Introduction

Classical swine fever (CSF) causes serious economic losses to the swine industry worldwide and outbreaks are notifiable to the World Organisation for Animal Health (OIE). Since 1990, prophylactic vaccination is banned in the EU and preventive slaughter of pig herds is a major control measure in the occurrence of an epidemic [1]. The high costs of mass slaughter of animals, together with ethical concerns and negative publicity [2,3], intensified the "vaccination to live" vs. pre-emptive slaughter debate.

Generally, an emergency vaccination scenario should target the animals at the earliest possible time point. If the sow has been vaccinated, offspring are protected by the maternally derived antibodies (MDAs) in the very first period of their life but later, as MDAs decline, they become susceptible to diseases including CSF. An optimal vaccination programme should build protection in the piglets towards the end of the MDAs immune protection window. In practice there is a period of overlap, as MDA levels decrease and vaccine-induced antibody (VIA) levels increase, when MDAs and VIAs could be present simultaneously. Vaccination during pregnancy is a feasible tool to control swine diseases such as swine influenza, pseudorabies or *Erysipelothrix rhusiopathiae* [4–7]. Vaccination of neonates is still a challenge for veterinary vaccine research because of the complication that MDAs interfere with the immune response triggered by the vaccine [4–9]. The potential outcomes of this interference between MDAs and VIAs are as follows:

i.) A reduction in vaccine efficacy, which may lead to increased animal-to-animal transmission of wild-type virus, as was

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observed in experiments with vaccination against pseudorabies virus [8].

ii.) Lower VIA levels induced by killed vaccines [4–8,10] or modified live C-strain vaccine [11,12] and weaker humoral immune response against CSFV.

iii.) Incoherent vaccine responses among MDA+ pigs, caused by neutralisation of the vaccine virus by MDAs, could result in very variable levels of protection with subgroups of poorly protected pigs that CSFV can circulate within (R0 > 1 within subgroups).

It is important to note that MDA+ animals are not expected in an emergency scenario within EU because preventative slaughter is a primary control measure, however, emergency vaccination is allowed under certain circumstances [13]. The currently available live attenuated vaccines cannot distinguish between infected and vaccinated animals so in vaccinated populations CSF-free status cannot be confirmed by serosurveillance. Marker vaccines, which allow for differentiation between infected and vaccinated animals (DIVA), could allow countries to use emergency vaccination without facing serious restrictions to their pig export markets. A chimeric Pestivirus CP7_E2alf marker vaccine candidate was developed using a CP7 bovine diarrhoea virus (BVDV) backbone that expresses the E2 glycoprotein of Alfort/187 CSFV strain [14]. The CP7_E2alf marker vaccine candidate only induces CSF-specific antibodies against E2 in vaccinated pigs so other immunogenic proteins, such as Erns, can be used to identify pigs that have been infected with wild-type virus. The immunogenic proteins of CSFV can be detected in commercial ELISA tests or other discriminatory tests such as a multiplex microsphere immunoassay [15].

The efficacy of the CP7_E2alf marker vaccine candidate in piglets with MDAs, either induced by C-strain [16] or by CP7_E2alf [17], has already been tested. Our study is the first to investigate the efficacy of a single dose of CP7_E2alf marker vaccine, administered either intramuscularly or orally, in 6-week-old domestic piglets with MDAs. The study is in accordance with the European Pharmacopeia (Ph.Eur.) Monographs 0065, “Swine-fever vaccine (live, prepared in cell cultures), classical” 7th Edition [18] and Ph.Eur Chapter 5.2.7. “Evaluation of efficacy of veterinary vaccines and immunosera” 7th Edition [19]. The requirements of Ph.Eur cannot apply fully to this study because the quoted Ph.Eur. lines demand tests on MDA-piglets.

2. Materials and methods

2.1. Experimental design and animals

Six pregnant Kahyb-bred sows were purchased from a local vendor (Pietkert Ltd, Hungary). Animals were moved to the animal facility at 75 days of gestation. The sows were vaccinated with CSFV strain “Thiverval” (Ceva-Phylaxia Co.) four weeks before farrowing. Piglets from the vaccinated sows were included in the study on basis of presence of MDAs. Serological tests were performed at 2 and 5 weeks of age using the commercial HerdChek® CSFV Ab ELISA Test Kit in order to determine the serological status of each piglet. A total of 40 six-week-old piglets were enrolled in the study and all were ELISA positive for CSFV antibodies at 2 and 5 weeks. The piglets were officially free of brucellosis, leptospirosis, porcine reproductive and respiratory syndrome (PRRS), and pseudorabies (SuHV-1). No pre-treatment was applied. Feed and water were provided *ad libitum*.

The study design was published by Lévai et al. [20]. Briefly, piglets were allocated to two treatment groups (TG, n = 15) and one control group (CG, n = 10) using a generalized randomized block design (GRBD) blocking on serological status and pen location. TG1 piglets received intramuscular (i.m.) administration and TG2 received oral administration. All 40 piglets were challenged with 2 ml of CSFV strain “Koslov” via the oronasal route at 14 days post vaccination.

Rectal body temperatures were measured daily using a manual thermometer from 3 days pre-vaccination until 7 days post vaccination and from 3 days pre-challenge until 21 days post-challenge. CG animals did not have rectal temperatures collected from days 1–14 post-vaccination. Fever was defined as a body temperature ≥40.0 °C for at least two consecutive days. Pigs with temperature ≥40 °C for two consecutive days were still enrolled, if they did not have any other abnormal clinical signs and appeared fit and healthy. Elevated body temperatures for one day were reported.

During the trial, animals were handled according to the welfare regulations and standards of Directive 2010/63/EU [21] and the internal SOPs of National Food Chain Safety Office (NFCSO). After completion of the study, all animals were disposed of by means of incineration or treated as contaminated tissues.

2.2. Vaccine, challenge and neutralisation virus

Two different batches of CP7_E2alf (5th passage of the Master Seed Virus) with minimum potency of 100 PD_{50} were produced under GLP conditions by Pfizer Olot S.L.U. (Spain) for i.m. and oral application. Vaccine candidate was formulated as 75% CP7_E2alf antigen+25% L2 stabilizer. For oral administration, a volume of 1.6 ml of Batch 191211 was administered at a 1:38 dilution. For i.m. administration, 1 ml of Batch VMRD-112-005 was applied at a 1:100 dilution. Unused vaccines were kept for the duration of the trial at the appropriate temperature (4 °C or −70 °C) and, thereafter, all remainders were autoclaved.

The piglets were challenged with 2 ml of the highly virulent Koslov strain (from the stock of Friedrich-Loeffler-Institut, Insel Riems, Germany), $10^{5.7}$ $TCID_{50}$/ml, which was administered oronasally to each animal (approximately 0.5 ml in each nostril and 1.0 ml in the mouth using a 2 ml syringe without needle). To obtain the real titres administered, both vaccine and challenge viruses were back-titrated.

The CSFV strain “Alfort 187”, which was used in the neutralisation tests, was kindly provided by the Community Reference Laboratory for CSF (TiHo Hannover, Germany).

2.3. Clinical examinations

Clinical signs were recorded according to the time schedule published by Lévai et al. [20]., the clinical signs were evaluated according to the Mittelholzer scoring system [22].

2.4. Sampling

From each animal a total amount of 10 ml blood, with and without anticoagulant, was taken from the *vena jugularis* or from the sinus *vena ophtalmica externa ventralis* using BD Vacutainer®. Each sample was identified with the number of the piglet, the study number and the date.

Serum samples for ELISAs and virus neutralisation were obtained from uncoagulated whole blood by centrifugation at 2000 × rpm at 4 °C for 10 min. Serum samples were aliquoted into pre-labelled cryotubes. Samples for virus isolation were obtained from Li-Hep blood by centrifugation at 6000 × g at 4 °C for 30 min. Subsequently, the samples were diluted tenfold in PBS containing antibiotics to a final volume 1.5 ml and were aliquoted into pre-labelled cryotubes.