University of Veterinary Medicine
Doctoral School

Efficacy studies on target animals with a newly developed marker vaccine candidate against classical swine fever

Summary of PhD theses

Réka Felföldiné Lévai

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Supervisor and members of the supervisory board:

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Dr. Attila Farsang
(Project manager)
Ceva-Phylaxia Ltd.
Supervisor

Prof. Dr. Tibor Soós
(Retired director)
National Food Chain Safety Office
Directorate of Veterinary Medicinal Products
Member of the supervisory board

Dr. Gábor Kulcsár
(Director)
National Food Chain Safety Office
Directorate of Veterinary Medicinal Products
Member of the supervisory board

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Réka Felföldiné Lévai

I. Introduction

Efficacy studies on laboratory and target animals are essential steps in developing human and veterinary vaccines. Investigating the biological features, safety and efficacy of vaccine candidate strains could be of crucial importance during the early stages of development and are obligatory to compile the registration dossier.

In my dissertation I make inquiries about the key aspects of vaccine development against classical swine fever, a significant veterinary pathogen from immunological, methodical, quality management and official point of view.

The new generation vaccines play an increasing role in preventing and controlling human and veterinary diseases. In this dissertation I demonstrate the efficacy studies in accordance with the European Pharmacopea with CP7_E2alf chimeric vaccine strain against classical swine fever.

II. Objectives

Our goals were:

i) to demonstrate the efficacy of one dose of CP7_E2alf marker vaccine candidate in challenge animal trials,

ii) to demonstrate the onset of immunity (OOI),

iii) to compare the effects evoked by the different application methods of the vaccine candidate in six-week-old maternally derived antigen negative (MDA-) and positive (MDA+) piglets,

iv) to investigate the success of immunisation in the shade of maternal antibodies.

II. Materials and methods

The two studies were performed under identical circumstances with identical settings at the closed animal facility in Gödöllő of National Food Chain Safety Office (NFCSO) Directorate of Veterinary Medicinal Products (DVMP). The only difference was the immunological status of the piglets: in the first study we use MDA- animals against pestiviruses while in the second study we use MDA+ piglets. In order to possess MDA+ piglets we purchased six KAHYB bred pregnant sows and on 75th days of their gestation they were moved into the closed animal facility. The sows were

vaccinated 4 weeks before farrowing with Thiverval CSFV strain. At 2 and 5 weeks of age serological tests were performed in order to see the serological status of the piglets. Only the seropositive animals were included in the MDA+ study.

A total number of 40 six-week-old piglets were included in both studies. They came by feed and water *ad libitum*.

The piglets were allocated into three different treatment groups: one control group (CG) contains 10 unvaccinated piglets and two vaccinated groups – TG1 contains 15 intramuscularly (im.) vaccinated piglets and TG2 contains 15 orally (p.o.) vaccinated piglets.

We registered the rectal temperatures and clinical signs regularly. An animal were considered feverish if its body temperature was above 40°C at least two consecutive days.

The piglets were handled during the studies in accordance with the 2010/63/EU guidelines and the animal welfare regulations of the DVMP. All piglets were euthanized in case of moribund condition or at the end of the study.

Piglets in TG1 were vaccinated with 1 ml of the CP7_E2alf vaccine candidate by intramuscular route while piglets in TG2 were vaccinated with 1.6 ml of the respective vaccine by oral route. The vaccine potency was 100 PD50 per dose. All piglets were challenged 14 days post vaccination with 2 ml of Koslov CSFV strain by oronasal route.

Before immunisation and after 14^{th}, 18^{th}, 21^{st}, 24^{th}, 28^{th} and 35^{th} days of vaccination serum samples were collected from the animals for E2 specific antibody ELISA (HerdChek® CSFV Ab ELISA [IDEXX Laboratories]), Erns specific antigen ELISA (HerdChek® CSFV Ag/Serum ELISA [IDEXX Laboratories]) and virus neutralisation tests. In order to isolate the virus from the whole blood samples virus isolation was performed. In the interest of detecting CSFV specific nucleic acid in the whole blood samples gel based conventional RT-PCR and for confirmation real-time RT-PCR were used.

Every dead and euthanized animal was subjected to post mortem examinations and for histopathological and immunohistochemical analysis organ samples were collected.

Vaccine efficacy (VE) was determined based on the following formula: VE = 1 – RR × 100 where RR (relative risk) = ARV (attack rate of vaccinated) / ARU (attack rate of unvaccinated).

III. Results and discussion

In the MDA- study all of the control animals showed CSF related clinical signs 4-5 days post infection and all of them died before the end of the study. Contrary to this in the MDA+ study only 6 control animals showed CSF related clinical sings 6 days post infection and only 70% of the control piglets died before the end of the study. Neither of the body temperatures of control animals reached 41.0°C in the MDA+ study. The moderate clinical signs and the lower mortality rate could be explained by the protection of MDAs.

In the MDA- study the challenge infection was successful as every blood sample taken from the control piglets was positive with antigen ELISA, 8 out of 10 with virus isolation and the 5 tested with RT-qPCR. The *post mortem* examinations performed on the control piglets proved the CSF specific changes and all the organ samples showed strong positive reactions during the immunohistochemical analysis.

It is important to be highlighted that 3 out of 10 control piglets from the MDA+ study were seroconverted: in their serum samples E2 specific antibodies were found after 10-14 days post infection and their neutralisation titers were slightly increased. Blood samples taken from the 7 spontaneously died piglets were positive with antigen ELISA, 6 out of this 7 with virus isolation and all of them with RT-qPCR. The *post mortem* examinations performed on the spontaneously died control piglets proved the CSF specific changes and the organ samples showed strong or mild positive reactions during the immunohistochemical analysis. However the blood samples taken from the 3 survived control piglets remained negative with antigen ELISA, virus isolation and immunohistochemical analysis. These findings demonstrate the role of the passive immunity and confirmed that our experimental model was well created as enough MDAs were presented in the piglets.

In the MDA- study mild clinical signs were observed after 4-5 days post infection in 5 from the TG1 piglets and in 6 from the TG2 piglets. However 1 out of these 6 sick TG2 piglets showed severe clinical sings and had to be euthanized before the end of the study.

Taking into consideration the negative serological and the positive antigen detecting results regarding this sick TG2 animal it could be conceivable that in this case the oral vaccination route was technically not fully proper. The TG1 piglets started to seroconvert after 14 days post vaccination (one week earlier than the TG2 piglets) and their neutralisation titers started to increase after 18 days post vaccination (one week earlier than the TG2 piglets). The blood samples taken from the treated animals (except for the previously discussed TG2 piglet) were negative with antigen ELISA and virus isolation. The vaccinated animals (except for the previously discussed TG2 piglet) did not show any severe clinical signs and except for two TG2 piglets no positive reaction was observed with immunohistochemical analysis.

Based on the above mentioned results it can be stated that the investigated vaccine candidate was efficacious in the MDA- study. Besides this all the serological and antigen detecting results, the clinical signs, the histological, histopathological and immunohistochemical findings obtained from the experimental animals were in line with each other. The experimental design and the applied challenge virus

strain were suitable to demonstrate the efficacy of a vaccine candidate.

However in the MDA+ study it is more difficult to draw an exact conclusion from the results while 3 out of 15 TG1 piglets showed mild and 9 out of 15 TG2 piglets showed mild and severe CFS related clinical signs and 7 out of this 9 TG2 piglets died spontaneously before the end of the study.

It can be stated that the im. applied vaccine gave appropriate protection in the piglets since it prevented the mortality, significantly reduced the clinical signs and the virus amount in the blood with the help of increasing the E2 specific antibody levels. However serum samples obtained from 9 out of 15 TG2 piglets were negative 14 days post vaccination with antibody ELISA and 6 out of this 9 sera remained negative until the death of these TG2 piglets. In addition the blood samples obtained from the 7 spontaneously died TG2 piglets were positive with antigen ELISA, with virus isolation and with RT-qPCR so we confirmed the virus presence.

Nowadays when African swine fever (ASF) an epidemiologically similar disease compared to CSF showed up and cause severe economic losses it

became more important to develop a suitable vaccine bait for wild boars.

Overall conclusions stated that this chimeric vaccine candidate against classical swine fever which formed the basis of my PhD theses could be a useful tool in controlling the disease. However it is only to be used in an outbreak situation in herds within restricted control zones.

IV. New scientific outcomes

We demonstrated first the efficacy of the CP7_E2alf chimeric vaccine candidate against classical swine fever using minimal dose.

The CP7_E2alf chimeric vaccine candidate gave appropriate protection with both application methods in piglets lack of pestivirus specific maternally derived antibodies. However the oral application compared to the intramuscular application gave only partial protection in piglets with pestivirus specific maternally derived antibodies.

We demonstrated that the CP7_E2alf chimeric vaccine candidate is suitable to eliminate the disadvantage of live attenuated C-strain based vaccines: the lack of DIVA potential.

V. List of publications

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