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Conference Title

Adjuvant formulations designed to improve swine vaccine stability: Application to PCV2 vaccines

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Abstract

Porcine circovirus associated diseases (PCVADs) are economically important diseases of domestic pigs caused by porcine circovirus type 2 (PCV2). PCV2 vaccination is usually performed with adjuvanted inactivated formulations and is necessary to control PCVADs and subclinical PCV2 related body weight losses in pig farming. An important issue with PCV2 vaccine formulation is that PCV2 antigenic media often have properties which destabilize vaccine formulations. Vaccine adjuvants are a key parameter in modern vaccination closely linked to galenic properties of vaccine formulations, and galenic stability is necessary to insure efficacy stability during vaccine shelf life. Here we show that especially designed formulations based on Montanide™ ISA 11R VG (Oil in water) and Montanide™ ESSAI Gel R (polymer) adjuvants are able to resist to very destabilizing antigenic media and conditions while keeping safety parameters and efficacy at requested levels.

Keywords: Adjuvant; Stability; PCV2; Swine vaccine; Montanide

1. Introduction

In order to control porcine circovirus associated diseases and reduce body weight losses in pig farming, adjuvanted inactivated or subunit PCV2 vaccines are applied to swine on a routine basis in all parts of the world [1]. Pigs are sensitive animals which need specific vaccine formulations to prevent local reactions and preserve meat quality at the injection site. Therefore, water continuous phase adjuvants such as oil-in-water (O/W) adjuvants, water-in-oil-in-water (W/O/W) adjuvants or polymeric adjuvants are usually preferred for injectable swine vaccination [2]. In specific cases, water-in-metabolizable oil adjuvants are also used to formulate one-shot vaccines.

Vaccine adjuvants are a key parameter in modern vaccination in order to increase the level of the induced immune response, the duration of the vaccinal protection and the orientation of the immune response. Most adjuvant formulations are composed of synthetic components combined to create a specific galenic antigen presentation. Moreover, most of the time, adjuvant components have intrinsic immunomodulator properties. The vehicle effect can be influenced by the evolution during storage of the

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organized system (e.g. emulsions) or by chemical degradations (oxidation, precipitations) of adjuvant components. More specifically, the stability of emulsions can be affected by the interaction of the adjuvant formulation with the antigenic media, and particularly during storage at room temperature. Some cell lines (e.g. PK-15) used to produce PCV2 antigen may also contain some components (e.g. enzyme) which disturb the emulsion's stability. One solution consists in decreasing antigen enzymatic activity by high temperature treatment [3] but this method is not compatible with sensitive immunogenic epitopes. During storage and transportation, stability issues of PCV2 vaccines have been reported (emulsion broken, high increase of viscosity…).

The Montanide™ range of adjuvants has been shown to be safe and efficient in diverse swine vaccines [4, 5, 6, 7]. We have developed new Montanide™ adjuvant formulations (polymer and O/W adjuvants) that are able to resist to very crude and concentrated destabilizing antigenic media and highly destabilizing storage conditions. Here we show that these adjuvants allow the conservation of stable PCV2 vaccines over the long term. We also show that the new adjuvanted PCV2 vaccines have safety and efficacy profiles that are comparable to standard PCV2 formulations.

2. Materials and Methods

2.1. New formulations: stability tests

First, new resisting adjuvants Montanide™ ISA 11R VG (O/W, ISA 11R) and Montanide™ ESSAI Gel R (polymer, ESSAI GEL R) were compared to reference adjuvants Montanide™ ISA 15A VG (O/W, ISA 15A) and Montanide™ Gel 01 PR (polymer, GEL) for galenic properties. To assess the stabilizing properties of resisting adjuvants, PCV2 vaccines were formulated with all adjuvants and stored at 4°C (normal conditions) or 20°C (destabilizing conditions). For these stability studies, two different PCV2 antigenic media (inactivated and recombinant) were tested respectively with O/W or polymer technologies. Stability of formulations was assessed after 6 months for polymers and 1 year of storage for O/W technologies by visual observation and default intensity quotation.

2.2. Model animals trials: Safety and efficacy

16 guinea pigs were separated in 4 groups of 4 animals. In groups 1 to 3, each animal received an intramuscular injection of 1ml of PCV2 vaccine formulated with the corresponding adjuvant. This injection was performed at D0 in the back left leg muscle. Guinea pigs from group 4 were not vaccinated and were used as a negative control. Vaccination groups were defined as follows:

- Group 1: Resisting adjuvant: ISA 11R adjuvanted PCV2 vaccine (1ml)
- Group 2: Resisting adjuvant: ESSAI GEL R adjuvanted PCV2 vaccine (1ml)
- Group 3: Commercial vaccine: "VERRES-CIRCO" by OOO "VETBIOCHEM", Moscow, polymer adjuvant technology (1ml)
- Group 4: Not vaccinated

Groups 1 and 2 received vaccines containing the same recombinant PCV2 antigen at the same antigenic dose. On D0 and D21, blood samples were taken from each animal, and PCV2 specific antibody titers were measured by enzyme-linked immunosorbent assay (ELISA). One animal in each group was euthanized at D21 and the injection site was dissected to observe any local reaction due to the vaccination.

2.3. Pigs trial: Vaccine groups

400 3-weeks old pigs (mixed Duroc, Landrace and Large white) of a farm susceptible to PCV2 infections were separated in 4 groups of 100 animals. All pigs were seronegative against circovirus at the

beginning of the trial. A non-vaccinated group was also analyzed as a control for natural infection. Pigs were randomly separated in 4 different vaccine groups.

On D0, each animal received an intramuscular injection in the neck of the corresponding vaccine:

- Group 1: Resisting adjuvant: ISA 11R adjuvanted PCV2 vaccine (1ml)
- Group 2: Resisting adjuvant: ESSAI GEL R adjuvanted PCV2 vaccine (1ml)
- Group 3: Commercial vaccine: "VERRES-CIRCO" by OOO "VETBIOCHEM", Moscow, polymer adjuvant technology (1ml)
- Group 4: Not vaccinated

Groups 1 and 2 received vaccines containing the same recombinant PCV2 antigen at the same antigenic dose.

2.4. Pigs trial: Safety assessment

Behavior of the animals was controlled on 3 days after vaccination. For 10 pigs in each group, body temperature and local reaction at the injection site was measured at 0, 4h, 24h and 48h post vaccination. At the end of the test, 10 pigs per group were slaughtered and local reactions at the injection sites were assessed after dissection.

2.5. Pigs trial: Efficacy assessment

At D0 (before vaccination), D30 and D120, blood samples were taken from 10 pigs in each group. Specific antibody titers in blood serum were assessed by specific ELISA titration (using commercial ELISA kit "CIRCO-SEROTEST" by OOO "VETBIOCHEM", Moscow).

To assess the protection conferred by the vaccine in field settings submitted to PCV2 infection, average daily body weight gain was assessed in each group to measure protection against subclinical PCV2 infection. The percentage of live pigs at the end of the trial was also assessed in each group of 100 pigs.

3. Results

3.1. Formulation properties

First, adjuvanted PCV2 vaccines stability was assessed over time in destabilizing conditions. New stabilizing adjuvants Montanide™ ISA 11R VG (oil in water) and Montanide™ ESSAI GEL R (experimental, polymer) were compared to reference O/W and polymer adjuvants.

1 year 4°C		**1 year 20°C**	
Standard O/W	Resisting O/W	Standard O/W	Resisting O/W
			
No Defect	No Defect	**Breakage Critical Defect**	**No Defect**

Fig. 1. Stability of PCV2 vaccines formulated with standard O/W or resisting O/W adjuvant (1 year at 20°C or 4°C).

Vaccines based on resisting adjuvants were perfectly stable over time, even in destabilizing conditions in which vaccines based on reference adjuvants showed stability defaults. Storage of standard O/W PCV2 formulation at 20°C for 1 year induced emulsion breakage. This defect was not observed when Montanide™ ISA 11R VG was used (Fig. 1.). Comparably, enhanced turbidity (non critical defect) was observed in the reference polymer vaccine after storage for 6 months at 4°C, whereas no stability defect was observed in the resisting polymer adjuvanted vaccine. These results show that these new adjuvants increase the stability of PCV2 vaccines. The resisting adjuvants were then tested for their safety and efficacy profiles in PCV2 vaccines.

3.2. Model animals results

The safety and efficacy of the vaccines were first assessed by vaccination of model animals. 4 guinea pigs in each group were vaccinated with 1ml of adjuvanted PCV2 vaccines: Group 1- ISA 11R (resisting O/W), Group 2- ESSAI GEL R (resisting polymer), Group 3- COMMERCIAL (polymer technology), Group 4- Not vaccinated.

No general reactions were observed after vaccination. At D21, no local reactions were observed after dissection of the injection site in any group. All animals were seronegative at D0. The vaccines based on resisting polymer adjuvant, the resisting O/W adjuvant and the commercial polymer adjuvanted vaccine showed similar antibody titers at D21 (Fig. 2.).

Following model animals trials, the efficacy and safety of the resisting PCV2 vaccines was assessed in a full scale field trial in a swine farm subject to PCV2 infection.

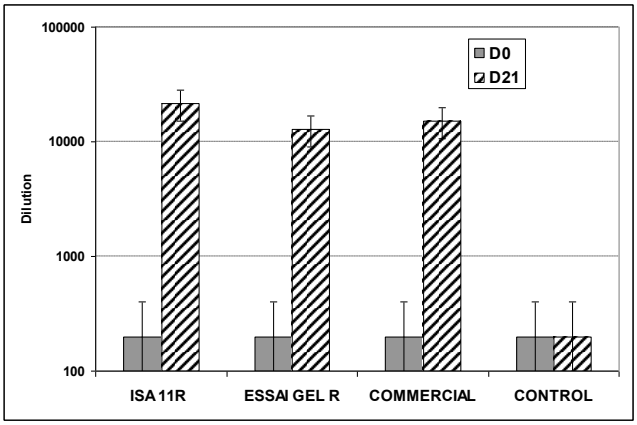

Fig. 2. Average antibody titers (dilution) after guinea pigs vaccination.

3.3. Swine field trial: Safety results

Groups	Transient oedema at injection: Frequency	Transient depressive state: Frequency
1- ISA 11R	3/10	12/100
2- ESSAI GEL R	1/10	6/100
3- COMMERCIAL	2/10	7/100

Table 1. Frequency of pigs showing depressive state and of local reactions at the injection site after vaccination.

Body temperature increase after injection was <1℃ in all groups. Some animals displayed slight depression after vaccination (Table 1), which disappeared after 3 days for all animals. Transient local reactions (small oedemas) at the injection site were observed at a low frequency in all vaccinated groups. All disappeared after 3 days. No lesions were observed at the slaughterhouse at the injection site. These results show that resisting adjuvants have an acceptable safety profile in swine which is similar to commercial formulations.

3.4. Swine field trial: Antibody titers

Titers at D0 show that all pigs were PCV2 seronegative at the beginning of the trial (Fig. 3). At D120, there was no difference in antibody titers between all 4 groups, including the non-vaccinated control group. This result shows that the animals have been as expected in contact with circovirus during the trial, and have therefore been submitted to a natural challenge. At D30, a lower variability among animals was observed in the commercial group compared to other vaccinated groups.

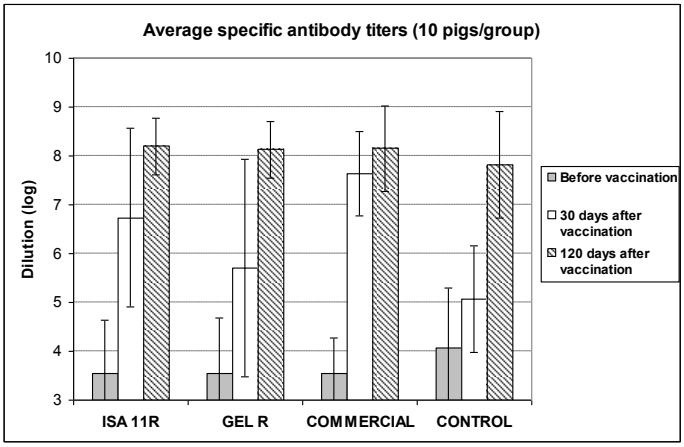

Fig. 3. Average IFA antibody titers (log of the dilution) after pigs vaccination (± sd)

3.5. Swine field trial: Body weight gain

As the animals have been submitted to PCV2 infection during the trial, average daily body weight gain and mortality rate of the animals are the best markers of performance of PCV2 vaccination (Table 2). Non-vaccinated control animals had reduced average body weight gain and lower probability of survival compared to vaccinated groups. The results show that resisting adjuvants induce a similar protection compared to commercial vaccine.

Groups	Average daily weight gain	% Alive pigs
1- ISA 11R	694 ± 7 g	96 %
2- ESSAI GEL R	697 ± 7 g	95 %
3- COMMERCIAL	698 ± 7 g	97 %
4- CONTROL	**664 ± 6 g**	**91 %**

Table 2. Average daily body weight gain (± s.e.m) and survival rate at D120

4. Discussion

Vaccine formulations can show stability defects over the long term, due to chemical degradations of the adjuvant (emulsions or polymer solutions) by enzymatic degradation or oxidation linked to the chemical properties of the antigenic media. These defects are usually enhanced and quickened by destabilizing storage conditions (especially in uncontrolled temperature conditions). We have shown that new resisting polymeric and O/W Montanide™ adjuvants increase the stability of swine vaccine formulations in presence of destabilizing antigenic media or in stressing storage conditions.

The goal of vaccine formulation is to produce vaccines that are robust and stable in a large range of conditions, have an acceptable safety profile, and induce a strong and specific disease protection. These parameters are interdependent and depend also strongly on the vaccine adjuvant. Adjuvant design should thus aim at reaching the best possible balance between stability, efficacy and safety of the final vaccine.

In this study we have shown that new resisting Montanide™ adjuvants have safety and efficacy profiles similar to reference standard adjuvant formulations in a PCV2 vaccine model. Resisting adjuvants Montanide™ ISA 11R VG and Montanide™ ESSAI GEL R are indeed able to induce high antibody titers in model animals and pigs, and show an acceptable safety level in swine. This new line of adjuvants will help to improve long term stability and thus long-term efficacy of pig vaccines which are based on destabilizing antigens or stored in stressing conditions.

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