Article

Montanide™ ISA 71R VG: A robust and flexible adjuvant formulation for potent and stable poultry vaccines

Juliette Ben Arous[a], Sebastien Deville[b], Svetlana Norkina[c], Vladimir Borisov[c], Tatiana Rozhdestvenskaia[c], Ivan Rozhdestvenskiy[c], Jérome Gaucheron[a], Laurent Dupuis[a]

a SEPPIC, 22 Terrasse Bellini, Paris La Défense, 92806 Puteaux Cedex, France
b SEPPIC, Knox Plaza, Mind Space, Off Chincholi Bunder Rd. Malad West, Mumbai 400064, India
c NPP AVIVAC, Leningradskaya area, village Gorbunki, 188 505 Saint Petersburg, Russian Federation
Contact: sebastien.deville@airliquide.com

Abstract:

Water-in-mineral oil emulsion adjuvants induce a strong long-term humoral immune response and are widely used in inactivated poultry vaccines. However, evolution and destabilization of vaccine emulsion formulations can be observed during storage when high concentration of enzymes are present in antigenic media or in stressing storage conditions. Resistant formulations are therefore needed to ensure stability of emulsions even in destabilizing conditions. Moreover, robust adjuvants that allow flexible ratio of oil and antigenic media in the vaccine are also needed for the formulation of multivalent vaccines. Here we show that the new resistant adjuvant Montanide™ ISA 71R VG can resist to destabilizing antigenic media and conditions, and can be used at flexible ratio in poultry vaccines. Using a Newcastle disease model vaccine, we demonstrate that this new adjuvant is also safe and can improve vaccine efficacy in poultry.

1. Introduction:

Vaccine adjuvants are added to vaccine formulations for multiple purposes, such as increasing the protection given to animals by veterinary vaccines, enhancing the protection duration of the vaccine, reducing the number of injections needed, reducing the antigen load in the vaccine, or orienting the immune response conferred by the vaccine. Diverse adjuvants technologies for veterinary vaccines have been developed in the last decades [1].

Water-in-mineral-oil (W/O) emulsion adjuvants are extensively used for the formulation of inactivated avian vaccines. These strong adjuvants induce long-term efficacy of poultry vaccines. They consist of a mix of a specific mineral oil of injectable grade and a surfactant system to stabilize the vaccine emulsion. It should be noted that a specific care has to be given to the quality of surfactants as well as to the nature of the oil to control safety issues [2].

The Montanide™ range of veterinary adjuvants include emulsion, micro-emulsion and polymeric adjuvants and have been used extensively for prophylactic veterinary vaccination in farm and companion animals [3].

Montanide™ W/O adjuvants for poultry are ready to use adjuvants that induce long lasting protection in chickens and other avian species [4, 5, 6].

Chickens receive multiple vaccinations during their life-time. Multivalent vaccines containing multiple antigens are therefore used frequently in the field to limit the number of injections. These vaccines can induce protection against diverse strains of the same pathogen, or against multiple pathogens. Multivalent antigenic media are complex media that contain multiple biological components that can influence the stability of vaccine emulsions.

Evolution and destabilization of vaccine emulsions during storage have indeed been observed and can be linked to high concentration of enzymes such as lipases and esterases in antigenic media, and to stressing storage conditions. Robust formulations are therefore needed to ensure stability of emulsions even in destabilizing conditions, and with flexible ratio of oil and antigenic media in the vaccine. These features are therefore especially important for the formulation of multivalent vaccines.

Here we show that Montanide™ ISA 71R VG, an innovative emulsion adjuvant dedicated to poultry vaccines, can be used at flexible ratios and is able to resist to destabilizing antigenic media and storage conditions. Moreover, we demonstrate that this adjuvant allows the formulation of safe and efficient inactivated vaccines against Newcastle disease. These results show that the safety and efficacy profiles of this robust adjuvant are comparable to reference water in oil adjuvants used in poultry vaccines.

2. Formulation trials: Methods and Results:

Montanide™ ISA 71 R VG is a water-in-mineral-oil adjuvant based on a specific mannide oleate based surfactant system that has been designed to increase vaccines stability, and can be used at flexible ratios (50 to 70% in the vaccine). In order to control the robustness of this new adjuvant, we developed a model destabilizing medium (MDM) enriched in enzymes, which is comparable to destabilizing poultry antigenic media in the field.

Montanide™ ISA 71 R VG and a standard water in mineral oil emulsion adjuvant were thus formulated at 70% with either buffer solution or with MDM, using the same high shear

emulsification protocol. 10g of each emulsion were formulated at high shear using a IKA DT20 tube and a IKA Ultra Turrax Tube Drive emulsification system. 3 x 3 ml of each emulsion were placed in 3 different haemolysis 5 ml tubes for stability studies at 4°C, 20°C and 37°C for 6 months. Vaccine emulsions are considered as stable if there is no defect or small reversible defects, such as oil releases smaller than 5% or creamings smaller than 15%.

When buffer solution was used, both resisting adjuvant Montanide™ ISA 71 R VG and classical W/O adjuvant allowed the formulation of emulsions that were stable over 30 days, even when stored at 37°C. On the other hand, in combination with destabilizing antigenic media, whereas vaccines based on resisting adjuvant showed no stability default in all temperature conditions, standard formulations showed breakage of the emulsion at 20°C and 37°C, as short as 7 days post formulation (Figure 1). Emulsions based on Montanide™ ISA 71R VG showed no defect 6 months after formulation.

Stability results 7 days after formulation

Formulations	4°C	20°C	37°C
Tween/Span - Placebo	stable	stable	stable
Tween/Span-MDM	creaming 20%	creaming 20%	phase separation 5%
ISA 71 R - Placebo	stable	stable	stable
ISA 71 R - MDM	stable	stable	stable

Stability results 30 days after formulation

Formulations	4°C	20°C	37°C
Tween/Span - Placebo	stable	stable	oil release 10%
Tween/Span-MDM	creaming 20%	Phase separation 15%	phase separation 20%
ISA 71 R - Placebo	ok	ok	ok
ISA 71 R - MDM	ok	ok	ok

Stability results of standard and resisting formulations after 6 months at 20°C

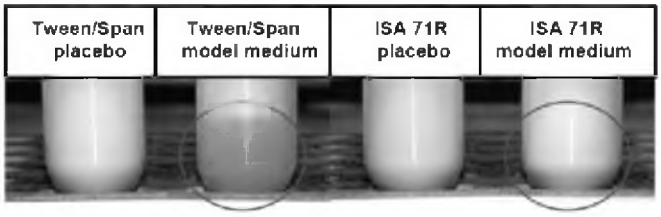

Figure 1: Results of stability study of Montanide™ ISA 71R VG and classical adjuvant formulations with buffer solution (Placebo) or MDM (model medium), 1 week, 1 month and 6 months post formulation.

3. Poultry trials: Methods and Results:

To assess safety and efficacy profiles of the new robust adjuvant Montanide™ ISA 71R VG in chickens, a vaccine against Newcastle disease was formulated using this adjuvant. This trial was performed in the facilities of NPP AVIVAC, Saint Petersburg, Russia.

a/ Vaccine groups and protocol

Inactivated Newcastle disease virus (NDV, La Sota strain, produced by NPP Avivac) was formulated either with reference W/O adjuvant Montanide™ ISA 70 VG (used at 70%), with robust adjuvant Montanide™ ISA 71R VG (used at 60%) or in PBS. All vaccines contained the same amount of antigen. Formulation with Montanide™ adjuvants was performed at high shear using a IKA 25T mixer and following Seppic's recommendations.

80 1-day old chickens (Hysex white) were randomly separated in 4 groups of 20 animals, and received at day 0 subcutaneously in the neck 0.1ml of the following vaccines:

- Group 1- ISA 71R:
  NDV Antigen + Montanide™ ISA 71R VG
  (60% adjuvant / 40% aqueous, weight/weight)
- Group 2- ISA 70:
  NDV Antigen + Montanide™ ISA 70 VG
  (70% adjuvant / 30% aqueous, weight/weight)
- Group 3- No adjuvant:
  NDV antigen + PBS, No adjuvant
- Group 4- Not vaccinated

All birds were slaughtered at D21, and local reactions at the injection site were assessed at slaughter.

Blood samples were taken at D14 and D21, and antibody titers in blood serum were assessed by hemagglutination inhibition test (HI).

b/ Safety results

In adjuvanted groups, small granulomas (smaller than 2mm, Figure 2) were observed in some animals (Table 1). This type of local reactions is minor and acceptable for poultry production. No significant difference was observed between robust and reference adjuvants.

These results show that all tested vaccines were safe in chickens, and that Montanide™ ISA 71 R VG has an acceptable safety profile in chicken, which is similar to reference adjuvant Montanide™ ISA 70 VG.

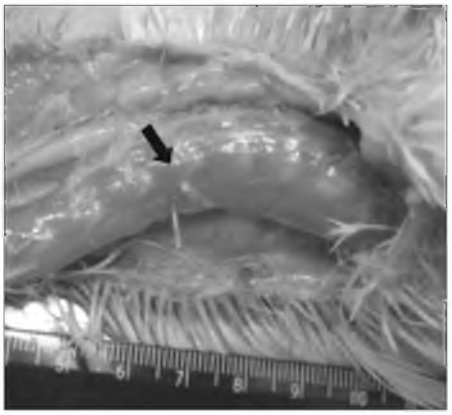

Figure 2: Representative local reaction observed with adjuvanted vaccines.

Adjuvant	Frequency of local reactions at slaughter
ISA 71R VG Resisting W/O	13/20
ISA 70 VG Reference W/O	11/20
No adjuvant	0/20

Table 1: Frequency of local reactions observed in chickens.

c/ Efficacy assessment: Antibody titers:

Specific antibody titers against inactivated NDV were measured by HI titration. Results are presented in Figure 3. Maternal immunity was still detectable at D14 post vaccination but had disappeared at D21.

At D21, significantly higher titers were observed in groups vaccinated with adjuvanted vaccines, compared to non adjuvanted vaccinated birds or non vaccinated birds. Both adjuvants induced comparable antibody titers. It should be noted that W/O reference adjuvant Montanide™ ISA 70 VG is used at 70% in the vaccine, whereas Montanide™ ISA 71R VG is used only at 60% in this trial.

These results show that this resisting adjuvant induced antibody titers that are comparable to a reference water in oil avian adjuvant, even when used in lower proportions.

4. Discussion:

The trend to develop multivalent vaccines gives new properties to antigenic media that destabilize avian vaccine formulations. Stability issues are often observed during storage and can induce the reduction of the efficacy of the vaccine. New resisting adjuvants will be therefore more and more needed to ensure proper stability profiles of vaccines.

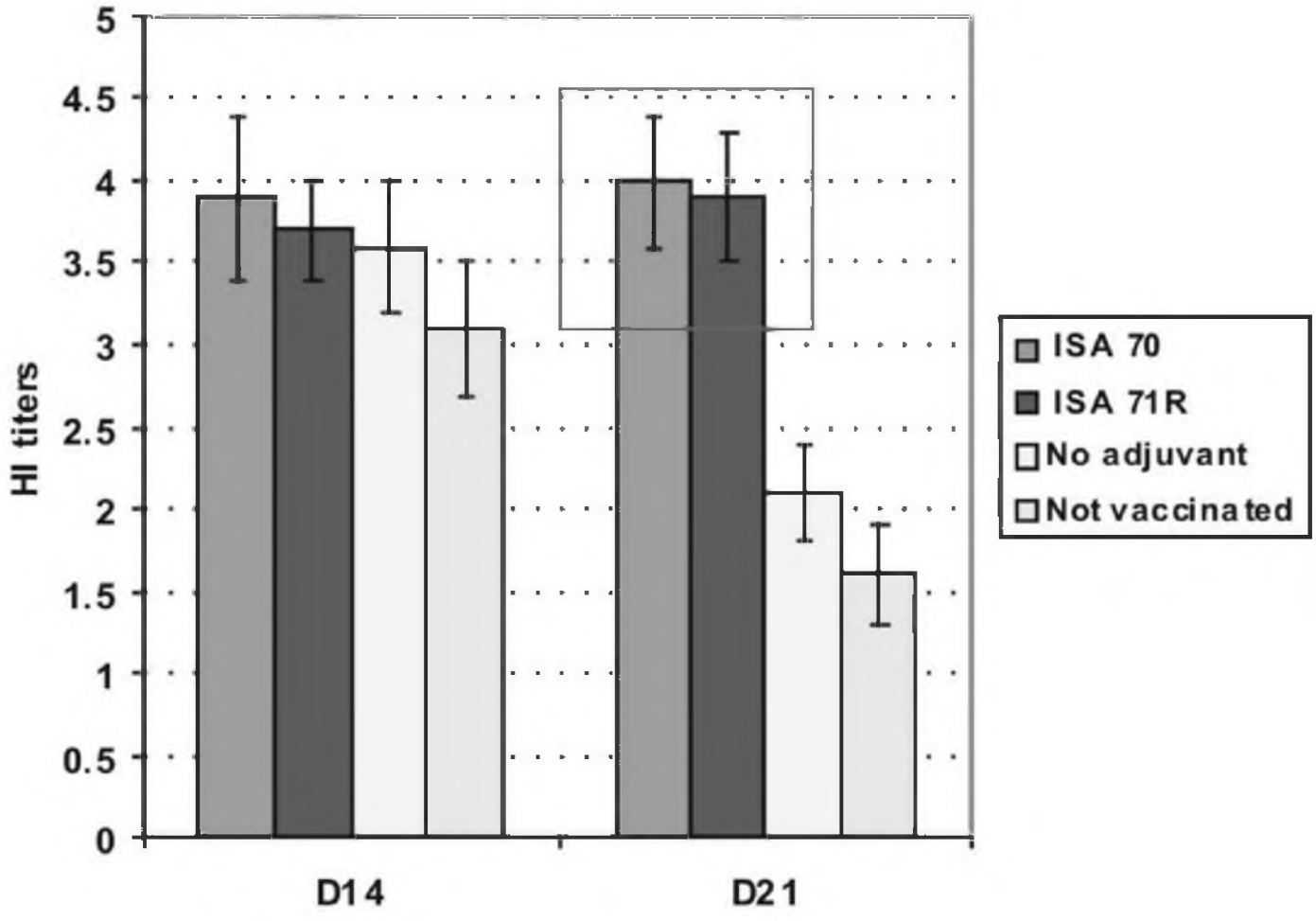

Figure 3: HI antibody titers after NDV vaccination

Moreover, the development of multivalent injectable vaccines requires flexible adjuvant formulations in which a larger volume of antigenic media can be included. In this study we have shown that we have developed a robust and flexible water in oil emulsion adjuvant able to resist to destabilizing antigenic media without impairing the safety and efficacy of avian vaccines. This adjuvant can be used at only 60% in the vaccine, which allows its use for multivalent vaccines.

In order to assess the efficacy and safety profile of this adjuvant, it was formulated with inactivated Newcastle disease virus antigen. Newcastle disease is an acute viral disease of domestic poultry and many other bird species, which presents primarily as a respiratory disease and is endemic in many countries. It is a worldwide problem for which clinical manifestations vary from high mortality to asymptomatic infections [7].

In order to control the disease, vaccination programs with inactivated or attenuated live vaccines are performed. For the formulation of inactivated vaccines, strong adjuvants are needed. Newcastle disease vaccine are often combined with other antigen valences to formulate multivalent vaccines.

As a proof of concept, we have shown that the use of Montanide™ ISA 71R VG adjuvant in combination with an inactivated Newcastle disease virus antigen allows the induction of high and protective antibody titers against Newcastle disease virus, a major pathogen of poultry stocks.

This new adjuvant has also been tested successfully in other vaccines for its ability to resist destabilization conditions, and its safety and efficacy performances have been confirmed. Such an adjuvant thus allows the development of stable and efficient multivalent vaccines for which long term emulsion stability is correlated with potency stability, and can also be adapted to other veterinary species.

5. References:

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