Adjuvant effect on humoral and cell mediated immune response in pigs vaccinated against *Mycoplasma hyopneumoniae*

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Introduction

Protection against *Mycoplasma hyopneumoniae (Mh)* infection is mediated by both the humoral and the cellular arms of the immune system, with more emphasis on the latter. Therefore, it is crucial for an efficient Mh vaccine to induce cell mediated immune response (CMI) in swine in addition to antibody response. Different adjuvants may drive the immune system towards Th1 (cell mediated) or Th2 type (antibody mediated) immune response which can be detected by cellular and serological assays, respectively. Here, we demonstrate the importance of adjuvant optimization by presenting the cellular and serological test results of blood samples obtained from pigs immunized with experimental *Mh vaccines*. These vaccines represent unoptimized and optimized adjuvant formulations, respectively. The optimized formulation has been used in the commercial vaccine, Hyogen®.

Materials and Methods

Three groups of 10 piglets each, seronegative to *Mh*, were immunized once at 3 weeks of age (D21) with vaccine formulations shown in Table 1. The antigen content of the vaccines used for immunization of Group 2 and 3 was kept constant, while the adjuvants consisted of variable amount of a non-toxic lipopolysacharide (LPS) derived from E. coli, J5, in oil-in-water formulations. The oil content of the formulations was also variable.

Table 1. *Vaccine formulations*

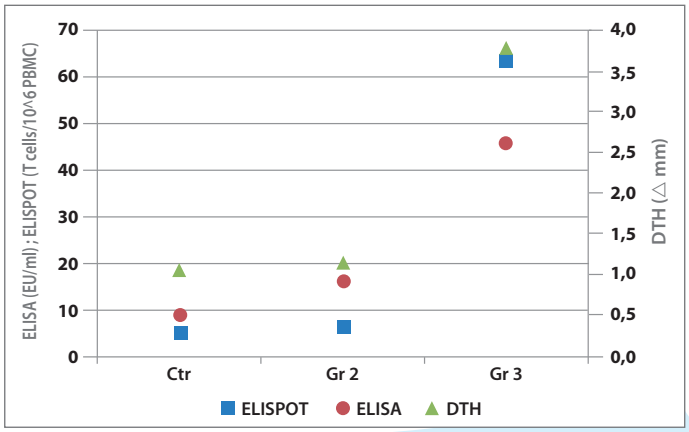
Group	*Mh* antigen	Adjuvant
ctr.	—	unoptimized
2	+	unoptimized
3	+	optimized

Delayed type hypersensitivity (DTH) test was performed on half of each group on D40 using intradermal injection of purified *Mh* antigen. Phytohaemagglutinin (PHA) was used as positive control. Skin thickness was measured with a pressure-sensitive digital caliper (Mitutoyo, Japan) after 24 hours post i.d. injection. Blood samples were taken from the second half of each group for interferon-γ (IFNγ) ELISPOT and antibody ELISA on D40. A porcine IFNγ ELISPOT kit (R&D, USA) and an ELISPOT reader (CTL, USA) were used for the quantification of antigen-specific cytokine secretion by in vitro stimulated T lymphocytes. *Mh-specific* serum antibody titer was determined with an indirect ELISA assay developed in our lab.

Results

Figure 1. shows the adjuvant effect on the cellular and humoral immune responses after a single shot vaccination.

Figure 1. *Humoral and cellular immune responses of vaccinated pigs*



Conclusion and Discussion

Here, we demonstrated the adjuvant effect of different vaccine formulations on cellular immune response and serum antibody titer measured by DTH, IFNγ ELISPOT and quantitative antibody ELISA, respectively. The optimized oil-in-water formulation combined with the proper amount of a non-toxic LPS adjuvant resulted in strong stimulation of both the cellular and humoral arms of the immune system in seronegative piglets after a single shot injection.

References

1 - Sibila *et al*, Vet J. 2009 181:221-231

2 - Herczeg *et al*, APVS, 2011

Hyogen® is a product of CEVA Santé Animale.

[1] Ceva-Phylaxia, Budapest, Hungary;

[2] Prophyl Ltd., Mohács, Hungary