Production of a vaccine against the swine pathogen *Mycoplasma hyopneumoniae*

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1. Introduction

Mycoplasma hyopneumoniae is the etiologic agent of enzootic pneumonia in swine. Enzootic pneumonia is a chronic disease that is an important cause of economic loss in the swine industry due to reduced weight gain and poor feed efficiency of host. The microorganism causes a chronic cough, dull hair coat, retarded growth and predisposition to secondary infections. The disease has almost no mortality but high morbidity.

M. hyopneumoniae attaches primarily to the ciliated epithelium of the trachea, bronchi and bronchioles. This attachment is a prerequisite for initiation of the infection. The colonization of the mucosal clearance by *M. hyopneumoniae* results in ciliostasis (disruption of the cilia on the epithelial surface). The microorganism modulates the immune system of the respiratory tract by altering alveolar macrophage function and lymphocyte function. This immonomodulation is the main responsible of ciliostasis and results in a significant reduction of the efficiency of protection against invading respiratory pathogens. The bronchopneumonia caused by coinfection of *M. hyopneumoniae* as primary pathogen and other bacteria as secondary pathogens is known as enzootic pneumonia.

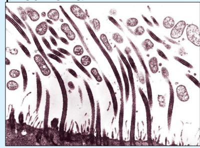

Figure 1. Micrograph of *Mycoplasma hyopneumoniae* attached to ciliated epithelium of the respiratory tract

Vaccination with commercial bacterins has become an important tool to control *M. hyopneumoniae* infections. These vaccines, produced from adjuvanted whole-cell or membrane preparations, are commonly used to induce partial protection and to control clinical signs of mycoplasmal pneumonia.

2. Initial hypothesis and objectives

The main hypothesis to be contrasted with this project is the next one: it is possible to produce a vaccine against *Mycoplasma hyopneumoniae* infection using an inactivated form of the pathogen, leading this way to an immune response able to develop an effective, but not fully, protection in pigs. In order to be able to demonstrate this hypothesis, it will be needed:

1. To study and understand the mechanism of infection of *Mycoplasma hyopneumoniae.*
2. To know the main types of vaccines and their characteristics and to relate this knowledge to the available information about the pathogen.
3. Once knowledge about the subject has been consolidated, to develop a feasible vaccine according to prior art.
4. To design an experiment capable of testing the effect of the vaccine in vivo.

3. Materials and methods

Vaccine design
This invention concerns a vaccine comprising a virulent *M. hyopneumoniae* strain, which has been inactivated with binary ethyleneimine (BEI), in a suitable amount to produce an effective immune protection in pigs using a single dose; a physiologically acceptable carrier; and an adjuvant as a potentiator of the immune response.

Vaccine production
M. hyopneumoniae Strain P-5722-3 will be grown in a suitable medium for mycoplasma growth. The production will be carried out through serially transferred batches until a 500 liters fermentation vessel is achieved.

The amount of *M. hyopneumoniae* is determined by DNA fluorometry using a Hoechst 33258 dye solution.

At the end of the growth period, pH is raised to 8.2 and a solution of 2-Bromoethylaminehydrobormide (BEA) is added. In the presence of the elevated pH, BEA is chemically changed to BEI, the inactivating agent. When inactivation is finished, BEI generated during inactivation is neutralized with sodium thiosulfate.

The inactivated bacterin is concentrated with an ultrafiltration and returned to an isotonic concentration with blood by diafiltration. Finally, the protector agents (thrimerosal and EDTA) and the adjuvant (Carbopol) are added to the final product and mixed for 30 minutes.

Clinical test
Study population: sixty healthy piglets free of *M. hyopneumoniae*, according to routine serological testing and study of clinical symptoms, are used in this experiment.

Experimental design: piglets are randomly divided in 3 groups: vaccinated and challenged with pathogen (G1), non-vaccinated and challenged (G2) and non-vaccinated and non-challenged (G3). Pigs in G1 where vaccinated at 7 days of age. Pathogen was administered in pigs in G1 and G2 at 42 days of age. G3 is used as negative control. Pigs were euthanized at the end of the experiment. At necropsy, lung samples were taken from both lungs.

Serology: blood samples were collected from all pigs weekly. Serum was obtained by centrifugation of blood. These samples were tested using a commercial HerdChek *M. hyopneumoniae* indirect ELISA assay (IDEXX Laboratories, Westbrook, Maine).

Quantification of M. hyopneumoniae DNA: DNA was extracted from bronchoalveolar lavage fluid (BALF) with the QIAamp DNA Mini Kit (Qiagen) and was quantified with a real-time PCR with primers based on the ABC transporter gene of *M. hyopneumoniae.*

Average daily weight gain (ADG): pigs are weighed periodically. The ADG (gram/pig/ day) is an important factor to be determined because *M. hyopneumoniae* disease has almost no mortality but high morbidity and reduction of weight gain is the main symptom of infection.

Feed conversion rate (FCR): FCR determines the efficiency of the animal in converting feed mass in body mass. It is the relation between the average daily feed consumption (AFC) and the ADG.

Pathologic examination: macroscopic examination of lungs was realized to determine the percentage of lung affected by pneumonia (each lobe is assigned with a score according to their approximate volume in whole lung).
Microscopic samples were taken from both lungs and were scored from 0 to 4 according to the severity of pneumonia.

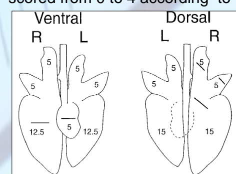

Figure 2. Gross pig lung lesion scoring system (bars represent areas where sections are taken for microscopic examination)

4. Results

Serology
It would be expected do not find antibodies against *M. hyopneumoniae* in pigs in the G1 before vaccination because in previous studies has been shown that vaccination with inactivated *M. hyopneumoniae* produces an anamnestic antibody response, so that pathogen administration is necessary to detect serological antibodies. No anti–*M. hyopneumoniae* antibodies should be detected in serum from negative control (G3) through all the experiment. In the other groups what would be expected to happen, due to the administration of the vaccine, is an enhanced humoral response of G1 (compared to G2) against *M. hyopneumoniae* once pigs have been challenged with the pathogen.

Quantification of *M. hyopneumoniae* DNA
To demonstrate the safety of the vaccine, G1 should not show positive results in BALF before inoculation because it would mean that vaccine has not been fully inactivated and maintains at least part of its virulence. In the same period, G2 should not show *M. hyopneumoniae* positive results. G3, as a negative control, should not have *M. hyopneumoniae* infected pigs to consider that experimental conditions are controlled.
In general, should be expected lower levels of *M. hyopneumoniae* DNA in vaccinated pigs (G1) than those challenged but not vaccinated (G2). It would be a demonstration of vaccine efficacy.

Average daily weight gain (ADG)
Normally, ADG depends on growth stage and it increases as time goes (from 0.2 to more than 1 kg per pig per day) until pig reaches adulthood. However, all pigs used in this study have the same age, so that it should not affect our results when comparing different study groups.

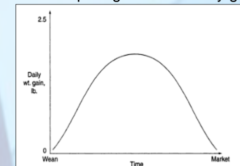

Figure 3. Changes in daily weight gain from weaning to market weight

Before inoculation pigs are not infected and all groups should grow in a similar way. Once G1 and G2 have been infected, both groups will have a reduced weight gain. G3 should gain weight faster than G1 and G2. However, G1 weight gain is expected to remain quite similar to G3 while G2 will have a more significant weight gain reduction.

Feed conversion rate
In the same way as ADG, FCR varies with pig's growth stage, but it should not influence the results. FCR results are expected to be quite similar to those found in ADG.
Pigs in G2 should experience reduced feed intake compared with G1 and G3 groups after inoculation. However, FCR will be reduced in G2 due to the lower ADG. G1 and G3 should remain quite similar between them all over the experiment.

Pathologic examination
In negative control group (G3), grossly visible pneumonia should not be detected in macroscopic examination (it would be expected a score near 0). G1 should not present macroscopic damage either (at least compared with G2 pathologic injury). Similar results are expected to be find through microscopic examination.

5. Conclusions

M. hyopneumoniae infections are spread all over the world and cause significant economic losses to the pig industry. In this context, it is important to make an effort to improve techniques to reduce the infection level and vaccines are an important tool in this field.

The present study has demonstrated the safety and the efficacy of this single dose vaccine. This inactivated vaccine has created an immune response which is able to control clinic symptoms and pulmonary lesions induced by *M. hyopneumoniae* infections. This way, pigs will benefit of an increased weight gain and conversion index. Although protection is incomplete and the vaccine does not prevent colonization, the quantity of microorganism in respiratory tract is reduced, as well as the incidence of infection.

In conclusion, vaccination with the designed vaccine in farms with significant levels of infection or with emerging clinical symptoms is a suitable and economically justified method of prevention.

6. Bibliography

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