

EVALUATION OF THE PROTECTION PROVIDED BY AN INACTIVATED TRIVALENT *PASTEURELLA MULTOCIDA* VACCINE AGAINST EXPERIMENTAL *PASTEURELLA* INFECTION AND PASTEURELLOSIS IN COMMERCIAL ENVIRONMENT

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ABSTRACT

Although chronic pasteurellosis and nasal *Pasteurella multocida* carriage still causes considerable losses of breeding females, preventive vaccination is not routinely used. Unsatisfactory field results might stay behind that, but observations from controlled field trials are lacking. In this study the protective effect of an inactivated trivalent *P. multocida* vaccine was evaluated in a heterologous challenge experiment and a field trial. The experimental vaccine contained three different *P. multocida* strains specific of the farm involved in the present study. Bacteria were inactivated with thiomersal and adjuvanted with Al(OH)₃. Repeated immunization raised up the specific antibody level four times in SPF rabbits. It conferred protection against challenge with heterologous strains in commercial rabbits which was proven by lower rectal temperature, fewer days with disease signs, smaller change of body weight, and increased antibody level. In the field study a positive effect of vaccination on nasal colonization with *P. multocida* was found. However, an adverse effect was observed: there was lower number of total born, lower proportion of alive new-born rabbits, and furthermore a higher mortality of sucklings in the case of immunized females. This might be related to the date (i.e. some days before artificial insemination) chosen for immunization.

Key words: Rabbit, *Pasteurella multocida*, Vaccine, Challenge, Field trial.

INTRODUCTION

Routine vaccination against pasteurellosis is not performed in most rabbit farms despite the frequent presence of *Pasteurella multocida* (*P.m.*) carriers and chronic forms of the disease. The prevention is based only on certain technical aspects of hygiene, i.e. immediate elimination of breeding rabbits when signs of chronic pasteurellosis were detected. This can limit the spread of the bacterium to the naïve animals, but apparently healthy carrier rabbits still remain in the stock exerting adverse effect on their own and on their stockmates' production. Within these circumstances the number of antigenically different *P.m.* strains is high what makes the prevention based on vaccination difficult (Coudert *et al.*, 2006). Since the extended use of hybrids in meat rabbit production, the result depends strongly on the *P.m.* carriage level of the parent stock purchased from breeding organizations. This is however rarely declared, therefore immunization may be performed when the parents are in the production stock and immediately before their first reproduction cycle. The previous experiments directed for vaccine development were performed mostly on younger rabbits and the effect was evaluated only by the protection efficacy against homologous challenge. Results of controlled field trials are lacking. The objective of this study was the evaluation of protection provided by a trivalent inactivated mixture of *P.m.* strains with adjuvant used in a heterologous challenge experiment and in a field trial.

MATERIALS AND METHODS

Animals and experimental design

Experiment 1

The protection provided by vaccination was studied in a challenge experiment with commercial hybrid (n=9) and SPF (Charles River, New Zealand White, n=3) rabbits weighing 3.4-4.1 kg. Commercial rabbits were screened for the colonization of nostrils with *P.m.* taking deep nasal swabs and for serum antibodies against *P.m.* with ELISA. Only negative ones were chosen for this experiment and subsequently housed in an isolated unit. The treated group (Tr, n=3) was vaccinated and challenged, the positive control group (PC, n=3) was not vaccinated but was challenged, and the negative control group (NC, n=3) was neither vaccinated nor challenged. A further group of SPF rabbits (SPF, n=3) was only vaccinated. Rabbits were housed in steel cages and were fed commercial rabbit pellet. Vaccine was inoculated intramuscularly and vaccination was repeated once at 2 weeks interval. Challenge inoculation was done 8 weeks after the first vaccination, and mortality, rectal temperature, feed consumption and body weight were checked during the next two weeks period. Serum was collected first on the challenge day and then at the end of the two weeks following challenge (day 56 and 70 after immunization). At the same time nasal swabs were also taken.

Experiment 2

Protection in the field was studied in a commercial rabbitry providing usual conditions of a conventional rabbit breeding unit. At first 102 nulliparous hybrid females were screened for the *P.m.* colonization of nostrils with deep nasal swabs. 78 nasal negative and 24 nasal positive rabbits were allocated to groups being vaccinated and not-vaccinated. The following four groups were formed in this way: nasal negative vaccinated (VN, n=37) nasal negative not vaccinated (NN, n=41), nasal positive vaccinated (VP, n=11) nasal positive and not vaccinated (NP, n=13). All rabbits were caged in alternate blocks of six vaccinated and six non vaccinated in the same row. First immunization paired with serum collection (day 0) was performed 3 days before the first artificial insemination (AI) of the females (day 3). Boost inoculation of the immunized rabbits and second serum collection from all rabbits was performed on day 46. Nasal swabs were taken on days 40 and 82. The last serum sampling was performed also on day 82. Kindling rate, litter parameters at birth and at 21 days of age, weaning weight, doe and pup mortality, *P.m.* colonization of nostrils and seropositivity turn-out were observed after the first AI.

Preparation of the vaccine and challenge inoculum

Three growing *P.m.* strains (exponential phase) isolated from the nostrils of healthy rabbits and previously characterized microbiologically, genetically and in mice experimental infection (Virág *et al.*, 2008a and 2008b) were used for vaccine preparation. The strains were cultured in TS broth at 36.5°C for 8 hours then plated to TSA to check their purity. The pure cultures were inactivated with 0.01% thiomersal. One inoculation dose contained 10¹⁰ inactivated cells and 3.5 mg Al (OH)₃ in 1 ml PBS. The challenge inoculum was prepared from two other virulent *P.m.* strains, which had been isolated from subcutaneous abscess and empyema of rabbits (Kulcsár *et al.*, 2008) and contained 10⁹ and 10⁷ CFU of each in 1 ml PBS.

Antigenicity measurement

Production of humoral antibodies was detected by ELISA with modified FlockCheck IDEXX *P.m.* Antibody Test Kit. Serum samples were diluted to 1:100, and the DakoCytomation P0448 Polyclonal Goat Anti-Rabbit Immunglobulins/HRP conjugate was used at a dilution of 1:250. Absorbance was read at 650 nm. ELISA value was calculated by dividing sample absorbance by conjugate control absorbance. In Experiment 1, change of the ELISA value following vaccination and challenge were presented as percentage of the ELISA value before immunization. In Experiment 2, change of the ELISA value following the first and the boost vaccination were presented as percentage of the ELISA value before vaccination.

Statistical analysis

All statistical analyses were performed with GenStat 8th software (VSN International Ltd., 2004) GLM module. In the challenge experiment the group factor, in the field study two factors, the group and the initial *P.m.* carriage and their interaction were included in the model. Qualitative traits were evaluated by general linear regression. Born alive/born total, suckling mortality between 1-21 and 21-35 days of age (expressed as number of dead/litter size born alive) were evaluated by the binomial proportions model. Bacteria colonization, doe mortality, litters with at least one died suckling until 21 days of age, and seropositivity were considered and evaluated as Bernoulli variables (only taking values 1 or 0). Results are presented as mean \pm s.e. for groups formed by the factors. Probability that the differences are caused by the treatments was found by F and Chi² tests.

RESULTS AND DISCUSSION

In experiment 1, the two immunizations with the vaccine resulted in high elevation of serum antibodies only in the group of SPF rabbits (Figure 1). In the vaccinated and challenged group the antibodies raised significantly only at day 70 post immunization i.e. at the end of the observation period after challenge. The antibody values did not change in non-vaccinated challenged and non-vaccinated not challenged groups.

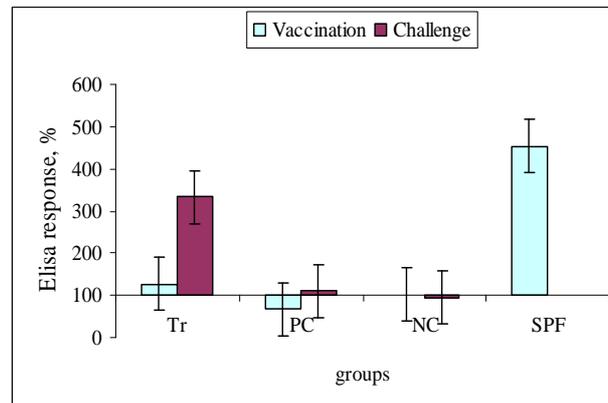


Figure 1: Serum antibody response after immunization and following the infection in the challenge experiment. Elisa response % after immunization and challenge were calculated as the percentage of the Elisa value before vaccination (=100%), Tr vaccinated and challenged, PC not vaccinated but challenged, NC neither vaccinated nor challenged, SPF vaccinated

Parameters related to the protection against a heterologous challenge are summarized in Table 1. Before challenge, rectal temperature was in normal range and was not different between groups ($39.4 \pm 0.12^\circ\text{C}$).

Table 1: Health parameters after challenge in rabbits immunized with the trivalent *P.m* vaccine

| Parameters | Groups | | | s.e. | Prob |
|---|-------------------|-------------------|-------------------|------|-------|
| | Tr | PC | NC | | |
| Body weight at day 0 (g) | 3862 | 3440 | 4123 | 252 | ns |
| Days with fever/days checked | 0.44 ^a | 1 ^b | 0.41 ^a | 0.08 | <0.01 |
| Rectal temperature ($^\circ\text{C}$) | 39.7 ^a | 40.9 ^b | 39.5 ^a | 0.2 | <0.01 |
| Body weight change (g) | -39 | -585 | 68 | 163 | ns |
| <i>P. multocida</i> nasal carriage | 0.67 | 0 | 0 | 0.05 | ns |
| Seropositivity | 1 | 0 | 0 | 0.06 | =0.01 |
| Mortality rate (%) | 0 | 0.67 | 0 | 0.09 | <0.1 |

Tr: vaccinated and challenged, PC challenged, NC: non-vaccinated not challenged

After challenge, in the non-vaccinated group, the rectal temperature was increased (40.9°C) during 12 days, a deep weight loss (-585 g), a high mortality rate (2/3) and a lack of antibody production were observed. In the vaccinated group the rectal temperature (39.7°C) was slightly increased during 12

days of the total 27 days checked, and the body weight decreased with 39 g only, all rabbits survived and all became seropositive after challenge. Strikingly however, *P.m.* nasal colonization was found after challenge in this group. Explanation of 0 nasal colonization in non-vaccinated challenged group is that 2 of the 3 rabbits died on day 59 of acute pasteurellosis, just 3 days after experimental inoculation.

In Experiment 2, the antibody level remained constant as expected in the non-immunized groups NN and NP, independently from their original nasal *P.m.* carriage. Surprisingly the same level was found in both immunized groups VN and VP after the first vaccination (Figure 2) but there was a slight increase in antibody level after the boost vaccination. That was however also observed in groups NN and NP, which were not vaccinated.

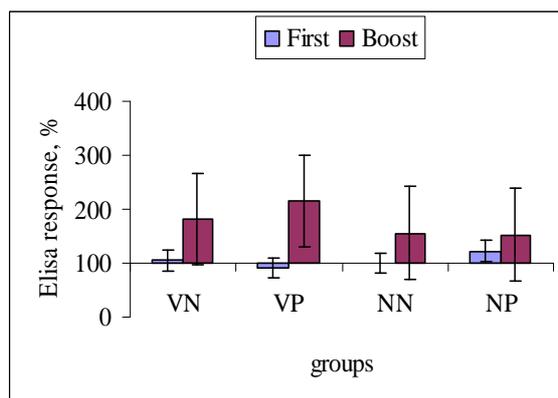


Figure 2: Serum antibody response to the first and to the boost vaccination in the field study. Elisa response % after the first and boost vaccination were calculated as the percentage of the Elisa value before vaccination (=100%), VN nasal negative vaccinated, VP nasal positive vaccinated, NN nasal negative non-vaccinated, NP nasal positive non-vaccinated

The proportion of nasal *P.m.* positive swabs taken on day 82 was significantly ($P < 0.05$) higher in the non-vaccinated NN (0.22) and NP (0.25) groups, than in the immunized VN (0.05) and VP (0.06) groups (Table 2).

Table 2: Results of the field protection test of rabbits immunized with the trivalent *P. multocida* vaccine

| | Groups | | | | s.e | Probability of effect | | |
|--|--------------------|--------------------|---------------------|--------------------|------|-----------------------|--------|----------|
| | VN | VP | NN | NP | | Vaccin | Pm I | Interact |
| <i>Parameters of protection</i> | | | | | | | | |
| <i>P. multocida</i> colonization rate ¹ | 0.054 ^a | 0.060 ^a | 0.22 ^b | 0.25 ^b | 0.08 | <0.05 | ns | Ns |
| Doe mortality rate | 0.21 | 0.38 | 0.20 | 0.37 | 0.09 | ns | =0.1 | Ns |
| Born alive/born total | 0.91 ^{ab} | 0.92 ^{ab} | 0.96 ^b | 0.87 ^a | 0.02 | ns | <0.1 | <0.05 |
| Suckling mortality rate, 2–21d of age ² | 0.14 ^a | 0.34 ^b | 0.13 ^a | 0.16 ^a | 0.04 | ns | <0.005 | <0.1 |
| Affected litter prevalence ³ | 0.50 ^a | 0.99 ^b | 0.48 ^a | 0.88 ^b | 0.08 | ns | <0.001 | ns |
| Pup mortality rate, 3 to 5 weeks of age | 0.03 ^a | 0.15 ^b | 0.07 ^a | 0.05 ^a | 0.03 | ns | ns | <0.05 |
| Seropositivity rate ³ | 0.57 ^b | 1 ^c | 0.20 ^a | 0.25 ^a | 0.19 | <0.05 | ns | ns |
| <i>Performance traits</i> | | | | | | | | |
| Kindling rate | 0.86 | 0.92 | 0.83 | 0.91 | 0.05 | ns | ns | ns |
| Litter size born total | 8.32 ^a | 7.6 ^a | 9.58 ^b | 9.55 ^b | 0.70 | <0.05 | ns | ns |
| Body weight at 35 d age | 867.8 ^b | 874.9 ^b | 811.3 ^{ab} | 757.4 ^a | 45.1 | <0.1 | ns | ns |

¹Positive nasal swabs/total nasal swabs on day 82, ²litter size at 21/litter size at 2 days of age, ³number of samples with increased antibody percentage/total number of serum samples, ³at least one suckling died until 21 days of age

VN: nasal negative vaccinated, VP: nasal positive vaccinated, NN: nasal negative non-vaccinated, NP: nasal positive non-vaccinated

The rate of seropositive rabbits was also higher ($P < 0.05$) in the vaccinated VN and VP groups (0.57 and 1, respectively) than in the non-immunized NN and NP groups (0.20 and 0.25, respectively). Doe mortality was slightly higher ($P = 0.1$) in the groups VP and NP, which were initially formed from nasal *P.m.* carrier rabbits. The ratio born alive / total new-born rabbits reached the highest value ($P < 0.1$) in

group NN (0.96) initially formed from *P.m.* negative rabbits and which was not immunized. The lowest value was found in group NP (0.87) showing that colonization of the nasal mucosa can cause defective foetus development. Suckling mortality rate between 2 to 21 days and 3 weeks to 5 weeks of age was the highest in the group VP perhaps surprisingly and this could be caused by some kind of interaction ($P<0.1$ and $P<0.05$, respectively) between initial nasal *P.m.* colonization and the immunization. Kindling rate was not significantly influenced by the treatments, though litter size (total neonates) was lower ($P<0.05$) in the immunized groups 8.32 in VN and 7.6 in VP. The latter could be due to the adverse effect of LPS that might be present in high amount in the vaccine.

CONCLUSIONS

Repeated immunization with the vaccine containing three different *P. multocida* strains specific to the farm involved in the present study triggered high antibody production only in the SPF rabbits of the New Zealand White breed. However, it conferred protection against challenge with heterologous strains in commercial rabbits in term of lower rectal temperature, reduced number of days with disease signs, smaller change of body weight, and increased antibody level. In the field study, the vaccine had a positive effect on nasal colonization with *P. multocida*. An adverse effect could be supposed on foetal development (reduced litter size) and a higher suckling mortality was observed especially in the group of *P.m.* carrier vaccinated females. This might be connected to the too short time between insemination (3 days) and immunization.

ACKNOWLEDGEMENTS

This study was supported by the grant 46025/2004 from the Hungarian Ministry of Agriculture and Rural Development

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