IMMUNOGENICITY OF EXPERIMENTAL PASTEURELLA MULTOCIDA VACCINE IN RABBITS

L.K. ABDEL-SAMIE

Colleague of at Veterinary Hospital, Zagazig Univ. Egypt.
Email: lamahsamie@gmail.com

ABSTRACT

Previously Pasteurella multocida was isolated and genotyped by the author from different outbreaks in rabbits at Sharkia Governorate, Egypt. In this work, development of a vaccine was carried out in the laboratory by sonication of the isolated bacteria genotype A, B and D. Two experiments were conducted for evaluation of the trialed vaccine. The first one was carried out for determination of the protective dose of the vaccine and the second experiment for testing of its immune competency with commercial vaccine. Immunogenic response was evaluated serologically by ELISA and protection percentage on challenge against infection. Results revealed that sonicated vaccine containing 4x10^9 colony forming unit (cfu)/ml provoked high level of immunity. On comparison with commercial vaccine, geometric mean antibody titre of the prepared vaccine was statistically higher along 4 weeks post vaccination. Moreover it produced 100% protection against challenge while commercial vaccine revealed 80% protection. Unvaccinated control group had zero protection. It is concluded that immunization of rabbits with vaccine prepared by sonication of genotyped field P multocida isolate would be supportive means for avoidance of pasteurellosis in rabbitaries.

Key words: Pasteurella multocida – Vaccine – Sonication – Immunogenicity – Rabbit

INTRODUCTION

Pasteurellosis caused by Pasteurella multocida is one of the most significant bacterial diseases of rabbits and causes considerable economic losses in large production units throughout the world (Takashima et al., 2001). In most cases, the likely site of initial infection is the upper respiratory tract. Transmission occurs readily through direct contact of susceptible rabbits with carrier animals, and airborne transmission does not occur after exposure periods of 3-8 weeks. Stressors such as crowding, transportation, and high ammonia concentrations in the air often stimulate latent P. multocida to proliferate and cause disease (Al-lebban et al., 1989, DiGiacomo et al., 1991 and Manning et al., 1989).

The disease is characterized by various clinical symptoms, including respiratory distress, genital infections, abscesses, otitis, and septicaemia, but infection by P. multocida can also appear without manifesting any clinical signs (DeLong and Manning, 1994). Serogroup A and, to a lesser extent, serogroup D strains of P. multocida have been considered as causative agents of rabbit pasteurellosis (Kawamoto et al., 1990 and Dabo et al., 1999). PCR – fingerprinting assessment separated the field isolates of P. multocida into 3 genotypes. The circulating P. multocida strain was genotype 1 conserved in 3 of the 4 examined outbreaks. So, the local vaccine strain should be prepared from the circulating strain in the region (Suelam and Abdel Samie, 2011).

Presently, prevention of pasteurellosis is carried out by vaccination using whole-cell bacterin, which confer serotype-specific protection, or with live vaccines composed of attenuated strains, which protects against both homologous and heterologous serotypes (Glisson et al., 1993 and Wang and Glisson, 1994). Publications on the specific prophylaxis of rabbit pasteurellosis are limited. In attempts to protect rabbits from infection with P. multocida, a variety of vaccines have been examined, including those composed of inactivated whole bacteria. (Al-lebban et al., 1989).

Although control measures such as optimizing the environment of the rabbitary and culling of symptomatic animals reduce morbidity, endemic infections represent a source of considerable potential loss. Results demonstrate the possibility of using vaccination to greatly limit infection and disease under field conditions. Several vaccines have
been tested under laboratory conditions, field trials of vaccines for the control of *P. multocida* in rabbits are few (Suckow et al., 2008).

The main objective of the study was preparation of vaccine from field isolates of *P. multocida* that genotyped by PCR and determination of its efficiency for protection of rabbits from infection.

**MATERIALS and METHODS**

**P. multocida:** Field isolates from 4 different outbreaks were previously identified and genotyped by PCR (Suelam and Abdel Samie, 2011). Genotype I (A, B and D) was used for development and assessment of the vaccine. The virulence of the bacterial isolates was tested by intra-peritoneal injection of 6 month old mice which died 24 hours post inoculation.

**Commercial vaccine:** Oil adjuvant formalin 0.5% inactivated *P. multocida* (A1, A3, A12 and D2) vaccine containing 4x10^7 c.f.u/ml produced by Veterinary Serum and Vaccine Research Institute, Cairo, Egypt.

**Vaccine preparation:** The bacteria were grown in brain heart infusion agar for 18h at 37°C. Cells were harvested, washed then centrifuged (8000 x g for 10 min. at 4°C), the supernatant was dialyzed extensively against a buffer containing 0.01 M Tris-HCl, 0.32 M NaCl, and 0.01% NaF (pH 8.0). The pellet was concentrated at 4x10^9 cfu/ml. The number of bacteria was determined by the optical density of the solution at 480 nm (Spectronic 70, Bausch and Lomb, USA) and compared with a calibrated standard. Then the bacterial suspension, was sonicated using ultrasonic disintegrator (MSE) at 1.2A for 15 min until a transparent solution was obtained, the lysate was filtered through a 0.45 sterile filter. Total protein concentration was 1.9 mg/ml. Sterility of the filtrates was confirmed by plating in blood agar containing 5% sheep blood. The Safety of the vaccine was carried out according to (OIE, 2008). These procedures took place in Biotechnology Lab., Faculty of Veterinary Medicine, Cairo University.

**Preparation of challenge organisms:** Isolated *P. multocida* cultured on blood agar containing 5% sheep blood at 37°C for 24 hours, harvested into broth and diluted to 10^6 organisms per 1ml. The challenge dose was 1ml per rabbit inoculated intranasal at 4weeks post last immunization for determination of vaccine efficacy. Protection% measurement was based on number of survivals and dead or rabbits show clinical signs.

**ELISA:** Commercial *Pasteurella multocida* ELIS Akit (ProFLOK, Snybiotic) was tested and used for screening of antibody level post vaccination in rabbits. Sera obtained from vaccinated and control rabbits were examined for the presence of *P. multocida* IgG antibodies on weekly intervals for one month. The titre was calculated according to the manufacturer Log10 titer = (1.464 x Log10 SP) + 3.197,

\[
\text{SP value} = \frac{\text{OD value of sample} - \text{Avg. OD Normal Control}}{\text{OD value of positive control} - \text{Avg. OD Normal Control}}
\]

Result interpretation: titer levels 0 (Negative), 148 and higher (Positive).

**Animals:** 65 weaned apparently healthy New Zealand white rabbit (Veterinary Hospital, Zagazig University) were deployed. Rabbits were divided into two experimental groups. All rabbits were housed in cages and fed on non medicated commercial feed. Tape water and feed were provided ad-libitum.

**Experimental design:**

**Experiment 1:** twenty rabbits of 60 days old were equally divided in four labeled cages. Rabbits of cage 1 were vaccinated twice subcutaneously with 0.5 ml (2x10^6 cfu), cage 2 with 1 ml (4x10^6 cfu) and cage 3 with 2 ml (8x10^6 cfu) of the prepared vaccine with 2 weeks interval while last cage4 was not vaccinated control group. After 3 weeks post last immunization sera were harvested from all animals for serological test then inoculated intranasal with 1ml of *P. multocida* broth contain 10^8 cfu and monitored for 14 days for morbidity and mortality.

**Experiment 2:** forty five rabbits of45 days old equally divided into three groups in separate cages. **Group A** was immunizedt wice with sonicated vaccine, **group B** immunized with commercial vaccine at the same age while **group C** was not vaccinated control. Immunized groups were vaccinated subcutaneously with 1ml containing 4x10^6 cfu at 45 and 60 days old. Blood samples were collected from all groups on weekly basisstarted with one week post last vaccination and lasted4 weeks for immune-assay. All groups were challenged intranasal with 1ml (10^6 cfu) of *P. multocida* at 88 days old.

**Statistical analysis:** Data were collected, organized and analyzed using one-way analysis of variance (ANOVA) through the general linear models (GLM) procedure of the Statistical Package for Social Sciences version 17.0 (SPSS for Windows 17.0, Inc., Chicago, IL, USA). Duncan multiple range test were used to separate means at p <0.05.

**RESULTS**

All experimental rabbits were tested negative for *P. multocida* before immunization. Experiment 1 results showed neither morbidity nor mortality among
rabbits of cage 2 and 3 reflecting 100% protection against challenge with virulent *P. multocida*. Cage 1 showed 2 mortalities and 1 with respiratory manifestation in the form of rhinitis, conjunctivitis and sneezing reflecting 40% protection while cage 4 had zero protection as all rabbits died. 3 weeks post last immunization, seroconversion results by ELISA recorded average antibody titer 738.40±24.74<sup>a</sup> and 760.20±25.82<sup>a</sup> for cage 2 and 3 respectively, with no statistically difference while cage 1 recorded 158.80±97.36<sup>b</sup> (Fig 1). The results of experiment 1 interpretative that dose of 1ml containing 4x10<sup>9</sup> cfu of sonicated *P. multocida* vaccine provide 100% protection and higher dose provoked the same degree of immunity while lower dose was unable to provide sufficient protection for immunized rabbits (Table 1).

Experiment 1 results revealed that rabbits immunized with sonicated *Pasteurella multocida* vaccine provoked not only higher antibody titre but also significantly difference than other rabbits immunized with commercial gel adjuvant formalized vaccine along 4 weeks post last vaccination (Table 2). The mean antibody titre of group A was 440.47±7.44<sup>a</sup>, 582.80±8.26<sup>a</sup>, 744.53±14.28<sup>a</sup> and 564.80±18.63<sup>a</sup> at 1<sup>st</sup> week, 2<sup>nd</sup> week, 3<sup>rd</sup> week and 4<sup>th</sup> week post second vaccination respectively, while it was 410.10±7.48<sup>b</sup>, 563.90±8.50<sup>b</sup>, 710.53±7.64<sup>b</sup> and 494.87±12.21<sup>b</sup> in group B respectively. Group C had negative results for antibody titre. At the end of the experiment challenge of all groups with pathogenic genotype of *P. multocida* reflected 100% protection in group A while it was 80% where 3 rabbits of group B showed signs of respiratory manifestation in the form rhinitis, lacrimation with conjunctivitis. All rabbits of group C died.

**Table 1: Results of experiment 1.**

<table>
<thead>
<tr>
<th>Grp</th>
<th>No of rabbit</th>
<th>Dose of the vaccine</th>
<th>Survivals</th>
<th>Death</th>
<th>Protection %</th>
<th>IgG titer*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cage 1</td>
<td>5</td>
<td>0.5 ml (2x10&lt;sup&gt;9&lt;/sup&gt; cfu)</td>
<td>2</td>
<td>3</td>
<td>40</td>
<td>158.80±97.36&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cage 2</td>
<td>5</td>
<td>1 ml (4x10&lt;sup&gt;9&lt;/sup&gt; cfu.)</td>
<td>5</td>
<td>0</td>
<td>100</td>
<td>738.40±24.74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cage 3</td>
<td>5</td>
<td>2 ml (8x10&lt;sup&gt;9&lt;/sup&gt; cfu.)</td>
<td>5</td>
<td>0</td>
<td>100</td>
<td>760.20±25.82&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cage 4</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0.00±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
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</table>

* Means within the same column carrying different superscript are significantly different at P value<0.05
This study investigated the immunogenic effect of sonicated vaccine prepared from field isolates of Pasteurella multocida in rabbits. The first OUT comereflected that the minimal dose of the vaccine that provoked the best immuneresponse in rabbits was1 ml containing 4×10⁹ cfu, in agreement with results (Ruzaukas, 2005) who recorded that clinical laboratory trial of the inactivated vaccine against rabbit pasteurellosis using rabbits as experimental and at the same time as target animals revealed that 100%of vaccinated rabbits survived after Pasteurella multocida infection when the vaccine concentration was4×10⁹ b.c./ml by immunization of rabbits with noless than 1 ml of the vaccine. On the contrary of (Arif et al., 2013) who prepared P. multocida vaccine containing 2×10⁹ cell/ml supplemented with 10% outer membrane protein and the total protein contents were maintained at 1mg/mlused for immunizing rabbits producing 88% protection on challenge at 21 days post vaccination.

On evaluation of the prepared vaccine, results showed that it was competent with other commercial vaccine that widely used in the Egyptian market, mean level of IgG was significantly higher and produced better protection against challenge. In spite of different methodology (Lee et al., 2007) who prepared outer membrane protein (OmpHs) P. multocida vaccine by sonication of the bacterial envelope and extracted the outer membrane and estimated its immunogenicity. Theyrecorded similar results on matching with other vaccines noting that the levels of anti body response in commercial vaccine and formalin-killed whole cell vaccines were relatively lower than those of the Omp Hs. This may be attributed to the use of genotyped bacterial isolates at certain locality in vaccine preparation and homologous challenge.

A 3rd week post initial vaccination antibodies was measured by ELISA in our experiment, unlikers result of (Suckow et al., 2008) recorded that after vaccination of rabbits with potassium thiocyanate extract (PTE) produced from P. multocida, ELISA was performed on samples obtained before, 5 and 24 week after initial vaccination. Both IN and SC vaccination with PTE stimulated humeral immunity by 5 week after initial vaccination this response decreased by 24 week afterward.

It concluded that methods of vaccine preparation and type of bacterial isolates responsible to great extend for its immunogenicity. Uses of sonicated vaccine from P. multocida isolated and genotyped from specific area would be a useful means for prevention of the disease in rabbits and improvement of its immune complex.

## REFERENCES


Kawamoto, E.; Sawada, T.; Suzuki, K. and Maruyama, T. (1990): Serotypes of Pasteurella multocida isolates from rabbits...


