

## STUDY OF PATHOGENICITY AND IMMUNOGENICITY OF *PASTEURELLA MULTOCIDA* RECENTLY ISOLATED FROM INFECTED RABBITS

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### Abstract

In this study, 150 samples were collected from rabbits in infected farms which show high mortality suspected to be affected with Pasteurellosis. Thirty of *Pasteurella multocida* isolates were obtained which were belonged to serotypes A:3, A:3,5, A:12, B:2, D:6 and uncommon types as A:4 and D:12. The determination of pathogenicity and immunogenicity of these isolates (in mice) indicated that some of these isolates were highly pathogenic, others are of moderate or low pathogenicity. The protective value was good in serotypes A:3, A:3,5, A:12, B:2, D:6 which gave high protective value (more than 2 log), when vaccinated mice were challenged with homologous strains. Rabbits vaccinated with currently used vaccine and challenged with homologous strains and heterologous recently isolates gave good immune response except with field serotypes B:2 and D:6.

### INTRODUCTION

Pasteurellosis remains a major problem in many commercial rabbitries. Manifestations of *Pasteurella multocida* (*P. multocida*) infections in rabbits include "snuffles", bronchopneumonia, middle ear infections, abscessation, conjunctivitis, mastitis, reproductive tract infections and acute fatal systemic infections (Percy and Bhasin, 1987). This disease causes serious lesions in small breeding and larger farms. Consequently, investigation on the development of effective control and preventive methods are of a vital importance.

*Pasteurella multocida* of rabbit origin had received many studies, of which are types A and D to be common in rabbits and others are types B and C which are most prevalent (Hagen, 1966). at the same time, a study reported from Japan, found capsular type A from rabbit in holding colony, while a report from United States found that capsular types A and D were most common in rabbits from the wide west region (Chengappa *et al.*, 1982). So, we need extensive study for *P. multocida* of rabbits and carrying out survey from time to time to determine any different capsular and somatic components in field isolates which may be found.

The aim of this study was done to determine the antigenic structure of recently isolated *P. multocida* from rabbits and to estimate its pathogenicity and

immunogenicity to select the ideal isolates to be added or not to the currently produced vaccine in the future.

## MATERIALS AND METHODS

Samples were collected from 150 diseased and recently dead rabbits in different farms of different localities in Egypt. The samples were in the form of nasal swabs, middle ear and internal organs. Rabbits in these farms were suffering from illness and high mortality which may be due to *Pasteurella* infection. The samples were subjected to bacteriological and biochemical identification.

### Serological typing of recent isolates of *P. multocida*

**1. Capsular typing of *P. multocida*** : This was performed according to Carter (1955).

**2. Somatic typing of *P. multocida*** : This was done according to Heddleston *et al.* (1972).

### Laboratory animals used

#### Mice

One thousand and seventy five 1675 healthy Swiss albino mice each weighing 18-22 g were obtained from the mice farm the at Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, and used for determination of *pathogenicity* and immunogenicity of recent rabbit *Pasteurella* field isolates.

### Determination of pathogenicity and immunogenicity of recently isolated *P. multocida* of rabbits origin

#### 1. Determination of the minimum lethal dose (LD<sub>50</sub>) of *P. multocida* in mice

Recent field isolates of *P. multocida* strains were cultivated on nutrient agar plates and incubated at 37°C for 24 hours, and the harvested cells were suspended in sterile saline in density equal to  $5 \times 10^9$  bacterial cell. The bacterial cell suspensions were serially ten fold diluted from  $10^{-1}$  to  $10^{-10}$  dilution using sterile saline. Each ten mice were injected S/C with 0.1 ml from each bacterial dilution, another ten mice were left as a control and injected with sterile saline. Mortalities were observed and LD<sub>50</sub> was calculated according to Reed and Muench (1938).

#### 2. Mean Death Time (MDT)

It was done to the recently isolated strains and calculated as described by **Hanson and Brandly (1955)** according to the following formula:

$$\text{MDT} = \frac{(\text{No. of dead at x hours}) \times (\text{x hour}) + (\text{No. of dead at y hour})}{\text{Total No. of dead}}$$

### 3. Vaccine preparation

The monovalent vaccine (inactivated formalized) was prepared for each serotype of recent isolates of *P. multocida* according to Borkowska *et al.* (1996).

#### Determination of log protection

The active mouse protection test was carried out as described by Ose and Muenster (1968). The mice were divided into six groups, 250 mice in each, designated A, B, C, D, E and F. Each group of mice from A to E was inoculated S/C by one of the monovalent 5 vaccines (inactivated formalized vaccine) in two doses at a dose of 0.2 ml/mice (2 weeks interval). The mice of group F were kept as control. Each group was further divided into 5 subgroups, 50 mice in each for receiving challenge homologous and heterologous inoculum (after 2 weeks from booster dose). On the day of challenge, groups of vaccinated and control were challenged with dilution from  $-1$  to  $-10$  log<sub>10</sub> at a dose of 0.1 ml / mice S/C in which the minimum lethal dose (LD<sub>50</sub>) was calculated for each group of mice, vaccinated and control groups based on accumulated death on the 7<sup>th</sup> day using Reed and Muench (1938) method. The (LD<sub>50</sub>) of the vaccinated mice was compared to the (LD<sub>50</sub>) of non-vaccinated mice. A requirement of 2 logs of protection is necessary to qualify the prepared vaccine as index of protection (Ose and Muenster, 1968).

#### Rabbits

Fifty-four rabbits with an average body weight of 2-2.5 kg were obtained from private farm and kept under hygienic measures. The rabbits were proved to be fully susceptible to the *P. multocida* by serological testing of random samples. These rabbits were vaccinated with 2 doses of currently vaccine at a dose of 2 ml/rabbit S/C (formalized inactivated vaccine) 2 weeks interval, then challenged with *P. multocida* homologous and heterologous strains (recent isolated strains) to determine the immunogenic properties of recent isolates in comparison with current vaccinal strains..

## RESULTS AND DISCUSSION

*Pasteurella multocida* infection is a constant serious disease problem in rabbits, and in fact it is the major cause of mortality in large rabbitaries through the world (Flatt, 1974). The incidence in rabbitaries varies greatly depending on sanitation, ventilation, genetic resistance and vaccination.

Thirty *P. multocida* isolates were obtained from 150 collected samples of infected and recently dead rabbits as proved by bacteriological examination and biochemical characterization. These isolates, as shown in Table 1, belonged to serotypes A, B and D in which 4, 2, 8, 7 and 6 isolates belonged to serotypes A:3,

A:3,5 , A:12, B:2 and D:6 respectively. Uncommon isolates belonging to serotypes D:12 (2 isolates) and A:4 (one isolate) were obtained. These results agreed with Karl (1966), Chengappa *et al.* (1982) and Mohamed (1998) who found that the most common field isolates were serotypes A:3, A:12, D:6, capsular types C and B. From the same Table, the results revealed that the cross-reaction occurred in a number of isolates (A:3,5 and A:3). These results were observed by Okerman and Spanoghe (1980), Carter and Chengappa (1981).

The investigation on immunity against *P. multocida* is frequently carried out on white mice because of their low price and high sensitivity to infection with the organism. The results illustrated in Tables (2 and 3) indicated the substantial differences in virulence for mice. Isolates A:3, A:3,5 and B:2 were highly pathogenic causing death within 18-24 hours, but those of types A:12 and D:6 showed moderate pathogenicity and killed mice within 36-72 hours. Meanwhile, types A:4 and D:12 are of low virulence and killed mice in more than 72 hours. These results are in agreement with Borkowska *et al.* (1995), Okerman and Spanoghe (1980), Okerman *et al.* (1978), Ose and Muenster (1968), who studied virulence of *P. multocida* strains isolated from rabbits. Such isolates obtained from septicemic cases revealed high pathogenicity, but those from typical catarrhal infections showed moderate or mild virulence. In this study, the strains of very low pathogenicity and did not cause any problem in field (A:4 and D:12) were ignored, and the comparative study was done with field strains that caused high mortality.

From data in Table 4, it was found that mice vaccinated with monovalent vaccine of recent isolates and challenged with homologous and heterologous *P. multocida* strains, gave good immunological value with homologous strains. Meanwhile, this vaccine failed to protect mice against heterologous challenge.

The previous results indicated that occurrence of partial cross-immunity is contributed to the presence of somatic type 3 (A:3,5 and A:3). These results are in agreement with Okerman and Spanoghe (1980) who found that the occurrence of partial cross-immunity is contributed to the presence of somatic antigen type 3 in some strains. Also, the results showed that the protective value of vaccines was not attributed to the capsular antigen of strains examined, but may be related with their somatic antigen, as previously confirmed by Borkowska *et al.* (1995).

From results reported in Table 5, it was noticed that the rabbits vaccinated with currently used vaccine and challenged with the same serotypes (homologous serotypes), gave good protection, and when challenged with heterologous serotypes gave various protection values. These results may be due to partial cross-protection among strains of various serotypes. Also, the results indicated that no protection was

noticed in vaccinated rabbits when challenged with recent isolated serotypes B:2 and D:6. This may be due to absence of these serotypes in the currently used vaccine.

In this work, we noticed that the appearance of disease in little farms in spite of the vaccination may be due to bad management or wrong vaccination. Also, isolation of *P. multocida* serotypes B:2 and D:6 in some farms causes pathological conditions in these farms. This may be due to presence of large animal farms near rabbit farms. This problem is very easy to treat by preparing autogenous vaccine. These results agreed with Borkowska *et al.* (1996) who found that the preparation of the vaccine must be prepared from most frequently isolated rabbit strains and selecting the strains with the most immunogenic properties.

From the previously obtained results, it could be concluded that the preparing of autogenous vaccine against rabbits *Pasteurella* infection is recommended as it is the best method to control the disease when showing any new immunogenic isolates even in sporadic cases.

Table 1. Recent capsular and somatic serotypes from rabbits field isolates of *P. multocida*.

| Somatic               | Capsular | Strain origin |    |            |         |      | No. of isolate |
|-----------------------|----------|---------------|----|------------|---------|------|----------------|
|                       |          | I             | II |            | III     |      |                |
|                       |          | NP            | NP | Middle Ear | Abscess | Lung |                |
| 3                     | A        |               | 2  |            |         | 2    | 4              |
| 3,5                   | A        |               | 1  |            |         | 1    | 2              |
| 12                    | A        |               | 3  | 2          | 1       | 2    | 8              |
| 4                     | A        | 1             |    |            |         |      | 1              |
| 2                     | B        |               | 2  |            | 1       | 4    | 7              |
| 6                     | D        |               | 2  |            | 1       | 3    | 6              |
| 12                    | D        | 2             |    |            |         |      | 2              |
| Total No. of isolates |          | 3             | 10 | 2          | 3       | 12   | 30 *           |

NP: Nasopharynx

I: Diseased animals with very low symptoms and less common types.

II: Diseased animals with sever symptoms.

III: Dead animals (recently dead and make PM).

\* These number of isolates isolated from 150 samples.

Table 2. The relationship between virulence of the organism (time of death in hours) and its serotype and the organ of isolation.

| Virulence             | Colony type | Time of death (hours) | No. of isolates | Serotype | Organ of isolation                    |
|-----------------------|-------------|-----------------------|-----------------|----------|---------------------------------------|
| Highly virulent       | Irresescent | Less than 24 hours    | 3               | A:3      | Nasopharynx<br>Internal organ         |
|                       |             |                       | 2               | A:3,5    |                                       |
|                       |             |                       | 5               | B:2      |                                       |
|                       |             | In 24 hours           | 2               | B:2      | Abscess                               |
|                       |             |                       | 1               | A:3      |                                       |
| Intermediate virulent | Irresescent | 36-48 hours           | 5               | A:12     | Nasopharynx<br>Internal organ         |
|                       |             |                       | 5               | D:6      |                                       |
|                       | Muroid      | In 72 hours           | 3               | A:12     | Ear<br>Abscess                        |
|                       |             |                       | 1               | D:6      |                                       |
|                       |             |                       | 1               | A:4      |                                       |
| Low virulent          | Blue        | More than 72 hours    | 2               | D:12     | Nasopharynx (with very mild symptoms) |

Table 3. Determination of minimum lethal dose (LD<sub>50</sub>) of recent *P. multocida* serotypes isolated from rabbits.

| Highly virulent |                    | Moderate virulent |                    | Low virulent |                    |
|-----------------|--------------------|-------------------|--------------------|--------------|--------------------|
| Serotyping      | LD <sub>50</sub>   | Serotyping        | LD <sub>50</sub>   | Serotyping   | LD <sub>50</sub>   |
| A:3             | 10 <sup>-9.4</sup> | A:12              | 10 <sup>-8.8</sup> | A:4          | 10 <sup>-6.1</sup> |
| A:2,5           | 10 <sup>-9.8</sup> | D:6               | 10 <sup>-8.9</sup> | D:12         | 10 <sup>-5.9</sup> |
| B:2             | 10 <sup>-9.5</sup> |                   |                    |              |                    |

Table 4. Protective value of monovalent vaccine prepared from different *Pasteurella multocida* serotypes (recently isolated strains) and challenged with homologous and heterologous serotypes in mice.

| Challenge strain | Immunization with monovalent vaccine of the following serotypes |         |         |         |         |
|------------------|---|---------|---------|---------|---------|
| Serotype         | A:3,5   | A:3     | A:12    | B:2     | D:6     |
| A:3,5            | 4.90  | 4.70    | 1.15    | 1.45    | 0.96    |
| A:3              | 4.68  | 4.84 ** | 1.26 *  | 1.40 *  | 0.88 *  |
| A:12             | 1.75*   | 1.65 *  | 3.19 ** | 1.59 *  | 0.98 *  |
| B:2              | 0.98*   | 0.92 *  | 0.79 *  | 4.98 ** | 0.73 *  |
| D:6              | 0.88*   | 0.98 *  | 0.88 *  | 1.15 *  | 3.85 ** |

\* Non-protective

\*\* Protective



Table 5. Results of challenge test in rabbits vaccinated with currently used vaccine and challenged with the field strains (heterologous strains) and vaccinal strains (homologous strains).

| Challenge strains       |       | Rabbits vaccinated with currently used vaccine |                                  |              | Non-vaccinated rabbits |                                  |              |
|-------------------------|-------|--|----------------------------------|--------------|------------------------|----------------------------------|--------------|
|                         |       | No. of rabbits                                 | No. of dead/ No. of total rabbit | Protection % | No. of rabbits         | No. of dead/ No. of total rabbit | Protection % |
| Recent isolated strains | A:3,5 | 4  | 0/4                              | 100 %        | 2                      | 2/2                              | 0 %          |
|                         | A:3   | 4  | 1/4                              | 75 %         | 2                      | 2/2                              | 0 %          |
|                         | A:12  | 4  | 2/4                              | 50 %         | 2                      | 2/2                              | 0 %          |
|                         | B:2   | 4  | 4/4                              | 0 %          | 2                      | 2/2                              | 0 %          |
|                         | D:6   | 4  | 4/4                              | 0 %          | 2                      | 2/2                              | 0 %          |
| Vaccinal strains        | 5:A   | 4  | 0/4                              | 100 %        | 2                      | 2/2                              | 0 %          |
|                         | 8:A   | 4  | 1/4                              | 75 %         | 2                      | 2/2                              | 0 %          |
|                         | 9:A   | 4  | 0/4                              | 100 %        | 2                      | 2/2                              | 0 %          |
|                         | D:2   | 4  | 1/4                              | 75 %         | 2                      | 2/2                              | 0 %          |

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## دراسة الضراوة والاستجابة المناعية لعترات الباستريلا مالتوسيدا المعزولة حديثاً من الأرانب المصابة

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الدقى - الجيزة

في هذه الدراسة تم تجميع 150 عينة من مزارع الأرانب والتي كانت تعاني من حالات  
ن فوق عالية يشتبه في إصابتها بميكروب الباستريلا مالتوسيدا حيث ثبت الإصابة في عدد ثلاثين من  
تلك الحالات (بنسبة 20%). وبإجراء اختبارات التصنيف السيرولوجي لتلك العينات تبين أن معظمها  
ينتمي لأنواع أ:3، أ:5.3، أ:12، ب:2، د:6 مع وجود بعض العترات الأخرى بنسبة ضئيلة وهي  
أ:4، د:12 وعند إجراء اختبار لوغاريم الحماية في الفئران وبمعرفة الخواص المناعية لتلك العترات  
وجد أن هناك عترات كانت عالية الضراوة وأخرى متوسطة وأخرى ضعيفة حيث كانت قوة الحماية  
جيدة في العترات أ:3، أ:5.3، أ:12، ب:2، د:6 بينما كانت العترات الأخرى قليلة الضراوة.

وقد تم في هذه الدراسة تحصين الأرانب باللقاح المحلى وإجراء اختبار التحدى بنفس  
عترات اللقاح ومرة أخرى بالعترات المعزولة حديثاً وجد ان اللقاح قد أعطى مناعة جيدة في الحالتين  
فيماعد العترتين المعزولتين حديثاً ب:2، د:6.

