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VACCINATION TRIALS IN RABBITS WITH CELL FREE CULTURE FILTURE FILTRATE OF PASTEURELLA MULTOCIDA

(With 4 Tables and 4 Figures)

By

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محاولات تحصين الأرانب بإستخدام الرشح الخالى من الخلايا لميكروب الباستيريلا

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فى هذا البحث تم إستخدام عدد ٨٠ أرنبا عمرها ٨ أسابيع قسمت إلى أربع مجموعات وأستخدم فى التحصين لقاح محضر من الرشيح الخالى من خلايا الباستريلا ملتوسيدا. المجموعة الاولى تم تحصينها عن طريق التنقيط فى العين ١رسم والثانية بالتنقيط فى الأنف (١ رسم) أما المجموعه الثالثه بالحقن ١/٢ سم تحت الجلد. والمجموعه الرابعة تم تحصينها عند عمر ١٢ أسبوع. وقد تم جمع عينات السيرم على فترات ٢١ يوم بعد الجرعة الأولى ، ٣٠ يوم بعد الجرعة الثانية من التحصين وأيضا تم جمع عينات السيرم من الأرانب عند ٤٠ ، ٦٠ ، ٩٠ يوم بعد إجراء أختبار تحدى المناعة الذى تم عند عمر ١٥ أسبوع. وقد تم أختبار جميع عينات السيرم بأستخدام أختبار الاليزا. ,أختبار تلازن الدم غير المباشر لقياس الأجسام المناعية بها. وقد تم إجراء أختبار التحدى وجد أن درجة الحماية كانت فى الأرانب المحصنه بلقاح عن طريق التنقيط فى الأنف ثم الحقن تحت الجلد ثم المحصنة عن طريق التنقيط فى العين ومن هذا يتضح أن لقاح الرشيح الخالى من خلايا الباستيريلا له تأثير مناعى قوى ضد ميكروب الباستريلا خصوصا فى الجزء العلوى من الجهاز التنفسى وقد تبين ذلك من فحص العينات هستوباثولوجيا.

SUMMARY

A total of 80 rabbits 8- week old were used in this experiment and a cell-free culture filtrate (CCF) of *P. multocida* was used as a vaccine. The rabbits were divided into four groups. The first group was vaccinated intraocularly (eye dreop) (0.1 ml). Second group intranasally (0.1 ml) and the third group

was inoculated S/C with o.5 ml. The fourth group was not vaccinated and served as a control. The second dose was given at 12 weeks of age. Sera were collected at 21 days after the first dose, 30 days after the second dose and 40, 60 and 90 days after challenge which was performed at 15 weeks of age. All sera were tested for anti *P. multocida* antibodies by using the enzyme linked immunosorbent assay (ELISA) and indirect haemagglutination test (IH). Challenge was conducted in vaccinated rabbits. Protection was superior in rabbits given CCP via intranasal route then the group vaccinated S/C came next. These results indicated that CCF could be considered as an effective immunogen when administered intranasally for protecting rabbits against Pasteurellosis and this was revealed by histopathological examination.

Key words: *Cell free culture filtrate vaccine*

INTRODUCTION

Pasteurellosis remains a common disease in commercially produced rabbits. Infected rabbits may become a symptomatic carrier or may manifest a variety of syndromes including chronic rhinitis, multiple abscesses, otitis media or genital infections (Flatt, 1974; Weisbroth and Scher, 1969 and Digiacome et al., 1983).

These conditions usually are non fatal but may render the animals unsuitable for biomedical research. Under stressful conditions, pneumonia and septicaemia may develop resulting in significant mortality (Flatt, 1974; Glorioso et al., 1982 and Digiacome et al., 1983).

The removal of *P. multocida* carriers and vaccination are the most likely means of controlling this disease. Thus a vaccine would be of great value in the production of rabbits. Previous attempts to vaccinate rabbits using *Past. multocida* bacterins were moderately successful in protecting against pasteurellosis in rabbits (Okerman and Spanoghe, 1981).

The successful application of cell free filtrates as a vaccine for turkeys arises a question whether this vaccine can be used in rabbits.

The aim of this work was to study the effect of vaccination with various routes by inoculation of *P. multocida* cell free culture filtrate, on performance and protection of rabbits against homologous challenge.

MATERIALS and METHODS

P. multocida strain:

Past. multodida serotype 12:A, was obtained from Vet. Serum and Vaccine research Institute, Abbasia, Cairo, Egypt.

Cell free culture filtrate of P. multocida vaccine:

CCF was produced in brain heart infusion (BHI) broth by incubating this organism for 72 hours at 37°C in a shaker water bath. Following incubation, the culture was centrifuged for 40 minutes at 1000 xg. The pellet was discarded. The supernatant was filtered twice through 0.45 um filter, and the resulting CCF was stored at 4°C until used. Sterility of CCF was confirmed by culturing small samples for 48 hours on blood agar plates at 37°C (Ficken et al., 1991b).

Experimental rabbits:

A total of 80 New Zealand rabbits (8 to 9 weeks old weighing 2.5 kg) were used. Rabbits were housed in wire rabbitary and observed for one week for any abnormal clinical signs of any disease before starting the experiments. Specimens were obtained for bacteriological cultures and serum was negative for *P. multocida* antibodies. Rabbits were divided into 4 groups (The first was vaccinated by eye drop (0.1 cc) the second group intranasally (0.1 cc) and the third was inoculated by 0.5 cc (S/C), and the fourth group was left as control without vaccination. A Second dose was given at 12 weeks of age.

Challenge:

All group of vaccinated rabbits were challenged with the virulent strain used in vaccine preparation by the same route of vaccine application. The fourth control group was challenged S/C with 0.1 ml of 100 LD_{50} of the virulent strain serotype 12A according to the mouse protection test.

Estimation of log protection in mice:

This was conducted by using the method of Ose and Muenster (1968). Briefly, 3 groups of mice (45 mice each) were vaccinated by the same route used in rabbits and the dose was one drop by instillation into the eye or the nose and 0.1 ml S/C. A fourth group 45 mice was left as non vaccinated controls.Nine serial dilutions (10^{-1} up to 10^{-9}) of virulent *P. multocida* strain used in vaccine preparation were inoculated I/P in groups of vaccinated and non vaccinated mice. LD_{50} was calculated in each group and log protection was estimated accordingly (Table. 4).

Collection of sera:

Sera were collected from vaccinated rabbits at 21, 30 days before challenge and at 40, 60, 90 days after challenge. All sera were tested for antibodies to *P. multocida* by enzyme linked immunosorbent assay (ELISA) as described by Marshall et al. (1981) and indirect haemagglutination test (IH) as described by Carter and Rappy (1962).

Histopathological changes:

Rabbits were monitored for 10 days post challenge or until death at which time they were necropsied. Samples from the lung, liver, heart, spleen were taken and fixed in 10% buffered formalin (Carletons, 1967). Paraffin sections were stained with haematoxylin and eosin and examined histopathologically.

RESULTS

It can be clearly seen from data illustrated in Table (1), that there was a significant rise in the mean optical density in sera of rabbits vaccinated by intranasal group as it was increased from 0.1 prevaccination to reach 2.3, 2.5 at 21 and 30 days post vaccination, respectively. Meanwhile, the mean optical density in sera of rabbit vaccinated S/C were 2.0 and 2.3 at the same previously mentioned time interval. The least optical density were noted in sera of rabbit vaccinated by eye drop, as it was 1.8 and to 2.06.

From the data illustrated in Table (2), it can be observed that the highest anti *P. multocida* antibody level was noted in sera of rabbits vaccinated intranasally as it reached a peak level (2500) at 30 days post vaccination. On the other hand, the lowest antibody titre (160) was recorded on testing sera of rabbits vaccinated by eye drop, at the same period of time post vaccination.

Lungs of rabbits vaccinated with CCF filtrate showed variable thickening of the alveolar walls by increased alveolar epithelium and increased cellular infiltration mainly by lymphocyte, macrophages, plasma cells and some neutrophils (Fig. 1-4).

The increased number of macrophages was seen either in loose contact with the alveolar walls or more commonly seen free in the alveolar and bronchial lumena.

The changes in liver were slight congestion of the sinusoids, the hepatocytes suffered from slightly variable degenerative changes, the cytoplasm became more eosinophilic and sometimes became faint with

hydropic vacuoles. Some hepatic vessels contained some rounded cells mainly plasma cells and lymphocytes.

Most of the cardiac blood vessels were dilated and engorged with blood, slight swelling of myocardial fibres and somewhat loss striations. Few lymphocytic infiltration and oedema between muscle bundle. Some renal capillaries were dilated and engorged with blood. Mild degeneration of the epithelial lining of the collecting tubules.

Generally speaking, spleen, kidney and heart were the least grossely affected organs.

DISCUSSION

Comparable results were obtained by using the IH and ELISA tests for testing sera of rabbits at different time intervals after immunization of CCF filtrate of *Past. multocida* given by eye drop, intranasal and S/C routes. The intranasal route gave the best antibody titre by both tests and come next S/C route and the eye drop route.

It was noted that rabbits inoculated via intranasal route had higher levels of antibodies that were developed at different intervals after vaccination as shown in Table (1). On the other hand, rabbits vaccinated via eye drop and S/C inoculation had lower antibody titres. Also, better resistance (85-95%) to challenge infection with the virulent *P. multocida* strain 12:A was noted in all groups that were vaccinated with CCF. Similar observations were previously noted by Ficken et al. (1991b) who noted that the CCF of *P. multocida* was an effective immunogen when administered via the lower respiratory tract (air sac injection) for protecting turkeys against Pasteurellosis when challenged either with the homologous strain or with a different strain of the same serotypes.

It was reported that CCF induced a transient air sacculitis in turkey (Ficken et al., 1991a) by 6 hours post inoculation. However, any air sac injury induced by intra air sac inoculation of CCF or its derivatives would appear to be minimal and these effects would have slight or no determental effects on bird performance (Ficken et al., 1991a).

Endotoxin content of CCF derived from *P. multocida* is responsible at least in part, for protection against challenge with virulent *P. multocida* of the same serotype (Kodama et al., 1983).

It allows accurate calculation of log protection, it includes the estimation of LD_{50} in vaccinated candidates in comparison with non vaccinated mice.

The pathological changes observed in the lung of rabbits vaccinated with CCF filtrate indicated that the presented pasteurella antigen stimulated the lung defense mechanisms as manifested by cellular proliferation and increased number of macrophages.

These observations are in accordance with that reported by several authors (Flatt and Hungworth, 1971; Watson et al., 1975 and Fahmy et al., 1985).

The change in liver was most probably due to the effectof the adsorbed pasteurella endotoxins. These findings are in partial agreement with that mentioned by Glavits and Magyar (1990) and Clarence et al. (1991) who only reported degenerated patches in the liver whereas Cheville and Rimler (1989) recorded liver cirrhosis after *Past. multocida* infection.

The spleen, kidneys and heart were the least affected organs, a nather which agreed with Hagen (1958) and Sigmund (1979).

These changes observed in different internal organs histopathologically were not degenerated and it can be regenerated and it is a transient reaction for endotoxin of *P. multocida* organisms.

From the previously obtained data, it can be deduced that CCF of *P. multocida* is an effective immunogen when administered intranasally at 8 weeks old and second dose at 12 weeks old for protecting rabbits against pasteurellosis.

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Table (1) : Antibody titre against Past. multocida detected by ELISA before and after vaccination of rabbits.

Route of inoculation	Days post vaccination					
	Pre - vaccination	21 D pre chall.	30 D pre chall.	40 D post challenge	60 D post challenge	90 D post challenge
1. Eye drop	0.1	1.8	2.060	1.245	2.32	2.58
2. Intranasal	0.1	2.3	2.584	2.124	2.86	3.14
3. S/C inoculation	0.1	2.0	2.384	2.431	2.56	2.74
4. Control	0.1	1.0	0.1	died	died	Died

The result is expressed as mean of optical density.

Table (2) : Antibody titre against Past. multocida type 12:A detected by IH test before and after vaccination of rabbits.

Route of inoculation	Days post vaccination					
	Pre - vaccination	21 D pre chall.	30 D pre chall.	40 D post challenge	60 D post challenge	90 D post challenge
1. Eye drop	5	20	160	80	320	640
2. Intranasal	5	80	2500	320	5000	10000
3. S/C inoculation	5	40	320	160	640	1280
4. Control	5	5	5	died	died	Died

Table (3) : Survival of vaccinated rabbits after challenge.

Route of vaccination	No. of rabbits	Dead / survive	Total No. of survive	% survive
1. Eye drop	20	3/20	17	85 %
2. Intranasal	20	1/20	19	95 %
3. S/C inoculation	20	2/20	18	90 %
4. Control unvaccinated	20	20/20	0	0 %

Log protection of vaccinated mice $10^{5.54}$.

Table (4) : Mice log protection.

Route of inoculation	Logs protection of mice
1. Eye Drop.	2.7
2. Intranasal	3.5
3. S/C inoculation	3.2
4. control unvaccinated	1.5

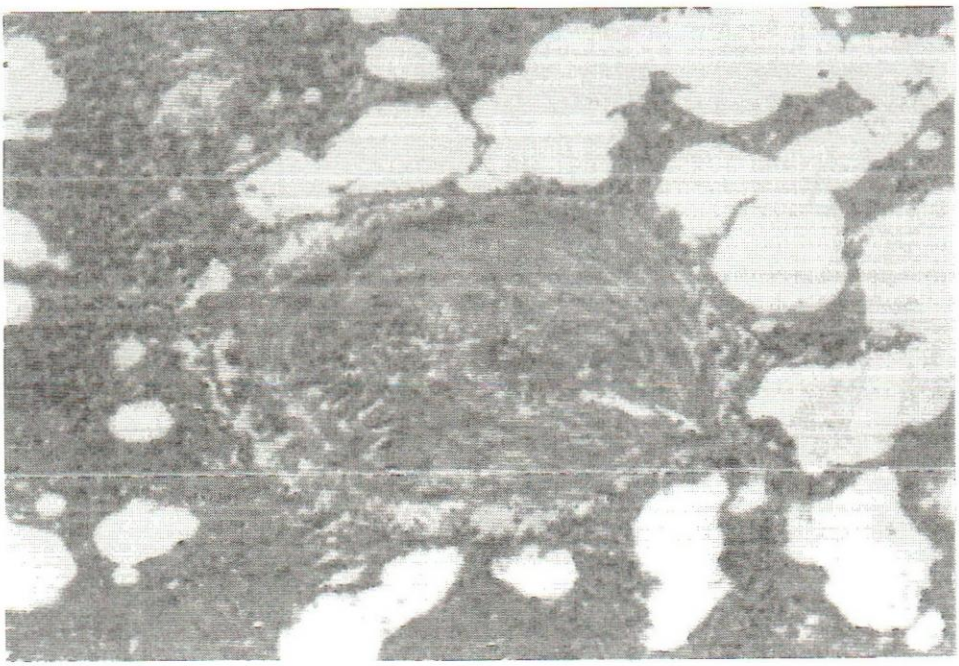

Fig. 1: Lung: Lining endothelium of the blood vessel is degenerated, the wall is thickened with few inflammatory cells.

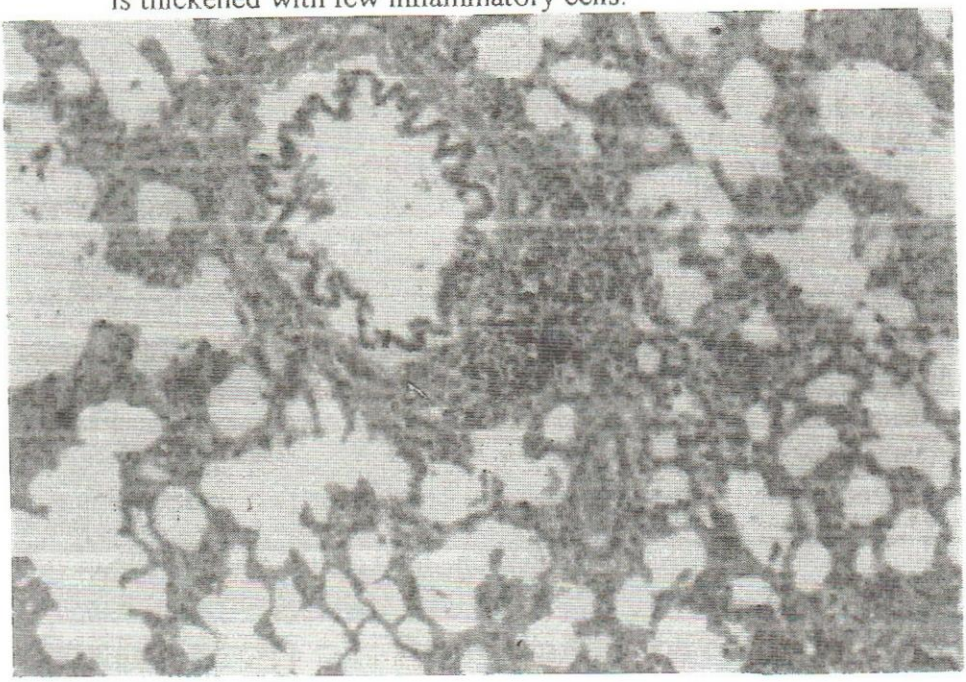

Fig. 2: Lung: Degeneration, focal desquamation of the bronchial epithelium.

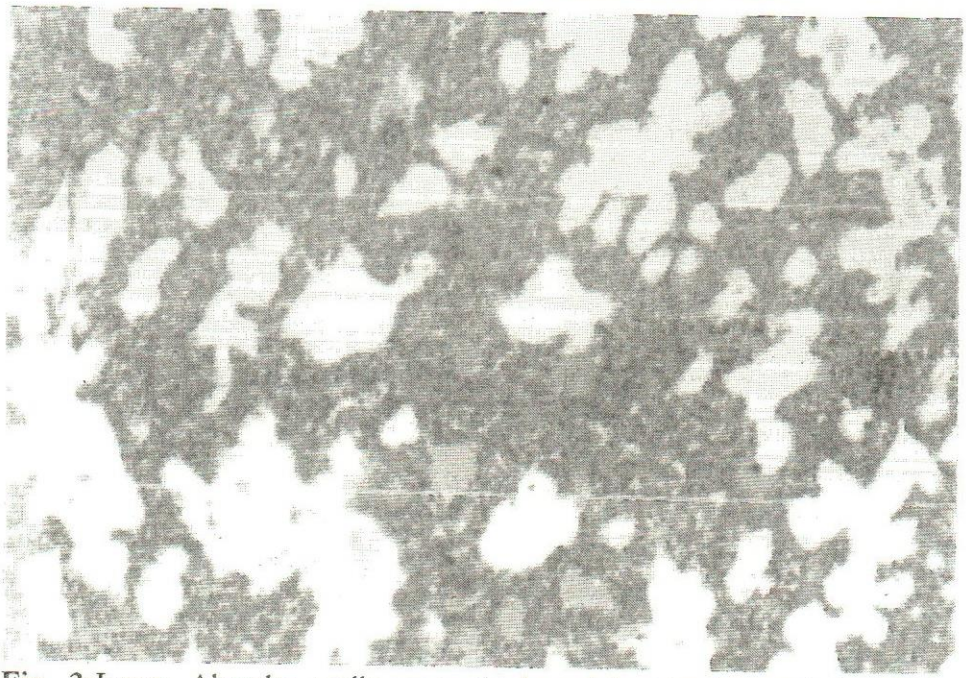

Fig. 3: Lung: Alveolar walls were thickened by the hyperplasia of their epithelial lining and infiltration with large number of mononuclear cells.

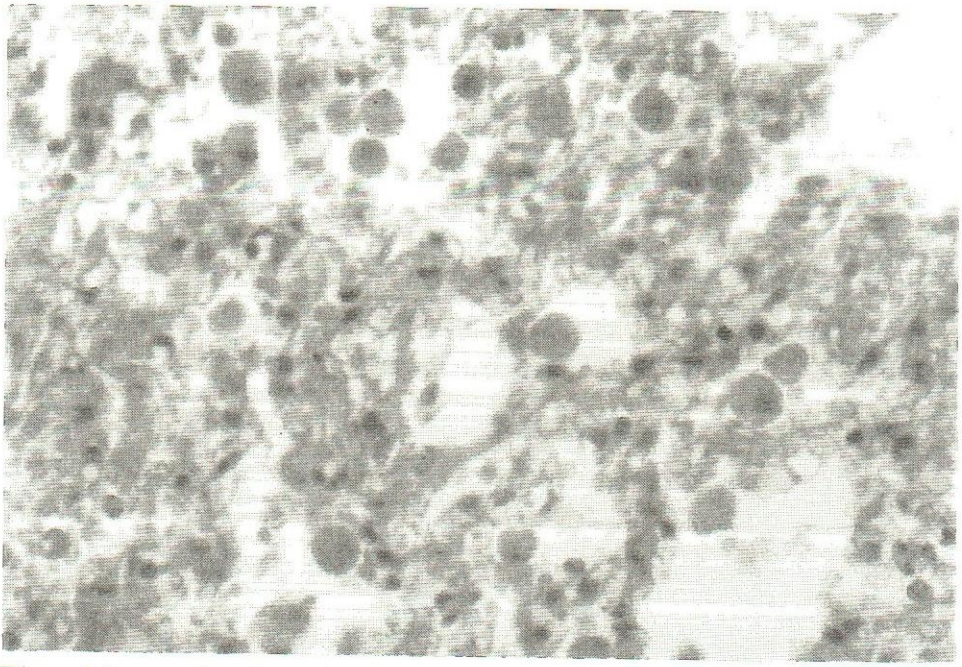

Fig. 4: Lung showing alveolar lumen and interalveolar septa filled with inflammatory cells mainly large macrophages and lymphocytes.