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IMMUNE RESPONSE OF CHICKEN TO STREPTOMYCIN-DEPENDENT MUTANT, CU AND OIL-ADJUVANT VACCINES OF PASTEURELLA MULTOCIDA

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SUMMARY : Streptomycin-dependent mutant was obtained from the local vaccinal strain of *Pasteurella multocida* by using the N-methyl-N'-nitro-N-nitrosoguanidine. The mutant was completely non-pathogenic to chicken and mice but it induced circulating and local humoral antibodies and provided protection against challenge with virulent strains of *P.multocida* through the palatine cleft of chickens. CU vaccine gave almost similar results. The oil adjuvant vaccine produced a higher titre of circulating but no local antibodies and its protective value was much inferior.

INTRODUCTION

Vaccination trials in poultry against pasteurellosis have been polarized in the last few years in the application of 3 types of vaccines: Oil adjuvant vaccine (Mall and Nilakantan, 1971; Matsumoto and Helfer, 1977), Clemsen University (CU)vaccine (Due and Maheswaran, 1978; Rice et al., 1978) and non-pathogenic mutant vaccines (Maheswaran et al., 1973;Michael et al., 1979; Derieux and Dick, 1980). The present work deals with comparative studies on these 3 vaccines with regard to their antibody response to these vaccines as measured by passive haemagglutination, ELISA and immunofluorescence tests. The pathogenicity and distribution of *P. multocida* strains used in these vaccines were also tried in chickens and mice.

MATERIALS AND METHODS

Animals : 658 nine week old healthy Newkelse chicken;having neither a history of fowl cholera infection or vaccination and 240 white mice were used.

Bacteria: *P. multocida* local strain was obtained from Vaccine and Serum Production Institute, Abbassia, Cairo Serotypes

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X-73, P-1059 and Liver-361 were supplied by the National Institute of Animal Health, Kodaira, Tokyo, Japan. Clemson University (CU) strain was obtained from Minnesota University, St. Paul, Minnesota, U.S.A.

Mutation of the local vaccine strain : Mutation was carried out with N-methyl-N'-nitro-N-nitrosoguanidine in order to obtain a streptomycin-dependent (Str.D.) mutant(Chengappa and Carter, 1979).

Pathogenicity test : 3 groups of 28 chickens each, were injected s.c. with 10-fold dilutions of 18 hours brain heart infusion broth cultures of the mutant, CU and virulent strains. The infected chickens were observed daily for 2 weeks. Three groups of 70 mice, each were injected s.c. with the mutant, CU and virulent strains in doses varying from 5×10^2 to 10^8. 30 mice were left as control.

Quantitation of *P. multocida* strains in the internal organs: 3 groups of 28 chicken each were injected i.v. with 1000 viable cells of mutant, CU and virulent strains. 4 chicken in each group were killed at 0,0.6, 3,21,24,48 and 72 hours and the number of Pasteurella organisms was counted per g of lung, liver and spleen or ml blood by the plate count technique using tryptose agar plates (Pabs-Garnon and Soltys, 1971).

Vaccination : 3 groups of 70 chicken each were vaccinated s.c. by 0.2 ml of 18 hours brain heart infusion broth cultures of mutant, CU and oil adjuvant vaccines. A second dose was injected after 4 weeks. A 4th group was left as a control. Tracheal secretions and serum samples were collected from 10 chickens from each group at intervals of 0,2,4,6,8,10 and 12 weeks after the second injection.

Detection of antibodies : Circulating antibodies in serum samples were detected by the passive haemagglutination test (Due and Panduranga Rao, 1978) and ELISA(Marshall et al., 1981). Local antibodies in tracheal secretions were demonstrated by the indirect immunofluorescence test (Due and Maheswaran, 1978).

Electrophoresis : Fractionation of serum globulins in chicken sera was carried out according to the method described by Bierer (1969).

Challenge : 3 groups of 60 chicken each, vaccinated by mutant, CU and oil adjuvant vaccines and 30 control chickens were challenged 3 weeks after the 2nd injection by swabing the palatine cleft with broth suspension of the serotypes X-73, P-1059 or liver-361 of virulent *P. multocida*. All birds were observed for 2 weeks.

RESULTS

Pathogenicity

The injection of the mutant strain in doses up to 10^8 organisms caused no death in chickens. Similar result was observed in case of CU strain with the exception of the group injected with 10^8 organisms where 1 out of 4 chicken died. On the other hand, the virulent strain caused 100 % mortality in doses of 10^5 - 10^8 viable bacteria.

The pathogenicity test in mice revealed that the mutant strain was also non-pathogenic to mice, whereas the CU strain caused 50 % mortality in a dose of 10^5 organisms and 100 % mortality in a dose of 10^7 - 10^8. The virulent strain caused death of 50 % of mice in a dose of 500 organisms and 100 % death in a dose of 10^3.

Quantitation of *P multocida* in chicken's body

As shown in Table 1, the virulent strain could be detected in the liver, lung and spleen 35 minutes after i.v. injection. The number of organisms increased reaching its maximum after 48 hours then dropped suddenly. The highest number was observed in the lung. It was detected in the blood 12-48 hours after injection then disappeared. The numbers of the CU organisms were very low in comparison with that of the virulent one and it disappeared from the internal organs after 72 hours. Only 10 - 20 organisms/ml were detected in the blood 12 - 24 hours after injection. The mutant strain could not be detected at all in the blood. It was detected

only in the lung and spleen during the first 3 hours then started to appear in the liver at 12 hours post inoculation. The highest number was recorded in the spleen after 3 hours.

Table 1: Number of *Pasteurella multocida* strains in various organs after intravenous inoculation with virulent, CU and mutant strains.

Strains	Hours After Infection*	Mean number of bacteria per gram			Bacteria per ml of blood
		Liver	Lung	Spleen	
	0**	0	0	0	0
	0.6	30	100	100	0
	3.0	400	1,000	1,000	0
Virulent	12	1,000,000	20,000	30,000	1,800
	24	22,000,000	10,000,000	300,000	5,000,000
	48	33,900,000	64,000,000	860,000	9,000,000
	72	3,000	25,000	18,000	0
	0	0	0	0	0
	0.6	70	10	40	0
	3	50 0	400	2,000	0
CU	12	3,000	2,000	20,000	20
	24	500	10,000	200	10
	48	20	1,000	100	0
	72	0	0	0	0
	0	0	0	0	0
	0.6	0	70	800	0
	3	0	40	950	0
Mutant	12	195	170	100	0
	24	70	30	20	0
	48	30	20	50	0
	72	0	0	0	0

* = Four birds were killed at each time.

**= 0: preinfection

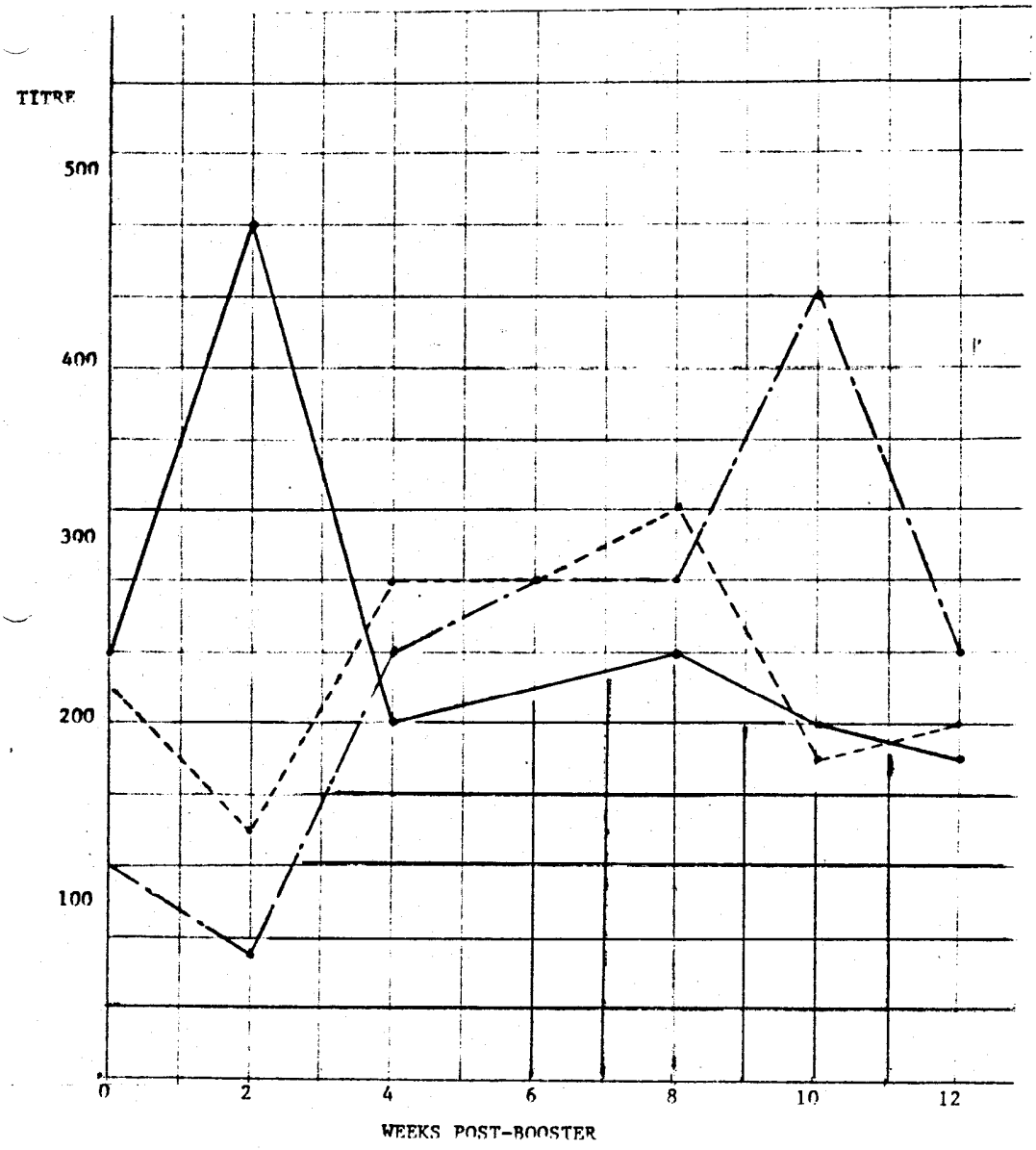

FIG.1. HAEMAGGLUTININ TITRES (MEAN) IN SERUM OF CHICKENS VACCINATED WITH VARIOUS VACCINES AT DIFFERENT INTERVALS.
(_____) CU, (-------) MUTANT, (__ __ __) OIL ADJUVANT

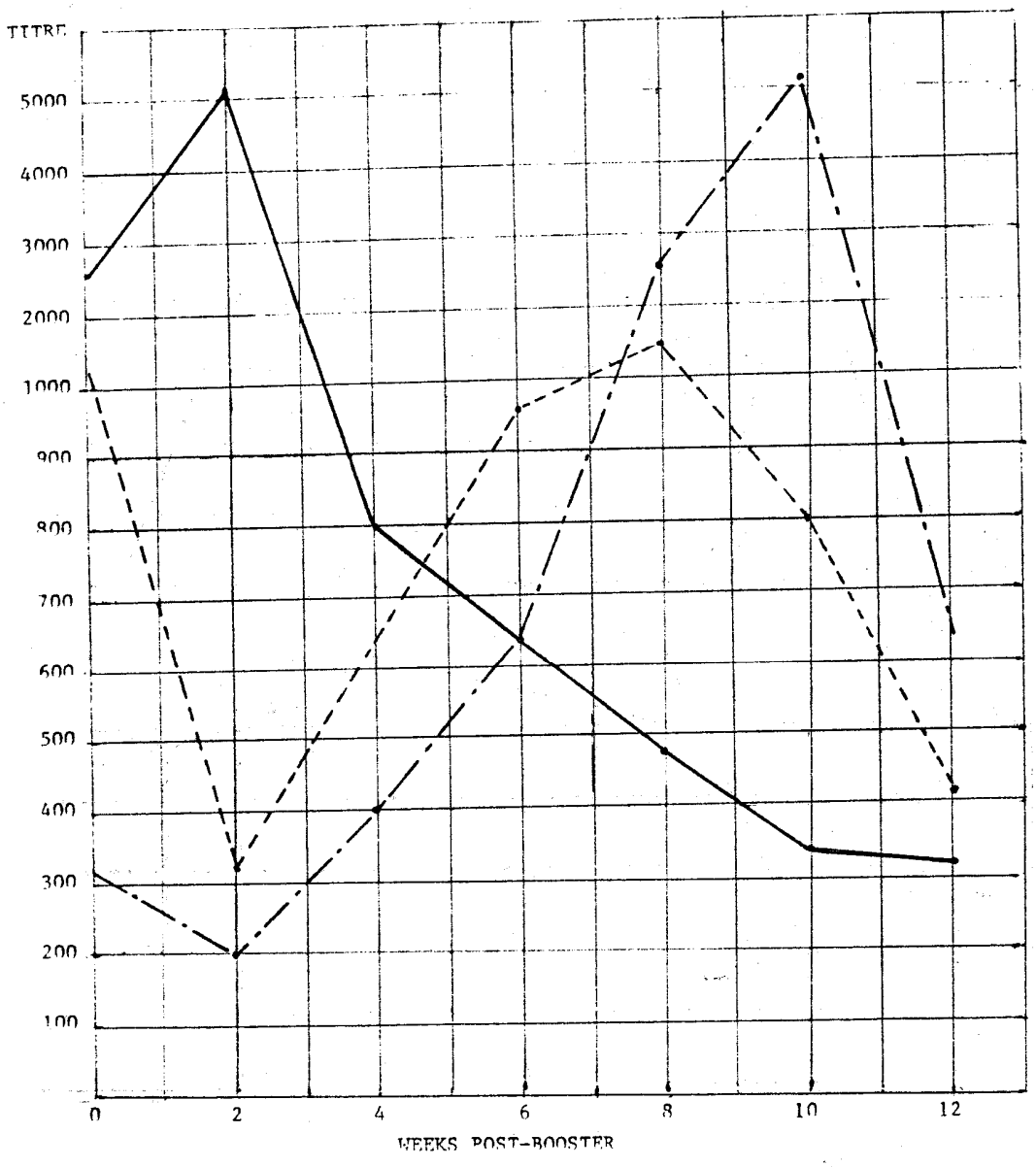

FIG.2. ANTIBODY TITRES IN SERUM OF CHICKENS VACCINATED WITH DIFFERENT VACCINES OF P. MULTOCIDA AS MEASURED BY ELISA TEST.
(_____) CU, (-------) MUTANT, (__ __ __) OIL ADJUVANT

Detection of antibodies

Systemic humoral antibodies

Figures 1 and 2 demonstrate the conformity of the results of both the passive haemagglutination and the ELISA test with regard to the pattern of systemic humoral antibodies. The CU antibodies reached their maximum on the 2nd week post-booster. The maximum antibody titre was observed on the 8th week in case of the mutant vaccine and on the 10th week in case of the oil adjuvant vaccine. The peak in all cases was followed by sudden and steep fall of antibodies.

The pattern of antibody curves is conforming to that of gamma globulins as determined by electrophoresis **(Table 2)**.

Table 2: Levels of total gammaglobuline in serum of chickens vaccinated with various vaccines as determined by electrophoresis.

Vaccines	Pre-booster	Time in weeks post-booster					
		2	4	6	8	10	12
Oil adjuvant	2.47*	1.03	2.42	2.43	2.46	2.97	2.34
Mutant	3.15	1.60	2.35	2.87	3.58	3.20	2.02
CU	3.52	5.78	1.16	3.24	3.20	3.24	2.57
Control	1.09	1.01	0.98	1.15	1.01	1.08	0.99

* = g. %

Local humoral antibodies

Local antibodies could be detected by the indirect immuno-fluorescence test in chickens injected with the mutant and CU vaccines from pre-booster and up to the 6th week post-booster. Chickens vaccinated with the oil adjuvant vaccine did not show any local antibodies during pre or post-booster intervals.

Challenge

As demonstrated in **Table 3**, the mutant vaccine gave the highest protection (70 - 90 %) to chickens challenged via the palatine cleft with the virulent strains, followed by the CU vaccine which protected 60 - 85 % of the challenged chickens. The oil adjuvant vaccine could protect only 40 - 50 % of the chickens.

DISCUSSION

It is clear from the obtained results in this work that, Str. D. mutant has lost its pathogenicity to chicken and mice and failed to multiply in high numbers in the internal organs as the virulent strain. Both the mutant and CU strains have not lost their invasive and disseminative characters completely as reported by Maheswaran et al. (1973) as they could be detected in the liver, lung and spleen though in small numbers.

Table 3: Results of challenge of chickens vaccinated with various vaccines

Type of vaccine	No.of birds	Challenge strains: X-73 Infected*/Challenged	X-73 Survival %	P-1059 Infected/Challenged	P-1059 Survival %	Liver-361 Infected/Challenged	Liver-361 Survival %	Total Survival No.	Total Survival %
Oil adjuvant	60	12/20	40	12/20	40	10/20	50	26	43
Mutant	60	6/20	70	2/20	90	4/20	80	48	80
CU	60	8/20	60	3/20	85	6/20	70	43	72
Control	30	10/10	0	10/10	0	10/10	0	0	0

* Including dead and moribund birds.

The results of the passive haemagglutination and ELISA are conforming with the pattern of the gamma globuline as determined by electrophoresis. In the 2nd week post-booster, the CU vaccine induced the highest level of serum antibodies in contrast to chickens vaccinated with the oil adjuvant and mutant vaccines, where the serum antibodies dropped to the lowest level during the whole experiment. However, the serum antibodies dropped rapidly in CU vaccinated chickens and reached on the 4th week post-booster a level lower than that of pre-booster. On the other hand, the antibodies in the mutant and oil vaccinated birds showed a slowly but increasing titres.

The fact that only the CU and mutant vaccines, i.e. living vaccines, but not the killed oil adjuvant vaccine, induced local antibodies as detected by the immunofluroescence test in the tracheal secretions, may explain the better protection against challenge in case of the live vaccines. This is of particular interest, as we used the palatine cleft in challenge, a route which is far more close to the natural infection than the i.m. or i.v. injection. The high protective value obtained by Bairey (1975) and Matsumoto and Helfer (1977) with the oil adjuvant vaccine is now understandable as they used the i.m. route for challenge, where the organisms met excessive amounts of circulating antibodies.

It is concluded that Str.D.mutant is a promising vaccine and it is worthy for further studies to determine the best conditions for field application.

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