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IMMUNE RESPONSE IN CHICKEN VACCINATED WITH COMBINED *E. COLI* AND *P. MULTOCIDA* VACCINE PRIVATE

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SUMMARY

Immunogenicity of an experimentally prepared combined oil emulsified *Escherichia coli-Pasteurella multocida* (*E. coli-P. multocida*) vaccine was evaluated in susceptible chickens. The immune responses of vaccinated birds against monovalent *E. coli, P. multocida* and combined *E. coli-P. multocida* vaccines as estimated serologically using indirect haemagglutination test and ELISA test revealed no substantial differences with respect to the protective values between the monovalent and combined vaccines. The results of challenge test showed that vaccinated chickens could be effectively immunized with combined *E. coli-P. multocida* vaccine against challenge with *E. coli* and *P. multocida* virulent strains. In conclusion, this locally prepared vaccine was safe, immunogenic and protect chickens against *E. coli* and *P. multocida* infection.

INTRODUCTION

Among the bacterial respiratory diseases of chickens, *P. multocida* and *E. coli* infection accounts for major economic losses to the industry through death weight loss, and condemnations during processing (Rosenberger et al., 1985 and OIE Manual, 1990).

E. coli respiratory disease in chickens appear to be secondary to a primary respiratory condition in which other pathogenic agents such as mycoplasma may be a primary pathogen. In addition there was enhancement susceptibility to *E. coli* infection when the respiratory tract of birds was previously affected either by bacterial or viral pathogen (Dozois et al., 1994). The most common clinical syndrome of *E. coli* infection is colisepticemia, which often begin as an upper respiratory infection followed by infiltrations of the blood vascular system and internal organs causing septicemia.

P. multocida infection (fowl cholera) usually occurs as an acute septicemic and chronic localized infection and considered as one of the costly bacterial respiratory diseases in poultry (Rimler and glisson, 1997). *P. multocida* enters through mucous membrane of upper respiratory tract and there presence was related to the severity of upper respiratory infection in chickens.

Both diseases are treated with expensive antibiotics or chemotherapeutic agents, often resulting in the subsequent development of resistant strains that prevent continued use of a formerly effective treatment, this consideration suggested that control by vaccination is of great value against each disease (Hussain, 1994). Nowadays new strategy has been established to use the combined vaccine against multiple infecting agent which have the advantage of providing protection against more than one disease at the same time.

Under field conditions, a combined vaccine against respiratory diseases is preferable for the protection of chickens. Taking into account the important role of the combined vaccine for protection against respiratory disease, for this we examined the efficacy of vaccination with a combined *E. coli-P. multocida* inactivated vaccine in protecting chickens against both infections, to report our continuing investigation on the usefulness of combined vaccines against multiple respiratory pathogens.

MATERIAL AND METHODS

1. Strains:

A. Virulent strain of Pasteurella multocida:

Pasteurella multocida serotype I was kindly supplied by National Animal Diseases Center, USA, Ames, Iowa.

B. Virulent Escherichia coli strain:

Escherichia coli serotype O2 isolated and identified locally according to Ibrahim (1997). Such strain has been the common cause of colibacillosis in chickens. The relative pathogenicity of this strain was re-evaluated in one day old susceptible chickens before vaccine preparation and pre-challenge.

2. Experimental birds:

Seventy-five Arber-Acres chickens, seven weeks old at time of vaccination were used in this experiment. They were free from all infectious disease and had neither a history of fowl cholera nor Escherichia coli infection. Random serum samples were tested for antibodies against Pasteurella multocida and *Escherichia coli*. They were used for evaluation of the locally prepared vaccines.

3. Vaccine preparation:

A. Inactivated fowl cholera vaccine:

Pasteurella multocida was cultivated in casamino acid medium (Bain, 1963) for 24 hours at 37°C with gentle aeriation. After sampies had been taken to check purity and determine colony-forming

unit (CFU) per ml, the culture was inactivated for 24 hours at 37°C with 0.5% formalin. The vaccine was standardized to contain 10^6 CFU/0.5 ml dose.

B. Inactivated *Escherichia coli* vaccine:

E. coli was seeded into tryptic soy broth medium containing 0.05% yeast extract and incubated at 37°C for 24 hours. The culture was adjusted at a concentration of 3.8 x 10^9 colony forming unit (CFU) per 0.5 ml (Panigraphy et al., 1983). The broth culture was taken to check purity, before inactivation with 0.5% formalin at 37°C for 24 hours.

C. Combined E. coli and P. multocida vaccine:

A combined vaccine of *E. coli-P. multocida* was prepared by mixing the previously prepared inactivated cultures (1:1) where each dose from the final mixture was equal to the same dose for each.

D. Addition of adjuvant:

According to Stone et al. (1978), the previously prepared inactivated vaccines (monovalent P. multocida, monovalent *E. coli* and combined vaccines) were emulsified in oil with an aqueous phase-to-oil phase ratio of 1:2. Mineral oil was used as an adjuvant and sorbitan monoleate and tween 80 respectively were used as oil phase and aqueous phase emulsifiers.

4. Quality control of the prepared vaccines:

The prepared vaccines were tested for sterility and safety following the standard international protocols as described by British Veterinary Codex (1970) and Code of American Federal Regulation (1985).

5. Vaccination:

Chickens were divided into four groups, three groups (1, 2 and 3) (15/each) were vaccinated subcutaneously at the age of 6 weeks with 0.5ml/bird with each of monovalent *E. coli, P. multocida* and combined vaccines respectively. Boostering with the same dose was carried out 4 weeks after initial vaccination. Group (4) (30 birds) was kept as unvaccinated control. Serum samples were collected at regular weekly interval for evaluation of immune response after vaccination for 16 weeks.

6. Serological tests for evaluation of humoral immune response:

ELISA test for E. coli and P. multocida antibodies:

It was applied according to method adopted by Marshall et al. (1981) and Leitner et al. (1990).

7. Indirect haemagglutination test (IHT):

The test was carried out according to Carter and Rappy (1962) and Leitner et al. (1990).

8. Challenge test:

A. For *P. multocida* vaccine:

The immunity of vaccinated and unvaccinated birds to *P. multocida* was tested by intramuscular challenge with 0.1ml of 24 hours old culture containing 10 LD_{50} of serotype 1 as suggested by Heddleston and Rebres (1968). Clinical signs, mortality rates and gross lesions were recorded for 7 days post challenge. Reisolation of viable organisms were also tried from liver, heart blood and bone marrow of dead challenged birds.

B. E. coli challenge test:

0.1ml of 24 hours brain heart infusion culture containing (1×10^8 colony forming units (CFU)/ bird of *E. coli* serotype O2 was inoculated via an intrathoracic route. Following challenge, all birds were kept under observation for 7 days and the mortality rate was recorded. All dead birds were subjected to post mortem examination of air sacs, liver and heart and lesions in these organs were scored from 0 to 4 according to severity (0 = no lesions, 1 = cloudy air sacs, pericarditis or perihepatitis, 2 = moderate air saculitis, pericarditis or perihepatitis 3 = bilateral air saculitis, pericarditis or perihepatitis and 4 = sever and extensive fibrinous air saculitis, pericarditis, or perihepatitis). The heart blood and liver specimens were cultured onto MacConkey media for *E. coli* reisolation.

Protective index (PIs):

Using the following formula described by Timms and Marshall (1989) protective indices (PIs) were assessed according to mortality (M) and PM lesions (PML)

$$PIs = \frac{\%\ (M \text{ and } PML) \text{ control} - \%\ \text{vaccinated}}{\%\ \text{control}} \times 100$$

RESULTS

Avian respiratory diseases is one of the most important diseases entities in commercial poultry. As in the case of respiratory diseases of other species, the etiology of avian respiratory diseases is complex. Among bacterial infections *E. coli* and *P. multocida* play the major rule in respiratory disease complex in chicken. Protection against these pathogens is the only mean of solving this problem.

Data presented in table (1) illustrate the GMT in sera of chicken following vaccination with monovalent *E. coli, P. multocida* vaccines as well as the combined vaccine prepared from both organisms. As can be deduced from this table no substantial difference in sera of chicken vaccinated with monovalent *E. coli, P. multocida* and combined *E. coli-P. multocida* vaccines. These parameters remained within the protective level till the 16th week post vaccination.

The same pattern of *E. coli* and *P. multocida* antibody response was also observed from the results of ELISA as shown in table (2). These data also showed that the combined *E. coli P. multocida* was capable of inducing seroconversion in sera of vaccinated chicken which could be detected at various intervals up to 16 weeks post vaccination.

Table (3) describes the lesion scores and persistence or elimination of *E. coli* in vaccinated and unvaccinated chicken after challenge with virulent *E. coli* strain. As could be seen from this table lower lesion scores were reported in chicken vaccinated with either the monovalent or combined vaccine in comparison with control non vaccinated chicken. Also lower recovery rates of 13.3% and 15% were observed after challenge versus 36.6% in controls.

The protective indices given in table (4) revealed that the protection index was 63% in *E. coli* vaccinated chicken while it was 72.4% in chicken immunized with the combined vaccine.

The data illustrated in table (5) explain the protective efficacy of the monovalent or combined vaccine in protection of chicken against virulent P. multocida challenge. This protection was 80% in chicken vaccinated with the monovalent *P. multocida* vaccine, while it was 86.6% in chicken immunized with the combined *P. multocida, E. coli* vaccine.

Table 1: Geometric mean antibody titers (GMT) in serum of chickens rollowing vaccination with different prepared vaccines as measured by indirect haemagglutination test (IHT).

Group	GMT of IHT/Weeks Post Vaccination											
	Antigen used	0	1	2	3	4	6	8	10	12	14	16
1. Vaccinated with monovalent *E. coli* vaccine	*E. coli*	5	11	25	46	53	61	92	121	181	368	279
2. Vaccinated with monovalent *P. multocida* vaccine	*P. multocida*	6	57	121	211	226	788	905	1024	1420	1280	997
3. Vaccinated with combined *E. coli P. multocida* vaccine	*E. coli*	6	21	45	49	65	86	113	197	345	245	226
	P. multocida.	6	49	115	181	197	422	970	1046	1372	1114	970
4. Control	*E. coli*	5	5	6	6	5	6	6	7	19	37	19
	P. multocida	6	7	7	5	6	5	6	6	5	5	5

Table 2: Humoral immune response of chicken following vaccination with different prepared vaccines using ELISA test.

Group	GMT of IHT/Weeks Post Vaccination										
	Antigen used	0	1	2	3	4	6	8	10	12	14	16
1. Vaccinated with monovalent *E. coli* vaccine	*E. coli*	0.08	0.38	0.52	1.02	1.04	0.10	1.20	1.30	1.11	1.02	0.95
2. Vaccinated with monovalent *P. multocida* vaccine	*P. multocida*	0.05	0.46	0.51	1.56	1.65	0.69	1.91	2.08	2.20	1.19	1.37
3. Vaccinated with combined *E. coli P. multocida* vaccine	*E. coli*	0.04	0.23	0.42	0.82	0.95	0.98	1.10	1.20	1.20	0.95	0.90
	P. multocida.	0.06	0.48	0.90	1.40	1.77	1.41	1.84	2.13	2.31	1.93	1.66
4. Control	*E. coli*	0.04	0.03	0.05	0.04	0.04	0.04	0.08	0.46	0.46	0.57	0.46
	P. multocida	0.06	0.06	0.05	0.04	0.04	0.05	0.04	0.06	0.06	0.06	0.06

Table 3: Lesion scores and persistance or elimination of *E.coli* in vaccinated and unvaccinated chicken after challenge with *E.coli* strain.

Group	GMT of IHT/Weeks Post Vaccination			% Recovery of *E.coli*
	Air sac	Pericardium	Liver	
Vaccinated with monovalent *E. coli* vaccine	0.40	0.20	0.60	13.3
Vaccinated with combined *E. coli P. multocida* vaccine	0.20	0.57	0.20	15
Control	1.4*	1.8	1.6	36.6

Table 4: Protection index assessment in chicken vacinated either with monovalent *E.coli* or combined *E.coli* - *P.multocida* vaccines following challenge with pathogenic *E.coli*

Group	Dead/Total	Survival with lesions	% of birds with lesions	Protective index
Vaccinated with monovalent *E. coli* vaccine	1/15	2/15	13.3	63%
Control	5/15	6/15	73.3	
Vaccinated with combined *E. coli P. multocida* vaccine	0/15	1/15	20	72.4%
Control	5/15	6/15	73.3	

Table 5: Efficacy of combined *E.coli* - *P. multocida* vaccine in protecting against challenge with *P.multocida* virulent strain

Group	No. of chicken challenged	No. of chicken died after challenge with P. multocida virulent strain			Total survivors	Protection %
		24 hours	28 hours	24 hours		
Vaccinated with monovalent *P. multocida* vaccine	15	0	2	1	12	80
Vaccinated with combined *E. coli P. multocida* vaccine	15	0	1	1	13	86.6
Control	15	13	2	0	0	0

DISCUSSION

Respiratory diseases complex involving a secondary infection by *E. coli* and upper respiratory infection caused by *P. multocida* are of the most common poultry diseases (Gross, 1956 and Rimler and Glisson, 1997). The control of such diseases by preventing the predisposing respiratory infection and vaccination has much more successful than treatment (Gross, 1956 and Formmer et al., 1994).

Therefore, this study was planned to investigate the possibility of producing a local combined inactivated vaccine against *E. coli* and *P. multocida* infection to induce simultaneous protective immunity against both of them.

The results of sterility test showed that the locally prepared vaccines were completely sterile from any bacterial, fungal and mycoplasmas contaminants. Also, the vaccines were safe when they were injected with double dose in chickens.

Data presented in table (1) revealed that there was no substantial differences found in the protective GMT antibody titres in sera of chicken vaccinated with monovalent *E. coli, P. multocida* and combined *E. coli-P. multocida* vaccines. These parameters remained within the protective level till 16th week. These results coincide with previous report of Heddleston et al. (1970) and Trampel and Grifth, 1997).

The same pattern of *E. coli* and *P. multocida* antibody response was also observed by ELISA test as shown in Table (2). The results showed that the combined *E. coli-P. multocida* vaccines was effective antibody producer and produce seroconversion which could be detected at different intervals up to 16 weeks. In addition, the results cleared that ELISA test was more sensitive than the indirect haemagglutination test and a high correlation was found between ELISA titres and protection against challenge with *E. coli* and *P. multocida* virulent strains. These results are confirmed with those observed by Marshall et al. (1981) and Leitner et al. (1990).

The above mentioned results were supported by challenge of all groups with virulent *E. coli* and *P. multocida* as shown in Tables (3, 4 and 5). Chickens vaccinated with monovalent *E. coli* and combined *E. coli-P. multocida* vaccines and challenged by E. coli virulent strain showed a stricting reduction in mortality rate. The gross lesions in the air sacs, pericardium and liver were so mild and *E. coli* was recovered from these vaccinated groups with low percentage. No significant differences were observed in these parameters within the two vaccinated groups. On the other hand, un-

vaccinated groups showed a higher mortality with average score lesions that were significantly ($P < 0.05$) higher than those observed in vaccinated groups which showed a good protection as demonstrated by protection index (PIs) assessment. These results were in agreement with Frommer et al. (1994). Regarding to the results of challenge test as shown in table (5), the immunity of chicken vaccinated either with monovalent *P. multocida* or combined *E. coli-P. multocida* vaccines and challenged with virulent *P. multocida* strain gave good protection. These protective percentages were found to be within the permissible limits as recorded by Matsumoto and Helfer (1977).

From the above mentioned results, it could be deduced that combining both *E. coli* and *P. multocida* vaccines has no adverse effect on the humoral immune response of chickens as detected either by IHT or ELISA test and the protective capacity remained within the permissible limits at challenge with virulent strains. No mutual interference between the two antigens could be observed. The obtained results agree with those observed by Sandhu and Layton, 1984).

In conclusion, it could be suggested that the locally prepared inactivated combined *E. coli-P. multocida* vaccine was a safe, and potent as an immunogen for protection against both infections.

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