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Immunity of Chicks Against Colibacillosis by Vaccination of Parent Breeders

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Abstract

To study in ovo transfer of immunity against *Escherichia coli* infection, three groups of hens were injected with different vaccines composed of *E. coli* components, including one experimental pilus vaccine and two commercial vaccines. Increases of specific antibodies to type 1 pili and lipopolysaccharides were found in vaccinated hens. Chicks hatched from eggs collected from vaccinated hens were examined for antibodies and challenged with virulent *E. coli* at 21 days of age; immunity was judged according to mortality and lesions of colisepticemia at 4 days after challenge. Chicks from vaccinated hens had low or no mortality, mild gross lesions in viscera, and hyperplasia of splenic lymphoid tissue. Chicks from unvaccinated hens had significantly greater gross lesions. Results of this study indicate that inoculation of laying hens with antigens of *E. coli* leads to immunity in chicks that is highly protective at 3 weeks of age.

Introduction

Escherichia coli infection in poultry may result in acute colisepticemia, subacute fibrinopurulent airsacculitis/pericarditis, or chronic granulomatous enteritis/hepatitis/pneumonitis. These syndromes may be caused entirely or partly by *E. coli.* They are responsible for major economic losses to the poultry industry. Colibacillosis is the most common subacte form of this disease. Losses usually occur among young birds, but may occur in birds of any age[2].

Avian *E. coli* strains that cause colisepticemia are sensitive to many drugs such as ampicillin, chloramphenicol, chlortetracyclin, streptomycin, nitrofurans, and sulfa drugs. Avian colibacillosis can be controlled in field conditions by treatment with these drugs. Prevention of avian colibacillosis may be accomplished by vaccination, e.g., vaccinated chickens eliminate *E. coli* faster than unvaccinated chickens[5, 6, 8]. In addition, parenteral inoculation of young birds with antigens of *E. coli* results in formation of serum antibodies that may be responsible for immunity because maternal antibodies are transmitted to the newly hatched chicks via the yolk sac[9, 11]. Antibodies to *E. coli* can be transferred from immunized hens to progeny chicks[7] and turkeys injected with hyperimmune serum can be passively protected from colibacillosis[1]. Recently, transferred immunity against colibacillosis has been achieved in progeny after vaccination of the parental broiler breeder hens with a formalin-inactivated, oil-emulsion *E. coli* bacterin[12], i.e., maternally derived antibody protected against mortality and/or lesions for as long as 2 weeks post-hatching.

The purpose of this study was to evaluate immunity against the avian colibacillosis

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in progeny of parent breeders vaccinated with commercial inactivated *E. coli* vaccines and with an experimental pilus antigens vaccine.

Materials and Methods

Hens were White Leghorns from the National Animal Disease Center flock in USA. *E. coli* serotype 078:K80:H9 was used as the virulent challenge strain; it had been isolated from a turkey flock with heavy death loss. Bacteria were harvested, washed three times in saline, and resuspended in saline to a concentration of approximately 10^8 CFU per ml.

Vaccines. Three different inactivated *E. coli* bacterins were used in this experiment. Vaccine A : *E. coli* pilus vaccine commercially prepared from inactivated *E. coli* strains 01, 02, and 078 in oil emulsion by Scherring Corp., Omaha, Nebraska. Vaccine B : *E.coli* vaccine without pilus antigen; formalin-inactivated, oil-emulsion bacterin composed of serogroups 02, 078, and 035, prepared commercially by Vineland Laboratory, Inc., Vineland, New Jersey. Vaccine C : *E. coli* pilus antigen in incomplete adjuvant; pili from *E. coli* serotype 078:K80:H9 were purified as previously described[4] and emulsified in Freund' incomplete adjuvant (Difco Laboratory, Detroit, Michigan) immediately before vaccination.

Serology. Antibodies against *E. coli* pilus and lipopolysaccharide in sera and egg yolks were measured by indirect haemagglutination (IH) assays[3, 4]. Type 1 pilus and lipopolysaccharide antigens were prepared from *E. coli* serotype 078:K80:H9 according to published procedures[4, 14]. Serological responses of hens were measured using blood collected by wing vein from hens at the time of first vaccination, and at 4 weeks after 2nd vaccination. Egg yolks were extracted with chloroform[10] and examined for the presence of maternal antibodies at the time of the first and 2nd vacciantion, and at 4 weeks after the 2nd vaccination of breeder hens. Serum antibodies of chicks were measured using blood taken by wing vein at 2 weeks after hatch.

Experimental design. Breeder chickens were separated in three groups of 12 females and 1 male, and housed by groups on litter in separate rooms. They were fed a commercial antibiotic-free laying mash and watered *ad. libitum.*

Three groups of breeder hens were vaccinated with vaccine A, B, and C; one unvaccinated control group was used. Hens in each group were injected into the breast muscle with 0.5 ml of *E. coli* vaccine, and were re-immunized 3 weeks later with the same *E. coli* vaccine.

Eggs were collected daily beginning when hens received the 2nd vaccination. Some eggs were opened and yolk antibody titers against *E. coli* pilus and lipopolysaccharide antigens were determined. Remaining eggs were placed in marked gauze bags and incubated.

Newly hatched chicks were divided into 4 groups according to each parental source and were housed in battery brooders in isolation rooms. They were fed a commercial diet free from antibiotics. Chicks in each group were bled from wing veins at 2 weeks of age, and serum antibody titers against *E. coli* antigens were measured by the IH tests. Chicks at 3 weeks of age were challenged intravenously with 10^7 CFU of virulent *E. coli* in 0.1 ml saline. After challenge, they were observed daily for signs of illness and mortality. Chicks were killed 4 days after challenge and examined for gross lesions. Tissues were collected and fixed in 10 % neutral buffered formalin. Sections were cut for micro-

scopy after routine paraffin-embedding and were stained with hematoxylin and eosin.

Gross and histologic lesions including pericarditis, peritonitis, pneumonitis, airsacculitis, and liver necrosis were scored on a scale of 0 to 4 : 0 (none), 1 (mild), 2 (moderate), 3 (marked), and 4 (severe). Histological alterations of spleen were examined for hyperplasia of lymphoid tissue, fibrinopurulent inflammation, and necrosis.

Results

Response of vaccination. Production and hatch ability of fertile eggs in the three vaccinated groups were depressed during the first two weeks after vaccination (Table 1).

Table 1. Reproductive performance in breeders after 2nd vaccination.

Group	Number of hens	Treatment	Weeks after 2nd vaccination	Number of eggs produced/hatched
A	12	Vaccine A	1	0/0
			2	4/2
			3	8/5
			4	11/11
B	12	Vaccine B	1	5/4
			2	12/9
			3	16/14
			4	11/11
C	12	Vaccine C	1	12/9
			2	28/25
			3	30/24
			4	21/21

Table 2. Antibodies to LPS and pilus antigens in sera of vaccinated hens.

Vaccine	Antibody titer against:					
	LPS			Pilus		
Group	Median	Range	Mean + SD	Median	Range	Mean + SD
Control*	640	320 - 1,280	640 ± 286.2	4,096	32 - 32,768	6,302 ± 313.0
A**	1920	1,280 - 10,240	3,200 ± 3,505.4	4,096	4,096 - 16,384	6,144 ± 5,016.6
B**	5120	1,280 - 10,240	4,864 ± 3,434.6	5,120	512 - 13,1072	26,453 ± 51,616.8
C**	640	640 - 1,280	768 ± 286.2	24,576	8,192 - 65,538	28,672 ± 20,561.8
Control**	480	320 - 640	480 ± 226.3	8,448	512 - 16,384	8,448 ± 11,223.2

* Sera collected before vaccination
** Sera collected 4 weeks after vaccination

These reactions were most severe in group A (receiving vaccine A). Reproductive performance gradually returned to normal. None of the vaccinated or nonvaccinated hens died. Antibody titers in sera of hens (Table 2) and in egg yolks (Table 3) were higher than titers in sera of chicks (Table 4 and 5). Hens immunized with vaccine A and B developed IH antibody to the lipopolysaccharide antigen of *E. coli* (Table 2). Hens immuniz-

Table 3. Antibodies to LPS and pilus antigens in yolks of eggs laid by vaccinated hens.

Vaccine Group	Antibody titer against: LPS Median	LPS Range	LPS Mean + SD	Pilus Median	Pilus Range	Pilus Mean + SD
A	2,560	640 - 10,240	4,000 ± 4,257.3	288	16 - 1,024	404 ± 469.8
B	1,280	160 - 5,120	1,960 ± 2,171.8	18	4 - 512	138 ± 249.7
C	80	10 - 320	105.7 ± 107.4	16,384	4,096 - 16,384	12,873 ± 5,995.9
Control	5	5 - 20	10 ± 7.07	10	2 - 32	13.2 ± 13.0

Table 4. IHA-detected antibody titer to LPS in chicks from vaccinated hens.

Vaccine	Weeks after vaccination: 1			2			3			4		
Group	Median	Range	Mean + SD	Median	Range	Mean + SD	Median	Range	Mean + SD	Median	Range	Mean + SD
A	ND*	ND	ND	80	80 - 80	80 ± 0	80	80 - 640	208 ± 244.0	80	20 - 160	65.5 ± 53.0
B	20	20 - 20	2 ± 0	20	20 - 40	22.2 ± 6.7	20	20 - 80	34 ± 25.0	40	20 - 320	78.3 ± 92.0
C	20	20 - 20	20 ± 0	20	20 - 20	20 ± 0	20	20 - 40	22 ± 6.3	20	20 - 80	27.3 ± 18.5
Control	ND	ND	ND	ND	ND	ND	20	20 - 20	20 ± 0	20	20 - 80	33.3 ± 24.2

*ND = Not determined

Table 5. IHA-detected antibody titer to pilus in chicks from vaccinated hens.

Vaccine	Weeks after vaccination: 1			2			3			4		
Group	Median	Range	Mean + SD	Median	Range	Mean + SD	Median	Range	Mean + SD	Median	Range	Mean + SD
A	ND*	ND	ND	128	128 - 128	128 ± 0	128	2 - 152	154.4 ± 209.6	32	2 - 128	40.5 ± 39.9
B	4	2 - 4	3.5 ± 1.0	8	2 - 16	7.3 ± 4.0	4	1 - 16	6 ± 4.2	4	2 - 16	5.5 ± 3.7
C	8	2 - 128	27.3 ± 40.1	16	2 - 1,024	206.5 ± 427.7	260	2 - 1,024	360.4 ± 419.6	128	2 - 2,048	571.4 ± 693.8
Control	ND	ND	ND	ND	ND	ND	8	4 - 64	17.3 ± 23.3	8	2 - 32	10.3 ± 10.9

*ND = Not determind

ed with vaccine B and C developed IH antibody to the pilus antigen of *E. coli* (Table 2). Serum antibody responses of *E. coli* bacterin inoculated hens were anamnestic, and there were similar increases in yolk antibody titer (Table 3). In addition, the ranges of yolk antibody titers were similar to those of breeder hens receiving *E. coli* bacterin.

Immune response in progeny. Passively acquired antibody was in progeny of the chickens receiving *E. coli* vaccine (Table 4 and 5). Chicks hatched from eggs of hens immunized with vaccines A and B developed weak IH antibody responses to lipopolysaccharide antigen of *E. coli* (Table 4). Chicks hatched from eggs in group A and C developed antibodies to pilus antigen of *E. coli* (Table 5).

On challenge of progeny at 21 days of age, progeny become sick, but mortality was usually none or low in chicks hatched each week after vaccination of hens (Table 6). In contrast, severe mortality occurred in challenged chicks from unvaccinated breeders.

Table 6. Results of challenged progeny chicks from vaccinated breeders.

Week of Egg Collection	Vaccine Given to Hen	No. of chicks	Challenge does (CFU/Bird)	Results of challenge	
				Died/Survived	Efficacy
1	B	4	5.94×10^7	0/4	100
	C	9		2/7	78
2	A	1	6.06×10^7	0/1	100
	B	9		3/6	67
	C	25		2/23	84
3	Control	24	6.80×10^7	19/5	21
	A	5		0/5	100
	B	14		0/14	100
	C	23		0/23	100
4	Control	13	6.50×10^7	12/1	8
	A	11		0/11	100
	B	13		0/13	100
	C	20		1/19	95

Pathology in progeny. Chicks in the 4 groups showed signs of illness such as ruffled feathers, white milky diarrhea, disclination to walk, and loss of appetite; mortality was usually high in chicks from unvaccinated breeder hens, and most deaths occurred within 48 hours. Progeny from twice-vaccinated breeders were resistant to intravenous challenge with the virulent *E. coli* (Table 6).

There were no significant differences in histologic lesions among the 4 groups (Table 7). Gross lesions included pericarditis, peritonitis, pneumonitis, airsacculitis, and liver necrosis. Lesion severity in chicks from unvaccinated breeders were higher than those in chicks from vaccinated breeders.

Gross lesions in viscera of progeny chicks from unvaccinated breeders included: extensive hyperemia and swelling of viscera, abundant serous fluid in body cavities, and hemorrhage and edema in the lung. Histologically, there were hyperemia, hemorrhage, edema, fibrino-purulent inflammation, and small foci of necrosis. In chicks that died within 24 hours after challenge, splenic white pulp was distorted by serofibrinous exudates, erythrocytes, and necrotic cells. Reticular sheaths were filled with proteinaceous fluid. The borders of reticular sheaths, lymphoid tissues, and pulp were indist-

Table 7. Mortality, gross lesion scores and microscopic distribution of splenic lesions.

Group*	Maternal treatment	No. of chicks	Mortality (%)	**Gross pathology index	Distribution of microscopic lesions in spleen (%)		
					Hyperplasia of lymphoid tissue	Heterophilic inflammation	Fibrinopurulent or necrotizing inflammation
1	B	4	0	1.50	75	25	0
	C	9	22	2.11	67	11	22
2	A	1	0	0.00	100	0	0
	B	9	33	2.33	45	33	22
	C	25	16	1.12	80	12	8
3	Control	24	79	3.79	17	0	83
	A	5	0	0.60	100	0	0
	B	14	0	0.64	64	36	0
	C	23	0	0.61	91	9	0
4	Control	13	92	3.85	8	0	92
	A	11	0	0.67	73	27	0
	B	13	0	1.00	62	38	0
	C	20	5	0.90	70	25	5

*Eggs were collected at 1, 2, 3, and 4 weeks after vaccination of hens.
**Gross lesion scores (0 to 4 scale) : 0 = mild; 2 = moderate; 3 = marked; 4 = severe

inct because of many erythrocytes in intercellular spaces. Many bacilli were in reticular sheaths and red pulp. In contrast, development of lesions in progeny from breeders vaccinated with vaccine A, B, and C were mild and progressive compared with the acute severe bacterial septicemia of control chicks. Few chicks died within 48 hours post challenge; they showed septicemia and moderate fibrinopurulent pericarditis. Often the pericardial sac was filled with slight white-yellowish fibrinous exudate. Histologically, there were many heterophiles and macrophages, but few lymphoid cells in the epicardium. In spleens of progeny that died within 48 hours after inoculation, the red pulp were expanded and filled with erythrocytes and heterophils. Heterophils were rare in or adjacent to reticular sheath. Spleens of many progeny killed at 4 days after challenge had lymphoid hyperplasia: periarteriolar and periellipsoidal lymphocytes had increased cytoplasm and large basophilic nuclei with prominent nucleoli. The response of lymphoid tissues was increased in poults of groups A, B, and C with survived.

Discussion

These experiments demonstrate the efficacy of inactivated *E. coli* vaccines when given to breeder hens by the intramuscular route in providing immunity against experimental virulent *E. coli* challenge. Although reproductive performance appears to be impaired by vaccination in this experiment, substantial protection against *E. coli* infection in young chicks was provided through breeder hen vaccination. Progeny from twice-vaccinated hens were more resistant than progeny from unvaccinated hens. Compared with progeny from unvaccinated hens, progeny from vaccinated hens had delayed onset of illness and clinical signs, less severe disease, and less mortality. Progeny chicks from breeder hens vaccinated twice with an inactivated *E. coli* vaccine A, B, and C were protected against the virulent *E. coli* challenge within the first 21 days after hatching. IH antibodies to pilus and lipopolysaccharide antigens appeared in vaccinated hens and were transmitted via the yolk to their progeny. The use of any one of the three inact-

ivated *E. coli* vaccines had desirable effects on experimental chicks. The insufficiency of protection against experimental virulent *E. coli* challenge in progeny from eggs collected at 1 and 2 weeks after vaccination may have resulted from the low levels of maternal antibody transferred to the chicks.

The finding of maternally derived protection in 21-day-old birds suggests that breeder vaccination will protect progeny during the growing age immediately post-hatching when chicks are the most susceptible to *E. coli* infections[13]. The ability to provide increased protection against *E. coli* infection in young chicks, especially when they come from highly immunized hens, may result in a period when active immunization could be established.

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