Vet.Med.J.,Giza. Vol.54, No.2. (2006):271-282.

COMPARATIVE STUDY OF THE CELLULAR IMMUNE RESPONSE OF CHICKENS AND TURKEYS VACCINATED WITH FOWL CHOLERA VACCINE

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Received: 11. 12. 2005
Accepted: 26. 12. 2005

SUMMARY

Differences were noted in the serologic and immunologic responses of chicken and turkeys to vaccination by living avirulent CU fowl cholera vaccine, administrated by wing web or drinking water routes.

Turkeys responded to vaccination by the two routes as they developed good humoral serologic response as revealed by IHA and ELISA tests. Local IgA antibodies were detected in tissue sections of lung and intestine of turkeys vaccinated via drinking water route and the protection was 87.5%, IgA was detected only in lung tissue sections of turkeys vaccinated by wing web route. In case of chicken vaccinated by drinking water route, a weak local and humoral response was noted and the protection was 50%. Meanwhile, in chicken vaccinated by the wing web route, a good humoral and local response was observed and 87.5% protection was noted.

In conclusion, turkeys responded to vaccination with avirulent *P. multocida* CU strain either via drinking water or wing web routes, while chicken respond to vaccination by the wing web route only but not by the oral route.

INTRODUCTION

Fowl cholera is peracute, acute or chronic septicaemic disease for chicken and turkeys, caused by P. multocida and characterized by rapid onset, high morbidity and mortality with few lesions in multiple organs, such as fibrinopurulent pneumonia, purulent synovitis, necrotic hepatitis, purulent encephalitis or peritonitis (Saif, 2003).

Epidemiological studies have revealed that the portal of entry of the organism is the pharynx and the upper respiratory tract (Arsov, 1968). The organism multiple in the lungs and then disseminated to the liver and spleen, where it cause pathological changes, therefore, it is quite conceivable

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that local and cell mediated immunity could participate in the defense mechanisms. Even though they have been considered separate components of the immune system, they could be participating in some unknown way to the stimulation of resistance to infection in immune birds (Dua and Maheshwaran, 1978).

Fowl cholera vaccination programs become numerous after the advancement of such vaccines, which varied in their potency and method of application. These vaccines that have been developed as prophylactic agents against fowl cholera include, inactivated adjuvanted bacterins and live avirulent vaccines. Live avirulent vaccines have the ability to fully mimic infection, bringing to live birds all immunity processes and thus provides a fully or chestrated immune response Schlink and Olson, 1987). Live vaccines cause turkeys to respond with secretory, humoral and cellular immunity. They are usually used orally or by wing web stabbing in turkeys (Hopkins and Olson, 1997).

The aim of the present study was to compare the immunologic and serologic response of chicken and turkeys to vaccination with the living avirulent CU vaccine administered by oral and wing web routes.

MATERIALS AND METHODS

Bacterial strain:

virulent P. multocida strain serotype A:3.

Fowl cholera living attenuated vaccine:

CU P. multocida vaccinal strain, belonged to serotype A:3, it was supplied by Scherring Plough animal health company, Omaha.

3. Fluorescein conjugated antiserum:

a. Polyclonal fluorescein conjugated antiserum for P. multocida serotype A:3 was prepared in rabbits according to the method of Narin (1976).
b. Fluorescein isothiocyanate conjugated IgG fraction anti-secretory IgA (heavy chain specific) was obtained from Cappel Laboratories, Inc., USA.

Fluorescent antibody technique:

It was applied on lung sections of vaccinated turkeys and chickens vaccinated by various routes and un-vaccinated controls according to the method of Narin (1976).

4. Avidin Biotin-peroxidase staining technique:

A standard Avidin Biotin immunoperoxidase method was used for detection of P. multocida in tissues as described by Su et al., (1981). The procedures of staining were performed according to the manufacturer instructions of the kit used (Vectastain, vector laboratories, Burlingame, Calif.). The counterstain used was haematoxylin which was obtained and the primary antibody from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

5. Experimental birds:

A total of 54 chickens and 54 turkeys, 6 weeks old were used in the experimental study. They were housed in separate pens and kept under strict hygienic measures of rearing and feeding. They were free from P. multocida antibodies as screened by ELISA test (Marshall et al., 1981).

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6. Experimental design:

Both 54 chicken and 54 turkeys were divided into two equal groups 24 each. The first group received the CU vaccine via drinking water route; the other received the vaccine via wing web route. The dose was estimated to be 1.5 x 109 organism/bird. 6 turkeys and 6 chickens were kept as control un-vaccinated group.

7. Collection of samples:

7.1. Serum samples:

Sera were collected from immunized birds every week up to 8 weeks, post vaccination.

Measurement of P. multocida antibodies:

1- ELISA as described by Marshal et al., (1981). The tires were determined as described by Breggs and Skeel (1984).

2- IHA by the method of Carter and Rappy (1962) were performed.

7.2. Tissue specimens:

Tissue specimens (lung and intestine) were collected from the sacrificed vaccinated birds at weekly intervals post vaccination, placed into 10% buffered formalin and then they were routinely processed according to Culling (1976), some of them stained with H and E and other were kept for immunoperoxidase and immunofluorsent techniques.

8. Challenge test:

Thirty eight birds were challenged by swabbing of the palatine cleft with 0.1 ml of 109 dilution of virulent A:3 P. multocida strain according to a standardized procedure of Heddleston and Rebers (1974).

RESULTS AND DISCUSSION

The present study was aiming to compare the immunological and serological response of chicken and turkeys to vaccination with the living avirulent CU (Clemson University) *P. multocida* strain administrated by different routes i.e. drinking water and wing web methods.

Heddleston and Watko (1965) compared the serological and immunological response of chicken and turkeys to the inactivated fowl cholera vaccine and they reported the existence of some differences between the two species.

From data given in Table (1) it can be deduced that by conducting the IFAT for detection of IgA in different tissue sections, lung and intestine of chickens and turkeys that a strong positive fluorescent reaction could be noted in intestine and lungs of turkeys given the CU vaccine via drinking water route. As well as turkeys and chickens vaccinated via the wing web stabling method, a strong fluorescent reaction was noted in lung sections (Photo 1). Meanwhile in chicken vaccinated via oral route, weak positive fluorescent reaction was seen in intestine and lung sections.

The results of immunohistochemical staining technique using Avidin Biotin Peroxidase are tabulated in Table (2). It is clear that the lung and intestine of turkeys showed a strong positive to

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moderate reaction at different intervals after vaccination with the CU vaccine via oral route. Meanwhile tissue sections of chicken were either weak positive or negative at different intervals after oral vaccination. A strong to mild reaction was noted in lung tissue sections of chicken and turkeys vaccinated by wing web stabling method, meanwhile, weak to moderate reaction was observed in chicken vaccinated by the same method. Control unvaccinated birds showed negative reactions. In case of turkeys vaccinated by the oral route, there was intense positive reaction in the intestine and lung tissues inside the macrophages in lamina propria of intestine (Photo 2) and a granular intra and extra cellular positive Avidin Biotin peroxidase staining in the macrophages in the interalveolar septa, (Photo 3). The intensity of reaction and the number of macrophages carrying the antigen were large in turkeys and chicken vaccinated by the wing web stabbing method but it was weak to negative in case of chicken vaccinated by drinking water. The above-mentioned results was agreed with these of Sulong and Maheshwaran (1976) who demonstrated the persistence of the avirulent CU strain in the lungs and splenic tissues of vaccinated turkeys by the indirect immunofluorescent technique. These tissues remained positive up to the fourth week post vaccination. No comparative study in chicken was performed by the authors. Derieux (1977) stated that chicken were much less resistant to challenge than turkeys after exposure to a virulent CU vaccine by drinking water. Moreover Rice et al., (1978) evaluated the immune response of chicken to various routes of vaccination with CU strain. He found that the subcutaneous route produced the greater degree of protection in all experiments and the differences were highly significant.

The results of serological immune response of chicken and turkeys as measured by IHA and ELISA are given in Tables (3 & 4) it is clear from these tables that the highest titres were detected in sera of turkeys vaccinated by drinking water and wing web routes. But in sera of chicken the wing web route gave significantly higher titres, than that for the drinking water route.

Table (5) described the results of challenge test of vaccinated turkeys and chicken by the nasal cleft swabbing method 87.5% protection was recorded in turkeys vaccinated by the oral drinking water or wing web method and in chicken vaccinated by the wing web method. While it was 50% in chicken vaccinated by drinking water route.

The aforementioned results documented the presence of local humoral antibodies in the lung of turkeys vaccinated by oral or wing web methods and also in chicken vaccinated with the wing web method.These antibodies were low in chicken vaccinated by the oral route, as they were unable to protect chicken against palatine cleft challenge. Dua and Maheshwaran (1978) also detected local humoral antibodies in tracheal secretions of turkeys vaccinated with CU strain of P. multocida in the drinking water. Their appearance and persistence were documented by the indirect fluorescent antibody technique.

Histopathological examination of lung tissue of turkeys vaccinated either by drinking water or

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wing web methods revealed that the alveolar lumina and the interstitial spaces were infiltrated with inflammatory cells including macrophages, lymphocytes and polymorphnuclear cells (Photo 4).

In case of chicken vaccinated by the wing web method, these changes were moderate; meanwhile it was weak in tissue sections of chicken vaccinated by drinking water route. Examination of intestinal sections of turkeys vaccinated by the drinking water route showed infiltration of the lamine propria with large number of inflammatory cells including lymphocytes, monocytes and heterophilis, hyperplasia of Pyer's patches.

Table (1): Detection of IgA in tissue sections of vaccinated turkeys and chickens by different routes using fluorescent antibody technique

Route of vaccination	Tissue section	Turkeys				Chicken			
		Weeks post vaccination				Weeks post vaccination			
		5th	6th	7th	8th	5th	6th	7th	8th
Drinking water	Lung	++	++	+++	++	+	++	+	-
	Intestine	+++	++	++	+	-	-	+	-
Wing web sticking	Lung	+++	++	+++	++	+++	+++	+++	-
Control	Lung	-	-	-	-	-	-	-	-
		-	-	-		-	-	-	-

Table (2): Detection of *P. multocida* antigen in tissue sections of vaccinated turkeys and chickens using Avidin Biotin Peroxidase reaction

Route of vaccination	Tissue section	Turkeys				Chicken			
		Weeks post vaccination				Weeks post vaccination			
		5th	6th	7th	8th	5th	6th	7th	8th
Drinking water	Lung	++++	+++	+++	++	+	+	+	-
	Intestine	+++	++	++	+	-	-	+	-
Wing web sticking	Lung	+++	+++	++	+++	+++	++	++	+
Control	Lung	-	-	-	-		-	-	-
	Intestine	-	-	-		-	-	-	-

\+ Weak positive staining
++ Mild
+++ Moderate
++++ Intense positive staining
\- Negative staining

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These changes were mild in chicken vaccinated via drinking water route. These results gave an additional evidence of the immunologic response of turkeys and chicken to immunization by the CU vaccine administration by the drinking water or wing web methods. It explains also the differences in response to vaccination by the CU vaccine in the two species of birds.

Photo (1): Strong +ve FA in lung section from turkeys vaccinated with fowl cholera CU vaccine via wing web route (note the fluorescent colour).

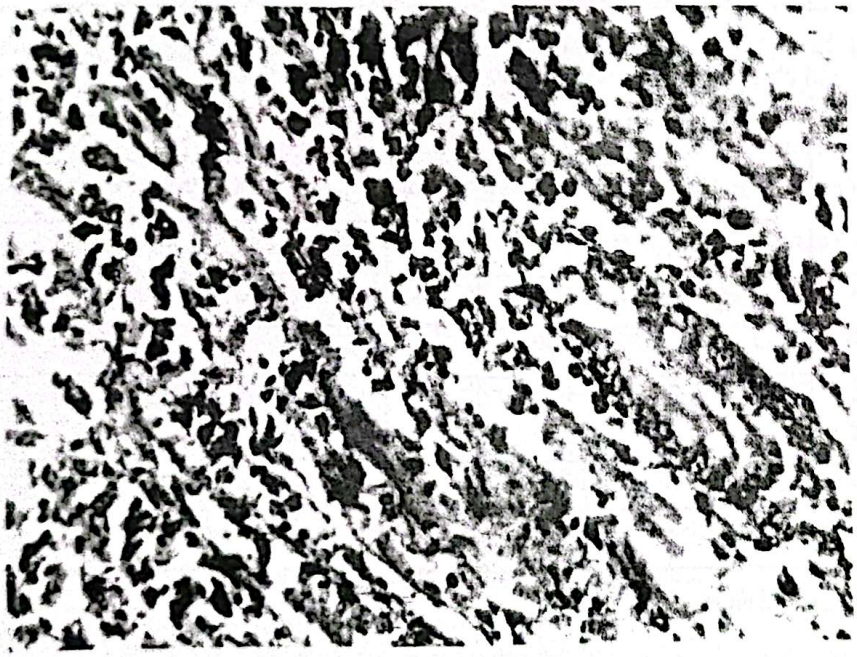

Photo (2): Intense +ve Avidin-Biotin peroxidase staining in lamina properia of intestine of turkey vaccinated via drinking water route (x132).

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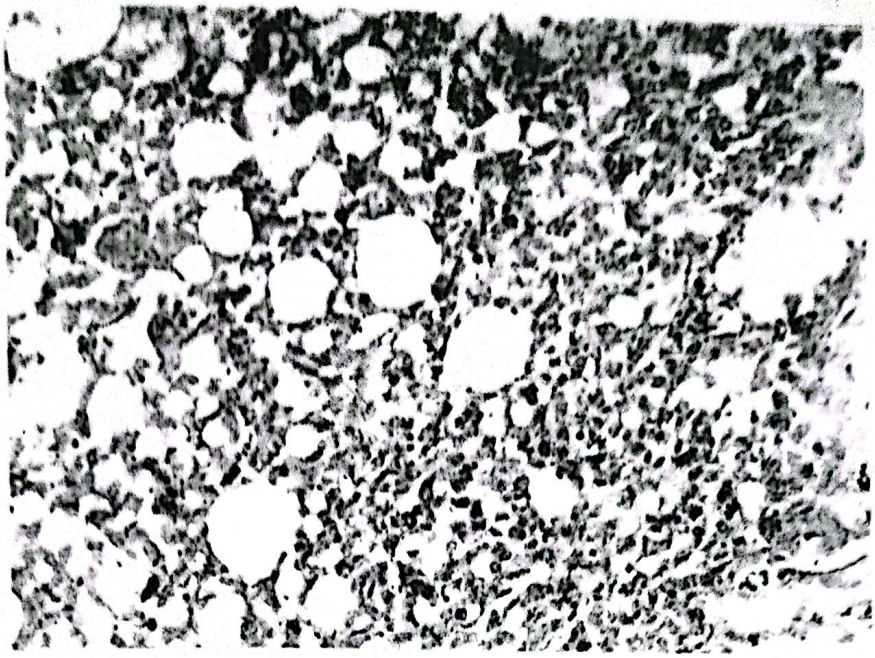

Photo (3): Intense +ve Avidin-Biotin peroxidase staining in the macrophages in intra-alveolar septa (x132)

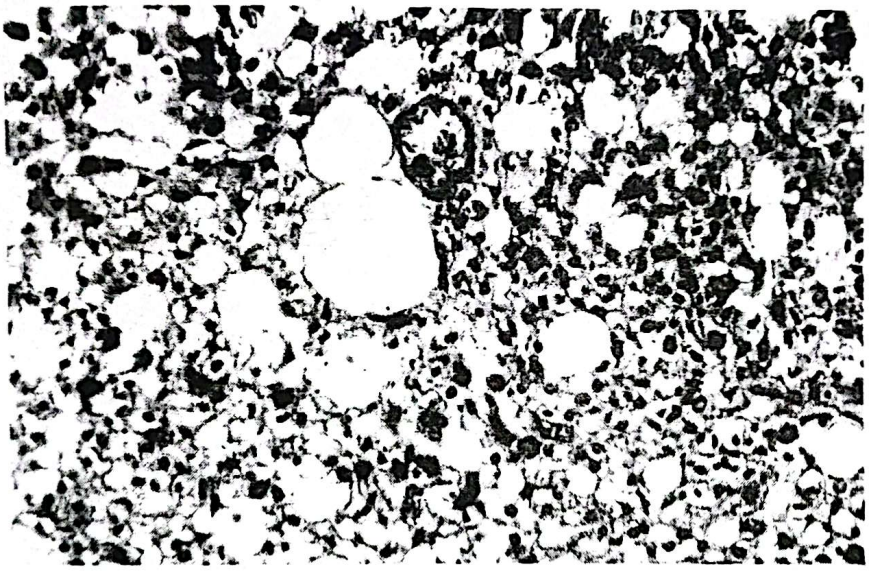

Photo (4): Lung of turkey vaccinated via drinking water showing thickening in the inter alveolar wall by leukocytes (H & E x132)

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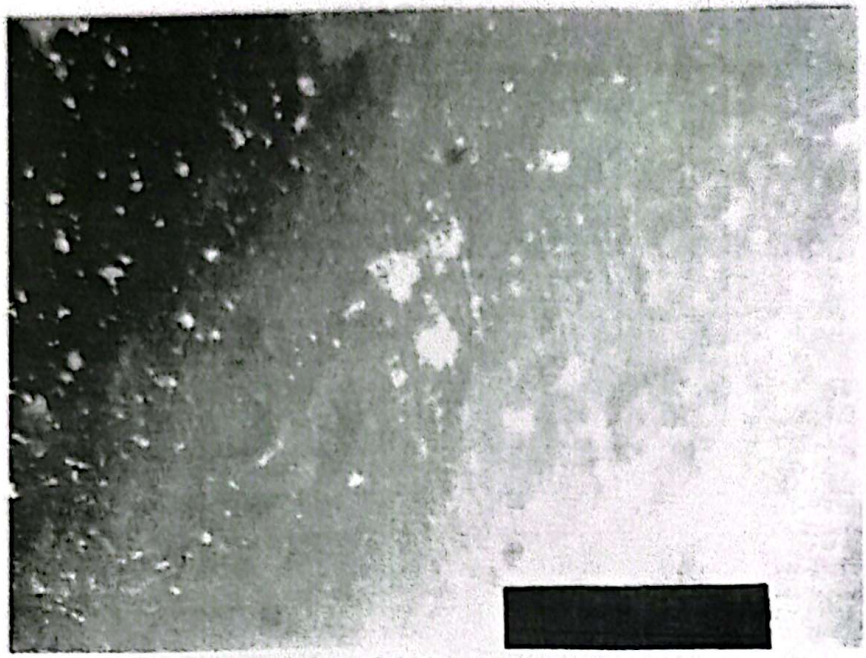

Photo (5): Mild positive FA in lung of chicken vaccinated via drinking water (Note the fluorescent colour) (H & E x33).

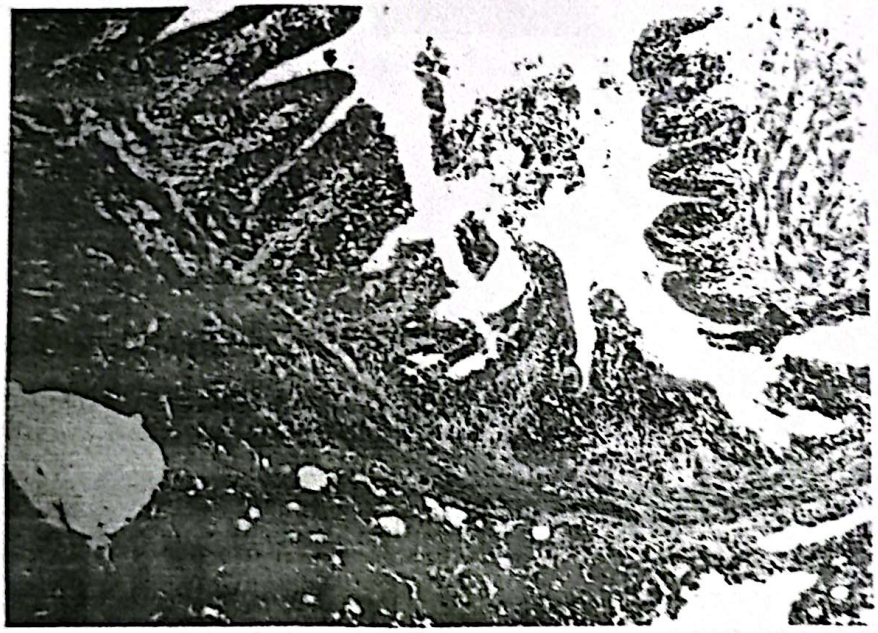

Photo (6): Lung of chicken vaccinated via wing web stabbing showing hyperplasia of the bronchial epithelum (H & E x16).

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Table (3): Comparison between antibody titres in sera of turkeys and chickens vaccinated with fowl cholera CU vaccine via oral and wing web sticking routes as determined by ELISA

Route of vaccination	Pre-vaccination	Turkeys								Chicken							
		Weeks post primary vaccination				Weeks post boostering				Weeks post primary vaccination				Weeks post boostering			
		1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Drinking water	30	798	890	1042	2120	1130	1480	2042	2480	236	455	322	350	470	383	320	480
Wing web sticking	40	860	990	1090	1113	1030	1320	2115	1958	721	890	1020	870	1350	1095	2012	1558
Control non-vaccinated	25	25	30	23	35	22	30	30	20	15	32	33	25	20	24	28	30

Table (4): Comparison between antibody titres in sera of turkeys and chickens vaccinated with fowl cholera CU vaccine via oral or wing web sticking routes as determined by indirect haemagglutination (IHA)

Route of vaccination	Pre-vaccination	Turkeys								Chicken							
		Weeks post primary vaccination				Weeks post boostering				Weeks post primary vaccination				Weeks post boostering			
		1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Oral	10	260	170	190	270	350	485	440	540	16	25	32	60	95	120	135	140
Wing web	12	60	170	90	182	490	512	320	432	40	150	187	220	210	330	256	410
Control non-vaccinated	10	11	10	14	12	8	11	10	13	8	5	7	11	10	8	9	8

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Table (5): Challenge exposure test results of vaccinated turkeys and chickens by various routes using virulent P. multocida serotype A:3.

	Route of vaccination	No. of challenged birds	Weeks post primary vaccination	Protection
Turkeys	Drinking water	8	7th	87.5%
	Wing web	8	7th	87.5%
Chickens	Drinking water	8	4th	50%
	Wing web	8	7th	87.5%
Control non-vaccinated		6	0%	0%

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دراسة مقارنة للمناعة المخاطية للدجاج والرومي المحصنة بلقاح كوليرا الطيور

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أوضحت هذه الدراسة وجود فروق في الإستجابة السيرولوجية والمناعية لكل من الدجاج والرومي عند التحصين بلقاح كوليرا الدواجن الذي الغير ضاري من نوع سي بو وذلك عند إعطاء هذا اللقاح عن طريق الوخز في الجناح أو عن طريق ماء الشرب.

وقد إستجاب الدجاج الرومي للتحصين بكلا الطريقتين حيث كانت الإستجابة السيرولوجية جيدة عند إجراء إختبار التلازن المعيق غير المباشر أو الإليزا. كما تم التعرف على وجود أجسام مناعية مصوصية من نوع IgA في الشرائح النسيجية للأمعاء والرئة للدجاج الرومي المحصن عن طريق ماء الشرب وكانت نسبة الحماية في إختبار التحدي ١٠٠٪ بينما كانت هذه الأجسام المناعية الوضعية موجودة في الشرائح النسيجية للرئة بالنسبة للدجاج الرومي المحصن عن طريق الوخز في الدجاج. أما في حالة الدجاج المحصن عن طريق ماء الشرب فقد كانت الإستجابة الوضعية والسيرولوجية منخفضة كما كانت نسبة الحماية عند إجراء إختبار التحدي ٢٥٪. بينما كانت الإستجابة المناعية السيرولوجية والوضعية جيدة في الدجاج المحصن عن طريق الوخز في الجناح وكانت نسبة الحماية ٩٠٪ عند إجراء إختبار التحدي.

ومن هنا نستخلص أن الدجاج الرومي يستجيب للتحصين بلقاح كوليرا الدواجن الذي من نوع

- ب سواء عن طريق ماء الشرب أو الوخز في الجناح بينما يستجيب الدجاج للتحصين بهذا اللقاح
- عن طريق الوخز في الجناح وليس عن طريق ماء الشرب.

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