EFFICACY OF INACTIVATED FOWL CHOLERA VACCINE IN QUAIL

Hanan, A. Ahmed

Central Laboratory for Evaluation of Veterinary Biologics, Cairo, Abassia

ABSTRACT

Pathogenicity of local Pasteurella multocida isolates for quails was studied. It was found that quails were highly susceptible to experimental infection with local P.mutocida serotypes A : 5, A : 8 and A : 9 resulted in death of all birds within 24-36 hours.

The immunogenicity of the local inactivated fowl cholera vaccine was evaluated in 4-weeks old quails. The immune response as measured by IHA showed high antibody titers at 6th weeks post vaccination. The results of challenge test revealed that vaccinated quails were successfully protected against challenge with virulent strains of P. multocida with protection rate reached to 85-90%. The obtained results revealed that vaccination of quails with local inactivated fowl cholera vaccine could protect quails against challenge with virulent strains of P. multocida.

INTRODUCTION

Pasteurella multocida infection, otherwise reffered to as fowl cholera, is the causative agent of heamorrhagic septicemia in a broad spectrum of animal hosts, and avian pasteurellosis has been recognized as a world wide disease for centuries **(Sander et al., 1998)**.

Fowl cholera is usually an acute or peracute disease. Lesions reported naturally occurring outbreaks are generally those of an acute septicemia, often with focal necrosis, in association with bacterial colonization in the liver, spleen and other organs **(Hunter and Wobeser, 1980)**.

Pathogenicity or virulence of P. multocida is complex and variable, depending on the strain type, the host species, variations among the strains and the host and conditions of contact between the two . Pasteurella multocida has 10 somatic serotypes in which serotype 1,3,4, 3 x 4 and 5 are the dominant serotypes belonging to capsular type A **(Roades and Rimler, 1990)**.

The need for an effective immunizing agent to prevent P. multocida infections has been in demand. Over the last several years, various immunoprophylactic agents that have been used to prevent fowl cholera include viable attenuated oral or subcutaneous vaccines and killed bacterins **(Robers and Heddleston, 1977 and Tatum et al., 2009)**.

The present work was planned to study the pathogenicity of local P. multocida strains for

quails and to evaluate the potency of inactivated fowl cholera vaccine in quails.

MATERIAL AND METHODS

1- Pasteurella multocida strains:

Local isolates of P. multocida serotypes, A:5, A:8 and A:9 were supplied from Aerobic Bacterial Vaccine Dept., Vet. serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

These isolates were used in the pathogenicity and challenge test.

2- Inactivated fowl cholera vaccine:

Inactivated local fowl cholera vaccine was kindly supplied by Veterinary Serum and Vaccine Research Institute, Abassia, Cairo. The vaccine was standardized to contain approximately $3x10^9$ CFU/ dose from each strain (A:5, A:8 and A:9).

3- Experimental hosts:

3.1-Quails:

One hundred and twenty 4-weeks' old quails were obtained from farm of Faculty of Agriculture, Egypt. These quails were used for the pathogenicity test and to evaluate the potency of inactivated fowl cholera vaccine. These birds were tested and found to be free from P. multocida infection and antibodies as determined serologically.

All birds were housed under hygienic measures in separate isolates receiving balanced ration and adequate water.

3.2- Mice:

A total of 250 weaned Swiss albino mice of about 25 gm body weights were used for passage and determination of the LD50 of P. multocida strains.

4- Pathogenicity test:

30 quails were divided into three groups (10/each) and each group was used to determine the pathogenicity of local P. multocida isolates (A:5, A:8 and A:9) for quails according to **(Miguel et al, 1998 and Goto et al, 2001)**. Quails that died post infection were examined and cultured to confirm diagnosis of P. multocida infection by taking smears from blood and organs (heart, liver and spleen) and stained with Giemsa stain to find the characteristic bipolarity of P. multocida microorganism.

5- Evaluation of the efficacy of the local inactivated fowl cholera vaccine:

5.1- Vaccination of quails with the local inactivated fowl cholera vaccine:

90 quails were divided as follows:

60 birds were vaccinated with the local inactivated fowl cholera vaccine twice subcut. with 3 weeks intervals with a dose of 0.3 ml/ bird **(Dabbert et al, 1996)**. 30 birds were kept as un-vaccinated control. All birds were housed in separate isolates under hygienic measures receiving adequate ration and water. Serum samples were obtained regularly on week intervals to follow up the induced antibody levels up to 6 weeks post the first vaccination.

5.2- Detection of the humoral immune response using indirect heamagglutination test(IHA):

IHA test was carried out according to **Carter and Rappy, 1962** using 1% sheep RBCs.

5.3- Challenge test:

0.1 ml of 24 hours old culture containing $4.3x10^4$ CFU/ml of A:5, A:8 and A:9 P. multocida local isolates were injected I.M. as suggested by **Dabbert et al,1996**.

RESULTS & DISCUSSION

Economically, the most important bacterial disease of poultry that continued to be the major cause of mortality is fowl cholera. Fowl cholera caused by gram negative bacterium, P. multocida has caused epizootic outbreaks on free-living birds **(Sawada et al,1999)**.

P. multocida serotype A:5 has been isolated from waterfowl and other free-living birds as recorded by **Pehlivanoglu et al, (1999),** also **Burns et al, (2003)** cultured P. multocida serotype A:8 and A:9 from liver and bone marrow of Japanese quails during an outbreak of the disease.

The pathogenicity testing of the local of P. multocida isolates for quails was performed as shown in Table (1) which revealed that P. multocida serotypes, A:5, A:8 and A:9 were highly pathogenic for quails causing 100% mortality within 18, 24 and 36 hours, respectively and P. multocida organism could be recovered from all dead birds, these findings came in agree with **Miguel et al, (1998)** who suggested that Pharoah quail were susceptible to P. multocida and were likely to develop subacute to chronic fowl cholera also, **Goto et al, (2001)** found that P. multocida serotypes, A:8 and A:9 caused acute septiceamia and death within few days after experimental infection of Japanese quails.

The humoral immune response of inactivated fowl cholera vaccine was evaluated in vaccinated quails by indirect heamagglutination test (IHA) as shown in Table (2).

The mean IHA antibody titers against P. multocida serotypes, A:5, A:8 and A:9 in sera of vaccinated birds increased from 8 prevaccination to reach (970), (905) and (844) at 6^{th} weeks post vaccination with inactivated fowl cholera vaccine, respectively, while the control un-vaccinated birds showed steady levels (5-11). These results confirmed earlier by **Dua and Mahsewaran (1978)** who recorded that P. multocida bacterin induced high level of systemic humoral immunity as measured by indirect heamagglutination test.

Table (3) illustrated that quails had been successfully vaccinated with inactivated fowl cholera vaccine which afforded highly significant protection rate. The results of challenge test were 90% protection when challenged with P. multocida serotypes, A:5, meanwhile, protection rate was 85% when challenged with P. multocida serotypes, A:8 and A:9.

Previously, **Comte (2001)** stated that inactivated vaccine against avian cholera provided sufficient protection of 90-100% against challenge for 4 months.

The results obtained in this study proved that quails are highly susceptible to infection with local P. multocida isolates, also, inactivated fowl cholera vaccine can protect quails against challenge with virulent P. multocida strains.

This is the first report on pathogenicity and vaccination of quails with fowl cholera in Egypt.

Table (1): Results of pathogenicity testing of local isolates of P. multocida for quails

P. multocida serotypes	Mean death time(hours)- No of dead /total				
	6	12	18	24	36
A:5	0/10	2/10	8/8	----	-----
A:8	0/10	1/10	5/9	4/4	------
A:9	0/10	0/10	4/10	3/6	3/3

Table (2): P. multocida mean antibody titers measured by indirect heamagglutination test (IHA) applied on the sera of vaccinated quails

Quail Groups	Strain used	P. multocida mean antibody titers /weeks post vaccination						
		Pre-V*	1	2	3	4	5	6
Vaccinated	A:5	8	32	106	226	520	755	970
	A:8	8	25	92	211	485	788	905
	A:9	8	30	98	197	422	680	844
Un-vaccinated control	A:5	7	10	8	11	10	8	7
	A:8	7	10	9	8	7	9	9
	A:9	7	7	5	6	11	10	10

*Pre-V= pre-vaccination

Table (3): Results of challenge test among quails vaccinated with inactivated fowl cholera vaccine.

Quails groups	Challenge Strain	No of quail Tested	No.of survived	Protection Rate	Lesion Score	Re-isolation of P. multocida
vaccinated	A:5	20	18	90%	+	-----
	A:8	20	17	85%	+	-----
	A:9	20	17	85%	+	-----
Un-vaccinated control	A:5	10	0	0%	+++	+
	A:8	10	0	0%	+++	+
	A:9	10	0	0%	+++	+

\+ : Slight lesions +++ : Sever lesions

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الملخص العربى

كفاءه لقـاح كوليرا الطيور المثبط فى السمان

حنان على أحمد

المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية - العباسية - القاهرة

تم دراسه مدى ضراوه عترات الباستيريلا ملتوسيدا المحلبه للسمان ووجد انها شديده الضراوة للسمان مسببه نسب نفوق ١٠٠% كما تضمنت الدراسه تقييم استجابه السمان للقاح كوليرا الطيور المثبط حيث انه اعطى مستويات عاليه من الاجسام المناعيه و ذلك بقياسها باختبار التلزن الدموى الغير مباشر كافيه للتغلب على العدوى بالميكروب الضارى عند اجراء اختبار التحدى بنسب حمايه تصل الى ٩٠% و٨٥% .

و على ذلك يمكن القول ان لقاح كوليرا الطيور المثبط يمكن ان يوفر حمايه جيده للسمان ضد العدوى بالميكروب الضارى.