JLSB
Journal of Life Science and Biomedicine

J. Life Sci. Biomed. 4(4): 252-256, 2014

ISSN 2251-9939

Efficacy of Local and Imported Vaccines Aainst Salmonella Entertiodes and A. Paragallinarium

Akeila MA[1] , Khalil S A[1] and Sedeik M[2]
[1.] Microbiology Dept. Fac. Of Vet. Med. Alex. University
[2.] Poultry and Fish Diseases Dept. Fac. Of Vet. Med. Alex. Univ

*Corresponding author's e-mail: **Seddeeklab@yahoo.com**

ABSTRACT: This study aimed to evaluation and comparing the efficiency of locally and imported vaccine against salmonella entrtidis and A. paragagallinarium.In this study, 270 SPF chickens were used to evaluate the efficacy of different imported and locally prepared inactivated pentavalent vaccines. The birds were divided into 3 experimental groups 1, and 2 (100 birds /each), each group was divided into 4 subgroups while Control group (3) had 70 birds and sub-divided into 4 subgroups as shown in the following figure.The Salmonella enteritidis it was taken and prepared locally in the Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, Cairo. The titer was 108 CFU / ml. These strains were used in vaccine and antigen preparation as well as in challenging vaccinated chickens. While, the avibacterium paragalinerium Serotypes A, B and C were prepared locally in the Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. The titer of each was 3X108 CFU / ml. These strains were used in vaccine and antigen preparation as well as in challenging vaccinated chickens.So our study concluded that, the local pentavalent vaccine (S.E. , IC) gave acceptable antibody titers and good protection levels in comparison with the imported pentavalent vaccine.
Key words: Efficacy , Local, Imported Vaccines, Salmonella Entertiodes, A. Paragallinarium

ORIGINAL ARTICLE
Received 13 Jan. 2014
Accepted 18 Mar. 2014

INTRODUCTION

Poultry represents an important and cheap source of protein throughout the world. Subsequently, the used vaccines and vaccination programs have also been developed.

Combined vaccines have the advantage of protection against more than one disease at the same time, beside, reducing vaccination expenses, number of vaccination performed and saving time. The most important problems facing the poultry industry in Egypt are the infection with avian salmonellosis, Infectious coryza. They cause economic losses, particularly in those parts of the world where the poultry industries are intensive and where open sided housing is common.

Infections with bacteria of the genus salmonellae are responsible for a variety of acute and chronic diseases in poultry. These diseases continue to cause economically significant losses in many nations and absorb a large investment of resources in testing and control efforts in others. Infected poultry nocks are also among the most important reservoirs of salmonellae that can be transmitted through the food chain to humans. Poultry and poultry products are consistently among the leading animal sources of salmonellae that enter the human food supply [1].

Avian salmonellosis is an inclusive term designating a large group of acute and chronic diseases of poultry caused by any one or more member of genus Salmonella. However, particular salmonella serovars may be encountered more frequently in one country than the other [2]. In Egypt several investigators [3,4]. Have isolated many Salmonella species particularly, S. enteritidis from poultry.

Infectious coryza (IC) is an acute respiratory disease of chickens caused by the bacterium Avibacterium paragallinarum. The greatest economic losses associated with infectious coryza result from poor growth performance in growing birds and marked reduction (10-40%) in egg production in layers [5].

Infectious coryza (IC) is an infectious contagious upper respiratory tract disease caused by Gram-negative bacterium Avibacterium (Haemophilus) paragallinarum [5] is a causative agent of avian infectious coryza. The disease causes retarded growth of young birds and reduced egg production (10% to more than 40%) in laying flocks [6] and subsequently, serious economic losses to the poultry industry annually worldwide . The most common clinical signs are a nasal discharge, conjunctivitis, and swelling of the sinuses and face. Birds may develop swelling of the wattles and diarrhea.

Many investigators have used various serological tests of S. enteritidis to detect the antibody in chicken. ELISA test also can be used to monitor the antibody levels following vaccination and may be to diagnose SE in the field Jouy et al. [7] The ELISA test, which is quick and simple, can replace other expensive and time consuming serologic tests.

So, the aim of this study is evaluation and comparing the efficiency of locally and imported vaccine against salmonella entrtidis and A. paragagallinarium.

To cite this paper: Akeila MA. And Sedeik M. 2014. Efficacy of Local and Imported Vaccines Aainst Salmonella Entertiodes and A. Paragallinarium. *J. Life Sci. Biomed.* 4(4):252-256.
Journal homepage:http://jlsb.science-line.com/

MATERIAL AND METHODS

A.Experimental Design

In this study, 270 SPF chickens were used to evaluate the efficacy of different imported and locally prepared inactivated pentavalent vaccines. The birds were divided into 3 experimental groups 1, and 2 (100 birds /each), each group was divided into 4 subgroups while Control group (3) had 70 birds and sub-divided into 4 subgroups as shown in the following figure.

B.Strain used:

- Bacterial strains

- Salmonella enteritidis:

It was taken and prepared locally in the Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, and Cairo. The titer was 108 CFU / ml. These strains were used in vaccine and antigen preparation as well as in challenging vaccinated chickens.

- Avibacterium paragalinerium

Serotypes A, B and C [1] were prepared locally in the Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. The titer of each was 3X108 CFU / ml. These strains were used in vaccine and antigen preparation as well as in challenging vaccinated chickens.

c. Laboratory chickens:

Specific pathogen free (SPF) chickens were obtained from Khom Oshem farm, El Fayoum, Egypt as one day old. They were reared and housed in positive pressure stainless steel isolation cabinets at CLEVB with continues light exposure till used.

D.Culture media:

Te following media were used For Salmonella Enteritidis:

-Salmonella Shigella agar medium (Oxoid ltd. Basing stake, Hamphire, England).

-Tryptose soya agar medium (Oxoid Ltd. Basing stake, Hampshire, England).

The media used for Avibacterium Paragallinarum:

•Tryptose phosphate broth (Code No. 0060 - 01 - Difco Laboratories, Detroit, Michigan, USA). It was used for rehydration of lyophilized strain.

Brain heart infusion broth (Code No. 152 - 00680 M - Difco LTD, Paisley, Scotland)

E.For testing vaccine sterility:

The different vaccines were inoculated on the following media for performing the sterility tests for bacteria and fungi according to the British pharmacopeia [8].

a) Thioglycolate broth for anaerobic bacteria which was inoculated and incubated at 37C for 72hours.

b) Nutrient agar plates for facultative anaerobic and aerobic bacteria which was inoculated and incubated at 37C for 72 hours.

c) Sabouraud's maltose broth and Sabouraud's maltose agar plates to test fungal sterility which was inoculated and incubated at 25C for 14 days.

1. Preparation of bulk culture from S. enteritidis according to Charles et al. [8].

2. Inactivation of S. enteritidis [8].

3. Safety (completion of inactivation for S. enteritidis)

It was carried out by culturing at nutrient broth to detect the presence or absence of growth and on sure complete inactivation.

f. Avibacterium paragallinarum:

1. Preparation of bulk culture from Avibacterium paragallinarum.

2. Inactivation of avibacterium paragallinarum cultures.

3. Safety (completion of inactivation for A. paragallinarum).

It was carried out by culturing at tryptose phosphate broth to detect the presence or absence of growth and on sure complete inactivation.

G.Evaluation of the produced vaccines:

It was done according OIE .

-Identity:

The identity of every component incorporated in the vaccine under test is carried out through testing of sera collected from vaccinated chickens (in conjunction with potency test).

-Completion of inactivation:

Ten SPF 9-11 day old ECE were inoculated each with the recommended dose of inactivated vaccine. The inoculated eggs were candled twice daily for six

Days the embryos that died after within first 25 hours were discarded. The embryos that died after the first 24 fours as well as the survivors, 6 days post inoculation were tested for the presence of haemagglutination activity using the rapid HA test. The harvested fluids were blindly passaged for 2 other passages before the vaccine being emulsified.

-Sterility:

It was carried out according to Allan et al. The prepared pentavalent inactivated vaccine was inoculated into different types of media for sterility testing:

a. Thioglycolate broth was inoculated and incubated at 370C for 72hours.

b. Nutrient agar plates were inoculated and incubated at 370C for 72 hours.

c. Sabouraud's maltose broth and Sabouraud's maltose agar plates were inoculated and incubated at 250C for 14 days. [9].

-Extraneous agent:

The test was run in conjunction with safety test. After 3 weeks, each inoculated bird with double dose was inoculated S/C with another one field dose from the tested vaccines. Serum samples were collected two weeks later and tested for antibodies to extraneous viral agents were performed.

Safety:

It was done accorded to [9].

Three groups of ten SPF chickens, one day old, were injected S/Cwith two doses from each tested vaccines. The vaccinated birds were observed 21 days post vaccination for the general appearance (behavior, appetite, and development status as weakness, dropping or any unexpected adverse events), body weight, performances and macroscopic examination of injection site

Potency:

It was done to demonstrate the antigenic capacity for each tested vaccine. SPF chickens, four weeks old, were vaccinated S/C with field dose. Blood samples were drawn weekly for 6 weeks (30 sample per week) and the serum samples were separated, inactivated at 56oC /30min and kept at -20oC till used. The serological analysis was done to antibody level against each component of the tested vaccines. Also, at 21 days post vaccination, 150 birds were challenged by local and virulent isolates corresponding to the vaccines component. The morbidity and mortality rates were recorded for each group till the end of the observation period to measure the protection %.

B. ELISA for IB and S. enteritidis using kits (biocheck kit) Haider et al. [10]

Reagent preparation: The microtiter plates coated with antigen were

Calculation of S/P ratio:

Calculation of Antibody Titer:

SP ratio

Log10 Titer=1.13Log (SP) +3.156

Antilog= Antibody titer.

I. bioassay test:

a. Salmonella enteritidis: Challenge was done using 0.5ml of containing 109 CFU strain S. enteritidis orally. The degree of protection was assessed according to the severity of the clinical signs, the mortality, and the post mortem lesions beside bacterial reisolation for 14 days sayed [9].

B. A. paragallinarum: Challenge was done using 0.5 ml of containing 107 CFU/ 0.5ml. By 0.25 ml inoculated into the infraorbital sinuses as well as via nostril. The birds were examined for any clinical manifestation of coryza beside bacterial reisolation for 14 days [11].

c. Newcastle disease virus: challenged was done using 0.5 ml containing 106 EID50 of VVNDV Intramuscular, the morbidity and mortality rates were recorded beside viral reisolation for 10 days [11].

RESULTS AND DISCUSSION

In the present work, it is noticed from Table 1 that the titres of antibody of chicken vaccinated with the locally prepared and imported inactivated Penta vaccines with SE component using ELISA test raised from 159.6 prevaccination among all groups, then reaching the maximum levels (2464.9 and 2587.6) at the 6th WPV in the two vaccinated groups, respectively, in comparison to the control group (186.6). The presence of ELISA reactors to SE inactivated vaccines supports the view that such serological test induced a satisfactory test for the evaluation of immune response to SE vaccines. Nearly, similar findings have been reported by Winterfield et al. [12].

Comparison between local and imported inactivated Penta vaccines as determined by challenge test is presented in Table 2. It is found that imported Penta vaccines elicited a high level of overall protection rates reaching 80 % each followed by local vaccines (73.3 %) when chicken vaccinated groups were challenged with virulent strain of SE. It can be concluded that all types of vaccines gave a good protection against S. entritidis disease. These results are in agreement with those of Yamamoto [13.

On the other hand the present study showed that, the challenge test for IC was performed in order to correlate the immune status of chicken vaccinated with either local or imported Penta vaccines using virulent A. paragallinarum serotypes. It could be seen from Table 3 it was found that inoculation of chicken with imported inactivated Penta vaccine and then challenged with virulent culture of A. paragallinarum "A" strain evoked a high degree of protection rate reaching 100 %, followed by those vaccinated with local vaccine (93.3 %). Infectious Coryza symptoms was observed in all chicken among control group and the respiratory signs which developed were either moderate or more severe and of longer duration than in the immunized chicken. These results agreed with the previous findings of Page et al. [14].

As shown in Table 4 it was found that, comparison between immunizing efficacy of local and imported inactivated Penta vaccines subjected to challenge with virulent "B" strain of A. paragallinarum, both vaccines evoked a high degree of protection reaching 80 % and 86.6 %, respectively. Thus, as pointed out by Marius et al. [15]. Local as well as imported trivalent inactivated vaccines can gave nearly overall mean protection percents to control Infectious Coryza in Egypt.

Resulting benefit of inactivated Penta vaccines is illustrated from results presented in Table 5 it could be deduced that no difference percent of protection between different vaccinated chicken groups with local and imported Penta vaccines (93.3% for each group) but present highly significant difference between vaccinated and control group when subjected to challenge with virulent "C" strain of A. paragallinarum. These findings are nearly coincide with the results obtained by Boots [16].

From Table 6 it could be concluded that slight significant differences between overall protection percents against challenge with virulent "A", "B" and "C" strains of A. paragallinarum can be seen. The overall protection percent reached 88.9% and 93.3% in chicken groups vaccinated with local and imported Penta vaccines, respectively. Thus, the local vaccine can give to somewhat a very close result as imported vaccine when challenged with the three virulent strains of A. paragallinarum "A", "B" and "C". Nearly, similar findings have been reported by Winterfield et al. [12].

So our study concluded that, the local pentavalent vaccine (S.E., IC) gave acceptable antibody titers and good protection levels in comparison with the imported pentavalent vaccine.

Table 1. Mean ELISA antibody titer against SE component of the local and imported inactivated polyvalent Penta vaccines in the sera of vaccinated birds groups

groups	Types of vaccines	No. of serum samples	ELISA antibody titers (Weeks post vaccination)						
			Pre.	1	2	3	4	5	6
1	Local	10	159.6	396.1	790.1	1097.8	1940.5	2254.8	2464.9
2	Imported	10	159.6	482.4	856.3	1187	2087.8	2354.7	2587.6
3	Control	10	159.6	163.6	166.1	171	176.9	177.8	186.6

Positive ELISA titre range: 654 or greater according to ELISA kit.

Table 2. Results of the efficacy of the local and imported inactivated polyvalent Penta vaccines in vaccinated birds against the challenge with virulent S. enteritidis at 4 weeks post vaccination

Groups	Type of vaccines	No. of birds	Daily examination for diseased (days post challenge)														Total diseased	Protection % *
			1	2	3	4	5	6	7	8	9	10	11	12	13	14		
1	**local**	15					2	2									4.15	73.3
2	**imported**	15					1	2									3.15	80
3	**control**	10			3	3	2	2									10.10	0

* Minimum protection % is 70% according Egyptian regulation

Table 3. Results of the efficacy of the local and imported inactivated polyvalent Penta vaccines in vaccinated birds against the challenge with virulent A serotype of A. Paragalinarum at 4 weeks post vaccination

Groups	Type of vaccines	No. of birds	Daily examination for diseased (days post challenge)														Total diseased	Protection % *
			1	2	3	4	5	6	7	8	9	10	11	12	13	14		
1	**local**	15						1									1.15	93.30
2	**imported**	15															0.15	100
3	**control**	10			7	3											10.10	0

*Minimum protection % is 80% according Egyptian regulation

Table 4. Results of the efficacy of the local and imported inactivated polyvalent Penta vaccines in vaccinated birds against the challenge with virulent B serotype of Avibacterium Paragalinerum at 4 weeks post vaccination

Groups	Type of vaccines	No. of birds	Daily examination for diseased (days post challenge)														Total diseased	Protection % *
			1	2	3	4	5	6	7	8	9	10	11	12	13	14		
1	**local**	15					1	1	1								3.15	80
2	**imported**	15					2										2.15	86.60
3	**control**	10				5	3	2									10.10	0

*Minimum protection % is 80% according Egyptian regulation

Table 5. Results of the efficacy of the local and imported inactivated polyvalent Penta vaccines in vaccinated birds against the challenge with virulent C serotype of A. Paragalinerum at 4 weeks post vaccination

Groups	Daily examination for diseased

To cite this paper: Akeila MA. And Sedeik M. 2014. Efficacy of Local and Imported Vaccines Aainst Salmonella Entertiodes and A. Paragallinarium. *J. Life Sci. Biomed.* 4(4):252-256.
Journal homepage:http://jlsb.science-line.com/

	Type of vaccines	No. of birds	(days post challenge)														Total diseased	Protection % *
			1	2	3	4	5	6	7	8	9	10	11	12	13	14		
1	**local**	15				1											1.15	93.30
2	**imported**	15					1										1.15	93.30
3	**control**	10			5	4	1										10.10	0

*Minimum protection % is 80% according Egyptian regulation

Table 6. Overall protection % of the local and imported inactivated polyvalent Penta vaccines in vaccinated birds against the challenge with virulent A, B and C serotypes of A. Paragalinerum at 4 weeks post vaccination

Groups	Vaccine types	No. of birds	Protection % for A Serotype	Protection % for B serotype	Protection % for C serotype	Overall protection % mean against A, B and C serotypes
1	**local**	15	93.3	80	93.3	88.9
2	**imported**	15	100	86.6	93.3	93.3
3	**control**	10	0	0	0	0

REFERENCES

1. Antunes, P., Rill, C., Sousa, E., Peixe, L. & Pestana, L. 2003. Incidence of Salmonella from poultry products and their susceptibility to antimicrobial agents. .1m. J. Food Microbio, 182:97-103.
2. Capita, R., Alvarez-Astorga, M., Alonso-Calleja, M.C., Moreno, B. & Del Camino Garcia-Fernandez, and M. (2003): Occurrence of salmonellae in retail chicken carcasses and their products in Spain. J. Food MicrobioI, 81:169-173.
3. EL-Agroudi, M.A. & Sadek, I.M. 1966.A rapid screening test for detection Salmonella typhimurium infection in poultry. J. Arab. Vet. Med. Assoc., (Cairo), 26: 235 -238
4. El-Agroudi, M.A. & Sadek, I.M. 1968. A survey on avian salmonellosis in UAR. J. Vet. Sci. (UAR), 5:11 -17.
5. Blackall, P.J., Christensen, H., Beckenham, T., Blackall, L.L. & Bisgaard, M. 2005. Reclassification of Pasteurella gallinarum, [Haemophilus] paragallinarum, Pasteurella avium and Pasteurella volantium as Avibacterium gallinarum gen. Nov. Int. J.Syst. Evol. Microbiol, 55:353–362.
6. Blackall, P.J., Yamaguchi, T. & Matsumoto, M. 1997. Infectiouscoryza. In: Calnek, B.W., Barnes, H.J., Beard, C.W., McDougald, L.R., Saif, Y.M. (Eds.), Diseases of Poultry. Iowa State University Press, Ames, 179–190.
7. Jouy, E., Proux, K., Humbert, F., Rose, V., Lalande, F., Houdayer, C., Picault, JP. & Salvat, G. 2005.Evaluation of a french ELISA for the detection of Salmonelle Enteritidis and Salmonella typhimurium in flocks of laying and breeding hens. Vet. Med, 71(1-2): 91-103.
8. Charles, S.D., Hussein, I., Nagraja, K.V. & Sivanadan, V. 1994. Adjuvanted subunit vaccines for the control of Salmonella enteritidis infection in turkeys. Am. J. Vet. Res., 55 (5): 636-642.
9. Sayed, R.H. 2010.Preparation and evaluation of combined inactivated vaccine against S. enteritidis, S. typhimurium, C. perfringens type A and C toxins in chickens. Master. Sci. Thesis, Fac. Vet. Med., Cairo Univ. Shouman, M.T. and Moustafa, F. (1972): Salmonellosis in chickens. Vet. Med. J., 20: 183 -192.
10. Haider, M.G., Rahman, M.M., Hossain, M.M., Rashid, M., Sufian, M.A., Islam. M.M. & Haque, A.H. 2007. Production of formalin killed fowel typhoid vaccine using local isolates Salmonella gallinarium in Bangladesh. Bangl. J. Vet. Med, (5): 33–38.
11. Mohamed, A.M. 1996. Trials for preparation and evaluation of a combined poultry vaccine composed of Newcastle disease virus and haemophilus paragallinarium. PhD. Sci. Thesis, Fac. Vet. Med., Cairo Univ OIE (2008): Evaluation of the produced inactivated vaccines.
12. Winterfield, R.W., Thacker, H.L. & badylack, S.F. 1984.Effects of subtype virations of the Holland strain of infectious bronchitis virus when applied as vaccine. Poultry Sci, 63 (2): 246-250.
13. Yamamoto, R. 1984.In: disease of poultry, 8th Ed. M.S. Hofstad, H.J. Barnes, B.W. Calnek, W.M. Reid and H.W. Jr. Yoder, Iowa, State Univ. Press, Iowa USA, 178-186.
14. Page, L.A., Rosenwald, A.S. & Price, F. C. 1963. Haemophilus infectious in chickens. IV. Results of laboratory and field trials of formalized bacterins for the preventions of disease caused by Haemophilus gallinarium. Avian. Dis., 7: 239-256.
15. Marius, V., Gillet, J.P., Hospitalier, R., Guittet, M. & Bennnejan, G.1983. Vaccination against infectious bronchitis in chicks, whether or not bearing maternal antibodies. Comp. Immunol. Microbiol. Infect. Dis. 6:115-134.
16. Boots, M.H. 1991.the cellular immune response to infectious bronchitis virus. Amodel for development of a peptide based vaccine. Thesis, Rijksuniversiteii to Ultrecht, Netherlands, 132.