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# Evaluation of Autogenous *Avibacterium paragallinarum* Bacterins in Chickens

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**Abstract:** In this investigation, a trial for preparation and evaluation of locally prepared (autogenous) bacterin against the infection with *Avibacterium paragallinarum* (*Avi. paragallinarum*) causing infectious coryza disease in layer chickens was done. Two types of adjuvant (aluminum hydroxide and mineral oil based) were compared. At 6 weeks of age, one hundred layer chickens were divided into equally distributed 4 groups each containing 25 birds. Group (1) was vaccinated with *Avi. paragallinarum* autogenous bacterin containing aluminum hydroxide, while group (2) received *Avi. paragallinarum* autogenous bacterin containing mineral oil. Both types of bacterins were given in a dose of 0.5 ml/bird and administered intramuscularly (IM). Booster dose of both types of the autogenous bacterins was given in group 1 and 2 at 9 weeks of age. Chickens of group (3) were kept without vaccination. Birds in groups 1, 2 and 3 were challenged with ($10^6$ CFU) of live *Avi. paragallinarum* culture by inoculation into the nasal sinus at 12 weeks old. Group (4) was left as blank control negative (not vaccinated or challenged). Birds after challenge were kept under complete daily observation for 7 days. Signs, mortalities, postmortem lesions, protection rate and reisolation rate of the organism were taken as criteria for bacterin evaluation, also agglutination test were performed on sera to determine the immune response to bacterin at the doses intervals. The results revealed that whatever the type of adjuvant, both different adjuvanted types' bacterines were effective and safe in prevention of infection against *Avi. paragallinarum* in layers when administered at 6 and boosted at 9 weeks of age.

**Key words:** *Avibacterium paragallinarum*, IC, autogenous vaccine, layers

## INTRODUCTION

Infectious Coryza (IC) is an acute respiratory disease of chickens caused by the bacterium *Avibacterium paragallinarum* (*Avi. paragallinarum*), once known as *Haemophilus paragallinarum* (Blackall *et al*., 2005). The disease was recognized as a clinical syndrome since the 1930s (Blackall *et al*., 1997) then it distributed worldwide. The economic importance of IC could be summarized as marked reduction (10-40%) in egg production in layers and breeders (Yamamoto, 1984) and increase the culling rate in broilers due to airsacculitis condemnation (Droual *et al*., 1990). Moreover, the losses due to IC are more significant when the infection is associated with *Mycoplasma gallisepticum* causing high mortalities (Kato, 1965; Sandoval *et al*., 1994).

Protection of chicken flocks against IC involves use of biosecurity, medication and vaccination. Effective biosecurity measures should prevent exposure of chickens to IC infection; however, in situations where the disease is endemic like in multi age farms, vaccination must be used to minimize the impact of IC (Philemon, 2009).

Early treatment of IC may be of value; however, the infected chickens continue to be carrier of the bacteria and relapse often occurs after treatment is discontinued (Yamamoto, 1991). In this respect, vaccination seems to be one of the best methods to prevent the occurrence of the disease (Reid and Blackall, 1983; Blackall and Reid, 1987).

Vaccination against coryza, is successful, is an ideal preventive method under current types of management where depopulation is not feasible. It should be noted that IC vaccination programs will not stop the birds from being infected but they lessen the signs of the disease and dramatically reduce shedding and spreading of the bacteria. Commercial vaccines for IC which that produced from standard internationally recognized strains of inactivated *Avi. paragallinarum* are widely used around the world (Blackall, 1999), but such vaccines are not protective against the local variants of *Avi. paragallinarum*. Therefore, there is a need to produce vaccines from local strains.

Therefore, several trials were conducted to produce IC bacterin from local bacterial strains either in aluminum hydroxide gel form (Matsumoto and Yamamoto, 1971; Davis *et al*., 1976; Kume *et al*., 1880; Zaki, 1985; Reid and Blackall, 1987; Yamaguchi *et al*., 1988; Blackall, 1991; Mouahid *et al*., 1991; Fernandez *et al*., 2005; Kridda *et al*., 2009; Philemon, 2009) or in mineral oil form (Page *et al*., 1963; Matsumoto and Yamamoto, 1975; Coetzee *et al*., 1982; Reid and Blackall, 1983; Blackall and Reid, 1987; Blackall *et al*., 1992; Jacobs *et al*., 1992; Terzolo *et al*., 1997).

The objective of the present investigation was preparation of *Avi. paragallinarum* autogenous bacterins containing either aluminum hydroxide or mineral oil adjuvant and evaluation of the efficacy of each bacterin in layer chickens.

## MATERIALS AND METHODS

**The bacterial strain:** The local strain of *Avi. paragallinarum* was provided kindly from isolated from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. The strain was isolated from IC outbreaks in laying flocks then it was identified as *Avi. paragallinarum* (Blackall *et al*., 2005). Also, the organism was tested to its ability to grow on McConkey agar, catalase activity, sugar fermentation and its ability to grow in the presence or absence of Nicotamide Adenine Dinucleotide (NAD) for the more identification of the strain (Horner *et al*., 1992; Mouahid *et al*., 1992; Bragg *et al*., 1993, 1996). This strain was used for preparation of vaccine, production of antigen for serology and as challenge bacteria.

**The challenge bacterial antigen:** For preparation of the challenge *Avi. paragallinarum* strain, 7-days old embryonated chicken eggs was inoculated through the yolk sac route after Page *et al*. (1963) and Reid and Blackall (1983).

**Haemagglutinating antigen preparation:** The method of preparation was described after Iritani *et al*. (1977) and 1980. Strain of *Avi. paragallinarum* used in agglutination test was grown in Chicken Meat Infusion (CMI) broth provided with 0.0025% NAD and incubated at 37°C for 48 h under microaerophilic conditions ($CO_2$ incubator). The bacterial antigen was collected from the broth culture after centrifugation at 6000 r.p.m for 30 h, washed 3 times in Phosphate Buffer Saline (PBS) containing 0.01% thiomersal and then the suspension was titrated and diluted in PBS to provide 4 haemagglutinating units in 0.025% of suspension. The antigen was stained with crystal violet in concentration of 0.02%.

**Preparation of the autogenous bacterins:** A broth vaccine was prepared from a culture of *Avi. paragallinarum* grown in Chicken Meat Infusion (CMI) broth with 0.0025% NAD and incubated at 37°C for 48 h in $CO_2$ incubator (Matsumoto and Yamamoto, 1975; Davis *et al*., 1976; Kume *et al*., 1980). For counting, aliquot (5 ml of the broth culture) had been removed, serially diluted in physiological saline and a droplet from each dilution was placed on CMI supplemented by 0.0025% NAD and incubated under previous conditions. The number of the Colony Forming Unit (CFU) was calculated in each dilution. The broth culture was adjusted to contain at least $10^8$ CFU/ml (Matsumoto and Yamamoto, 1975). The culture was inactivated by adding formalin to a final concentration of 0.25% (Rimler *et al*., 1975; Coetzee *et al*., 1982). Aluminum hydroxide gel adjuvant was added at concentration of 25% of the final volume (Matsumoto and Yamamoto, 1971; Boycott *et al*., 1977; Blackall and Reid, 1987; Reid and Blackall, 1987; Blackall *et al*., 1992) and another bacterin was prepared by addition of mineral oil (Freund's incomplete adjuvant) at a concentration of 50% of the final volume (Davis *et al*., 1976). Inactivation of bacteria was confirmed by streaking a 0.1 ml sample on blood and chocolate agar plates and incubated for 24 h at 37°C in 10% $CO_2$ that showed no growth of the bacteria. Both types of bacterins were stored at 4°C till used.

**Experimental chicks:** One hundred, day old LSL layer pullets were obtained from a commercial hatchery. Chicks were kept in separate thoroughly cleaned and disinfected pens and were fed on starter and grower ration *ad libitum*. The birds were checked clinically, bacteriologically and serologically for the absence of *Avi. paragallinarum* and other infectious agents just before grouping or vaccination.

**Experimental design:** At 6 weeks of age, one hundred layer chickens were divided into equally distributed 4 groups each containing 25 birds. Group (1) was vaccinated with *Avi. paragallinarum* autogenous bacterin containing aluminum hydroxide, while group (2) received *Avi. paragallinarum* autogenous bacterin containing mineral oil. Both types of bacterins were given in a dose of 0.5 ml/bird and administered Intramuscularly (IM) (Blackall and Reid, 1987). Booster dose of both types of the autogenous bacterins was given in group 1 and 2 at 9 weeks of age. Chickens of group (3) were kept without vaccination. Birds in groups 1, 2 and 3 were challenged with 0.2 ml ($10^6$ CFU) of live *Avi. paragallinarum* culture by inoculation into the nasal sinus at 12 weeks old (Yamaguchi *et al*., 1988). Group (4) was left as blank control negative (not vaccinated or challenged). Birds after challenge were kept under complete daily observation for 7 days.

**Criteria for evaluation of the prepared autogenous bacterins**

**Signs, mortalities, gross lesions and the protection rate:** All the chickens in the experimental groups were observed for typical IC signs (nasal discharge, sneezing, conjunctivitis, swelling of sinuses and facial oedema) (Yamamoto, 1980) and mortalities daily after challenge and all the birds were necropsied 7 days after challenge (end of the study). Chickens were considered protected if they showed no signs of coryza clinically or at necropsy.

**Bacterial reisolation:** Swabs were collected from the infraorbital sinuses from birds with signs, dead birds as well as necropsied ones for reisolation of the challenge organism (Rimler *et al*., 1975). Each swab sample was streaked onto CMI agar with 0.0025% NAD and incubated at 37°C for 48 h in $CO_2$ incubator. Birds were considered protected if they showed no growth of *Avi. paragallinarum* on cultures.

**Serological monitoring:** Blood samples were collected from ten birds in each group just before vaccination and weekly intervals for 3 weeks after vaccination in order to determine the extent of seroconversion of birds to the bacterins.

The agglutination test was conducted as Iritani *et al*. (1977) and Thornton and Blackall (1984). Briefly, the blood samples were collected from the wing veins of birds, left to clot, centrifuged and the serum samples were separated. The antibody response was determined by a micro plate agglutination test which was performed in 96-well V-shaped micro plates. Serum sample from each chicken was diluted in twofold steps in PBS. Twenty-five micro liters of both *Avi. paragallinarum* antigen suspension and the serum sample dilutions per well were mixed together and incubated for 18 h at 37°C. The results were recorded as $\log_2$ x of the reciprocal of the highest serum dilution which showed agglutination then geometric mean antibody titers were calculated. Positive and negative sera were included as controls.

**Statistical analysis of the data:** The collected data were tested using the method of Snedecor and Cochran (1980). The serological results were analyzed by analysis of variance and by Student's t-test. Statistical significance was determined at the 0.05 level of probability.

## RESULTS

The obtained *Avi. paragallinarum* strain failed to grow on McConkey agar and it was catalase negative and fermented mannitol, fructose, mannose, sucrose, glucose and sorbitol. Moreover, it failed to produce acid from arabinose, dulcitol and lactose. The organism revealed satellites growth on CMI agar and it was NAD-dependent. From these results, it could be ensure that the strain was *Avi. paragallinarum*.

No signs were recorded in the blank non vaccinated 0 challenged group (4). Clinical signs as nasal discharge, sneezing, conjunctivitis and swelling of the infraorbital sinuses were observed in non vaccinated and challenges (group 3) at the 2nd day post challenge, whereas signs appeared in the vaccinated and challenged groups (1 and 2) at the 4th day after challenge.

Table 1 showed the efficacy of the two types of autogenous *Avi. paragallinarum* bacterins in layer chickens one week after challenge. It was observed although there was no significant ($p<0.05$) differences between vaccinated groups (1 and 2) but the significant ($p<0.05$) differences was between the vaccinated and the non-vaccinated ones (3 and 4). Chickens vaccinated with autogenous aluminum hydroxide adjuvant bacterin showed percentage of clinical signs (8%), dead birds (4%) and protection rate (92%), while they were 12%, 4% and 88%, respectively in birds vaccinated with autogenous mineral oil adjuvant bacterin. The percentage of clinical signs, dead birds and protection rate were 72%, 16% and 28%, respectively in the challenged non vaccinated group.

The post mortem lesions of dead as well as sacrificed birds 7 days after challenge were catarrhal rhinitis, sinusitis and laryngitis with the presence of subcutaneous oedema in the tissues around the infraorbital sinuses. The severity of lesions was more increase in the vaccinated challenged than the non-vaccinated challenged birds.

Considering the reisolation rate of *Avi. paragallinarum* from birds with signs, dead and necropsied ones at the end of the observation period. Table 1 revealed that *Avi. paragallinarum* recovered from the infraorbital sinuses of all birds in the non-vaccinated and challenged group (3), while it was not reisolated from the blank non vaccinated or challenged group (4). The reisolation rate of the organism was 12% in chickens received autogenous aluminum hydroxide adjuvant bacterin and 16% in layers given autogenous mineral oil adjuvant bacterin.

What was mentioned in Table 2 indicated that there was gradual increase in the Geometric Mean Titer (GMT) of antibodies from 3.4 at the 1st week to reach 6.2 $\log_2$ at the 3rd week post vaccination in chickens vaccinated with autogenous bacterin in aluminum hydroxide gel, as well this titer was elevated from 2.9 to 5.3 $\log_2$ at the previous intervals in layers vaccinated with autogenous bacterin in mineral oil. Additionally, non-vaccinated chickens did not develop antibodies.

Table 1: The efficacy of the two types of autogenous *Avi. paragallinarum* bacterins in layer chickens one week after challenge

| | | | | | Criteria for evaluation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Group No. | No. of birds | Alum. hydroxide adjuvant | Mineral oil adjuvant | Chall. | Respiratory signs | Sinus swelling | % of clinical signs[C] | % of dead birds | Protection rate (%)[C] | Reisolation rate (%) |
| 1 | 25 | + | - | + | 1 | 1 | 2/25 (8%) | 1/25 (4%) | 92% | 3/25 (12%) |
| 2 | 25 | - | + | + | 2 | 1 | 3/25 (12%) | 1/25 (4%) | 88% | 4/25 (16%) |
| 3 | 25 | - | - | + | 11 | 7 | 18/25 (72%) | 4/25 (16%) | 28% | 25/25 (100%) |
| 4 | 25 | - | - | - | 0 | 0 | 0/25 (0%) | 0/25 (0%) | 100% | 0/25 (0%) |

All the vaccinated groups were differ significantly from the non vaccinated groups ($p<0.05$). Chall. = Challenge

Table 2: The antibody response at the different intervals after vaccination of layer chickens with autogenous *Avi. paragallinarum* bacterin

| Group No. | Alum. hydroxide adjuvant | Mineral oil adjuvant | Challenge | Antibody titers at different weeks interval[a] 1st wk PV | 2nd wk PV | 3rd wk PV |
|---|---|---|---|---|---|---|
| 1 | + | - | + | 3.4 | 5.1 | 6.2 |
| 2 | - | + | + | 2.9 | 4.9 | 5.3 |
| 3 | - | - | + | 0 | 0 | 0 |
| 4 | - | - | - | 0 | 0 | 0 |

Wk PV = Week Post Vaccination. [a]Geometric Mean Antibody Titers (GMT) expressed as $\log_2$ x (number of birds = 10)

## DISCUSSION

*Haemophilus paragallinarum* which is now called *Avibacterium paragallinarum* (*Avi. paragallinarum*), the causative agent of IC in chickens can cause economic losses to poultry operations throughout the world. The disease is a highly contagious upper respiratory disease, characterized by foamy conjunctivitis, sinusitis, nasal discharges, depression, lethargy and birds may develop swelling of the wattles and diarrhea. Affected flocks in lay can suffer drops in egg production from 5 to 10% and in some cases, from 40 to total loss 100% of eggs (Mouahid *et al*., 1991). Despite the widespread use of vaccines against IC, the disease remains a serious problem in the poultry industry. Several inactivated vaccines have been used to prevent IC, but there have been outbreaks reported after using those vaccines (Davis *et al*., 1976). It has been well established that there is no cross protection between different serogroups (Rimler *et al*., 1977; Kume *et al*., 1980). Thus, the use of autogenous bacterins appears to be more effective in control of IC if they contain serovars which are present in a country or locality (Blackall and Reid, 1987; Mouahid *et al*., 1991). Moreover, in order to be successful, these bacterins can only be used before the birds go into productions, i.e., vaccination done at 12-18 weeks of age.

In the present study, double doses of autogenous IC bacterins (aluminum hydroxide and mineral oil adjuvanted bacterins) against *Avi. paragallinarum* was tested serologically using agglutination test and both types provoked production of protective immunity in layer chickens. Similarly, Mouahid *et al*. (1991) and Glisson (1998) found that chickens vaccinated with autogenous bacterin showed a very broad immune response and protection against *Avi. paragallinarum* infection. Previous studies by Otsuki and Iritani (1974) and Nakamura *et al*. (1994) reported a close correlation of antibody titer and protection in chickens vaccinated against IC. In this work, although the titer of antibodies was higher in aluminum hydroxide adjuvanted bacterin than mineral oil one but there was no significant difference between them. Davis *et al*. (1976) reported accord results. Also, Matsumoto and Yamamoto (1971); Boycott *et al*. (1977); Kume *et al*. (1880); Blackall and Reid (1987) and Blackall *et al*. (1992) observed that not only IC aluminum hydroxide adjuvanted bacterin was protective but also not induce adverse reaction on the vaccination site.

The protection rate from IC signs and the clearance of *Avi. paragallinarum* from the sinuses were taken as criteria for evaluation of IC bacterin. By applying these criteria, we clearly showed that better protection was obtained when using two shots of the autogenous bacterins regardless to the type of adjuvant (Yamamoto, 1984; Blackall and Reid, 1987; Blackall, 1988). Controversial results were obtained by Matsumoto and Yamamoto (1975); Kume *et al*. (1980); Coetzee *et al*. (1982); Zaki (1985) and Philemon, (2009) who demonstrated that a single dose of aluminum hydroxide gel based adjuvant gave protection for layer birds up to nine months after vaccination. Blackall (1995) concluded that for longer term protection against IC, aluminum hydroxide vaccines should be given at two doses, at least three weeks a part. Autogenous bacterins containing the prevalent serotypes in an area and incorporating aluminum hydroxide gel as an adjuvant appear to be more effective in controlling IC (Reid and Blackall, 1983; Blackall and Reid, 1987).

Blackall *et al*. (1992) stated that vaccination programs for layers and breeders against IC usually consist of a two-dose regime, the first begins from 6 to 8 weeks of age to protect pullets against the symptoms, but mainly function more as a primer for a second vaccination, 6-8 weeks later. For these early vaccinations, poultry producers prefer the aluminum hydroxide-based vaccines, due to the minimal local reaction around the injected site. This reduces the chances of stressing the pullets at such an important period of feed intake and proper carcass development. A good vaccination program with a good primer and booster should provide long-lasting protection.

In conclusion, both autogenous (aluminum hydroxide and mineral oil adjuvants) *Avi. paragallinarum* bacterins were found to be effective and safe when given as double I/M shots in layer chickens at 6 and 9 weeks of age.

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