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CROSS-PROTECTION BETWEEN *HAEMOPHILUS PARAGALLINARUM* STRAINS AND EVALUATION OF INFECTIOUS CORYZA VACCINES

(With 7 Tables)

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دراسة الحماية التصالبية بين عترات الهيموفيلاس باراجالينيرم وتقييم لقاحات مرض الزكام المعدى

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أثبتت النتائج أن الحماية التصالبية بين عترات الهيموفيلاس باراجالينيرم التابعة لنفس النوع كانت متباينة. بعض العترات كانت عالية مناعيا حيث أعطت حماية جيدة بينما العترات الأخرى وجد أنها ضعيفة مناعيا و أعطت حماية ضعيفة نسبيا ضد إختبار تحدى المناعة بالعترات المختلفة من نفس النوع. على الجانب الآخر لم توجد مناعة تصالبية بين الأنواع الثلاثة المختلفة من الهيموفيلاس باراجالينيرم حيث أن اللقاحات الأحادية أحدثت حماية ضد العدوى بنفس النوع فقط مدللة على أن مناعية هذه الأنواع مختلفة. بالإضافة إلى أن هناك حماية تصالبية جزئية بين عترات الهيموفيلاس باراجالينيرم التابعة لنفس النوع. تم تقييم فعالية لقاح ثلاثي يحتوى على ثلاث عترات معزولة محليا وذات المناعية العالية والممثلة للأنواع أ، ب، و س من الهيموفيلاس باراجالينيرم ومقارنته بالنوع التجاري الثنائي. وجد أن اللقاح الثلاثي أحدث أحسن حماية للدجاج المحصن ضد العدوى بأي من الأنواع الثلاثة للهيموفيلاس باراجالينيرم وكانت نسبة الحماية الناتجة عن اللقاح هي ١٠٠% ضد اختبار تحدى المناعة بأى من الأنواع أ، ب، و س للهيموفيلاس باراجالينيرم. على الجانب الآخر أحدث اللقاح التجاري الثنائي المحتوى على النوعين أ ، س حماية ضد إختبار تحدى المناعة ضد النوعين أ (٨٠%) ، س (٧٠%) في حين أنه فشل في حماية الدجاج من العدوى بالنوع ب. وجد أن هناك توافق بين مستوى الأجسام المناعية المثبطة لتلازن الدم والحماية ضد العدوى بالأنواع المختلفة. من النتائج السابقة يمكن استنتاج أن لقاحات مرض الزكام المعدي يجب أن يحتوى على الثلاثة أنواع أ ، ب ، س لكن يوصى باستخدام العترات المعزولة محليا في تحضير اللقاحات ضد مرض الزكام المعدي.

SUMMARY

The cross-protection between *H. paragallinarum* strains within the same serovar was variable. Some strains were found highly immunogenic providing a good protection, while others were weakly immunogenic and afforded a relatively poor protection against heterologous challenge. On the other hand, no cross-protection was found between the three serovars of *H. paragallinarum*, where the monovalent vaccines provided protection only against homologous challenge indicating that the immunogenicity between them was different. The efficacy of inactivated trivalent coryza vaccine prepared from three highly immunogenic locally isolated strains representing serovars A, B and C of *H. paragallinarum* was evaluated and compared with that of a commercial bivalent vaccine. The trivalent vaccine provided the best protection (100%) against challenge with any of the reference strains of serovars A, B, and C of *H. paragallinarum*. On the other hand, the commercial bivalent coryza vaccine containing *H. paragallinarum* serovars A and C conferred protection only against serovars A (80 %) and C (70 %) challenge, while it failed to protect against serovar B challenge. A good correlation was found between HI titers and protection against challenge. It could be concluded that coryza vaccines should contain strains representing all the three serovars A, B, and C of *H. paragallinarum*, and the inclusion of locally isolated strains in preparation of coryza vaccines is recommended.

Key words: *Haemophilus paragallinarum – Vaccine – Cross- Protection*

INTRODUCTION

Infectious coryza is one of the most important respiratory diseases caused by *Haemophilus paragallinarum* (*H. paragallinarum*), and characterized mainly by by serous to mucoid nasal discharge and facial oedema. The greatest economic losses are due to marked reduction (10-40 %) in egg production in laying chickens and increased percentage of culls in growing birds (Yamamoto, 1991). In developing countries, coryza is commonly complicated by other infections, resulting in severe and prolonged disease (Blackall, 1999).

Rimler et al. (1977) reported that *H. paragallinarum* exists in three different immunotypes that correlate with the serovars A, B, and C as determined by Page's scheme (Page, 1962). Chickens vaccinated with

a bacterin prepared from one serovar were protected only against homologous challenge (Blackall and Reid, 1987).

Several studies have indicated that inactivated infectious coryza vaccines with different adjuvants can protect chickens against the disease (Matsumoto and Yamamoto, 1975; Davis et al., 1976; Kume et al., 1980; Reid and Blackall, 1987). Most of the commercially available coryza vaccines are bivalent containing serovars A and C of *H. paragallinarum*. Therefore, these types of vaccines conferred type-specific immunity against those Page's serovars included in the vaccine (Rimler et al., 1977; Kume et al., 1980). Moreover, the type-specific immunity produced by infectious coryza vaccines occurred within the Page's serovars, but no cross-protection was found between serovars (Rimler et al., 1977). A fairly recent study by Yamaguchi et al. (1991) has shown that cross-protection within Page's serovar B is only partial.

On the other hand, Sandoval et al. (1994) reported that the vaccine failures in Argentina occurred because the commercial coryza vaccines did not include local isolates of the three serovars of *H. paragallinarum* present in the target population

Although the majority of the poultry farms in Egypt use commercial coryza vaccines in their programs, outbreaks of infectious coryza continue to occur. Thus, the purpose of this study was to investigate the cross-protection between different serovars as well as between strains within the same serovar of *H. paragallinarum*, in order to develop a vaccine against infectious coryza with higher immunogenicity and broader protection, covering all *H. paragallinarum* serovars and compare its efficacy with a commercial bivalent vaccine.

MATERIALS and METHODS

***H. paragallinarum* reference strains:**

The strains 221, Spross and H-18 representing *H. paragallinarum* serovars A, B, and C, respectively, were kindly supplied by Dr. Chuzou Ushimi, National Institute of Animal Health, Tokyo, Japan. All strains were in freeze-dried form, and were reconstituted in chicken meat infusion broth before use.

***H. paragallinarum* locally isolated strains:**

Fifteen local isolates of *H. paragallinarum* have been isolated and identified after Page's scheme (1962) from outbreaks of the disease in layer chicken flocks (Aly, 2000), and were used in this study. Each of serovars A, B, and C of *H. paragallinarum* was represented by 5

isolates. These strains were designated as L1, L2, L12, L14, and L22 (serovar A); L5, L8, L13, L17 and L19 (serovar B); and L3, L6, L9, L10 and L21 (serovar C).

Commercial bivalent vaccine:

Inactivated bivalent commercial vaccine (*Rhone Merieux Comp.*) against infectious coryza potentiated with aluminum hydroxide containing serovars A and C of *H. paragallinarum* was used in this study.

Experimental chickens:

Ten-week-old male Hy-Line type chickens obtained from El-Menia Poultry Farm were used for evaluation of the efficacy of different coryza vaccines as well as for cross-protection studies. Chickens proved to be negative for *H. paragallinarum*, *Mycoplasma gallisepticum*, and *Mycoplasma synoviae* infection as a result of serological and cultural examinations.

Preparation of different infectious coryza vaccines:

A trivalent vaccine and monovalent vaccines against infectious coryza were prepared from local isolates according to the method described by Yamaguchi et al. (1991). Briefly, the strains of *H. paragallinarum* were cultured in chicken meat infusion (CMI) broth (Matsumoto and Yamamoto, 1971), containing 1 % (vol/vol) sterile chicken serum, incubated at 37 C for 18 hours and then inactivated by the addition of 0.01 % (vol/vol) thimerosal. Aluminum hydroxide gel was added (50 % vol/vol) as an adjuvant. The monovalent vaccines were prepared from each of the reference strains 221, Spross, and H-18 as well as from each of the 15 local isolates of *H. paragallinarum* in a concentration of 10^8 colony forming units (CFU)/ml. The trivalent vaccine contained three local isolates, which proved by pretesting to provoke relatively broader cross-protection with heterologous local isolates and reference strain of the same serovars. These isolates were L12 (serovar A), L13 (serovar B) and L9 (serovar C) of *H. paragallinarum*, each at a concentration of 10^8 CFU/ml. All vaccines were tested for their sterility, safety and stability and preserved at 4 C until use.

Vaccination procedure:

Each vaccine was administered to chickens by the intramuscular route (in the thigh) in a dose of 0.5 ml per bird. All vaccines were administered in two doses, the first dose at 10 weeks of age and the booster dose at 13 weeks of age. Vaccinated chickens were observed for one week post-vaccination for any adverse reaction after vaccination.

Serum samples:

Five serum samples were collected from each group vaccinated with either trivalent or bivalent vaccine weekly after the first vaccination up to 18 weeks of age and examined by the HI test. For cross-HI reactivity between reference serogroup strains of *H. paragallinarum*, five serum samples were collected from the respective vaccinated groups at 4 weeks after the booster vaccination.

Challenge exposure:

The challenge was done 4 weeks after the booster vaccination. Each of vaccinated or nonvaccinated challenged control birds was inoculated directly into the right infraorbital sinus with 200 µl of infected egg yolk containing 10^8 CFU/ml of virulent *H. paragallinarum* organisms.

Clinical observation post-challenge:

All challenged chickens were observed for typical clinical signs of infectious coryza (swollen sinuses and/or nasal discharge) for one week post-challenge. Birds were killed and necropsied 7 days after challenge. At necropsy, sinuses were examined carefully for presence of exudate and swabs from the sinuses were made.

Chickens were considered protected if they showed no signs or lesions of infectious coryza clinically or at necropsy and yielded no *H. paragallinarum* on culture. The protection rate of each vaccine group was calculated as the number of protected chickens in the group expressed as percentage relative to the number of chickens in the vaccinated group.

Bacterial reisolation:

Sinus swabs obtained from chickens were streaked on tryptose blood agar plates and cross-streaked with a feeder culture of *Staphylococcus epidermides*. Plates were incubated at 37 C for 24 hours in a Candle Jar, then examined for satellite growth and characteristic colonies of *H. paragallinarum*.

HA antigens:

The serovar-specific HA antigens were prepared from each reference strain of *H. paragallinarum* as described by Yamaguchi et al. (1990) and used for cross-HI reactivity studies between the three serovars.

For estimation of the seroconversion in groups vaccinated with trivalent or bivalent vaccine using HI test, the HA antigen was prepared from reference strain 221 of *H. paragallinarum*.

Hyaluronidase treatment of HA antigens:

The simple washed bacterial cells were treated with hyaluronidase after the method described by Yamaguchi et al. (1989). The adjusted bacterial cells were centrifuged, resuspended in an equal volume of hyaluronidase solution (50 units/ml) in PBS (pH 6.0), and incubated in a water bath at 37 C for 2 hours. After being washed twice with PBS (pH 7.4), the treated cells, termed hyaluronidase-treated HA antigen, were resuspended in the original volume of PBS and used in the HI test after adjustment to 4 HA unites.

Haemagglutination inhibition (HI) test:

The HI titers were determined in the serum samples as described by Yamaguchi et al. (1989). Twofold serial dilutions of sera (50 µl) were made in phosphate buffered saline (PBS; pH 7.2), using round bottom microtiter trays. An equal volume (50 µl) of hyaluronidase-treated HA antigen containing 4 HA units was added to each serum dilution. After mixing, the trays were held at room temperature for 10 minutes, an equal volume of 0.5 % formalinized chicken erythrocytes (50 µl) was added to each well. After incubation for 45 minutes at room temperature, the HI titer was determined as the reciprocal of the maximum serum dilution that completely inhibited haemagglutination. The formalinized chicken erythrocytes were prepared according to the method described by Jacobs et al. (1992).

Experimental design:

Cross-protection of *H. paragallinarum* strains within the same serovar:

Groups of 10-week-old male Hy-Line type chickens, of 10 birds each, were immunized twice at 10 and 13 weeks of age with each of the locally isolated strains (5 of each serovar) or the reference strains of *H. paragallinarum*. Four weeks after the booster vaccination, all birds were challenged with either the homologous or heterologous strains within the same serovar. A group of 10 birds served as nonvaccinated-nonchallenged control. All groups were observed for clinical signs and mortalities for one week post-challenge. After the end of the observation period, all birds were killed, necropsied and sinuses were examined.

Cross-protection between reference strains of different serovars of *H. paragallinarum:*

Ten groups (1-10) of 10-week-old male Hy-Line type chickens, each contained 10 birds were used in this experiment. Each group was vaccinated with one type of monovalent vaccines. Groups 1-3 were vaccinated with strain 221 vaccine, groups 4-6 were vaccinated with

strain Spross vaccine and groups 7-9 were vaccinated with strain H-18 vaccine. Each vaccine was administered in two doses at 10 and 13 weeks of age by intramuscular route. Group 10 served as nonvaccinated nonchallenged control. Four weeks following the booster vaccination, all vaccinated groups were challenged with virulent *H. paragallinarum* organisms as follows; groups 1, 4, and 7 were challenged with strain 221, groups 2, 5, and 8 were challenged with strain Spross and groups 3, 6, and 9 were challenged with strain H-18. All groups were observed for clinical signs and mortality for one week post-challenge. Serum samples were collected 4 weeks following the booster vaccination and subjected to cross-HI test using serovar specific HA antigens. One week post-challenge, all birds were killed, necropsied and sinuses were examined.

Evaluation of locally prepared trivalent coryza vaccine in comparison with commercial bivalent vaccine:

Ten groups (1-10) of 10-week-old male Hy-Line type chickens of 10 birds each, except groups 7-10, each contained 5 birds were used. Groups 1, 2, and 3 were vaccinated twice at 10 and 13 weeks of age with the trivalent vaccine containing highly immunogenic local strains L12, L13 and L9 representing *H. paragallinarum* serovars A, B and C, respectively. Groups 4-6 were vaccinated twice with the commercial bivalent vaccine. Groups 7, 8, and 9 served as nonvaccinated-challenged controls, while group 10 served as nonvaccinated- nonchallenged control. Four weeks following the booster vaccination, all groups, except group 10, were challenged as follows; groups 1 and 4 were challenged with strain 221, groups 2 and 5 with strain Spross and groups 3 and 6 with strain H-18. The control groups 7, 8, and 9 were challenged with strains 221, Spross, and H-18, respectively. All groups were observed for clinical signs and mortality for one week post-challenge. Serum samples were collected weekly post-vaccination and subjected to HI test. One week post-challenge, all birds were killed and necropsied and sinus swabs were obtained for reisolation of challenged organisms.

RESULTS

Cross-protection of *H. paragallinarum* strains within the same serovar:

Tables (1, 2 and 3) show the protection rates afforded by monovalent vaccines prepared from local and reference strains of *H. paragallinarum* after homologous and heterologous challenge with strains within the same serovar. All strains showed 100% protection

against homologous challenge, while variable protection rates were observed after heterologous challenge with strains belonging to the same serovar. Two locally isolated strains of serovar A (L12 and L22) and one strain of each serovar B (L 13) and C (L 9) were found highly immunogenic, where they induced the best protection against both homologous and heterologous challenge. The nonvaccinated-nonchallenged control group remained healthy during the experiment.

Cross-protection and cross-HI reactivity between serovars A, B, and C reference strains of *H. paragallinarum*:

As shown in Table (4), no cross-protection was found between serovars A, B, and C strains of *H. paragallinarum*. The monovalent vaccines induced variable protection (80-90%) in vaccinated chickens against homologous challenge but not against heterologous challenge. The nonvaccinated-nonchallenged control group remained healthy during the experiment.

The cross-HI test results (Table, 5) indicated that a very little cross-HI reactivity was found between serovar A and C strains, while no cross-HI reactivity was found between serovar B and either serovar A or C strain.

Efficacy of locally prepared trivalent vaccine in comparison with commercial bivalent vaccine:

As shown in Table (6), the protection rates afforded by the trivalent vaccine was 100 % against challenge with any of serovars A, B or C of *H. paragallinarum*. The commercial bivalent vaccine induced protection rates of 80 % and 70 % against challenge with strains 221 (serovar A) and H-18 (serovar C) respectively, while it failed to protect against challenge with strain Spross of serovar B (0.0 %). The HI response (Table, 7) could be detected in vaccinated groups two weeks after the first vaccination, increased at subsequent intervals, and peaked 3 weeks after the booster vaccination. A correlation was found between protection rates and the level of HI titers. The unprotected chickens showed typical signs of infectious coryza observed 2-5 days post-challenge and the challenging organisms could be reisolated.

DISCUSSION

Concerning the cross-protection studies, results indicated that the cross-protection between *H. paragallinarum* strains within the same serovar was variable after heterologous challenge. Some strains were weakly immunogenic and afforded relatively poor protection against

heterologous challenge. On the other hand, two strains of serovar A and one strain of either serovar B or C were found highly immunogenic against both homologous and heterologous challenge. These results are similar to those reported by Matsumoto and Yamamoto (1975), Rimler et al. (1977) and Yamaguchi et al. (1991) who demonstrated that vaccines prepared from different strains belonged to the same serovar of *H. paragallinarum* yielded only partial cross-protection among themselves. These results also confirmed the findings of Terzolo et al. (1997) and Sandoval et al. (1994) suggesting that vaccine failure occurred because the commercial vaccines, although they may contain different serovars, did not include local isolates of *H. paragallinarum* present in the target population.

Results of cross-protection test between serovars A, B and C reference strains of *H. paragallinarum* indicated that the monovalent vaccines conferred only type-specific immunity and therefore no cross-protection was found between the three serovars. Similarly, Rimler and Davis (1977) reported that Page's serovars A, B, and C represent three immunotypes. On the other hand, Sawata et al. (1980) reported that Page's serovar B strains only had common antigen of the species and were therefore considered variants of serovars A and C. Whatever the contradictory reports about the serovar B strains, the results reported here altogether with those reported by Yamaguchi et al. (1990) and Jacobs et al. (1992) confirmed that serovar B strains are truly a distinct serovar and are typically pathogenic for chickens.

The loss of cross-protection among different serovars of *H. paragallinarum* was accompanied with a very little cross-HI response existed only between serovars A and C strains, however it was very low to protect against challenge.

Most of commercial coryza vaccines are prepared from serovars A and C of *H. paragallinarum*. For this and because of isolation of serovar B strains from outbreaks of infectious coryza, it becomes essential to include the B serovar in vaccine preparation. Also the choice of a highly immunogenic strain from each serovar of *H. Paragallinarum* was essential in order to garantee full protection against the prevalent strains in the area where the vaccine is used.

In the present study, the efficacy of prepared trivalent coryza vaccine from the highly immunogenic previously isolated local strains representing the three serovars of *H. Paragallinarum* was evaluated and compared with a commercial bivalent vaccine. From our results, it is evident that the trivalent vaccine was the best in protection against

challenge (100%) with any of the three serovars of *H. Paragallinarum*. The commercial bivalent vaccine failed to protect against serovar B challenge and even the protection rates afforded against serovars A and C strains (80 % and 70 %, respectively) were lower than that induced by the trivalent vaccine. These results are similar to those reported by Jacobs et al. (1992), who found that a trivalent coryza vaccine induced a good protection against challenge with all serovars of *H. paragallinarum* even at 55 weeks post-vaccination in comparison with a bivalent vaccine. In the same manner, Terzolo et al. (1997) reported that all tested commercial bivalent vaccines containing serovars A and C did not protect against serovar B challenge.

Furthermore, the HI titers in chickens vaccinated with trivalent vaccine were higher than those induced by the bivalent vaccine. However, in both vaccines, the HI response detected at 2 weeks after the first vaccination and peaked at 2 weeks after the booster vaccination was correlated with the protection rates in all groups, which extended the results obtained by Otsuki and Iritani (1974); Yamaguchi et al. (1988) and Jacobs et al. (1992).

It could be concluded that coryza vaccines protect only against serovars used in vaccine preparation and the three serovars should be included. The choice of a strain representing each serovar of *H. paragallinarum* should be carefully selected, which may vary from area to another according to the immunogenicity of the strain and protection afforded against other strains within the same serovar.

REFERENCES

Aly, M. (2000): Characteristics and pathogenicity of Haemophilus paragallinarum isolates from Upper Egypt. Assiut Vet. Med. J. Vol. 43 No. 85: 319-338.

Blackall, P.J. (1999): Infectious coryza: overview of the disease and new diagnostic options. Clin. Microbiol., 12: 627-632.

Blackall, P.J. and Reid, G.G. (1987): Further efficacy studies on inactivated, aluminum-hydroxide-adsorbed vaccines against infectious coryza. Avian Dis., 31: 527-532.

Davis, R.B.; Rimler, R.B. and Shotts, E.B. (1976): Efficacy studies on *Haemophilus gallinarum* bacterin preparations. Am. J. Vet. Res., 37: 219-222.

*Jacobs, A. C.; Cuenen, W., and Storm, P. K. (1992):*Efficacy of a trivalent *Haemophilus paragallinarum* vaccine compared to bivalent vaccines. Vet. Microbiol., 32: 43-49.

Kume, K.; Sawata, A., and Nakase, Y. (1980): Relationship between protective activity and antigenic structure of *Haemophilus paragallinarum* serotypes 1 and 2. Am. J. Vet. Res., 41: 97-100.

Matsumoto, M. and Yamamoto, R. (1971): A broth bacterin against infectious coryza: Immunogenicity of various preparations. Avian Dis., 15: 109-117.

Matsumoto, M. and Yamamoto, R. (1975): Protective quality of an aluminum hydroxide-adsorbed broth bacterin against infectious coryza. Am. J. Vet. Res., 36: 579-582.

Otsuki, K. and Iritani, Y. (1974): Preparation and immunological response to a new mixed vaccine composed of inactivated Newcastle disease virus, inactivated infectious bronchitis virus, and inactivated *Haemophilus gallinarum*. Avian Dis., 18: 297-304.

Page, L.A. (1962): Haemophilus infection in chickens. 1. Characteristics of 12 *Haemophilus* isolates recovered from diseased chickens. Am. J. Vet. Res., 23: 85-95.

Reid, G.G. and Blackall, P.J. (1987): Comparison of adjuvants for an inactivated infectious coryza vaccine. Avian Dis., 31: 59-63.

Rimler, R.B. and Davis, R.B. (1977): Infectious coryza: in vivo growth of *Haemophilus gallinarum* as a determinant for cross-protection. Am. J. Vet. Res., 38: 1591-1593.

Rimler, R.B.; Davis, R.B. and Page, R.K. (1977): Infectious coryza: Cross-protection studies, using seven strains of *Haemophilus paragallinarum*. Am. J. Vet. Res., 38: 1587-1589.

Sandoval, V.E.; Terzolo, H.R. and Blackall, P.J. (1994): Complicated infectious coryza outbreaks in Argentina. Avian Dis., 38: 672-678.

Sawata. A.; Kume, K. and Nakase, Y. (1980): Biologic and serologic relationships between Page's and Sawata serotypes of *Haemophilus paragallinarum*. Am. J. Vet. Res., 41: 1901-1904.

Terzolo, H.R.; Sandoval, V.E. and Gonzalez, P. (1997): Evaluation of inactivated infectious coryza vaccines in chickens challenged by serovar B strains of *Haemophilus paragallinarum*. Avian Pathol., 26: 365-376.

Yamaguchi, T.; Blackall, P.J.; Takiami, S.; Iritani, Y., and Hayashi, Y. (1990): Pathogenicity and serovar-specific hemagglutinating antigens of *Haemophilus paragallinarum* serovar B strains. Avian Dis., 34:964-968.

Yamaguchi, T.; Blackall, P.J.; Takiami, S.; Iritani, Y. and Hayashi, Y. (1991): Immunogenicity of *Haemophilus paragallinarum* serovar B strains. Avian Dis., 35:965-968.

Yamaguchi, T.; Iritani, Y. and Hayashi, Y. (1988): Serological response of chickens either vaccinated or artificially infected with *Haemophilus paragallinarum*. Avian Dis., 32:308-312.

Yamaguchi, T.; Iritani, Y. and Hayashi, Y. (1989): Haemagglutinating activity and immunological properties of *Haemophilus paragallinarum* field isolates in Japan. Avian Dis., 33: 511-515.

Yamamoto, R. (1991): Infectious coryza. In: Diseases of Poultry, 9th ed. Calnek, B. W.; Barnes, H. J.; Beard, C. W.; Reid, W. M., and Yoder, Jr. (eds). Iowa State University Press, Ames, Iowa. pp. 186-195.

Table 1: Cross-protection between *H. paragallinarum* serovar A strains at 4 weeks post-vaccination.

Vaccine strain	Protection rate*					
	Challenge strain					
	L1	L2	L12	L14	L22	Strain 221
L1	100%	70%	90%	70%	80%	80%
L2	90%	100%	70%	80%	70%	70%
L12	100%	90%	100%	100%	100%	90%
L14	80%	80%	70%	100%	80%	80%
L22	90%	100%	100%	90%	100%	90%
Strain 221	90%	70%	80%	70%	90%	100%

* Calculated for groups of 10 chickens each vaccinated with 2 doses of inactivated strain/bird at 10 and 13 weeks of age.

Table 2: Cross-protection between *H. paragallinarum* serovar B strains at 4 weeks post-vaccination.

Vaccine strain	Protection rate*					
	Challenge strain					
	L5	L8	L13	L17	L19	Strain Spross
L5	100%	70%	90%	70%	80%	80%
L8	80%	100%	70%	80%	70%	70%
L13	100%	90%	100%	100%	100%	90%
L17	80%	80%	70%	100%	70%	80%
L19	90%	70%	70%	80%	100%	70%
Strain Spross	70%	70%	80%	70%	90%	100%

* Calculated for groups of 10 chickens each vaccinated with 2 doses of inactivated strain/bird at 10 and 13 weeks of age.

Table 3: Cross-protection between *H. paragallinarum* serovar C strains at 4 weeks post-vaccination.

Vaccine strain	Protection rate*					
	Challenge strain					
	L3	L6	L9	L10	L21	Strain H-18
L3	100%	70%	80%	70%	80%	80%
L6	80%	100%	70%	80%	70%	70%
L9	100%	90%	100%	100%	100%	90%
L10	70%	80%	70%	100%	80%	80%
L21	80%	80%	70%	90%	100%	80%
Strain H-18	80%	70%	80%	90%	70%	100%

* Calculated for groups of 10 chickens each vaccinated with 2 doses of inactivated strain/bird at 10 and 13 weeks of age.

Table 4: Cross-protection between *H. paragallinarum* reference strains of serovars A, B, and C.

Group	Vaccine Strain (serovar)	Challenge Strain (serovar)	No. of Diseased birds	% of protection
1	221 (A)	221 (A)	1/10 *	90
2	221 (A)	Spross (B)	10/10	0.0
3	221 (A)	H-18 (C)	10/10	0.0
4	Spross (B)	221 (A)	10/10	0.0
5	Spross (B)	Spross (B)	2/10	80
6	Spross (B)	H-18 (C)	10/10	0.0
7	H-18 (C)	221 (A)	10/10	0.0
8	H-18 (C)	Spross (B)	10/10	0.0
9	H-18 (C)	H-18 (C)	1/10	90
10	None	Nonchall.	0/10	-

* No. of birds with signs /Total No. of birds.

Table 5: Cross-HI reactivity between reference strains of serovars A, B, and C of *H. paragallinarum* at 4 weeks after the booster vaccination.

Antisera of Vaccinated groups	Serovar	Mean HI titers (n=5) Against different antigens		
		221	Spross	H-18
221	A	1024	0	8
Spross	B	0	256	0
H-18	C	8	0	512
Non-vacc. control	-	0	0	0