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# CHARACTERISTICS AND PATHOGENICITY OF *HAEMOPHILUS PARAGALLINARUM* ISOLATES FROM UPPER EGYPT

(With 7 Tables)

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## خصائص وضراوة عترات الهيموفيلاس باراجالينيرم المعزولة من مصر العليا

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تمت دراسة خصائص ٢٦ عترة من ميكروب الهيموفيلاس باراجالينيرم تم عزلها من ٣٦ وباء من مرض الزكام المعدى في قطعان الدجاج البياض في صعيد مصر خلال الفترة من ١٩٩٥ حتى ١٩٩٩. كل العترات المعزولة كانت مطابقة تماماً في الخواص البيوكيميائية لميكروب الهيموفيلاس باراجالينيرم. تباينت العترات المعزولة في حساسيتها للمضادات الحيوية المختلفة. لم تحدث العترات تلازن لكرات الدم الحمراء للدجاج الغير معاملة في حين أن ١٠٣ عترة أحدثت تلازن لكرات الدم الحمراء للدجاج المعاملة بالفورمالدهيد بدون معاملة أنتيجين تلازن الدم بواسطة أنزيم الهيالورينيديز. في حين أن عشرة عترات إحتاجت إلى معاملة بانزيم الهيالورينيديز حتى تحدث تلازن لكرات الدم الحمراء. تم تصنيف العترات سيرولوجيا بإستخدام إختبار تلازن الدم الإحباطى ومقارنته بإختبار التلازن السريع. وجد أن هناك تطابق بين الإختبارين بالنسبة لعدد ١٨ عترة حيث أن ٨ عترات تتبع صنف (أ) ، ٤ عترات تتبع الصنف (ب)، ٦ عترات تتبع الصنف (س) من الهيموفيلاس باراجالينيرم. أما باقى العترات الثمانية والتى لم يمكن تصنيفها بإختبار التلازن السريع فقد تم تصنيف خمسة عترات منها بواسطة إختبار تلازن الدم الإحباطى حيث أن عترتين تتبع الصنف (أ)، عترة واحدة تتبع الصنف (ب)، عترتين تتبع الصنف (س). وجد أيضا أن هناك ثلاثة عترات لم يتم تصنيفها بإستخدام الإختبارين السابقين. وبإستخدام الأجسام المناعية الأحادية ضد الصنف (أ) والصنف (س) أثبتت النتائج أن كل العترات التابع للنوع (ب) لم تتفاعل مع كلا من الأجسام المناعية الأحادية للنوعين (أ) و (س). وجد أيضا أن كل العترات التابعة للنوع (س) تفاعلت مع الأجسام المناعية الأحادية ضد النوع (س) ولكنها لم تتفاعل مع الأجسام المناعية الأحادية ضد النوع (أ). أما بالنسبة لعترات النوع (أ) وجد أن سبعة عترات فقط منها تفاعلت مع الأجسام المناعية الأحادية للنوع (أ) وجميعها لم تتفاعل مع الأجسام المناعية

الأحادية للنوع (أ) وجميعها لم تتفاعل مع الأجسام المناعية الأحادية للنوع (س). أما بالنسبة لضراوة العترات المعزولة وجد أنها جميعها ضارية بالنسبة لأجنة بيض الدجاج وكان المتوسط الزمنى لموت الأجنة يتراوح بين ١٤ و ٣٤ ساعة، في حين أن ٢٣ عترة منهم تباينت في ضراوتها للدجاج ووجد أن الثلاثة عترات المتبقية غير ضارية للدجاج.

## SUMMARY

The characteristics of 26 *Haemophilus paragallinarum* (*H. paragallinarum*) isolates recovered from 36 outbreaks of infectious coryza in layer chicken flocks in Upper Egypt during 1995-1999 were investigated. All isolates had the typical biochemical properties of *H. paragallinarum*. A variable susceptibility to different antimicrobial agents was existed among isolates. None of the isolates showed haemagglutination (HA) activity against fresh chicken erythrocytes, while 13 isolates had HA activity against formaldehyde-fixed erythrocytes without hyaluronidase treatment of the HA antigen. Ten isolates required hyaluronidase treatment of the HA antigen. Three isolates did not have a detectable HA activity despite hyaluronidase treatment. Isolates were serotyped according to the Page's scheme using haemagglutination inhibition (HI) test in comparison with plate agglutination method for serotyping. A complete correlation was found between the two tests for 18 isolates, 8 were serovar A, 4 were serovar B, and 6 were serovar C of *H. paragallinarum*. The remaining 8 isolates could not be serotyped by agglutination method but 5 of them could be serotyped by HI test, 2 as serovar A, 1 as serovar B, and 2 as serovar C. Three isolates were nonserotypeable by both methods. The use of monoclonal antibodies (MAbs) 4-D and 11-E (specific for serovar A) and F2E6 (specific for serovar C) indicated that all serovar B isolates did not react with either serovar A or C specific MAbs. All serovar C isolates reacted with serovar C MAbs but not with serovar A MAbs. Only 7 of 10 serovar A isolates could be recognized by serovar A MAbs. All isolates were pathogenic for chicken embryos with a mean death time (MDT) ranged from 14 to 34 hours, and 23 isolates of them varied in their virulence for chickens. Three isolates were found nonpathogenic for chickens.

***Key words: Haemophilus paragallinarum***

## INTRODUCTION

*Haemophilus paragallinarum* (*H. paragallinarum*) is the causative agent of infectious coryza, an acute upper respiratory disease of chickens. The disease can occur in both growing and layer chickens, causing an increased culling rate in meat type chickens and a reduction in egg production (10-40 %) in laying and breeder hens, particularly on multiple age farms (Yamamoto, 1991).

The most widely used serotyping scheme for *H. paragallinarum* is the Page's scheme (Page, 1962), who used agglutination test to recognize three serovars termed A, B, and C.

An alternative scheme was developed by Kume et al. (1983), which based on KSCN-extracted and sonicated antigens detected in haemagglutination inhibition (HI) test. This scheme recognized three serogroups termed I, II, and III and a total of seven serovars, termed HA-1 to HA-7.

On the other hand, Sawata et al. (1978) serotyped *H. paragallinarum* isolates using agglutination test into two serovars, designated 1 and 2. In a further study, Sawata et al. (1980) found that Sawata's serovars 1 and 2 were equivalent to Page's serovars A and C respectively. They also suggested that Page's serovar B strains lacked the serovar-specific agglutination antigen and they were non pathogenic for chickens, suggesting that they might be variants of serovars A and C and not a true serovar.

In contrast, Rimler et al. (1977) reported that serovar B strains 0222 and Spross were pathogenic for chickens and Thronton and Blackall (1984) found that strain 0222 produced serovar-specific agglutinating antibodies in hyperimmunized rabbits. In the same manner, Yamaguchi et al. (1990-a) showed that serovar B strains possess serovar-specific haemagglutinating antigen.

In Egypt, the disease still occur despite the use of infectious coryza vaccines. In previous studies, Zaki (1983) and Abd-Elmotelib (1985) found that all isolates of *H. paragallinarum* belonged to serovar A, wlile Aly (1987) found that all isolates belonged to serovar B.

The objectives of this study were to investigate the characteristics of 26 field isolates of *H. paragallinarum* recovered from recent outbreaks of infectious coryza in Upper Egypt. Also to investigate the Pathogenicity of these isolates for chicken embryos as well as for chickens.

## MATERIAL and METHODS

**History of outbreaks:**

A number of 36 outbreaks of infectious coryza were investigated during 1995-1999. These outbreaks were located in provinces of Beni-Suef (9 outbreaks), El-Menia (10 outbreaks), Assiut (11 outbreaks) and Sohag (6 outbreaks). All affected chicken flocks were subjected to clinical, postmortem and bacteriological examination. Data of these outbreaks including, age of affected flocks, morbidity, mortality, egg production and history of vaccination were collected.

***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.

**Monoclonal antibodies (MAbs):**

A panel of MAbs against *H. paragallinarum* were kindly supplied by the National Vet. Assay Lab., Ministry of Agriculture, Forestry and Fisheries, Tokura, Kokubunji, Tokyo, Japan. The MAbs 4-D and 11-E (specific for serovar A) and F2E6 (specific for serovar C) were preserved at –80 C until used in the HI test.

**Embryonated chicken eggs:**

Fertile chicken eggs were obtained from the Poultry Farm of the Fac. of Agriculture, Assiut University and incubated at 37 C until used at 6 days of age for propagation of *H. paragallinarum* strains as well as for testing the pathogenicity of field isolates.

**Experimental chickens:**

A number of 145 male Hy-Line chickens of 10 weeks old were kindly supplied by El-Menia Poultry Farm, and kept in isolation in the Dept. of Poultry Diseases, Fac. of Vet. Med, Assiut University. All birds were negative for *H. paragallinarum, Mycoplasma gallisepticum* and *Mycoplasma synoviae* organisms or their antibodies after cultural and serological examination. Birds were used for testing the pathogenicity of *H. paragallinarum* field isolates and reference strains.

**Culture media:**

**A) Chicken meat infusion broth (CMI):**

CMI broth supplemented with 0.5 % NaCl, 1 % polypeptone, 0.5% chicken serum and 0.01 % nicotinamide adenine dinucleotide (NAD) was prepared according to the method described by Matsumoto

and Yamamoto (1971). It was used for propagation of *H. paragallinarum* and preparation of antigens.

**B) Tryptose blood agar:**

Tryptose agar (Difco) supplemented with 5-10 % sterile sheep blood was used (Yamamoto, 1991).

**Bacterial isolation:**

Sinus swabs were collected from chickens suffering from infectious coryza and streaked onto tryptose blood agar plates containing inactivated chicken serum and then cross-streaked with a feeder culture of *Staphylococcus epidermides*. Plates were incubated at 37 C for 24 to 48 hours in a 5-10% CO2 atmosphere using a Candle Jar. *H. paragallinarum*-like colonies were selected and subcultured for purification, then Gram stain reaction and cell morphology were determined (Yamamoto, 1991).

**Growth characteristics and growth factor requirements:**

The satellitic growth phenomenon was tested using tryptose blood agar and a nurse colony of *Staphylococcus epidermides* (Yamamoto, 1991). The requirement for X and V-factors was tested using Oxoid discs on test medium agar according to the method described by Blackall and Farrah (1985).

**Preparation of *H. paragallinarum* antisera:**

Antisera against *H. paragallinarum* reference strains were prepared according to the method described by Kume et al. (1983). The antigens for rabbit inoculation were prepared and adjusted as previously described in HA antigens. A portion (1 ml) of a suspension containing equal volumes of the adjusted antigen and Freund's complete adjuvant was inoculated subcutaneously into rabbits on two occasions with an interval of 3 days. Subsequent injections were given intravenously at 3 days intervals and consisted of 0.5, 0.5, 1.0, 1.0, 2.0, 2.0, 4.0, and 4.0 ml of adjusted antigens. The rabbits were exanguinated 7 days after the last inoculation, blood was collected and serum was separated. Sera were stored at –20 C until used in HI and plate agglutination tests.

**Preparation of HA antigens:**

The HA antigens were prepared as described by Yamaguchi et al. (1990-a). Briefly, the bacterial cells were grown in CMI broth containing 1% (vol/:vol) sterile chicken serum at 37 C for 18 hours, collected by centrifugation at 8000 Xg for 20 minutes, washed twice in phosphate-buffered saline (PBS; pH 7.2) containing 0.01% (wt/:vol) thimerosal. The number of bacterial cells was adjusted to 10 times the optical

density of 1.0 at 660 nm (approximately 3 x $10^{10}$ cells/ml). The antigens were stored at 4 C until used. This referred to as untreated HA antigen.

**Hyaluronidase treatment of HA antigen:**

When no HA activity could be detected in the simple washed bacterial cells, the cells were treated with hyaluronidase as 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 antigen, were resuspended in the original volume of PBS.

**Preparation of agglutination antigen:**

The agglutinating antigen used in rapid plate agglutination test was prepared according to the method described by Sawata et al. (1978).

**Biochemical identification:**

Carbohydrate fermentation, catalase, indol and oxidase tests were performed on each isolate according to the method described by Blackall and Reid (1982) and Blackall (1983). For sugar fermentation tests, tubes of CMI broth containing phenol red supplemented with 1 % (wt/vol) of galactose, glucose, lactose, manitol, sorbitol, sucrose, maltose, arabinose, trehalose and Xylose were each inoculated with 100 µl of the overnight cultures. All tubes were incubated at 37 C and observed daily for up to 7 days for utilization of the carbohydrates.

**Formaldehyde-fixed chicken erythrocytes:**

Formalinized chicken erythrocytes were prepared by incubating chicken erythrocytes (washed twice in PBS) with PBS with 3 % formaldehyde for 18 hours. Subsequently, the erythrocytes were washed once in PBS and suspended in PBS to make 50 % suspension, which was diluted to 0.5 % just before use (Jacobs et al., 1992).

**Haemagglutination test:**

The HA activity of all isolates was determined as the method described by (Yamaguchi et al., 1989) using a microdilution method with fresh or formalinized chicken erythrocytes. For the isolates failed to induce HA activity, the hyaluronidase-treated HA antigen was used. Twofold dilutions of 50 µl of antigen were made with PBS (pH 7.2) and the same amounts of 0.5 % chicken erythrocytes were added to each dilution. After the HA plates were incubated for one hour at room temperature, the HA titer was determined as the reciprocal of the highest dilution that showed complete HA

**Haemagglutination inhibition (HI) test:**

The test was performed using rabbit raised antisera as well as MAbs specific for serovars A and C of *H. paragallinarum*. The test was done according to the method described by Yamaguchi et al. (1989). Twofold serial dilutions of the serum in PBS (pH 7.2) containing 0.1 % bovine serum albumin and 0.001 % (wt/vol) gelatin were placed in a round bottom microtiter trays. An equal volume of HA antigen containing 4 HA units as established in the HA test was added to each serum dilution, and the plate was then mixed on a shaker. After the mixture stood at room temperature for 10 minutes, an equal volume of 0.5 % chicken erythrocyte suspension was added to the mixture. The trays were held at room temperature for 45 minutes, and the HI titer was determined as the reciprocal of the maximum serum dilution that completely inhibited haemagglutination.

**In vitro sensitivity to antimicrobial agents:**

The antimicrobial susceptibility patterns were carried out on *H. paragallinarum* field isolates using 12 chemotherapeutic discs (Oxoid Basingstoke, England), including Enrofloxacin (5 µg), Danofloxacin (5 µg), Norfloxacin (10 µg), Amoxycillin (25 µg), Doxycycline hydrochloride (30 µg), Lincomycin (15 µg), Gentamicin (10 µg), Streptomycin (10 µg), Erythromycin (15 µg), Spectinomycin (10 µg), Oxytetracycline (30 ug), and Sulphamethoxazole/Trimethoprim (25 µg). Interpretation of the results was recorded according to Castle and Elstub (1971).

**Pathogenicity testing in chicken embryos:**

Each *H. paragallinarum* isolate was inoculated in 5 embryonated chicken eggs via yolk sac at 6 days of age. A group of 5 eggs served as noninfected control. All embryonated eggs were incubated at 37 C and candled daily. Dead embryos were kept at 4 C until examined. The mean death time (MDT) in chicken embryos was determined by dividing the summation of hours at which the embryos dead on the number of eggs per group.

**Pathogenicity testing in chickens:**

Each field isolate of *H. paragallinarum* was inoculated in 5 of 10-week-old chickens via intranasal route in a dose of 0.2 ml (containing $10^8$ CFU/ml) per bird. A group of 5 chickens served as noninfected control. All groups were kept in isolation and observed daily post-infection for clinical signs and mortality. Reisolation from infected birds was done by culturing of sinus swabs on tryptose blood agar from all groups one week post-infection.

## RESULTS

**Epidemiological features of outbreaks:**

Data concerning the investigated outbreaks of infectious coryza are summarized in Table (1). In all outbreaks, the disease started acute (facial swelling) and persisted up to 4-6 weeks. All affected flocks showed drop in egg production ranged from 15 to 35 % and in some flocks, the expected maximum standard egg production was not reached, and afterward lay was irregular for long period. Most of outbreaks occurred in winter season and at beginning of egg season. Out of the 36 affected flocks, 23 flocks had a history of vaccination with commercial infectious coryza vaccines.

**Recovery of *H. paragallinarum* isolates:**

Table (1) shows that 26 field isolates of *H. paragallinarum* could be recovered from 36 outbreaks of infectious coryza. These isolates were 7 from Beni-Suef, 8 from El-Menia, 7 from Assiut and 4 from Sohag provinces. All isolates were identified biochemically and serologically as *H. paragallinarum.*

**Biochemical and physiological characterization:**

All isolates were gram-negative, pleomorphic coccobacilli or rods that were non-motile. All isolates were identical to the *H. paragallinarum* reference strains in that they were catalase-negative, failed to produce indol, had oxidase activity and reduced nitrate. They fermented glucose, maltose and sucrose, but failed to ferment arabinose, galactose, lactose, xylose, or trehalose. Isolates varied in fermentation of sorbitol and mannitol (Table 2). All isolates required V-factor except 2 isolates were found V-factor-independent, and all did not require X-factor for their growth. The organisms showed a satellitic phenomenon on tryptose blood agar with the use of a nurse colony of *Staphylococcus epidermides.*

**HA activity:**

As shown in Table (3), none of the isolates had HA activity against fresh chicken erythrocytes, while 13 isolates had HA activity against only formaldehyde-fixed chicken erythrocytes using untreated HA antigen, and 10 isolates had HA activity only after hyaluronidase treatment of HA antigen and formaldehyde treatment of chicken erythrocytes. Three isolates lacked detectable HA activity, even after hyaluronidase treatment of HA antigen and use of formaldehyde-fixed chicken erythrocytes. All reference strains of *H. paragallinarum* showed

HA activity against formaldehyde-fixed chicken erythrocytes except strain 221 showed HA activity against fresh chicken erythrocytes with the use of hyaluronidase-treated HA antigen. The HA activity was increased with hyaluronidase treatment of HA antigen.

**Serotyping:**

Results of serotyping (Table, 4), indicated that there was a complete agreement between HI and plate agglutination tests for 18 isolates which serotyped as serovar A (8 isolates), serovar B (4 isolates) and serovar C (6 isolates) of *H. paragallinarum*. Of the 8 isolates, which could not be serotyped by plate agglutination method, 5 of them could be serotyped by the HI test as serovar A (2 isolates), serovar B (1 isolate), and serovar C (2 isolates). Three isolates were untypable by both methods.

**HI assay with MAbs:**

As shown in Table (5), all serovar B isolates did not react with either serovar A or C MAbs. All serovar C isolates reacted with serovar C specific MAbs but not with serovar A MAbs. For serovar A isolates, only 7 isolates could be recognized by serovar A specific MAbs and all the 10 serovar A isolates did not react with serovar C specific MAbs. The three untypable isolates could not be recognized by any of the MAbs used.

**Pathogenicity for chicken embryos:**

All field isolates of *H. paragallinarum* were found pathogenic for chicken embryos when inoculated at 6 days of age. Dead embryos showed a severe congestion and presence of peticheal haemorrhages on the extremities. Oedema was observed in some embryos especially with highly virulent isolates. The MDT (table, 6) in chicken embryos ranged from 14 to 34 hours. All reference strains of *H. paragallinarum* were pathogenic for chicken embryos with a MDT of 15, 18 and 17 hours of strains 221, Spross and H-18, respectively.

**Pathogenicity testing in chickens:**

As shown in Table (6), isolates of *H. paragallinarum* varied in their pathogenicity to chickens from low (4 isolates), to high (13 isolates). Six isolates were moderate in their pathogenicity. The highly virulent isolates induced severe clinical signs typical to infectious coryza observed as facial swelling and profuse nasal discharges which started serous and became tenacious by time. In moderately virulent isolates the signs were as that observed in highly virulent isolates but less severe. Isolates with low virulence induced mild signs observed as slight

swelling of the infraorbital sinuses and nasal discharges. Three isolates (untypable) were found nonpathogenic for chickens.

**Reisolation:**

All pathogenic *H. paragallinarum* isolates were isolated at 7 days post-infection from all affected chickens (table, 6).

**In vitro sensitivity to antimicrobial agents:**

As shown in Table (7), the in vitro sensitivity to different antimicrobial agents were variable among 26 field isolates of *H. paragallinarum*. All isolates were highly susceptible to Fluoroquinolones, Gentamicin, streptomycin and spectinomycin. Other antimicrobial agents were variable in their efficiency among isolates.

## DISCUSSION

The present study appears to represent the first extensive characterization of *H. paragallinarum* field isolates in the last years in Upper Egypt. The characterization of *H. paragallinarum* isolated from field from time to time provide an important base of knowledge for the recent isolates and developing programs for the prevention and control of infectious coryza (Yamamoto, 1991).

Twenty-six isolates of *H. paragallinarum* recovered from 36 outbreaks of infectious coryza during 1995-1999 in layer chicken flocks were investigated in this study. The affected flocks suffered from severe economic losses, mainly due to drop in egg production (15-35 %) as well as from increased mortalities. The most important data for these flocks was that despite vaccination of 23 flocks against infectious coryza, using commercial vaccines, outbreaks were occurred and *H. paragallinarum* could be isolated. This indicated that these types of vaccines may not be providing the appropriate coverage for the types of *H. paragallinarum* that infect local chicken flocks. Rimler et al. (1977) and Kume et al. (1980) reported that coryza vaccines provided protection only against those serovars included in the vaccine.

As reported here, the biochemical and physiological properties of *H. paragallinarum* isolates from Upper Egypt were broadly similar to the characterization studies performed in other countries (Page, 1962; Hinze, 1973; Blackall and Reid, 1982 and Terzolo et al., 1993). All isolates were identical to *H. paragallinarum* reference strains in their biochemical properties except for sorbitol and mannitol fermentations which were variable among field isolates. Two isolates were only found to be NAD-independent. The NAD-independent isolates of *H.*

*paragallinarum* were isolated previously in other countries. Bragg et al. (1997), in South Africa, reported on isolation of NAD-independent isolates belonged to serovars A, B, and C of *H. paragallinarum*.

One of the most important properties of *H. paragallinarum* is the HA activity which was first reported by Kato et al. (1965). Results of the present study indicated that none of the isolates showed HA activity against fresh chicken erythrocytes but some of them showed this activity against only formaldehyde-treated erythrocytes. All isolates showed HA activity after hyaluronidase treatment of HA antigen with increasing in the HA titers. On the other hand three isolates had no HA activity despite hyaluronidase treatment of antigen.

The presence of HA activity in many isolates representing *H. paragallinarum* serovars A, B, and C, as reported here, without any need for enzyme treatment or sonication of *H. paragallinarum* cells is in contrast with results of Sawata et al. (1980) who reported that the HA activity in simple washed bacterial cells was present only in organisms of serovar A of *H. paragallinarum*. However, Yamaguchi et al. (1989) showed that hyaluronidase-treated *H. paragallinarum* cells of serovars A and C possessed HA activity against formaldehyde-fixed chicken erythrocytes.

According to the Page's scheme for serotyping, results of HI serotyping in comparison to the agglutination method indicated that there was a complete correlation between the two tests for serotyping of 18 isolates assigned to serovar A, B or C of *H. paragallinarum*. On the other hand, five isolates could not be serotyped by agglutination method but could be serotyped by HI test. Three isolates were untypable by both tests. The ability of HI serotyping method to serotype isolates that agglutination method failed, confirmed the findings of Eaves et al. (1989) and Yamaguchi et al. (1989) that HI test is considered a convenient method for serotyping *H. paragallinarum* that it overcomes the problem of nontypable isolates when agglutination test is used. In addition, these results support the findings reported by Blackall et al. (1991) and Yamaguchi et al. (1990-a) that *H. paragallinarum* serovar B isolates have a serovar-specific antigen and are considered a true serovar. In contrast, Sawata et al. (1980) failed to detect serovar-specific agglutination antigen in serovar B strains.

As expected, all isolates of serovar B did not react with either serovar A or C MAbs in HI test. All serovar C isolates reacted with serovar C specific MAbs but not with serovar A specific MAbs. The unexpected result was that 7 isolates only of the 10 serovar A isolates

reacted with serovar A specific MAbs. These results confirmed that MAbs represent an attractive alternative approach in serotyping and determination of antigenic variation among *H. paragallinarum* isolates (Yamaguchi et al., 1990-a; Blackall et al.,1991, and Zhang et al., 1999). However, the failure of some isolates of serovar A to be recognized by serovar A specific MAbs indicated that these isolates may be antigenically distinct from those found in other countries (Rimler et al., 1977).

All these findings mirror the situation of *H. paragallinarum* in Upper Egypt, where all serovars have been demonstrated.

The variation in the antimicrobial susceptibility patterns among *H. paragallinarum* isolates may be due to the improper use of antimicrobial agents resulting in development of drug resistance.

Regarding the pathogenicity of *H. paragallinarum* isolates, a correlation was found between the MDT in chicken embryos and virulence in chickens. Interestingly, the three nonpathogenic isolates for chickens were those lacked the HA activity, suggesting that HA antigen may be one of virulence factors of *H. paragallinarum* and may be involved in attachment process, (Yamaguchi et al., 1990-b). Results also confirmed that serovar B isolates were pathogenic for chickens that are in agreement with Rimler et al. (1977) but in contrast with the results obtained by Sawata et al. (1980) who reported that serovar B strains were nonpathogenic for chickens.

This study concluded that the three Page's serovars of *H. paragallinarum* have been recognized and responsible for recent outbreaks of infectious coryza and serovar A was the most prevalent, suggesting that coryza vaccines should contain all *H. paragallinarum* serovars. Lastly, the HI test with polyclonal or MAbs is more reliable than agglutination test for serotyping of *H. paragallinarum*.

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Table (1): Data concerning 36 outbreaks of infectious coryza and recovery of 26 H. paragallinarum isolates.

| Province | No. of outbreaks | No. of [a] Vaccinated Flocks | Age in weeks | % of morbidity | % of [b] mortality | Drop in egg prod. | No. of recovered isolates |
|---|---|---|---|---|---|---|---|
| Beni-Suef | 9 | 4 | 20-60 | 40-50 | 1.5-2.5% | 15-30% | 7 |
| El-Menia | 10 | 8 | 15-40 | 25-35 | 1.1-2% | 20-30% | 8 |
| Assiut | 11 | 7 | 10-50 | 20-40 | 1.2-2.5% | 15-35% | 7 |
| Sohag | 6 | 4 | 20-70 | 15-45 | 1-2.3% | 15-25 | 4 |
| Total | 36 | 23 | | | | | 26 |

a) Chickens vaccinated twice with commercial inactivated coryza vaccines.
b) Cumulative mortality during the course of outbreak (4-6 weeks).

Table (2): Biochemical properties of field isolates and reference strains of H. paragallinarum.

| Characteristics | H. paragallinarum | |
|---|---|---|
| | Reference strains [a] | Field isolates [b] |
| Catalase | -ve | -ve |
| Oxidase | +ve | +ve |
| Nitrate | +ve | +ve |
| Indol | -ve | -ve |
| Acid from: | | |
| Glucose | +ve | +ve |
| Mannitol | +ve | V [c] (+ 21) |
| Arabinose | -ve | -ve |
| Galactose | -ve | -ve |
| Lactose | -ve | -ve |
| Sucrose | +ve | +ve |
| Sorbitol | -ve | V (+ 4) |
| Trehalose | -ve | -ve |
| Maltose | +ve | +ve |
| Xylose | -ve | -ve |
| V-factor | dependent | 24 (dependent)<br>2 (independent) |
| X-factor | independent | independent |
| Motility | nonmotile | nonmotile |

a) Reference strains 221, Spross and H-18 of H. paragallinarum.
b) Numbers in parentheses are the numbers of field isolates positive.
c) V= variable reaction.

Table (3): HA activity of H. paragallinarum field isolates and reference strains.

| Isolates | Serovar | HA titers | | | |
|---|---|---|---|---|---|
| | | Untreated HA antigen | | Treated HA antigen | |
| | | Fresh Erythrocytes | Fixed erythrocytes | Fresh erythrocytes | Fixed erythrocytes |
| L1 | A | < 2 | 16 | < 2 | 64 |
| L2 | A | < 2 | < 2 | < 2 | 32 |
| L3 | C | < 2 | 16 | < 2 | 64 |
| L4 | A | < 2 | 32 | < 2 | 128 |
| L5 | B | < 2 | < 2 | < 2 | 16 |
| L6 | C | < 2 | < 2 | < 2 | 16 |
| L7 | Untypable | < 2 | < 2 | < 2 | < 2 |
| L8 | B | < 2 | 8 | < 2 | 32 |
| L9 | C | < 2 | 16 | < 2 | 64 |
| L10 | C | < 2 | < 2 | < 2 | 32 |
| L11 | C | < 2 | < 2 | < 2 | 16 |
| L12 | A | < 2 | 32 | < 2 | 128 |
| L13 | B | < 2 | < 2 | < 2 | 8 |
| L14 | A | < 2 | 16 | < 2 | 32 |
| L15 | Untypable | < 2 | < 2 | < 2 | < 2 |
| L16 | C | < 2 | 8 | < 2 | 32 |
| L17 | B | < 2 | < 2 | < 2 | 8 |
| L18 | A | < 2 | < 2 | < 2 | 32 |
| L19 | B | < 2 | 16 | < 2 | 64 |
| L20 | A | < 2 | 16 | < 2 | 128 |
| L21 | C | < 2 | < 2 | < 2 | 16 |
| L22 | A | < 2 | 64 | < 2 | 128 |
| L23 | C | < 2 | < 2 | < 2 | 8 |
| L24 | A | < 2 | 32 | < 2 | 64 |
| L25 | Untypable | < 2 | < 2 | < 2 | < 2 |
| L26 | A | < 2 | 16 | < 2 | 128 |
| Strain 221 | A | < 2 | 128 | 16 | 256 |
| Strain Spross | B | < 2 | 8 | < 2 | 32 |
| Strain H-18 | C | < 2 | 16 | < 2 | 64 |

Table (4): Comparison of serotyping of H. paragallinarum isolates according to the Page's scheme by agglutination and HI tests.

| Agglutination serovar | HI serovar | | | |
|---|---|---|---|---|
| | A | B | C | NT |
| A | 8 | - | - | - |
| B | - | 4 | - | - |
| C | - | - | 6 | - |
| NT | 2 | 1 | 2 | 3 |
| Total | 10 | 5 | 8 | 3 |

NT = Nontypable.

Table (5): HI test of H. paragallinarum isolates using serovars A and C MAbs.

| Serovar | No. of isolates | HI activity | |
|---|---|---|---|
| | | Serovar A MAbs | Serovar C MAbs |
| A | 10 | 7/10* | 0/10* |
| B | 5 | 0/5 | 0/5 |
| C | 8 | 0/8 | 8/8 |
| NT | 3 | 0/3 | 0/3 |

*No. of isolates reacted with MAbs / Total No. of isolates.
NT = Nontypable.

Table (6): MDT in chicken embryos and Pathogenicity of H. paragallinarum isolates for chickens.

| Isolates | Serovar | MDT in Chicken embryos | No. of Diseased birds | Clinical signs | Reisolation | % of infection |
|---|---|---|---|---|---|---|
| L1 | A | 21 | 5/5 * | Severe | 5/5 * | 100% |
| L2 | A | 30 | 2/5 | Mild | 2/5 | 40% |
| L3 | C | 19 | 5/5 | Severe | 5/5 | 100% |
| L4 | A | 18 | 4/5 | Moderate | 4/5 | 80% |
| L5 | B | 20 | 5/5 | Severe | 5/5 | 100% |
| L6 | C | 30 | 3/5 | Mild | 3/5 | 60% |
| L7 | Untypable | 32 | 0/5 | - | 0/5 | 0.0% |
| L8 | B | 28 | 5/5 | Moderate | 5/5 | 100% |
| L9 | C | 25 | 5/5 | Severe | 5/5 | 100% |
| L10 | C | 17 | 5/5 | Severe | 5/5 | 100% |
| L11 | C | 23 | 5/5 | Severe | 5/5 | 100% |
| L12 | A | 16 | 5/5 | Severe | 5/5 | 100% |
| L13 | B | 31 | 4/5 | Moderate | 4/5 | 80% |
| L14 | A | 32 | 3/5 | Mild | 3/5 | 60% |
| L15 | Untypable | 34 | 0/5 | - | 0/5 | 0.0% |
| L16 | C | 21 | 5/5 | Severe | 5/5 | 100% |
| L17 | B | 14 | 5/5 | Severe | 5/5 | 80% |
| L18 | A | 20 | 5/5 | Severe | 5/5 | 100% |
| L19 | B | 26 | 4/5 | Severe | 4/5 | 80% |
| L20 | A | 25 | 3/5 | Moderate | 3/5 | 60% |
| L21 | C | 23 | 3/5 | Mild | 3/5 | 60% |
| L22 | A | 17 | 5/5 | Severe | 5/5 | 100% |
| L23 | C | 20 | 4/5 | Severe | 4/5 | 80% |
| L24 | A | 19 | 4/5 | Moderate | 4/5 | 80% |
| L25 | Untypable | 26 | 0/5 | - | 0/5 | 0.0% |
| L26 | A | 28 | 4/5 | Moderate | 4/5 | 80% |
| Strain 221 | A | 15 | 5/5 | Severe | 5/5 | 100% |
| Strain Spross | B | 18 | 5/5 | Severe | 5/5 | 100% |
| Strain H-18 | C | 17 | 5/5 | Severe | 5/5 | 100% |

* = No. of diseased birds or positive in reisolation / Total No. of birds.

Table (7): In vitro sensitivity of 26 H. paragallinarum isolates to different antimicrobial agents.

| Antibiotics | Sensitive | | Moderate | | Resistant | |
|---|---|---|---|---|---|---|
| | No. of isolates | % | No. of isolates | % | No. of isolates | % |
| Enrofloxacin | 24 | 92.3 | 2 | 7.7 | - | 0.0 |
| Danofloxacin | 22 | 84.6 | 4 | 15.4 | - | 0.0 |
| Norfloxacin | 20 | 76.9 | 6 | 23.1 | - | 0.0 |
| Doxycyclin | 12 | 46.2 | 9 | 34.6 | 5 | 19.2 |
| Gentamicin | 23 | 88.5 | 3 | 11.5 | - | 0.0 |
| Lincomycin | 4 | 15.4 | 7 | 26.9 | 15 | 57.7 |
| Spectinomycin | 22 | 84.6 | 4 | 15.4 | - | 0.0 |
| Streptomycin | 18 | 69.2 | 8 | 30.8 | - | 0.0 |
| Oxytetracycline | 7 | 26.9 | 11 | 42.3 | 8 | 30.8 |
| Erythromycin | 9 | 34.6 | 7 | 26.9 | 10 | 38.5 |
| Amoxycillin | 6 | 23.1 | 11 | 42.3 | 9 | 34.6 |
| Sulphamethoxazole/ Trimethoprim | 13 | 50 | 10 | 38.5 | 3 | 11.5 |