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# COMPLICATED INFECTIOUS CORYZA IN BROILER AND LAYER CHICKENS IN UPPER EGYPT

(With 4 Tables)

By

***R.S. IBRAHIM; S. MOUSA; M. ALY and WAFAA EID ABDEL-NASER****

* M.V.Sc., Vet. Medicine Section, Assiut Governorate

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## مرض الزكام المعدى المعقد فى بدارى التسمين والدجاج البياض فى محافظات صعيد مصر

*رجب سيد ابراهيم ، صلاح عبد الرحمن موسى ، محمد محمود احمد على وفاء عيد عبد الناصر*

تمت دراسة مدى انتشار مرض الزكام المعدى المعقد فى بدارى التسمين والدجاج البياض فى محافظات صعيد مصر. فى حالة بدارى التسمين ظهرت الاعراض على شكل اعراض تنفسية شديدة وسجلت نفس الاعراض فى الدجاج البياض بالاضافه الى انخفاض معدل انتاج البيض حتى٤٠%. تراوحت نسبة الاصابة فى كلا من بدارى التسمين والدجاج البياض بين 10-30% & 60 -30 % على التوالى، كما تراوح معدل النفوق من 0.5-2% & 1-10% على التوالى. اتسعت الفترة الزمنية لمسار المرض فى الدجاج البياض من 6-3 اسابيع ومن 4-2 اسابيع فى بدارى التسمين. اظهر الفحص التشريحى المرضى وجود التهابات فى الجيوب الانفية والجهاز التنفسى العلوى بالاضافه الى التهابات فى الاغشية السيروزية والاكياس الهوائيه مع التهابات قناة البيض والمبيض. تم اختيار عدد 22 عترة مورفولوجيا لدراسة الاختبارات البيوكيميائية والسيرولوجية والبيولوجية. أظهرت 15 عترة خاصية تلازن الدم مع الخلايا السابق معاملتها بالفورمالين. أثبتت الاختبارات السيرولوجية وجود الانواع A &B & C بالترتيب بينما لم يتم التعرف على بعض العترات. تم عمل اختبار الضراوة فى أجنة البيض والدجاج. وعند محاولة عزل الميكروبات المشاركة فى مرض الزكام المعدى المعقد تم التعرف على عدة ميكروبات مثل الميكروب القولونى بنسبة 29% & الكلبسيللا بنسبة 8.2% والسيودوموناس بنسبة 10% والبروتياس بنسبة 11% والميكوبلازما جالبسيتكم بنسبة 25%. وأخيرا تم احداث العدوى الصناعية بميكروب هيموفيلاس باراجالينيرم على حدة ثم مع الميكروب القولونى ثم مع الميكوبلازما جالبسيتكم ثم تحت ظروف بيئية وصحية سيئة. كانت النتائج حدوث اعراض الزكام المعدى البسيط مع ميكروب هيموفيلاس باراجالينيرم على حدة بينما تشابهت الاعراض والآفات التشريحية فى

الحـــالات الأخـــرى علـــى شكل الزكام المعدى المعقد مما يدلل على اشتراك عدة انواع من البكتـــيريا مع الهيموفيلاس بارا جالينيرم فى تفاقم الاصابة وزيادة حدة المرض بالاضافة الى الخسائر الاقتصادية الناجمة عنه.

## SUMMARY

Screening the prevalence of complicated infectious coryza in broiler and layer chickens in Upper Egypt was tried. The examined broiler chickens showed severe respiratory signs. Similar clinical signs were observed in layer chickens in addition to decreased egg production (3-40%). The morbidity rate was (10-30%) and (30-60%) in broiler and layer chickens respectively, while mortality rate was (0.5-2%) and (1-10%) in broiler and layer chickens respectively. The course of the disease was prolonged in layers (3-6 weeks) while it was ranged from 2-4 weeks in broilers. Post mortem examination revealed conjunctivitis, infra-orpital sinusitis, tracheitis, air sacculitis, fibrinous pericarditis, peritonitis and enteritis. In layers salbingitis and oophoritis were observed. Twenty-two morphologically selected isolates were applied for biochemical, serological and biological investigations. Fiveteen out of 22 isolates had haemagglutination activity against only formaldehyde-fixed chicken erythrocytes. Serological testing proved the presence of *Haemohpilus paragallinarum* serotypes A, C and B in order. Pathogenicity testing of identified isolates were carried-out in embryonating chicken eggs and layer chickens. *E. coli* (29%), *Klebsiella species* (8.2%), *Pseudomonas species* (10%)and *Proteus species* (11%) were recovered in association with *Haemohpilus paragallinarum*. *Mycoplasma gallisepticum* was also isolated from cases of chronic or complicated coryza (25%). Induction of complicated coryza was done. The group, which infected with *Haemophilus paragallinarum*, only displayed signs of simple coryza in addition to decreased egg production within 24-72 hours, while the course of the disease extended between 7-10 days. The group, which infected with *Haemophilus paragallinarum* and *Mycoplasma gallisepticum* showed severe clinical signs (coryza, swollen wattles, dyspenea, rales and decreased body weight) after 24 hours and the egg production decreased to 40-50%. Post mortem lesions were sinusitis, tracheitis, air sacculitis, fibrinous pericarditis, peritonitis, salbingitis, presence of caseated materials in the oviduct and oophoritis. While the group, which was infected with *Haemophilus paragallinarum* and *E. coli* and the group, which was infected with *Haemophilus*

*paragallinarum* and kept under bad hygienic conditions showed a similar clinical and post mortem picture to the last mentioned group.

***Key words:*** *Haemohpilus paragallinarum, complicated coryza, chickens, decrease in egg production.*

## INTRODUCTION

Infectious coryza is an acute respiratory disease of chickens. The clinical syndrome has been recognized since the 1930s (Blackall *et al.*, 1997). The disease occurs worldwide and causes economic losses due to increased number of culls and a marked drop of egg production, particularly on multi-age farms. Early workers identified the causative agent as "*Haemohpilus gallinarum*," an organism that required both X (hemin) and V (NAD) factors for growth in vitro. However, from the 1960s to the 1980s, all isolates of the disease producing-agent have been shown to require only V factor and have been termed *Haemohpilus paragallinarum* (Blackall *et al.*, 1997). V-factor-independent Haemohpilus paragallinarum isolates have been encountered in the Republic of South Africa since 1989 (Mouahid *et al.*, 1992). Thus, the causative agent of infectious coryza is regarded as *Haemohpilus paragallinarum*, an organism that can be either V-factor dependent or independent (Blackall *et al.*, 1999). This work was done to study the complex nature of infectious coryza outbreaks in area of Upper Egypt, where other disease agents and/or stress factors are important complicating factors.

## MATERIALS and METHODS

**Screening the prevalence of complicated infectious coryza in chickens:**

A total of 205 broilers and 162 layers chicken both alive and freshly dead of different ages and breeds suspected to be suffering from complicated coryza were obtained from different farms at Beni-suif, El-menia, Assiut and Sohag Governorates. Samples were subjected to clinical, necropsy and bacteriological examinations.

**Standard strains and antisera:**

*Standard strains of Haemophilus paragallinarum* {strain 221, Spross and H-18} representing serovars A, B, and C respectively.

*E. coli reference antisera* were produced by Behring Werka, Marbuge, Iham, Germany. Sera were supplied as two sets of polyvalent sera. I

(anti-OK-B-26, 55, 78, 86, 111, 114 and 129) and II (anti-OK-B-124, 126, 127 and 128).

*Reference Mycoplasma gallisepticum (M.G.) antisera and liposomal antigens* for RPA testing were kindly supplied by Dr. S.H.Kleven, Poultry Dis. Res. Center, Georgia University, Athens, Georgia, U.S.A.).

**Isolation and identification of *Haemophilus paragallinarum*:**

Isolation was done from each organ (different parenchymatous organs, ovary, oviduct, spleen, intestine, respiratory passages and air sacs) and deeply from infraorpital sinus or by squeezing nasal cleft after surface sterilization by 70% alcohol. The swabs were streaked on nutrient blood agar or trypticase blood agar (5% sheep blood), which were then cross-streaked with *Staph epidermidis*. Blood agar supplemented with 2.5 ug/ml of reduced form of nicotinamide adenine dinucleotide (NADH) as V factor was also used for isolation. The seeded plates were incubated at 37C for 24-48 hours under 10% CO2 tension using candle jar (Page 1962).

Identification tools included colonial, cellular morphology, motility on semisolid agar, biochemical testing and sugar fermentation. Catalase, indol production, urease, nitrate reduction, haemolysis on blood agar and haemagglutination tests were carried out according to Page, 1962; Kume *et al.*, 1978 and Hinz, 1980. Sugar fermentation test was done for glucose, lactose, mannose, arabinose, dextrin, galactose, sorbitol, salicin and mannitol using phenol red broth base containing 1% NaCl, 0.0025% NADH, 1% chicken serum and supplemented by aforementioned sugars in concentration of 0.5% according to Cruichshank *et al.*, 1975.

*Haemagglutination (HA) test*: HA activity of suspected isolates was determined as method described by Yamaguchi *et al.*, 1989 using a microdilution method with fresh or formalinized chicken erythrocytes.

**Serology:**

*Rapid plate agglutination test*: This was carried out by using standard antiserum diluted as 1:5 in phosphate buffered saline (PBS) as described by Kume *et al.*, 1978.

*Haemagglutination inhibition (HI) test:* Two-fold serial dilutions of the serum in PBS (PH, 7.2) containing 0.1% bovine serum albumin (BSA) and 0.001% (w/v) gelatin were placed in a round micro-titer trays. An equal volume of HA antigen containing 4 HA unites followed by shacking for 10 minutes at room temperature. An equal volume of 0.05% chicken RBCs suspension was added. Incubated at room temperature for 45 minutes, HA titer was determined as the reciprocal of

the maximum serum dilution that completely inhibited HA (Yamaguchi *et al.*, 1989).

**Pathogenicity tests for *Haemophilus paragallinarum*:**

*Chicken embryo*: Six-day-old embryonating chicken eggs were used for propagation and testing pathogenicity of isolated *Haemophilus paragallinarum* according to Kume *et al.*, 1978. Eggs were obtained from breeding flock proved to be free from *Haemophilus paragallinarum* and their specific antibodies through cultural and serological examination. Each egg was inoculated with 0.1 ml of 24 hours broth culture containing $10^8$ CFU/ml via yolk sac route. Control was inoculated with 0.1 ml of sterile broth. All inoculated eggs were incubated at 37C in humid conditions and candled twice daily for two days. Deaths were recorded and the yolk materials were harvested.

*Chickens*: Two hundred and thirty, 65-week-old chickens proved to be free from *Haemophilus paragallinarum* and their specific antibodies through cultural and serological examination were used for pathogenicity testing of isolated *Haemophillus* isolates. Birds were divided into 24 equal groups of 10 birds each. Each isolate was inoculated intranasally in 5 birds and itrasinuslly in other 5 birds. The inoculum was 0.2 ml of broth culture containing $10^8$ CFU/ml. Control group was inoculated with 0.2 ml of sterile broth via same route. All birds were observed daily for a period of 10 days for clinical signs. After $10^{th}$ day birds were sacrificed. Signs and lesions were recorded frequently and reisolation subsequently was carried out.

**Isolation and identification of associated bacterial agent/s:**

Parallel to *Haemophillus* isolation, samples were subjected for isolation of associated bacterial agent/s. Specimens for isolating bacteria other than *Mycoplasma species* were taken from all internal organs in addition to sinus. Samples were cultured in tryptose broth, and the obtained growth then subcultured on nutrient and MacConky's agar plates under aerobic conditions.

Samples for isolating *Mycoplasma species* were obtained from lung, trachea and air-sacs, inoculated in brain heart infusion broth containing (yeast extract, horse serum, penicillin G sodium, 10% thalium acetate, and phenol red) and incubated at 37C for 3-5 days until evidence of growth (slight turbidity or pH change), then subcultured on BHI agar which was incubated at 37C for further 3-5 days under reduced oxygen tension and increased humidity. Plates were examined microscopically for presence of the common fried-egg-like colonies (Yoder, 1980).

Identification of associating bacterial agents was done according to Edwards and Ewing, 1972 & Cruickshank *et al*., 1975 and Collins and Lyne, 1991.

Identification of *Mycoplasma species* was done using growth inhibition test (Clyde, 1964) and serologically by using rapid plate agglutination test (Adler and Yamamoto, 1956) and HI test (Yoder, 1980).

**Induction of complicated coryza:**

Fifty, 65-week-old chickens were divided into five groups, each of ten birds. The infecting dose was 0.2 ml of broth culture containing $10^8$ CFU/ml in case of *Haemophilus paragallinarum*. The first group was inoculated intranasally only with *Haemophilus paragallinarum*. The second group was inoculated intranasally with *Haemophilus paragallinarum* and *Mycoplasma gallisepticum* (0.1 ml of broth culture containing $10^8$ CFU/ml). The third group was inoculated intranasally with *Haemophilus paragallinarum* and orally with *E. coli* (0.1 ml of broth culture containing $10^8$ CFU/ml). The fourth group was inoculated intranasally with *Haemophilus paragallinarum* and kept under bad hygienic conditions (bad ventilation, malnutrition and overcrowdness). The fifth group was kept as non-treated control. Birds were observed for 8 weeks for clinical examinations, and at the end of $8^{th}$ week, the survivors were sacrificed and necropsy findings were recorded.

## RESULTS

**Screening the prevalence of complicated infectious coryza in chickens:**

The examined broiler chickens showed severe respiratory signs as nasal discharges, conjunctivitis, facial edema, lacrimation, sneezing, infraorpital sinusitis, dyspenia and abnormal respiratory sounds, decreased food consumption, diarrhea and retarded growth. Similar clinical signs were observed in layer chickens in addition to decreased egg production (3-40%), results are illustrated in tables 1& 2.

The morbidity percentage was (10-30%) and (30-60%) in broiler and layer chickens respectively, while mortality percentage was (0.5-2%) and (1-10%) in broiler and layer chickens respectively. The course of the disease was prolonged in layers (3-6 weeks) while it was ranged from 2-4 weeks in broilers.

Post mortem examination revealed presence of caseous plugs in nasal passages, conjunctivitis, infraorpital sinusitis, tracheitis, air

sacculitis, fibrinous pericarditis, peritonitis and enteritis. In layers salbingitis, presence of caseated materials in the oviduct and oophoritis manifested with follicular degeneration were observed.

**Isolation and identification:**

Out of 367 examined cases 121 (33%) cases were positive for isolation of organism suspected to be *Haemophils paragallinarum*. Colonies were tiny, smooth, dewdrop and iridescent. Culture stained smears revealed Gram-negative pleomorphic coccobacilli, while bacteria were stained bipolar pleomorphic coccobacilli in nasal exudates smear and recent culture. Out of 121 morphologically suspected cultures, 22 selected colonies were applied for biochemical, serological and biological investigations. The biochemical reactions of the isolates were positive for catalase, oxidase, and nitrate reduction, while they were negative for indole and urease production. Sugar fermentation tests revealed glucose, sucrose, fructose and maltose fermentation without gas production, while xylose, galactose, trihalose and arabinose could not be fermented.

**Haemagglutination activity:**

Non of the isolates had haemagglutination activity against fresh chicken erythrocytes, while 15 isolates had haemagglutination activity against only formaldhyde-fixed chicken erythrocytes, and only 7 isolates lacked detectable haemagglutination activity.

**Serology:**

Indicated that 9 isolates belonged to serotype A; 3 isolates belonged to serotype B; 7 isolates belonged to serotype C, and three isolates were untypable, results are shown in table 3.

**Pathogenicity test:**

*Chicken embryo:*

All isolates caused death of the embryos within 24-48 hours after yolk sac inoculation. The dead embryos showed severe congestion as well as embryonic membranes.

*Chickens:*

In case of intrasinus inoculation the birds showed typical signs of infectious coryza after 24-48 hours in all inoculated cases, while the intranasal inoculation produced longer incubation period (4-5 days), and clinical signs occurred in 80% of tested birds. Necropsy findings were observed as congestion and increased exudates in mucous membranes of the sinuses, facial edema. Catarrhal tracheitis and lung congestion were recorded in some cases.

**Isolation and identification of associated bacterial agent/s:**

*E. coli* {95/328 (29%)}, *Klebsiella species* {27/328 (8.2%)}, *Pseudomonas species* {33/328 (10%)} and *proteus species* {36/328 (11%)} were recovered in association with *Haemohpilus paragallinarum*. *Mycoplasma gallisepticum* was also isolated from cases of chronic or complicated coryza {82/328 (25%)}.

**Induction of complicated (chronic) coryza:**

Results are illustrated in table 4. The first group, which infected with *Haemophilus paragallinarum* only displayed signs of simple coryza in addition to decreased egg production within 24-72 hours, while the course of the disease extended between 7-10 days. The second group (infected with *Haemophilus paragallinarum* and *Mycoplasma gallisepticum*) showed severe clinical signs (coryza, swollen wattles, dyspenea, rales and decreased body weight) after 24 hours and the egg production decreased to 40-50%. Post mortem lesions were sinusitis, trachcitis, air sacculitis, fibrinous pericarditis, peritonitis, salbingitis, presence of caseated materials in the oviduct and oophoritis. The third group, which was infected with *Haemophilus paragallinarum* and *E. coli* and the fourth group, which was infected with *Haemophilus paragallinarum* and kept under bad hygienic conditions showed a similar clinical and post mortem picture as described in the second group. The control group showed no clinical signs.

## DISCUSSION

Infectious coryza is an acute respiratory disease of chickens caused by *Haemohpilus paragallinarum*. The disease has two forms, simple and complicated form although the simple form typically causes only mild clinical signs. The complicated form shows tragic picture represented by severe clinical signs accompanied with economic losses as high and persistent mortality, increased culling rate in broilers, drop in egg production (10-40%) in layers and high costs of medication (Sandoval *et al.*, 1994). In the present work, some affected flocks showed persistent signs of infectious coryza, which get complicated with other pathogens. Morbidity rate in layers and broilers were increased and ranged from (30-60%) and (10-30%) respectively, while mortality rate during the course of the disease was ranged from (1-10%) and (0.5-2%) in layers and broilers respectively. The course of the disease was prolonged in layers (3-6 weeks) while it was ranged from 2-4 weeks in broilers. The examined broiler chickens showed severe respiratory signs as nasal discharges, conjunctivitis, facial edema,

lacrimation, sneezing, infraorpital sinusitis, dyspenia and abnormal respiratory sounds, decreased food consumption, diarrhea and retarded growth. Similar clinical signs were observed in layer chickens in addition to decreased egg production (3-40%). The mentioned results were similar to those reported by Gordon and Jordan (1982); Barr *et al.*, (1986); Sandoval *et al.*, (1994); Aly *et al.*, (1995) and Blackall (1999). On contrast, Roberts *et al.*, (1964) considered that *Haemohpilus paragallinarum* as non-important pathogen in the respiratory disease complex in Great Britain. Post mortem examination revealed presence of caseous plugs in nasal passages, conjunctivitis, infraorpital sinusitis, tracheitis, air sacculitis, fibrinous pericarditis, peritonitis and enteritis. In layers salbingitis, presence of caseated materials in the oviduct and oophoritis manifested with follicular degeneration were observed. These results were greatly supported by Nakamura *et al.*, (1993); Sandoval *et al.*, (1994) and Aly *et al.*, (1995).

Isolates have been recovered from flocks with percentage of 57%. The difficulty of isolation originates from growth requirements restrictions demanded by *Haemohpilus paragallinarum* and its slow growth rate. The order, which give chance for other complicating bacteria to over grow and obscure the haemophilus growth (Chen *et al.*, 1996).

The biochemical reactions and sugar fermentation tests of the isolates revealed that all culture positive isolates were belonged to *Haemohpilus paragallinarum*. These results were similar to those described by Hinz, (1973); Hinz (1980); Terzolo *et al.*, (1993) and Aly (2000).

Serological studies indicated that 9 isolates belonged to serotype A; 3 isolates belonged to serotype B; 7 isolates belonged to serotype C, and three isolates were untypable. This result proved that all serovars of *Haemohpilus paragallinarum* are distributed in Upper Egypt, which is similar to that reported by Aly (2000).

Concerning the isolation of associated bacterial agents; *E. coli*, *Klebsiella species*, *Pseudomonas species* and *proteus species* were recovered in association with *Haemophilus paragallinarum*. *Mycoplasma gallisepticum* was also isolated from cases of chronic or complicated coryza. Several authors described more or less similar findings, Aly (1987); Droual *et al.*, (1990); Nakamura *et al.*, (1993), Sandoval *et al.*, (1994) and Aly *et al.*, (1995).

Concerning the pathogenicity of *Haemohpilus paragallinarum* to chicken embryo, all isolates caused death of the embryos within 24-48

hours after yolk sac inoculation. The dead embryos showed severe congestion as well as embryonic membranes. This result comes in agreement with Zaki (1983) and Blackall *et al*., (1997).

Regarding to pathogenicity to chickens, in case of intrasinus inoculation the birds showed typical signs of infectious coryza after 24-48 hours in all inoculated cases, while the intranasal inoculation produced longer incubation period (4-5 days), and clinical signs occurred in 80% of tested birds. Necropsy findings were observed as congestion and increased exudates in mucous membranes of the sinuses, facial edema. Catarrhal tracheitis and lung congestion were recorded in some cases.

The pathogenesis of complicated infectious coryza was studied in 65 week-old layer chickens. The group, which infected with *Haemophilus paragallinarum* only displayed signs of simple coryza in addition to decreased egg production within 24-72 hours, while the course of the disease extended between 7-10 days. While other groups, which were experimentally infected with *Haemohpilus paragallinarum* and other associating agents and bad hygienic conditions displayed signs of coryza (swollen wattles, dyspenea, rales and decreased body weight) after 24-48 hours, high morbidity (80%), high mortality rate (20-40%) and the egg production decreased to 10-60%. Post mortem lesions were sinusitis, tracheitis, air sacculitis, fibrinous pericarditis, peritonitis, salbingitis, presence of caseated materials in the oviduct and oophoritis. Several reports agreed with the formerly mentioned results, Raggi *et al*., (1967), Barr *et al*., (1986), Linizitto *et al*., (1988), and Blackall (1999). Hinz, 1996 stated that, although *Haemohpilus paragallinarum* is non-invasive bacteria with strong tropism for ciliated cells, it can migrate into the internal organs only after synergistic interaction with other infectious agents and or when encouraged by immun-suppression. In this study, extension of *Haemohpilus paragallinarum* from the upper respiratory tract to lower organs and to the enteric organs were investigated and demonstrated and explained by presence of associating bacteria and devitalizing conditions which gave the power of *Haemohpilus paragallinarum* to invade tissues other than respiratory organs.

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**Table 1:** Shows epidemiology of complicated infectious coryza in broiler Chickens

| Localities | Breed | Total No. | Age in weeks | No. examined | Morbidity rate | Mortality rate |
|---|---|---|---|---|---|---|
| Beni-suif | Hubbard | 4500 | 2 | 20 | 10% | 1.8% |
| El-menia | Ross | 4000 | 4 | 40 | 20% | 1.8% |
| El-menia | Hubbard | 8000 | 4 | 30 | 30% | 2% |
| El-menia | Hubbard | 14000 | 6 | 15 | 15% | 1.2% |
| Assiut | Hubbard | 7000 | 3 | 25 | 25% | 1.5% |
| Assiut | Hubbard | 3000 | 4 | 20 | 30% | 2% |
| Assiut | Avian 43 | 5000 | 6 | 15 | 20% | 0.5% |
| Assiut | Arber Acres | 12000 | 1 | 10 | 20% | 1.4% |
| Sohag | Ross | 10000 | 2 | 20 | 25% | 1.25% |
| Sohag | Hubbard | 6000 | 5 | 10 | 15% | 1.7% |

Mortality rates during the course of the diseases (2-4 weeks).

**Table 2:** Shows epidemiology of complicated infectious coryza in layer Chickens

| Localities | Flock | Breed | Age (weeks) | Examined No. | Morbidity rate | Mortality rate | Drop in egg production |
|---|---|---|---|---|---|---|---|
| Beni-suif | 1 | Hyline | 50 | 7 | 40% | 5% | 15% |
| | 2 | Hyline | 35 | 10 | 35% | 5.5% | 40% |
| | 3 | L.S.L | 35 | 9 | 42% | 3% | 25% |
| El-menia | 1 | Hyline | 40 | 12 | 45% | 1% | 25% |
| | 2 | Hyline | 45 | 10 | 30% | 3% | 10% |
| | 3 | Hyline | 30 | 5 | 20% | 1.4% | 30% |
| | 4 | Hyline | 32 | 9 | 50% | 2% | 30% |
| Assiut | 1 | Hyline | 55 | 4 | 60% | 10% | 25% |
| | 2 | Hyline | 50 | 12 | 65% | 3.5% | 40% |
| | 3 | Hyline | 33 | 10 | 34% | 4% | 35% |
| | 4 | Hyline | 28 | 13 | 62% | 8% | 30% |
| | 5 | Hyline | 30 | 5 | 55% | 2% | 20% |
| | 6 | L.S.L | 35 | 8 | 61% | 5.4% | 5% |
| | 7 | L.S.L | 45 | 12 | 43% | 2.5% | 10% |
| | 8 | L.S.L | 42 | 13 | 50% | 4% | 10% |
| Sohag | 1 | L.S.L | 51 | 10 | 50% | 1% | 3% |
| | 2 | Hyline | 34 | 1 | 60% | 2% | 10% |
| | 3 | Hyline | 40 | 5 | 55% | 1% | 35% |
| | 4 | Hyline | 45 | 7 | 40% | 1% | 30% |

Mortality rates during the course of the diseases (3-6 weeks).

**Table 3:** Shows comparison of serotyping of *Haemophilus Paragallinarum* isolates according to the Page's scheme and HI tests

| Agglutination Serovars | HI Serovars | | | |
|---|---|---|---|---|
| | A | B | C | Non-typable |
| A | 9 | - | - | - |
| B | - | 3 | - | - |
| C | - | - | 7 | - |
| Non-typable | - | - | - | 3 |

**Table 4:** Shows results of experimental infection of 65-week-old chickens with *Haemophilus paragallinarum* and associated bacteria

| Group | Infective agent/s | Rout | Incubation period | Course of diseases | Morbidity rate | Mortality rate |
|---|---|---|---|---|---|---|
| 1 | *Haemophilus paragallinarum* | Intrasinus | 24-48 hrs | 7-10 days | 50% | 10% |
| 2 | *Haemophilus paragallinarum* & *M.G.* | Intrasinus | 24 hrs | 5 weeks | 80% | 40% |
| 3 | *Haemophilus paragallinarum* & *E. coli* | Intrasinus & orally | 24 hrs | 5 weeks | 80% | 30% |
| 4 | *Haemophilus paragallinarum* & Bad hygien | Intrasinus | 24-48 hrs | 6 weeks | 70% | 20% |

N.B. drop in egg production was observed in most of the infected cases from (10-60%), while in some cases complete cessation of egg production was observed.