

BACTERIOLOGICAL STUDIES ON HAEMOPHILUS PARAGALLINARUM IN CHICKEN IN DAKAHLIA GOVERNORATE

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ABSTRACT

One hundred & eighty samples collected from commercial layers, broiler, breeders and native breeds farms to detect the incidence of infectious coryza in Dakahlia Governorate. 12 isolates of Haemophilus paragallinarum (HPG) were isolated, these isolates could be identified morphologically, biochemically and serologically into 8 isolates belonged to serotype A and the other 4 isolates belonged to serotype C. The antibiogram sensitivity test proved that the enrofloxacin was the most effective antibiotic followed by ampicillin, ciprofloxacin, amoxicillin, and the lowest one was penicillin.

INTRODUCTION

Infectious coryza is an acute upper respiratory disease of chickens characterized by sneezing, nasal discharge, facial swelling, lacrimation, sinusitis, decrease food and water consumption and retarded growth in broilers and reduce egg production in layers flocks from 10 to 40% leading to increase number of culls particularly in multiages farms (**Blackall, 1999**).

Early workers identified the causative agent of infectious coryza as *Haemophilus gallinarum*, an organism that required both X and V factors for growth, however all isolates of the organisms producing agent required only V factor and named *Haemophilus paragallinum* (HPG) (*Chen et al., 1996 and Bragg et al., 1997*).

HPG is delicate and extremely fastidious microorganism not easy to grow in pure culture in vitro requiring specific growth condition for isolation (*Chen et al., 1996 and Bragg et al., 2004*). HPG is Gram-negative, non motile, bipolar bacterium, coccobacilli or short rods, 1-3µm in length and 0.4-0.5 µm in width. The capsule may be present in a virulent strain (*Hinz 1973, Sawata et al., 1981 and Richarad and Gretchen, 2004 and Garcia et al, 2004*).

MATERIAL AND METHODS

Materials:

Samples:

One hundred & eighty alive and freshly dead chickens at age of 18 to 180 days were collected from layers and broilers farms scattered all over the Dakahlia Governorate. These birds suffering from sneezing, nasal discharge, lacrimation, facial swelling and anorexia.

Media:

- a- Brain heart infusion broth and agar (*Blackall 1989, and Horner et al 1992*).
- b- Blood agar.
- c- Chocolate blood agar (*Koneman et al., 1998*).
- d- Tryptose blood agar (*Rimler, 1979*).

- e- MacConkey agar (*Cruickshank et al., 1975*).
- f- Sugar media.
- g- Urea, Citrate, Nitrate media .
- h- Phosphate buffer salin, used for antigen preparation.
- i- Nicotineamide adenine dinucleotide (NAD). (Sigma).
- j- Merthiolate 0.01% were used for antigen preservation.

Standarded strains:

- a- HPG 0083, 0222 and Modesto strains were used for antigens preparation. These strains were kindly provided from Dr. R. Rimler College of Vet. Med. Poultry Disease Research Center, Department of Avian Medicine, University of Georgia, USA. They were stored as described by **Iritani et al., (1977)**.
- b- Staphylococcus epidermidis strain were kindly provided from Bacteriology, Mycology and Immunology Department of Faculty of Vet, Med, Zagazig University, & was used as feeder culture to HPG & to study the satellitism phenomena.

Stains:

- a- Gram's stain. *Cruickshank et al., (1975)*.
- b- Crystal violet 0.002% for antigen staining (**Iritani et al., 1977**).

Embryonated chicken eggs: used for preservation & propagation of suspected *Haemophilus strains*.

Antimicrobial discs: used for antibiogram sensitivity test.

Methods:

a- Bacteriological swabs: infraorbital sinuses and tracheal swabs were taken from the collected chickens under aseptic conditions. Then cultivated onto blood agar, tryptose agar, chocolate blood agar and brain heart infusion agar plates with the feeder culture of *Staphylococcus epidermidis* as a source of NAD for HPG isolation. The plates were incubated at 37C for 24-48 hr in the presence of 10% CO₂(candel jar) MacConkey's agar media used for other bacterial isolation. The growing colonies were examined for their shape, size and hemolysis onto blood agar.

b- Biochemical identification: Investigated according to *Cruickshank et al.,(1975)and Terezol et al.,(1993)*:Catalase,indole,nitrate reduction test, urease test, citrate test and sugar fermentation test were applied.

Serological identification:- Antigen preparation for the suspected colonies and standared strains according to *Rimler et al., (1977)*.

Antisera preparation according to *Page (1962)*.

Slide agglutination test according to *Irritani et al., (1977)*.

Antibiogram sensitivity pattern of HPG carried out according to *Rimler (1979)*.

RESULTS AND DISCUSSION

The results of bacteriological isolation in broiler breeders and native breeds were shown in table (1) total of 118 samples yield only 5 strains of HPG with a total incidence of 4.23% ,E. Coli 42 isolates in incidence of 36%, Staph 50 isolates in incidence of 42.37% and Pseudomonas24 isolates in incidence of 20.39%.

Table (1): Incidence of HPG in broiler breeders and native breeds chickens in Dakahlia Governorate.

Locality	Breed	Age/day	No.of samples	Bacteriological isolation			
				HPG	E.Coli	Staph	Pseudomonas
Belkas	Hubbard	23	13	1	3	6	3
Talkha	Native breed	33	10	2	3	3	5
Basandila	Hubbard	30	25	1	7	11	6
Batra	Cubb	40	24	1	8	12	3
Sherbin	Native breed	30	8	-	4	4	-
El-Tawila	Native breed	50	6	-	6	-	-
El-Maesara	Hubbard	31	11	-	4	4	3
Dekernes	Cubb	25	21	-	7	10	4
-	-	Total	118	5 =4.23%	42 =36%	50 =42.37%	24 =20.39%

The results of bacteriological isolation in layers were shown in table (2) total 62 samples yield only 7 isolates of HPG in incidence of 11.3%, 20 isolates of E.Coli in incidence of 32.26%, 25 isolates of Staph in incidence of 40.32% and 11 isolates of Pseudomonas in incidence of 17.74%.

Table(2): Incidence of HPG in layer chicken in Dakahlia Governorate.

Locality	Age/day	No.of samples	Bacteriological isolation			
			HPG	E.Coli	Staph	Pseudomonas
Belkas	150	13	1	3	6	3
Talkha	140	11	2	4	4	1
Sherbin	160	12	2	4	4	3
El-Tawila	180	12	1	5	6	-
El-Maesara	165	14	1	4	5	4
-	Total	62	7 =11.3%	20 = 32.26%	25=40.32%	11=17.74%

Total of 180 samples revealed,12 isolates HPG, 62 E.coli,75 Staph, 35 Pseudomonas. These results were supported by *Awad Alla (1989)* who idntified15 isolates of HPG (incidence 5.8%), in addition to 33 isolates of E.coli,5 isolates of Salmonella, 9 isolates of Pseudomons, one isolate of Arizona and 14 isolates of Staph from 256 samples in Sharkia Governorate. While *Zaki (1983)* isolated 11 isolates of HPG from 190 examined samples at Giza Governorate with incidence 5.78%.

The suspected colonies were tiny dew drops like colonies up to 0.3-0.5 mm in diameter when incubated for 24-48 hours in the presence of 10% CO₂ and feeder culture. They were non hemolytic on blood agar and didn't grow onto MacConkey's agar media and when grow onto chocolate agar with presence of feeder culture (Staph. Epidermidis) the growth was abundant near the feeder culture. These colonies were Gram negative, coccobacilli or short rods, bipolar bacterium 1-3um in length and 0.4-0.8 um in width and non spore forming bacteria these results agree with *Hinz (1973)*, *Sawata et al (1981)*, *Awadalla (1989)*, and *Richard and Gretchen, 2004 and Garcia et al., (2004)*.

The biochemical identification revealed that Catalase, Indol, Urease and Citrate tests were negative, while Nitrate reduction was positive. Concerning to sugar fermentation maltose, mannitol were positive, galactose was negative, meanwhile lactose and sucrose showed variable results, 5 isolates showed positive reaction and 7 isolates showed negative reaction. These results supported with the results recorded by *Kume et al., (1978)*, *Narita et al., (1978)*, *Sawata et al., (1979)*, *Rimler (1979)*, *Horner et al.,(1992)*, *Terzolo et al., (1993)*, *Blackall et al.,(1994) and (2005)*.

The results of serological identification by using rapid slide agglutination test showed formation of definite clumps within 1-2 minutes ,8 out of these isolates belonged to serotype A(0083),while the other 4 isolates belonged to serotype C (Modesto). These results were similar to *Awad alla (1989)* who isolated 15 isolates of HPG collected from different localites at Sharkia Governorate and identified 10 of them as serotype A and 5 as serovare C. While *Zaki (1983)* identified 11 isolates of HPG from 190 examined samples at Giza Governorate as serovare 1. Meanwhile

Shahata et al (1988) who identified his isolates from upper Egypt as serotype 2 and *Aly and Mousa (2000)* who identified 18 isolates of HPG also in upper Egypt as 8 isolates belonged to serotype A, 4 isolates belonged to serotype B and 6 isolates belonged to serotype C.

Regarding to the antibiogram sensitivity pattern of HPG the results were shown in table (3).

Table(3): Antibiotic sensitivity test of 12 isolates of HPG :-

Chemotherapeutic	Symbol and concentration	No.of sensitive isolates	%of activity
Enrofloxacin	AVT(10µg)	11	91.66
Ampicillin	AMP(10µg)	9	75
Ciprofloxacin	CIP(5µg)	9	75
Amoxycillin	AML(10µg)	9	75
Norfloxacin	NOR(10µg)	8	66.66
Kitasamycin	KT(70µg)	7	58.33
Gentamycin	CN(10µg)	5	41.66
Sulfamethoxazole	SXT(25µg)	5	41.66
Erythomycin	E(15µg)	4	33.3
Neomycin	N(30µg)	3	25
Streptomycin	S(10µg)	3	25
Pencillin G	P(10I.U)	2	16.66

These results were nearly agreed with **Horner et al.(1992)** who recorded that enrofloxacin and ampicillin were highly potent antibiotic with an activity percentage of 100% for each while sensitivity to pencillin, neomycin and streptomycin gave variable percentage of activity. In the

same time *Peornomo et al.,(2000)* indicated that 11 isolates from 18 isolates were resistant to erythromycin and streptomycin, 10 isolates were resistant to neomycin, 3 isolates were resistant to sulfamethoxazole trimethoprim but only one isolate was resistant to ampicillin. On the other hand, *Banani et al., (2007)* proved that all isolates were sensitive to ciprofloxacin with an activity percentage of 100%, 3 isolates were sensitive to enrofloxacin and streptomycin with an activity percentage of 75% for each other, 2 isolates were sensitive to ampicillin with an activity percentage of 50% and one isolate was sensitive to gentamycin and amoxicillin with an activity percentage 25% for each of them, while all isolates were completely resistant to penicillin. The obtained results were contradicted with *Muhammad et al.,(1998)* who indicated that gentamycin was the most effective antibiotic against HPG isolates.

Our results were supported to some extent with (*Rimler, 1979; Blackall, 1988 and Zaini et al, 1991*) who found that, all strains were sensitive to ampicillin, gentamycin, erythromycin, chloramphenicol, kanamycin, tetracycline. Also *Soriano et al, (2001)* reported that 96.8% of the studied microorganisms were sensitive to enrofloxacin and variable susceptibility to gentamycin, amoxicillin, trimethoprim, oxitetracycline and phosphomycin. *Blackall et al, (1990)* suggested that resistance of *H. paragallinarum* serotypes to streptomycin still predominant and this confirms our results.

We can conclude that HPG infection has economic significance in broilers as well as in layers. The most pathogenic serotypes are A and C. The most effective antibiotics are enrofloxacin followed by ampicillin, amoxicillin and ciprofloxacin.

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دراسات بكتريولوجية على ميكروب الهيموفلس باراجالينرم فى الدجاج بمحافظة الدقهلية

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**معمل المنصورة

قد تم تجميع 180 عينة من دجاج مختلف السلالة من مزارع حكومية وأهليه منتشرة في أماكن مختلفة في محافظه الدقهليه وتم إجراء الفحص لبكتريولوجي والاختبارات البيوكيميائية والسيرولوجيه وكذلك تم عمل اختبارات الحساسية لهذه العترات ضد أنواع مختلفة من المضادات الحيويه وكانت النتائج كالاتي تم عزل 12 عتره من عترات الهيموفلس باراجالينرم وقد تم تصنيفها علي اساس الفحص المورفولوجي وقابليتها لصبغه الجرام والاختبارات البيوكيميائية والسيرولوجيه إلى 8 عترات تنتمي إلى العتره وأربع عترات تنتمي إلى العتره وبإجراء اختبار الحساسية تبين أن انروفلوكساسين هو اكثر المضادات الحيويه فاعليه 91.66 % يليه الامبسلين والسبروفلوكساسين بنسبه 75% لكل منهم واثبت الاختبار أن اقل المضادات الحيويه تأثيرا هم الاستربتومايسين والنيومايسين والأموكسيسيلين بنسبه 25% وأخيراً البنسلين بنسبه 17%.