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PREVALENCE OF *PASTEURELLA MULTOCIDA* IN WATERFOWL WHICH SUFFERING FROM RESPIRATORY DISORDER AND ITS SENSITIVITY TO SOME DIFFERENT ANTIBIOTICS

(With 6 Tables)

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مدى تواجد ميكروب الباستيرلا ملتوسيدا فى الطيور المائية والتى تعانى من مشاكل تنفسية وحساسيته لبعض المضادات الحيوية المختلفة

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اجريت الدراسة على عدد 250 طائر مائى (150 بطة و 100 اوزة) تعانى من اعراض تنفسية ونفوق عالى من مزراع وقطعان فى محافظة الاسكندرية بالاضافة الى 30 عينة ماء و30 عينة طمى من اماكن تواجد البط والأوز وذلك للفحص البكتريولوجى لعزل ميكروب الباستيرلا ملتوسيدا وكانت الأعراض الظاهرية للطيور المفحوصة فقدان الشهية وخمول واعراض تنفسية واسهالات واظهرت الصفة التشريحية عن وجود انزفة دموية على الكبد والطحال والقلب والامعاء واحتقانات فى الرئة والقصبة الهوائية مع وجود افرازات مخاطية بها وتم اخذ عينات من دم الطيور المصابة للقحص الميكروسكوبى وعينات من الأعضاء الداخلية للفحص البكتريولوجى وقد اظهرت الخواص المورفولوجية والتفاعلات البيوكميائية الى عزل 134 عترة باستيرلا ملتوسيدا من 300 عينة بنسبة (43،2%) منها 92 من عينات البط بنسبة (61،3%) 36 عترة من عينات الأوز بنسبة (36%) و4 عترات من عينات الماء بنسبة (13،3%) و 2عترة من الطمى بنسبة (6،7%) وقد ثبت أن هنالك تفاوت فى نسبة العزل فى الطيور السليمة ظاهريا والمريضة والنافقة حديثا حيث كانت (7،5%) و(53,6%) و(71,4%) على التوالى0 وبفحص ضراوة بعض العترات المعزولة على فئران التجارب عن طريق الحقن البروتونى وبالعدوى عن طريق الفم والحقن فى القصبة الهوائية فى البط الى حدوث نفوق تراوحت نسبته من 20 الى 100% حيث كان هنالك تفاوت فى شدة الضراوة بين العترات المعزولة من الحالات السليمة ظاهريا والمريضة والنافقة وقد سجلت الأعراض الإكلينيكية والآفات التشريحية ووجدت أنها تشبه إلى حد كبير تلك التى سجلت في العدوى الطبيعية وتم عزل الميكروب مرة أخرى منها وتم عمل اختبار حساسية لميكروب الباستيرلا المعزول بأستخدام المضادات الحيوية المختلفة والتى ثبت فعاليتها لكل من البنسيللين واللنكومايسين والأوكسى تتراسيكلين وم قاومته لكل من الكلورامفينوكول والنيومايسين

وستربتومايسين وتم مناقشة الأهمية الصحية من تواجد ميكروب الباستيرلا ودوره فى حدوث النفوق العالى فى الطيور المائية

SUMMARY

A total of 250 waterfowl (150 ducks and 100 geese), having a history of respiratory disorders and mortality, additional to 30 water and 30 sediments samples which waterfowl income were examined in the present study. Samples were collected from different private farms and flock's ducks and geese in Alexandria Province for P.M and bacteriological examination. The clinical sings showed exhausted birds, drowsy, loss of appetite, swim in circles, lameness, swollen wattles, difficult breathing, cyanosis, watery green yellowish diarrhea, ruffled feathers, mucous discharge from the mouth, while postmortem lesions revealed hemorrhages of various sizes on the heart, liver, gizzard and intestines, also white spots and necrotic foci were present on the liver and spleen. Bacteriological examination of these samples for the prevalence of *P. multocida* according to morphological characters and biochemical reactions, revealed 134 *P. mulotcida* isolates out of 360 samples with incidence of (43.2%). 92(61.3%) of *P. mulotcida* isolates from ducks, 36 (36%) and geese samples, 4 (13.3%) isolates from water samples and 2 (6.7%) isolates from sediments samples. There are variations in the prevalence rate of *P. multocida* isolates from apparently healthy, diseased and freshly dead in both ducks and geese examined. It was found 3 (7.5%) in apparent healthy, 75 (53.6%) in diseased birds, while was 50 (71.4%) in dead birds. Results of experimental infection of *P. multocida* isolates in mice and ducks revealed difference in mortality rate between strains isolated which ranging from 20% to 100% due to difference in the virulence of these strains. The clinical symptoms and post-mortem pictures of experimentally inoculated ducks are similarly to those observed in naturally. Sensitivity test revealed that *P. multocida* isolates were more sensitive to Penicillin**,** Lincomycin and Oxytetracycline, while were resistant to Chloramephenicol, Neomycin and Streptomycin.

Key words: *P. multocida - Fowl cholera, waterfowl, ducks and geese*

INTRODUCTION

Fowl cholera (avian pasteurellosis) is a commonly occurring avian disease that can affect all types of birds and is often fatal (Derieux, 1978). The first outbreak of fowl cholera occurred in a flock of Muscovy

ducks (Cairina moschata) in Okinawa Prefecture of Japan in November 1990. Fifty (25%) of 200 birds in a farm died of an acute disease (Nakamine *et al.* (1992). Also Richard *et al.* (1967) reported that more than 1,000 geese died in one night suffering from fowl cholera.. Avian cholera has become one of the most important causes of infectious disease causing mortality among North American wildfowl (Stout and Cornwall, 1976; Friend, 1999).

Avian cholera is an infectious disease caused by *Pasteurella multocida*, a Gram-negative, rod-shaped bacterium, with a bipolar staining characteristic (Rimler and Glisson, 1997).

Pasteurella multocida subspecies multocida is the most common cause of fowl cholera, although *P. multocida* subspecies septica and gallicida may also cause fowl cholera-like disease to some extent Christensen and Bisgaard (2000)

Blanchong *et al.* (2006) reported that avian cholera, caused by *Pasteurella multocida*, affects water birds across North America and occurs worldwide among various avian species. Once an epizootic begins, contamination of the wetland environment likely facilitates the transmission of *P. multocida* to susceptible birds

The viability of *P. multocida* has varied with physical and chemical characteristics of water and wetlands. (Bredy and Botzler, 1989 and Price *et al.*, 1992 and Titche, 1979). Wetlands have long been suspected to be an important reservoir for *Pasteurella multocida* and therefore the likely source of avian cholera outbreaks. *P. multocida* has been isolated from the water and sediment of wetlands avian cholera epizootics and the bacteria can persist in wetland soil and water (Price and Brand, 1984; Backstrand and Botzler, 1986; Bredy and Botzler, 1989 and Samuel *et al.*, 2003). Also Samuel *et al.* (2004) isolated *P. multocida* from 20 of 44 wetlands, including 7% of the water and 4.5% of the sediment samples Lehr *et al.* (2005) studied patterns in avian cholera mortality, the presence of *Pasteurella multocida* in the water or sediment, and water chemistry characteristics in 10 wetlands. They recovered *P. multocida* from water and sediment samples in six of the 10 study wetlands.

The duck may be a better carrier of *P. multocida* under scavenging system than chickens. This contact cross transmission may be playing a role in the maintenance of the bacterium at the village level (Mbuthia *et al.*, 2005)

Aim of the study

The aim of the present study:

1- The incidence of *Pasterulla multocida* in domestic waterfowl (ducks and geese), water and sediment of wetlands and ponds which waterfowl income
2- Describing the clinical signs and necropsy lesions of *Pasterulla multocida* infection in ducks and geese
3- Antimicrobial susceptibility of *Pasteurella multocida* isolates
4- Studying the pathogenicity of *P. multocida* isolates by using experimental animals and birds.

MATERIALS and METHODS

1- Sources of specimens:

Samples were collected from different private farms and flock' of ducks and geese in Alexandria Province

2- Collection of samples

A total of 250 domestic waterfowl included 150 ducks and 100 geese with an average from 4 to 12 months old aged, in additional to 30 water and 30 sediment samples were collected from wetlands and ponds which waterfowl income. Bacteriological examination of water and sediment samples for isolation of *P. multocida* using the methods of (Cruickshank *et al.*, 1982 and Samuel *et al.* 2003). The healthy status of waterfowl examined were 20 apparently healthy, 90 diseased birds and 40 freshly dead for ducks and 20 apparently healthy, 50 diseased birds and 30 freshly dead for geese. These birds were subjected to postmortem and bacteriological examination for *Pateurella multocida* investigation. Samples were collected from visceral organs such as lung, liver, spleen, air sacs, intestine, bone marrow, and heart blood of birds. The liver should be removed and placed in a separate bag; Refrigerate these samples as soon as possible after collection and insure that they are kept cool during shipment. Care must be exercised during carcass collection to minimize the amount of fluid discharged into the environment from noses and mouths of diseased birds. Bags of carcasses should always be securely closed before being removed from the area.

3- Isolation and identification of *Pasteurella multocida*

Primary isolation by inoculated samples on dextrose starch agar, blood agar, and trypticase-soy agar at 37^0C for 24-48 hours. Isolation may be improved by the addition of 5% heat-inactivated serum. Suspected colonies were examined for their colonial morphology (shape, color and size). A liver impression smear and blood stained with Giemsa and Wright's stain for bipolar rods, through a microscopic examination.

Identification of *P. multocida* is based on the results of biochemical tests, which include carbohydrate fermentation, enzyme production, and selected metabolite production, were carried out on the isolated strain according to Richard and Glisson (1997) and Glisson (2003). Commercial media and biochemical test kits are available. (Oxoid, 1982).

4- Pathogenicity test of *P. multocida* isolates:

a) Pathogenicity test of *P. multocida* in vivo in experimental animals:

The pathogenicity of 3 strains of P.multocida isolated from ducks and geese were used. One strain isolated from each of apparently healthy, diseased birds and freshly dead was used and 20 Swiss mice (4-6-week-old,) were divided into 4 groups each group contain 5 mice, the last group used as control. One strain for each group was injected into the intraperitoneal of mice, with 5ml (10^7 cfu/ml) of 24hr broth culture. The plate count technique was used for determination of the viable count of cell per ml of suspension of *Pasteurella multocida* isolated accordaning (Cruickshank *et al.*, 1982), while last group inoculated by 5ml sterile normal saline and unlimited foods and water were provided. All inoculated mice were kept under observation, dead mice were recorded and trial for re-isolation of inoculated organisms was conducted.

b) Pathogenicity test of *P. multocida* in experimental birds

The same isolated previous, three strains of *P. multocida* were used. One strain for each group. Forty-five ducks (3-4 months-old) were obtained from farm and private flock's ducks in Alexandria Province were used in the pathogenicity and experimental studies.

Experimental infection design:

The experiment was performed to study the pathogenicity of the isolated *Pasteurella multocida* through oral administration and I/T inoculation by (10^7 cfu/ml) of 24hr broth culture of *Pasteurella multocida* isolate**,** while control ducks well be gave oral adminstration and I/T inoculation by sterile normal saline., unlimited foods and water are provided. The birds were kept in cages and observed for a period a week. A random samples of five ducks were slaughtered and exposed to post-mortem, parasitological and bacteriological examination, which proved their healthy status and free from diseases, the other birds were classified into 8 groups, each group contain 5 birds., the last group was kept as control. All inoculated ducks were kept under observation for 4-30 days. Clinical signs and post mortem lesions of dead birds were

recorded and trial for re-isolation of the inoculated organisms was conducted.

5- In vitro sensitivity of *Pasteurella multocida* isolates to different antimicrobial agents:

Antimicrobial susceptibility patterns were carried out using 10 isolates of *Pasteurella multocida* which isolated from examined samples and 10 chemotherapeutic discs produced by (Oxoid, 1982). The discs included Penicillin (1.5/iu), Ampicillin (10/ug) Lincomycin (15/ug), Ciprofloxacin (10/ug), Chloramphenicol (30/ug), Erythromycin (15/ug), Oxytetracycline (30/ug), Neomycin (30/ug), Gentamycin (10/ug) and Streptomycin (10/ug) One ml of 24hr. broth cultures was spread on the surface of Mueller-Hinton agar in accordance with the National Committee for Clinical Laboratory Standard (Bergan and Norris,1978). Chemotherapeutic discs were placed on the surface seeded agar. Plates were incubated aerobically at 37^0C for 24hr. The sensitivity judged according to the diameter of clearance zone around the discs according to Koneman *et al.* (1988) and Quinn *et al.* (1994).

RESULTS

Table 1: Percentage of *P. multocida* isolated from Waterfowl, water and sediment samples.

Source of samples	No of samples	No. of positive isolates	
		No	%
Waterfowl:	250	128	51.2
Ducks	150	92	61.3
Geese	100	36	36.0
Water	3o	4	13.3
Sediments	30	2	6.7
Total	310	134	43.2

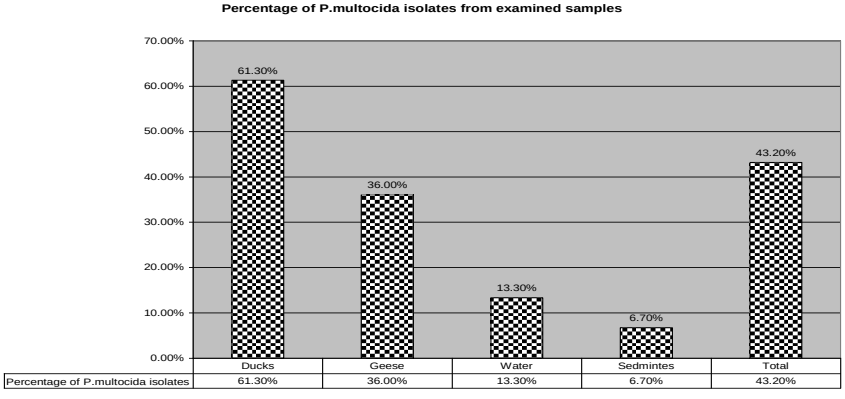

Table 2: Percentage of *P. multocida* isolated from waterfowl, (ducks and geese) according to healthy status.

Waterfowl								
healthy status	Ducks			geese			Total No of *P.multocida* isolate	
	No. of examined birds	No of *P.multocida* isolate		No. of examined birds	No of *P.multocida* isolate			
		No.	%		No.	%	No.	%
Apparently healthy	20	2	10	20	1	5.0	3	7.5
Diseased birds	90	60	66.7	50	15	30	75	53.6
Freshly dead birds	40	30	75.0	30	20	66.7	50	71.4
Total	150	92	61.3	100	36	36.0	128	51.2

Percentage of *P. multocida* isolates calculated according to number of healthy statue of each type

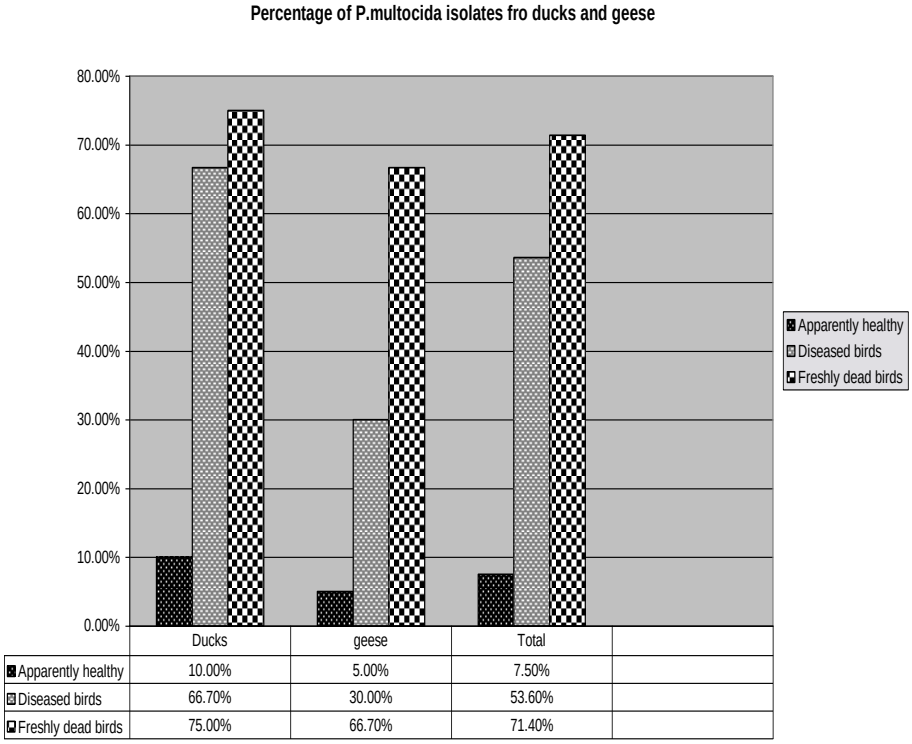

Table 3: Results of biochemical Tests used for identification of *Pasteurella multocida*

Test	*P. multocida*
Haemolysis on blood agar	-
Growth on MacConkey's agar	-
Indole production	+
Gelatin liquefaction	-
Hydrogen sulphide	+
Voges-Proskauer	-
Methyl red	-
Urease production	-
Glucose fermentation	+
Maltose fermentation	-
Lactose fermentation	-
Sucrose fermentation	+
Ornithine decarboxylase	+
Catalase production	+
Oxidase production	+
Motility	-

Table 4: Showing the results of pathogenicity of *P. multocida* on Swiss mice

Groups	No. of Swiss mice	Source of strain	Type of inoculation	Rout of infection	Does of inoculums	Mortality rate	
						No.	%
Group (1)	5	Apparently healthy	*p. multocida*	intraperitoneal	5ml (10^7 cfu/ml)	1	20.0%
Group (2)		Diseased birds				4	80%
Group (3)		Freshly dead birds				5	100%
Group (4) control			Normal saline		5ml	0	0.0%

Table 5: Showing results of pathogenicity of *P. multocida* in ducks

Groups	No of birds	Source of isolates	Type of inoculation	Rout of infection	Does of inoculums	Daily deaths post infection						No. of survivors	No. of death	Mortality rate
						1-4	5-15	16-20	20	25	25-30			
Group 1	5	Apparently healthy	*p.multocida*	Oral	1X10^7 cfu/ml)	0	0	0	0	0	0	5	0	0.0%
	5			I/T		0	1	0	0	0	0	4	1	20.0%
Group 2	5	Diseased birds		Oral		2	1	0	0	0	0	2	3	60.0%
	5			I/T		3	1	0	0	0	0	1	4	80.0%
Group 3	5	Freshly dead birds		Oral		4	0	0	0	0	0	1	4	80.0%
	5			I/T		5		0	0	0	0	0	0	100%
Group 4 control	5			Oral		0	0	0	0	0	0	0	0	0.0%
	5			I/T		0	0	0	0	0	0	0	0	0.0%

Table 6: In vitro sensitivity of *Pasteurella multocida* isolates (No.10) to different antimicrobial agents.

Antibacterial agent	*P. multocida* (No.5)					
	Sensitive		Intermediate		Resistance	
	No	%	No	%	No	%
Penicillin (1.5/iu)	5	100	0	0.0	0	0.0
Ampicillin (10/ug)	3	60	2	20	0	0.0
Lincomycin (15/ug)	4	80	0	0.0	1	20
Ciprofloxacin (10/ug)	3	60	2	40	0	10
Chloramephenicol (30/ug)	0	0.0	0	0.0	5	100
Erythromycin (15/ug)	4	80	1	10	0	0.0
Oxytetracycline (30/ug)	4	80	1	20	0	0.0
Neomycin (30/ug)	0	0.0	0	0.0	5	100
Gentamycin (10/ug)	1	20	1	20	3	20
Streptomycin (10/ug)	0	0.0	1	20	4	80

DISCUSSION

Pasteurella sp. is normal flora of the respiratory and gastrointestinal tract of many species of domestic and wild birds so it has primary or secondary role in pneumonia of poultry and the virulence of pasteurella is related to the polysaccharide capsule that allow the organism to resist phagocytosis. (Bailey and Scott' s., 1994)

The severity and incidence of *P. multocida* infections may vary considerably depending on several factors associated with the host including species and age of the infected birds, the environment and the bacterial strain. No single virulence factor has been associated with the observed variation in virulence among strains. (Christensen and Bisgaard, 2000)

Diagnosis depends on isolation and identification of the causative bacterium, *Pasteurella multocida*. Presumptive diagnosis may be based on the occurrence of typical signs and lesions and/or on the microscopic demonstration of numerous bacteria in blood smears, or impression smears of tissues such as liver or spleen

Clinical signs and gross lesions of the examined ducks and geese showed:

Sick birds appear exhausted, drowsy, loss of appetite other birds have convulsions, swim in circles, loss of appetite,, lameness, swollen and edematous wattles and combs, difficult breathing, cyanosis, watery yellowish or green diarrhea, ruffled feathers, mucous discharge from the

mouth, Similar Symptoms reported by Friend. (1987), Takahashi *et al.* (1996) and Woo and Kim (2006) they also showed that similar signs.

The most prominent lesions were hemorrhages of various sizes are found on the heart, liver, gizzard, and intestines. The liver may enlarged and darkened in color; a small number of necrotic and inflammatory foci were also discovered on the liver surface, heart and spleen. The lower portions of the digestive tract commonly contain thickened yellowish fluid, mild ascites and firm material collection in joints. Similar symptoms were recorded by Friend. (1987) and Kwon and Kang (2003) they reported clinical sings of Fowl cholera in all examined birds, including multifocal necrotic foci in the liver with enlargement, petechial hemorrhages on the heart, and mucoid exudates in the duodenal mucosa. Microscopically, there were hepatocytic necrosis with bacterial colonization, hemorrhage and necrosis in the myocardium, and hemorrhagic enteritis. Also Woo and Kim (2006) reported similar symptoms included orofacial edema, wattle and comb swelling, and respiratory symptoms. The cardiac air sac was filled with inflammatory materials, and serious pneumonic lesions were also found at the end of the lung lobe

Microscopical examination of blood samples and tissue smears of organs revealed bipolar staining bacillus and the pure colonies on blood agar plates showed transparent, glossy, and big colonies gave off a characteristic and sweet smell, Colonies range from 1 to 3 mm in diameter after 18-24 hours of incubation and are discrete, circular, convex,, translucen*t* which is characteristic of *P. multocida.* Results of biochemical test of pure isolate illustrated on Table (3). These results agree with those reported by (Bisgaard *et al.,* 1991; Murray *et al.,* 1995; and Woo and Kim, 2006). They identification of Pasteurella species included Gram staining, motility test, glucose fermentation test, oxidase and catalase reactions. The strains obtained were typed by using the following reactions; ornithine decarboxylation, urease production, indole formation and production of acid from sucrose, maltose, mannitol, dulcitol, and sorbitol.

Bacteriological examination of samples according to the morphological characters, biochemical reactions of the recovered *Pasteurella multocida* were Tabulated in Table (1) revealed 134 (43.2%) out of 310 samples were positive to *P. multocida.* The positive samples included, 128 (51.2%) out of 250 waterfowl, 4 (13.3%) out of 30 water samples and 2 (6.7%) out of 30 Sediment samples.

Wetlands have long been suspected to be an important reservoir for *Pasteurella multocida* and therefore the likely source of avian cholera outbreaks *P. multocida* has been isolated from the water and sediment of wetlands experience avian cholera epizootics and the bacteria can persist in wetland soil and water (Price and Brand 1984; Backstrand and Botzler, 1986)

The incidence of *P. multocida* in both water and Sediments samples in our study were (13.3%) and (6.7%) respectively. These results are higher than that obtained by Samuel *et al.* (2004) They attempted to isolate *P. multocida* from 440 water and 440 sediment samples collected from 10 wetlands during winter and spring seasons. They could not be isolate *P. multocida* from envy samples. In contrast, they could isolated *P. multocida* from 20 of 44 wetlands, including 7% of the water and 4.5% of the sediment samples on the other side Lehr *et al.* (2005) could not be isolated *P. multocida* from 786 water and 786 sediment samples during winter 1997, while during winter 1998 could be isolated *P. multocida* serotype 1 from three enzootic and three reference wetlands at percentage of 14 out of 396 (3.5%) water samples and one out of 396 (0.3%) sediment samples .

Table (2) revealed 128 *P. multocida* isolates from all waterfowl samples (ducks and geese) with allover incidence of (51.2%). Out of these 92 (61.2%) from ducks and 36 (36%) from geese examined samples. We could be isolated *P. mulotcida* from apparent healthy, diseased, and freshly dead ducks at percentages of (10.0%). (66.7%) and (75.0%) respectively while from geese were (5.0%), (30.0%) and (66.7) respectively.

Our results are higher than that obtained by Nakamine *et al.* (1992) they isolated *Pasteurella multocida* from Fifty (25%) of 200 Muscovy ducks during outbreak of fowl cholera in Okinawa Prefecture of Japan, Samuel *et al.* (1997) isolated *P. multocida* serotype 1 from an adult male goose and *P. multocida* serotype 3 from an adult female goose out of 298 lesser snow geese and also higher than recorded by (Muhairwa *et al.*, 1999) they detected of *P. multocida* in ducks at percentage of (7%) in all three zones investigated, Mbuthia *et al.* (2007) they could be isolated *P. multocida* from only four chickens out of 123 birds examined, while could not be isolated from 24 ducks examined on the basis of biochemical characterization. Samuel *et al.*, (2003) could be isolated *P. multocida* from apparently healthy geese. Also Lehr *et al.* (2005) found that a small proportion of 322 wild birds (<5%) were carriers of pathogenic *P. multocida.* On the basis of serology, an

additional group of these birds (<10%) were survivors of recent avian cholera infection. They also found these birds were carriers of P. multocida even in the absence of disease outbreaks while, our results are lower than that obtained by Takahashi *et al.* (1996) they isolated Nine strains of *Pasteurella multocida* from Muscovy ducks, 5 to 6 dead ducks at percentage of (83.3%) and 4 of 8 ducks with percentage of (50.0%) from ducks have clinical of subacute disease characterized by lameness, corneal turbidity, and depression, and Leotta *et al.* (2006) they could be isolated Fifty-five *P. multocida* gallicida, type A:1 from Eighty-six dead birds and one from water samples

Pathogenicity in vivo on mice

Results for the virulence of 3 strains of *P. multocida* on 3 groups of mice (each group contain 5 mice) within 24-48 hours after inoculation, recorded 20% mortality. by strain isolated from apparent healthy, 80% mortality with strain isolated from diseased birds and 100% with strain isolated from freshly dead birds, while no death from control group Table (4).

From direct smear, specimens of heart blood from dead mice were observed through the microscopic examination, bipolar staining bacillus were observed in all the specimens. This shows that there is a difference in mortality rate between strains isolated from examined ducks and geese due to difference in virulence of the strains.

Pathogenicity in ducks:

Results of experimental infection of the susceptible ducks, instullated in Table (5) that the *P. mulotcida* isolates were pathogenic on experimental ducks through oral administration and I/T inoculation with a variety of mortality rates. It showed that, the first group which inoculated with strain isolated from apparent healthy cases was revealed 0 to 20 % mortality, and the second group which, inoculated with strain isolated from diseased birds was ranged from 60 to 80%, while was 80 to 100% in the third group which inoculated with strain isolated from freshly dead birds, while no death recorded in control group.

The clinical symptoms, direct smear of heart blood from dead birds revealed gram-negative, bipolar staining bacillus and post-mortem pictures of dead birds, are similarly that showing in examined ducks and geese samples in our work and agree with those reported by the author Samuel *et al.* (2003) they found that virulence of *P. multocida* isolates from wetlands and Pekin ducks, ranged from (0 to 100%). In contrast, the virulence of isolates collected during the winter of 1997 from other wetlands ranged from (67% to 100%) and agree with reported those by

Wobeser (1997) who reported that virulence among strains within the same *P. multocida* serotype may be different. Samuel *et al.* (1997) reported that pathogenicity of the serotype 1 isolate was confirmed by inoculation in Pekin ducks (Anas platyrhynchos). while; serotype 3 isolate was non-pathogenic in Pekin ducks and could be isolated from pathogenic *P. multocida* serotype 1 from apparently healthy wild snow geese.

Antimicrobial drugs susceptibility

The extensive use of antibiotics as growth promoters and prophylactic agents for disease control in veterinary medicine has undoubtedly been responsible for large numbers of bacteria that have become resistant to different antibiotics. In-vitro tests the proper antimicrobial agents against *P. multocida* isolates, illustrated in Table (6) five strains were tested against 10 antimicrobial drugs. These strains somehow acquired a significantly high level of resistance to 3 types of antimicrobial drugs: Chloramephenicol (100%), Neomycin (100%) and Streptomycin (80%), while highly sensitive to 4 types of antimicrobial drugs which included, Penicillin (100%), Lincomycin (80%), Oxytetracycline (80%) and Ampicillin (80%). Some of these results are agreed with those obtained by (Morishita *et al.*, 1996) they reported that P. multocida isolates were susceptible to Penicillin G, Sulfisoxazole, Tetracycline, and Trimethoprim-sulfamethoxazole. While, Woo and Kim (2006) they found that *P. multocida* was of high level of resistance to 7 types of antimicrobial drugs: Kanamycin, Neomycin, Oxytetracycline, Tetracycline, Tobramycin, Doxycycline, and even Gentamicin.

Conclusion:

From the abovementioned results, it can be concluded that a variation in virulence and survival of *P. multocida* might explain why avian cholera outbreaks were observed without detecting pasteurellae in the environment. Virulence among strains within the same *P. multocida* serotype may vary.

Strict control on entry of feed bags, equipment and personnel is necessary to exclude infection with fowl cholera. As well as, rodents need to be controlled and free-flying birds excluded. Carcass collection to reduce bacterial contamination in wetlands is the current method used to control avian cholera in waterfowl. Other tools to control avian cholera have been suggested, including treatment of water chemistry to reduce the survival of *P. multocida* (Friend, 1999).

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