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**BACTERIAL AGENTS ASSOCIATED
WITH SINUSITIS IN WATERFOWLS AND TURKEYS
IN ASSIUT AND EL-MINIA GOVERNORATES**
(With 4 Tables and 1 Figure)

By

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**العوامل البكتيرية المصاحبة لالتهاب الجيوب الأنفية
في الطيور المائية والرومي في محافظتي أسيوط و المنيا**

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أجريت هذه الدراسة على تجمعات صغيرة من البط والرومي ذات أعمار مختلفة في عدة قرى في محافظتي أسيوط والمنيا وكانت كلها تعاني من التهاب الجيوب الأنفية . كانت البكتريا المعزولة من البط تتمثل في الميكروب القولوني (٢١,٦ %)، رايمريلا أناتيبستفر بمعدل ٥ % وميكوبلازما جاليسيبتكم (٥%). أما في حالة البط النامي الذي تراوح عمره بين ١ - ١٠ أسابيع فقد تم عزل عدة ميكروبات هي رايمريلا أناتيبستفر (١٠.٩%) والميكروب القولوني (٣,٦%). كان الميكروب القولوني هو البكتريا الوحيدة التي تم عزلها من الأوز بمعدل ٢٠%. في حالة الرومي البالغ تم عزل ميكروبات الميكوبلازما جاليسيبتكم (٤٢%) والميكروب القولوني (١٠%) والباستريللا المذيبة للدم (٤%). بينما تعددت العوامل البكتيرية التي تم عزلها من بداري الرومي وكانت بالترتيب ميكوبلازما جاليسيبتكم (١٨,٧%) ، سودوموناس (٧,١%)، رايمريلا أناتيبستفر (٥,٧%) بروتئوس مورجاني (٤,٢%) وأخيرا الميكروب القولوني بمعدل (٢,٩%). هذا وقد فشلت محاولة أحداث التهاب الجيوب الأنفية بميكروب الريمريلا أناتيبستفر في البط الصغير عن طريق التنقيط في الأنف بينما تبين فقط ظهور إفرازات من الأنف والعيون وتبع ذلك التهاب الأغشية السيروزية. عند إجراء اختبار حساسية البكتريا المعزولة للمضادات الحيوية المختلفة ظهرت الحساسية العالية عند استخدام مركبات الأموكساسيلين، نورفلوكساسين، أنروفلوكساسين، فليموكوين، وسيفالوسبورين في حالة الرايمريلا أناتيبستفر أما معزولات الميكروب القولوني فكانت عالية الحساسية لمركبات نورفلوكساسين، أنروفلوكساسين، و فليموكوين .

SUMMARY

Small collections from ducks and turkeys of variable ages in several villages at Assiut and El-Minia governorates were suffering from infraorbital sinusitis. The bacteria isolated from adult ducks were *E.coli* (21.6%), *Rimerilla anatipestifer* (5%) and *Mycoplasma gallisepticum* (5%). Bacteriological examination of growing ducks aged from 1-to-10 weeks of age revealed isolation of *R.anatipestifer* (10.9%) and *E.coli* (3.6%). *E.coli* was the only bacterium isolated from geese (20%). In case of adult turkeys, *Mycoplasma gallisepticum* (42%) was frequently recovered followed by *E.coli* (10%) and *Pasteurella haemolytica* (4%). Several bacterial agents were recovered from turkey poults and growing turkeys. These were *Mycoplasma gallisepticum* (18.7%), *Pseudomonas fluorescense* (7.1%), *Rimerilla anatipestifer* (5.7%), *Proteus morganii* (4.2%) and *E.coli* (2.9%). Intranasal inoculation of *R.anatipestifer* in ducklings resulted in sticky oculo-nasal discharges and subsequent serositis. *In vitro* sensitivity testing showed high susceptibility of *R.anatipestifer* to amoxicillin, norfloxacin, enrofloxacin, flumequine and cephalosprin, while *E.coli* isolates were highly susceptible to the action of norfloxacin, enrofloxacin and flumequine.

Key words: Bacterial agents, sinusitis, waterfowls, turkeys.

INTRODUCTION

Several infectious agents are considered to be the causes of several diseases in waterfowl. Infraorbital sinusitis, a common problem in waterfowls and turkeys, was observed in several villages in Upper Egypt. In turkeys, sinusitis is due to *Mycoplasma gallisepticum* (Kleven,1998), while in ducks, there are many agents incriminated in such problem and described in few reports (Ibrahim,1991; Aly,1995; Leibovitz,1972 and Bendheim *et al.*,1978). According to our field experience during the last few years, we have observed treatment failure and recurrence of disease conditions in spite of the use of the most common antimicrobials. Therefore, this study was done to isolate the possible associating bacterial agents and study their *in vitro* sensitivity.

MATERIALS and METHODS

Birds:

Different species of birds of different age groups were investigated in this study (Table. 1). Birds suffered from respiratory signs as oculo-nasal discharge and infraorbital sinusitis ranging from mild to severe, soft in early stages to hard in late stages, either unilateral or bilateral (Fig. 1). Samples were obtained from clinically diseased birds from several small collections in different villages of Assiut and El-Minia governorates.

Table 1: Birds investigated for sinusitis in Assiut and El-minia Governorates.

Species	Breed	Age (week)	No. Examined
Ducks	Native	25-35	60
Ducklings	Native	1-10	110
Geese	Native	22-30	10
Turkeys	Native	30-40	50
Turkey Poults	Native	3-7	70

Isolation and identification:

The fluids of sinus were aspirated by sterile syringe and examined by methylene blue stained smear. Culturing was done from exudates using tryptose broth (TB, Oxoid). Swabs from sinus were cultured on brain heart infusion broth (BHI, Oxoid) containing penicillin, thallium acetate and horse serum for the isolation of *Mycoplasma*. TB cultures were outplated on dextrose starch agar (DSA, Difco) and MacConkey's agar plates. BHI cultures were subcultured from broth enrichment for 3-5 days to BHI enriched agar medium. The dominant colonies were subjected for cellular and colonial morphology, serological, biochemical and sugar fermentation tests according to Cruickshank *et al.* (1975). Results are illustrated in table 2. Glucose fermentation, phosphatase test and arginine hydrolysis were done for *Mycoplasma* identification.

Experimental infection:

Five 7-day-old ducklings were inoculated intranasally using 10^8 CFU/ml of *Rimerilla anatipestifer* tryptone soy broth culture. Inoculated birds were kept for observation. Control birds were inoculated with sterile broth.

In vitro sensitivity test:

The antibacterial agents used in this study are described in Table 4.

RESULTS

Clinical picture:

Diseased birds showed respiratory signs. Sometimes, conjunctivitis and sinusitis were noticed. Unilateral or bilateral sinusitis was always

recorded with variable degree. Lacrimation and nasal exudate were usually noticed in acute or early stages followed by severe swelling of the sinus which was easily aspirated. By time, progressive caseation may occur to the swollen sinus (Fig.1)

Isolation and identification:

In acute or recent cases especially in growing ducks, methylene blue stained film of exudate showed bipolar short coccobacilli in heavy population. Sometimes, mixed population of bacteria were noticed. Identification was done using different criteria. Results are shown in Table 2. Bacterial species isolated from different species under investigation are described below.

Duck: Bacteria isolated were *E.coli*, *Mycoplasma gallisepticum* and *R. anatipestifer*.

Growing ducks: The most frequently isolated bacteria were *R. anatipestifer* and *E.coli*.

Geese: *E.coli* was the only isolated bacteria.

Turkeys: Frequently isolated bacteria were *Mycoplasma gallisepticum*, *Pasteurella haemolytica* and *E.coli*.

Turkey poult: Several bacterial agents were isolated but the most frequently isolated bacteria were *Mycoplasma gallisepticum*, *Pseudomonas fluorescense*, *Rimerilla anatipestifer*, *Proteus morganii* and *E.coli*. Results are listed in Table 3.

Experimental infection:

Signs started to appear as sticky oculo-nasal discharges and conjunctivitis. Sinusitis did not develop but necropsy revealed fibrinous inflammation of the serous membranes of the liver, heart and abdominal air sac.

In vitro sensitivity:

E.coli isolates were highly susceptible to the action of enrofloxacin, norfloxacin, danofloxacin, flumequine and lincospectin. *Rimerilla anatipestifer* was susceptible to amoxicillin, norfloxacin, enrofloxacin, flumequine and cephalosporin. Complete susceptibility to all tested antibiotics by *Pasteurella haemolytica* were noticed. High resistance was recorded by *Pseudomonas fluorescense*. Results are illustrated in Table 4.

DISCUSSION

Sinusitis or infraorbital swelling is a problem or disease condition frequently observed in waterfowls and turkeys at the area of Upper Egypt (Assiut and El-Minia governorates). Little effort was given to such a problem. Ibrahim (1991) and Aly (1995) studied this condition as one of the most important problems observed in ducks. In the current study, the

authors tried to study this problem in regard to the species of birds (ducks, geese and turkeys), age of birds (growing and adult) as well as isolation of the possible bacterial agents. Adult ducks showing sinusitis revealed isolation of *E.coli*, *R.anatipestifer* and *Mycoplasma gallisepticum*. *R.anatipestifer* and *E.coli* were isolated from growing ducks. Few cases of geese were studied, and *E.coli* only was isolated. *M.gallisepticum*, *E.coli* and *P.haemolytica* were isolated from adult turkeys. Several bacterial agents were isolated from turkey poults. These agents were *M.gallisepticum*, *Pseudomonas fluorescense*, *R.anatipestifer*, *Proteus morgani* and *E.coli*. It is clear that *Mycoplasma gallisepticum* infection occurs mostly and of first order in turkeys, both adult and growing. Also *R.anatipestifer* is one of the major aetiological agents in growing and breeding ducks. *E.coli* is one of the most important bacterial pathogens associated with such a problem while *Pseudomonas* is unusual pathogen associating sinusitis referring to the former literatures. Most of the literature confirmed the isolation of *M.gallisepticum* from turkey sinusitis (Jordan and Amin, 1980; Kleven and Glisson, 1997 and Kleven, 1998). *Mycoplasma anatis* was isolated by many authors from clinical cases of duck sinusitis (Goldberg *et al.*, 1995 and Samuel *et al.*, 1996). Isolation of *Mycoplasma gallisepticum* from duck sinusitis was supported by Yamada and Matsuo, 1982; Cookson and Shivaprasad, 1994 and kleven and Glisson, 1997). In Egypt, Shahata (1977) was successful in isolating *P. anatipestifer* from ducks. Ibrahim (1991) and Aly (1995) reported on isolation of *Pasteurella anatipestifer* from duck sinusitis. Few literatures described *P.anatipestifer* isolation from ducks and turkeys suffering from sinusitis (Leibovitz, 1972; Bendheim *et al.*, 1978; Ibrahim, 1991), while several authors isolated *P.anatipestifer* from ducks and turkeys suffering from respiratory signs and sinusitis (Sandhu and Layton, 1985; Chudhury and Mahanta, 1985 and Hatfield and Morris, 1988;). Several infectious agents are considered to be the causes of the disease in waterfowls (Astogra *et al.*, 1994). Therefore, many bacterial agents were recovered in this study, in which *E.coli* was the most frequently isolated. No available literature cited on *E.coli* isolation from cases of sinusitis but some of them indicated the association of *E.coli* in respiratory diseases in different birds. Droual and Woolcock (1994) recovered *E.coli* from lesions of trachea and sinus from commercial broiler flocks. Also Riddell *et al.* (1992) isolated *E.coli*, *Strept.*, *Staph.* and *Proteus* from excess mucoid material in the conjunctival sacs of goslings. John Barnes and Gross (1997) stated that *E.coli* often infects respiratory tracts of birds concurrently infected with various combinations of infectious agents including mycoplasmas. Isolation of *Pasteurella haemolytica* only from

turkeys with sinusitis represents infrequent finding. Heddleston (1975) stated that *P. haemolytica* has been isolated from turkeys with respiratory signs but some predisposing factors or concurrent disease are required before it becomes a problem. Nicolet and Fey (1965) and Hacking and Pettit (1974) could isolate *P. haemolytica* from different respiratory tissues such as lungs, sinus, trachea and air sac of diseased poultry. The present work is supported by John Barnes (1997) who stated that *Pseudomonas* is an important opportunist that produce respiratory infection including sinusitis in turkeys and conjunctivitis in chicks. Moreover, it associates with multicausal respiratory diseases of chicken and turkeys. Some authors found that *Proteus morganii* has been associated with respiratory diseases in chickens. Sinusitis is a multicausal problem due to isolation of different aetiological agents. Furthermore, it is difficult to reproduce by experimental inoculation as the authors found that experimental infection with *R.anatipestifer* isolated as a single pathogen from ducklings failed to reproduce sinusitis, but only conjunctivitis and nasal discharges. Kleven and Glisson (1997) stated that environmental factors play a significant role in interacting with infectious agents in the production of respiratory diseases in poultry.

It is clear that most of the isolated bacteria, except *Pseudomonas*, were completely susceptible to fluoroquinolones followed by lincospectin, cephalosporin, amoxicillin and gentamicin.

The present work may throw some light on the possible bacterial agents associated with sinusitis in waterfowls and turkeys. *R.anatipestifer*, *E.coli*, *Pseudomonas*, *Proteus* and *P.haemolytica* are several pathogens not frequently described in former literatures. These results may be helpful for antibacterial use to treat and control such a problem.

REFERENCES

- Aly, M. (1995): Epidemiological studies on sinusitis in ducks. Alex. J. Vet. Sci., 11 (4): 485-496.
- Astorga, R.J.; Cubero, M.J.; Leon, L.; Maldonado, A.; Arenas, A.; Tarradas, M.C. and Perea, A. (1994): Serological survey of infections in waterfowls in the Guadalquivir marshes (Spain). Avian Dis., 38 (2): 371-375.
- Bendheim, U.; Shoshan, A.E.; Baroutchieva, M. and Plessner, O. (1978): Pasteurella anatipestifer, a pathogenic agent in turkeys: Etiology, epizootiology and pathogenicity. Proceeding and abstracts of the XVI World Poultry Congress, Rio de Janeiro, vol., II, 1461-1467.

- Chudhury, B. and Mahanta, S. (1985):* Studies of the pathology of infectious serositis in ducklings. *Indian Vet. J.*, 62: 552-553.
- Cookson, K.C. and Shivaprasad, H.L. (1994):* Mycoplasma gallisepticum infection in chukar partridges, pheasants and pea fowl. *Avian Dis.*, 38 (4): 914-921.
- Cruichshank, R.; Duguid, J.P.; Marion, B.P. and Swain, R.H.A. (1975):* Medical microbiology, 12th ed. Vol.II. Published by Churchill Livingstone, Edinburgh, London and New York.
- Droual, R. and Woolcock, P.R. (1994):* Swollen head syndrome associated with E.coli and infectious bronchitis virus in the central valley of California. *Avian Pathology*, 23: 733-742.
- Goldberg, D.R.; Samuel.M.D.; Thomas, C.B.; Sharp,P.; Krapu, G.;Robb, J.R.; Kenow, K.P.; Korschgen, C.E.; Chipley, W.H. and Conroy, M.J. (1995):* The occurrence of Mycoplasmas in selected wild North American waterfowl. *J.wild.I.Dis.*, 31 (3): 364-371.
- Hacking, W.C. and Pettit, J.R. (1974):* Cited by Heddleston, K.L. and Rhoades, K.R. (1978). *Avian Pasteurellosis*, In: *Diseases of Poultry*, 7th ed.M.S. Hofstad, H.J. Barnes, B.W. Calnek, W.M. Reid and H.W. Yoder. Iowa State University Press, Ames, Iowa. pp.181-197.
- Hatfield, R.M. and Morris, B.A. (1988):* Influence of the route of infection of Pasteurella anatipestifer on the clinical and immune responses of white peking ducks. *Vet. Sci.*, 44: 208-214.
- Heddleston, K.L. (1975):* Pasteurellosis. In: *Isolation and identification of avian pathogens*. 1st ed.(Hitchner, S.P., Domermuth, C.H., Purchase, H.G. and Williams, J.E.) Am. Ass.of Avian Pathologists, college station, Texas. pp. 38-51.
- Ibrahim, R.S. (1991):* Some studies on avian pasteurellosis. M.V.SC., Dept. of Poultry Dis. Fac. Vet. Med., Assiut University.
- John Barnes, H. (1997):* Other bacterial diseases. In: *Diseases of poultry*. 10th ed. B.W. Calnek, H. John Brnes, C.W. Beard, L.R. McDougald and Y.M. Saif. Iowa State University Press, Ames, Iowa. pp.291-292.
- John Barnes, H. and Gross, W.B. (1997):* Colibacillosis. In: *Diseases of poultry*. 10th ed. B.W. Calnek, H. John Brnes, C.W. Beard, L.R. McDougald and Y.M. Saif. Iowa State University Press, Ames, Iowa. pp.131-141.
- Jordan, F.T.W. and Amin, M.M. (1980):* a survey of Mycoplasma infections in domestic poultry. *Res. Vet. Sci.*, 28: 96-100.

- Kleven, S.H. (1998):* Mycoplasmas in the aetiology of multifactorial respiratory disease. *Poultry Science*, 77 (8): 1146-1149.
- Kleven, S.H. and Glisson, J.R. (1997):* Multicausal respiratory diseases. In: *Diseases of poultry*. 10th ed. B.W. Calnek, H. John Brnes, C.W. Beard, L.R. McDougald and Y.M. Saif. Iowa State University Press, Ames, Iowa. pp.1008-1009.
- Leibovitz, L.B.A. (1972):* A survey of the so called anatipestifer syndrome. *Avian Dis.*, 16: 836-851.
- Nicolet, I. And Fey, H. (1965):* Role of *Pasteurella haemolytica* in salpingitis of fowls. *Schweizer Arch. Tierheilk.*, 107: 329-334.
- Riddell, C.; Hurk, J.V.; Copeland, S. and Wobeser, G. (1992):* Viral tracheitis in goslings in Saskatchewan. *Avian Dis.*, 36: 158-163.
- Samuel, M.D.; Goldberg, D.R.; Thomas, C.B.; Sharp, B.; Robb, J.R.; krapu, J.L.; Nersessian, B.N.; Kenow, K.P.; Korschgen, C.E.; Chipley, W.H. and Conroy, M.J. (1996):* Exposure of wild waterfowl to *Mycoplasma anatis*. *J. Wild I. Dis.*, 32 (2): 331-337.
- Sanhu, T.S. and layton, H.W. (1985):* Laboratory and field trials with formaline inactivated *E.coli* (O78)- *Pasteurella anatipestifer* bacterin in white pekin ducks. *Avian Dis.*, 29 (1): 128-135.
- Shahata, M. A. (1977):* Studies on fowl cholera in ducks and its control. M.V.Sc., Dept. of Poultry Diseases, Faculty of Vet. Med., Assiut University.
- Yamada, S. and Matsuo, K. (1982):* Experimental infection in ducks with *Mycoplasma gallisepticum*. *Avian Dis.*, 27 (2): 405-408.

Table 2: Physiological, biochemical and sugar fermentation tests.

Test	E.coli	R.anatipestifer	P.haemolytica	Proteus	Pseudomonas
Morphology	-ve	-ve coccobacilli	-ve	-ve	-ve
Motility	+	-	-	swarmig	+
Haemolysis	-	-	+	-	V
MacConkey	F	-	NF	NF	NF
H ₂ S	-	-	+	+	-
Urease	+	+	-	+	ND
Catalase	+	+	ND	ND	+
Indole	+	-	-	+	-
EMB	+	ND	ND	ND	ND
MR	+	-	-	ND	-
Citrate	+	-	ND	-	ND
LM	ND	Alkaline	Acidic	ND	ND
Gelatin liq.	+	+	ND	-	+
Glucose	+	-	+	-	-
Arabinose	+	-	-	ND	ND
Mannitol	+	-	+	-	ND
Mannose	+	-	+	ND	ND
Lactose	+	-	+	-	-
VP	-	ND	ND	ND	-
NO ₃	ND	-	ND	ND	+

F: lactose fermenter. NF: non-lactose fermenter. V: variable. ND: not determined.

-ve: Gram negative bacilli.

Table 3: Frequency of different bacterial agents isolated from different birds showing sinusitis.

Species	Ducks		Ducklings		Geese		Turkeys		Poults	
	No.	%	No.	%	No.	%	No.	%	No.	%
<i>M.gallisepticum</i>	3/60	5	-	-	-	-	21/50	42	13/70	18.7
<i>P.haemolytica</i>	-	-	-	-	-	-	2/50	4	-	-
<i>R.anatipestifer</i>	3/60	5	12/110	10.9	-	-	-	-	4/70	5.7
<i>E.coli</i>	13/60	21.6	4/110	3.6	2/10	20	5/50	10	2/70	2.9
<i>Proteus</i>	-	-	-	-	-	-	-	-	3/70	4.2
<i>Pseudomonas</i>	-	-	-	-	-	-	-	-	5/70	7.1

Table 4. In vitro sensitivity testing.

	<i>E.coli</i>	<i>R.anatipestifer</i>	<i>P.haemolytica</i>	<i>Proteus</i>	<i>Pseudomonas</i>
Enrofloxacin	S	S	S	S	I
Norfloracin	S	S	S	I	I
Flumequine	S	S	S	I	R
Oxalinic acid	I	I	S	I	R
Amoxicillin	I	S	S	I	R
Lincospectin	I	I	S	I	I
Gentamicin	I	I	S	I	R
Cephalosporin	I	S	S	I	I
Trimethazole	R	I	S	R	R

S: susceptible. I: intermediate. R: resistant.

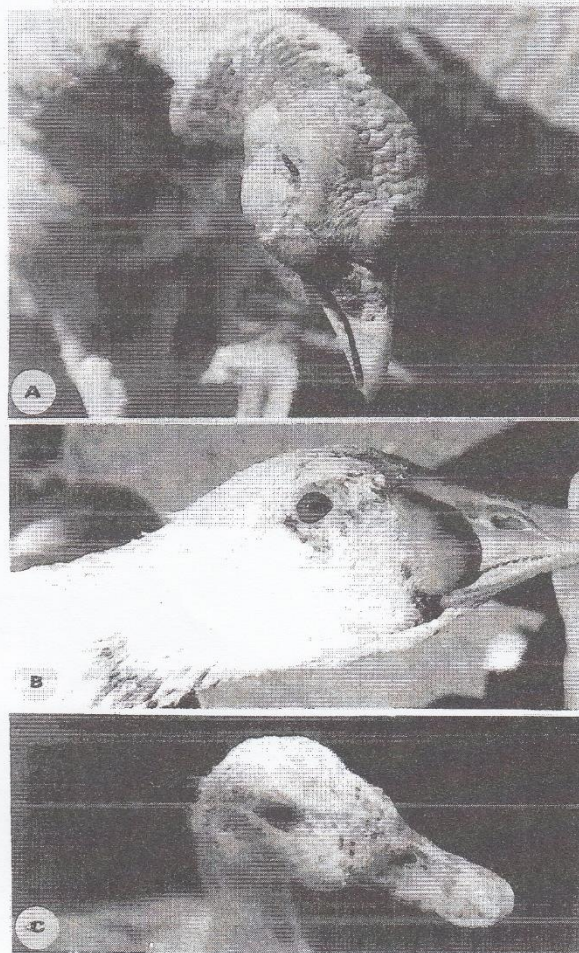


Fig.1. Sinusitis in turkeys (A), geese (B) and ducks (C).