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EPIDEMIOLOGICAL STUDIES ON BACTERIOLOGICAL ASPECTS OF AIR SACCULITIS IN CHICKENS

(With 5 Tables and 4 Figures)

By

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دراسات وبائية على المسببات البكتيرية لالتهاب الأكياس الهوائية في الدواجن

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أجريت هذه الدراسة الوبائية على خمس مزارع دواجن بمحافظة الإسماعيلية وكانت الاعراض المميزة أعراض تنفسية مصحوبة بالتهابات معوية. كما أسفر التشريح المرضي للطيور المصابة عن وجود صورة من التسمم الدموي. وبإجراء الفحص البكتيري تم عزل الميكروب القولوني (٦٠ & ٤٠%)، بينما عزل ميكروب الاورنيسو بنسبة (٢٦,٦ & ٢٠%) من كتاكيت التسمين والدجاج البياض على التوالي. كما أسفرت نتائج تصنيف الميكروب القولوني إلى ٨٥ عتره وكانت كالأتي : سبعون صنفوا إلى ٦ أنواع مختلفة و١٥ عتره غير محدده. وكل العترات المعزولة من الميكروب القولوني قد تم تقييم مدى ضراوتها باستخدام صبغة الكونجو والنشاط التحليلي للدم والقدرة على حدوث التسمم. كما تم استخدام المضادات البكتيرية على الميكروبات المعزولة. وقد أجريت العدوى التجريبية لعتره الاورنيسو التي تم فحصها وتسجيل نتائجها أسبوعيا". وقد أسفر العلاج في التجربة بالانروفلوكساسين عن نجاحه وحدوث الشفاء من الأعراض التنفسية. ومن تلك النتائج يمكن استنتاج أن التهاب الأكياس الهوائية يكون منتشرا في كتاكيت التسمين والدجاج البياض بمحافظة الإسماعيلية. وان الميكروب القولوني هو الميكروب الأول في حدوث المرض ويليه ميكروب الاورنيسو. لذلك يجب إتباع الأسلوب الأمثل في النظافة لتقليل عدد الميكروبات المحيطة بالطيور.

SUMMARY

This study was carried out on five poultry farms at Ismailia Governorate suffering from respiratory symptoms accompanied with gastroenteric findings. Post mortem findings revealed septicemia. Bacteriological examination revealed the isolation of *E. coli* (60 %& 40%) while ORT

was (26.6% & 20%) and *E. coli* concomitant with ORT were (18.6% & 12%) in broilers and layer respectively. The eighty-five *E. coli* isolates were as follow: seventy were identified to six different serotypes, O78, O26, O1, O2, O157 and O111, with different frequencies and fifteen untypable. All *E. coli* isolates tested for evaluation of it's virulence by Congo red binding assay, Hemolytic activity, Invasiveness assay and Enterotoxins assays. The in vitro Antimicrobial resistance patterns of the isolates were reported. Experimental infections of *O. rhinotracheale* were examined and scored weekly. Recovery from respiratory disease was overall most successful after Enrofloxacin treatment. It could be concluded that air sacculitis is prevalent among broiler and layers chickens at Ismailia province. *E. coli* is the most commonly incriminated agent and *Ornithobacterium rhinotracheale* stands as the second most important pathogen. Therefore, management and sanitation practices designed to reduce the number of these types of organisms in the birds' environment are necessary.

Key words: *Poultry, E.coli, Ornithobacterium rhinotracheale, and Air sacculitis*

INTRODUCTION

"Air sacculitis" means any infection or inflammation of the bird air sacs, which mainly appear in chronic cases. Poor blood supply to the air sacs makes removal of inhaled microorganisms very difficult by the bird's immune system. Signs of air sacculitis may be present at rest or only after flying, coughing, wheezing, and labored respiration (Pesek, 2000). Air sacculitis is a leading mortality and a significant cause of carcass condemnation in broilers. Air sacculitis is a respiratory disease of poultry, frequently caused by *E. coli*. However, outbreaks of air sacculitis caused by *E.coli* acting as a primary pathogen have been documented (Stebbins *et al.*, 1992). It is characterized by thickened, inflamed air sacs with fibrinous exudates, pericarditis and perihepatitis as sequalae of a coli- septicemia (Gross, 1994). Biological and environmental stresses such as viral infection, overcrowding, and poor ventilation predispose birds to *E. coli* infections (Mellata *et al.*, 2003). Escherichia coli causes a variety of diseases in poultry, including respiratory tract infection, Omphalitis, swollen-head syndrome, enteritis, septicemia, and cellulites (Gross, 1994 and Norton, R.A. 1997) and these diseases are responsible for major economic losses in the chicken industry.

Ornithobacterium rhinotracheale (ORT), which is a respiratory pathogen, has been described by Vandamme *et al.* (1994). It was initially regarded as a Pasteurella-like organism. ORT can cause severe respiratory clinical signs in turkeys and chickens (Hinz *et al.*, 1994 and Sprenger *et al.*, 1998). ORT was proven to be a primary pathogen in broilers (Van Veen *et al.*, 2000) and potentially pathogenic for fowl (Sprenger *et al.*, 1998; Van Empel *et al.*, 1999 and McMullin, 2004). Although ORT infections are frequently associated with other respiratory diseases, ORT has sometimes been isolated from asymptomatic flocks (Charlton *et al.*, 1993 and McMullin, 2004). *ORT* is often overgrown by *E. coli*, Proteus spp. and Pseudomonas spp., which have a rapid growth rate, especially in materials taken from hens (*Türkyilmaz, 2005*). Blood-stained mucus in the mouth is an occasional finding. The organism has been isolated from the partridge, pheasant, pigeon, rook, quail duck, ostrich, goose, guinea fowl, chicken and turkey (Vandamme *et al.*, 1994; Van Empel *et al.*, 1999; Charlton *et al.*, 1993 and McMullin, 2004). The disease has been described in many countries (Charlton *et al.*, 1993 and Erganifl *et al.*, 2002 and McMullin, 2004). In Turkey, ORT isolations were first obtained from two commercial pullets aged 12 and 15 weeks *(Türkyilmaz, 2005)*. The most common post mortem lesions were pneumonic lungs, pleuritis with accumulation of creamy "yoghurt-like" exudates (Hafez, 2002).

The aim of this work to investigate the bacterial causes of air sacculitis in broiler and layer at Ismailia province and their associated with antimicrobial susceptibility in affected chickens and application of antibiotic of choice.

MATERIALS and METHODS

1-History and clinical examination:

A total number of 125 chickens (moribund and freshly dead) were clinically examined in this study. Broiler samples (75 broilers) were collected from three broiler chicken farms (aged from 17 to 35 days). Layer samples (50 layers) from two layers farms (aged from 18-24 months). The collected samples were mainly from birds suffering from respiratory signs and swollen head accompanied with gastroenteric findings and cyanosed comb and wattles.

2-Samples:

Tracheal swabs, lungs and air sacs, liver, and heart blood were collected from 75 broilers, and 50 layers for bacteriological examination

3-Bacteriological examination:

3.1- Isolation of *E. coli*: was carried out according to Quinn, *et al.* (2002). Where the serological typing of the *E. coli* isolates was carried according to Edwards and Ewing (1972).

3.2- Isolation of ORT: Tracheal swabs were aseptically inoculated into brain heart infusion broth supplemented with 10 µg/ml Gentamycin according to Back *et al.* (1997) and incubated at 37°C for 24 -48 hours., under 7.5–10% CO2 tension by using gas bags in candle jar according to Vandamme *et al.* (1994); Traverse *et al.* (1996) and Zorman-Rojs *et al.* (2000). Then that loop full from the cultured broth was streaked onto blood agar supplemented with 7% sheep blood and 10 µg/ml gentamycin (to inhibit growth of other bacteria). The plates were incubated in a 7.5–10% CO2 atmosphere at 37°C for at least 48 hours. Storage of isolates was according to Chin *et al.* (2003).

3.3- *E. coli* serotypes were tested for evaluation of its virulence by application of:

A- Congo red binding assay (Berkhoff and Vinal, 1986). Tested strains were examined for its growth on Congo red medium. The reaction appeared after 24 hrs of incubation at 25°C and left for 4 days the CR positive was in-dicated by the development of bright or orange red colonies, and the negate-ve isolate appeared white colonies.

B- Hemolytic activity (hemolysin assays) according to Beutin *et al.* (1989).

C- Invasiveness assay according to Feingold and Baron (1986): The ability of *E. coli* strain to invade epithelial cell were tested in rabbit eye model "Sereny Test". Cultures were grown onto nutrient agar plates and were washed off in phosphate buffer saline to give a suspension of approximately 10^8 cfu/ml. A single drop from the suspension was allowed to fall into the eye of a rabbit while the eye lid was held a part. The other eye was dropped by phosphate buffer saline and kept as a control. The invasive organism was allowed to penetrate the epithelial cells, causing a purulent and oxidative conjunctivitis and compared with the other normal eye.

D- Enterotoxins assays: The ability of the isolated strains of *E. coli* to produce enterotoxins was assayed by the infant mouse test according to Erganis *et al.*, (1986). A volume of 0.1 ml of culture filtrates of *E. coli.* Isolates was injected through the abdominal walls into the milk-filled stomach of infant mice, which were 2 to 4 days old. Also, there were two infant mice injected by 0.1 ml of saline and were used as negative control. After 4 hours, the mice were sacrificed and the entire intestine

was removed. The intestine and the remaining body were weighed to calculate the ratio of intestine weight/ remaining body weight. A ratio greater than 0.085 was recorded as positive test for enterotoxins.

4- Antimicrobial susceptibility tests: The Bauer–Kirby technique (Bauer *et al.*, 1966) was adopted. Muller–Hinton agar (Oxoid, Basingstoke, UK) was prepared in a uniform thickness (4 mm) for testing of *E. coli* isolates, whereas 5% sheep blood agar was prepared for *ORT*. The following anti-microbial agents (ARCOMEX, co.), which represent the commonly used antibiotics, were tested: Enrofloxacin (5 µg), Ofloxacine (5µg), Amikacine (30µg), Neomycin (30µg), Flumequine (30µg), Amoxicillin (10), Tetracycline (30 µg), Gentamycin (10µg), Oxytetracycline (30 µg), Danofloxacin (5µg), Sulfamethoxazole-trimethoprim (5 µg), Cholormphenicol (30 µg), and Ampicillin (10 µg).

5- Pathogenicity test of *O. rhinotracheale*. Forty five healthy Cobb chickens 21 days old were used for that trails. Five birds from them were tested before experiment and proved free from ORT organism. The other birds were equally divided into four groups: The 1st group challenged by aerosol with 1ml of whole culture of brain heart infusion count of approximately 10^8 c.f.u/ml according to El-Sukhon *et al.* (2002). The second and the third groups were inoculated with aerosol route and treated with Amoxicillin, Enrofloxacin. The fourth group was inoculated with saline and kept as negative control. Drugs in the second and the third groups were applied into drinking water for five successive days. Chickens kept under observation for 3 weeks post infection for clinical signs and mortality. Two chicks were sacrificed at 7, 14 and 21 days post infection and all dead chicks were examined for score lesions. Re-isolation of ORT from experimentally infected birds was done. Score lesions post infection was done according to Van Veen *et al.*, (2000). As follow: Lung = 0 (no abnormalities), 1=unilateral pneumonia, 2=bilateral pneumonia. for Trachea=0 (no abnormalities), 1= Exudates in trachea, 2=trachea filled with exudates, Air sacs 1= fibrinous exudates

6- Therapeutic drugs: Enrofloxacin (Baytrail®10%) Bayer 10mg/kg body weight. Amoxicillin 0.1% (Pfizer) were added in drinking water.

RESULTS

The clinical signs and post mortem findings: among the affected birds varied according to the severity of the disease. Facial edema, conjunctivitis, swollen sinuses beside closed eyes, diarrhea,

conjunctivitis and cyanosed comb and wattles were the most common signs. Other signs as emaciation, dullness, inappitance and recumbence before death were recorded. Moreover, some respiratory signs in the form of sneezing, coughing and gasping were observed in some birds. Postmortem findings from naturally infected dead chicks or layers revealed air sacculitis, pneumonia, tracheitis, edema in face and sinus and the head appear cyanotic, inflammation of oviduct and a picture of septicemia, congestion of the liver and lung with enlargement of the heart and thick pericardium were seen. Morbidity in farms ranged from 4-7% and mortality ranged from 5-12% (Table 1).

Bacteriological examination showed that 65 (45 broiler chicks and 20 layer) E. *coli* isolates from air sacculitis cases. 30 strains were identified as *O. rhinotracheale* (10 broiler chicks and 20 layers). 20 samples (14 broiler chicks and 6 layers) were showed to be mixed *E. coli* with *O. rhinotracheale* (Table 2). Seventy from the 85 *E. coli* isolates were identified and belonged to 6 different serotypes, namely O78,O26, O1,O2, O157and O111, with frequencies of 24, 16, 12, 10, 5and 3 respectively (Table 3). However, the remaining 15 *E. coli* isolates were untypable.

Biological characteristics and virulence factors of *E. coli* isolated from diseased, dead and slaughtered birds were tabulated in Table (3). Enterotoxins assays revealed that two *E. coli* strains recovered from birds were entero-toxigenic and caused accumulation of fluid in the intestinal tract of infant mice belonged to serotype O26 (16/70) 22.86% and O157 (4/70) 5.71%. The results of antibiogram for isolated microorganisms recovered from diseases, dead and slaughter birds were illustrated in Table (4).

Pathogenicity test of *O. rhinotracheale*: The results of clinical signs in experimental infection with ORT non treated group revealed the same signs as natural infection, figure (1, 2, 3, and 4) during the observation period (21 days). Mortality was 20% (2/10) and significant decrease in body weight after 14 days of infection. While in-group 2 (infected and treated with Amoxicillin) the mortality was 10% (1/10) without significant decrease in body weight, while the third treated group with Enrofloxacin (Baytrail®10%) showed improvement in health condition, no mortality and non significant decrease in body weight. The results of postmortem lesion were similar to the natural infection (air sacculitis, pneumonia, pericarditis and liver congestion). The re-isolation trials were positive from trachea, air sacs, and lungs from the infected non treated group.

Table 1: Bird's morbidity %, and mortality % from affected flocks in Ismailia province.

Flocks	No. of bird/flock	Age	Morbidity %	Mortality %
1) Broiler	15.000	17-18 days	6%	5%
2) Broiler	10.000	25 days	5%	10%
3) Broiler	25.000	35 days	4%	12%
4) Layer	20.000	18 month	7%	6%
5) Layer	24.000	24 month	5%	8%

Table 2: Incidence of *E.coli* and ORT from different organs from both broiler and layer flocks.

Species / Organs	Broiler n=75			Layers n=50		
	E.coli	ORT	*E.coli*+ ORT	*E.coli*	ORT	*E.coli* + ORT
Tracheal swabs	6	7	2	0	2	2
Lung and Air sacs	8	13	8	0	8	3
Liver	9	0	0	5	0	0
Heart	22	0	4	15	0	1
Total	45	20	14	20	10	6
Percent	60%	26.6%	18.6%	40%	20%	12%

Table 3: Biological characteristics of *E.coli* (85 isolates) sero-groups isolated from diseased, dead and slaughter birds.

Serogroup	Total no. of isolates		No. of isolates				Congo red assay		Hemolysin production					
	No.	%	Chicks N= 55		Layer N=30				α- hemolysin		β- hemolysin		Negative hemolysin	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
O78	24	28.2	15	27.27	9	30	24	100	0	0	19	79.17	5	27.7
O26	16	18.8	10	18.1	6	20	16	100	4	25	10	62.5	2	11.1
O1	12	14.1	9	16.36	3	10	11	100	0	0	10	71.43	1	5.5
O2	10	11.8	7	12.7	3	10	11	100	0	0	6	60	5	27.7
O157	5	5.88	4	7. 3	1	3.3	5	100	0	0	2	50	3	16.6
O111	3	3.5	2	3.6	1	3.3	3	100	0	0	1	50	2	11.1
untypable	15	17.6	8	14.5	7	23.3	--	--	--	--	--	--	15	100
Total	85	100	15	68.5	30	31.4	85	100	4	7.3	48	56.47	33	38.8

No. =positive number percentage was calculated according to number of examined *E.coli*

Table 4: Antibiogram for isolated microorganisms

Antibiotic disc	Conc.	Sensisitivity of *E.coli* Serotyping						Sensitivity of ORT
		O_{78}	O_{26}	O_{157}	O_{111}	O1	O2	
Enrofloxacin	5 µg	SS	SS	SS	SS	SS	SS	SS
Ofloxacine	5 µg	S	S	S	SS	S	S	R
Amikacine	30 µg	SS	SS	SS	SS	SS	SS	R
Neomycin	30 µg	S	S	R	R	R	R	S
Flumequine	30 µg	S	SS	S	S	S	S	R
Amoxicillin	30 µg	R	R	R	R	S	S	SS
Tetracycline	30 µg	R	R	R	R	R	R	SS
Gentamycin	10 µg	SS	S	SS	S	S	S	R
Oxytetracycline	30 µg	R	R	R	R	R	R	S
Danofloxacin	5 µg	R	R	R	R	R	R	SS
Sulfamethoxazole - Trimethoprim	5 µg	S	S	R	R	R	S	R
Cholormphenicol	30 µg	S	S	R	R	S	S	S
Ampicillin.	10 µg	R	R	R	R	R	R	S

Legend: SS=highly sensitive S =sensitive R= resistant

Table 5: Experimental aerosol inoculation for 21 –day old chicks with ORT

Groups	No. of chicks	Dose	Lesion score						Mortality	
			1st week		2nd w		3rd w			
			lung	trachea	lung	trachea	lung	trachea	No.	%
(1)	10	$1x10^8$	1	1	1	2	o	1	2	20%
(2)	10	$1x10^8$	0	1	1	1	0	1	1	10%
(3)	10	$1x10^8$	0	0	0	1	0	0	0	0%
(4)	10	-	0	0	0	0	0	0	0	0%

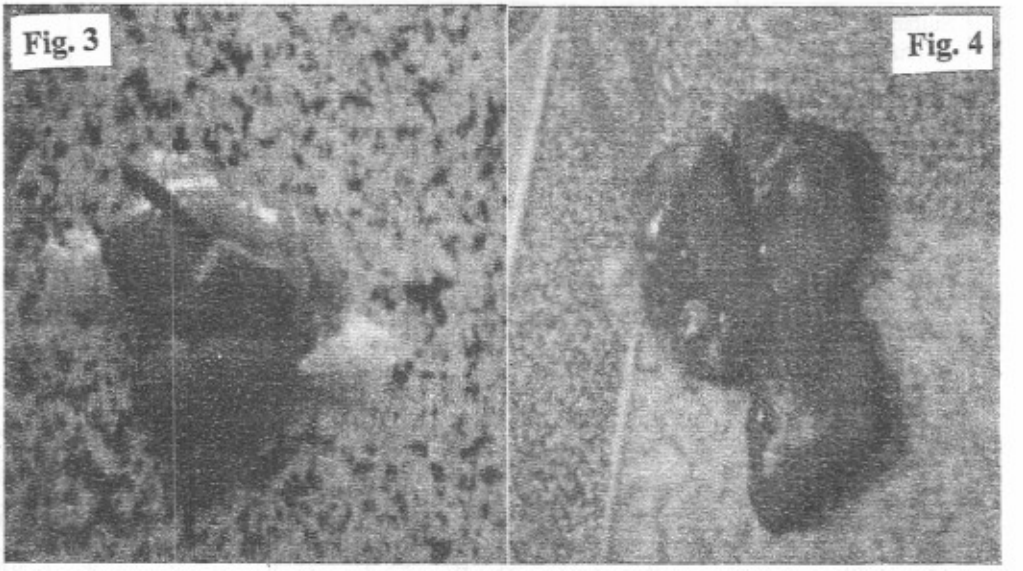

Fig. 1, 2: showed cyanosed head, facial edema, conjunctivitis, swollen sinuses beside closed eyes.

Fig. 3: showed sever congestion of liver, very clear pericarditis and perihepatitis.

Fig. 4: showed lung congestion pneumonia white exudates and air sacculitis.

DISCUSSION

Respiratory diseases are one of the most common disease problems affecting poultry world wide. Respiratory diseases result in serious economic losses to the poultry industry worldwide (Rosenberger, 1985). Various bacterial and viral pathogens are encountered in respiratory disease in domestic poultry (Chin and Droual 1997 and El-Sukhon *et al.* 2002). Colibacillosis is a respiratory diseases causing major problems, which face poultry industry. It is responsible for drastic chicken morbidity and mortality in Egypt.

Ornithobacterium rhinotracheale has been isolated in 1994 by Vandamme *et al.* (1994) and named ORT. It has recognized in many countries and is considered an important agent in respiratory diseases in chickens and turkeys (Joubert *et al.*, 1999). In Egypt, El Gohary and Awaad (1998) found that ORT has been incriminated as possible causative agent in respiratory diseases complex either alone or in synergy with other infectious or non infectious agent.

Table (2) showed the incidence of *O. rhinotracheale* from broiler chickens and layers suffering from airsacculitis was (26.6%), and (20%), respectively. ORT isolation in association with the isolation of *E. coli* represent 18.6 % and 12% respectively. Our results agreed with such an association of *O. rhinotracheale* with *E. coli* was reported by Sakai *et al.* (2000) and El-Sukhon *et al.* (2002).

Isolation of *E. coli* in a much higher percentage (60% & 40%), more than that of *O. rhinotracheale* (26.6% & 20%), because *E. coli* is common in the upper respiratory tract of chickens, and the stress factors may facilitate its spread with possible subsequent air sacculitis (Rosenberger *et al.*, 1985). The obtained results were consistent with the results recorded in some other parts of the world (Lambie *et al* 2000, Sakai *et al.*, 2000 and El-Sukhon *et al.*, 2002). Different serotypes of *E. coli* associated with respiratory infection in chickens were reported by Allan *et al.* (1993) and Chin *et al.* (2003). In this study, serotype O78 was the most common (28.2%) followed by serotypes O26 (18.8%), O1 (14.11%), O2(11.76%), O157 (5.88%). and O111 (3.5%). These results agreed to some extent, with some other studies (Al-Tarazi, 1983; Allan *et al.*, 1993 and Chin and Droual, 1997).

Isolated strains of *E. coli* , which recovered from diseased birds, could bind Congo red (100%), thus the use of Congo red dye was proposed with the objective of distinguishing between pathogenic and non-pathogenic microorganism, our results agreed with Berkhoff and

Vinal (1986); Nagwa *et al.* (2001) and Amany and Ebtehal (2002). Pathogenic *E. coli* have some products associated with virulence of organism, they include β-hemolysin which was observed in 48 out of 85 isolates (56.47%). our results agreed with Holland *et al.*, (1996) and Amany and Ebtehal (2002) which used β-hemolysin producer as a virulence factor.

Our results of antibiogram in vitro showed 100% the *E. coli* isolates were resistant to Amoxicillin, Tetracycline, Oxytetracycline, Danofloxacin and Ampicillin. Moderate sensitive to Gentamycin and Flumequine but meanwhile, high sensitive to Enrofloxacin, Amikacine and Ofloxacine. The obtained results agree with other reported results by Lambie *et al.* (2000); El-Sukhon *et al.* (2002) and Marien *et al.* (2007). Enrofloxacin is frequently, used in the treatment of *E. coli* infection in poultry (Gross 1984). ORT isolates were highly sensitive to Enrofloxacin, Tetracycline, and Amoxicillin moderate to Ampicillin, Neomycin, and Streptomycin and as resistance to Flumequine, Amikacine and Gentamycin. These results agree totally or partially, with some reports (Abdul-Aziz and Weber 1999 and Zorman-Rojs *et al.*, 2000) Also these result agreement with Marien *et al.* (2007) who reported the successful Enrofloxacin effect on ORT and Shahata *et al.* (2006) who reported the ORT sensitivity to Flumequine and Gentamycin. On the other hand these results disagreed with Türkyilmaz (2005) how reported that the ORT resistance to Enrofloxacin.

Ornithobacterium rhinotracheale was determined, in broiler and layer. Similar results were reported in different parts of the world (Van Empel *et al.*, 1997; Joubert *et al.*, 1999; Sakai *et al.*, 2000 and El-Sukhon *et al.*, 2002). The natural infection with ORT was accompanied by decreased water and feed in take (Van Empel *et al.*, 1999), dyspnea, mucous discharge, and high mortality (Sakai *et al.*, 2000; Sprenger *et al.*, 2000 and El-Sukhon *et al.*, 2002).

The experimental infections of ORT revealed growth retardation, cyanosed head, facial edema, conjunctivitis beside closed eyes (Figure 1, 2) and mortality which recorded in two bird after an aerosol inoculation with ORT isolate. These results were in agreement with other studies recorded by Van Empel *et al.* (1996). The Mortality and lesion score tabulated in Table (5) were 20%, 10% and 0% in the infected none treated, treated with Amoxicillin, and treated with Enrofloxacin groups respectively. This results is similar to that reported by Goovaerts *et al.* (1998) and Shahata *et al.* (2006). Postmortem findings were (air sacculitis, pneumonia and pericarditis, and liver necrosis) as shown in

figure (3, 4) were in agreement with other results obtained by Van Veen *et al.* (2000). Nevertheless, not all of the clinical signs and/or postmortem findings that were observed in the natural outbreak could be recorded experimentally (Van Empel *et al.*, 1996), possibly because of some environmental field stress conditions or infection with other pathogens (Zorman-Rojs *et al.*, 2000). Treatment trails based on the results of in vitro antibiogram. Treated group with Enrofloxacin was superior over non treated group and followed with Amoxicillin treated group.

We conclude that air sacculitis in Ismailia province is common among broiler chickens and layers. *Escherichia coli* is the most commonly incriminated agent and its serotypes O78, O26, O1, O2, O157 and O111 were the most commonly encountered serotypes. *Ornithobacterium rhinotracheale* stands as the second most important pathogen. So, management and sanitation practices designed to reduce the number of these types of organisms in the birds' environment are necessary. In addition, reducing stress factors and other disease agents can enhance the ability of birds to defend against harmful infections.

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