Dept. of Poultry Diseases
Animal Health Research Institute, Ismailia

STUDIES ON CHICKENS ORNITHOBACTERIUM INFECTION AT ISMAILIA PROVINCE

(With 6 Tables and 8 Figures)

By

M.K. MOURSI and KAWTHER H. SABAH*

* Dept of Bacteriology, Animal Health Research Institute, Ismailia,

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دراسات على الإصابة بعدوى ميكروب الاورنيثوباكتيريا فى الدجاج بمحافظة الاسماعيلية

محمد كمال مرسي دسوقى ، كوثر حسين احمد

أجريت هذه الدراسة لعزل وتصنيف ميكروب الاورنيثوبكتيريا (الأنف والرغام) من دجاج التسمين وأمهات دجاج التسمين في محافظة الاسماعيلية ودراسة مدى استجابتها للمضادات الحيوية المختلفة بالإضافة إلى إجراء العدوى الاصطناعية بالميكروب المعزول. وقد تم تجميع عدد ١١٥ عينة من دجاج مريض وأخر نافق حديثا من مختلف المزارع التي تعاني من أعراض تنفسية ومصحوبة بتورم في الوجه وذلك من دجاج التسمين وأمهات دجاج التسمين فى أعمار مختلفة. وقد تم إجراء وتسجيل العلامات المرضية الظاهرة على الدجاج وكذلك الصفة التشريحية. ولإجراء الفحص والعزل البكتيرى تم تجميع عينات من (الرئة، القلب، القصبة الهوائية والأكياس الهوائية) من الدجاج. وقد أسفرت النتائج عن أن ٢٧ عينة من أصل ١١٥ كانت ايجابية للعزل لميكروب الاورنيثوبكتيريا وذلك بنسبة ٢٣،٤٧ ٪ وتمثلت فى (٦٤/١٦ من دجاج التسمين بنسبة ٢٥ ٪ و٥١/١١ من أمهات الدجاج بنسبة ٢١،٥٦ ٪). وعند إجراء العدوى الاصطناعية لكتاكيت عمر ٤ أسابيع وذلك بطرق عدوى مختلفة بالحقن فى الوريد والحقن فى القصبة الهوائية والحقن فى الأكياس الهوائية – أدى ذلك إلى انخفاض في كمية الغذاء وتأخر النمو وتمثل ذلك إحصائيا فى نقص معنوى فى أوزان الطيور المصابة عن الطيور الغير مصابة. وكانت الإعراض عبارة عن انكماش واكتئاب، صعوبة في التنفس، التهاب الملتحمة وتورم العين، العطس وانتفاخ الوجه. وفي حين تفاوتت الآفات التشريحية في شدتها وفقا لطريقة العدوى وعموما كانت عبارة عن التهاب الأكياس الهوائية والقصبة الهوائية ووجود غشاء فبرينى على الكبد والقلب بالإضافة إلى التهابات فى الرئة مع وجود بعض المواد المتجبنة فى الأكياس الهوائية فى بعض الطيور. وسجلت حالتين فقط للنفوق فى العدوى عن طريق الحقن فى الوريد وذلك بمعدل ١٣,٣٣%. وقد أمكن إعادة عزل الميكروب المحقون من أنسجة الطيور المصابة بشكل تجريبي. واظهر ميكروب الاورنيثوباكتيريا استجابة كانت حساسة لكل من السفتيفيور وكبريتات الكولستين والاريثروميسين والتتراسكلين وبينما كانت المقاومة للجنتاميسن، والانروفلوكساسين والسيبروفلوكساسين وكذلك الترای میثوبريم.

SUMMARY

A total of 115 diseased and freshly dead broilers and broiler breeders' chickens were collected from different farms at different ages. They were suffering from respiratory signs and facial swelling. Complete clinical and postmortem examinations were recorded. Samples from lung, heart, trachea and air sacs were collected for bacteriological isolation. Antibiotic sensitivity test as well as experimental infection were applied. The results showed that 27 out of 115 (23.47%) were positive for isolation of *O. rhinotracheale* (16/64 from broilers 25% and 11/51 from broiler breeders 21.56 %). Experimental infection of 4 weeks old chickens via intravenous, intra-tracheal and intra-air-sac leads to decrease in feed intake and growth retardation (The mean of body weight in all infected groups were significantly ($P < 0.05$) lower than control group, depression, ruffling difficult breathing, conjunctivitis, sneezing, nasal discharge followed by facial edema. Postmortem lesions varied in severity according to inoculated routes and generally were tracheitis, arisaculitis, pneumonia, pericarditis, and perihepatitis, caseated material in air sac of some birds and arthritis. *O. rhinotracheale* could be reisiolated from affected organs of experimentally infected birds. Mortality was recorded in two birds only (13.33%) after intravenous inoculation. The isolated *O. rhinotracheale* were sensitive to amoxicillin, ceftiofur colstin sulphate, erythromycin and tetracycline while were resistant to gentamycin, enrofloxacin, ciprofloxacin and trimethoprim.

Key words: *Ornithobacterium O. rhinotracheale, broilers, antibiotic sensitivity*

INTRODUCTION

Respiratory infections are still a major problem in poultry and accompanied by heavy economic loss due to increase mortality, increase medication costs, increase condemnation rates, drops in egg production, reduction of egg shell quality and decrease hatchability (Van Epmel and Hafez, 1999).

Since December 1991 respiratory manifestation with different clinical courses have been observed in poultry flocks in different countries (Charlton *et al.,* 1993; Hafez *et al.,* 1993; Hinz *et al.,* 1994; Van Beek *et al.,* 1994). It was initially regarded as pasteurella like organism and finally *O. rhinotracheale* has been proven to be the primarily pathogen in broiler (Van Veen *et al.,* 2000 a & b). The organism has been isolated from the partridge, pheasant, pigeon, rook, quail duck, ostrich, goose, guinea fowl, chicken and turkey (Charlton *et*

al., 1993; Vandamme *et al.,* 1994 and van Empel *et al.,* 1999). *O. rhinotracheale* (ORT) is a Gram-negative, non-motile, pleomorphic, rod-shaped, non-spore forming bacterium. Most strains grow under aerobic, micro-aerophilic, and anaerobic conditions. Optimal growth occurs on 5% sheep blood agar (Van Empel and Hafez, 1999).

Ornithobacterium rhinotracheale infection is an acute highly contagious disease of chickens and turkeys. The severity of clinical signs, duration of the disease and mortality are extremely variable and are influenced by many environmental factors such as poor management, inadequate ventilation, high stocking density, poor litter conditions, poor hygiene, high ammonia level, concurrent diseases and the type of secondary infection (Hafez, 1996). It is very sensitive to antibiotics and chemical disinfectants (Hafez and Schulze, 1998) but it can acquire resistance against antibiotic easily (Van Emple, 1998), Currently *Ornithobacterium rhinotracheale* infections may be appeared to become an endemic features, since it can affect every new restocking even in previously cleaned and disinfected houses especially in areas with intensive poultry production with multiple age farms (Hafez, 1996, Van Empel and Hafez, 1999).

O. rhinotracheale were able to induce the same kind of respiratory inflammations and weight-gain losses in chickens as well as turkeys after experimental infection Maja *et al.* (2006a) and Van Empel *et al.* (1996).

This study aimed to:

- Isolation and identification of *O. rhinotracheale* and its incidence in broilers and broilers breeders at Ismailia governorate.
- Experimental infection of isolated bacteria to susceptible chicks via different routes.
- Investigation of antibiotic sensitivity test of isolated bacteria to some chemotherapeutic agents.

MATERIALS and METHODS

Birds and Samples:

A total No. of 115 (64 broilers and 51 broilers breeders' chickens) were collected from different farms at Ismailia province, they were suffering from respiratory signs and facial swelling. Besides decreased eggs production in broiler breeders. Birds were subjected for clinical and postmortem examinations. Samples from lung, heart, trachea and air sacs were collected for bacteriological isolation.

Bacteriological examination:

Bacterial isolation:

Lungs, heart, trachea and tracheal swabs were inoculated into brain heart infusion broth supplemented with gentamycin as 5-10 µg/ml and incubated at 37°c for 24 - 48 hr., under 5% CO2 tension by using gas bags in candle jar. Then loopfull from cultured broth were streaked on Blood agar with 7% sheep blood either supplemented with gentamycin 10 µg/ml (to inhibit other bacterial growth) or without gentamycin and on MacConkey Agar. The plates were incubated at 37^{o}C under aerobic conditions as well as at 37^{o}C under anaerobic conditions in 5% CO_2 enriched environment by using gas bags in candle jar for 2-3 days according to Vandamme *et al.* (1994) Traverse *et al.* (1996) and Rojs *et al.* (2000).

Identification:

All suspected colonies were subjected for identification by colonial morphology (shape- colour- size- odour) and films were stained by gram stain and conventional biochemical tests were also applied (Van Empel *et al.,* 1997).

Biochemical characterization:

ORT isolates were subjected to standard biochemical tests, including catalase, indole, motility, hydrogen sulfide, carbohydrates fermentations, phenylalanine deaminase, Lysine decarboxylase, β –Galactosidase, Ornithine decarboxylase methyl red, Voges Proskauer, citrate, urease and gelatine liquefaction, described by (Van Empel *et al.*, 1997).

In vitro sensitivity test:

Determination of in vitro sensitivity pattern of the isolated organism against different chemotherapeutic discs were done. Interpretation of the results of susceptibility findings was done according to a standard protocol for antibiotic sensitivity tests described by the National Committee for Clinical and Laboratory Standards (NCCLS, 2002) and Malik *et al.* (2003).

Pathogenicity test:

Fifty six, days-old healthy chickens were used and reared at hygienic conditions, food and water were available ad libitum. By the age of 4 weeks; five randomly selected birds were sacrificed and bacteriologically tested before experiment to prove that they were free from ORT. The other birds (60) were divided into 4 groups each of 15 and housed separate as follow:

Group 1: chickens were inoculated intravenously with 1ml of a whole culture of brain heart infusion broth adjusted to account of

approximately 1 X 10^7 colony forming units/ml according to Saeb *et al*. (2002).

Group 2: chickens were inoculated intra-air-sac with 1ml of inoculum of ORT according to Saeb *et al.* (2002).

Group 3: chickens were inoculated intratracheally with 1ml of a whole culture of brain heart infusion broth adjusted to account of approximately 1 X 10^7 colony forming units/ml according to Saeb *et al.* (2002).

Group 4: chickens non-inoculated and served as control.

Parameters of infection: During 7 to 14 days after *O. rhinotracheale* challenge, morbidity and mortality were recorded, the daily weight gain after challenge was determined, macroscopical lesions were recorded at postmortem examination of the birds, and attempts to reisolate the challenge bacteria were carried out. At postmortem examination, a scoring system for the observed lesions was used according to (Van Veen *et al.*, 2000 a & b) as follows:

For air sacs:

0 = no abnormalities

1 = one air sac seriously affected by fibrinous airsacculitis or limited pin-head sized foci of fibrinous exudate in both air sacs

2 = both air sacs seriously affected by fibrinous airsacculitis;

For lungs:

0 = no abnormalities

1 = unilateral pneumonia

2 = bilateral pneumonia.

For trachea:

0 = no abnormalities.

1 = some exudates in the tracheal lumen

2 = lumen of the trachea filled with exudates.

Statistical analysis: The statistical analyses for the weights and weight gains were performed by using Student's *t*-test (Snedecor & Cochran, 1967).

RESULTS

Clinically affected bird showed depression, roughled feathers, decreased feed intake, reduce weight gain, sneezing, nasal discharge and facial edema. while the postmortem examination revealed uni- or bilateral consolidation of the Lungs fibrinous pericaditis airsacullitis tracheitis in some cases swelling of the liver and spleen

Bacterial examination:

Small pinpoint grey colonies, sometimes with a reddish glow, non hemolytic varied in diameter were observed after 24 or 48 hr of incubation on blood agar. Growth was observed under both aerobic or microaerophilic conditions. No growth on MacConkey agar was seen. All isolates were gram negative, pleomorphic, rod-shaped bacteria.

Biochemical and enzymatic results obtained with routine laboratory reagents were uniform among isolates, showing oxidase positive, ferments glucose, lactose, galactose and fructose but doses not ferment sucrose and maltose. It was negative for catalase, methyl red, voges proskauer, gelatin liquefaction, citrate utilization, indole, nitrate reduction and triple sugar iron (Table 2). According to the cellular and colonial morphology of the organism and based on biochemical and enzymatic characteristics, by laboratory procedures, all isolates were identified as *O. rhinotracheale*.

The incidence of *O. rhinotracheale* isolation was 23.47% from the total examined samples of (lung, trachea, airsac). 27 out of 115 were positive for isolation of *O. rhinotracheale* (16/64 from broilers 25% and 11/51 from broiler breeders 21.56 %) as shown in Table (1). Table (3) showed the results of antibiotic sensitivity test of isolated *O. rhinotracheale*.

Experimental studies:

The most prominent clinical signs 1st & 2nd weeks after experimental infection appeared to be decreased feed intake and growth retardation (The mean of body weight in all infected groups were significantly ($P < 0.05$) lower than control group) Table (6), depression, ruffling difficult breathing, mild conjunctivitis, sneezing, nasal discharge lacrimation followed by facial edema (Fig. 1), unilateral swelling of infraorbital sinuses (Fig. 2).

Post-mortem lesions were tracheitis, uni or bilateral pneumonia (Fig. 3), airsacculitis, slightly congested and enlarged liver & spleen, fibrinous airsacculitis, tracheal exudates, fibrinous percarditis & perhepatitis (Fig. 4), accumulation of fibrin and caseated materials in abdominal cavity (Fig. 5), patchy areas of fibrin on lungs surface. Some variations in signs and postmortem lesions were found between the inoculated routes (Table 4 & 5). Moreover I/V inoculation resulted in airsaculitis, pneumonia and severe arthritis in some birds and the bird could not stand (Fig. 6). The inoculated bacteria could be reisolated at day 7 postinoculation from the heart, liver, spleen, air sacs, and lung (Table 4).

The intratracheal challenge resulted in airsacculitis, hydropericardium, pneumonia and mild arthritis. The inoculated bacteria could be reisolated from the trachea, lung, and air sac of inoculated chickens (Table 5).

The intra-air sacs challenge resulted in severe airsacculitis, accumulation of fibrin in abdominal cavity, pericarditis, perihepatitis, liver and heart adhesion. Bacteria were reisolated from the heart, lung, and air sac (Table 5). The mortality rate was recorded only in two birds (13.33%) after I/V inoculation. No deaths were observed in other groups.

Table 1: Incidence of *O. rhinotracheale* isolation from different examined flocks.

Flock no.	Type	Age	No. Of examined Samples	Results of isolation			
				Positive		Negative	
				No	%	No	%
1	Broilers	4 weeks	20	5	25	15	75
2	Broilers	5 weeks	23	6	26.08	17	73.92
3	Broilers	6 weeks	21	5	23.80	16	76.20
4	Broiler breeders	25 weeks	17	4	23.52	13	76.47
5	Broiler breeders	30 weeks	18	3	16.66	15	83.34
6	Broiler breeders	34 weeks	16	4	25	12	75
Total			115	27	23.47	88	76.53

Table 2: Biochemical prosperities of isolated *O. rhinotracheale*

Test Reaction	Result
Oxidase	+
Catalase	-
Nitrate reduction	-
Indole	-
Growth on MacConkey	-
Arginine dehydrolase	+
Lysine decarboxylase	-
β -Galactosidase	+
Ornithine decarboxylase	-
Phenylalanine deaminase	-
Urease	+
Voges Proskauer	+
Acid from carbohydrates:	
Glucose	+-
Mannose	+
Lactose	+
Sucrose	+
Sorbitol	-
Maltose	+
Dulcitol	-
Fructose	+

Table 3: In vito sensitivity test of *O. rhinotracheale*

Antimicrobial disk	Antibiotic disc/conc. (µg)	Sensitivity of ORT			
		S		R	
		No.	%	No.	%
Enrofloxacin	(Enr)-10 µg	10/27	37	17/27	63
Ciprofloxacin	(Cip)-5 µg	11/27	40.74	16/27	59.26
Gentamycin	Gn- 10 µg	0/27	0.0	27/27	100
Neomycin	N – 15 µg	14/27	51.85	13/27	48.15
Erythromycin	E – 15 µg	25/27	92.59	2/27	7.41
Ceftiofur	CT–30 µg	24/27	88.88	3/27	11.12
Colistin sulphate	Col – 10 µg	27/27	100	0/27	0.0
Tetracycline	TE – 30 µg	26/27	96.29	2/27	3.71
Amoxycillin	AM – 10 µg	27/27	100	0/27	0.0
Penicillin G.	P - 10 µg	16/27	59.25	10/27	40.75
Trimethoprime / Sulphamethoxazol	SXT 25 µg	9/27	33.33	18/27	66.67

Table 4: The pathological score lesion of experimentally infected chicks with *O. rhinotracheale*

Route of inoculation	N	Pathologic lesions scores		
		Air sac	Trachea	Lung
Intravenous	15	1	0	2
Intratracheal	15	2	2	1
Intra-air-sac	15	2	1	1
Control	15	0	0	0

Table 5: The postmortem lesions observed 2wk post-experimental infection via three inoculation routes with *O. rhinotracheale* (ORT) and their reisolation.

Route of infection	No. of chickens	Postmotem findings					Reisolation at end of experiment					
		A/S	P	Ar	HP	PH/PC	Airsac	lung	Trachea	Heart	Liver	Spleen
Intravenous	15	4	7	3	0	2	+	+	-	+	+	+
Intratracheal	15	9	3	0	2	1	+	+	+	-	-	-
Intra-air-sac	15	7	0	0	1	7	+	-	+	+	+	-
Control	15	0	0	0	0	0	0	0	0	0	0	0

A/S = airsacculitis; P = pneumonia; Ar = arthritis, swollen joint with caseous material; HP = hydropericardium; PH/PC = fibrinous perihepatitis and pericarditis with adhesions.

Table 6: The effect of *O. rhinotracheale* infection on average body weight and average daily weight gain.

Route of inoculation	No. of chickens	Body weight			Average daily weight gain between 21-35
		Day 21	Day 28	Day 35	
Intravenous	15	845 (±60)	1080 (± 76)[b]	1295 (±122)[c]	30(±5)[c]
Intratracheal	15	805 (±81)	1085 (±85) [b]	1330 (±127) [c]	35 (±1)[c]
Intra-air-sac	15	815 (±109)	1120 (±132) [b]	1400 (± 130)[b]	39 (± 2)[b]
Control	15	795 (±120)	1160 (±91)[a]	1575 (±103)[a]	52 (±8)[a]

Within columns, averages having different letter are significantly different ($P<0.05$)

LIST OF FIGURES


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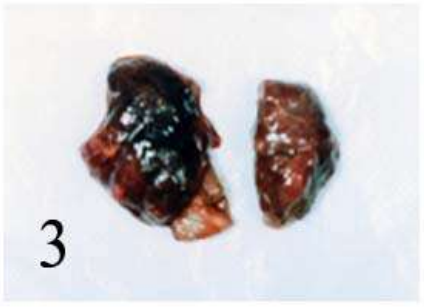
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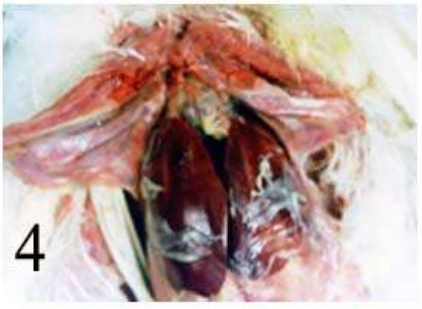
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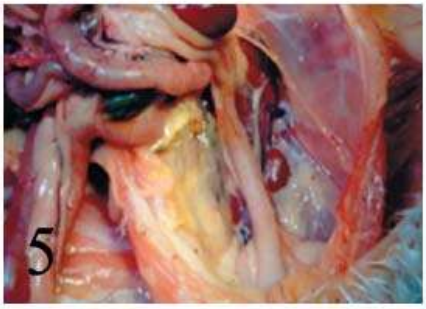
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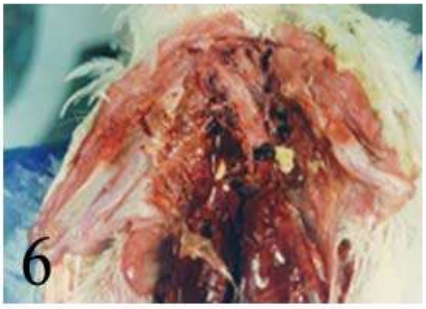
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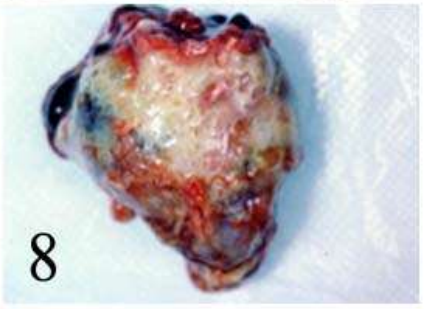
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DISCUSSION

Respiratory disease conditions due to bacterial and viral agents are continuing to cause heavy economic losses in the poultry industry worldwide**.** *Ornithobacterium rhinotracheale* has been recognized in many countries worldwide and incriminated as a possible additional causative agent in respiratory disease complex. (Hafez, 2002).

In this study the clinical signs of naturally affected birds were depression, roughled feather, decreased feed intake, reduced weight gain, sneezing, nasal discharge and facial edema while the gross lesions in general were severe pneumonia, airsaculitis, tracheitis, oedema, uni- or bilateral consolidation of the lungs with fibropurulent exudates, pericarditis, airsacculitis, peritonitis. These findings are similar to those reported by Hafez, (2002) and Soriano *et al.* (2002)

Bacteriological examination revealed that the isolation of Small pinpoint grey colonies, sometimes with a reddish glow, non hemolytic varied in diameter were observed after 24 or 48 hr of incubation on blood agar. All isolates were gram negative, pleomorphic, rod-shaped bacteria showing oxidase positive, ferments glucose, lactose, galactose and fructose but doses not ferment sucrose and maltose and for other tests it was negative for catalase, methyl red, voges proskauer, gelatin liquefaction, citrate utilization, indole, nitrate reduction and triple sugar iron.

All isolates could be identified as *O. rhinotracheale* by bacteriological laboratory tests. This result was completely agreed with biochemical and enzymatic properties of *O. rhinotracheale* reported by Charlton *et al.* (1993), Van Empel *et al.* (1997) Soriano *et al.* (2002) and Canal *et al.* (2005).

Ornithobacterium rhinotracheale was isolated from lung, air sac and trachea of examined birds as that reported by Saeb *et al.* (2002) and Soriano *et al.* (2002).

The incidence of *O. rhinotracheale* isolation in this study was 23.47% (27/115) from total examined samples (16/64 from broilers, 25% and 11/51 from broiler breeders, 21.56 %) this result is much less than that reported by Abden and lotfy (2006) they reported that *O. rhinotracheale* was isolated by 75% of total examined chicks while, Shahata *et al.* (2006) isolated *O. rhinotracheale* at 34% from lung, trachea and air sac of examined chickens and layers. In contrast Shihata and Ibrahium (2004) isolated *O. rhinotracheale* by 11.72%. while Saeb *et al.* (2002) recoverd *O. rhinotracheale* by 8.8%. Also Türkyilmaz,

(2005) isolated *O. rhinotracheale* from broiler breeders of 37, 42 and 46 week old by 1.2%.

Since a standard protocol for antibiotic sensitivity tests for *O. rhinotracheale* does not exist we depended upon the method described by the Clinical and Laboratory Standards Institute (CLSI, 2002) for fastidious Gram-negative organisms.

With regard to antibiotic sensitivity testing, the isolated *O. rhinotracheale* were sensitive to amoxicillin, ceftiofur colstin sulphate, erythromycin and tetracycline while were resistant to gentamycin, enrofloxacin ciprofloxacin and trimethoprim. This results are in agreement with these reported by Malik *et al.* (2003), Türkyilmaz, (2005) and Maja *et al.* (2007). Maja *et al.* (2006 a) reported that Acquired fluoroquinolone resistance is commonly encountered in ORT isolates. On the other hand, Shahata *et al.* (2006) found that amoxicillin, tetracycline and enrofloxacin were the most effective drugs against isolated ORT in vitro. Moreover, Saeb *et al.* 2002, found that *O. rhinotracheale* isolates were sensitive to tetracycline and to lesser extent to other antibiotics. Such differences in the antibiotic susceptibility pattern may be due to misuse and /or frequent exposure of the bacteria to antibiotics in field.

The results of experimental infections of *O. rhinotracheale* revealed that the three inoculated routes resulted in growth retardation, depression, ruffling, difficult breathing, conjunctivitis, sneezing, nasal discharge, lacrimation followed by facial edema, unilateral swelling of infraorbital sinuses. These results are in agreement with the studies of Travers *et al.* (1996) and Saeb *et al.* (2002) .Van Empel *et al.* (1996) reported that the most prominent clinical signs after experimental infection appeared to be the growth retardation, dyspnoea, mucus discharge and mortality.

In this study mortality was recorded in only two birds (13.33%) after an intravenous injection with *O. rhinotracheale*. Goovaerts *et al.* (1998) found that only an intravenous challenge was able to induce 20% mortality in experiemntally *O. rhinotracheale* chicks, while Shahata *et al.* (2006) observed that the mortality was 30% in experimentally infected birds. Also Shihata and Ibraheem (2004) found that moralities were 10% and 20% in hubbard and balady chicks respectively after experimental infection.

The result of gross lesion revealed development of different records of postmortem findings post-experimental infection via three inoculation routes. These findings were airsacculitis, pneumonia and

pericarditis perihepatitis, and liver and heart adhesion and joint affection which are in agreement with the results obtained when similar inoculation routes were applied by Saeb *et al.* (2002) and Shahata *et al.* (2006). Also Ryll *et al.* (1997a); Sprenger *et al.* (1998) reported that airsacculitis, pneumonia and increased mortality were observed after aerosol, intra-tracheal, intravenous and/or intra-thoracic infection.

Nevertheless, not all of the clinical signs and/or postmortem findings that were observed in the natural outbreak could be reproduced experimentally. This discrepancy between natural and experimental *O. rhinotracheale* infection might be explained by differences in aggravating and predisposing factors such as stress, high stock density, poor ventilation, presence of other bacteria, or high ammonia levels in field conditions.

The obtained result in this study indicated obviously variations in pathogenicity of *O. rhinotracheale* among the inoculated routes. Similar conclusions were reported by other researchers (Van Empel *et al.,* 1996 and Travers *et al.*, 1996) they found that the severity of clinical signs and lesions could be referred to route of infection and variations in the virulence and pathogenicity of *O. rhinotracheale*.

In conclusion, our results indicate that *O. rhinotracheale* was isolated by 23.47% from examined cases of broiler and broiler breeders at Ismailia province. Challenge test suggests that this bacterium was able to produce respiratory signs with various postmortem lesions in experimentally infected chicks and considered as a primary pathogen for chickens.

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