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SOME STUDIES ON THE INCIDENCE OF ORNITHOBACTERIUM RHINOTRACHEALE INFECTION IN CHICKEN EMBRYOS AND LAYERS

(With One Table and 8 Figures)

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بعض الدراسات عن نسبة الإصابة ببكتريا الأورنيثو فى أجنة البيض والدجاج البياض

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تم جمع ٨٠ عينة من كتاكيت فاقسة ونافقة حديثاً و ١٤٠ عينة من دجاج بياض ونافق حديثاً عمر ٨ – ١٢ شهراً (٣٥ عينة من المفصل ، ٣٥ عينة من قناة البيض و ٣٥ عينة من المخ، ٣٥ عينة من الرئة والقصبة الهوائية والأكياس الهوائية) وذلك من مزارع مختلفة بمحافظة أسيوط. وقد أمكن عزل ميكروب الأورنيثو بنسبة ٤٠% من الكتاكيت الفاقسة حديثاً وبنسبة ١٧% ، ٢٢,٨% ، ٨,٥% ٣٤% من كل من المفصل وقناة البيض والمخ والرئة والقصبة الهوائية والأكياس الهوائية على التوالى. وقد تم إجراء عدوى صناعية بالميكروب المعزول على كتاكيت عمر ٣٠ يوم عن طريق الحقن فى الوريد والحقن فى الأكياس الهوائية والذى أدى إلى إنكماش الطيور ونقص فى الوزن وتورم العين وصعوبة التنفس وإفرازات بالأنف والبعض أظهر بعض الحركات العصبية قبل النفوق الذى وصلت نسبته إلى ٣٠% وذلك فى اليوم الثالث والرابع بعد إجراء العدوى الصناعية أما فى الإسبوع الثانى من الحقن ظهرت بعض الحالات بها عرج وعدم القدرة على الوقوف أما الصفة التشريحية أظهرت التهابات بالأكياس الهوائية وغشاء فيبرينى على الكبد والقلب بالإضافة إلى التهابات بالرئة وبعض الطيور كانت بها مواد صفراء متجبنة بالأكياس الهوائية فى منطقة البطن والبعض الأخر أظهر احتقانات بالمخ. وقد أظهرت الكتاكيت التى حُقنت عن طريق الأكياس الهوائية نفس الأعراض والصفة التشريحية السابقة ولكن بعد فترة أطول. تم إعادة عزل الميكروب من الحالات المعداة صناعياً. بإجراء اختبار الحساسية لميكروب الأورنيثو المعزول وجد أن الأموكسسيلين والتتراسيكلين والأنروفلوكساسين هم الأدوية الأكثر تأثيراً.

SUMMARY

Eighty samples were collected from freshly dead hatched chicks, also one hundred and forty freshly dead layers 8 –12 month old age were collected (35 samples from joint, 35 from oviduct, 35 from brain and 35 from lung, trachea and air sac) from different farms in Assiut Governorate. Ornithobacteriun rhinaotracheale organism was isolated at a rate of 40% from newly hatched chicks and at a rate of 17%, 22.8%, 8.5% and 34% from joint, oviduct, brain and lung, trachea and airsac respectively. Experimental infection of 30 days old chicks by isolated organism through intravenous and intraairsac inoculation was done. Intravenous inoculation leads to depression, ruffling feather of the infected birds and decreased body weight, eye affection, difficult in breathing, nasal secreation and some birds showed nervous signs before death which reached to 30% in the 3rd and 4th day after infection but in the 2nd week postinfection some birds revealed arthritis and could not stand. Postmortem examination revealed airsacculitis, perihepatitis, pericarditis, pneumonia and oedema of the lung. Some birds showed caseous, yellow material on the abdominal airsacs, and others revealed congestion in the brain. Birds which inoculated intra airsac showed also the same previous signs and lesions but it appeared at longer time. Reisolation of the organism from experimentally infected birds was succeeded. Sensitivity test revealed that amoxycillin, tetracycline and enrofloxacin were the most effective drugs.

The aim of this work is designed to cover the following points:

- Isolation and identification of the Ornithobacterium rhinotracheale organism and its incidence in the newly hatched chicks and layers.
- Experimental infection of the isolated organism to 30 days-old chicks.
- In vitro sensitivity test of the isolated organism to some antimicrobial drugs.

Key words: *Ornithobacterium rhinotracheale, hatched chicks and layers*

INTRODUCTION

Ornithobacterium rhinotracheale (ORT) has been repeatedly found to be involved in cases of respiratory disease in broiler chickens (Charlton *et al;* 1993 – Hafez *et al;* 1993 and Leorat *et al;* 1994).

ORT is a gram – negative, non motile, pleomorphic rod, non sporulating bacterium of the rRNA super family V, in the taxonomic

neighbourhood of the genera Cytophaga, Riemerella, Flavobacterium, Sporocytophaga and Capnocytophaga.

Osteitis, meningitis and joint infections, can be induced by intravenous application with ORT. The infection can be transmitted horizontally by aerosol, as well as vertically through eggs, which probably accounts for its rapid and worldwide spread. Twelve serotypes can be distinguished within the species ORT, of which serotype A is the most prevalent, therapeutic treatment of the disease can be difficult because acquired resistance against the regular antibiotics is very common within the genus (Vandamme *et al.*, 1994).

Travers *et al.* (1996) were able to induce the disease by inoculation ORT isolates via the coudal abdominal airsac into 28-days old broiler. They noted that the ORT isolates were capable of causing primary disease, with statistically significant resultant mass loss, respiratory and arthritic symptoms were reproduced but no sinusitis was observed. Also they observed a highly significant reisolation of ORT from the brains of broilers challenged with the isolates.

Van Empel *et al.* (1997) could isolated the ORT from the respiratory tract, liver, joints and brains of diseased birds as well as from egg shells and yolk sac of one day old chicks from various countries.

Meningitis, osteitis were observed in experimentally infected SPF chickens by intravenous challenge with 20% mortality but no airsacculitis as seen in the field (Goovaerts *et al.*, 1998).

Nagaraja *et al.* (1998) noted that the ORT was able to survive in the ovary and oviduct of experimentally infected breeder hens without clinical signs.

Mild respiratory symptoms and growth retardation, increased mortality were reported by Van Empel *et al.* (1999). Postmortem investigations showed a foamy, yellow-white "yoghurt like" exudate predominatly in the abdominal airsacs and sometimes accompanied by a purulent pneumonia often unilateral and / or exudate in the trachea.

In layers, ORT has been associated with outbreaks of respiratory disease, decreased egg production and mortality in South Africa and Egypt. Many chickens were found dead without antemortem signs, postmortem findings were tracheitis, airsacculitis, pericarditis, ruptured ovarian follicles and egg yolk peritonitis (Stephanie *et al.*, 2000)

Treatment can be difficult because acquired resistance against the regular antibiotics is common in ORT isolates and an inactivated vaccine for broiler breeders has been developed (Van Veen, 2000)

Van Veen *et al.* (2001) observed that ORT was able to induce lesions after aerosol challenge without a previous priming with virus and thus ORT was proven to be a primary pathogen.

Van veen *et al.* (2004) observed in an experiment in SPF broiler chickens that the respiratory tract lesions at postmortem (P.M) examination were positive for ORT by bacteriological and immunohistological examination.

MATERIALS and METHODS

Samples:

A total of 80 dead balady chicken embryos and 140 samples from freshly dead layers were collected (35 samples from joints, 35 samples from oviducts, 35 samples from brain and 35 samples from airsacs, lungs and trachea) collected from different farms at Assiut Governorate and were subjected for bacteriological examination.

Media used:

Sheep blood agar, trypticase soy agar, brain heart infusion broth, trypticase soy broth, triple sugar iron agar, glucose phosohate broth, gelatin agar media, koser's citrate medium, christensen's media, nitrate broth, sugar fermentation media and pepton water (2%).

Reagents and solutions :

Kovac's reagent, methyl red reagent, nitrate reagent, mercuric chloride solution, α – Naphthol 5%, 40% potassium hydroxide in absolute ethyl alcohol, 1% solution of tetramethyl -P- phenylens diamine dihydrochloride and 30% hydrogen peroxide.

Stain used: Gram's stain.

Experimental birds:

Twenty five, 30 days old balady chickens obtained from the faculty of Agriculture Assiut University poultry farm were used in our experiment.

Antibiotic sensitivity discs:

Include: Enrofloxacin (5μg). Ampicillin (10μg), Chloramphenicol (30 μg), Amoxycillin (10μg), Gentamycin (10μg), Trimethoprim (5μg), Neomycin (30 μg), Oxytetracycline (30μg), Danofloxacin (5μg), Streptomycin (10μg), Spectinomycin (25μg), Tetracycline (30μg) and lincomycin (2μg).

Methods:

I – Isolation

a- Samples from dead chicken embryos were taken from lung, trachea, liver and yolk sac and cultured into brain heart infusion broth supplemented with gentamycin as 10µg/ml media according to Back et al. (1996), and incubated at 37°c for 24 – 48 hr., under 7.5 – 10% Co_2 tension by using gas bags in candle jar according to Vandamme *et al.* (1994) Traverse *et al.* (1996) and Rojs *et al.* (2000). Then a loopfull from the cultured broth was streaked onto sheep blood agar plates supplemented with 10µg gentamycin /ml media to inhibit growth of enterobacteriaceae. Plates were incubated at 37°c for 48 hr. under 7.5 – 10 % Co_2.

b- Dead laying chickens were subjected to p.m. examination. Swabs taken from joint, oviduct, brain, airsac, lung and trachea were cultured as mentioned before.

II – Identification of the isolated organism

The suspected colonies were examined for their morphology (shape – colour – size- odour) and films from suspected colonies were stained by Gram's stain.

- Biochemical reactions: The most important biochemical reactions of ORT in comparison to these of Kingella spp. and the gram – negative rods related to the family Pasteurellaceae potentially pathogenic for fowl and also be differentiated from other pathogenic gram – negative rods in the families Enterobacteriaceae and Neisseriaceae were done according to Van Empel *et al.* (1997).

Pathogenicity test:

Twenty five, 30-days-old healthy chickens were used and 5 birds from them were tested before experiment and proved to be free from ORT organism. The other birds were divided as follow:

- 1^{st} group: ten, 30 days old chickens inoculated intravenous with 1ml of a whole culture of brain heart infusion broth adjusted to a count of approximately 10^9 colony forming units/ml according to Saeb *et al.* (2002).
- 2^{nd} group: five, 30 days old chickens inoculated intra airsac with 1ml of inoculum of ORT according to Saeb *et al.* (2002)
- 3^{rd} group,: five – 30 days old chickens were left as control.

Reisolation of ORT from experimental birds were done.

In vitro sensitivity test:

The determination of sensitivity of the isolated organism against different antibiotic discs was done according to the technique of Bauer – Kirby *et al.* (1966).

RESULTS

Postmortem examination of naturally infected freshly dead layers revealed airsacculitis, pneumonia, tracheitis and inflammation of the oviduct.

Bacteriological examination showed that the suspected ORT colonies were pin point size, greyish white and sometimes with a reddish glow appearance and a distinct odour, similar to the odour of butyric acid.

Gram's stain revealed gram negative, pleomorphic plump short rods.

Biochemical reactions revealed that the isolated organism was oxidase positive, ferments glucose, lactose, galactose and fructose but dose 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.

According to the cellular and colonial, morphology of the organism as well as the biochemical reactions, the frequency of the isolated ORT was 40% from dead chicken embryos while in dead layers it was 34% from the samples of (lung, trachea, airsac) and was 8.5% from the brain, 17% from joint and was 22.8% from the oviduct samples.

Pathogenicity test:

Chickens in group I inoculated intravenously showed in the 1st week postinoculation (PI) decrease in the body weight, depression, ruffling feather (Fig. 1) eye affection, difficult in breathing and nasal exudate (Fig. 2). In addition three birds showed nervous signs before death (30% mortality) in the 3rd and 4th day P.I (Fig. 3). In the 2nd week PI, some birds revealed joint affection and the bird could not stand (Fig. 4). Postmortem examination showed airsacculitis perihepatitis, pericarditis (Fig. 5), Pneumonia and oedema of the lung (Fig. 6) while the other birds showed yellow caseous material in the abdominal air sacs (Fig. 7) and others showed congestion of the brain (Fig. 8).

Chickens in group II inoculated intraairsacs also showed signs similar to those in group I but without deaths and revealed the same

gross lesions as in group I but appeared later than those observed in group I.

Neither signs or lesions or deaths were recorded in bird of group III.

Reisolation of ORT from lung, airsac, joint and brain in experimentally infected birds was successful.

Sensitivity test:

The effect of the different antibiotics on the isolated ORT are illustrated in Table I.

Table I: Illustrates the results of the in vitro sensitivity test:

Antibiotic discs	Conc.	Sensitivity of ORT isollates
Amoxycillin	10 µg	+++
Tetracycline	30 µg	+++
Enrofloxacine	5 µg	+++
Chloramphenicol	30 µg	++
Ampicillin	10 µg	++
Streptomycin	10 µg	++
Oxytetracycline	30 µg	++
Neomycin	30 µg	+
Danofloxacin	5 µg	+
Lincomycin	2 µg	+
Spectinomycin	25 µg	+
Trimethoprim	5 µg	-
Gentamycin	10 µg	-

+++ Sensitive (zone size of inhibition is above 26 mm).
++ moderate sensitive (zone size of inhibition is equal or less than 26 mm).
+ week sensitive (zone size of inhibition is above 18 mm).
- resistant (zone size of inhibition is less than 18 mm).

Fig. 1: Experimentally infected chicken showing depression and ruffling feathers.

Fig. 2: Eye affection and difficult in breathing in experimentally infected chicken.

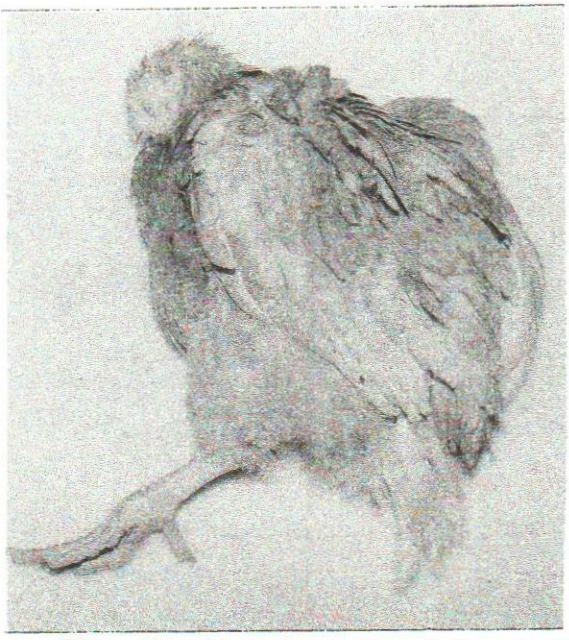


Fig. 3: Experimentally infected chicken showing nervous signs before death.

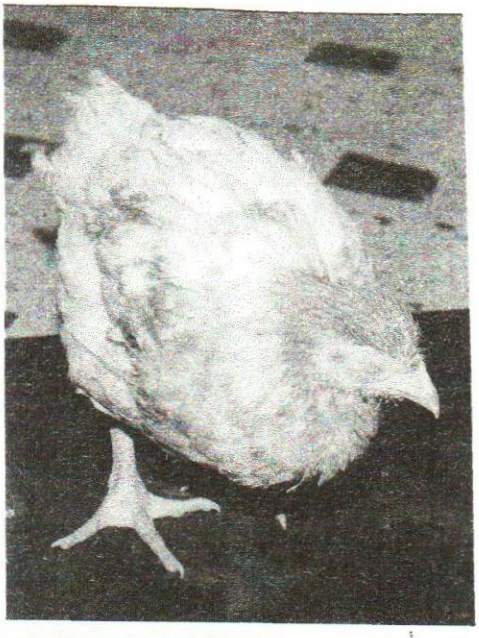
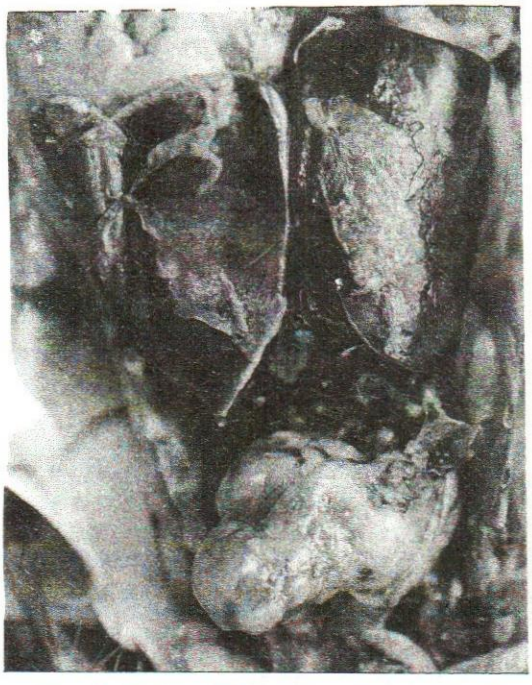


Fig. 4: Experimentally infected chicken showing joint affection and the bird could not stand



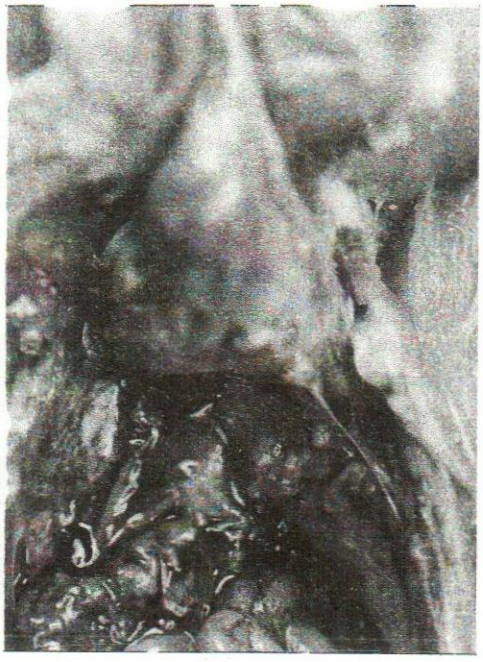


Fig. 5: Pericarditis and perihepatitis of experimentally infected chicken.

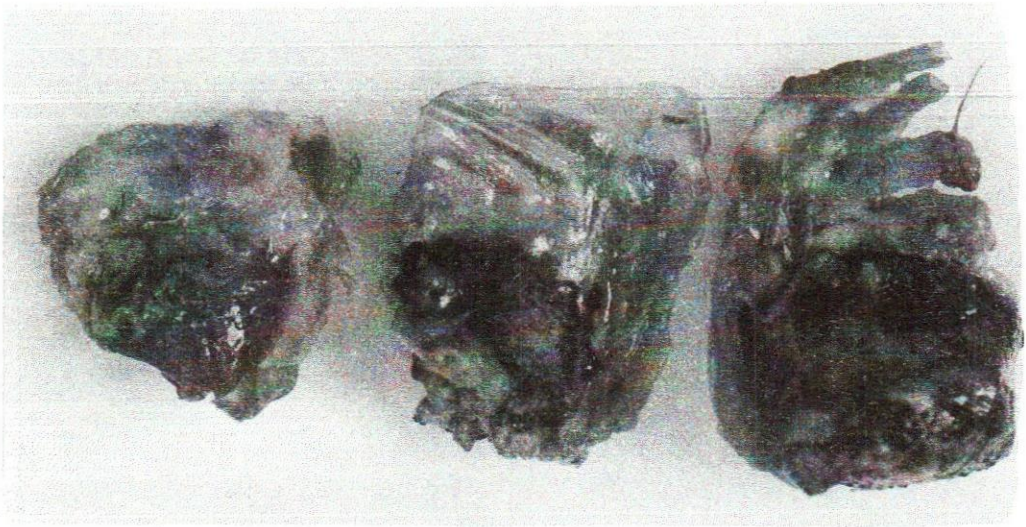


Fig. 6: Pneumonia, congestion and oedema of the lung of experimentally infected chicken.

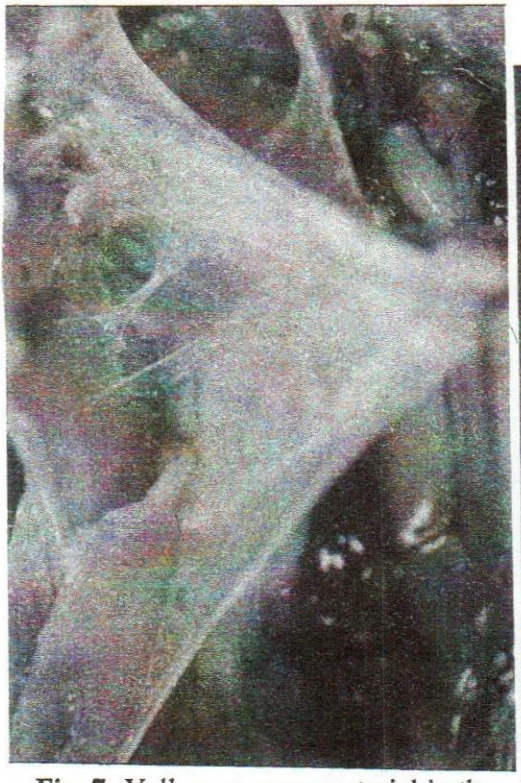


Fig. 7: Yellow caseous material in the abdominal air sacs of experimentally infected chicken.

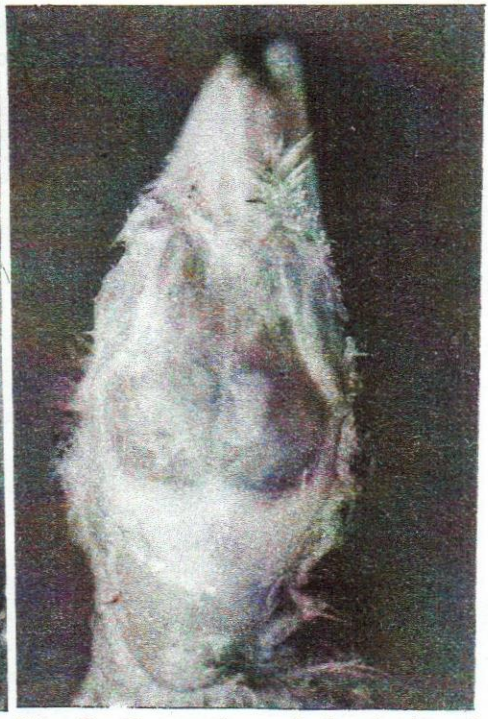


Fig. 8: Congestion of the brain of experimentally infected chicken.

DISCUSSION

Respiratory infections are the most serious group of diseases affecting poultry and are accompanied by heavy economic losses due to increased mortality, increased medication costs, increased condemnation rates, drop in egg production, reduction of eggshell quality and decreased hatchability. ORT has been identified as newly emerging respiratory bacterial pathogen that has caused significant economic losses to the poultry industry with a reported incidence that ranged from 30 – 35% (Travers *et al.*, 1996 and Malik *et al.*, 2003).

In this study, postmortem examination of freshly dead layers showed peritonitis, tracheitis, airsacculitis, pericarditis and ruptured of ovarian follicles, this finding is similar to that reported by Stephanie *et al.* (2000) and Soriano *et al.* (2002).

Bacteriological examination revealed that ORT was recovered from airsac, lung and trachea as that reported by Van Empel *et al.* (1999), Saeb *et al.* (2002) and Soriano *et al.* (2002). Also we could isolate the organism from the joint, ovary and brain, this finding is inagreement with the result obtained by Van Empel *et al.* (1997).

Also we were able to recover ORT from the yolk sacs of newly hatched chicks and this result is similar to that obtained by Stephanie *et al.* (2000).

Experimental infection of the isolated organism to 30 days old chickens either intravenously or intraairsac revealed decrease of the weight gain, depression, ruffling feather, eye affection, difficult in breathing and nasal exudate. Nervous signs, meningitis and arthritis were also reported in some birds. These findings are similar to those observed by Travers *et al.* (1996), Goovaerts *et al.* (1998) and Saeb *et al.* (2002).

The gross lesions showed airsacculitis, perihepatitis, pericarditis, pneumonia and oedema of the lung, this result goes hand in hand with that observed by Travers (1996), Ryll *et al.* (1997), Sprenger *et al.* (1998) and Saeb *et al.* (2002).

In our experiment, we showed 30% mortality in the infected birds, this result is somewhat similar to that reported by Goovaerts *et al.* (1998) who recorded 20% mortality rate in their experiment.

The obtained results in our experiment and those that reported by other authors (Leorat *et al.*, 1994 and Travers *et al.*, 1996) differ with the data mentioned by Van Empel *et al.* (1996) who said that ORT culture

was only capable of reproducing the clinical features of the disease if the birds had been previously infected with respiratory viruses.

The most prominent gross lesions seen in some birds was foamy white, yoghurt-like' exudate in the airsacs this result is similar to that observed by Van Empel and Hafez (1999).

We were successful in reisolation ORT from experimentally infected birds and this result is inagreement with those observed by Van Beek *et al.* (1994), Nagaraja *et al.* (1998) and Soriano *et al.* (2002).

In vitro sensitivity test reevealed that amoxyicillin, tetracycline and enrofloxacin were the most effective drugs against the isolated organism, our result is relatively similar to that observed by Hafez (1996) who found that amoxycillin, tetracycline and chloramphenicol were effective to ORT isolates, but Saeb *et al.* (2002) found that tetracycline was the only drug which was sensitive to ORT isolates Amal (2002) recorded that amoxycillin, chloramphenicol and ampicillin were the most effective drugs.

Our conclusion in this study proved that ORT can be transmitted vertically through eggs as well as horizontally and ORT can be regarded as a primary pathogen in chickens to cause respiratory symptoms.

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