Zagazig Veterinary Journal
Volume 43, Number 3, p. 82-94, 2015

DOI: 10.21608/zvjz.2015.28445

An Experimental Co-Infection of Broilers with Local Isolates of *Ornithobacterium rhinotracheale* and *Escherichia coli*

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Article History: Received: 6/12/2015 Received in revised form: 29/12/2015 Accepted: 30/12/2015

Abstract

Ornithobacterium rhinotracheale is a Gram-negative bacterium associated with respiratory diseases in many avian species and it causes variable economic losses to the poultry industry. In this study, single aerosol infection of broiler chickens with three PCR confirmed local isolates of *Ornithobacterium rhinotracheale* (ORT) from Sharkia Governorate was found to cause growth retardation together with mild respiratory manifestations and 5-10% mortalities. Mild tracheitis, airsacculitis and pneumonia were observed post aerosol administration. Co-infection with *E. coli* was found to have triggered effect on ORT infection in broiler and cause a higher degree of pathogenicity, higher mortalities and severe growth retardation than the single infection. The three different isolates were found to cause nearly the same degree of inflammatory response. ORT infection alone resulted in minimal microscopic lesions in the trachea and air sacs. Mixed infection (ORT with *E. coli*) resulted in more severe lesions than those by ORT alone as well as dense lymphocytic infiltrations in tracheas, lungs, air sacs and hearts were shown. Amoxicillin was successfully improving the clinical signs and body weight gain of ORT infected birds.

Keywords: *Ornithobacterium rhinotracheale*, *Escherichia coli*, Sharkia, Aerosol

Introduction

Respiratory tract infection considered one of the serious problems in the intensive poultry production. Hence, It is accompanied by heavy economic losses due to increase mortality, medication costs, condemnation rates, a drop in egg production, reduction in egg shell quality and decrease hatchability [1]. Several pathogens are indicated as a possible etiology of respiratory tract affection either alone, in-synergism with other microorganisms, or in combination with poor management practices [1].

Ornithobacterium rhinotracheale (ORT) can be a primary or secondary avian respiratory pathogen. It can cause highly infectious disease in poultry, but the severity of clinical symptoms, duration of the disease and mortality have been described to be highly variable [1,2]. In postmortem examination, ORT infection associated with tracheitis, pericarditis, sinusitis, exudative pneumonia and Yoghurt-like exudates in the abdominal air sacs [3].

The disease spread horizontally by direct and indirect contact. Vertical transmission was proven, since some researchers isolated ORT at a very low incidence from reproductive organs, hatching eggs, infertile eggs and dead embryos [4].

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The microbiological isolation and identification have been performed by several scientists, however currently many reports discussed the laboratory diagnosis of ORT using molecular identification techniques such as polymerase chain reaction (PCR) and 16S ribosomal gene sequencing [5,]. On the other hand, still the isolation of the bacterium is necessary for serotyping, determining the antimicrobial susceptibility for an effective therapy and producing autogenous vaccines [7,8].

The present study aimed to experimentally investigate whether or not ORT infection with locally isolated strains can induce disease alone or need a trigger. Additionally, to perform therapeutic trials in experimentally infected birds as animal model.

Material and Method

Birds

One hundred and sixty, one-day-old, Cobb chicks were reared on floor based system and fed on commercial balanced ration free from antibiotic, anticoccidial and mycotoxin. They were housed in isolators at the Experimental Animals Research Unit (EARU) at the Faculty of Veterinary Medicine, Zagazig University, Egypt, with food and water *ad-libitum*. They were tested to be free from ORT infection.

Ornithobacterium rhinotracheale inoculum

The ORT challenge inoculums were prepared from 3 selected previously isolated ORT isolates [9] from 3 different broiler flocks located in different districts at Sharkia Governorate, Egypt, according to Van Empel *et al*. [10]. The challenge inoculums suspensions were adjusted to contain 10^9 CFU/ml according to Microbiology-International Standard Organization [11].

Escherichia coli inoculum

E. coli (O_{11}) challenge inoculums were prepared from preserved glycerol stock kindly obtained from Dr. Ashraf Hamed, Avian and Rabbit Medicine Department, Faculty of Veterinary Medicine, Zagazig University, Egypt [12]. The bacteria were cultured onto MaCconkey's agar and incubated for 24 hours at 37°C. After 24 hours of incubation, 3-5 ORT specific colonies were transferred into 5 ml BHI broth, incubated for 24 hours at 37ºC and then 5 ml were taken and centrifuged at 4000 rpm for 20 minutes. After discarding the supernatant, the sediments were re-suspended with 5 ml PBS and compared with MacFerland No. 1. The challenge inoculum suspensions were adjusted to contain 10^9 CFU/ml according to Microbiology-International Standard Organization [11].

Antibiogram of the Ornithobacterium rhinotracheale challenge inoculum

Muller Hinton agar supplemented with 10% sheep blood agar was prepared for determining detecting the *in vitro* sensitivity of ORT to different antimicrobial agents [13]

Experimental infections of broiler chickens with ORT isolates and reference strain of E. coli

At the third day of age, one hundred and sixty chicks were divided into eight groups, 20 chicks each. The first three groups were infected with three different ORT field isolates; ORT_1, ORT_2 and ORT_3 [9]. Groups No 4, 5 and 6 were infected with ORT triggered with *E. coli* strain (O_{11}) as ORT_1 with *E. coli*, ORT_2 with *E. coli* and ORT_3 with *E. coli*.

Group No. 7 was infected with *E. coli* alone and group No. 8 was used as a blank control. ORT infection was performed via aerosol route using commercial paint sprayer (Particle size 7.50 um). The dose of infection was 10^9 CFU per ml in 100 ml of ORT. The developed mist was maintained in the isolators for at least 10 min after closing the air circulation [10]. While, *E. coli* infection was performed via subcutaneous injection of 10^9 CFU/bird and the clinical signs and mortality of the birds had been observed.

Parameters and confirmation of infection

All the birds were weighed weekly and postmortem examination was performed for two birds from each group at 3, 6, 9, 12, 15 and up to 27 days PI. Lesion scores of lungs, trachea, airsac, heart and liver were calculated [14]. During the post-mortem examination samples of livers, hearts, lungs, air sacs, tracheas were collected and subjected to re-isolation trials. Re-isolation was confirmed by microscopic evaluation (Gram's stain reaction) and PCR technique.

Reisolation and morphological and biochemical identification of the experimentally infected Ornithobacterium rhinotracheale

Loopfuls were taken from the tracheas, lungs and airsacs and were directly inoculated onto 10% sheep blood agar media and brain heart infusion agar supplemented with gentamycin sulphate 10 µg/ml, for primary isolation of ORT.

The plates were incubated at 37ºC under microaerophilic using carbon dioxide bags under 5 to 12 % CO_2 tension for 24-48hours [15]. The same media were used also for subcultural trials. The suspected grown single and well separated ORT colonies were examined for its morphology (shape, size, odor, appearance and elevation).

Films were prepared from the suspected pure colonies, stained with Gram stain and then examined microscopically according to Cruickshank *et al*. [16].

DNA extraction and PCR amplification

Three hundred microlitters from each ORT cultured broth were used for DNA extraction using Gene-spinTM DNA/RNA extraction kit (iNTRON) following the manufacturer's instructions. The PCR assays were performed using primers which were previously reported by Sharifzadeh *et al.* [17], OR16S-F1 (5´- TGG CAT CGA TTA AAA TTG AAA G) and OR16S-R1 (5´- CAT CGT TTA CTG CGT GGA CTA C) in order to amplify a 625-bp DNA fragment within the 16S ribosomal RNA region. The amplification was performed using i-Taq^{TM} PCR Master Mix (2x) (iNTRON).

Analysis of DNA fragments was performed by 1% ethidium bromide stained agarose gel electrophoresis in 1x Tris acetate EDTA (TAE) buffer and 1kb plus DNA ladder (Tiangen biotech) was used as a standard. Positive control was included in the reaction, ORT strain, was kindly provided by Bacteriology, Mycology and Immunology Department (BAMI), Faculty of Veterinary Medicine, Zagazig University, Egypt.

Histopathology

Samples from trachea, lungs, airsacs, liver and heart were collected from recently dead and sacrificed birds during the observation period. The samples were fixed in 10% buffered neutral formalin solution and 5 um sections were stained with Hematoxlin and Eosin (H&E) according to Suvarna *et al*. [18].

Statistical analysis

The statistical analysis was carried out using SPSS (version 10, Richmond, VA, USA) [19]. Body weights and weight gains were performed through one-way analysis of variance (ANOVA). The results were expressed as mean± S.D. (Standard Deviation) Duncan's Multiple Range test was used to compare between means at $P \leq 0.05$.

Table 1: Lesion scores of experimentally infected broiler chickens with the isolated ORT strains and/ or *E. coli* infection as a trigger

G	Lesion scores																											
	3rd day PI					6th day PI					9th day PI					12 days PI					15th days PI					27th days PI				
	AS	Tr.	L.	Li.	H.	AS	Tr.	L.	Li.	H.	AS	Tr.	L.	Li.	H.	AS	Tr.	L.	Li.	H.	As	Tr.	L.	Li.	H.	As	Tr.	L.	Li.	H
1	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	1	2	1	1	0	1	2	1	1	0	1	2	1	1	0
2	0	0	0	0	0	1	1	1	0	0	1	1	2	0	0	1	1	2	0	0	1	1	2	0	0	1	1	2	0	0
3	0	0	0	0	0	0	1	0	0	0	1	1	2	0	0	1	1	2	0	0	1	1	2	0	0	1	1	2	0	0
4	0	0	0	0	0	0	2	1	1	1	0	2	1	1	1	0	2	1	1	1	0	2	1	1	1	0	2	1	1	1
5	0	0	0	0	0	1	3	2	1	1	1	3	2	1	1	1	3	2	1	1	1	3	2	1	1	1	3	2	1	1
6	0	0	0	0	0	1	1	1	2	2	1	1	2	2	2	1	1	2	2	2	1	1	2	2	2	1	1	2	2	2
7	0	0	0	0	0	1	1	2	2	2	1	1	2	2	2	1	1	2	2	2	1	1	2	2	2	1	1	2	2	2
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

G: Group Number

PI= post infection, AS =Air sacs, Tr =Trachea, L =Lung, Li =Liver, H = Heart

Group No. 1 =Infected with ORT strain 1, Group No. 2= Infected with ORT strain 2, Group No. 3= Infected with ORT strain 3, Group No 4= Infected with ORT strain 1 with E-coli O_{11}, Group No 5= Infected with ORT strain 2 with E-coli O_{11}, Group No. 6= Infected with ORT strain 3 with E-coli O_{11}, Group No 7= Infected with E-coli O_{11} alone, Group No. 8= Negative control.

Lesion scores were calculated as follow [10, 14, 22]:

Air sacs, 0= no abnormalities, 1= Slight airsacculities, 2= Moderate airsacculities with limited pinheaded foci of fibrinous exudate, 3= one air sac seriously affected by fibrinous airsacculitis or limited pin-head sized foci of fibrinous exudate in both air saces, 4= severe fibrinous airsacculitis.

Trachea, 0= no abnormalities, 1= slight exudate in tracheal lumen, 2= moderate exudate in tracheal lumen, 3= lumen of trachea filled with exudate.

Lungs, 0= no abnormalities, 1= unilateral congestion, 2= bilateral congestion, 3=consolidation.

Liver, 0= no abnormalities, 1= mild congestion, 2= severe congestion, enlargement, perihepatitis.

Heart, 0= no abnormalities, 1=turbidity of pericardium, 2= hydropericardium, fibrinous pericarditis.

Results

Clinical signs and mortality rate

Infection with ORT alone revealed mild clinical signs in the form of ruffled feathers, dullness, nasal discharge, decrease food intake, mild conjunctivitis and mild rales. The clinical signs appeared as early as 6 days post infection (PI) and persisted during the whole observation period, except in case of ORT_3 infected group. The clinical signs were in the form of nasal discharge, decrease food intake and conjunctivitis as early as 9 days PI.

Simultaneous infection of three different ORT isolates and *E. coli* as a trigger led to the development of more apparent clinical signs in the three groups such as nasal discharge, rales, ruffled feathers and diarrhea at 6 days PI. Depression and decrease food intake were observed in the *E. coli* infected group. Aerosol infection with ORT only resulted in no mortality in group 1, 5% mortality in group 2 and 10% mortality in group 3. In case of triggering with *E. coli*, 10% mortality was recorded in either group 4 or 5 and 25% mortality in group 6. On the other hand, single infection with *E. coli* resulted in 15% mortality.

Gross lesions

Trachietis was detected in the both infected groups with ORT only or in combination with *E. coli*. However, the lesion in the trachea was earlier and more evident in the dually infected groups. Lesions in the either ORT infected lungs or in combination with *E. coli* varied from unilateral to bilateral congestion. Mild airsacculitis was observed, 6-9 days PI, in case of exposure to ORT only or ORT and *E. coli* as a trigger (Table 1).

Appearance of slight frothy yellow exudate in the abdominal air sacs was existed in case of exposure to ORT_1 and triggered by *E. coli* (O_{11})

(Figure 1a). There was mild congestion in the examined livers at 12, 15 and 27 days PI. When ORT triggered by *E. coli*, marked pericarditis and perihepatitis could be seen in some cases. Postmortem examination of birds infected with *E. coli* only revealed mild tracheitis, congested lung, airsacculitis, hepatic congestion and fibrinous pericarditis.

Reisolation of experimentally infected Ornithobacterium rhinotracheale

The reisolation patterns of the infected ORT field isolates and *E. coli* from the different organs are listed in Table 2a and b. Reisolation was persisted up till 27 days PI and were confirmed via PCR (Figure 1b). Ninety two ORT isolated were obtained from 480 specimens (infected with ORT only or both ORT and *E. coli*).

ORT isolates were 33/96, 29/96 and 30/96 from the trachea, lungs and air sacs, respectively with an incidence of 34.37%, 30.20% and 31.25%, respectively. There was no ORT isolates obtained from the liver neither from the heart.

Body weight

At three and six days PI, there was no significant difference in the average body weights between the differently treated groups. One week later, at 12 and15 days PI, there was a significant difference between either the ORT and ORT and *E. coli* infected groups compared with the control non-infected group. At 15 days PI, there was a marked decrease in the average body weight of the ORT and *E. coli* infected groups compared with the ORT infected groups.

This decrease was more evident and statistically significant in group 1 (ORT_1 infection) compared with group 4 (ORT_1 and *E. coli* infection). Twenty seven days post infection, there was a significant decrease in the average body weight of group 1 (ORT_1 infection) and group 3 (ORT_3 infection) compared with group 4 (ORT_1 and *E. coli* infection) and group 6 (ORT_3 and *E. coli* infection), respectively (Table 3).

Table 2a: Bacteriological investigations of ORT from different organs of the experimentally infected broiler chickens

Days P/I		3rd day PI*								6th day PI*								9th day PI*								12 days PI*								15th days PI*								27th days PI*							
	G	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Organs	Tr	+	-	-	-	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
	L	-	-	-	-	-	+	-	-	-	+	+	+	+	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
	AS	-	-	-	-	+	+	-	-	-	+	+	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
	Li	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

***** Blood agar with gentamycin was used for the reisolation

G: Group Number

PI= post infection, Tr =Trachea, L =Lung, AS =Air sacs, Li =Liver, H = Heart

Table 2b: Bacteriological investigations of E. coli from different organs of the experimentally infected broiler chickens

Days P/I		3rd day PI*								6th day PI*								9th day PI*								12 days PI*								15th days PI*								27th days PI*							
G		1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Organs	Tr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	L	-	-	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-
	AS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Li	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-
	H	-	-	-	-	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	-

***** MacConkey agar was used for the reisolation
G: Group Number
PI= post infection, Tr =Trachea, L =Lung, AS =Air sacs, Li =Liver, H = Heart

Histopathological findings of experimentally infected chicks with either ORT alone or triggered with E. coli

After aerosol infection with ORT, the tracheas of most cases were normal except for a few extravasated erythrocytes and edema in the lamina propria (Figure 2a). When triggered with *E. coli*, focal necrosis in the mucosa with lymphocytes and heterophils aggregations besides extravasated erythrocytes and edema in the lamina propria (Figure 2b).

ORT infection led to severe lung congestion besides perivascular and interlobular edema (Figure 2c). After co-infection with *E. coli*, thickening of the interlobular septa with serofibrinous exudates and heterophils infiltrations besides collapse in the adjacent air capillaries. The bronchi showed severe catarrhal bronchitis with mucinous degeneration and desquamation in the lining epithelium, congested capillaries and leukocytes infiltrations of predominantly heterophils (Figure 2d).

The air sacs were slightly thickened with severe congested blood vessels (Figure 2e). The air sacs of experimentally infected chicks with the dual infection were thickened and firmly adhered to the lung tissue with caseous necrosis, fibrinous exudates and round cells and heterophils infiltrations (Figure 2f).

Therapeutic trial of the experimentally infected broilers

The results of antibiogramme test indicated that the three different isolates were sensitive to amoxicillin, ampicillin and doxycycline but were resistant to gentamycine, norofloxacin, ciprofloxacin, cefotaxim, sulphamethoxazole-trimethoprim and colistin sulphate. Therefore, amoxicillin was used in the treatment of experimentally infected birds 10mg/kg weight for two successive days. Three days later, after treatment two birds from each group were sacrificed and samples were collected in order to be subjected to bacteriological examination. No suspected ORT colonies was able to grow on blood agar with gentamycin from either tracheas, lungs or air sacs of infected birds with ORT and treated with Amoxicillin.

Discussion

In this study, we performed experimental and therapeutic trials for three different local isolates of ORT alone or triggered with *E. coli*. *Ornithobacterium rhinotracheale* is a Gram negative bacterium of the rRNA superfamily V, and the name *rhinotracheal* was suggested for the species associated with respiratory disease in domesticated and wild birds [1].

The pathogenicity of the isolated ORT strains was adopted in three-days-old chickens and/or triggered with *E. coli* infection. In our study, the most characteristic finding was the ORT infection alone caused only mild respiratory manifestations with no recorded mortality in group 1, 5% mortality in group 2 and 10% mortality in group 3. Mild to moderate gross lesions in the respiratory system were the most evident findings for the single aerosol infection with ORT such as mild trachietis, unilateral pneumonia and mild to moderate airsacculitis mainly at 15-27 days PI.

Aerosol administration of ORT, without triggering with viral or bacterial infection, for specific pathogen free chickens, usually did not accompany by inflammatory changes in the respiratory system [1]. Similar to the present study, airsacculitis and pneumonia usually developed when commercial chickens were used for aerosol administration of ORT due to the incomplete confirmation of the bird's microbiological status [10,20,21].

Table 3: Weekly average body weight (gm) /bird of experimentally infected broiler chickens with ORT and *E. coli*

G7	G6	G5	G4	G3	G2	G1	Control	**A/W**	**A/D**	**DPI**
65	60.25	65.05	60.3	60.6	60.8	65.25	60	1st	3	0 day
±2.11	±1.78	±3.21	±1.56	±2.11	±1.54	±3.12	±2.10	week		
140.09	130.31	130.18	135.16	145.16	149.05	150.11	160.33		6	3 days
±3.56^{c}	±4.97^{d}	±5.62^{d}	±6.84^{d}	±3.64^{bc}	±2.21^{b}	±3.56^{b}	±3.65^{a}			
355.21	345.71	344.93	340.56	360.25	365.37	368.18	380.31	2nd	9	6 days
±6.45^{c}	±7.56^{d}	±6.56^{d}	±8.24^{d}	±4.67^{b}	±5.34^{b}	±6.56^{b}	±6.52^{a}	week		
425.69	400.81	405.41	400.76	440.76	448.38	450.07	475.35		12	9 days
±19.56^{c}	±15.65^{d}	±17.96^{d}	±17.49^{d}	±6.23^{b}	±7.89^{b}	±9.56^{b}	±8.97^{a}			
465.76	435.9	430	420.38	482.38	490.38	495	545.07	3rd	18	15 days
±6.98^{c}	±6.56^{d}	±5.65^{d}	±5.98^{d}	±8.79^{b}	±9.96^{b}	±9.48^{b}	±22.32^{a}	week		
770.75	735	730.37	720.75	780.25	790.66	780.3	800.5		21	18 days
±11.36^{c}	±9.56^{d}	±10.45^{d}	±16.34^{d}	±13.56^{b}	±13.54^{b}	±11.36^{b}	±23.56^{a}			
1220	1120	1130.66	1150.83	1300.83	1360.71	1355	1550.71	4th	30	27 days
±27.34^{c}	±25.22^{d}	±24.31^{d}	±19.87^{d}	±19.34^{b}	±24.56^{b}	±22.87^{b}	±26.31^{a}	week		

The means within the same row for the same day with different superscripts differ significantly at ($P \leq 0.05$)
A/W: Age/Week; A/D: Age/Day; DPI: Day Post Infection

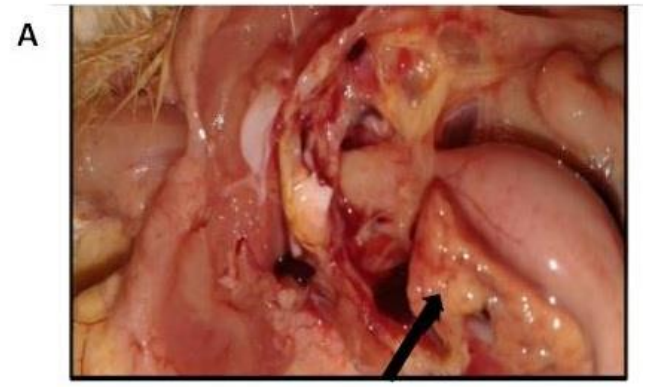

Figure 1a: Air sac of 15 days old chicken experimentally infected with ORT_1 with E- coli showed air sacculities with yellow exudate in thoracic air sac.

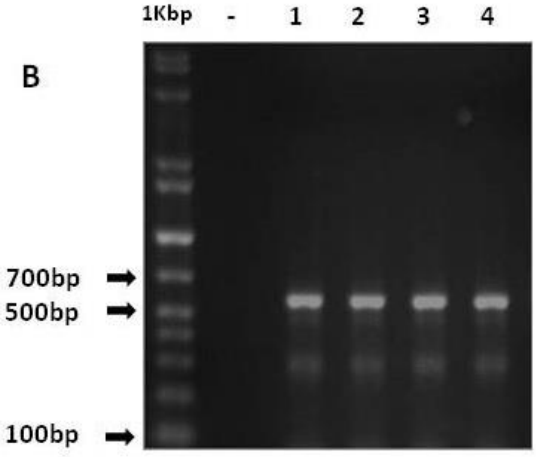

Figure 1b: PCR screening of re-isolation of ORT from experimentally infected chickens: 1% ethidium bromide stained agarose gel showed 625bp fragments of 16S ribosomal RNA gene of ORT, Lane – negative control (water), Lane 1 positive control (ORT strain from BAMI department), Lane (2, 3 and 4) lung ORT isolates at 12 days PI.

Ornithobacterium rhinotracheale serotype A was recorded in Egypt for the first time from an outbreak of respiratory disease in broilers concomitantly with *E. coli* O55.K59 [22]. Therefore, in the present study three groups were triggered with *E. coli* (O_{11}) which showed more severe respiratory signs and higher mortalities, up to 25 %. The birds necropsy revealed moderate tracheitis, uni- or bilateral pneumonia and frothy yellow exudates in the abdominal airsacs, especially in group 5, as well as marked pericarditis and perihepatitis. These results argued that ORT might act as a primary pathogen and bacteria such as *Bordetella avium* and *E. coli* can act as triggers for ORT infection [23,24]. The latter was concurrent with the results of several researchers [4,22,25,26].

Microscopically, in case of ORT infection most histological lesions can be seen in the lungs, pleura and air sacs. Therefore, in our study, the tracheas in case of single infection with ORT were normal except for a few extravastaed erythrocytes and edema in lamina propria. On contrary, Chin *et al*. [27] found congested lungs with macrophage, heterophils and lymphocyte infiltrations lying free within the lumen of air capillaries and parabronchi in naturally infected birds with ORT. However, in the present study, similar findings including, lungs congestion beside heterophils and lymphocyte infiltrations were noticed only after co-infection of ORT with *E. coli*.

The difference can be attributed to the difference between the conditions of field and experimental infections. Similarly, in the present study, fibrinous inflammation and heterophils infiltration of air sac was observed only after co-infection with *E. coli*.

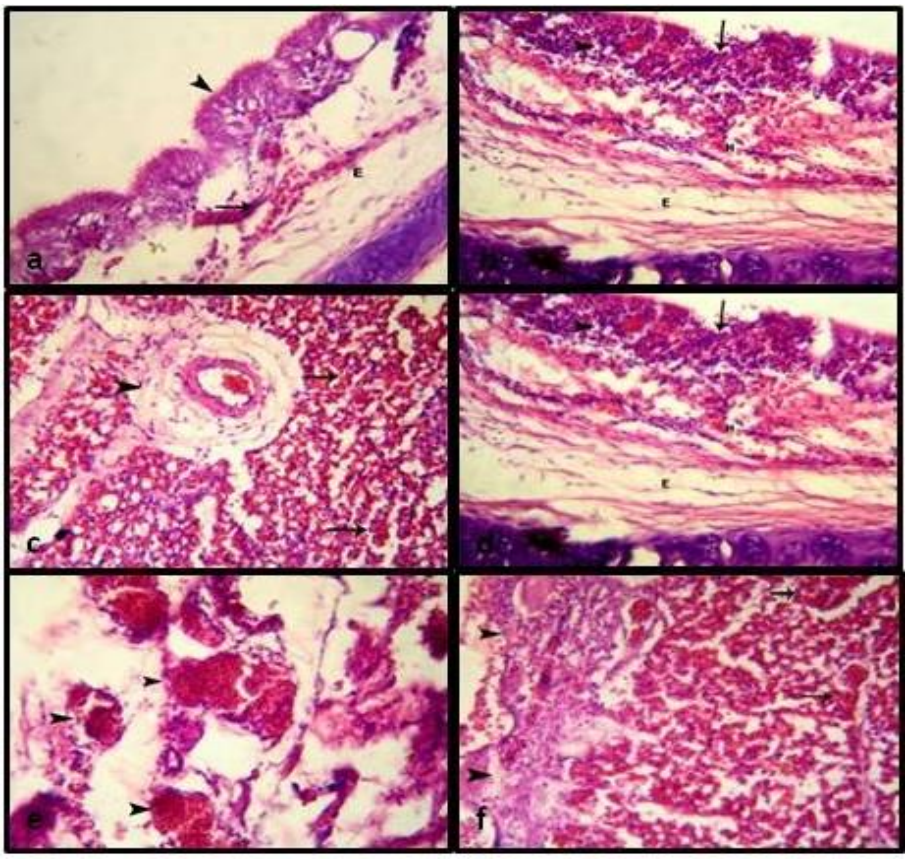

Figure 2a: Trachea of ORT experimentally infected chicken showed normal mucosa (arrowhead) with few extravasated erythrocytes (arrow) and edema (E) in the lamina propria, HE x 400. Figure 2b: Trachea of *E. coli* and ORT experimentally infected chickens showed focal necrosis in the mucosa with lymphocytes and heterophils aggregation besides extravasated erythrocytes (H) and edema (E) in the lamina propria, HE x 400. Figure 2c: Lung of ORT experimentally infected chicken showed severe congestion (arrows) besides perivascular and interlobular edema (arrowhead), HE x 400. Figure 2d: Lung of *E. coli* and ORT experimentally infected chicken showed thickening of the interlobular septa with serofibrinous exudates and heterophils infiltrations besides collapse in the adjacent air capillaries, HE x 400. Figure 2e: Air sac of ORT experimentally infected chicken showed congestion of blood vessels (arrowheads) with no evidence of inflammation, HE x 400. Figure 2f: Air sac of *E. coli* and ORT experimentally infected chicken showed inflamed airsac adhering with the lung by fibrinous exudates infiltrated with round cells (arrowheads), HE x 400.

Ornithobacterium rhinotracheal infection was positively correlated with loss in the body weight gain [28, 29]. One of the most prominent findings after the experimental infection appeared to be the growth retardation of the infected birds, which exacerbated in case of the dual infection [10].

In vitro, the sensitivity test revealed that the ORT isolates were more sensitive to amoxicillin, ampicillin and doxycycline and the isolates were resistant to colistin and ciprofloxacin. Several studies were consistent with our results [15, 30, 31], while others showed different sensitivity patterns [32, 33].

The treatment trial of broiler chickens experimentally inoculated with ORT and treated with amoxicillin revealed that the treated groups showed increases in body weight gain, decreased the mortality rate to zero and the rate of re-isolation of ORT from experimentally infected and non-treated group reached 70% and zero in infected and treated groups. These results were in agreement with Awaad *et al*. [34] and Nagaraja *et al*. [35]. The most characteristic ORT histological lesions usually might be confined to lungs, pleura and air sacs.

The investigation in this study confirmed the finding in a previous experimental study of Van Empel *et al.* [10] who concluded that aerosol exposure of chicken with ORT without triggering with another pathogen did not result in severe inflammatory lesions in the respiratory tract.

Conclusion

In conclusion, ORT may act as a primary pathogen for broiler associated with mild respiratory disease and adversely affects bird performance. Dual infection with bacteria such as *E. coli* increased the severity of the respiratory disease, mortality rate and loss in the weight gain. Amoxicillin could be used as an effective therapy to clear ORT infection and decrease respiratory problems in broilers.

Conflict of interest

None of the authors have any conflict of interest to declare.

Acknowledgment

We are thankful to Prof. Dr. Mohamed Hamed Mohamed, Pathology Department, Faculty of Veterinary Medicine, Zagazig University, Egypt, for his help and guidance in histopathological examination and imaging. Also, we would like to acknowledge Prof. Dr. Mohamed Wafek, Biochemistry Department, Central Laboratory for Aquaculture Research, Abbasa, Egypt, for his help in statistical analysis.

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الملخص العربي

العدوي المصاحبة التجريبية لبداري التسمين بالمعزولات المحلية من الأورنيثوباكتريم رينوتراكيال و الأيشرشيا كولي

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الأورنيثوباكتريم رينوتراكيال هي بكتريا سالبة الغرام متلازمة مع أمراض تنفسية في العديد من أنواع الطيور وتسبب خسائر إقتصادية متفاوتة لصناعة الدواجن. في هذه الدراسة تم اجراء العدوي الهوائية المنفردة في بداري التسمين بثلاث معزولات محلية من محافظة الشرقية بالأورنيثوباكتريم رينوتراكيال والمؤكدة بتفاعل انزيم البلمرة المتسلسل. وجد أنها تسببت في تثبيط النمو مع أعراض تنفسية معتدلة ونفوق من ٥ الى ١٠%. لوحظ بعد العدوي الهوائية وجود التهاب معتدل في الحنجرة الهواية وإلتهاب في الأكياس الهوائية وإلتهاب رئوي. العدوي المصاحبة بالأيشرشيا كولي وجد انها احدثت اصابة أشد حدة عن العدوى المنفردة بميكروب الأورنيثوباكتريم رينوتراكيال في بداري التسمين وأدت الى مزيد من الألتهابات والنفوق وكذلك التثبيط الحاد لنمو الطيور. الثلاث معزولات المختلفة وجدوا انهم يسببوا نفس الدرجة من الأستجابات الألتهابية تقريبيا. العدوي بميكروب الأورنيثوباكتريم رينوتراكيال فقط والتى نتج عنها تغيرات هستوباثولوجية طفيفة بسيطة في القصبة الهوائية والأكياس الهوائية. بينما أدت العدوي المختلطة (الأورنيثوباكتريم رينوتراكيال والأيشرشيا كولي) الي اصابات اشد حدة مقارنة بميكروب الأورنيثوباكتريم رينوتراكيال منفردا مع ملاحظة وجود ارتشاح ليمفاوي كثيف في القصبات الهوائية والرئات والأكياس الهوائية.