

Incidence of *Ornithobacterium Rhinotracheale* (ORT) in Broiler Breeders at North Sinai Governorate

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ABSTRACT

This study was performed on broiler breeders during different season of the year at North Sinai Governorate. Bacteriological examination, of nasal, trachea, lung, air sacs, heart blood, spleen and liver swabs revealed ORT which was commonly isolated from lung, sinus, trachea, and air sacs. The results of isolation trials revealed that 5 ORT positive cases out of 100 diseased and freshly dead birds.

Pathogenicity of some ORT isolated strains were carried out in Hubbard chicken at 15 days of age (1×10^8 CFU/ ml) aerosol challenge, clinical signs, post mortem and re-isolation were recorded. The mean of body weight in infected groups were significantly lower than control groups ($P < 0.05$).

In vitro anti-biogram test against some antimicrobial agent and isolated strain revealed that isolates were sensitive to Amoxicilin, Colistin – sulphate, Ampicilin, and Oxytetracycline, moderately sensitive to Penicillin, Neomycin and Norxin. The tested isolates were resistant to Erythromycin, Streptomycin, Gentamycin, Spectam and Lincospectin. This study investigates the role of ORT as etiological agent in field respiratory problems among broilers.

INTRODUCTION

Ornithobacterium Rhinotracheale (ORT) infection is a contagious disease of birds that causes respiratory distress mortality and decreased growth rate. It has been isolated from chicken, ducks, turkeys and pigeons. The severity of the clinical signs, duration of the disease and mortality are extremely variable and influenced by environmental factors, (1-3). The clinical signs of ORT in broilers generally appeared between 2nd and 3rd week of age and is the most common in 24 to 52 week of age of chickens during peak of egg production, together with mortality rate ranged from 2- 10 %. The gross lesions noticed in ORT infection of broiler were facial oedma, cyanosis head, pneumonia mostly unilateral and air sacculites containing creamy exudates, congestion and enlarged of liver, spleen lung and kidney, fibrinous exudates in peritoneum and arthritis. (3- 5). In old birds slight depression and gasping with expectoration of mucous and cyanoses of bare area of head before death.(6). The aim of the present study was to investigate the role of ORT in filed problems among broilers breeders with particularly emphasis on clinical signs their pathological lesion beside isolation and

identification of causative agent. Study the pathogenicity of some isolates to 2 week old broilers (Hubbard), in addition the sensitivity test to various antibacterial agents *in vitro* and *in vivo*.

MATERIAL AND METHODS

Material

Samples, this study was obtained from diseased and freshly dead (100 broiler breeders) birds (6 to 18 month old) suffering from respiratory signs and swollen head. Under aseptic condition, a loop-foul from sinuses, trachea, lung, liver heart blood and spleen, were streaked on 10 % sheep blood agar containing 10 ug gentamycin / ml of media. The plates incubated aerobically (10% CO₂) at 37 C° for 48 h. The suspected colonies were picked up, identified morphologically and biochemically using API20 NE Micro Test Strip Bio –Merieux, France (7).

Media: blood agar media (10% sheep blood)- Brain heart infusion broth media (10).

Gram stain

Birds

Sixty Hubbard broilers birds, one day old, from commercial farm kept under aseptic

condition until 2 weeks old for experimental infection

Methods

Bacterial examination, under anaerobic condition isolated colonies were identified morphologically and biochemical tests were applied. The field strain of ORT isolated from broiler breeder was used for pathogenicity test by aerosol challenge (3). A loopful of suspected colonies was inoculated into brain heart infusion broth for serial ten fold dilution of the organisms; growth was adjusted by turbidity (1×10^8 CFU/ml) (6,8 and 9)

Clinical signs and post mortem examination had been done to experimental infected birds

Experimental birds

Sixty, 2 weeks - old of Hubbard chicks were used for Pathogenicity test. Random tracheal swabs from these birds proved to be free from ORT. The birds were divided into three groups each of 20 birds. Group 1 kept as control, while group 2 were challenged with ORT, and group 3 were challenged with brain heart infusion broth containing (1×10^8 CFU/ml) ORT using paint sprayer. All birds were kept under close observation for 3 weeks for clinical signs, post mortem examination, and re-isolation trials. Group 3 treated with Amoxicillin (0.1%) for 5 successive days after infection in drinking water. Live body weight were recorded every 7 days post challenge with ORT up to 3 weeks to study the effect of ORT on body weight.

Pathogenicity parameters

Post mortem examination was carried out at 7 days post challenge of ORT. Gross lesions were scored as follow(2,10) :- lungs L, 0 = no abnormalities, 1 = unilateral pneumonia, 2 = bilateral pneumonia, trachea ; T, 0 = no abnormalities, 1 = some exudates in the tracheal lumen, 2 = lumen of tracheal filled with exudates, air sacs ; AS, 0 = no abnormalities, 1 = fibrinous air sacculities, 2

= both air sacs seriously affected by fibrinous air sacculitis,

Sensitivity test - Strains of ORT were tested in agar gel diffusion test for their sensitivity to certain antibacterial drugs using disc diffusion technique (8). Using commercial sensitivity discs obtained from Oxide. Statistical analysis were carried out (11)

RESULTS

ORT was isolated from 5 out of 100 of examined birds (Table 1). The highest prevalence was recorded in trachea, lung, air sac, sinus, liver, spleen and heart blood (Table 2). Samples from nasal sinuses, trachea, lungs and liver inoculated into blood agar containing 10 % sheep blood at 37 C° an aerobically (10% CO₂) showed pin point small circular colonies, opaque to Gray in colour and convex appeared into surface of media after 48 hours. Isolates were identified by biochemical reaction which tabulated in (Table 3). Re-isolation of ORT organisms at the end of experiment was recorded. Post mortem lesion in experimental infected group was recorded in (Table 4). *In vitro* the result were recorded in (Table 5).

Clinical signs and post -mortem findings

The affected chicken showed facial oedema, conjunctivitis and swollen sinus beside closed eyes. Moreover, some respiratory signs in the form of sneezing, coughing and gasping could be seen in some birds. The affected chicken's revealed reduction in feed and water intake and death reached to 5 % in affected birds, Post mortem examination showed oedema in the face and sinuses particularly infra-orbital sinus beside mucous exudates usually seen in nares and trachea.

The mean of body weight in all infected groups were significantly ($P < 0.05$) lower than control group (Table 6).

Table 1. Results of ORT Isolation from broiler breeders and 6-18 months

Species	Age	No. of examined birds		No. of positive		%	Season
		No. of diseased birds	No. of freshly dead birds	Diseased birds	Freshly dead birds		
Broiler breeders	6 - 18 month	15	15	1	1	6.6 %	Winter
		10	10	1	0.	5 %	Autumn
		20	20	1	1	5 %	Winter
		5	5	0	0	0 %	Autumn
Total		50	50	3	2	6% - 4% Respectively	

Table 2. Incidence of ORT isolation among examined organs of broiler breeders

Sample	Broiler breeder		Percentage %
	No. of examined	No. of positive	
Trachea	75	5	6.6%
Lungs	65	4	6.1%
Sinus	70	2	2.8%
Air sac	50	3	6%
Spleen	60	2	3.3%
Liver	90	4	4.4%
Heart blood	90	3	3.3%
Total	500	23	4.6%

Table 3. Biochemical reaction of the isolated bacteria from examined birds

Test	Results	Test	Results
Urea production	+	Motility	-
Acid phosphatase	+	Catalase	-
Estrase lipase	+	Oxidase	+
α - chemotrypsin	+	Gram stain	-
α - Mannosidase	-	H ₂ S	+
Alkaline phosphatase	+	Lipase	-
β -galactosidase	+	Trypsin	+
β - glucosidase	-		

Table 4. Experimental infection of 2 week's old Hubbard chicks with ORT isolated strain

group	No. of birds	Dose of ORT/ml	treatment	Lesion score after infection									mortality	
				1 st week			2 nd week			3 rd week			No.	%
				L	T	as	L	T	as	L	T	as		
1	20	0	Control	0	0	0	0	0	0	0	0	0	0	0
2	20	1x10 ⁸	Infected	0	0	0	1	1	1	2	2	2	1	5%
3	20	1x10 ⁸	Infected and treated	0	0	0	1	1	1	0	0	1	-	0

Table 5. Results of *in vitro* sensitivity test of isolated ORT to different antibacterial agents

Antibacterial agent	Potency of disc (ug)	Standard sensitivity zone MM)	No. of tested isolates	No. of sensitive isolates	%
Amoxicillin (AMX)	25	23>31	5	5	100%
Ampicillin (AML)	30	---	5	4	80%
Penicillin (P)	10	21>29	5	3	60%
Erythromycin (E)	20	14>18	5	0	0%
Oxytetracycline (OT)	30	13>16	5	5	100%
Gentamycin (GM)	30	15>19	5	0	0%
Neomycin (N)	30	13>17	5	3	60%
Lincospectin	-	10>15	5	0	0%
Norxin (NOR)	10	---	5	2	40%
Spectam (SH)	40	12>15	5	0	0%
Streptomycin (S)	10	17>22	5	0	0%
Colisitin - sulphate	10	13>18	5	5	100%

Table 6. Average body weight (gm) of 2 week old Hubbard chicks experimentally infected with ORT (N=5), at three different periods.

Group	Treatment	Days post challenged with ORT		
		7 th D	15 th D	21 st D
G1	Control	1150	1550	1860
G2	Infected with ORT	890	1170	1390
G3	Infected and treated	1000	1410	1680

Significant ($P < 0.05$)

DISCUSSION

The isolated bacteria in the present work was ORT Gram negative bacteria, the growing colony appeared as grey to grey white colonies of convex shape on blood agar under anaerobic conditions. This result is completely agreement with those previously recorded (12, 13), which mentioned that ORT is a gram negative non motile non sporulating bacteria. The biochemical reaction included alkaline phosphates, esterase, lipase, acid phosphates, trypsin and α - Mannosidase confirmed the ORT.

In the present work mortality rate was 5 % among the examined bird. The mortality among broiler infected with ORT were 2-10 % (5,14). The clinical signs included swollen

head with closed eye, coughing, sneezing and gasping with reduction in feed and water intake with 5% mortality rate. Nasal discharge and drop in feed and water intake with mortality rate of 1,5 - 5% was the initial symptoms of an out - break of ORT (8,15). Also mild conjunctivitis, sneezing, nasal discharges, lacrimation followed by facial oedema, off food and depression were recorded (5).

The predominant lesions in our work included oedema and exudates in the face and sinuses particularly infraorbital sinus and trachea, these lesion were responsible for the predominant clinical signs. The target organ of ORT infection is sinuses (2). Moreover, pneumonia and air sacculitis usually seen accompanied with congestion of the viscera

beside necrotic foci were seen on liver surface which confirm previous record (13, 14, 16). ORT was isolated from broilers breeder as well as turkeys and ducks (12).

The highest prevalence was recorded in trachea 6.6%, followed by lungs 6.1%, air sac 6%, liver 4.4%, spleen 3.3%, heart blood 3%, and sinuses 2.8%. It has been recorded that trachea and lung are the two organs from which ORT most commonly isolated and also cultured from sinuses, air sacs, liver, spleen, heart blood and ovary (2,12).

Post - mortem lesions in sacrificed and dead bird in infected group were trachitis or bilateral pneumonia, air sacculitis, slight congestion and enlarged liver, spleen and trachea. Fibrin on surface lung and uni or bilateral lung consolidation with fibrinopurulent exudates were recorded. Similar result previously recorded by several investigators (2, 5, 8).

In vitro all tested strains of ORT were sensitive to ampicillin, amoxicillin, oxytetracycline, penicillin, norxin, and were resistant to gentamycin, streptomycin and lincospectin these result confirm previous study (15).

In vivo the treatment is based on the results of the *in vitro* antibiogram of the isolated organism. The clinical signs disappeared in group which treated with amoxicillin than group which was infected untreated respectively. Post - mortem lesions in treated group disappeared (8). The body weight of infected bird was decreased, on the other hand ORT infection significantly reduce of growth of infected birds. The drug has considerable effect in treatment of infection with ORT in broiler breeders.

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الملخص العربي

مدى أنتشار ميكروب الاورنيثوباتيريم رينوتراكيا ليز في قطعان أمهات التسمين بمحافظة شمال

سيناء

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أجريت هذه الدراسة على أمهات التسمين على فترات مختلفة من العام بمحافظة شمال سيناء تم جمع العينات من حوال 100 طائر مابين المريضة او الميته حديثا وكانت الاعراض المرضيه هي وجود اعراض تنفسيه مع ورم في منطقة الراس . واعمارها مابين 6 - 18 شهر كانت الاعراض الاكلينيكيه لتلك الدواجن المصابة هي تورم في منطقة الوجه والتهاب في اغشية العين وغلقها ومن دراسة الصفة التشريحيه للحالات المصابه تبين وجود ارتشاح اوديومي تحت جلد الوجه مع وجود افرازات بالجيوب الانفيه احتقان في القصبه الهوائيه ووجود افرازات في الجيوب الانفيه واحتقان في الرنتين والكبد والطحال. تم اخذ مسحات من الجيوب الانفيه والقصبه الهوائيه، الرنتين، الكبد، ودم القلب وذلك للعزل البكتريولوجي. ولقد تم عزل ميكوب الاورنيثوباتيريم رينوتركيا ليز وتم التعرف عليه من الشكل المرولوجي والاختبارات البيوكيميائيه وبإجراء الاختبارات على معدل النمو وجد ان اليكروب له تاثير على وزن الطيور.

وبإجراء العدوى الصناعيه على كتاكيت التسمين عمر اسبوعين وجد ظهور الاعراض الاكلينيكيه. وبإجراء الصفة التشريحيه كانت الاعراض الاكلينيكيه في العدوى الصناعيه مشابهه للعدوى الطبيعيه. هذه الدراسه ابرزت دور الميكروب في ظهور الاعراض التنفسيه واثر ذلك على الانتاج. تم تسجيل الاعراض الاكلينيكيه والصفة التشريحيه وكذلك اعاده العزل للميكروب من الطيور المصابه , تم اجراء اختبار العترات المعزوله للمضادات البكتيرييه وظهرت النتائج تاثر الميكروب بمركب الاموكسيلين والامبيسلن, الاوكسيتراسيكلين والنيروكسين ولم يتاثر بالاستربتومايسين والجنتاميسين والاسيكتام واثناء العلاج قل ظهور الاعراض الاكلينيكيه عنها في المجموعه المصابه والتي لم تعالج .