Animal Health Research Institute, Zagazig Branch.

SOME STUDIES ON ORNITHOBACTERIUM RHINOTRACHEALE INFECTION IN BROILER FLOCKS AT SHARKIA GOVERNORATE

(With 5 Tables)

By

H.I.R. EL-BANNA; E.A. MASOUD and M.K. RIZK*

*Animal Health Research Institute, Mansoura Branch

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بعض الدراسات عن عدوى الاورنيثوباكتريم رينوتراكيال في بداري التسمين بمحافظة الشرقية

حلمي إبراهيم رجب البنا ، السيد السعيد مسعود ، مدحت كمال رزق

تم أخذ عينات من 200 طائر تعاني من أمراض تنفسية من بعض المزارع في محافظة الشرقية، وتم إجراء الفحص الظاهري وعمل صفة تشريحية لهذه الطيور وتم أخذ عينات من الأحشاء الداخلية للزرع البكتيري. تم عزل 24 ميكروب من عترة ORT بنسبة (12%) من القصبة الهوائية، الرئتين، الأكياس الهوائية والجيوب الأنفية بنسب 10.5% و8% و5.5% و1% علي الترتيب، بينما لم يتم عزل ORT من الكبد. تم تصنيف العترات بيوكيميائياً وكذلك سيرولوجياً ووجد أنها جميعاً تنتمي للفصيلة A. وتم عمل اختبار حساسية لمعرفة مدى حساسية العترات المعزولة للمضادات الحيوية ووجد أن جميع العترات حساسة للأموكسيسيلين والأوكسي تتراسيكلين والأمبسيلين ووجد أنها غير حساسة للجنتاميسين والكولستين والدانو فلوكساسين. وتم عمل العدوى الصناعية بالعترات المعزولة بالرش في دجاج تسمين عمر 3 أسابيع ووجد أن الميكروب استطاع إحداث عدوى في الدجاج مع إحداث نفوق يصل إلي 5%. وكانت أهم الآفات التشريحية إلتهاب في الأكياس الهوائية والتهاب رئوي والتهاب القصبة الهوائية.

SUMMARY

The present study was carried out on 200 broiler chickens which had respiratory troubles on different farms of different districts at Sharkia Governorate, to throw a spot light on the clinical and microbiological investigations as well as prevalence rate of ORT infection in broiler flocks. Bacteriological examination revealed that 24 isolates (12%) of ORT were isolated from the examined birds. ORT was isolated from trachea, lungs, air sacs and sinuses in a percentage of 10.5%, 8%, 5.5% and 1% respectively. No ORT was isolated from liver. Serotyping of

ORT using AGPT proved that all the isolates were belonged to serotype A. Antibiogram studies showed that all the isolates were highly sensitive to Amoxycillin, Ampicillin, Oxytetracyclin, and resistant to Gentamicin, Danofloxacin and colistin. Experimental infection with ORT evoked respiratory signs with mortality rate reached to 5%.

Key words: *Ornithobacterium rhinotracheale, broiler, respiratory troubles.*

INTRODUCTION

Respiratory diseases due to infectious agents are an important problem in the broiler chicken industry (Turan and Akcadag, 2003). Ornithobacterium rhinotracheale (ORT) is a pathogenic agent causing respiratory diseases especially in chickens and turkeys (Hadimli and Erganis, 2004 and Kaukonen *et al.*, 2004). Ornithobacterium rhinotracheale is a recent described species of bacterium associated with respiratory diseases, growth retardation, mortality and decreased egg production in chickens and turkeys. Pneumonia, pleuritis and air sacculitis characterize the infection (Canal *et al.*, 2005 and Veen *et al.*, 2005).

Ornithobacterium rhinotracheale (ORT) has been firstly isolated from broiler chickens by DuPreez (1991). Recently, Hafez (2001) isolated ORT from ducks, goose, ostrich, pheasants, pigeons, quails, rook and turkeys.

In many countries of the worlds, ORT has been incriminated as a possible additional causative agent in respiratory disease complex in poultry. The organism cause substantial financial 1osses due to high rates of contamination up to 50% in slaughtered affected flocks (Charlton *et al.*, 1993; Hinz *et al.*, 1994; Dudouyt *et al.*, 1995; Back *et al.*, 1997; Hafez and Sting, 1999 and Van Empel, 1999).

MATERIALS and METHODS

(1) Samples:

A total of 200 diseased and freshly dead birds suffering from respiratory troubles were collected from different farms of different districts at Sharkia Governorate. The examined birds were subjected to clinical signs and postmortem examination.

(2) Isolation and identification:

Loopfuls from trachea, lungs, air sacs, sinuses and liver were inoculated into defibrinated 10% sheep blood agar media containing 10ug gentamicin sulphate/ml blood agar (Back *et al.,* 1996 and Rahimi and Banani, 2007) and incubated at 37^{o}C for 48 hours under 7.5 – 10% Co_2 tension by using gas bags (Oxoid) in candle jar according to Vandamme *et al.* (1994) and examined for suspected ORT colonies.

The obtained pure cultures of ORT were identified biochemically according to Hafez *et al.* (1993). The isolates were serotyped by using agar gel precipitation test (AGPT) according to Van Empel, (1997).

(3) Sensitivity test:

It was carried out according to the technique described by Back *et al.* (1997).

(4) Pathogenicity test:

This experiment was done to study the effect of ORT isolates on three weeks old broiler chickens.

One hundred and fifty, one day old broilers obtained from Al-Brmawy Company. Ten of them sacrificed and were subjected to postmortem and bacteriological examination. These birds were free from infection with ORT. The remaining 140 chicks were grouped into four equal groups (A, B, C and D).

All birds were vaccinated with Lasota ND vaccine. First group (A) remained as control not infected and not treated while groups (B, C and D) infected with ORT. The Group (B) was remained not treated but group (C) was treated with Amoxycillin 10mg/kg b.wt. for 3 days. Group (D) was treated with Amoxycillin 10mg/kg b.wt. for 5 successive days. The dose of ORT was 100ml of cultured brain heart infusion broth (BHI), each ml of culture contain x 10^9 c.f.u. The ORT was inoculated by coarse spray (Van Empel *et al.,* 1996).

Birds were kept under observation for 4 weeks post infection. Clinical signs, postmortem lesions and reisolation trials and mortality were recorded.

RESULTS

Table 1: Results of isolation of ORT from naturally infected chicken in different farms at Sharkia Governorate.

Case No.	Districts	Total number of birds in farm	No. of examined birds	Result of examined birds for ORT		Result of bacterial isolation for ORT
				No.	%	
1	Hehia	20000	8	2	25	+ve
2	Al-Hussaynia	10000	12	1	8.33	+ve
3	Kanayat	15000	15	3	20	+ve
4	El-Salhia	25000	15	1	6.67	+ve
5	Abo-Kabier	22000	5	2	40	+ve
6	Belbis	30000	30	1	3.33	+ve
7	Dearb Negm	10000	22	2	9.09	+ve
8	Menia El- Kamh	10000	24	3	12.5	+ve
9	Abo Hamad	10000	22	4	18.18	+ve
10	Abo Hamad	15000	14	3	21.43	+ve
11	Belbis	18000	13	1	7.69	+ve
12	Fakous	12000	20	1	5	+ve
Total		197000	200	24	12	

Table 2: Biochemical character for isolated ORT.

Biochemical test	Reaction
Oxidase	+
Catalase	-
Urease	+/-
Indole	-
Nitrate reduction	-
Methyl red	-
Voges proskauer	-
Citrate utilization	+
Sugar fermentation	+
Glucose	+
Sucrose	-
Maltose	-
Lactose	+
Fructose	+
Galactose	+

Table 3: Isolation of ORT from internal organs of naturally infected birds.

Flock No.	Number of +ve cases	Isolation of the internal organs				
		Trachea	Lungs	Air sacs	Sinuses	Liver
1	2	2	2	1	1	-
2	1	1	1	1	1	-
3	3	3	2	2	-	-
4	1	1	1	-	-	-
5	2	1	1	-	-	-
6	1	1	1	1	-	-
7	2	2	1	1	-	-
8	3	3	2	1	-	-
9	4	3	2	1	-	-
10	3	2	1	1	-	-
11	1	1	1	1	-	-
12	1	1	1	1	-	-
Total	24	21	16	11	2	-
	12%	10.5%	8%	5.5%	1%	0%

Table 4: Result of in vitro sensitivity test of ORT isolates to different antimicrobial agents.

No	Antibiotic disc	Discpotancy	Standard sensitivity	
1	Amoxicillin	25ug	>13 <16	+++
2	Oxytetracycline	30 ug	>13<16	+++
3	Ampicillin	10 ug	>11<13	+++
4	Ciprofloxacin	10 ug	>13<15	++
5	Streptomycin	25 ug	>15<18	++
6	Enrofloxacine	54 ug	>15<18	++
7	Neomycin	19 ug	>13<16	-
8	Spectinomycin	10 ug	>13<15	-
9	Gentamicin	10 ug	>15<18	-
10	Colistin	10 ug	>13< 16	-
11	Danofloxacin	5 ug	>11<13	-

Table 5: Experimental infection of 3 weeks-old broiler chickens with ORT isolates

Group	No. of birds	Infected with ORT	Treatment with Amox	P.M. lesion	Morbidity rate	Mortality rate	Reisolation of ORT
(A)	35	-	-	-	-	-	-
(B)	35	+ve	-	Pneumonia Exudate in air sacs Severe congestion of trachea	57%	5%	4/5
(C)	35	+ve	+ve	Pneumonia Exudate in air sacs	22%	1%	1/5
(D)	35	+ve	+ve	Pneumonia	10%	0%	1/5

DISCUSSION

Natural infection of ORT in chickens at 4 weeks which was suffering from relatively mild respiratory signs (nasal exudates, sneezing, wetting of eyes, swelling of infraorbital sinus, retarded growth) accompanied by increased mortalities. The course of the disease was about 10- 14 days.

These finding were concurrent with findings of Van Beek *et al.* (1994); Sakai *et al.* (2000) and Heyla *et al.* (2005).

The main postmortem lesions in examined birds were congestion of respiratory passage and trachea, pneumonia, exudate in the air sacs, predominantly in the abdominal air sacs were the most striking feature. Similar findings were observed by Hafez *et al.* (1993) and Abd El-Ghany Wafaa, (2000).

The isolation of 24 (12%) isolates of ORT from 200 diseased and freshly dead chickens were collected from different farms of different districts at Sharkia Governorate were reflected their wide distribution through out these province (Table 1).

The distribution of ORT in internal organs showed that the highest incidence were observed in trachea (10.5%), lungs (8%), air sac (5.5%), sinuses (1%) and liver (0%) (Table 3). These findings were supported with the results reported by Chin (1996); Cauwerls *et al.* (2002) and El-Gohary and Sultan (2002).

Morphologically, the ORT colonies were observed on 10% sheep blood agar as non-haemolytic, non-pigmented grayish white colonies.

Microscopical examination of ORT colonies, stained with Gram stain revealed highly pleomorphic Gram negative rods, non-motile, non-spore former and non-capsulated. These findings inconformity with those obtained by Hafez *et al.* (1993); Van Empel *et al.* (1996) and Hanan *et al.* (2008).

The biochemical reaction of the ORT isolates in Table (2). These findings agree with those described by Charlton *et al.* (1993); Hinz, *et al.* (1994); De Rosa *et al.* (1996) and El-Gohary *et al.* (1998).

Serotyping of 24 isolates of ORT using agar gel precipitation test with specific antisera against 18 ORT serotypes (A-R) revealed that all tested isolates were belonged to serotype A. These findings were similar to the previous results recorded by Hafez, (1996); El-Gohary and Awaad, (1998) and Abd El-Ghany, Wafaa (2000).

The result of sensitivity test for ORT isolates against antimicrobial agents, by using disc diffusion technique (Table 4) revealed that the isolates were sensitive to Amoxycillin, Oxytetracycline and Ampicillin while were variably to Ciprofloxacine, Streptomycin and

Enrofloxacine and resistant to Neomycin, Spectinomycin, Gentamicin, Colistin and Danofloxacin. These result agreed partially with those described by Hinz *et al.* (1994).

Experimental infection with ORT are given in Table (5). Birds in group (B) "infected with ORT and not treated", the ORT was able to reproduce the respiratory disease as the same characteristics as seen in the natural outbreak and the mortality was 5% and morbidity rat was 57%. The birds in group (C) "infected with ORT and treated with Amoxycillin for 3 days", the ORT was cause light respiratory disease and mortality rate was 1% and morbidity rate was 22%. While the birds in group (D), the mortality rate was 0.0% and morbidity rate was 10%. Similar results observed by Hinz *et al.* (1994).

The result of treatment of experimentally infected broiler revealed that the treatment with Amoxycillin 10mg/kg b.wt. for 5 days decreased the mortality and morbidity rates than those treated with Amoxycillin 10mg/kg b. wt. for 3 days. Reisolation rate of ORT from experimentally infected and not treated broilers was 80% but 20% reisolation rate was recorded in infected and treated birds. These results were in agreement with those obtained by Hafez *et al.* (1993) and Awaad *et al.* (2002).

It is concluded that the ORT infection are incorporated in complicated respiratory infection and the utmost need for use of sensitive antibiotic for control measures considered an important direction to avoid loss of body weight, respiratory signs and economic losses.

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