Vet. Med. J., Giza. Vol. 45, No. 1. (1998): 67-75.

CONCOMITANT ORNITHOBACTERIUM RHINOTRACHEALE (ORT) AND E.COLI INFECTION IN CHICKEN BROILERS

By

A.A ELGOHARY* and M.H.H. AWAAD**

* Faculty of Veterinary Medicine, Tanta University.

** Faculty of Veterinary Medicine, Cairo University.

Received: 5.8.1997

Accepted : 29.12.1997.

SUMMARY

The first identification of Ornithobacterium rhinotracheale (ORT) in Egypt was undertaken from an outbreak of respiratory disease in 5000 broilers ageing 38 days with 7.12% mortality during 20 days. The recorded clinicopathological picture was similar to that reported from broilers in Europe, USA and South Africa as being associated with ORT. ORT concurrently isolated with E.coli from lungs and air sacs of examined cases.

INTRODUCTION

Respiratory diseases are a major problem facing poultry industry not only on national but also on international bases as well. DuPreez (1991) in South Africa described a respiratory disease with relatively mild respiratory symptoms, accompanied by increased mortality and poor performance with post mortem lesions of foamish "Yoghurt-like" exudate in the air sacs with pneumonia. Bacteriological examination revealed a slowly growing, pleomorphic, gram negative-rods (PGNR) (Charlton et al. 1993), which could not be classified into one of the known species. This bacterium initially was named Pasteurella-like or Kingella-like and Prof.M. Pisgaard stated that the organism could be classified within a group of bacteria he named Taxon 28 (van Empel, 1996). By carrying out further identification of isolates obtained from the respiratory tract of different avian species from different countries using genetic taxonomic methods ultimately the name Ornithobacterium rhinotracheal (ORT) gen.nov., sp.nov. was suggested for this new species by Vandamme et al. (1994). ORT could repeatedly be isolated from airsacculitis and pneumonia of meat turkeys and broilers (Hafez, 1994; van Beek et al., 1994; van Emple, 1996 and Odor et al., 1997).

The present work describes concomitant ORT and E.coli infection in broilers.

MATERIAL AND METHODS

Specimens:

Samples of lung, air sacs, trachea, liver, heart blood and bone marrow were collected from 15 birds out of 5000, 38 day-old broilers. The flocks was suffering of respiratory manifestations and mortality.

Bacteriological examination:

Samples were cultured on MacConkey's agar, 5% sheep blood agar, brain heart infusion broth, peptone water , and Pasteurella broth . All cultured media were incubated at 37°C and 42°C for at least 48 hours. Isolated bacteria were identified morphologically using gram stain, biochemically using catalase test and API 20 E strip microtest system (Bio-Merieux, France). Serological identification of the isolates suspected to be E.coli was conducted using O-K antiserum (Behring Werk, Ag. Marburg, Lahn, Germany). ORT suspected isolates were kindly serologically identified by Prof. H.M. Hafez (Institut fur Geflugelkrankheiten, Berlin, Germany) using agar gel precipitation (AGPT) test against polyvalent antisera of ORT serotypes A-H.

Swabs from hock joints, trachea and air sacs were inoculated on modified Frey's broth and agar (Frey et al., 1968) for isolation of Mycoplasma species.

Mycological examination:

Samples of lungs from selected birds were cultured on Sabouraud's dextrose agar and incubated at 37°C for 24 hours followed by incubation at room temperature for 4 weeks fungal isolation.

Virological examination:

Pooled samples of trachea and lungs treated gentamycin were inoculated into chorioallan and yolk sacs of embryonated chicken eggs isolation of viral agents. Inoculated emb were candled daily for mortality and alllan fluid was examined for haemagglutina activity.

Antibiogramme:

The antibiogramme of isolated bacterial isol was investigated against 15 antimicrobial age using disc diffusion technique according Cruickshank et al. (1975). The test procedure that recommended by the National Committee Clinical Laboratory Standards (1990).

Pathogenicity test:

Eighty-five, day-old commercial broilers w floor reared and used for pathogenicity testi Five out of these birds were randomly sacrific and exposed for bacteriological examination prove their freedom from bacterial infection. A weeks of age the other 80 birds were divided i equal groups consisting of 20 each (1-4). Chic of group 1 were aerosolised with 100ml peptone water containing 10^9 CFU/ml of O using a commercial paint sprayer. The develop mist was maintained in the isolator for at least minutes with air circulation closed (van Empel al., 1996). Those of group 2 were oral inoculated with 10^9 CFU of the isolated E.c serogroup 055:K59 per bird (Calanek et a

1991). Chickens of group 3 were similarly infected with both ORT and E.coli strains. While those of the last group (4) were kept without infection as control. Birds of all groups were kept for 3 weeks observation period during which clinical signs and mortality were recorded. Dead as well as sacrificed birds at the end of observation period were subjected to post mortem and bacteiological examinations. A scoring system for the lesions post ORT infection was adopted after van Empel et al. (1996).

RESULTS

The observed clinical symptoms in the studied broielr flock were respiratory signs including gasping, coughing, sneezing, and sinusitis. The birds also suffered from depression, ruffled feathers, anorexia, retardation in growth and in 7.12% mortality within 20 days course of illness.

Concerning postmortem lesions severe congestion of lungs with acute exudative or fibrinous pneumonia, tracheitis, pericarditis, airsacculitis (serofibrinous or fibrinous), enlarged liver, enlarged spleen and sinusitis were; the most stricking lesions in dead birds.

Bacteriological examination revealed only isolation of a lactose fermenter gram-negative bacteria suspected to be E.coli from trachea, lungs and air sacs together with a pleomorphic gram-negative rods from lungs and air sacs on blood agar (Table 1). The colonial morphology of the latter was 1-2mm, grey, convex, circular pin point shaped colonies. These colonies were able to grow on peptone water and Pasteurella broth and grow on triple sugar iron agar (TSI) slant with no change in the but portion.. They also were unable to grow on MacConkey's, S.S., Gassner agar or brain heart infusion broth oragar. The colonies had a distinct butyric acid odour. Biochemical characters of the isolated bacteria are shown in Table (2). Serological examination of the isolated ORT and E.coli isolates clarified that they belong to serotype A and O55: K59 respectively. Results of virological and mycological examinations were negative.

Results of senitivity testing of isolates to antimicrobial agents revealed that all ORT isolates were 100% sensitive to chloramphenicol, kitassamycin and amyoxycillin; 75% for lincospectin and danofloxacin; 50% for erythromycin and oxytetracycline; 37.5% for enrofloxacin and streptomycin; 25% for ampicillin, colistin and flumequine and were resistant to gentamycin, neomycin and sulfamethazole trimethoprim. While all E.coli isolates wre 100% sensitive to danofluxacin; 80% for gentamycin lincospectin and kitassamycin; 60% for enrofloxacin and streptomycin; 40% for amoxycillin, flumequine, colistin, , chloramphenicol and erythromycin; 20% for neomycin and sulfamethaxazole trimethoprim; and were resistant to ampicillin and oxytetracycline.

Pathogenicity testing of ORT to 2 week-old broilers revealed only mild airsacculitis at 2nd and 3rd weeks post infection (PI). Moderate airsacculitis with limited pinheaded foci of fibrinous exudate accompanied with mild tracheitis were recorded at the 4th week PI

Table 1 : Frequency of isolation of bacteria from various organs of chicken broiler flock .

Birds		Frequency of isolation			
	No of birds	Organs	No. of culture	No. of ORT isolates	No. of E. coli isolates
Commercial Chicken Broilers (5000, 38 day old)	15	Trachea	15	0	1
		Lungs	15	6	3
		Air-sacs	10	2	1
		Pericardium	15	0	0
		Sinuses	8	0	0
		Liver	15	0	0
		Spleen	15	0	0
Total	15	7 organs	93	8	5
Rate of isolation	-	-	-	8.6 %	5.4 %

Table 2 : Biochemical characteristics of the isolated bacteria from broiler chickens .

Tests		Pin point non haemolytic colonies on blood agar								Lactose fermenting colonies on MacConkey agar				
		1	2	3	4	5	6	7	8	1	2	3	4	5
API 20 E*	ONPG	+	+	+	+	+	+	+	+	+	+	+	+	+
	ADH	+	+	+	+	+	+	+	-	-	-	-	-	-
	LDC	-	-	-	-	-	-	-	-	-	-	-	-	-
	ODC	-	-	-	-	-	-	-	-	+	+	+	+	+
	CIT	-	-	-	-	-	-	-	-	-	-	-	-	-
	H_2S	-	-	-	-	-	-	-	-	-	-	-	-	-
	URE	+	+	+	+	+	+	+	+	-	-	-	-	-
	TDA	-	-	-	-	-	-	-	-	-	-	-	-	-
	IND	-	-	-	-	-	-	-	-	+	+	+	+	+
	VP	-	-	-	-	-	-	-	-	-	-	-	-	-
	GEL	-	-	-	-	-	-	-	-	+	+	+	+	+
	GLU	-	-	+	-	-	+	-	-	+	+	+	+	+
	MAN	+	-	-	+	-	-	-	+	+	+	+	+	+
	INO	+	-	-	+	-	-	+	-	-	-	-	-	-
	SOR	-	+	-	+	-	-	+	-	+	+	-	-	+
	RHA	+	-	-	+	-	+	-	+	+	+	-	-	+
	SAC	+	-	-	+	-	+	-	-	+	+	+	+	+
	MEL	-	-	-	+	-	-	+	+	+	-	-	+	+
	AMY	+	-	-	+	-	+	-	-	+	+	-	+	+
	ARA	+	-	+	+	-	-	-	+	+	+	+	-	+
	OX	+	+	+	+	+	+	+	+	-	-	-	-	-
Catalase test		-	-	-	-	-	-	-	-	not done				
The isolated pathogens		Ornithobacterium rhinotracheale								Escherichia coli				

* Code number : 4056127 (13)

ONPG = Beta - galactocidase .

ADH = Arginine hydrolase .

LDH = Lysine decarboxylase .

ODC = Ornithine decarboxylase .

CIT = Citrate utilization .

OX = Cytochrome oxidase .

H_2S = H_2S production .

URE = Urease .

TDA = Tryptophane desaminase .

IND = Indole production .

VP = Acetoin production .

GEL = Gelatinase .

GLU = Glucose [fermentation / oxidation] .

MAN = Manitol [fermentation / oxidation] .

INO = Inositol [fermentation / oxidation] .

SOR = Sorbitol [fermentation / oxidation] .

RHA = Rhamnose [fermentation / oxidation] .

ARA = Arabinose [fermentation / oxidation] .

SAC = Sucrose [fermentation / oxidation] .

MEL = Meliliose [fermentation / oxidation] .

AMY = Amygtalin [fermentation / oxidation] .

Table 3 : Experimental challenge of commercial broiler chickens with the isolated strains of Ornithobacterium rhinotracheale (ORT) and Escherichia coli .

Group No.	Lesion scores *												Mortalities				Reisolation	
	1st. week P.I.			2 nd. week P.I.			3 rd. week P.I.			4 th. week P.I.			1st. week P.I.	2 nd. week P.I.	3 rd. week P.I.	4 th. week P.I.	No.	%
	Tr.	L.	AS	Tr.	L.	AS	Tr.	L.	AS	Tr.	L.	AS						
1	0	0	0	0	1	0	0	1	1	1	0	2	-	-	-	-	6/20@	30 %
2	0	0	0	1	0	0	1	0	1	1	0	1	-	-	1	-	17/20	85 %
3	0	0	0	1	0	1	1	1	2	1	2	2	-	1	1	-	4+5/20	20+25%
4	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	0/20	0 %

1 = Challenge with Ornithobacterium rhinotracheale isolates .
2 = Challenge with Escherichia coli isolates .
3 = Challenge with Ornithobacterium rhinotracheale + Escherichia coli isolates
4 = Blank control

Tr. = Trachea . L. = Lung . AS. = Air sac

@ No. of positive / total No. of examined birds

* lesion scores are calculated After van Emple et al. (1996) as follows:

Trachea : 0 = No abnormality. 1 = Slight exudate. 2 = moderate exudate. 3 = Lumen filled with exudate.
Lungs : 0 = No abnormality. 1 = Unilateral pneumonia. 2 = Bilateral pneumonia. 3 = Consolidation.
Air sacs : 0 = No abnormality. 1 = Slight airsacculitis. 2 = Moderate airsacculitis with limited pinheaded foci of fibrinous exudate.
3 = Severe fibrinous airsacculitis.

without mortality and 30% rate of reisolation. Pathogenicity testing of E.coli showed slight tracheitis at 2nd week PI., slight tracheitis and airsacculitis at 3rd and 4th weeks PI respectively with 5% mortality and 85% rate of reisolation.

Pathogenicity testing of both ORT and E.coli resulted in slight tracheitis and airsacculitis at the 2nd week PI. On the 3 d week; there was slight exudate in trachea with unilateral pneumonia and moderate airsacculitis with limited pin headed foci of fibrinous exudate. While on the 4th week there was slight exudate in trachea, bilateral pneumonia and moderate airsacculitis with pin headed foci of fibrinous exudate. A rate of mortality of 5% was recorded at 2nd and 3rd weeks post infection with 20 % and 25% rate of reisolation for ORT and E. coli were recorded respectively (Table 3).

DISCUSSION

Very recently Ornithobacterium rhinotracheale (ORT) was isolated in various countries from turkeys and chickens suffering from respiratory infections (Charlton et al., 1993; Hafez et al., 1993; Hinz et al., 1994; Van Beek et al., 1994; Tanyi et al., 1996 and Odor et al.,1997).

In the present study an outbreak in a commercial broielr flock characterised by respiratory manifestation in El-Gharbia province was investigated . Bacteriological studies revealed the isolation of 8 isolates of ORT serotype A from lungs and air sacs together with 5 isolates belonging to E.coli serogroup O55 :K59 out of which 4 isolates were concurrently isolated with ORT from lungs and air sacs. Awaad (1972) isolated E.coli serogroup 055: K 59 from outbreaks of colisepticaenua in borilers. Hafez (1993) mentioned that ORT serotype A is the most common strain identified in chickens.

Regarding our results of ORT sensitivity in vitro; Hafez (1996) recorded that all tested isolates (100%) showed high sensitivity to amoxicillin and chloramphinicol and none of the isolates was susceptible to gentamycin, neomycin and sulfonamide trimethoprim which completely accords with out results.

In the present investigation, experimental infection of 2 week-old chickens with ORT and/ or E.coli could reproduce the respiratory disease with at least in part the same characteristics as seen in the clinical outbreak. It is evident that concomitant E.coli infection increased the severity of the lesions associated with ORT (Table 3). Experimental infection proved that ORT isolated strain was capable of inducing airsacculitis, and the infection appeared to be aggravated by administration of E.coli. Traver (1996) reported that concomitant ORT and ND infection led to a significantly more severe respiratory disease syndrome in the affected broilers. The most prominent clinical signs after experimental infection appeared to be mild respiratory signs. However, other clinical signs seen in natural outbreak such as sinusitis were not observed after experimental infection. With regard to pathology, the most prominent lesions after experimental infection of either ORT or E.coli was the development of mild airsacculitis

with 5% mort ality in E.coli infected group. Moreover; concomitant infection with both pathogens yielded lesions of mild to moderate airsacculitis with limited pinheaded foci of fibrinous exudate, mild tracheitis and unilateral or bilateral pneumonia, accompanied with 5% mortality. These post mortem features were also observed in the clinical outbreak additionally to fibrinous airsacculitis, pericarditis, congestion and haemorrhages on heart and liver. The discrepancy between natural and experimental infection might be explained by differences in predisposing and aggravating factors. Where under field conditions other factors can also be of importance, such as stress, high stock density, poor ventilation, or high ammonia levels (Van Empel et al., 1996).

Ryll et al. (1997) failed to reproduce respiratory score lesions in chickens experimentally infected with ORT and concluded that it does not appear to be a primary, but secondary respiratory pathogen in broilers. Obtained results of ORT pathogenicity accord with the conclusion of van Empel et al. (1996) who stated that although not all clinical signs and post-mortem findings observed in clinical outbreaks could be reproduced after experimental ORT infection, this micro-organism is a true infectious agent in turkeys and broiler chickens.

In conclusion ; from the epizootiological stand point of view; as ORT is claimed to be a new pathogen firstly isolated in Egypt; further study about mode of infection, transmission and source of infection is extremely commendable.

ACKNOWLEDGEMENT

The authors are indebted to Prof. H.M. H
(Institute of Poultry Diseases, Berlin, Germa
for serotyping ORT isolated strains.

REFERENCES

Awaad, M.H.H. (1972): Studies on colisepticaemi
chickens. M.V.Sc. thesis, Faculty of Veteri
Medicine, Cairo University.

Charlton, B.R. S.E. Channing, Santiago, A.A. Brick
C.J. Cardona, R.P. Chin, G.L. Cooper, R. Droual,
Jeffry, C.U. Meteyer, H.L. ShivapraSad, and
Walker (1993): Preliminary characterisation
pleomorphic gram-negative rod associated with a
respiratory disease, J.Vet. Diagn. Invest., 5; 47-51.

Criuckshank, R.; Dugid, J.R. Morimon, B.P.; and Sw
R.H.A. (1975): Medical Microbiology. 12th Ed.
Livingstone limited, Edinburgh, London, New York

Duprees,J. (1991): Cited from van Empel et al. (1996).

Devrise, L.A.J. Hommez; P. Vandamme; K,Kersters; an
Haesebroul (1995): In vitro antibiotic sensitivity
Ornithobacterium rhinotrachheale strain from pou
and wild birds. Vet. Rec. 137: 435-436.

Frey, M.L. R.P. Ganson, and D.P. (1968): A medium for
isolation of avian mycoplasmas. Am. J. Vet. Res.
2163-2171.

Hafez, H.M.; W. Kruse; J. Emele; and R.Sting (1993): E
Atemwegsinfektion bei Mastputen durch Pasteur
ahnliche Erreger: Klinik, Diagnostik; und Thera
Deutsch Veterinar Medicinische Gesellsgafte.
Frankfurter strasse 89, D-35392 Postdam, 28.07-3
7.pp. 105-112.

Hafez, H.M. (1994): Respiratory disease conditions in meat turkeys caused by Ornithobacterium rhinotracheale: Clinical signs , diagnostics and therapy. proc. 43 rd. Western Poultry Disease Conference, Sacramento, California.

Hinz, K.H., C. Blome, and M. Ryll. Acute exudative pneumonia and airsaculitis associated with Ornithobacterium rhinotracheale in turkeys. Vet. Rec: 233-234. 1994.

National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests, 4th ed. Approved standard . NCCLS document M2-A4, Villanova, Pa. 1990.

Odor, E.M.; M. Salem; B. Sample; C.pope Jr.; and M.Murphy 1997; Diagnostic dilemmas of Ornithobacterium rhinotracheal Presented October 17, 1997, at the 32nd, National Meeting on poultey health and processing , Ocean City maryland.

Ryll, M.K., K.H. Hinz, V. Neumann and H.Salisch (1997): Pathogenicity of Ornithobacterium rhinotracheale for chicken under experimental conditions. XIth International Congress of the World Veterinary Poultry Association pp: 47.

Tanyi, J.; Bistyak; E. Kaszanyitzky; F. Vetesi and M. Dobos-Kovacs (1995): Isolation of Ornithobacterium rhinotracheale from chickns, hens and turkeys showing respiratory symptoms. Magyar Allatrovosk Lapja, 50 (6): 328-330.

Traver, F. (1996): Cited from Chin, R.P.; Droual and B.R. Charlton (1996): Ornithobacterium rhinotracheale infection in turkeys. Proc. of The Turkey ORT Symposium, September 4-6, 1996 Minnrapolis, Minnesota, pp:68.

Van Beek, P.N.G.M., P.C.M. Van Empel, G. Van den Bosch, P.K. Storm, J.H. Bongers, and J.H. DuPreex. Ademhalings problem groeivetaging gewrichtssontssteking Bij Kalkoenen en Vleeskuikens door een Pasteurella-achtige bacterie Ornithobacterium rhinotracheale of "Taxon 28" Tijdschr. Diergeneeskd. 119: 99-101. 1994.

Vandamme, P., P. Segers, M. VanCanneyt, K. Van Hove, R. Mutters, J. Hommez, F. Dewhirst, B. Paster, K. Kersters, E. Falsen, L.A. Devviese, M. Bisgaard, K. H. Hinz, and W. (1994). Mannheim Ornithobacterium rhinotracheale gen nov., sp. nov., isolated from the avian respiratory tract. Int. J. Syst. Bacteriol. 44: 24-37, 1994.

Van Empel, P.;H. Van den Bosch; D. Goovaerts, and P. Storm (1996): Experimental infection in turkeys and chickens with Ornithobacterium rhinotracheale. Avian Dis. 40: 858-864.