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ORNITHOBACTERIUM RHINOTRACHEALE (ORT) ASSOCIATED WITH HATCHING PROBLEMS IN CHICKEN AND TURKEY EGGS.

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SUMMARY

Isolation and identification of *Ornithobacterium rhinotracheale* (ORT) from infertile eggs, dead embryos, dead in shell chicks and turkeys as well as day-old chicks and turkey poults obtained from native hatcheries suffering from increased embryonic mortality, dead in shell birds and high mortality in newly hatched chicks and turkey poults was undertaken for the first time. Salmonella give (S. give could also be concomitantly isolated in certain occasions. Pathogenicity testing of the isolated ORT and/or S. give was investigated in 3 day-old chicks. The clinicopathological picture in experimentally infected chicks was described.

INTRODUCTION

Ornithobacterium rhinotracheale (ORT) gen. nov., sp. nov. has been described by Vandamme et al. (1994). ORT could repeatedly be isolated from airsacculitis and pneumonia of meat turkeys and broilers (Hafex, 1994; van Beek et al., 1994; van Emple, 1996, Odor et al., 1997 and El-Gohary and Awaad 1997). ORT constitutes a distinct species and genus placed in taxonomic neighbourhood of the genera Flavobacterium, Cytophaga, Capnocytophoga and Riemerella within the rRNA super family V (Hinz et al., 1994).

The present work describes isolation and identification of ORT in native hatcheries in Egypt.

MATERIAL AND METHODS

Specimens:

A total of 220 specimens constituting 55 infertile eggs, 110 dead embryos (55 early and 55 late deaths) as well as 55 dead in shell chicks and turkey poults were used. Additionally; samples of

lungs, trachea, liver, gall bladder, spleen, heart blood, pericardial fluid, and yolk sac contents were collected from 26 and 15; one-day-old hatched chicks and turkey poults respectively. All collected specimens were representing 9 native hatcheries complaining of low hatchability and high mortality in newly hatched chicks and turkey poults. The specimens were subjected to bacteriological examination.

Bacteriological examination:

Samples were cultured on MacConkey's agar, 10% sheep blood agar, brain heart infusion broth, peptone water, and Pasteurella broth. All cultured media were incubated at 37°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 identificaiton of the isoaltes suspected to be Salmonella was conducted using polyvalent and monovalent salmonella antisera (Difco lab., Detroit, Michigan, USA). ORT suspected isolates were serologically identified with the agar-gel precipitation (AGPT) test against antisera of ORT serotypes A, B, C, D, E, G and H according to methods described by Van Emple et al. (1997) and Hafez and Sting (1997).

Pathogenicity test:

Eighty-five, day-old commercial broilers were floor reared and used for pathogenicity testing. Five out of these birds were randomly sacrificed and exposed for bacteriological examination to prove their freedom from bacterial infection. At 3 days of age the other 80 birds were divide equal groups consisting of 20 each (1-4). of group 1 were sprayed with 100 ml of water containing 10^9 CFU/ml of ORT commercial sprayer (particle size 250 U dveloped mist was maintained in a com sprayer (particle size ⊇ 50 U). The develop was maintained in the isolator for at l minutes with the air circulation closed Empel et al., 1996). Those of group 2 were inoculated with 10^9 CFU of the isolated per bird (El-Gohary, 1992). Chickens of g were kept without infection as control. Bird groups were kept for 3 weeks observation during which clinical signs and mortality recorded. Dead as well as sacrificed birds end of observation period were subjec post-mortem and bacteriological examinati scoring system for the lesions post ORT inf was adopted after Van Empel et al. (1996).

RESULTS

Obtained results are shown in Tables 1-4. *Bacteriological* examination revealed isola a non-lactose fermenter gram negative b suspected to be Salmonella species from yo heart blood and liver of day-old chicken turkeys and from dead embryos w pleomorphic gram negative rods on bloo (Tables 1 and 2). The colonial morphology latter was 1-2 mm, grey, convex, circul point shaped colonies. were able to gro peptone water and Pasteurella broth and gr triple sugar iron agar (TSI) slant with no c

in the but portion. They also were unable to grew on MacConkey's, S.S., Gassner agar or brain heart infusion broth or agar. the colonies have a distinct butyric acid odour. Biochemical characters of the isolated bacteria are shown in Table (3) Serological examination of all ORT and Salmonella isolates clarified that they belong to serotype A and S. give (El; 3, 10; I, V; 7) respectively.

Pathogenicity testing of ORT to 3-day-old chicks revealed only mild airsacculitis at 2nd. week post infection (PI). Mild airsacculitis, mild tracheitis and unilateral pneumonia were recorded at the 3rd. and 4th week PI with 15% mortality and 35% rate of reisolation.

Pathogenicity testing of S. give showed slight airsacculitis at 2nd week PI. Unilateral pneumonia and mild tracheitis at 3rd. week PI. While on 4th. week mild tracheitis, mild airsacculitis and unilateral pneumonia were recorded accompanied with 20% mortality and 85% rate of reisolation.

Pathogenicity testing of both ORT and S. give resulted in slight airsacculitis at the 1st. week PI. On the 2nd week; there was slight tracheitis with unilateral pneumonia and moderate airsacculitis. On the 3rd and 4th weeks; there was moderate tracheitis, moderate airsacculitis and bilateral pneumonia. The mortality reached 25%. The rate of reisolation of ORT and S. give was 30% and 80 respectively (Table 4).

Table (1): Isolation and identification of ORT and S.give from hatching eggs and newly hatched chickens and turkeys.

No. of hatch	species	Samples					Total	Rate of isolation	
		I.E	ED	LD	D.Sh	D-O		ORT	S. give
1	Turkey	10	10	10	10	10	50	9	-
2 a	Turkey	5	5	5	5	5	25	2	1
2 b	Chicken	5	5	5	5	5	25	5	10
3	Chicken	5	5	5	5	3	23	1	-
4	Chicken	5	5	5	5	3	23	-	4
5	Chicken	5	5	5	5	3	23	-	-
6	Chicken	5	5	5	5	3	23	-	-
7	Chicken	5	5	5	5	3	23	2	4
8	Chicken	5	5	5	5	3	23	-	2
9	Chicken	5	5	5	5	3	23	-	-
Total 9	Turkey& chicken	55	55	55	55	41	261	19	21

I.E= Infertile eggs.
E.D= Early deathes.
L.D= Late deathes.
D.Sh = Dead in shell embryo.
D-O = Day-old chicks.

Table(2): Frequency of isolation of ORT and S.give from day old chicks and turkey poults.

Organs	Frequency of isolation			
	Day-old chicks		Day-old turkey poults	
	ORT	S.give	ORT	S.give
Yolk sac	2	6	3	1
Heart blood	-	3	1	-
Pericardium	-	-	-	-
Liver	-	3	-	-
Gall bladder	-	-	-	-
Spleen	-	-	-	-
Lung	1	-	1	-
Trachea	-	-	-	-
Intestine	-	1	-	-
Total	3	13	5	1

Table (3) : Biochemical characteristics of the isolated bacteria from hatching eggs and newly hatched chickens and turkeys.

Tests		Pin point non haemolytic colonies on blood agar	Non-Lactose fermenting colonies on S.S. agar
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		-	+
The isolated pathogens		Ornithobacterium rhinotracheale	S. give

* Code number : 4155159 (40)

ONPG = Beta - galactocidase .
ADH = Arginine hydrolase .
LDC = 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 (4) : Experimental challenge of three days-old chicks with the isolated strains of Ornithobacterium rhinotracheale (ORT) and S.give.

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.	Total		ORT	%
	Tr.	L.	AS	Tr.	L.	AS	Tr.	L.	AS	Tr.	L.	AS					No.	%		
1	0	0	0	0	1	1	1	1	1	1	1	1	-	1	1	1	3	15	7/20 Ω	35
2	0	0	0	0	1	1	1	1	1	1	1	1	-	1	2	1	4	20	0/20	0
3	0	0	1	1	2	2	2	2	2	2	2	2	-	2	2	1	5	25	6/20	30
4	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	0	0	0/20	0

1 = Challenge with Ornithobacterium rhinotracheale isolates .
2 = Challenge with S. give isolates .
3 = Challenge with Ornithobacterium rhinotracheale + S. give isolates
4 = Blank control
Tr. = Trachea . L. = Lung . AS. = Air sac
Ω 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.

DISCUSSION

Ornithobacterium rhinotracheale (ORT) was isolated in different areas allover the world from turkeys and chickens suffering from respiratory infection (Charlton et al., 1993; Hafez et al., 1993; Hinz et al., 1994; van Beek et al., 1994; Tanyi et al., 1995, Odor et al. (1997) and El-Gohary and Awaad 1997).

Nine hatcheries suffered from a problem of increased embryonic mortality (early and late deaths) as well as increased number of dead in shell chicks and turkey poults with high mortality in newly hatched birds were subjected to the present investigation.

Two out of the 9 examined hatcheries wsa incubating turkey eggs, one of them was also incubating chicken eggs in the same hatchery. The remaining 7 hatcheries were incubating chicken eggs.

As one out of 9 examined native hatcheries were incubating both chicken and turkey eggs together; ORT could be isolated from both species. Moreover, it was noly ont isolated from dead embryos but also from hatched day old chicks and turkey pouts.

The obtained results clarified that 11 out of 75 examined turkey speciemens (14.7%) were ORT positive, while only 8 out of 186 examined chicken specimens (4.3%) were ORT positive. This result indicated the magnified role played by ORT in turkey eggs hatchability probelms.

All ORT isolates belonged to the serotype A. Serotype A appears to be the most common ORT serotype in chickens and turkeys (Van Empel, 1994 and Hafez and Beyer 1997).

Several infectious diseases appeared to be involved in drop of egg production. In turkey flocks Chin (1996) reported on drop in egg production after ORT infection in turkey breeder flock. Same observation was made by Hafez (1997) in broiler breeder flocks with antibodies to both ORT and avian pneumovirus (Turkey rhinotracheitis).

S. give could concurrently be isolated with ORT from 4 out of the 9 examined hatcheries.

Experimental infection of 3 days-old chicks with the isolated ORT and/or S.give developed respiratory disease with at least in part the same characteristics as seen in the natural outbreak described by aforementioned authors.

It is apparent that concomitant S.give infection increased the severity of the lesion associated with ORT (Table 3). Traver (1996) reported that concomitant ORT and ND infection led to a significantly more severe respiratory disease syndrome in the affected broilers, El-Gohary and Awaad (1997) concluded that experimental infeciton of E. coli aggravated ORT post-mortem lesions. The most prominent clinical signs after experimental infection appeard to be mild respiratory signs. The most prominent lesion after experimental infection of either ORT or S. give was the development of mild airsacculitis.

Moreover; concomitant infeciton with both pathogens yielded lesions of mild to moderate airsacculitis with unilateral or bilateral pneumonia. Similar postmortem freatures had also been observed in the natural outbreaks and in experimental infections of ORT (Hafez et al.; 1993, and El-Gohary and Awaad; 1997).

Contrary to our results, 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.

Eventually; in the light of the obtained result, critical questions are emerging such as what is ORT status of SPF birds ? and are living vaccines free from bacterial (ORT) contamination?. Such quesitons are urgently in need of further inverstigations.

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