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Pathogenic bacteria related to respiratory diseases in poultry with reference to *Ornithobacterium rhinotracheale* isolated in India

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ABSTRACT

Isolation and identification of pathogenic bacteria, with special reference to *Ornithobacterium rhinotracheale* associated with respiratory diseases, were performed from a total of 253 biomaterials collected from 125 layers of 35 commercially reared layer farms in Namakkal of Tamil Nadu state. In total, 27 (51.9%), 18 (34.6%), 5 (9.6%) and 2 (3.8%), isolates were identified as *Escherichia coli*, *Ornithobacterium rhinotracheale*, *Pasteurella multocida* and *Haemophilus paragallinarum* respectively. *Ornithobacterium rhinotracheale*, one of the causative agents of the emerging respiratory diseases of poultry could be isolated either singly or concurrently with other bacteria such as *Escherichia coli* and *Haemophilus paragallinarum,* indicating its possible etiological role in respiratory disease.

Key words: isolation, *Ornithobacterium rhinotracheale*, poultry, respiratory diseases

Introduction

Respiratory infection is the most serious disease affecting poultry and causes heavy economic losses in the poultry industry worldwide. In avian host, several microorganisms

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of the genus *Pasteurella* (*P. multocida, P. gallinarum, P. haemolytica* and *P. anatipestifer), Bordetella* (*B. avium*) and *Haemophilus* (*H. paragallinarum*) were involved in respiratory diseases complex (HAFEZ, 2002). *Escherichia coli* associated with respiratory infection in chickens has also been reported (EL-SUKHON et al., 2002). *Ornithobacterium rhinotracheale* has recently been identified as a pathogen causing respiratory tract infections in poultry and other birds (VANDAMME et al., 1994; CHIN et al., 2003). Tracheitis, exudative pneumonia, pleuritis, air sacculitis, pericarditis, sinusitis, characterize the infection (ZORMAN-ROJS et al., 2000; CANAL et al., 2005). *Ornithobacterium rhinotracheale* has been isolated from chicken, turkeys, quails, ducks, geese, ostriches, guinea fowl, pheasants, rooks and pigeons. There were reports of *O. rhinotracheale* infections in the United States, Germany, South Africa, The Netherlands, France, Israel, Belgium, Hungary, Japan, the United Kingdom, Turkey, Canada, Jordan and Brazil (TRAVERS, 1996; JOUBERT et al., 1999; VAN EMPEL and HAFEZ, 1999; CHIN et al., 2003).

Namakkal, one of the most leading chicken egg-producing pockets in India, is constantly facing the threat of emerging respiratory diseases even after vaccinating for the known viral and bacterial diseases. This study was aimed at isolation and identification of bacteria associated with sinusitis, pneumonia and air sacculitis with special reference to *O. rhinotracheale*.

Materials and methods

Necropsy was conducted on 125 layer chicken (both dead and ailing birds) from 35 commercially reared layer farms showing respiratory disease symptoms.

The clinical signs noticed in the ailing birds associated with respiratory disease, were weakness, gasping, pump handled respiration, dyspnoea, mucous discharge and increased mortality in acute cases, especially in growers of 8-2 weeks of age. The other clinical signs in infected layers were swelling of sinuses, facial oedema, drop in egg production and poor egg quality.

A total of 253 biomaterials, such as tracheas, lungs, air sacs, exudates of infra orbital sinus, heart blood, and liver samples, were collected for isolation of pathogenic bacteria.

The collected samples were streaked onto the media of 10% sheep blood agar (Hi Media laboratories, Mumbai) without or with Gentamicin (10 μg per mL), Dextrose starch agar(Hi Media laboratories, Mumbai) with 1% (v/v) filter sterilized and heat inactivated chicken serum, Chocolate agar (Hi Media laboratories, Mumbai) with supplementation of 0.01% reduced nicotinamide adenine diphosphate and MacConkey's agar (Hi Media laboratories, Mumbai). The culture plates were incubated for at least 48 hr at 37 °C in anaerobic or aerobic conditions with or without CO_2.

Following incubation, the growth characteristics and colony morphology were studied. Smears for Gram staining were made from colonies, collected directly from respective culture plates. Biochemical identification tests and IMViC tests for confirmation of bacteria were carried out by conventional methods (BARROW and FELTHAM, 1993; QUIN et al., 1994; VAN EMPEL et al., 1997).

Results

The gross findings at necropsy were sinusitis, tracheitis, yoghurt like exudates in air sacs and severe bronchopneumonia (Fig. 1). Accumulation of blood in the trachea and severe congestion of lungs with haemorrhagic foci were noticed on the lungs. Accumulation of fibrin was noted on the pleural tissue and on the surface of the lungs. Fibrinous exudates filled the pericardium in some cases.

Table 1. Bacteria isolated from poultry showing respiratory disease syndrome

Bacteria isolated	N° of isolates	Trachea	Air sac	Lungs	Infra orbital sinus exudates	Liver	Heart blood	%
E. coli	15	1	-	5	4	1	4	28.8
ORT	8	3	2	2	1	-	-	15.4
P. multocida	2	1	-	1	-	-	-	3.8
H. paragallinarum	1	-	-	-	1	-	-	1.9
E. coli + ORT	9 + 9	2 + 2	2 + 2	5 + 5	-	-	-	34.6
E. coli + *P. multocida*	3 + 3	-	-	-	-	2 + 2	1 + 1	11.5
E. coli + *H. paragallinarum*	-	-	-	-	-	-	-	0
ORT + *P. multocida*	-	-	-	-	-	-	-	0
ORT + *H. paragallinarum*	1 + 1	-	-	-	1 + 1	-	-	3.8
P. multocida + *H. paragallinarum*	-	-	-	-	-	-	-	0
Total	52	9	6	18	8	5	6	-

According to the results of the growth characteristics, colony morphology, biochemical reactions and carbohydrate fermentation reaction patterns, the bacterial isolates were identified as *E. coli*, *O. rhinotracheale*, *P. multocida*, and *H. paragallinarum*.

Small pinpoint non-haemolytic grey colonies with a reddish glow and a butyrous odour were observed after 48 hr of incubation on sheep blood agar culture plates. Growth

was observed under both aerobic and anaerobic conditions. Staining by Gram method revealed gram negative, highly pleomorphic, rod shaped bacteria and identified as *Ornithobacterium rhinotracheale* (Fig. 2).

Table 2. Biochemical properties of various bacteria isolated

Test	ORT	*P. multocida*	*E. coli*	*H. paragallinarum*
Oxidase	+	+	-	-
Catalase	-	+	+	-
Indole production	-	+	+	-
Urease	+	-	-	-
MR test	-	-	+	-
VP test	+	-	-	-
Nitrate reduction	-	+	+	+
Haemolysis on blood agar	-	-	+	-
Growth on MacConkey's agar	-	-	+	-
Lysine	-	-	+	-
Ornithine	-	+	+	-
Arginine	+	-	-	-
Fermentation of Fructose	+	-	+	+
Lactose	+	-	+	-
Maltose	+	+	+	+
Galactose	+	+	+	-
Glucose	+	+	+	+
Sucrose	-	+	+	+
ONPG	+	-	+	+
H_2S production	-	-	-	-
Citrate	-	-	-	-
Motility	-	-	+	-
Gelatin liquification	-	-	-	-
Phenylene deaminase	-	-	-	-

Smooth, circular, convex and smooth colonies were observed on the dextrose agar plates and the colonies were iridescent under obliquely transmitted light. Staining by Gram method revealed gram negative, coccobacillary organisms and identified as *Pasteurella multocida.*

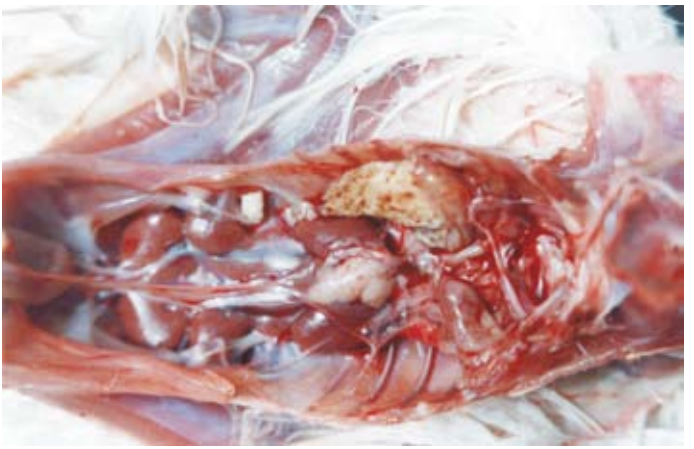

Fig. 1. Air sac showing deposition of yoghurt like exudates in *O. rhinotracheale* infected bird

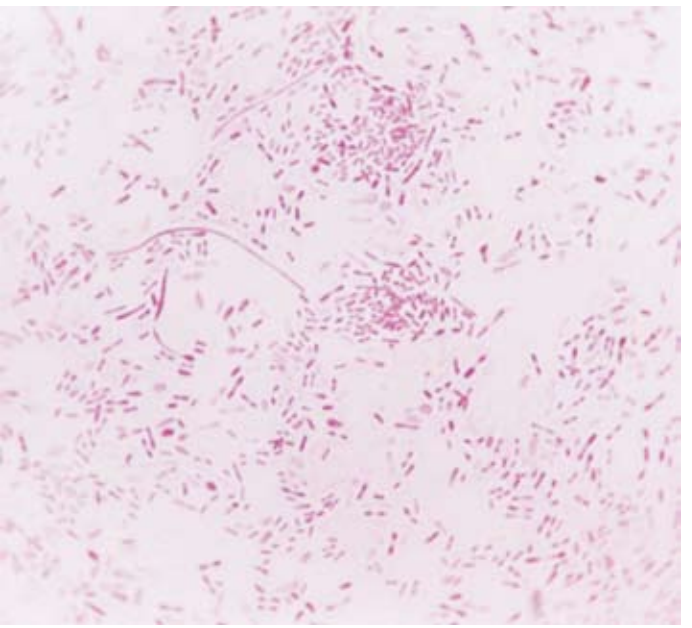

Fig. 2. Gram staining of *O. rhinotracheale* bacteria from a 48h culture plate showing characteristic pleomorphism. ×1000.

Tiny dewdrop colonies were noticed on the chocolate agar plates under anaerobic conditions and the colonies showed satellitism on the sheep blood agar plates near the

Staphylococcus aureus streak line. Staining by gram method revealed gram-negative short rods with a few coccobacillary forms, occurring in single or occasionally in short chains, and identified as *Haemophilus paragallinarum.*

Bright pink colonies observed as a result of fermentation of lactose on MacConkey's agar were identified as *Escherichia coli,* and confirmed by the IMViC test.

The results of the biochemical reactions of the isolates are presented in Table II. *O. rhinotracheale* could be differentiated from other pathogenic bacteria by biochemical reactions. The notable biochemical reactions of *O. rhinotracheale* were catalase negative, oxidase positive, indole negative, ONPG positive, no growth on MacConkey's agar, no reaction on triple sugar iron agar. No motility of the bacteria was noticed.

In total, 27 (51.9%), 18 (34.6%), 5 (9.6%), 2 (3.8%), isolates were identified as *E. coli*, *O. rhinotracheale*, *P. multocida* and *H. paragallinarum* respectively. The *E. coli* was isolated in pure culture from 15 cases, from 9 cases together with *O. rhinotracheale*, and 3 cases with *P. multocida*. *O. rhinotracheale* was recovered in pure culture from 8 cases, and 1 isolate with *H. paragallinarum*. *P. multocida* was isolated in pure culture in 2 cases and *H. paragallinarum* was isolated purely from a single case.

Discussion

Respiratory diseases in poultry associated with *O. rhinotracheale* have been described in various clinical reports and the bacteriological properties of *O. rhinotracheale* have been reviewed, allowing information to be used for identification and characterization purposes (VAN EMPEL and HAFEZ, 1999; HAFEZ, 2002; CHIN et al., 2003). The clinical symptoms noticed and necropsy findings reported in this study were in agreement with the findings reported by ZORMAN-ROJS et al. (2000).

In the present study, out of 18 *O. rhinotracheale* isolates from different organs, 16 were from the trachea, lung and air sacs of infected birds. The organism could also be isolated in pure culture in specimens from the trachea, lung or air sac exudates and these findings were in accordance with VANDAMME et al. (1994) and HINZ et al. (1994).

The frequency of isolation of *O. rhinotracheale* from various organs indicated that the isolates were most commonly recovered from the lungs, trachea and airsacs. In one bird *O. rhinotracheale* was isolated from the infraorbital sinus, in association with *H. paragallinarum*. *O. rhinotracheale* was most frequently isolated from the respiratory tract of poultry, whereas *P. multocida* could be isolated from various organs, notably the liver. But *O. rhinotracheale* was not recovered from any of the liver or heart blood samples. The results were consistent with DE ROSA et al. (1996). The isolation of *E. coli* in a much higher percentage (51.9%), than that of *O. rhinotracheale* (34.6%), *P. multocida*

(9.6%) and *H. paragallinarum* (3.8%) were consistent with the results reported by EL-SUKHON et al. (2002).

In the present study, *O. rhinotracheale* was isolated from several organs, suggesting that systemic spread might occur. These findings were in confirmation with the results reported by BACK et al. (1998) except that it was not isolated from liver samples in the present study as reported by DE ROSA et al. (1996).

The lesions, such as yoghurt like deposits in air sacs, fibrinopurulent pneumonia, air sacculitis, found in the clinical cases associated with the isolation of *O. rhinotracheale,* correlate with those reported elsewhere, by SPRENGER et al. (1998), DE ROSA et al. (1996), ROEPKE et al. (1998), VAN EMPEL et al. (1996).

JOUBERT et al. (1999) reported a pure culture of *O. rhinotracheale* colonies obtained from enrofloxacin treated birds, whereas cultures from untreated birds presented an overgrowth with colonies of *E. coli* and *Staphylococcus* spp. In the present study also, a pure culture of *O. rhinotracheale* could be isolated in a few cases, whereas in other cases concomitant infection with other bacteria was reported and this could be due to treatment with antibiotics.

Isolation of various pathogenic bacteria in this study probably indicates that frequent indiscriminate uncontrolled use of antibiotics in layer poultry farms might result in resistance to antimicrobial agents among the pathogenic bacteria, particularly for *E. coli* and then *O. rhinotracheale* (RYLL et al., 1996; EL SUKHON et al., 2002). Moreover, under field conditions many factors, such as stress, high stock density, poor ventilation, the presence of other bacteria or high ammonia levels, could aggravate *O. rhinotracheale* infection. But in experimental *O. rhinotracheale* infection prior administration of certain avian viruses aggravated *O. rhinotracheale* infection in turkeys or chicken (VAN EMPEL et al., 1996).

O. rhinotracheale is usually associated with other respiratory pathogens (CHARLTON et al., 1993; DE ROSA et al., 1996) and it is likely to be overgrown by other bacteria, particularly E. *coli,* thereby making it difficult to identify by the routine methods used in most diagnostic laboratories. *O. rhinotracheale* was isolated with *E. coli* in nine cases in the present study. In a few cases *O. rhinotracheale* was isolated with other respiratory pathogens such as *P. multocida* and *H. paragallinarum*. These findings probably indicate concomitant infection with *E. coli, P. multocida* and *H. paragallinarum* increasing the severity of infections associated with *O. rhinotracheale* as reported by DE ROSA et al. (1996).

The clinical signs, gross lesions observed and various organs from which pathogenic bacteria were isolated in the present study confirmed other earlier reports. *O. rhinotracheale* was most frequently isolated from the respiratory tract of poultry (HINZ et

al., 1994) where as *P. multocida* could be isolated from most organs, notably bone marrow, liver and lungs (GLISSON et al., 2003) and *H. para gallinarum* primarily affects the nasal passages and could be isolated particularly from infra orbital sinus exudates (BLACKALL and MATSUMOTO, 2003).

In conclusion, *O. rhinotracheale*, one of the causative agents of the emerging respiratory diseases of poultry could be isolated either singly or concurrently with other bacteria, such as *E.coli* and *H. paragallinarum,* indicating its possible etiological role in the respiratory disease complex of poultry. In poultry, mixed or concomitant bacterial infections are significant but the synergistic role between *O. rhinotracheale* and other bacterial pathogens is yet to be ascertained.

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MURTHY, T. R. G. K., N. DORAIRAJAN, G. A. BALASUBRAMANIUM, A. M. DINAKARAN, K. SARAVANABAVA: Patogene bakterije povezane s dišnim bolestima peradi u Indiji s posebnim osvrtom na vrstu *Ornithobacterium rhinotracheale*. Vet. arhiv 78, 131-140, 2008.

SAŽETAK

Od ukupno 253 uzoraka biološkoga materijala prikupljenih od 125 nesilica iz 35 komercijalnih uzgoja u Namakkalu u državi Tamil Nadu izdvojene su i identificirane patogene bakterije povezane s dišnim bolestima s posebnim osvrtom na vrstu *Ornithobacterium rhinotracheale*. Ukupno je 27 (51,9%) izolata identificirano kao *Escherichia coli*, 18 (34,6%) kao *Ornithobacterium rhinotracheale*, 5 (9,6%) kao *Pasteurella multocida* i 2 (3,8%) kao *Haemophilus paragallinarum*. *Ornithobacterium rhinotracheale*, kao jedan od uzročnika emergentnih dišnih bolesti peradi, bio je izdvojen zasebno ili zajedno s ostalim bakterijama poput vrsta *Escherichia coli* i *Haemophilus paragallinarum*, što upućuje na njegovu moguću etiološku ulogu kod dišnih bolesti peradi.

Ključne riječi: *Ornithobacterium rhinotracheale*, perad, dišne bolesti