ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 1(5): 114-118, 2002

Diagnosis of *Ornithobacterium Rhinotracheale*

Hafez Mohamed Hafez
Institute of Poultry Diseases, Free University Berlin
Koserstr.21, 14195 Berlin, Germany
E- mail: hafez@zedat.fu-berlin.de

Abstract: Respiratory disease conditions are continuing to cause heavy economic losses in the poultry industry. Since Dec. 1991 respiratory manifestation with different clinical courses have been observed in poultry flocks in different countries. Bacteriological examinations have resulted in isolation of pleomorphic gram-negative rods (PGNR). The detected bacteria were designated as *Ornithobacterium rhinotracheale* gen. nov., sp. nov. in the rRNA-Superfamily V. The present paper reviews the literatures related to ORT current situation on isolation and identification, serotyping of ORT as well as differential diagnosis from other similar bacterial infections.

Key Words: Respiratory disease, *Ornithobacterium rhinotracheale*

Introduction

Respiratory disease conditions are continuing to cause heavy economic losses in the poultry industry worldwide. Since December 1991 respiratory manifestation with different clinical courses have been observed in poultry flocks in different countries (DuPreez, 1992; Charlton *et al*., 1993; Hafez *et al*., 1993; Hinz *et al*., 1994; Van Beek *et al*., 1994). Bacteriological examinations have resulted in isolation of pleomorphic gram-negative rods (PGNR). The detected bacteria were designated as *Ornithobacterium rhinotracheale* gen. nov., sp. nov. in the rRNA-Superfamily V (Vandamme *et al*.,1994).

Ornithobacterium rhinotracheale (ORT) has been recognised in many countries worldwide and incriminated as a possible additional causative agent in respiratory disease complex. Although, ORT has been proved to be highly sensitive to different chemical disinfectants (Hafez and Schulze, 1998), currently, ORT infection appears to have become endemic and can affected every new restocking even in previously cleaned and disinfected houses especially in areas with intensive poultry production as well as in multiple age farms.

The disease is spread horizontally by direct and indirect contact. Vertical transmission is suspected, since some recent research has isolated ORT at very low incidence from reproductive organs and hatching eggs, infertile eggs and dead embryos (Tanyi *et al*., 1995; Van Empel, 1997; Nagaraja *et al*., 1998; El-Gohary, 1998). It is however, not yet known if this vertical transmission is caused by ovarial or cloacal contamination. ORT has been isolated from chicken, chukar partridge, duck, goose, guinea fowl, gull, ostrich, partridge, pheasant, pigeon, quail, rook and turkey. Within this bacterial species several serotypes and isolates with different virulence seem to be exist (Ryll *et al*., 1996; Travers, 1996; Van Empel *et al*., 1996).

ORT is an acute highly contagious disease of chickens and turkeys. The severity of clinical signs, duration of the disease and mortality are extremely variable and are influenced by many environmental factors such as poor management, inadequate ventilation, high stocking density, poor litter conditions, poor hygiene, high ammonia level, concurrent diseases and the type of secondary infection (Hafez, 1996). Initial symptoms are nasal discharge, sneezing coughing and sinusitis followed in some cases by severe respiratory distress, dyspnoea, prostration and mortality. The symptoms are accompanied with a reduction in feed consumption and water intake. The lesions can include rhinitis, tracheitis, oedema, uni- or bilateral consolidation of the Lungs with fibropurulent exudates. Pericarditis, airsacculitis, peritonitis and enteritis could be detected (Van Empel and Hafez, 1999).

Laboratory diagnosis: Clinical signs and lesions are of little value in diagnosis, since many other infectious diseases can produce similar clinical signs and post mortem lesions. Accurate diagnosis must be substantiated by direct detection or isolation of the causative bacteria and /or indirectly through detection of antibodies using serological examination (Fig. 1).

Detection of the bacteria:

Polymerase chain reaction (PCR): A specific PCR can be performed using the primer combination OR16S-F1 (5'-GAG AAT TAA TTT ACG GAT TAA G) and OR16S-R1 (5'-TTC GCT TGG TCT CCG AAG AT). This combination amplifies a 784 bp fragment on the 16S rRNA gene of O. rhinotracheale, but not of other closely related bacteria with which *O. rhinotracheale* could be confused (van Empel, 1998; Hung and Alvarado, 2001). In future PCR assays can also be optimised for the demonstration of ORT in tracheal swabs, eggs and environmental

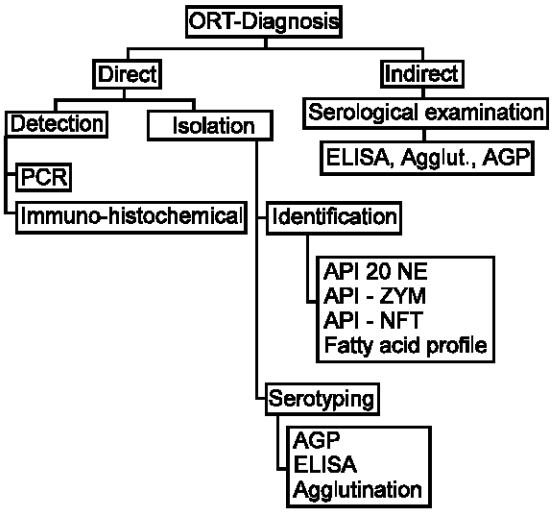

Fig. 1: Laboratory diagnosis of ORT (Hafez, 1998, modified)

samples.

Immuno-histochemical staining: In field trials, using a sensitive immuno - histochemical staining, it was found that *O. rhinotracheale* was the cause of 70% of the cases with respiratory symptoms in broiler chickens, while through bacteriology and/or serology only 30% of the cases could be connected to *O. rhinotracheale* (Van Empel *et al.*, 1999; Van Veen *et al.*, 2000).

Isolation: Samples for bacterial culture should be collected at early stage of the disease. The ORT can usually be isolated from trachea, tracheal swabs, lungs and air sacs. Culture of heart blood and liver tissue under field condition has revealed negative results (Hafez *et al.*, 1993). However, the bacteria could be isolated from those organs as well as joints and brains, ovary and oviduct after experimental infections. (Van Beek *et al.*, 1994; Back *et al.*, 1998b). Blood agar with 10% sheep blood is commonly used for primary isolation. The incubation of the plate at 37°C for 48 hours under anaerobic or micro aerobic condition is recommended. The organisms grow readily also on tryptose soy agar as well as in peptone water and Pasteurella broth aerobically and anaerobically. In contaminated samples with fast growing bacteria such as *E. coli, Proteus or Pseudomonas*, ORT colonies may be overgrown and therefore cannot be detected in routine investigation. Since it has been shown that the most of ORT isolates are resistant to gentamicin (Hafez *et al.*, 1993). Back *et al.* (1998b) recommended the use of 10μg of gentamicin per ml of blood agar medium in aim to isolate ORT from contaminated samples. Also using blood agars that contain 5 μg per ml of gentamicin and polymyxin seem to be very effective (van Empel, 1997).

Identification: On blood agar the colonies are small, grey-white, opaque, non hemolytic and differ in diameter (1-3 mm). ORT cells are gram-negative pleomorphic rods. There is no growth on MacConkey agar. Isolated organisms produce oxidase but not indole. All isolates are β-galactosidase (ONPG) positive, catalase negative and most of them reacted positively in urease test. Recently, however, Günther *et al.* (2002) were able to isolate and identification of a cytochrome-oxidase negative strain of ORT from turkeys.
Biochemical identification using commercial biochemical test-kit (API 20 NE, Bio-Mérieux, France or API 20 NFT, USA) showed that 99.5 % of ORT strains give a reaction code of 022 000 4 (61 %) or 002 000 4 (38.5 %) in this system (Van Empel, 1998). Further identification could be carried out using API ZYM, or fatty acid profile (Charlton *et al.*, 1993). Another commercial identification system, the RapID NF Plus system (Innovative Diagnostics, USA), did give high identification scores (Biocodes 4-7-2-2-6-4, 4-7-6-2-6-4, 6-7-6-2-6-4 or 6-7-2-2-6-4) when investigating 110 ORT strains (Post *et al.*, 1997).

Typing of the isolates:
Serological typing: The confirmation could be carried out using serological examination with known positive antisera in agar gel precipitation (AGP), ELISA (Van Empel, 1998; Hafez and Sting, 1999) or rapid slide agglutination (Bock *et al.*, 1997; Back *et al.*, 1998a). Currently 18 serotypes designated (A to R) seem to be exist (Van Empel, 1998 and personal communication). Most of the chicken isolates belong to the serotype A and the turkey isolates are more heterogeneous and belongs to serotype A, B and D. (Van Empel *et al.*, 1996; Hafez, 1998; Van Empel, 1998).

Molecular biological typing: A further possibility for the typing is the using of PCR (S. detection) or using the random-amplified-polymorphic-DNA (RAPD). Using the primers M13 (5'-TAT GTA AAA CGA CGG CCA GT- 3) and ERIC 1R (5'- ATG TAA GCT CCT GGG GAT TCA C -3'), variations were found between all tested serotypes PCR fingerprints with M13 and ERIC 1R primers are a useful tool for typing and epidemiological investigation of *O. rhinotracheale* isolates (Hafez and Beyer, 1997; Hung and Alvarado, 2001).
Using pulsed-field gel electrophoresis (PFGE) discrimination between the tested 17 standard serotypes (A-Q) could be detected, however, testing field isolates of serotype A originated from German turkeys wide variation was observed. On the other hand, serotype B isolates are identical. Comparing isolates from different countries high similarity within the isolates

Table 1: Differential characteristic of some bacteria involved in respiratory disease in poultry

Test	Result							
	ORT	Pasteurella					Ba	Hp
		Pm	Ra	Pg	Ph	Pp		
Hemolysis	-	-	-	-	+			-
MacConkey agar	-	-	-	-	-	+/-	+	-
Oxidase	+	+	+	+	+	-	+	-
β - galactosidase (PNPG)	+	+/-	-	+/-	-	+		+
Indole	-	+	+/-	-	-	-		-
Urea	+/-	+	+/-	+	+	+	-	-
Arginine dihydrolase	-/+	-	+	-			-	-
Nitrate reduction	-	+	-	+	+	+/-	-	+
Catalase	-	+	+	+	+	+		-
Acid from carbohydrate								
- Lactose	-/+	+/-	-	-	+/-	-		-
- Maltose	-/+	-/+	-	+	+	+		+
- Galactose	-/+	+	-	+		+		-
- Fructose	-/+	+	-	+		+		+

ORT = Ornithobacterium rhinotracheale; Pm = Pasteurella multocida; RA = Riemerella anatipestifer; Pg = Pasteurella gallinarum
Ph = Pasteurella haemolytica; Pp =Pseudotuberculosis; Ba = Bordetella avium ; Hp = Haemophilus paragallinarum

of the same serotype, despite the origin of the isolate (chicken/turkey), was observed. The primarily result suggests the existence of relationships between the geographic origin, the serotype and the DNA fingerprint pattern (Popp and Hafez, 2001).

Serological examinations: Serological examination for detection of antibodies can be carried out using slide agglutination test prepared from different serotypes (Bock *et al*., 1997; Back *et al*., 1998a Erganis *et al*., 2002), ELISA-tests (Hafez and Sting, 1996; Van Empel, 1994) or DOT- Immunobinding assay (Erganis *et al*., 2002).

The serotype specificity of the ELISA depends on the method of antigen extraction used for coating the ELISA plates. Boiled extract antigens are serotype-specific (van Empel *et al*., 1997), while Antigen extraction with sodium dodecyl sulphate (SDS-antigen) result in more cross-reactions (Hafez and Sting, 1999). Self made ELISA (SDS-extraction) as well as two commercial available ELISA-kits (Biocheck and IDEXX) are able to detect antibodies against all tested ORT serotypes. In addition, examination of serum samples collected from commercial flocks in all three systems showed similar results on flock bases using these ELISA-testes, however some minor variations on sample bases (Ballagi *et al*., 2000; Hafez *et al*., 2000). Generally, using ELISA, antibodies against ORT can be detected in serum and egg yolk shortly after infection and titres will peak between 1 to 4 weeks post infection (van Empel *et al*., 1996). Because titres decline rapidly after peaking, serum samples for flock screening should be taken frequently.

The advantage of the serological tests over bacteriological examination is that antibodies persist for several weeks after infection and the bacterial shedding is short. However, ORT excretion and antibody response may also be affected by a number of factors such as antibiotic therapy and vaccination. The influence of antibiotic therapy on the serological response to ORT remains unclear. Popp and Hafez (2002) carried out investigation in aim to determine the effect of drug therapy using amoxicillin on the antibody kinetics after experimental infection. Amoxicillin was confirmed to be very effective against most isolates tested in vitro (Hafez *et al*., 1993). Three groups of SPF layers each of 10 birds were experimentally infected with an ORT strain at 36 weeks of age intravenously. Each bird received $5x10^8$ cfu. Group 1 was kept as infected non-treated control. Group 2 was infected and treated immediately with amoxicillin at dose level of 250 ppm via drinking water for 5 days. Group 3 was infected as mentioned above and received amoxicillin for 5 days started at 7^{th} day post infection. An additional group (Group 4) was kept as non-infected non-treated control. Blood samples were collected at 5 day intervals till 50^{th} day post infection and tested for antibodies against ORT using ELISA. The results showed that immediately treatment did not influence the antibody response. While the treatment started at 7^{th} day post infection resulted in lower antibody response compared to infected control.

Differential diagnosis: In avian host several microorganisms of the genus Pasteurella include P. multocida, P. gallinarum, P. haemolytica and P. anatipestifer as well as pseudotuberculosis (Yersinia pseudotuberculosis, which previously was included in genus Pasteurella) are involved in respiratory diseases complex. Except P. multocida and P. anatipestifer, other bacteria are of less economic important in avian species. Further bacterial agents such as Bordetella avium and Haemophilus paragallinarum were frequently

isolated from respiratory outbreaks in poultry. As clinical sings, post mortem lesions are similar to other bacterial and virus infections differential diagnosis and isolation and identification of the causative agent are essential (Table 1).

References

Back, A., D. Halvorson, G. Rajashekara and K. Nagaraja, 1998a. Development of a serum plate agglutination test to detect antibodies to *Ornithobacterium rhinotracheale*. J. Vet. Diagnostic Invest., 10: 84-86.

Back, A., G. Rajashekara, R. Jeremiah, D. Halvorson and K. Nagaraja, 1998b. Tissue distribution of *Ornithobacterium rhinotracheale* in experimentally infected turkeys. The Vet. Rec., 143: 52-53.

Ballagi, A., G. Holmquist, M. Odmark and V. Leathers, 2000. ELISA test for the detection of *Ornithobacterium rhinotracheale* infection in chickens and turkeys. Proceedings of the 49th Western Poultry Disease Conference, Sacramento, California. p: 50.

Bock, R., P. Freidlin, M. Manoim, A. Inbar, A. Frommer, P. Vandamme and P. Wilding, 1997. *Ornithobacterium rhinotracheale* (ORT) associated with a new turkey respiratory tract infectious agent in Israel. Proceedings of the 11th International Congress of the World Veterinary Poultry Association, Budapest , p: 120.

Charlton, B. R., S. E. Channing-Santiago, A. A. Bickford, C. J. Cardona, R. P Chin, G. L. Coopeer, R. Droual, J. S. Jeffrey, C. U. Meteyer, H. L. Shivaprasad and R. L. Walker, 1993. Preliminary characterization of a pleomorphic gram-negative rod associated with avian respiratory disease. J. Vet. Diagnostic Invest., 5: 47 - 51.

DuPreez, J., 1992. *Ornithobacterium rhinotracheale* in broiler in South Africa. Personal communication.

El-Gohary, A. A., 1998. *Ornithobacterium rhinotracheale* (ORT) associated with hatching problems in chicken and turkey eggs. Vet. Med. J. Giza., 46: 183-191.

Erganis, O., H. H. Hadimili, K. Kav, M. Corlu and D. Öztürk, 2002. A comparative study on detection of *Ornithobacterium rhinotracheale* antibodies in meat-type turkeys by dot immunobinding assay, rapid agglutination test and serum agglutination. Avian Path., 31: 201-204.

Günther, R., M. Ryll, K. H. Hinz and H. M. Hafez, 2002. New variety of *Ornithobacterium rhinotracheale*. Proceedings of the 4th international symposium on turkey diseases, Berlin. (Ed. H. M. Hafez). In press.

Hafez, H. M., 1996. Current status on the role of *Ornithobacterium rhinotracheale* (ORT) in respiratory disease complexes in poultry. Archiv für Geflügelkunde, 61: 208-211.

Hafez, H. M., 1998. Current status on the Laboratory diagnosis of *Ornithobacterium rhinotracheale* "ORT" in poultry. Berliner und Münchener Tierärztliche Wochenschrift, 111:143-145.

Hafez, H. M. and W. Beyer, 1997. Preliminary investigation on *Ornithobacterium rhinotracheale* (ORT) isolates using PCR-fingerprints. In Proceedings of the XIth International Congress of the World Veterinary Poultry Association, Budapest, p: 51.

Hafez, H. M. and D. Schulze, 1998. Efficacy of clinical disinfectants on *Ornithobacterium rhinotracheale* in vitro : Short communication. In Proceedings of the 1st international symposium on turkey diseases, Berlin. (Ed. H.M. Hafez and A. Mazaheri), ISBN 3-930511-53-3. P: 146-150.

Hafez, H. M. and R. Sting, 1996. Serologic surveillance on *Ornithobacterium rhinotracheale* in poultry flocks using self-made ELISA. Proceedings of the 45th Western Poultry Disease Conference, Cancun, p: 163-164.

Hafez, H. M., W. Kruse, J. Emele and R. Sting, 1993. Eine Atemwegsinfektion bei Mastputen durch Pasteurella-ähnliche Erreger: Klinik, Diagnostik und Therapie. Proceedings of the International Conference on Poultry Diseases, Potsdam, p:105 - 112.

Hafez, H. M. and R. Sting, 1999. Investigations on Different *Ornithobacterium rhinotracheale* "ORT" Isolates. Avian Dis., 34: 1–7.

Hafez, H. M., A. Mazaheri and R. Sting, 2000. Efficacy of ELISA for detection of antibodies against several *Ornithobacterium rhinotracheale* serotypes. Deutsche Tierärztliche Wochenschrift, 107: 142 -143.

Hinz, K. H., C. Blome and M. Ryll, 1994. Acute exudative pneumonia and airsacculitis associated with *Ornithobacterium rhinotracheale* in turkeys. The Vet. Rec., 135: 233-234.

Hung, A. L. and A. Alvarado, 2001. Phynotypic and molecular characterization of isolates of *Ornithobacterium rhinotracheale* from Peru. Avian Dis., 45: 999-1005.

Nagaraja, K., A. Back, S. Sorenger, G. Rajashekara and D. Halvorson, 1998. Tissue distribution post-infection and antimicrobal sensitivity of *Ornithobacterium rhinotracheale*. Proceedings of the 47th Western Poultry Disease Conference, Sacramento, p: 57-60.

Post, K., S. Murphy, J. Boyette and P. Resseguie, 1997. Evaluation of the rapid NF plus system for the identification of *Ornithobacterium rhinotracheale*. Proceedings of the 40th Annual meeting of the American Association of Veterinary Diagnostics, Louisville, p: 88.

Popp, C. and H. M. Hafez, 2001. *Ornithobacterium rhinotracheale*: Comparison between serotypes and puls gel electrophoresis muster isolates of different sources and origin. Proceedings of the XII International Congress of the World Vet. Poult. Assoc. Cairo, Egypt, p: 269.

Popp, C. and H. M. Hafez, 2002. Investigations on *Ornithobacterium rhinotracheale*. Proceedings of the 4th international symposium on turkey diseases, Berlin. (Ed. H.M. Hafez). In press.

Ryll, M., K. H. Hinz, H. Salisch and W. Kruse, 1996. Pathogenicity of *Ornithobacterium rhinotracheale* in turkey from turkey poults under experimental conditions. The Vet. Rec., 137: 19.

Tanyi, J., A. Bistyak, E. Kaszanyitzky, F. Vetesi and M. Dobos-Kovacs, 1995. Isolation of *Ornithobacterium rhinotracheale* from chickens, hens and turkeys showing respiratory symptoms. Preliminary report. Magyar Allatorvosok Lapja, 50: 328-330.

Travers, A.F., 1996. Concomitant *Ornithobacterium rhinotracheale* and Newcastle disease infection in broilers in South Africa. Avian Dis., 40: 488-490.

Van Beek, P. N., Van P. Empel, Van den G. Bosch, P. Storm, J. H. Bongers and J. H. DuPreez, 1994. Ademhalingsproblemen, groeivertraging en gewrichtsontsteking bij kalkoenen en vleeskuikens door een Pasteurella-achtige bacterie: *Ornithobacterium rhinotracheale* of "Taxon 28". Tijdschrift voor Diergeneeskunde, 119: 99 - 101.

Van Empel, P., 1994. *Ornithobacterium rhinotracheale*: Isolation, identification and experimental infection results. Paper given at Poultry Veterinarian Study group of the EU held in Amsterdam 11th November, 1994.

Van Empel, P., 1997. *Ornithobacterium rhinotracheale*: An update. Proceedings of Fachgruppe "Geflügelkrankheiten" der Deutschen Veterinärmedizinischen Gesellschaft and German branch of the World's Veterinary Poultry Association, 52nd Semi-annual Meeting on Poultry Diseases, Hannover, Germany, p: 418-421.

Van Empel, P., 1998. *Ornithobacterium rhinotracheale*. Thesis, University of Utrecht, ISBN 90-393-1574-4.

Van Empel P. and H. M. Hafez, 1999. *Ornithobacterium rhinotracheale*: a review. Avian Pathology, 28: 217-227.

Van Empel, P., H. van den Bosch, D. Goovaerts and P. Storm, 1996. Experimental infection in turkeys and chickens with *Ornithobacterium rhinotracheale*. Avian Diseases, 40: 858-864.

Van Empel, P., H. van den Bosch, P. Loeffen and P. Storm, 1997. Identification and serotyping of *Ornithobacterium rhinotracheale*. J. Clin. Microbiol., 35: 418-421.

Van Empel, P., M. Vrijenhoek, D. Goovaerts and H. van den Bosch, 1999. Immuno-histochemical and serological investigation of experimental *Ornithobacterium rhinotracheale* infection in chickens. Avian Path., 28: 187-193.

Van Veen, L., E. Gruys, K. Frik and P. van Empel, 2000. Increased condamnation of broilers associated with *Ornithobacterium rhinotracheale*. Vet. Rec., 147: 422-423.

Vandamme, P., P. Segers, M. Vancaneyt, K. van Hover, R. Mutters, J. Hommez, F. Dewirst, B. Paster, K. Kersters, E. Falsen, L. Devrieze, M. Bisgaard, K. H. Hinz and W. Mannheim, 1994. Description of *Ornithobacterium rhinotracheale* gen. nov. sp. nov. isolated from the avian respiratory tract. Int. J. Sys. Bacteriol., 44: 24-37.