

Animal Health Research Institute, Dokki, Giza, Egypt.
El-Mansoura Province Lab.

**PATHOLOGICAL STUDIES ON
ORNITHOBACTERIUM RHINOTRACHEALE
INFECTION OF BROILER AT SHARKIA PROVINCE**
(With 2 Tables and 8 Figures)

By

SH. ABDEEN and O.O. LOTFY

*The Veterinary Clinic, Fac. Vet. Med., Zagazig University
(Received at 22/3/2006)

دراسات باثولوجية علي ميكروب الأورنيسوباكثيريم رينوتراكيا
في دجاج التسمين في محافظة الشرقية

شاكر عابدين حسنين ، عمر عثمان لطفى

أجري هذا البحث علي دجاج من سلالة الهيرد مربى في أربعة مزارع تسمين بمحافظة الشرقية (٢٠,٠٠٠، ٢٤,٠٠٠، ٢٥,٠٠٠، ٣٠,٠٠٠ طائر). هذا الدجاج يعاني من ظاهرة الأعراض التنفسية وتورم الرأس وكانت أعمارها تتراوح من ٣٠ إلى ٤٤ يوم. أجريت الدراسات البكتريولوجية والباثولوجية لتلك المزارع للوصول إلي مسبب المرض ودراسته باثولوجيا وكانت الأعراض الإكلينيكية لتلك الدواجن المصابة هي تورم الوجه والتهاب في أغشية العين وغلقها ومن دراسة الصفة التشريحية للحالات المصابة تبين وجود ارتشاح أوديومي تحت جلد الوجه والرأس مع تخلل الخلايا الالتهابية بين الأنسجة مع وجود إفرازات بالجيوب الأنفية. وقد أسفرت الدراسة البكتريولوجية عن عزل ميكروب الأورنيسوباكثيريم (ORT) وتم تأكيده بالتفاعلات البيوكيميائية. وقد أظهر الفحص الهستوباثولوجي تغيرات باثولوجية بجلد الرأس والقصب الهوائية والحويصلات الهوائية عبارة عن التهابات وارتشاحات وتخلل للخلايا الالتهابية وخاصة خلايا الهيتروفيل. أيضا أظهر الفحص الباثولوجي للكبد وجود نخر نخري مع ارتشاح لخلايا الدم البيضاء في المناطق البابية خصوصا خلايا الهيتروفيل والخلايا اللمفاوية وخلايا الميكروفاج. وأظهر الطحال ضمور ليمفاوي ونخر في النسيج الأبيض. هذه الدراسة أبرزت دور ORT في المشاكل التنفسية لمزارع دجاج التسمين والتي تسبب خسارة كبيرة.

SUMMARY

This study was performed on 4 broiler chicken farms (20,000, 24,000, 25,000 and 30,000 bird) in Sharkia province. Birds of these farms suffered from respiratory manifestation and swollen head. Their ages ranged from 30 to 44 days. Bacteriological and pathological

examinations were done. Clinically, the affected examined chickens were suffered from facial edema, conjunctivitis, closed eyes and swollen head. Macroscopically, edema in the face and sinuses, cyanotic head, mucous exudate in nares and trachea was seen. Lungs were congested with area of consolidation. Bacteriological examination revealed the isolation of *Ornithobacterium rhinotracheale* (ORT) which was identified by biochemical reactions. Histopathologically, face skin was suffered from edema with leukoytic infiltration mainly heterophils, lymphocytes and macrophages. The trachea showed tracheitis together with fibrinous or mucous exudates. The tracheal mucosa was thickened by inflammatory cells mainly heterophils with exudates within tracheal lumen. Bronchitis of the secondary bronchi were common. The lungs showed pneumonia with the presence of air sacculitis. Pericarditis, with leukocytic aggregation and edema was seen. Liver showed coagulative necrosis and portal leukocytic infiltration. Lymphoid depletion of the spleen also was noticed. This study investigate the role of ORT as a field respiratory problems among broilers.

Key words: Ornithobacterium rhinotracheale, broilers, histopathology

INTRODUCTION

Ornithobacterium rhinotracheale infection is a contagious disease of birds that causes respiratory distress mortality and decreased growth rate. The severity of the clinical signs, duration of the disease and mortality are extremely variable and influenced by environmental factors (Saif *et al.*, 2003).

Van Beck *et al.*, (1994) reported *O. rhinotracheale* infection in broilers showing respiratory distress with unusual clinical signs. It was initially designated as pasteurella like organism and later named (ORT) which was the name proposed by Van damme *et al.*, (1994).

ORT appears to spread horizontally by direct and indirect contact through aerosols or drinking water. Experimental infections in 5 weeks old chickens, ORT infected the respiratory organs within 2 days post infection (PI) and clinical signs were seen after 4 days (Van Beek *et al.*, 1994).

Many researchers proved the clinical signs in broiler chickens (Franz *et al.*, 1997 and Odor *et al.*, 1997), others mentioned that ORT infection can be accompanied with mortality rates of up to 50% (Back *et al.*, 1998 and Derosa *et al.*, 1996).

Hafez, (1996) mentioned that the clinical signs of ORT in broilers generally appeared between 3rd and 4th week of age together with mortality rate ranged from 2-10%.

The gross lesions noticed in ORT infection of broiler chickens were pneumonia mostly unilateral and air sacculitis containing creamy exudates (Yoghurt like) (Hafez, 1996). Facial oedema and cyanosis of head together with the presence of exudates were recorded as gross lesions (Nesma, 2004).

The microscopical findings of ORT in broiler chickens were dermatitis, sinusitis, tracheitis, congested lungs with fibrinous exudates and oedema in the interstitial tissue and within pulmonary tissue (Saif *et al.*, 2003 and Nesma, 2004). Inflammatory changes were seen also in liver and spleen (Nesma, 2004).

The aim of the present study was to investigate the role of ORT in field problems among broilers with particularly emphasis on clinical signs and their pathological lesions beside isolation and identification of the causative agent.

MATERIALS and METHODS

A) Samples:

This study was applied on broiler chicken (their ages varied from 30 to 44 days old) suffered from respiratory signs and swollen head. They were obtained from 4 farms (20.000, 24.000, 25.000 and 30.000) in Sharkia province.

Table 1: Summarized examined flocks and their numbers, age, morbidity and mortality rates during the course of disease and season of infection.

No. of flock	No. of bird/flock	Age/day	Morbidity during course of disease	Mortality during course of disease	Season
1	20.000	30	3%	4%	Winter
2	25.000	35	4%	3.5%	Autumn
3	24.000	28	5%	3.5%	Autumn
4	30.000	44	3.5%	5%	Winter

Samples from contents of sinuses, trachea, lung, liver, heart blood and spleen were collected for isolation and identification of the causative agent.

B) Bacteriological examination:

80 samples from nasal sinuses, trachea, lungs liver, heart blood and spleen were directly inoculated onto 10% sheep blood agar according to Vandamme *et al.*, (1994) under anaerobic conditions. Colonial morphology and biochemical tests were applied according to Cruickshank *et al.*, (1980).

C) Pathological examination:

Postmortem examination had been done to the necropsied broilers. Specimens from skin of face, nasal sinuses, trachea, lungs, heart, liver, and spleen were collected and fixed in 10% neutral buffered formalin. Paraffin sections of 5 μ thickness were prepared and stained with H & E for microscopical examination (Bancroft *et al.*, 1996).

RESULTS

Bacteriological findings:

80 samples from nasal sinuses, trachea, lungs and liver were inoculated into blood agar containing 10% sheep blood at 37°C anaerobically (7.5-10% CO₂). Pin point small circular colonies (less than 1mm diameter), opaque to gray in color and convex appeared onto the surface of the used media after 48 hours.

60 isolates were identified by biochemical reactions which tabulated in Table (2).

Table 2: Revealed the biochemical reaction of the ORT

Test	Result
Alkaline phosphatase	+
Esterase lipase	+
Acid phosphatase	+
B-Galactosidase	+
Trypsin	+
α -chemotrypsin	+
Lipase	-
β -Glucosidase	-
α -mannosidase	-

Pathological findings:

Clinical signs:

The affected chickens showed facial edema, conjunctivitis and swollen sinuses beside closed eyes (Plate I₁). Moreover, some respiratory signs in the form of sneezing, coughing and gasping could be seen in some birds. The affected chickens revealed reduction in feed and

water intake beside body gain and deaths reached to 5% in some affected flocks which persisted for several days (course of the disease).

Post-mortem examination:

Edema in the face and sinuses particularly infraorbital sinus beside mucous exudates usually seen in nares and trachea. The head appeared cyanotic.

The affected upper respiratory mucous membranes usually congested. The lungs were congested or pneumonic with area of consolidation and some chicks had turbid air sacs. Thickened pericardium was seen in some birds. Liver and spleen were congested with petechiae on coronary fat.

Microscopic examination:

Dermatitis of skin face characterized by edema and aggregation of numerous leukocytes mainly heterophils and lymphocytes (Plate I,₂). The mucous membranes of the sinuses were thickened with edema and inflammatory cells mainly heterophils, lymphocytes and macrophages with partial or complete destruction of their epithelial lining (Plate I,₃).

Sometimes the epithelial lining became hyperplastic forming folds with hyperplastic nasal glands and interglandular heterophilic aggregations (Plate I,₄). The blood vessels of nasal mucosa were congested with presence of serofibrinous exudate and destructed lining epithelium.

The tracheal mucosa was thickened by inflammatory cells mainly heterophils with exudate and leukocytes within the tracheal lumen (Plate II,₅). Other areas revealed fibrinous exudates or mucus inside tracheal lumen. The tracheal glands became hyperplastic in some areas.

Pneumonic areas and bronchitis of the secondary bronchi were common. Edema and leukocytic infiltration were encountered in the interstitial tissue and within the pulmonary tissue (Plate II,₆). The lining epithelium and glands of secondary bronchi were hyperplastic and sometimes showed metaplasia to goblet cells. Air sacculitis characterized by exudate, leukocytic infiltration and dilated capillaries were seen. Pericarditis manifested by leukocytic aggregation mainly heterophilic infiltration or aggregations and edema (Plate II,₇).

The inflammatory reactions sometimes extended to the deeper muscle fibers which later on become necrotic or degenerated with intermuscular edema.

The liver capsule was thickened due to peritonitis and sometimes area of coagulative necrosis usually seen in the hepatic tissue. Interstitial

and portal leukocytic infiltrations mainly heterophils beside portal hyperplastic bile ductules could be seen.

Lymphoid depletion and necrosis in the white pulp of the splenic tissue were noticed (Plate II-8). Thickened splenic capsule by hyalinized fibrinous tissue could be seen with subcapsular hemorrhages.

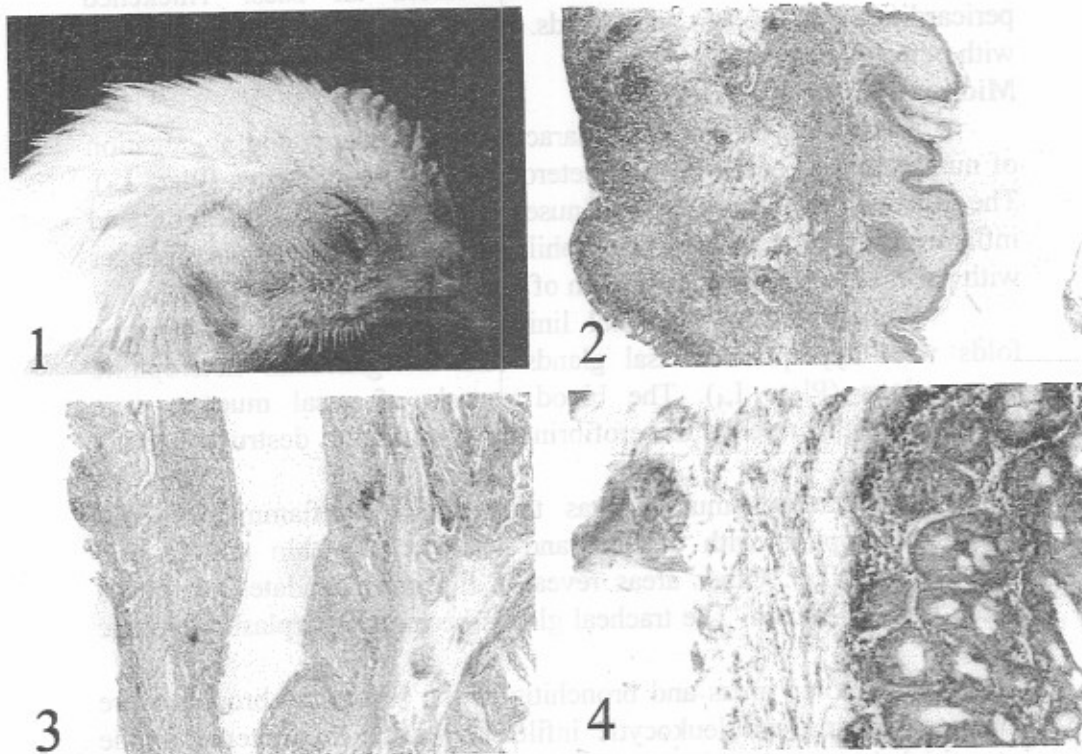


Plate (I,1): Chicken showed facial edema, conjunctivitis with closed eyes.

Plate (I,2): Skin of the face showing edema and aggregation of numerous leukocytes (H & E X 300).

Plate (I,3): Nasal sinus showing thickened mucus membran with edema and inflammatory cells with partial or complete destruction of epithelial lining (H & E X 300)

Plate (I,4): Nasal sinus showing hyperplastic nasal glands and interglandular heterophilic aggregations (H&E X300)

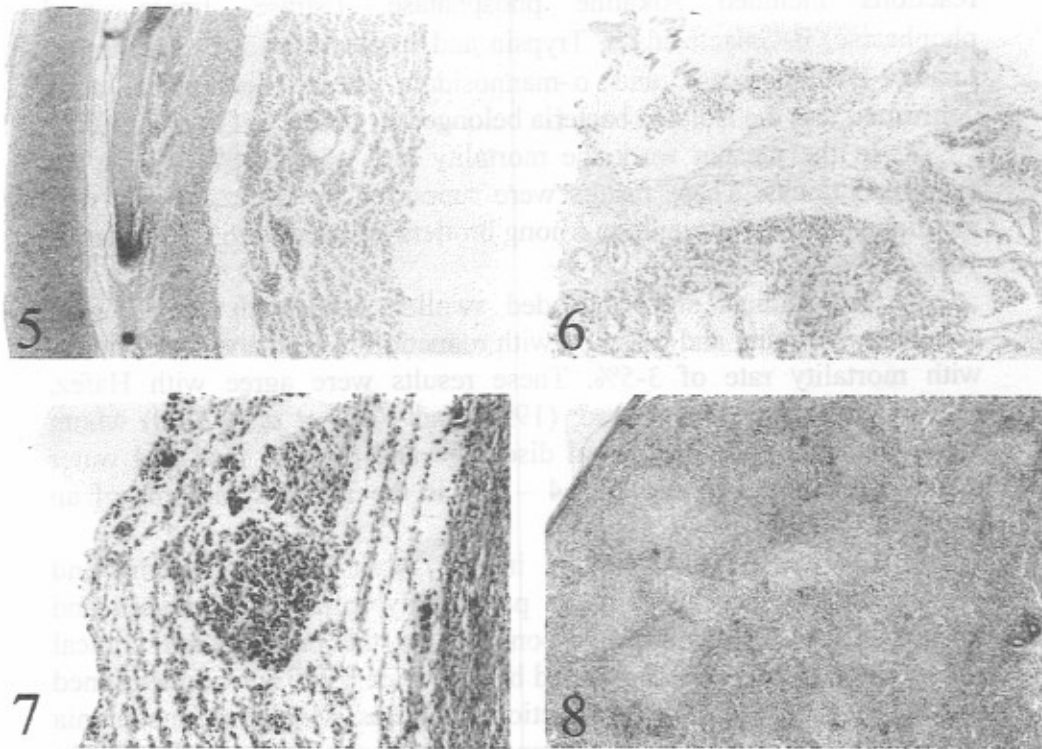


Plate (II,₅): Trachea showing thickened mucosa by inflammatory cells mainly heterophils with exudate and leukocytes within the tracheal lumen (H & E X 300).

Plate (II,₆): Lung showing edema and leukocytes in the interstitial tissue and within pulmonary tissue (H & E X 300).

Plate (II,₇): Heart showing leukocytic aggregation mainly heterophil and edema (H & E X 300).

Plate (II,₈): Spleen showing depletion of splenic lymphoid tissue of the white pulp (H&E X120)

DISCUSSION

The isolated bacteria in the present work was ORT Gram negative bacterium. The growing colonies appeared as gray to gray white colonies of convex shape on blood agar under anaerobic conditions. This result is completely agreement with those noticed by Leroy-Setrin *et al.*, (1998) who mentioned that ORT is a Gram negative, non motile, non sporulating bacteria. Also Saif *et al.*, (2003) mentioned that ORT develop very small non hemolytic colonies that are circular, gray to gray-white and convex with entire edge. The biochemical

reactions included Alkaline phosphatase, Estrase, lipase, acid phosphatase, B-Galactosidase, Trypsin and α -chemotrysin in addition to lipase, B-Glucosidase and α -mannosidase these biochemical tests confirmed that the isolated bacteria belonged to ORT (Saif *et al.*, 2003).

In the present work the mortality rate was 3.5-5% among the examined flocks. These results were supported by Hafez, (1996) who mentioned that the mortalities among broilers infected with ORT were 2-10%.

The clinical signs included swollen head with closed eyes, coughing, sneezing and grasping with reduction in feed and water intake with mortality rate of 3-5%. These results were agree with Hafez, (1994); El-Gohary and Awaad, (1998) and Veen *et al.*, (2000) whom mentioned that coughing, nasal discharge and drop in feed and water intake with mortality rate of 1.4 – 5% is the initial symptoms of an outbreak of ORT.

The predominant lesions in our work included edema and exudate in the face and sinuses particularly infraorbital sinuses and trachea. These lesions were responsible for the predominant clinical signs. These results were supported by Saif *et al.*, (2003) who mentioned that the target organ of ORT infection is sinuses. Moreover, pneumonia and air sacculitis usually seen accompanied with congestion of the viscera beside necrotic foci 0.1-0.2mm diameter were seen on liver surface. Similar results were reported by El-Shamy *et al.*, (2000); Saif *et al.*, (2003) and Nesma, (2004).

The microscopic findings in the skin of the face showed dermatitis which characterized by edema and aggregation of neumerous leukocytes mainly heterophils and lymphocytes. These result were in agreement with (Nesma, 2004) who described dermatitis due to ORT which characterized by oedema and neumerous leukocytic infiltration mainly heterophils and lymphocytes particularly in dermal layers.

Tracheitis, bronchitis, pneumonia and air sacculitis were common lesions in ORT infection due to localization of the causative agent in these organs. The causative agent produce toxins which affect on the permeability of blood vessels causing exudation in these organs. The predominant inflammatory cells were heterophils and lymphocytes which considered as positive chemotactic for bacteria and their toxins. These results were in agreement with Odor *et al.*, (1997), who described the lesion of ORT which included lymphocytic tracheitis with diffuse epithelial hyperplasia and pleuropneumonia.

Also our result was supported by Calnek *et al.*, (1997) and El-Shamy *et al.*, (2000) who mentioned that ORT causing pneumonia and air sacculitis with infiltration of leukocytes mainly heterophils.

Moreover pericarditis with leukocytic aggregation mainly heterophils were seen. This results agreed with Abdul-Aziz and Weber, (1999) and Nesma, (2004), who noticed serofibrinous pericarditis together with subepicardial oedema.

Hepatitis with thick liver capsule and area of coagulative necrosis beside interstitial and portal leukocytic infiltration with noticed in the present work were agree with Derosa *et al.*, (1996) and Jonbert *et al.*, (1999) who mentioned that ORT causing hepatitis with area of coagulative necrosis in addition to lymphocytic and heterophilic portal infiltration.

The lymphoid depletion of splenic white pulp with subcapsular haemorrhage were agree with Joubert *et al.*, (1999) and Nesma, (2004) who declared lymphoid depletion of white pulp of spleen with subcapsular haemorrhage due to ORT infection.

REFERENCES

- Abdul-Aziz, T.A. and Weber, L.J. (1999): *Ornithobacterium rhinotracheale* infection in turkey flock in Ontario Canal. Vet. J. 40: 349-350.
- Back, A.; Nagaraja, K.; Rajjachekara, G.; Heremain, R. and Halvorsor, D. (1998): Tissue distribution of *Ornithobacterium rhinotracheale* in experimentally infected turkeys. Vet. Rec., 143: 52-53.
- Bancroft, J.D.; Stevens, A. and Turner, D.R. (1996): Theory and practice of histological technique. 4th Ed. Churchill, Livingston, New York, London, San Francisco, Tokyo.
- Calnek, B.W.; Barnes, H.J.; Beard, C.W.; McDougald, L.R. and Saif, Y.M. (1997): Diseases of Poultry. 10th Ed. Editorial Board for the American Association of Avian Pathologists. Mosby-Wolfe.
- Cruickshank, R.; Duguid, J.P.; Marminon, B.P. and Swan, R.H.A. (1980): "Medical Microbiology" 12th Ed. Vol. 11. Reprinted Churchill Livingstone & Robert Stevenson Edinburgh, England.
- Derosa, M.; Droual, R.; Chin, R.; Shivaprasad, H. and Walker, R. (1996): *Ornithobacterium rhinotracheale* infection in turkey breeder. Avian Dis., 40: 865-874.

- El-Gohary, A.A. and Awaad, M.H.H. (1998): Contaminant Ornithobacterium rhinotracheale (ORT) and E. coli infection in chicken broiler. Vet. Med. J., Giza. 46 (1): 67-75.*
- El-Shamy, S.A.; Shair, Z.N. and Bayoumi, A.H. (2000): Morphological and aetiological studies on pneumonia in turkey: Ornithobacterium rhinotracheale infection. Assiut Vet. Med. J., 43 (86): 338-346.*
- Franz, G.; Hein, R.; Bricker, J.; Walls, P.; Odor, E.; Salem, M. and Sample, B. (1997): Experimental studies in broilers with a Delmarva O. rhithobacterium isolate. Proc 46th Western Poultry Disease Conference. 46-48.*
- Hafez, H.M. (1994): Respiratory disease conditions in meat turkeys caused by Ornithobacterium rhinotracheale: clinical signs, diagnostic and therapy. In Proceeding 43rd Western Poultry Disease Conference, Sacramento, California, pp. 113-114.*
- Hafez, H.M. (1996): Current studies on role of (ORT) in respiratory disease complexes in poultry. Arch. Geflugelk, 60 (5): 208-211.*
- Joubert, P.; Higgins, R.; Iaperle, A.; Mikalion, I.; Van, D. and Silim, A. (1999): Isolation of ornithobacterium rhinotracheale from turkeys in Quebec, Canada, Avian Dis., 43:622-626.*
- Leroy-Sterin, S.; Flaujac, G.; Thenaisy, K. and Chaslus, E.; Dancla, (1998): Genetic diversity of Ornithobacterium rhinotracheale strains isolated from poultry in France. Letters in Applied Microbiology. 26: 189-193.*
- Majo, N.; Allan, G.M.; O'loan, C.J.; Pages, A. and Ramis, A.J. (1995): A sequential histopathological and immunocytochemical study of chickens, turkey poults and broiler breeders experimentally infected with turkey rhinotrachietis virus. Avian Dis., 39: 887-896.*
- Nesma, R.M. El-Awady, (2004): Pathological studies on swollen head syndrome of chickens. M.V.Sc. Thesis (Pathology). Fac. Vet. Med., Zagazig Univ., Egypt.*
- Odor, E.; Salem, M.; Pope, C.; Sample, B.; Prim Vance, K. and Murphy, M. (1997): Case report: Isolation of Ornithobacterium rhinotracheale from commercial broiler flocks on the Delmarva Peninsula. Avian Dis., 41: 257-360.*
- Saif, Y.M.; Barnes, H.J.; Glissons, J.R.; Fadly, A.M.; McDonglad, L.R. and Swayne, D.E. (2003): Diseases of poultry. 11th Edition. Iowa State press. P. 683-689.*

- Van Beek, P.; Van Empel, P.; Van Den Bosch, G.P. and Rn Du Prez, J. (1994):* Ademhalings problemen, groeivetragingen gewrichtsontstekingen bij katkoenen en vleeskuikens dooreen *Pasteurella actinobacterium* bacterie. *Ornithobacterium rhinotracheale* of taxon 28. Tijdsch Diergeneeskd. 119: 99-101.
- Van-damme, P.; Segers, P.; VanCneyt, M.; Van Hover, K.; Mulders, R.; Hommez, J.; Dewirst, F.; Paster, B.; Kersters, K.; Falsen, E.; Devrieze, L.; Bisgard, M.; Hinz, K.H. and Mannheim, W. (1994):* Description of *Ornithobacterium rhinotracheale* gen-ove-sp-nov isolated from the avian respiratory tract. Inter. J. Sys. Bacteriol. 44: 24-37.
- Veen L. Van; Gruys, E.; Frik, K.; Empel, P. Van; Van Veen, L. and Ban Empel, P. (2000):* Increased condemnation of broilers associated with *Ornithobacterium rhinotracheale*. Vet. Rec., 147 (15): 422-423.