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Research Paper

Clinico-pathological changes related to *Ornithobacterium rhinotracheale* in broiler chicken

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Abstract

Infections of the respiratory system are the most serious illnesses that affect the poultry industry. The pathogen *Ornithobacterium rhinotracheale* (ORT), which is most well-known for causing respiratory tract illnesses in birds worldwide, including pneumonia and air sacculitis, has caused a serious problem in Egypt's intensive chicken production. One hundred samples of tracheal swabs were taken from five various flocks of broilers in Sharkia Governorate, with or without respiratory symptoms. Twenty isolates from tracheal swabs were recognized as ORT based on the findings of culture and biochemical testing; however, molecular characterization revealed that Four ORT isolates tested positive in the PCR test and produced the anticipated 784 bp amplification product. Applying the disk diffusion technique a panel of commonly used antimicrobial drugs was utilized to evaluate the drug resistance profiles of ORT isolates. According to an antimicrobial susceptibility test, ORT strains were resistant to the majority of aminoglycosides (gentamicin and amikacin), polymyxins (colistin), and sulfonamides (sulfamethoxazole trimethoprim). On the other hand, tetracyclines (doxycycline and tetracycline) and beta-lactams (amoxicillin, clavulanic acid, and oxacillin) were found to be effective against ORT strains. Macrolides (erythromycin) and quinolones (ciprofloxacin and nalidixic acid) showed varying antibiotic susceptibility

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patterns. Study revealed significant decrease RBCs count, Hb concentration, PCV, heterophils and monocytes while TLC and lymphocytes measures showed significant increase in diseased birds. MDA and NO showed significant increases while SOD, IL2, IL6 and GPX revealed significant decreases in diseased birds compare with apparently healthy. Histopathology revealed tracheal lesions represented in moderate to severe inflammation with submucosal hemorrhage and edema while lung suffered pneumonia, endotheliosis and hemorrhage. Heart showed focal myomalacia and disorganization of cardiac fiber with edema and hemorrhage. The purpose of this investigation was to isolate, identify, and assess ORT's susceptibility to antibiotics in flocks of broiler chickens according to molecular and culture testing and investigation of clinical and pathological changes related to ORT infection.

Introduction

Respiratory diseases have a significant concern in commercial chicken farming. Carcasses condemned at processing facilities because of air sacculitis in diseased hens could result in further financial losses for the producers. (Rubio and Salazar 2010). Numerous infections, either primary or secondary, have been found to cause respiratory diseases. A recently identified pathogen, *Ornithobacterium rhinotracheale* (ORT), is a rod-shaped, pleomorphic, Gram-negative bacterium linked to respiratory illness, growth retardation, death, and reduced egg output in hens (Chin and Charlton 2008). Germany, the US, South Africa, France, the Netherlands, Slovenia, Belgium, Italy, England, Ireland, Canada, Peru, Iran, India, Turkey, Thailand, Taiwan, Malaysia, Korea, China, and Indonesia are among the countries where ORT has been reported and is suspected to have a worldwide distribution (Lopes *et al.*, 2002). ORT can result in highly contagious infections, however there is considerable variation in the severity of clinical symptoms, disease duration, and mortalities depending on environmental factors, such as poor management, inadequate ventilation, high stocking density, poor litter conditions, poor hygiene, high ammonia levels, concurrent diseases, and the type of secondary infection (Van Empel and Hafez., 1999). ORT was isolated from domesticated fowl in a number of nations with diverse geographic origins shortly after it was initially discovered, a lot of work has gone into isolating and characterizing ORT in order to comprehend its population structure and answer epidemiological queries (Chou *et al.*, 2009).

The main characterized symptom observed was face edema and head swelling, which were caused by inflammatory exudates accumulation beneath the head's skin (Abdelmoez *et al.*, 2019). Additional symptoms mentioned were rales, coughing, and trouble breathing, conjunctivitis, profound depression, and enlarged infraorbital and supraorbital sinuses was crucial to correctly identify the ORT strain based on these symptoms. Exudative pneumonia, sinusitis, pericarditis, and tracheitis and yogurt-like exudate in the abdominal air sac are the most common post-mortem lesions linked to ORT infection (Banani *et al.*, 2001). Macrophages infiltrate the interstitial space in a noticeable and diffuse manner, while heterophils are less prevalent. There are widespread, coalescing foci of necrosis that are frequently located in the parabronchial lumen and extend into the nearby parenchyma. Small clusters of bacteria may be sporadic inside the necrotic foci, which are typically filled with thick aggregates of necrotic heterophilic infiltration or exudate. Distended blood capillaries that are packed with fibrin thrombi are possible (Hauck *et al.*, 2015).

Chicken meat accounts for about 36% of global meat output and is the second most popular meat. As a result, respiratory illnesses in poultry affect not only the largest producers but also the entire world, Therefore this review focused on the clinical, pathological, bacteriological, emergence of antibiotic resistance, and genetic traits of *Ornithobacterium rhinotracheale* in order to discuss the clinicopathological changes associated with this agent in chicken.

Materials and Methods

Collection of samples

One hundred tracheal swab samples were taken from various flocks of broiler chickens in El-Sharkia governorate, with or without respiratory signs. Samples were transferred to Animal Health Research Institute, Zagazig branch in sterile ice-filled containers and test tubes with transport medium to preserve the germs and keep swabs from drying up, according to (Tsai and Huang, 2006).

Bacteriological examinations

On 5% sheep blood agar with 10 µg/mL gentamicin, samples were streaked. Plates were kept in an incubator at 37 °C for 24 to 48 hr (Markey *et al.*, 2013). The colonies that were small, round, opaque to grayish, nonhemolytic, and had a diameter of one to three millimeters were chosen. Colonies exhibiting ORT features were detected biochemically and stained using Gram staining.

Biochemacal identification

Biochemical characterization was assessed through oxidase, catalase, H₂S production, triple sugar iron (TSI) agar (Merck), indole production, urease, nitrate reduction, gelatinase and motility tests. Some carbohydrate fermentation tests such as sucrose, glucose, sorbitol, lactose, arabinose and maltose were also implemented. Brain heart infusion (BHI, Merck) broth containing 30% glycerol was used to preserve suspected ORT isolates at -70°C (Fawole and Oso, 2004).

Susceptibility to antimicrobials

The ORT isolates were tested using the disk-diffusion method in accordance with the guidelines provided in the Clinical and Laboratory Standards Institute paper (CLSI, 2008). The antibiotics used were as follows: amikacin (30µg), amoxicillin clavulanic acid (30µg), oxacillin (1µg), ciprofloxacin (5ug), nalidixic acid (30µg), colistin (10ug), doxycycline (30ug), erythromycin (15ug), gentamicin (10µg), sulphamethoxazole-trimethoprim (23.75 + 1.25µg), and tetracycline (30µg). Bacterial isolates were obtained from 24-hour blood agar culture plates, the isolated colonies from agar plates were directly suspended in

tryptic soy broth (TSB; Merck) to create the inoculums, which were then applied to the Mueller-Hinton agar (Merck) surface using sterile cotton swabs. This test assessed the isolates' sensitivity to ten distinct antimicrobial agents. Following a 20-hour incubation period at 37°C, the inhibition (halo) zones were measured and the data were interpreted in accordance with the guidelines outlined in (CLSI: M31-A3).

Confirmation of *Ornithobacterium rhinotracheale* and its virulence and resistance gene by PCR

The manufacturer's instructions were followed in order to extract DNA from 20 likely isolates, in order to use the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) to detect the 16S rRNA of *Ornithobacterium rhinotracheale*, *vapD* virulence gene and *mecA*, *mcr-8* resistance genes. 200µl of the sample suspension, 200µl of lysis buffer, and 10µl of proteinase K were incubated for ten minutes at 56°C. Following that, 200µl of 100% ethanol was added to the lysate. The manufacturer's recommendations were followed when rinsing and centrifuging the sample. Elution buffer (100µl) included in the kit was used to elute the nucleic acid. The primer sets were purchased from **Biobasic (Canada)**. The cycling parameters are shown in (Table 1). The results of the PCR were separated by electrophoresis using gradients of 5V/cm on a 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature. Twenty microliters of the PCR products were put into each gel slot for the gel inspection. The Gelpilot 100 bp DNA Ladder (Qiagen, Germany, GmbH) was used to determine the sizes of the fragments. The gel photo was taken using a gel documentation system (Alpha Innotech, Biometra). The data was analyzed using computer software.

Table (1). Primer sequences, target genes, and amplicon sizes of *Ornithobacterium rhinotracheale*

Target gene	Primers sequences	Amplified segment (bp)	Reference
<i>16S rRNA</i>	GAGAATTAATTACGGATTAAG	784	Hafez. (2002)
	TTCGCTTGGTCTCCGAAGAT		
<i>vapD</i>	ATGTACGCAATAGCATTGACGCG	223	Yusefinejad et al. (2024)
	GCTCTGATATCTCTTACAGACGCG		
<i>mecA</i>	CCA ATT CCA CAT (2006) TGT TTC GGT CTA A	310	McClur et al. (2006)
	CCA ATT CCA CAT TGT TTC GGT CTA A		
<i>mcr-8</i>	TTGTCGTCGTGGGCGAAAC	514	Gorecki et al. (2022)
	CTGTCGCAAGTTGGGCTAAAG		

Blood samples:

Blood samples were collected from wing vein puncture from healthy and diseased birds with aseptic measures. For the hematological analysis, the first sample was one milliliter of blood obtained on EDTA. Three milliliters of blood were drawn for the second sample without the use of an anticoagulant in a clean and dry centrifuge tube, left to clot at room temperature and centrifuged at 3000 rpm for 5 min. Serum samples were putted in dry clean capped tubes and kept in deep freeze at -20°C for biochemical analysis.

Hematological studies:

Blood samples with anticoagulant were subjected for detection of cellular blood constituents according to (Feldman *et al.*, 2000).

Biochemical assay:

Malondialdehyde (MDA) according to (Sato, 1987), superoxide dismutase (SOD) was estimated according to (Aebi, 1984), Interleukin 2 (IL2) and interleukin 6 (IL6), were measured using Ray Bio® ELISA Kits protocol. Nitric oxide level according to method described by (Ramadan and Attia, 2003). Antioxidant activity: Glutathione (Gpx) activity was measured according to (Molavian *et al.*, 2015).

Pathological examination:

Specimens from trachea, lungs and hearts of freshly dead three chickens were collected and fixed in 10% buffered neutral formalin. Hematoxylin and eosin were used to stain paraffin sections of two to three microns in thickness, which were then inspected under a microscope (Suvarna *et al.*, 2013).

Statistical analysis:

The T-test was used to statistically assess the data from this study in accordance with (Tamhane and Dunlop, 2000).

Results**Clinical Sign**

Coughing, sneezing, nasal discharge, reduced intake of food and water, severe depression, and mild conjunctivitis.

Postmortem lesions

Pneumonia, pleuritis, and abdominal air sacculitis with frothy, white yogurt-like exudates were the most frequent macroscopic abnormalities in broiler hens; thoracic and/or abdominal air sacculitis, pericarditis, and peritonitis have also been reported. There have been reports of joint infections, liver and spleen swelling, and heart muscle deterioration.

Bacteriological examinations

The isolation of *Ornithobacterium rhinotracheale* (ORT) from broiler trachea caused numerous challenges arose since the ORT colonies were obscured by overgrowth of fast-growing bacteria. ORT colonies on blood agar were non hemolytic, small circular convex with round edges, opaque greyish white with a strong odor similar to butyric acid and showed poor adhesion to the agar surface. Colonies grew on BHI broth and trypticase soy agar, but not on MacConkey agar or nutrient agar media. 20 ORT isolates appeared as Gram negative, pleomorphic, non-motile and non-sporulated, arranged in clusters or fat short rods.

Biochemical identification

Biochemical characterization revealed that *Ornithobacterium rhinotracheale* isolates tested negative for catalase, H₂S generation, Tsi, Indol, nitrate, and gelatinase but positive for oxidase and urease. The results of sugar fermentation ORT were negative for sorbitol and positive for sucrose, glucose, lactose, arabinose and maltose.

Antimicrobial susceptibility

It was interesting to note that all ORT strains were resistant to 4-6 antimicrobial groups (multidrug resistance). According to the antibiotic susceptibility test utilizing the KirbyBauer standard disk diffusion method, ORT strains were resistant to the majority of aminoglycosides [gentamicin: 18/20(20%) and amikacin: 17/20(85%)], polymyxins [colistin: 15/20 (75%)], and sulfonamides [sulfamethoxazole trimethoprim:14/20(70%)]. On the other hand, ORT strains were susceptible to tetracyclines [tetracycline: 16/20(80%) and doxycycline: 15/20(75%)] and beta-lactams [amoxicillin-clavulanic acid:14/20(70%) and oxacillin:12/20(60%)]. Macrolides (erythromycin) and quinolones (ciprofloxacin and nalidixic

acid) showed moderate level of antibiotic susceptibility on isolated ORT estimated by 10/20 (50%) and 11/20 (55%) respectively.

PCR Detection of *Ornithobacterium rhi-notracheale* and virulence, resistance factor

As shown in **Fig (1)** out of 20 positive ORT isolates by conventional method, 4 samples were positive for the presence of 16S rRNA (784bp) by PCR, one of them was positive for the presence of Vap D virulence gene(223bp). Also PCR was applied to four confirmed isolates for the detection of the *mecA* and *mcr-8* resistance genes and results showed that this genes were detected in 2 of 4 examined isolates and gave a characteristic band at (310 bp) and (514bp) respectively (**Fig 2**).

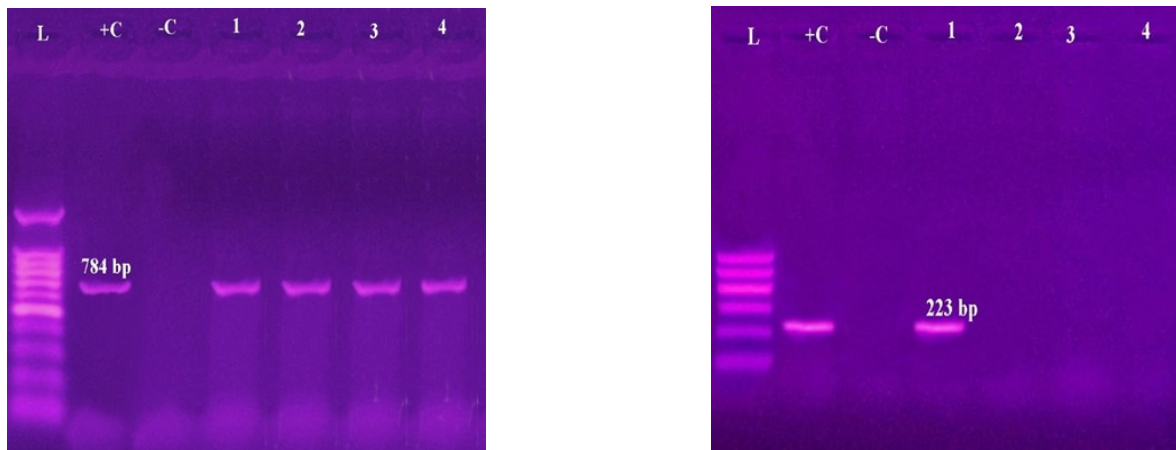


Fig. (1). PCR was applied to ORT isolates for the detection of the 16SrRNA gene and results showed its detection in 4 samples and gave a characteristic band at (784 bp), while the Vap D virulence gene was detected in one of the examined isolates at the expected product size (223bp).

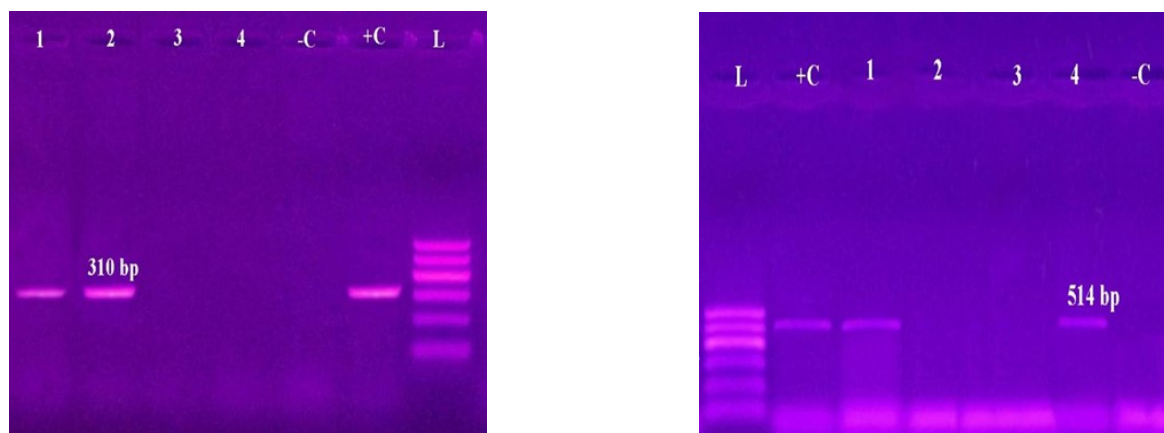


Fig. (2). PCR was applied to four confirmed isolates for the detection of the *mecA* and *mcr-8* resistance genes and results showed that this genes were detected in 2 of 4 examined isolates and gave a characteristic band at (310 bp) and (514bp) respectively

Hematological findings

The changes in Erythrogram and leukogram parameters were demonstrated in table (2) showed that the diseased broilers resulted in

significant decrease RBCs count, Hb concentration, PCV, heterophils and monocytes while TLC and lymphocytes measures showed significant increase in diseased birds (n=5).

Table (2). Hematological parameters (Mean values \pm S.E) of healthy control and diseased chickens (n=5).

Parameters	RBCs $\times 10^6 / \mu\text{l}$	Hb (gm/dl)	PCV %	TLC $\times 10^3 / \mu\text{l}$	Heterophils $\times 10^3 / \mu\text{l}$	Lymphocytes $\times 10^3 / \mu\text{l}$	Monocytes $\times 10^3 / \mu\text{l}$	Eosino- phils $\times 10^3 / \mu\text{l}$
Apparently healthy Birds	2.67 ± 0.02	13.3 ± 0.25	30.25 ± 2.35	21.19 ± 1.10	6.63 ± 0.12	10.85 ± 0.38	2.65 ± 0.04	1.06 ± 0.02
Diseased Birds	2.18** 0.07 \pm	11.15** 0.18 \pm	27.80* ± 0.32	22.85* ± 1.15	5.87** ± 0.12	13.50*** 0.25 \pm	2.38* 0.08 \pm	1.10 0.01 \pm

*: Significant at $P < 0.05$; **: Significant at $P < 0.01$; ***: Significant at $P < 0.001$

Biochemical analysis

Diseased birds exhibited the higher levels of MDA and NO in table (3) while SOD, IL2, IL6

and GPX revealed significant decreases compared with apparently healthy (n=5).

Table (3). Serum biochemical parameters (Mean values \pm S.E) of healthy control and diseased chickens (n=5).

Parameters	MDA $\mu\text{mol}/\text{mg}$	SOD μ/mg	No $\mu\text{mol}/\text{L}$	IL2 (pg m/L)	IL 6 (pg m/L)	GPX u/ml
Apparently healthy Birds	6.40 ± 0.18	96.00 ± 1.45	90.82 ± 1.80	48.20 ± 1.78	66.00 ± 2.05	114.30 ± 2.59
Diseased Birds	10.39*** ± 0.28	83.80*** ± 1.53	105.25*** ± 2.75	32.25*** ± 2.18	54.40*** ± 2.25	95.30*** ± 3.46

*: Significant at $P < 0.05$; **: Significant at $P < 0.01$; ***: Significant at $P < 0.001$

Pathological findings:

Microscopical findings

The trachea of infected chicken shown figure (3) exhibited different lesions with variable severity. Tracheitis was recognized by vascular congestion and inflammatory cells infiltration with focal mucosal erosion detected in some chickens (fig.3 a, b). Some chickens showed partial tracheomalacia in addition to tracheitis (fig. 3 c). Few cases revealed submucosal edema with cystic dilation of some mucosal glands (fig.3 d). Hemorrhage with perichondral edema was also detected (fig. 3e). Focal vacuolation of some mucosal cells was seen (fig. 3f). Heart of infected chicken showed moderate to severe lesions as in fig (4) the lesions were represented by severe congestion of large blood vessels (fig. 4a). Focal myomalacia of

myocardium with intermuscular edema (fig. 4b). Perivascular edema with endotheliosis were also detected (fig. 4 c&d). Myocardial hemorrhage and disorganization of cardiac muscle fiber were also detected consequently (fig 4e & 4f). lung of infected chickens were severely affected and exhibited, severe pneumonia associated with hemorrhage (fig 5a). Hyperplasia of bronchial epithelium was showed in (fig 5b). Endotheliosis with perivascular edema and pneumonia were detected in some chickens (fig 5c). Some cases showed diffuse proliferative pneumonia (fig 5d). Pulmonary and perivascular hemorrhage were observed in fig (5e).

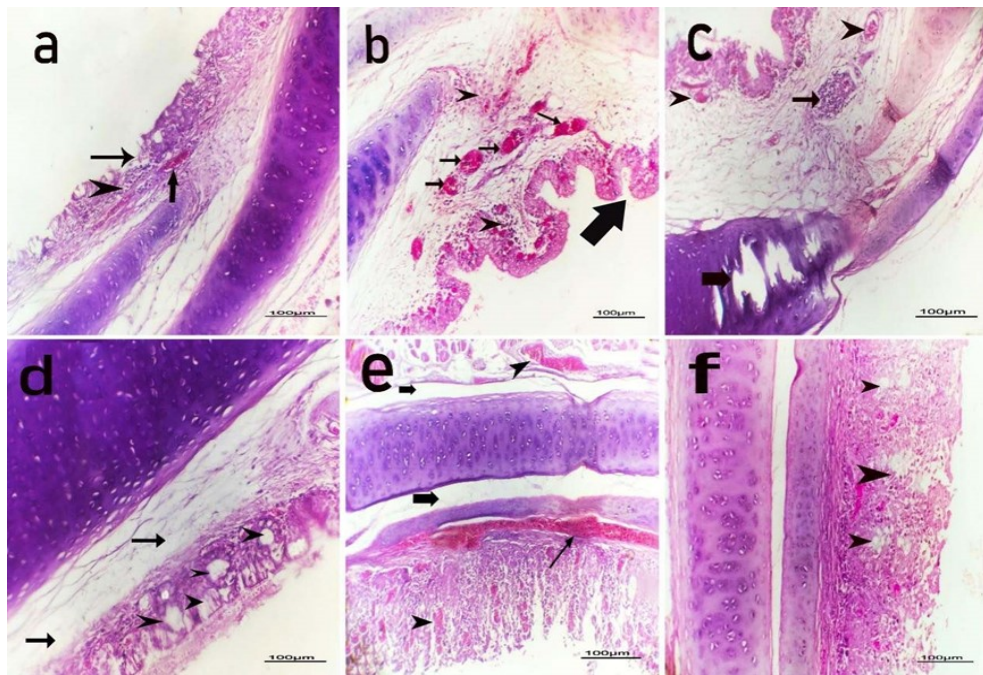


Figure (3). Photomicrograph of chicken trachea infected with *Ornithobacteria* showing: a) Tracheitis represented in vascular congestion (thick arrow) and inflammatory cells infiltration (arrowhead) with erosion of mucosal epithelium (thin arrow) . b) Corrugated hyperplasia of lining epithelium, mucosal and submucosal edema, inflammatory cells infiltration (arrow head) and congestion (thin arrows). c) Partial Tracheomalacia (thick arrow) with vascular congestion (thin arrow) and focal inflammatory cells infiltration (arrowhead). d) Submucosal edema (arrows) with cystic dilation of mucosal glands (arrows head). e) Submucosal hemorrhage (thin arrow) with perichondrial edema (thick arrows) and vascular congestion (arrowhead). f) Focal vacuolation of mucosal glands (arrowshead) (Scale bar = 100 µm).

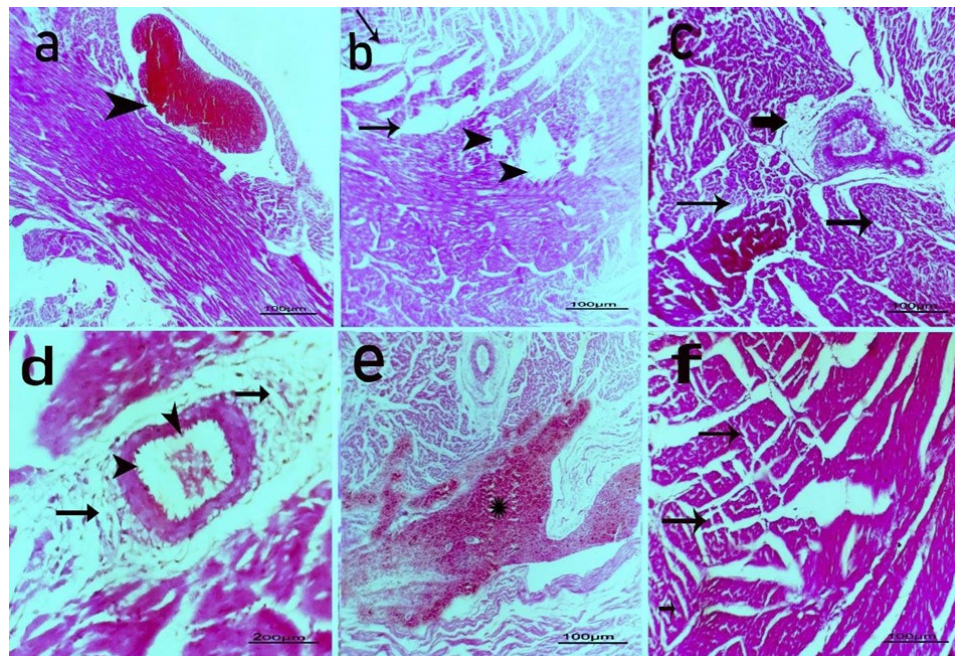


Figure (4). Photomicrograph of chicken heart infected with *Ornithobacteria* showing: a) severe congestion of large blood vessels (arrowhead). b) focal myomalacia of myocardium (arrowhead) with intermuscular fibers edema (arrow) .c) perivascular edema (thick arrow), congestion (arrowhead) and disorganized cardiac fibers (thin arrows). d) perivascular edema (arrows) and endotheliosis (arrowheads). e) myocardial hemorrhage (star). f) disorganization of cardiac fibers (arrows). (Scale bar,a, b, c, e = 100 µm, d =200 µm).

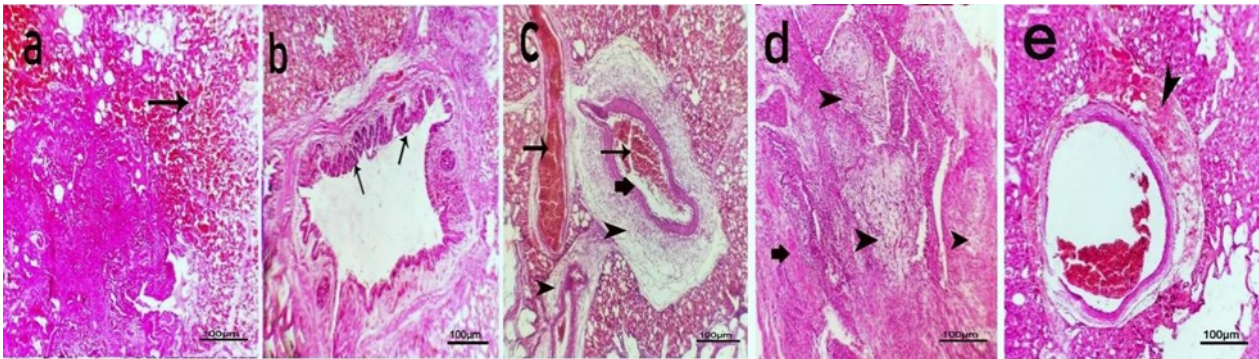


Figure (5). Photomicrograph of chicken lung infected with *Ornithobacteria* showing: **a)** Severe hemorrhagic pneumonia (arrow). **b)** Hyperplasia of bronchial epithelium (arrow). **c)** Severe congestion of pulmonary blood vessels (thin arrows) with endotheliosis (thick arrow) and perivascular edema (arrowhead). **d)** Diffuse proliferative pneumonia (arrowheads) with vascular congestion (thick arrow). **e)** Severe pulmonary hemorrhage (star). **f)** Perivascular extravasated erythrocytes (arrowhead). (Scale bar = 100 µm)

Discussion

Particularly in developing nations, respiratory illnesses in chickens result in large financial losses for the sector. *Ornithobacterium rhinotracheale*, a novel bacterial respiratory illness in chickens, was observed in South Africa (Vandamme *et al.*, 1994). The ORT incidence rate was 20% , this rate was more than the previously reported rates 7.27% (Elbestawy, 2010) and 8.5%–10% (Seyyed *et al.*, 2012). The increased experience with this bacterial pathogen since 2009, as well as the rise in infectious bronchitis virus and respiratory viral illnesses in the Egyptian poultry production industry, may be the cause of this higher isolation rate when compared to earlier investigations. The isolation of *Ornithobacterium rhinotracheale* (ORT) from broiler trachea caused numerous challenges arose since the ORT colonies were obscured by overgrowth of fast-growing bacteria as shown by (Hafez, 1998). Our findings differed with the organism's frequencies in other investigations, (Ozbey *et al.*, 2004) analyzed 250 lung and trachea samples from ten flocks of slaughtered broiler chickens who had respiratory symptoms, and found that five (1.50%) of the chickens' tracheas had ORT. Additionally, it has been reported that ORT was isolated from tracheal swab and tracheal tissue samples taken from broiler chickens (0.40% and 1.20%, respectively) (Erganis *et al.*, 2002 and Turkyilmaz, 2005). Three out of 300 (1.0%) total lung and tracheal tissue samples from broiler chickens at slaughterhouses and dead birds of broiler flocks with

respiratory illness symptoms, and one out of 150 (0.60%) tracheal swab samples from Iran's north, west, and center were isolated and characterized as ORT.

(Hassanzadeh *et al.*, 2010). Similarly, using the cultural technique, 3 out of 290 (1.03%) tracheal swab samples from 29 slaughtered broiler chicken flocks in the northern Iranian province of Guilan tested positive for ORT (Asadpour *et al.*, 2011). Additionally, (Barin *et al.*, 2008) used a cultural approach to investigate tracheal swab samples taken from 38 broiler chicken flocks in Babol city (north of Iran) that had respiratory problems, they found that only 1 flock (2.60%) tested positive for ORT. According to (Seifi, 2012), biochemical tests were used to identify 12 ORT isolates from 450 tracheal samples (2.60%) taken from 45 broiler flocks in Mazandaran region (north of Iran). (Ghaemmaghami *et al.*, 2007) used biochemical tests to analyze 173 trachea and lung samples from broiler chicken flocks exhibiting clinical respiratory symptoms in Markazi province (central Iran), they found that ORT was isolated from 17 (9.80%) of the samples. Similarly, biochemical and cultural testing revealed that Out of 254 tracheal swab samples from slaughtered broiler chicken flocks in the province of Khuzestan, 22 (8.66%) were positive for ORT (Jamshidian and Mayahi, 2008). In contrary, (Banani *et al.*, 2001) found that 59 (59.00%) isolates were identified as ORT from tracheal samples taken from the carcasses of 100 broilers, broiler

breeders, and layer flocks with respiratory illnesses. The majority of the isolates reported in the literature (**Vandamme *et al.*, 1994**, **Hinz *et al.*, 1994** and **Travers *et al.*, 1996**) exhibited biochemical traits that were present in suspected isolates. The more often used antibiotics in the source of the isolation determine the antibiotic resistance profile of the various ORT strains. The origins of the strain and commonly used antibiotics in the region influence the sensitivity pattern of ORT strains. The strains investigated in this study have been demonstrated to be resistant to some of the major antibiotics, similar to the ORT strains isolated in other studies. This could be because antibiotics were improperly used to treat secondary infections linked to the prevalence of respiratory diseases complex in broiler chicken farms. The majority of ORT isolates in Germany and the Netherlands are resistant to ciprofloxacin (**Van Beek, 1994**). Similarly, pure ORT has been recovered from birds treated with ciprofloxacin in Canada (**Joubert, 1999**). However, the majority of ORT strains obtained in Belgium were found to be susceptible to quinolones, particularly enrofloxacin (**Devriese *et al.*, 1995**). Additionally, the susceptibility of Mexican isolates of ORT to amoxicillin, ciprofloxacin, and oxytetracycline varied (**Soriano *et al.*, 2003**). The majority of the recovered ORT organisms in Iran were shown to be susceptible to florfenicol, enrofloxacin, and trimethoprim-sulfamethoxazole but resistant to colistin (**Ghaemmaghami *et al.*, 2007**).

Asadpour *et al.*, (2011) reported that all ORT isolates were susceptible to ceftriaxon and resistant to ciprofloxacin, erythromycin, and tetracycline; however, two isolates were moderately sensitive to amoxicillin and florfenicol. The examined ORT isolates were shown to be resistant to colistin, tetracycline, and gentamicin (**Barin *et al.*, 2008**). Additionally, it was demonstrated by (**Jamshidian and Mayahi, 2008**) that all ORT isolates were sensitive to ampicillin but resistant to gentamicin and trimethoprim-sulfamethoxazole. Due to its difficulties in isolation and biochemical characterization since it is typically overgrown by other bacteria, the PCR technique is thought to be a helpful laboratory tool for the quick identifica-

tion of suspected ORT isolates (**Churria *et al.*, 2012**). To identify and describe ORT bacterial strain, PCR is a quick, sensitive, and specific technique (**Ozbey *et al.*, 2004**; **Hassanzadeh *et al.*, 2010**). Four positive ORT strains were verified by PCR in this investigation, in contrast, 21 out of 100 (21.00%) tracheal swabs taken from commercial broiler flocks in Jordan's southern and northern regions that had respiratory diseases tested positive for ORT in a PCR test (**Roussan *et al.*, 2011**).

The study showed a significant decrease in RBCs count, Hb, PCV and lymphocytic counts with a significant decrease in total leukocytic, heterophils and monocyte counts may be due to the ORT stress on the bird which agreed with (**El-Abasy *et al.*, 2016**) who detected the same results accompanied by non regenerative normocytic normochromic anemia and macrocytic hypochromic anemia due to chronic renal failure supported by increase in urea and creatinine while the increase of leukocytic and heterophilic count may revealed to a primary bacterial infection response of respiratory tract as the same obtained by (**Ahamefule *et al.*, 2006**) who reported it usually associated with microbial infection or foreign bodies or antigens in the circulatory system.

Our study revealed a significant decrease in SOD and GPX with a significant increase in MDA and NO values which may be as a result of infection that induced inflammatory response that increased oxidative stress in birds with pneumonia with a production of high levels of ROS that are toxic to the cell, particularly the cell membrane, in which these radicals interact with the lipid bilayer and produce lipid peroxides and decomposes high concentrations of H₂O₂ agreed with (**Benzer and Yilmaz 2022**) who explained that broilers infected with ORT have increased oxidative stress by increased LPO in plasma, trachea and lung concluded that lung infection with ORT adversely affected the tissue oxidant-antioxidant status, while NO elevation may be due to increase expression of INOS which were associated with respiratory tract inflammatory illnesses in respiratory epithelium and inflammatory-immune cells, and significantly raise NO production,

most likely as an extra host defense against viral or bacterial infections.

On histopathological examination, the distinct features of the microscopic lesions were changes involving tracheal tissues of birds which showed infiltration of lymphocytes, plasma cells, and macrophages in the lamina propria. Lymphoid aggregates were present in severely affected tracheas, which caused the mucosa to bulge into the lumen. A few of the glands were dilated and empty and the cilia were disrupted (Lopes *et al.*, 2002). The histological findings were consistent with those of (Chin *et al.*, 2008), who said that pneumonia and tracheitis are typically the most typical ORT histopathology lesions. ORT was found to be a component of a complex of various respiratory agents, including avian paramyxovirus type 2, avian coronavirus, Mycoplasma species, *Escherichia coli*, Pasteurella species, and Syngamus trachea, indicating synergism with these agents (Welchman *et al.*, 2013) which explain the respiratory lesion in the infected chickens in both lung and tissue that lead to respiratory distress. Also (Pavle *et al.*, 2016) found that the histopathological findings in most samples were indicative of catarrhal inflammation in the trachea and larynx. Epithelial hyperplasia was observed in a number of samples, and desquamation and degenerative necrotic lesions were also observed in certain mucosal regions. There was congestion in the blood vessels of the lamina propria of the tracheal and laryngeal mucosa. Erythrocytes are found in the lungs. Heterophil-rich exudate is seen in the bronchus lumen, whereas lymphocytes and heterophils are infiltrated in the lamina propria. Thickening of the air sac wall and epithelial necrosis as a result of mononuclear inflammatory cell infiltration in the lungs, hyperaemia was the most common histological identification. There were clogged lungs' blood vessels.

Conclusion

In Egypt, ORT infection is currently prevalent in older chickens, good management techniques and biosecurity measures are necessary for efficient disease control in the absence of an ORT vaccination. The results will allow the

development of a program to control ORT. Future work will be aimed to generate information about the economical losses due to ORT, characterization of antimicrobial susceptibility, pathogenicity and eventually vaccine strains. Our findings indicated that the simultaneous use of both cultural and molecular techniques results in more outcomes in the isolation and identification of the organism. One of the main veterinary concerns is the ORT strain's emergence of multidrug resistance.

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