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Seroprevalence of *Ornithobacterium rhinothracheale* Infection in Commercial Layers in Tunisia

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ABSTRACT

Ornithobacterium rhinotracheale (ORT) infection is a worldwide disease in commercial poultry production. It is caused by a gram-negative bacterium, inducing respiratory and articular infections. The present study investigated the seroprevalence of ORT in commercial laying chicken farms in the Sfax region of South-eastern Tunisia. Diagnosis of ORT is based on the clinical signs such as respiratory distress, sneezing, cough, and limping, gross pathology in post-mortem examination (fibrinous tracheitis, fibrinous pneumonia, caseous arthritis, and tendinitis), and laboratory investigations, including isolation and identification, molecular and serological analysis. In the current study, 470 serum samples were collected from 25 commercial layer flocks (18 to 20 blood samples/flock) and tested using an indirect enzyme-linked immunosorbent assay (ELISA), which revealed that all 25 flocks (100%) demonstrated evidence of past or recent ORT infection. Seropositivity of the tested sera indicated the presence of antibodies against ORT, ranging widely from 7.69% to 100% within individual layer hens flocks. The average antibody levels varied considerably among flocks, from 1425.67 to 15233 in different flocks. The current findings indicated widespread ORT exposure in commercial layer farms in the Sfax region of South-eastern Tunisia, signifying the potential for horizontal transmission of ORT, particularly in multi-age layer integrations where older flocks can act as a source of infection for younger pullets.

Keywords: Biosecurity, Commercial layer, Enzyme-linked immunosorbent assay, *Ornithobacterium rhinotracheale*

INTRODUCTION

Ornithobacterium rhinotracheale (ORT) is a gram-negative bacterium that causes a highly contagious respiratory illness in poultry. This bacterium's shape can vary (pleomorphic), and it cannot move independently (nonmotile). The organism has a rod shape and does not produce spores for survival (Van Empel and Hafez, 1999). The ORT belongs to rRNA superfamily V and shares some genetic characteristics with other bacteria, such as *Cytophaga*, *Riemerella*, and *Flavobacterium* (Van Empel and Hafez, 1999; Canal et al., 2005). The severity of ORT infection depends on different factors, such as environmental conditions, including ambient temperature, humidity, and ammonia, the bacterium's ability to form protective biofilms, and the presence of co-infections with other pathogens such as *Avian Metapneumoviruses* and *Avibacterium paragallinarum* (Marien et al., 2005; De la Rosa-Ramos et al., 2015; Stępień-Pyśniak et al., 2024).

Over 18 serotypes of ORT, designated from A to R, have been identified through studies (Hafez and Sting, 1999; De la Rosa-Ramos et al., 2018). Serotype A of ORT stands out for its high dominance, infecting 94% of chickens and 57% of turkeys (Siddique et al., 2008; Kursa et al., 2022). Furthermore, several studies revealed variations in virulence and adherence properties among serotypes A, B, C, D, and E (Chansiripornchai et al., 2007; De Haro-Cruz et al., 2013).

While ORT is susceptible to common disinfectants such as chloride, phenols, and glutaraldehyde, it can become endemic in poultry houses, persistently infecting new flocks even after poor cleaning and disinfection protocols of the poultry houses. Persistence of ORT is particularly problematic in multi-age layer farms (Hafez and Schulze, 2003).

Ornithobacterium rhinotracheale primarily transmits horizontally among chickens, which means the infection spreads through inhalation of respiratory droplets, direct contact with infected chickens, or indirectly through contaminated feed, equipment, and drinking water. However, vertical transmission appears to be limited (Boulianne et al., 2020). While ORT was isolated from ovary and oviduct, infertile eggs, hatching eggs, and even dead embryos, it is unclear if this pathogen is viable or contributes significantly to chicken infection (Shahata et al., 2006; Boulianne et al., 2020).

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The presence of ORT is confirmed in a wide range of domestic and wild birds suffering from respiratory issues, including chickens, turkeys, ducks, chukar partridges, geese, quail, ostriches, guinea fowl, rooks, pheasants, and pigeons (Boulianne et al., 2020).

Clinical signs of ORT infection are more readily apparent in meat birds (broilers and turkeys) compared to laying hens (Van Veen et al., 2000). Turkey flocks can be exposed to ORT and seroconvert without exhibiting any outward signs of illness (Back et al., 1998a). The age of susceptibility to infection differs among bird types. Broilers typically experience infection between three and four weeks of age, while turkeys are more susceptible from 14 weeks until slaughter age (Charlton et al., 1993; Back et al., 1998b; Van Veen et al., 2000). The pathogenicity of ORT as a primary pathogen in layer chickens is poorly understood. However, severe respiratory signs were described in layer hens' flocks in Japan aged from 26 to 30 weeks (Umali et al., 2018).

Outbreaks of ORT have been reported in breeder farms, causing similar clinical signs to those observed in broiler chickens and turkeys, along with secondary economic consequences (Charlton et al., 1993). Economic losses include decreased egg production, decreased eggshell quality, and reduced hatchability (Charlton et al., 1993). Breeder hens seemed to be most susceptible before or during their peak laying period (Hafez, 1996). The ORT infection in commercial laying hens has been associated with clinical illness, manifesting as increased mortality, respiratory signs, and decreased egg production (Joubert et al., 1999; Lopes et al., 2002).

Diagnosing ORT infection requires a multi-pronged approach due to the challenges associated with culturing the bacteria. While cultural methods are available for isolating and identifying the causative agent, ORT's slow growth (more than 48 hours) in liquid media, such as blood agar or soybean casein agar, incubated at 37°C for 24 to 48 hours under anaerobic or microaerophilic conditions, makes the process challenging (Mayahi et al., 2016). Serological tests offered a more practical alternative for large-scale testing and surveillance programs (Back et al. 1998a). Serotyping of ORT isolates has been carried out using specific antisera against 18 serotypes, employing an enzyme-linked immunosorbent assay (ELISA) and an agar gel precipitation test (Hassan et al., 2020). Real-time polymerase chain reaction (PCR) was also performed for the definitive diagnosis of ORT infections and/or bacterial and viral respiratory co-infections, particularly in cases where cultural or serological methods were not available or were inconclusive (Hashish et al., 2022; Nguyen et al., 2023; Krylova et al., 2024).

The scarcity of published data on ORT prevalence within Tunisia's commercial poultry sector, particularly laying hens, highlighted the need for the present study. The present study aimed to investigate the seroprevalence of ORT infections in commercial layer farms located in the Sfax region of south-eastern Tunisia.

MATERIALS AND METHODS

Ethical approval

The experiment was approved by the Institution of Agricultural Research and Higher Education, National School of Veterinary Medicine of Sidi Thabet, University of Manouba, Tunisia.

Study region

The current study was carried out on twenty-five commercial layer flocks, from November 2020 to May 2021, located in the governorate of Sfax, Tunisia. The governorate of Sfax is located in the Southeast of the country on the eastern coast of Tunisia (Latitude: 34° 44' 26.02" N; Longitude: 10° 45' 37.01" E (Figure 1). Sfax is bounded by the Mediterranean Sea to the east, the governorate of Mahdia to the north, and the governorates of Kairouan, Sidi Bouzid, and Gafsa to the west. The climate of Sfax, where the current study was conducted, is Mediterranean, characterized by mild, relatively rainy winters and hot, sunny summers. Although winter is mild, there can sometimes be cold periods, during which the temperature drops to around 0°C (32°F) and remains at around 10°C (50°F). In summer, during invasions of hot air from the desert, which have become more frequent in recent years, the temperature can exceed 40°C (104°F). The present study focused on commercial layer farms in the Sfax governorate, Tunisia. According to the latest poultry establishment census conducted by Tunisian veterinary authorities between 2015 and 2016, Sfax boasted a particularly high concentration of layer hen flocks, accounting for 70% of Tunisia's total. Furthermore, Sfax is home to an estimated 88 percent of the nation's pullet farms, representing 295 flocks of pullets (ONAGRI, 2021).

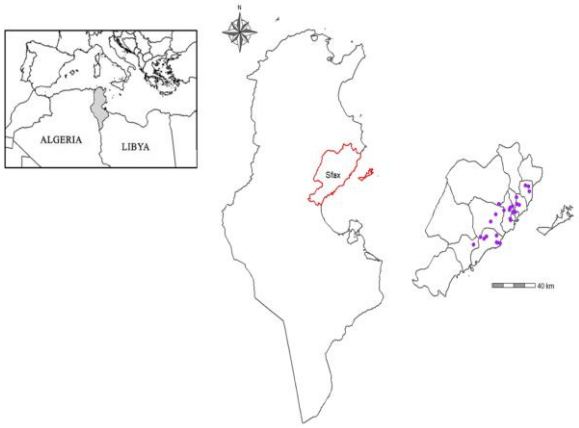

Figure 1. Geographical situation of the governorate of Sfax and distribution of sampled poultry houses. purple points represent flocks' locations.

Flocks and blood samples

The current study involved twenty-five commercial layer flocks, numbered from 1 to 25, chosen randomly across three age groups (20 to 30 weeks, 31 to 55 weeks, and over 55 weeks, Table 1). Data collection utilized a standardized survey form administered at each farm which focused on biosecurity measures implemented, including vehicle disinfection, footbath, distance between houses, pest control and results of bacteriological analysis of environmental samples for controlling disinfection (the supervising veterinarian of the farm carried out swabs of surfaces and equipment), medication history (date of treatment, molecules, method of administration, and duration of treatment), and the overall health status of the flocks (history of pathologies and cumulative mortality).

To assess seroprevalence, blood samples were randomly collected from 18 to 20 chickens in each flock, yielding 470 serum samples. Blood samples were collected from the wing vein and centrifuged at 1500 rpm for ten minutes to separate the serum. Aliquots of the whole sera were stored in clean tubes at -20°C until further analysis (Xue et al., 2020). Serological testing (indirect ELISA) to detect antibodies against ORT was subsequently performed in the Microbiology Laboratory of the National Veterinary Medicine School of Sidi Thabet, Tunisia.

Table 1. Individual seroprevalence of *Ornithobacterium rhinotracheale* infection according to age group and flock size in commercial layers in Tunisia from November 2020 to May 2021

Factor	Category	Flock (Number)	Tested sera (Number)	Positive sera (Number)	Seroprevalence (%)	P value
Age group	20-30 weeks	5	154	113	73.4%	0.001*
	31-55 weeks	10	112	76	67.9%	
	Above 55 weeks	10	204	175	85.8%	
Flock size	Small: < 10 000	7	121	86	71%	0.001*
	Medium: 10 001-30 000	13	233	172	73.8%	
	Big: Above 30,000	5	116	106	91.9%	

*: Significant difference in each column ($p < 0.05$)

Enzyme-linked immunosorbent assay

An indirect ELISA was employed to detect antibodies against ORT in the collected sera, using commercially available kits (ID-VET, ID Screen® ORT Indirect, France), and strictly adhered to the manufacturer's instructions. Following incubation and washing steps, the plates were read at a wavelength of 450 nm using an ELISA reader (Thermofisher®, USA). Samples were categorized as positive or negative based on a pre-determined sample-to-positive ratio (S/P) cut-off value. An S/P ratio less than or equal to 0.4 (titer ≤ 844) was considered negative, while values exceeding 0.4 (titer > 844) were considered positive. Flock-level ORT prevalence was determined by designating a flock as positive if at least one chicken within the flock tested positive for indirect ELISA. Notably, none of the sampled chickens in the present study had been previously vaccinated against ORT.

Statistical data

Statistical analyses were performed using R software (version 4.3.3; R Core Team, 2024) to assess group differences. Specifically, potential associations among age, flock size, and the mean ELISA titers were investigated using the Pearson chi-square test to identify statistically significant variations ($p < 0.05$).

RESULTS

The current epidemiological investigation indicated that 23 of the 25 visited flocks (92%) belonged to multi-age farms. Biosecurity measures varied across farms, with five flocks (aged from 20 weeks to 26 weeks) demonstrating satisfactory practices, 17 with acceptable measures (aged from 32 weeks to 52 weeks), and the remaining three exhibiting low biosecurity levels (aged above 55 weeks). Disinfection before pullet entry, a crucial control measure, was only implemented by 12 flocks (aged from 20 weeks to 48 weeks). The efficacy of disinfection was evaluated by 18 farms based on laboratory analyses of environmental samples (swabs of surfaces, soil, and equipment). The origin of the pullet supply was considered a potential risk for contamination. Only four flocks were sourcing pullets from a single origin (hatchery in Sfax). The remaining 21 flocks obtained pullets from multiple sources (hatcheries in Sfax and Tunis), which could introduce new pathogens.

The present serological analysis revealed a high prevalence of ORT exposure in the studied flocks. A significant majority (77.45%, n = 364) of the 470 collected serum samples tested positive for antibodies against ORT. Notably, positive samples were detected in all 25 flocks investigated (Figure 2A). Seropositivity varied considerably among

flocks, ranging from 7.69% (flock 25) to 100% (Flocks 14, 15, and 16). Furthermore, ELISA mean titers significantly varied across all flocks ($p < 0.05$). The ELISA titers ranged from 1425.67 (flock 12) to a much higher level of 15233 (flock 19; Figure 2B; $p < 0.05$). The variation in antibody levels was further substantiated by the coefficient of variation (CV) values, which ranged from 30.94% to 93.40% and indicated substantial variability in antibody responses among chickens within each flock.

Seroprevalence of ORT infection varied across different age groups (Table 1). Young hens (20-30 weeks old) illustrated a seroprevalence of 73.4%, while seroprevalence increased to 85.8% in older layers (above 55 weeks, $p < 0.05$). Additionally, flocks between 31 and 55 weeks had a slightly lower seroprevalence of 67.9%. The statistically significant difference ($p < 0.05$) exhibited a potential association between age and exposure to ORT. However, the correlation between layer age and ELISA mean titers was low positive ($r = 0.351$; Figure 3). Seroprevalence also exhibited variation based on flock size (Table 1). Smaller flocks (under 10,000 chickens) had a seroprevalence of 71%, while this seroprevalence increased to 91.4% in large flocks (over 30,000 chickens). Medium-sized flocks from 10,001 to 30,000 chickens illustrated an intermediate seroprevalence of 73.8%. The statistically significant difference ($p < 0.05$) suggested a potential link between flock size and exposure to ORT. Furthermore, a positive correlation ($r = 0.467$) was observed between ELISA mean titers and flock size (Figure 3), which indicated that larger flocks may tend to have higher average antibody levels, potentially reflecting a greater possibility of exposure in the flock.

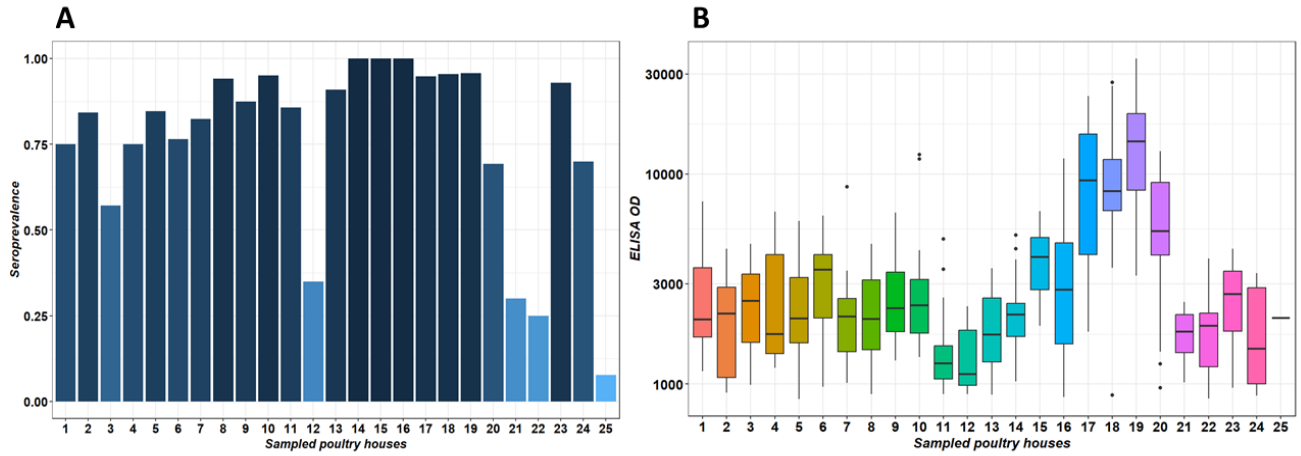

Figure 2. Seroprevalence frequency and enzyme-linked immunosorbent assay titer variation among sampled poultry houses with ages varied from 20 weeks to 107 weeks in the governorate of Sfax, Tunisia. **A**: Seroprevalence frequency, **B**: ELISA titer; each bar corresponds to a flock. Upper limit is the highest titer, lower limit is the lowest titer, horizontal line of the bar is the average antibody titer against *Ornithobacterium rhinotracheale.*

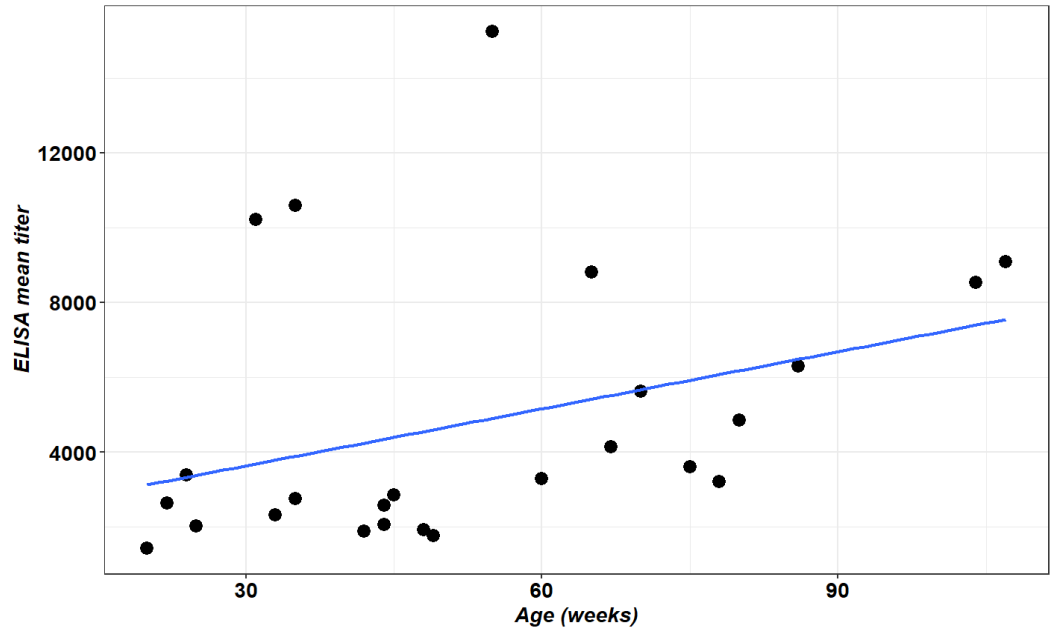

Figure 3. Correlation between layer age flocks (age varied from 20 weeks to 107 weeks) and *Ornithobacterium rhinotracheale* enzyme-linked immunosorbent assay mean titer, in the governorate of Sfax, Tunisia.

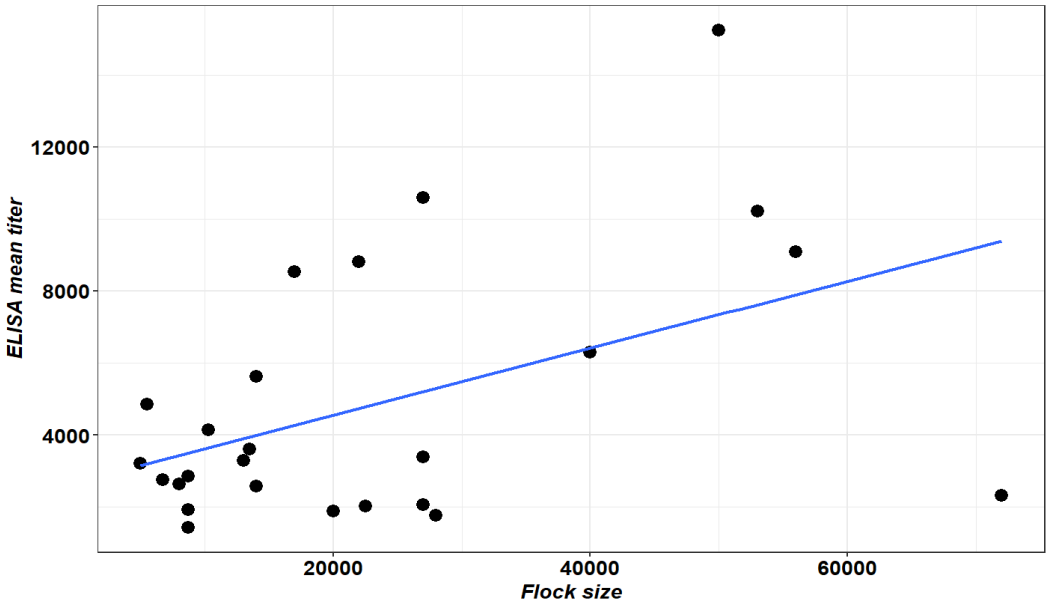

Figure 4. Correlation between flock size (age varied from 20 weeks to 107 weeks) and *Ornithobacterium rhinotracheale* enzyme-linked immunosorbent assay mean titer, in the governorate of Sfax, Tunisia.

DISCUSSION

Ornithobacterium rhinotracheale is a bacterial pathogen responsible for contagious respiratory and articular infections in poultry, particularly affecting turkeys, broilers, and laying hens (Boulianne et al., 2020). Susceptibility to ORT infection can vary depending on several factors, including bird species, the age of the animal, and overall farming conditions (Boulianne et al., 2020). The immune system typically responds to ORT exposure by producing specific antibodies (immunoglobulins M, G, and A). These antibodies can be detected as early as 5 days post-exposure through experimental aerosol inoculation (Eröksüz et al., 2006). The early detection capability of serological tests makes them valuable tools for identifying and monitoring ORT infections in poultry flocks.

The current study investigated the seroprevalence of ORT infection in commercial layer flocks located in the Sfax governorate, southeastern Tunisia. A total of 470 serum samples were collected from 25 randomly selected flocks, all of which were in the laying period. Utilizing an indirect ELISA test, found that 364 samples (77.45%) were positive for antibodies against ORT, indicating individual-level seroprevalence. Notably, positive samples were detected in all 25 flocks, resulting in a 100% flock-level prevalence. The high seroprevalence in the present study aligned with previous findings, which reported a 61.5% seroprevalence (eight seropositive flocks out of 13 flocks) in meat turkey farms within the governorate of Sfax (Ben Mbarek, 2009).

The current findings indicated a 100% flock-level seroprevalence for ORT in Tunisian layer flocks, consistent with previous reports from other countries. Similarly, 100% was reported in commercial layers from the North Central Region of the United States (Heeder et al., 2001) and South Korea (Jung, 2020). However, seroprevalence rates varied across studies. For instance, a seroprevalence of 96% was reported in India (Sumitha et al., 2015), while a lower prevalence of 17.4% in commercial layer flocks was found in different regions of Turkey (Aras et al., 2016).

These discrepancies might be attributed to several factors, including sample size and selection methods for participating farms, geographic location and potential regional variations, seasonal variations in ORT circulation patterns, management practices employed on the farms, such as biosecurity measures and vaccination protocols, the nature of the poultry farming systems, such as free-range versus intensive housing, farm density within the study area, potentially influencing transmission risks, antibiotic usage patterns on the farms, environmental factors that may influence ORT persistence and transmission, and the specific serological tests utilized (Ali et al., 2022).

The present study indicated a trend of increasing seroprevalence with flock age. The highest prevalence (85.78%) was observed in layers older than 56 weeks. Additionally, a moderate positive correlation ($r = 0.351$) was found between age and antibody titers, indicating a potential link between longer lifespans and higher exposure to ORT. The results of the current study aligned with the reports in Iran by Allymehr (2006) and in India by Baksi et al. (2017), where the highest prevalence was detected in older broiler breeders. Similarly, older chickens had higher infection rates and stronger antibody responses in China (Xue et al., 2020). However, no significant association was confirmed between age and ORT antibodies in broiler breeders in southern Brazil (Canal et al., 2003). These contrasting findings suggested that

the influence of age on ORT exposure may depend on different factors, warranting further investigation.

CONCLUSION

The high seroprevalence of *Ornithobacterium rhinotracheale* (ORT) antibodies (ranging from 1425.67 in flock 12 to 15233 in flock 19) detected in the current study, coupled with the absence of vaccination against ORT in Tunisia, strongly suggested widespread exposure to ORT in the commercial layer flocks (aged from 20 weeks to 100 weeks) of the Sfax governorate, Tunisia. The current findings highlighted the need for comprehensive prevention strategies to mitigate the impact of ORT on poultry health and productivity in the study region. Future studies could explore the most effective biosecurity measures, vaccination programs, and management practices to minimize ORT circulation and associated economic losses in different locations.

DECLARATIONS

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Ethical considerations

This article was originally written by the authors and has not been published elsewhere. The authors checked the text of the article and confirmed that the article is written based on their original scientific results.

Authors' contributions

Khaled Kaboudi performed methodology, investigation, data analysis, and original draft writing. Imen Hamadi provided sampling, laboratory analysis, and data analysis. Atef Walha concluded experimental design, investigation, and sampling. Aymen Mamlouk provided laboratory analysis, statistical data, and review. Adel Souissi performed sampling and field supervision. All the authors confirmed the final edition of the manuscript before submission to the journal.

Competing interests

The authors have not declared any conflict of interest.

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Availability of data and materials

The data that support the findings of the present study are available from the corresponding author upon reasonable request.

REFERENCES

Ali MZ, Islam S, Hafez MH, and Javed MT (2022). Seroprevalence and risk factors of *Ornithobacterium rhinotracheale* in poultry farms in Bangladesh. Journal of Advanced Veterinary Research, 12(5): 547-552. Available at: https://www.advetresearch.com/index.php/AVR/article/view/1025#google_vignette

Allymehr M (2006). Seroprevalence of *Ornithobacterium rhinotracheale* infection in broiler and broiler breeder chickens in West Azerbaijan Province, Iran. Journal of Veterinary Medicine: Series A, 53(1): 40-42. DOI: https://www.doi.org/10.1111/j.1439-0442.2006.00782.x

Aras Z, Sayin Z, and Sanioglu G (2016). Serologic prevalence of *Ornithobacterium rhinotracheale* infection in commercial layers. Eurasian Journal of Veterinary Science, 32(1): 22-25. DOI: https://www.doi.org/10.15312/EurasianJVetSci.2016115445

Back A, Rajashekara G, Jeremiah RB, Halvorson DA, and Nagaraja KV (1998a). Tissue distribution of *Ornithobacterium rhinotracheale* in experimentally infected turkeys. Veterinary Record, 143(2): 52-53. DOI: https://www.doi.org/10.1136/vr.143.2.52

Back A, Halvorson D, Rajashekara G, and Nagaraja KV (1998b). Development of a serum plate agglutination test to detect antibodies to *Ornithobacterium rhinotracheale*. Journal Veterinary Diagnostic Investigation, 10(1): 84-86. DOI: https://www.doi.org/10.1177/104063879801000116

Baksi S, Rao N, and Chauhan P (2017). Seroprevalence of *Ornithobacterium rhinotracheale* in broiler breeders in India. PSM Veterinary Research, 2(2): 29-32. Available at: https://psmjournals.org/index.php/vetres/article/view/151

Ben Mbarek H (2009). Étude de la seroprevalence d'*Ornithobacterium rhinotracheale* dans des élevages de dindes en Tunisie [Study of *Ornithobacterium rhinotracheale* seroprevalence in turkey flocks in Tunisia]. Thesis of Veterinary Medicine Doctorate, National School of Veterinary Medicine of Sidi, Thabet.

Boulianne M, Blackall PJ, Hofacre CL, Ruiz JA, Sandhu TS, Hafez HM, Chin RP, Register KB, and Jackwood MW (2020). Pasteurellosis and other respiratory bacterial infections. In: D. E. Swayne (Editor), Diseases of poultry, 14th Edition. Wiley, pp. 831-889. DOI: https://www.doi.org/10.1002/9781119371199.ch19

Canal CW, Leão JA, Ferreira DJ, Macagnan M, Pippi Salle CT, and Back A (2003). Prevalence of antibodies against *Ornithobacterium rhinotracheale* in broilers and breeders in Southern Brazil. Avian Diseases, 47(3): 731-737. DOI: https://www.doi.org/10.1637/6090

Canal CW, Leão JA, Rocha SLS, Macagnan M, Lima-Rosa CAV, Oliveira SD, and Back A (2005). Isolation and characterization of *Ornithobacterium rhinotracheale* from chickens in Brazil. Research in Veterinary Science, 78(3): 225-230. DOI: https://www.doi.org/10.1016/j.rvsc.2004.10.003

Chansiripornchai N, Wanasawaeng W, and Sasipreeyajan J (2007). Seroprevalence and identification of *Ornithobacterium rhinotracheale* from broiler and broiler breeder flocks in Thailand. Avian Diseases, 51(3): 777-780. DOI: https://www.doi.org/10.1637/0005-2086(2007)51[777:SAIOOR]2.0.CO;2

Charlton B, Channing-Santiago S, Bickford A, Cardona CJ, Chin RP, Cooper GL, Droual R, Jeffrey JS, Meteyer CU, Shivaprasad HL et al. (1993). Preliminary characterization of a pleomorphic gram-negative rod associated with avian respiratory disease. Journal Veterinary Diagnostic Investigation, 5(1): 47-51. DOI: https://www.doi.org/10.1177/104063879300500111

De Haro-Cruz MJ, Ixta-Avila L, and Guerra-Infante FM (2013). Adherence of five serovars of *Ornithobacterium rhinotracheale* to chicken tracheal epithelial cells. British Poultry Science, 54(4): 425-429. DOI: https://www.doi.org/10.1080/00071668.2013.800942

De la Rosa-Ramos MA, Rodríguez-Cruz M, López-Villegas EO, Castro-Escarpulli G, and Guerra-Infante FM (2015). Conditions that induce biofilm production by *Ornithobacterium rhinotracheale*. Avian Pathology, 44(5): 366-369. DOI: https://www.doi.org/10.1080/03079457.2015.1059923

De la Rosa-Ramos MA, Muñoz-Solís K, Palma-Zepeda M, Gutierrez-Castillo AC, López Villegas EO, Guerra-Infante FM, and Castro-Escarpulli G (2018). Adherence of *Ornithobacterium rhinotracheale* to chicken embryo lung cells as a pathogenic mechanism. Avian Pathology, 47(2): 172-178. DOI: https://www.doi.org/10.1080/03079457.2017.1390208

Eröksüz H, Özbey G, Çevik A, Gencer Tarakci B, and Balik DT (2006). Immuno-histochemical, pathological, enzyme linked immunosorbent assay and polymerase chain reaction analysis of experimental *Ornithobacterium rhinotracheale* infection in quails (*Coturnix coturnix japonica*). Revue de Médecine Vétérinaire, 157(4): 197-202. Available at: https://scispace.com/papers/immuno-histochemical-pathological-enzyme-linked-37y851zpjw

Hafez HM (1996). Current status on the role of *Ornithobacterium rhinotracheale* (ORT) in respiratory disease complex in poultry. Arch Guflügelkunde, 60(5): 208-211. Available at: https://www.european-poultry-science.com/artikel.dll/1996-60-208-211_guydkobugy2a.pdf

Hafez HM and Schulze D (2003). Examinations on the efficacy of chemical disinfectantson *Ornithobacterium rhinotracheale in vitro*. European Poultry Science, 67(4): 153-156. Available at: https://www.european-poultry-science.com/artikel.dll/s-153-156_g44danq.pdf

Hafez HM and Sting R (1999). Investigations on different *Ornithobacterium rhinotracheale* ORT isolates. Avian Diseases, 43(1): 1-7. Available at: https://pubmed.ncbi.nlm.nih.gov/10216753/

Hashish A, Sinha A, Sato Y, Macedo NR, and El-Gazzar M (2022). Development and validation of a dew TaqMan real-time PCR for the detection of *Ornithobacterium rhinotracheale*. Microorganisms, 10(2): 341. DOI: https://www.doi.org/10.3390/microorganisms10020341

Hassan A, Medhat MA, Shahata M, and Bekheet A (2020). Phenotypic and molecular characterization of *Ornithobacterium rhinotracheale* isolates in broiler chickens. Journal Advances Veterinary Research, 10(4): 193-199. Available at: https://advetresearch.com/index.php/AVR/article/view/548

Heeder CJ, Lopes VC, Nagaraja KV, Shaw DP, and Halvorson DA (2001). Seroprevalence of *Ornithobacterium rhinotracheale* infection in commercial laying hens in the North Central Region of the United States. Avian Diseases, 45(4): 1064-1067. DOI: https://www.doi.org/10.2307/1592890

Joubert P, Higgins R, Laperle A, Mikaelian I, Venne D, and Silim A (1999). Isolation of *Ornithobacterium rhinotracheale* from turkeys in Quebec, Canada. Avian Diseases, 43(3): 622-626. Available at: https://pubmed.ncbi.nlm.nih.gov/10494438/

Jung K (2020). High seroprevalence of *Ornithobacterium rhinotracheale* in layer chickens in Gyeonggi province, South Korea. Korean Journal of Veterinary Service, 43(4): 257-259. DOI: https://www.doi.org/10.7853/kjvs.2020.43.4.257

Krylova E, Bogomazova A, Kirsanova N, Putintseva A, Gorbacheva N, Prasolova O, Soltynskaya I, and Ivanova O (2024). Development and validation of PCR diagnostic assays for detection of *Avibacterium paragallinarum* and *Ornithobacterium rhinotracheale*. Veterinary Science, 11(1): 7. DOI: https://www.doi.org/10.3390/vetsci11010007

Kursa O, Tomczyk G, and Sawicka-Durkalec A (2022). Occurrence of *Ornithobacterium Rhinotracheale* in Polish turkey flocks. Journal of Veterinary Research, 66(1): 77-84. Available at: https://pmc.ncbi.nlm.nih.gov/articles/PMC8959689/pdf/jvetres-66-077.pdf

Lopes VC, Back A, Shin HJ, Halvorson DA, and Nagaraja KV (2002). Development, characterization, and preliminary evaluation of a temperature sensitive mutant of *Ornithobacterium rhinotracheale* for potential use as a live vaccine in turkeys. Avian Diseases, 46(1): 162-168. DOI: https://www.doi.org/10.1637/0005-2086(2002)046[0162:dcapeo]2.0.co;2

Marien M, Decostere A, Martel A, Chiers K, Froyman R, and Nauwynck H (2005). Synergy between avian pneumovirus and *Ornithobacterium rhinotracheale* in turkeys. Avian Pathology, 34(3): 204-211. DOI: https://www.doi.org/10.1080/03079450500096414

Mayahi M, Gharibi D, Ghadimipour R, and Talazadeh F (2016). Isolation, identification and antimicrobial sensitivity of *Ornithobacterium rhinotracheale* in broilers chicken flocks of Khuzestan, Iran. Veterinary Research Forum, 7(4): 341-346. Available at: https://pmc.ncbi.nlm.nih.gov/articles/PMC5251358/

Nguyen VG, Cao TB, Le VT, Truong HT, Chu TT, Dang HA, Nguyen TH, Le TL, and Huynh TM (2023). A multiplex PCR method for simultaneous detection of infectious laryngotracheitis virus and *Ornithobacterium rhinotracheale*. Veterinary Science, 10(4): 272. DOI: https://www.doi.org/10.3390/vetsci10040272

Observatoire National de l'Agriculture (ONAGRI) (2021). La filière avicole en Tunisie [National observatory of agriculture: The poultry sector in Tunisia], pp. 1-19. Available at: http://www.onagri.nat.tn/uploads/filieres/avicole/FICHE-AVICULTURE-2021.pdf

Shahata MA, Abd El-Motelib TY, and HebatAllah AM (2006). Some studies on the incidence of *Ornithobacterium rhinotracheale* infection in chicken embryos and layers. Assiut Veterinary Medicine Journal, 52(110): 243-257. DOI: https://www.doi.org/10.21608/avmj.2006.177400

Siddique M, Zia T, and Rehman SU (2008). Outbreak of *Ornithobacterium rhinotracheale* (ORT) infection in chickens in Pakistan. Archiv für Geflügelkunde, 72: 202-206. Available at: https://www.european-poultry-science.com/artikel.dll/m07-35mk_gqzdcobvgq2q.pdf

Stępień-Pyśniak D, Dec M, Hauschild T, Kursa O, Marek A, Wilczyński J, and Brzeski M (2024). Case reports involving coinfection with *Avibacterium paragallinarum* and *Ornithobacterium rhinotracheale* in broiler chickens and *Avibacterium endocarditis* in broiler breeding hens in Poland. Avian Pathology, 53(4): 291-302. DOI: https://www.doi.org/10.1080/03079457.2024.2323029

Sumitha P, Moorthy M, Sukumar K, Mani K, and Arthanari Eswaran M (2015). Prevalence of *Ornithobacterium rhinotracheale*, *Mycoplasma gallisepticum* and *Mycoplasma synoviae* antibodies in layers. Indian Veterinary Journal, 92(8): 78-79.

Umali DV, Shirota K, Sasai K, and Katoh H (2018). Characterization of *Ornithobacterium rhinotracheale* from commercial layer chickens in eastern Japan. Poultry Science, 97(1): 24-29. DOI: https://www.doi.org/10.3382/ps/pex254

Van Empel PC and Hafez HM (1999). *Ornithobacterium rhinotracheale*: A review. Avian Pathology, 28(3): 217-227. DOI: https://www.doi.org/10.1080/03079459994704

Van Veen L, Gruys E, Frik K, and Van Empel P (2000). Increased condemnation of broilers associated with *Ornithobacterium rhinotracheale*. Veterinary Record, 147(15): 422-423. DOI: https://www.doi.org/10.1136/vr.147.15.422

Xue J, Lv C, He P, Xu M, and Zhang G (2020). Research note: Serological investigation of *Ornithobacterium rhinotracheale* infection in China. Poultry Science, 99(10): 4814-4817. DOI: https://www.doi.org/10.1016/j.psj.2020.06.045