



## Detection of *Ornithobacterium rhinotracheale* antibodies in broiler by ELISA in Mosul city

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### Abstract

Respiratory infections in poultry are considered one of the most acute infections that negatively affect the poultry industry and cause severe economic losses worldwide. *Ornithobacterium rhinotracheale* (ORT) is one of the fundamental causes of poultry respiratory diseases due to misdiagnosis with other respiratory pathogens and the absence of ideal diagnostic protocols. Accordingly, this study aimed to detect the presence of antibodies against ORT using indirect ELISA. For this purpose, serum samples n=368 were collected from 16 small broiler farms in Mosul city and adjacent areas. The results showed that 32.1% (118/368) of the tested samples were seropositive for ORT antibodies. Furthermore, only (18.8%) 3/16 of the farms follow standard management practices, while 13/16 (82.2%) have poor management practices. Additionally, the study showed that only 2/16 (12.5%) of the farms use antibiotics after performing an antibiotic sensitivity test, while 14/16 (87.5%) randomly use antibiotics without testing antibiotic sensitivity. Finally, 62.5% (10/16) of the studied farms have a previous history of respiratory infections, while only 37.5% (6/16) of the farms have no previous history of respiratory infections. In conclusion, detecting seropositive against ORT in the studied broiler farms with poor management and arbitrary use of antibiotics might suggest a significant role of ORT in developing respiratory infections and finally contribute to the massive financial losses.

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### Introduction

*Ornithobacterium rhinotracheale* (ORT) is a Gram-negative, pleomorphic, rod-shaped bacteria that mainly affect the respiratory system (1,2). ORT has been isolated from bird species worldwide, including chickens, geese, ducks, turkeys, pigeons, and other domestic and wild birds (3-5). Infection with ORT is frequently associated with growth retardation, reduced and low-quality egg production, and mortality in chickens (4). Many factors can affect ORT to be a primary or secondary pathogen, such as the virulence of the strain, unfavorable environmental factors, the immune status of the flock, and infections with other pathogens (6). The infections by ORT can be spread by horizontal transmission, including direct or indirect contact methods. However, the vertical transmission was also suggested as a

rapid means for spreading infection (3). The laboratory diagnosis of ORT infection is mainly performed based on bacterial isolation and identification (7), serological tests (8,9), and molecular detection by polymerase chain reaction (10,11). Nevertheless, bacterial isolation is time-consuming and requires special conditions for successful growth (1,12). On the other hand, Molecular methods require expensive equipment and well-trained personnel. Nevertheless, large genetic variation of species or subspecies within the genus *Ornithobacterium* renders genetic characterization of this cumbersome bacteria work for routine detection (2). Therefore, primarily ELISA-based tests and serological methods are adequate for rapidly diagnosing ORT infections. Moreover, the ELISA tests can be performed on a large scale, giving an advantage over other tests (8,13). In Iraq, the respiratory infections of broiler flocks have overshadowed

their impact and led to significant economic losses for this vital industry. Many recent studies focused mainly on viral causes of infections, including avian influenza virus (AI), Newcastle disease virus (NDV), infectious bronchitis virus (IB) (14-16), or *Mycoplasma* infections (17). The first study in Iraq was conducted by Al-Hasan *et al.* (18), which directed to isolate and identify ORT in broiler farms in the South of Iraq. However, there is no published data related to infection by ORT, especially in poultry in Mosul city, and to the best of our knowledge, this is the first study to detect the presence of ORT in broilers. Accordingly, this study aimed to detect the presence of antibodies against ORT in broilers using ELISA.

## Materials and methods

### Sampling

A total of 16 small broiler farms aged 4-6 weeks were selected randomly from different areas of Mosul city and adjacent areas from April to December 2021. The areas include; Al-Rashidia, Sada and Bawiza, Gogjali, Bashiqa, Bartilla, and Al-Hamdania. All broilers have previous vaccination history with Newcastle disease (ND), infectious bronchitis (IB), and infectious bursal disease (IBD). Blood samples (n=368) were collected from the wing vein of the birds. Samples were submitted to the Department of Microbiology (College of Veterinary Medicine, Mosul University). After collection, serum samples were separated and stored at -20 °C until assayed (19). Also, data collected from these farms include; the type of management of broiler farms (standard or non-standard sanitary and biosecurity practices), antibiotic administration according to sensitivity test results, and presence of a previous history of respiratory infections using a particular questionnaire form.

### Serology

A Commercial indirect enzyme-linked immunosorbent assay (ELISA) kit for detecting *Ornithobacterium rhinotracheale* (ORT) antibodies were purchased from BioChek, UK. Sera were tested according to the manufacturer's instructions. Briefly, 100 µl of diluted sera (1:100), positive and negative controls were added to a coated microplate and incubated for 60 min. Then the plate was washed with 350 µl/well 1X wash buffer solution using an automated microplate washer (BioTek, 50 TS, USA). After that, 100 µl of the conjugate solution was added to each well and incubated for 30 min. Then, the plate was rewashed, as mentioned previously. Finally, 100 µl of substrate solution was added and incubated for 15 min, followed by 100 µl of stop solution. The optical density (OD) was measured at 450 nm using a microplate reader (BioTek 800TS) with Gen5™ software. OD readings were transformed to serum/positive percentage (S/P%) according to a specific equation cited by the manufacturer. The sample considered positive if it gives S/P % >70%, while 60% < S/P % < 70% considered

doubtful, and S/P % < 60% considered negative.  $S/P\% = (OD \text{ sample} - OD_{NC}) / (OD_{PC} - OD_{NC})$  (20). Chi-square ( $\chi^2$ ) test was performed to compare *P* values using Statistical Package for Social Sciences (SPSS) software version 22.  $P < 0.05$  was considered significant.

## Results

This study showed that 32.1% (118/368) of the tested samples were seropositive for ORT antibodies. However, the highest seropositivity values were recorded in Al-Rashidia at 46.8% (22/47), followed by Sada and Bawiza at 43.2% (19/44). At the same time, the lowest seropositivity was recorded in Al-Hamdania, 17.6% (12/68), followed by Bashiqa, 23.7% (22/93) (Table 1). On the hand, only 18.8% (3/16) of the farms followed standard management practices, while 82.2% (13/16) of the farms had poor management practices (Figure 1). Furthermore, the study showed that 12.5% (2/16) of the farms use antibiotics after obtaining the results of the antibiotic sensitivity test, while 87.5% (14/16) of the farms use antibiotics randomly without performing an antibiotic sensitivity test (Figure 2). Finally, the results showed that 62.5% (10/16) of the studied farms have a previous history of respiratory infections, while 37.5% (6/16) of the farms have no previous history of respiratory infections (Figure 3).

Table 1: Seropositivity of ORT according to areas

Area	Farms	Samples	Positive (%)
Bashiqa	4	93	22(23.7)*
Al-Hamdania	3	68	12(17.6)*
Bartilla	3	75	26(34.7)
Al-Rashidia	2	47	22(46.8)
Sada and Bawiza	2	44	19(43.2)
Gogjali	2	41	17(41.5)
Total	16	368	118(32.1)

*P* value of  $\chi^2 = 0.002$ , \* indicates significant differences.

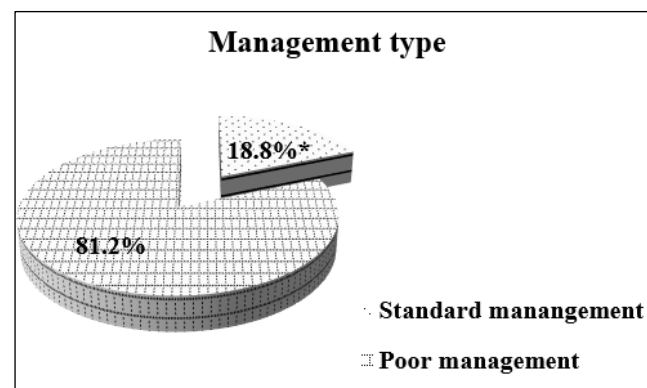


Figure 1: Type of management of broiler farms. \* Indicates significant differences,  $P < 0.05$ .

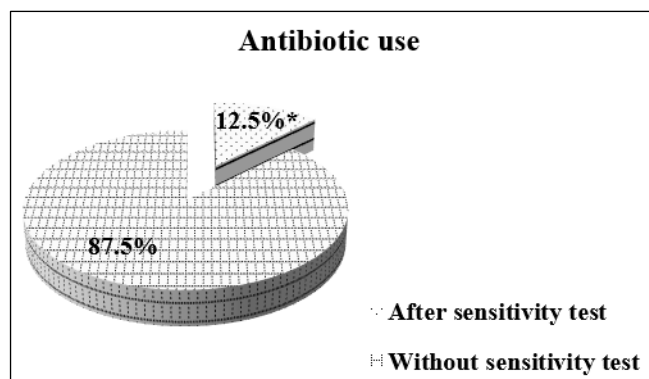


Figure 2: Antibiotic use according to sensitivity test. \* Indicates significant differences,  $P < 0.05$ .

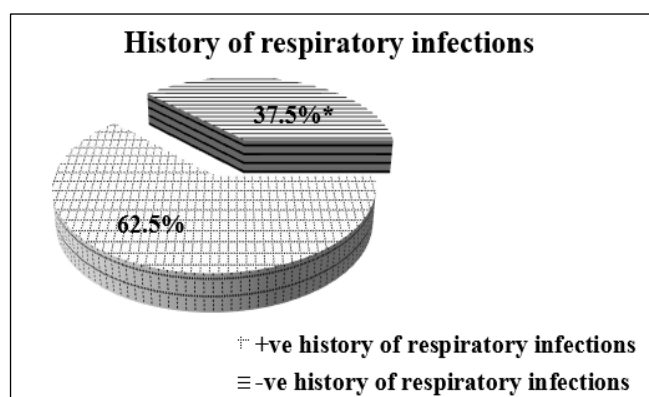


Figure 3: Presence of respiratory infections history. \* Indicates significant differences,  $P < 0.05$ .

## Discussion

In the current study, ORT antibodies were investigated in serum samples of small broiler farms in Mosul city. The results indicated that 32.1% (118/368) of broilers have circulating antibodies against ORT. Other studies also reported the ORT in poultry worldwide, Eid *et al.* (21) reported that 80% of the tested broiler chickens' flocks were seropositive for ORT, and 46/49 (93.9%) of selected sera found positive for ORT in chickens that were suffering from respiratory infections. Also, Xue *et al.* (19) reported 44.06% seroprevalence of ORT among the tested chickens. In contrast, Bhuiyan *et al.* (9) recorded high seropositivity of 43.3% (156/360) of ORT in broilers in Bangladesh.

Similarly, Gullu and Erbas (13) evaluated the presence of ORT in Turkey and found that the seropositivity rate of the disease was 232/420 (55.2%) by ELISA. Finally, Baksi *et al.* (8) found that 354/476 (74.37%) were seropositive for ORT while testing Broiler breeder farms in India. The high seropositivity of ORT infection is directly correlated to a significant economic impact on the poultry industry since infections with ORT might trigger the co-infection with

other pathogens (9,22). Our study revealed that Bashiqa and Al-Hamdania broiler farms recorded significant differences, with the lowest seropositivity at 22/93 (23.7%) and 12/68 (17.6%) values, respectively. Al-Rashidia, Sada, and Bawiza recorded the highest seropositivity at 22/47 (46.8%) and 19/44 (43.2%) values, respectively. This might be due to the type of management adopted by these small broiler farms, and this was confirmed by this study with 81.2% of these farms were reported with poor management systems, including insufficient ventilation with high ammonia levels, poor hygienic conditions such as poor-quality litter and overcrowding (8,19).

On the other hand, only 18.8% of the studied farms followed a standard management system.

Another stress factor that affects the poultry industry is the arbitrary administration of antibiotics. Our study revealed that only 12.5% of the studied farms followed the standard method of antibiotic administration after, i.e., after performing an antibiotic sensitivity test. While 87.5% of the farms did not follow standard methods of antibiotic administration and only relied on arbitrary use of different types of commercial antimicrobial agents that might be of low quality of manufacturing to compensate for lousy management system followed and poor biosecurity regimes. The misuse of massive doses of antibiotics for long periods and insufficient diagnosis could lead to resistant pathogenic strains of bacteria, leaving few choices for treatment shortly after (18,23). A recent study by Al-Hasan *et al.* (18) reported the highest resistance 100% to antimicrobial agents such as azithromycin, colistin, erythromycin, gentamicin, oxacillin, tilmicosin, and amoxicillin in broiler farms, and this indicates dangerous levels of resistance that could be easily transmitted to human through the food chain and finally could lead to the emergence of resistant bacterial strains (23). Other studies also confirmed these results (3,4,24). Another factor that affects ORT infections is a history of respiratory infections (25). Although all studied broiler farms have a previous history of vaccination with primary viral pathogens such as ND, IB, and IBD, we found that 62.5% of the studied farms have previous respiratory infections with prominent signs such as sneezing, nasal secretions and swelling of the sinuses. This actually might reflect the failure of the vaccine or vaccination process to protect the flock and render them vulnerable to other respiratory pathogens (1,9). Many viral, bacterial, and fungal agents play a crucial role in exacerbating the respiratory problem with the presence of ORT, which leads to the development of co-infection with such pathogens and finally results in extensive economic losses, especially with the absence of adequate vaccination against ORT (22,26,27).

## Conclusion

Significant seropositive antibodies against ORT were detected in different small broiler farms in Mosul city,

mainly with poor hygienic and management practices, arbitrary use of antibiotics, and the risk of other respiratory pathogens, all rendering the investments in such an important industry vulnerable to severe economic losses.

### Acknowledgments

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### Conflict of interest

No competing interests have to be declared by the authors.

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الأنفية البلعومية باستخدام تقنية الاليزا غير المباشرة. تم جمع عدد= ٣٦٨ عينة مصل من ١٦ حقل للفروج اللحم في مدينة الموصل والمناطق المحيطة بها. أظهرت النتائج إن ٣٢,١% (٣٦٨/١١٨) من العينات الكلوية المفحوصة أظهرت أجسام مضادة موجبة ضد جراثيم الاورنيثو الأنفية البلعومية. إضافة الى ذلك فقد تبين أن ١٨,٨% (١٦/٣) فقط من الحقول كانت تتبع طرق الإدارة القياسية في حين كانت ٨٢,٢% (١٦/١٣) من الحقول كان لديها سو في الإدارة. إضافة إلى ذلك فقد كانت ١٢,٥% (١٦/٢) من الحقول تجري اختبار فحص حساسية للمضادات الحيوية، في حين كانت ٨٧,٥% (١٦/١٤) من الحقول تستخدم المضادات الحيوية بشكل عشوائي ولا تجري اختبار فحص الحساسية. وأخيرا فقد كانت ٦٢,٥% (١٦/١٠) من الحقول لديها إصابات تنفسية سابقة، في حين كانت ٣٧,٥% (١٦/٦) لم تسجل إصابات تنفسية مسبقاً. يستنتج من هذه الدراسة أن الكشف عن وجود مصول موجبة ضد جراثيم الاورنيثو الأنفية البلعومية في حقول الفروج اللحم والتي تم دراستها مضافاً اليه سو الإدارة والاستخدام العشوائي للمضادات الحيوية قد يشير الى الدور المهم الذي تلعبه جراثيم الاورنيثو الأنفية البلعومية في تطور الإصابات التنفسية والتي بالتالي تساهم في حدوث خسائر مالية كبيرة.

## الكشف عن الأجسام المضادة لجراثيم الاورنيثو الأنفية البلعومية في دجاج اللحم باستخدام الاليزا في مدينة الموصل

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### الخلاصة

تعد إصابات الجهاز التنفسي في الدواجن واحدة من بين اهم الإصابات التي تؤثر سلبيا على صناعة الدواجن عالميا وتسبب خسائر اقتصادية واسعة. تعتبر جراثيم الاورنيثو الأنفية البلعومية أحد الأسباب المهمة والمتهمة لإحداث الأمراض التنفسية في الدواجن، ويعود السبب في ذلك الى التشخيص الخاطئ لهذه الجرثومة مع باقي المسببات المرضية التنفسية وغياب الطرق القياسية لتشخيصها. لذلك هدفت الدراسة الى التحري عن الأجسام المضادة الخاصة بجراثيم الاورنيثو