SURVEY AND MOLECULAR DETECTION FOR INFECTIOUS CORYZA OF POULTRY IN IRAQ

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ABSTRACT

The current study was aimed at case recording and molecular detection of infectious coryza in chickens. Samples were collected and identified for *Avibacterium paragallinarum* from about 68 poultry farms for eight months from different five provinces Baghdad, Diyala, Babil, Salahuddin, and Wasit from different flock layers and broilers. Diagnosis of bacterial colonies was first done by isolation and microbiological and biochemical tests. From 15 nasal samples of respiratory sinus cases of chicken,6 isolates were *A. paragallinarum*, additionally, *PCR* technique was used to confirm *A. paragallinarum*, the results for these bacteria in this study were detected in 6 isolates from 15 samples of poultry, Then, after, the result of sequences of *A. paragallinarum* serogroup C Sequence ID: KJ867498.This study concluded that *A. paragallinarum is* known to be a significant pathogen causing respiratory infection and economic losses.

Key words: *Avibacterium paragallinarum*, PCR, Sequencing bacteria.

المسح الميداني والكشف الجزيئي عن عدوى كوريزا الدواجن في العراق

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المستخلص

هدفت الدراسة الحالية إلى تسجيل والكشف الجزيئي لمرض الزكام المعدي في الدواجن. تم جمع وتشخيص بكتيريا *Avibacterium paragallinarum* من حوالي 68 مزرعة دواجن لمدة ثمانية أشهر من خمس محافظات مختلفة بغداد، ديالى، بابل، صلاح الدين وواسط من قطعان مختلفة دجاج بياض وفروج لحم. تم تشخيص المستعمرات البكتيرية أولاً عن طريق العزل والاختبارات الميكروبيولوجية والكيميائية الحيوية. عزلت 15 عينة لحالات الجيوب التنفسية للدجاج، 6 منها كانت *A. paragallinarum*، بالإضافة إلى ذلك، تم استخدام تقنية PCR للتأكد من وجود بكتيريا *A. paragallinarum*، وقد تم الكشف عن نتائج هذه البكتيريا في هذه الدراسة في 6 عزلات من 15 عينة من الدواجن، ثم بعد العزل،نتيجة تسلسلات البكتريا *A. paragallinaru* معرف التسلسل: KJ867498. وخلصت هذه الدراسة إلى أن بكتيريا *A. paragallinarum* من مسببات الامراض المهمة التي تسبب عدوى الجهاز التنفسي وخسائر اقتصادية.

الكلمات المفتاحية: أفيبكتريم باراگالينيرم، تفاعل البلمرة المتسلسل، تسلسل البكتريا.

Received:13 /6/2023, Accepted:29/10/2023, Published:December 2025

INTRODUCTION

Infectious coryza "IC" is a highly infectious illness of the respiratory system brought on by the gram-negative bacterium *Avibacterium paragallinarum A. paragallinarum* that affects chickens, the disease has been reported to have a global prevalence, with a significant impact on the poultry industry (13,46). The clinical signs of IC include facial edema, conjunctivitis, and nasal discharge, IC morbidity rates are high, with a low mortality rate, and the severity of the disease can range from acute to chronic (10,42). Infectious coryza infection in poultry can lead to significant economic losses, including decreased egg production (10-40%), increased mortality rate in hens, lower body weight, and increased medical expenses (1,6,19,45). Control and prevention of IC have been achieved through vaccination, biosecurity measures, and antibiotic treatments (39,50). Infectious coryza (IC) can spread laterally with in flocks through inhalation of aerosols and direct contact with infected birds, although evidence of vertical transmission between parents and offspring has not yet been shown, the occasional excretion of *Avibacterium paragallinarum* from carrier birds with subclinical illnesses is critical to the disease's propagation (18,40,48). The IC's morbidity and mortality rates can be exacerbated by concurrent bacterial infections, including *Mycoplasma synoviae, Mycoplasma gallisepticum, Ornithobacterium rhinotracheale, Pasteurella multocid,* and *Gallibacterium anatis* as well as viral infections such as infectious bronchitis, infectious laryngotracheitis, and fowl pox virus (11,34,37). Clinical symptoms of IC include anorexia, facial edema, lacrimation, conjunctivitis, and nasal discharge, the disease results in decreased egg production (10-80%), delayed development, and higher mortality rates (2-10%), negatively impacting global economies (32,49). Sinusitis, characterized by serous to mucoid nasal discharge, infraorbital sinus enlargement, facial edema, conjunctivitis, and lacrimation, is the most prominent manifestation of IC, an acute to chronic inflammation of the upper respiratory tract (5,12). Accurate diagnosis is essential for the prevention and management of coryza in poultry. Clinical symptoms are used for a preliminary diagnosis, while biochemical testing is used for confirmation (35). The diagnosis of IC using polymerase chain reaction(PCR) has gained considerable attention due to its accuracy and speed, PCR is recommended as a confirmatory test over traditional culture studies for infectious coryza diagnosis (37).

MATERIALS AND METHODS

Samples collection: Samples collection and Identification for *Avibacterium paragallinarum* from about (68) poultry farms for eight months from different five provinces (Baghdad, Diyala, Babil, Salahuddin, and Wasit) from different flocks (layers and broilers) appear with respiratory signs. Recorded all case history and clinical manifestations, age, number of birds per farm, location of the farm, morbidity and mortality rate, and postmortem lesions. All samples were taken from (lung, trachea and swab) and collected in sterile cups for PCR test for *A. paragallinarum*, stored in deep freeze -20°c until detection. For the isolation of *A. paragallinarum*, we used sterile cotton swabs to collect samples aseptically from the infraorbital sinus of birds suffering from respiratory symptoms such as swelling in one side of the head.

DNA extraction: Bacterial DNA was extracted by using a Kylt RNA/DNA purification kit and according to the manufacturer's instructions. The extracted DNA can immediately be used for applications such as PCR or kept in deep freezing at -20°c till use.

Molecular identification of *Avibacterium paragallinarum:* Conventional PCR is used for the detection of *Avibacterium paragallinarum* DNA using Kylt Coryza kits according to the manufacturer's instructions.

Bacteriological isolation and purification: In this study we need (NAD) nicotinamide adenine dinucleotide for feeder *A. paragallinarum*. For isolation and identification of *A. paragallinarum*, was needed (NAD) from *S. aureus*. The nasal swab samples were aseptically inoculated into the nutrient broth for both bacteria and incubated overnight at 37°c, On the next day to obtain microbial growth for *S. aureus* culture in blood

agar, gram staining and biochemical assays were used to diagnose both *A. paragallinarum* and *S. aureus* (8,33).

Biochemical detection: Biochemical tests including the carbohydrate fermentation test were carried out using suspected isolates by inoculating a loopful of a nutrient broth culture of the bacteria into the tubes containing five basic sugars like galactose, maltose, sucrose, mannitol, and glucose, incubated at 37°c for 24 hrs.=

Sequencing: After microbiological tests pure bacteria or bacterial colonies of *A. paragallinarum* that growth on blood agar with *S. aureus* were obtained for sequencing. Sequences of *A. paragallinarum* serogroup C in AniCon lab. GmbH, *Avibacterium paragallinarum* strain TW07 Hmtp210 (hmtp210) gene.

RESULTS AND DISCUSSION

Detection of infections coryza: Respiratory diseases are the most detrimental diseases to the poultry industry and need to be addressed because of their major economic losses. In the current study total of 68 poultry flocks including layer, broilers from different provinces (Wasit, Baghdad, Salah Uddin, Babil, Diyala), in general the affected flocks reported clinical signs of off-feed, gasping, sneezing, coughing, swelling head, drowsiness, decreasing output and varying degree of mortality. Since infectious coryza is almost commonplace, we are unaware of any data regarding the disease's exact prevalence in commercial breeding flocks or backyard chickens in Iraq. The multi-age poultry production system and partial immunity resulting from the serovar content of commercial vaccines against regionally prevalent Av serovars are thought to be the reasons why IC agents persist in poultry farms. *Paragallinarum,* in addition to the role that backyard chickens play in spreading IC to nearby chicken flocks (15). IC spreads in large areas of the world, and its infection is frequent in hot areas and is more severe in relation to infection in the summer (2,4,44).

Isolation of *Avibacterium paragallinarum:* The samples from the trachea and swabs from suspected Infectious coryza (IC) cases of layer poultry 15 samples collected from field cases were subjected to cultural and biochemical investigation for the isolation of causal agents, molecular test, and pathological study of the affected tissue. The results obtained were 6 samples positive and 9 samples negative. It has been observed that all turkey and chicken ages have a readiness for infection, with matured ages being the readiest for infection (9,19,21), and that it infects all types of birds, with the least resistant birds causing a high rate of mortality. This illness disengages the causative specialist and studies its qualities in this study, a few reports of IC from no sub-atomic observing information exist to depict the study of disease transmission of IC in the adjoining nations of Iraq (25, 28, 41).

Culture of *Avibacterium paragallinarum:* For the successful growth of *A. paragallinarum* additional NAD is needed, however, *S. aureus* growth in blood agar can produce additional NAD for the growth of *A. paragallinarum* figure (1). All samples cultured in blood agar that show tiny dew drop colonies were positive for *A. paragallinarum* (30). Gram's stain of *A. paragallinarum* colony in blood agar media was stained with Gram’s stain, and bacteria of all the suspected two colonies showed Gram-negative, red color, rod-shaped bacilli.

Figure 1. *A. paragallinarum* colonies in blood agar media

Biochemical identification of *Avibacterium paragallinarum:* Biochemical tests for samples from blood agar, a series of biochemical tests especially selective for *A. paragallinarum* were performed with the positive culture and Gram-negative rod-shaped bacteria, the results were furnished below Sugar fermentation test of *A. paragallinarum*. All six isolates fermented four basic sugars (lactose, maltose, sucrose, and mannitol) was indicated by the color change from reddish to yellow, other biochemical tests of *A. paragallinarum* of two isolates were then subjected to different biochemical tests such as methyl-red test(MR), vokasproskaur test (VP) and indole test, all the isolates were MR test negative, VP test negative and indole test negative Table (1) show the results.

Table 1. Results of biochemical characteristics of *A. paragallinarum*

Biochemical test	Lactoe	Sucrose	Maltose	Mannitol	Indole	MR	VP	Catalase	Oxidase
Result	+	+	+	+	-	-	-	+	+

IC in adjoining nations of Iraq such as Iran have likewise been epidemiologically detailed in layer and reproducer ranches not withstanding lawn chicken, Further examinations ought to be led to explore the pervasiveness of IC among various kinds of chicken and its conceivable job in complex respiratory disorders in Iran, the event of IC in business poultry runs of Iraq has not been formally revealed by the Iraq Veterinary directorate/Service of rural which could beat some point a decent result of immunization conventions performed by the imported antibodies against serogroups A, B, and C excepted one concentrate in Badosh (14,24). In this study clinical image of IC has been determined in some layer ranches as IC was bacteriologically affirmed by the detachment of causative bacteria (20,29). The fermentation of sugars has changed the medium's color from reddish to yellow to indicate acid production (22,26,47).

Molecular detection of *A. paragallinarum:* Conventional PCR was used for the detection of *A. paragallinarum*. The result was shown that from 15 tested samples, the bacteria was detected in 6 samples figure (2).

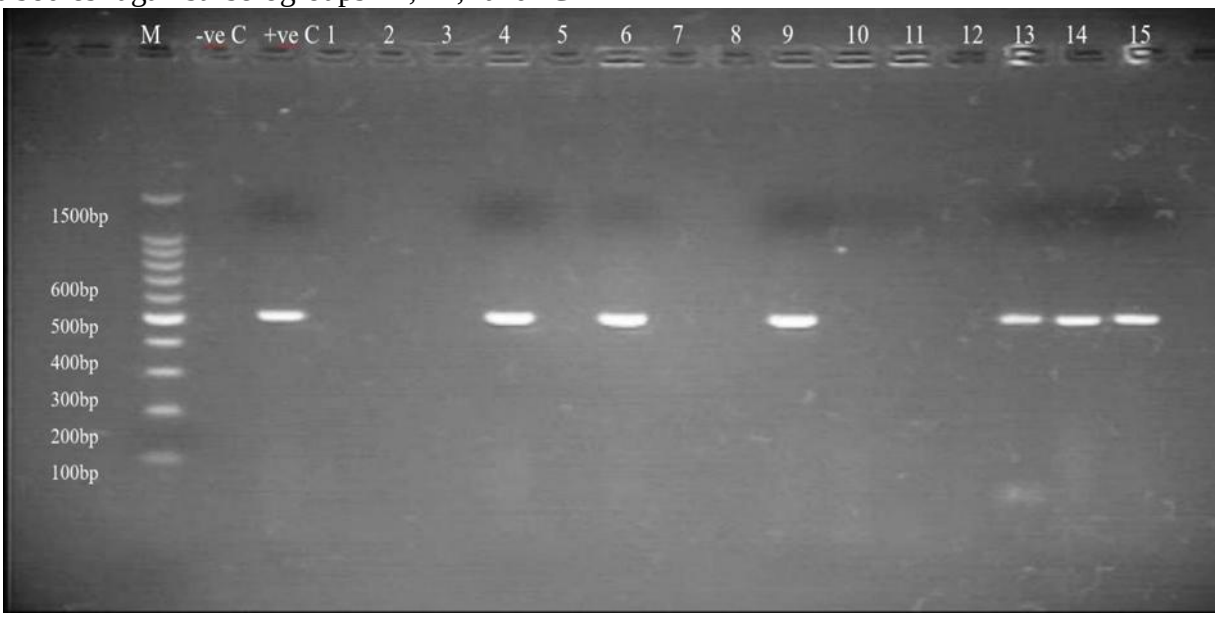

Figure 2. Agarose gel electrophoresis for PCR product. The product was electrophoresis on 1.5% agarose. M: DNA ladder (500bp). –ve c: negative control, +ve C: positive control, 1-15: samples

To the best of our knowledge, there is no information on the exact prevalence of the disease among commercial breeding herds in Iraq. Disease spreads from one region to another through migratory birds (29,31, 38). After the isolation and identification of bacteria PCR was used to affirm the results. Then after effects of bacterial culture 6 examples of a positive outcome, and 9 examples of a negative outcome, the level of tainted ranches was 40%, in Iraq most layer poultry ranches inoculated against IC to be controlled IC, this outcome comparative to (7,15,23,36). In addition to backyard chickens,

epidemiological reports of IC in neighboring countries like Iran and Turkey (19) have also been made in layer and breeder farms. Further research is needed to determine the prevalence of IC among various types of chicken and its potential role in Iran's complex respiratory syndrome.

Sequence of *Avibacterium paragallinarum:* The result of sequences of *A. paragallinarum* serogroup C according to a report of AniCon lab. Sequence ID: KJ867498.1Length: 6255Number of Matches. Table (2) and Figure (3) represent the polymorphism of *Avibacterium paragallinarum*.

Table 2. Represent type of polymorphism of *Avibacterium paragallinarum*; Hmtp210 (hmtp210) gene

Source: *Avibacterium paragallinarum*; Hmtp210 (hmtp210) gene Sequence ID with compare: ID: KJ867498.1							
No. of sample	**Type of substitution**	**Location**	**Nucleotide**	**Nucleotide change**	**Amino acid change**	**Predicted effect**	**Identities**
1	Transvertion	364	T\A	TAT\AAT	Y(Tyrosine)\N(Asparagine)	Missense	99%
	Transition	503	G\A	CGT\CAT	R(Arginine)\H(Histidine)	Missense	
	Transvertion	883	A\C	AGA\CGC	R(Arginine)\ R(Arginine)	Silent	
	Transvertion	885	A\C	AGA\CGC	R(Arginine)\ R(Arginine)	Silent	

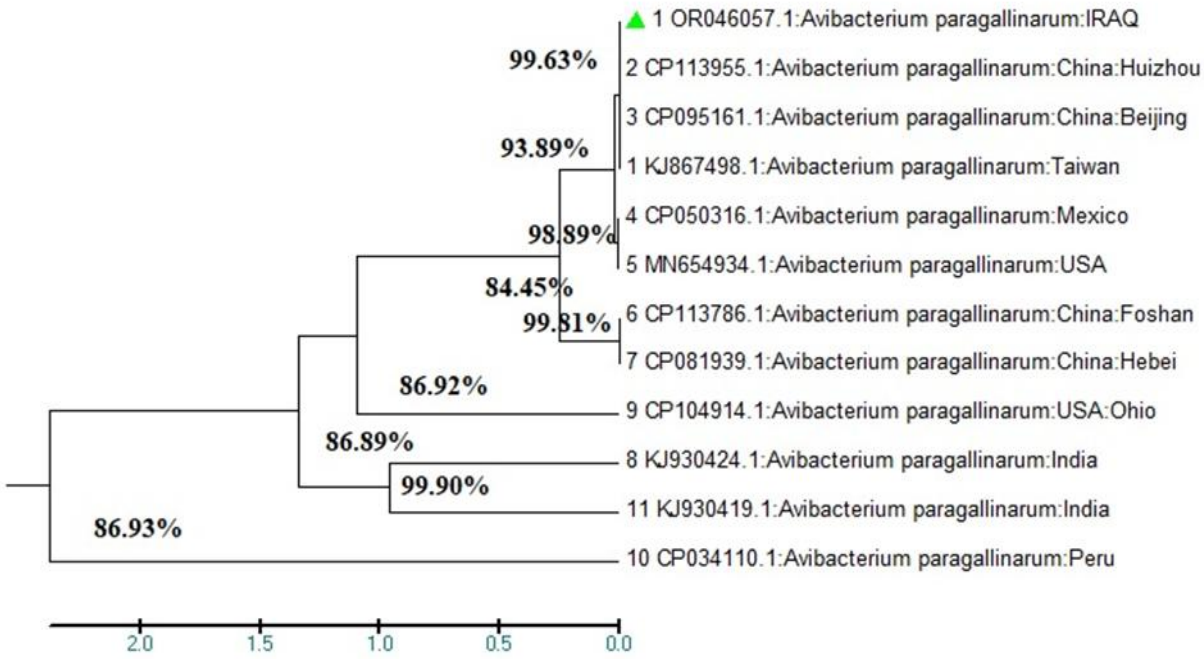

Figure 3. Neighbor-joining tree *Avibacterium paragallinarum*

The results of the sequence are comparable to those of (16,17,27,43). They were related to strains reported from Taiwan (KJ867498) (98.89 percent), China (CP113955) (99.63 percent), and India (KJ930424, KJ930419) by serotype C KJ867498. The infection entered through the import of poultry into Iraq (3).

Conclusion

The performance of poultry is heavily influenced by respiratory infections. In this study conducted in Iraq *Avibacterium paragallinarum* was isolated and identified from chickens. this bacterium is known to be a significant pathogen causing respiratory infection and economic losses. Isolation and identification of these bacteria are crucial for understanding their prevalence.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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