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Animal Diseases

CASE REPORT

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Avibacterium paragallinarum: an emerging birds pathogen in Qinling wildlife conservation center, China

Honglin Xie[1], Hui Li[1], Chenfei Yu[1], Yongqiang Miao[1], Yaping Wu[2], Ruoyi Jia[2], Qiang Zhang[2], Guanglin Pan[2], Qingyi Ma[2], Kangsheng Jia[2] and Xinglong Wang[1*]

Abstract

The bacterium *Avibacterium paragallinarum*, previously known as *Haemophilus paragallinarum*, is responsible for causing infectious coryza (IC) in chickens and other avian species. In this case report, an outbreak of *Avibacterium paragallinarum* occurred in the Qinling area of China, resulting in clinical symptoms of facial swelling in several bird species, including Golden pheasant, Temminck's tragopan, and Peafowls, and three Golden pheasants died due to prolonged infection. Specific PCR results confirmed the presence of the pathogen in the infected birds. The report describes the clinical symptoms and pathological changes observed in the affected birds, as well as the isolation and identification of *Avibacterium paragallinarum*. Whole-genome sequencing and phylogenetic analysis were performed, and this is the first report of inter- and intra-species transmission of infectious coryza among wild birds in China.

Keywords Infectious coryza, *Avibacterium paragallinarum*, Avian diseases, Pathogen, Wlidlife, Case report

Introduction

Infectious coryza (IC), caused by *Avibacterium paragallinarum* (*A. paragallinarum*), is a widely occurring upper respiratory system in chickens (Blackall 1999; Blackall and Argas 2020). IC is a bacterial infection that primarily affects the upper respiratory tract in poultry and is characterized by symptoms such as inflammation, facial swelling, conjunctivitis, nasal discharge, diarrhea, and anorexia (Blackall et al. 2005; Guo et al. 2022). The disease is considered economically significant in the poultry industry, causing a decline in egg production in the layer and poor growth performance in broilers (Blackall 1999). Severe and prolonged infections can lead to more complex conditions, such as chronic respiratory disease, swollen head syndrome, airsacculitis, tarsal arthritis, and septicemia (Caballero-Garcia et al. 2022; Guo et al. 2022). When other pathogens are associated with the infection, mortality rates may increase, making early diagnosis and treatment essential for effective management. The presence of IC outbreaks has been reported in many countries and has also increased in China in recent years, but mainly occurs in commercial birds.

Qinling is the geographical boundary between the northern and southern China.. This area is a major biodiversity hotspot in China with many unique and rare plant and animal species.

In addition, several natural reserves have been established in this region, and the local government has invested significant resources in conservation, including various strategies to monitor the population of precious wildlife and technical programs for captive breeding. Overall, the Qinling region is particularly

*Correspondence:
Xinglong Wang
wxlong@nwsuaf.edu.cn
[1] College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi, China
[2] Qinling Giant Panda Research Center, Shaanxi Rare Wildlife Rescue Base, Shaanxi Province, Zhouzhi, Xi'an, China

significant for this study, as it provides habitat for many endemic threatened species, and the outbreak of *A. paragallinarum* infection in wild birds could have implications for the conservation of these species.

Here, we report on the recent outbreak of *A. paragallinarum* infection in wild birds in the Qinling region and describe the clinical, pathologic, and whole-genome sequencing of the first isolated strains from wild birds in this geographical area. These findings are crucial for understanding the impact of *A. paragallinarum* infection on wild birds and developing effective prevention and control strategies to protect both commercial and wild birds.

Fig. 1 Infectious coryza causing Temminck's tragopan (**A**) Peafowl (**B**) and Golden pheasants (**C**) with severe infraorbital sinus swelling, and Peafowl showed with catarrh discharges in the infraorbital sinuses; **C** The macroscopic appearance of deceased Golden pheasant; **D** A mass of caseous sediment found in infraorbital sinuses, due to prolonged infection

Case presentation

Case history

In May 2022, the first case of facial edema and discharge in the infraorbital sinuses was observed in a Golden pheasant. By July 2022, there was a sharp increase in the number of wild birds with similar clinical signs at the Qinling wildlife rescue center. These birds, rescued from a natural reserve, were quarantined and treated symptomatically. However, the outbreak continued to spread, affecting three wild bird species, including Temminck's tragopan (Fig. 1A), and Peafowl (Fig. 1B), Golden pheasant (Fig. 1C). The similarity in symptoms between the affected bird species suggests a possible outbreak of disease, and local veterinarians and staff conducted a numerical count of the affected birds. The number of bird infections, death rates, and specific symptoms are presented in Table 1. Overall, a significant number of birds exhibited facial swelling associated with the infection, including 18 Golden pheasants (3 of which died), 2 Peafowl, and 57 Temminck's tragopan.

Samples form three species were taken to the Laboratory of Infectious Disease Control, Northwest A&F University, China. Postmortem analysis was carried out on dead Golden pheasants.

Clinical findings

The dead birds showed noticeable physical changes, including facial swelling and stunted growth (Fig. 1C). Upon postmortem examination, characteristic changes were observed in the affected birds' appearance, with the accumulation of a mass of cheesy caseous exudate in the infraorbital sinuses (Fig. 1D) and slight hemorrhaging in the trachea. However, no significant lesions were observed in the lungs. These observations suggest that the infected birds may have experienced impaired vision, which could have hindered their ability to feed normally.

Specimen preparation

The diagnosis of classic infectious coryza is performed by bacteriological culturing of mucus samples taken from the infraorbital sinus or the nasal turbinates (Caballero-Garcia et al. 2022). In this case, caseous and mucosal

Table 1 Whole case record of *A. paragallinarum* outbreak in Qinling

Species	Diseased (n)	Clinical symptom	Specific-PCR result	Dead (n)	Mortality rate (%)	Recovery (n)
Golden pheasant	18	Facial edema with discharges	+	3	16.7	15
Peafowls	2	Facial edema with discharges	+	0	0	2
Temminck's tragopan	57	Facial edema and open-mouth breathing	+	0	0	57
Crested Ibis	/	/	-	/	/	/

The Crested Ibis was not found to have an *A. paragallinarum* infection. +,—indicates positive and negative

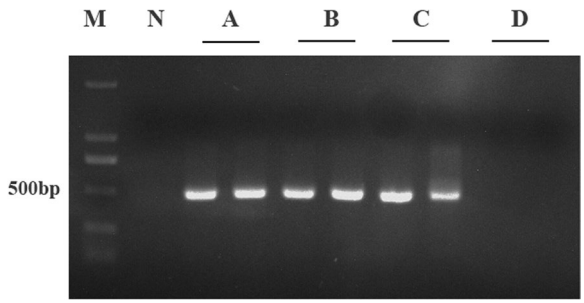

Fig. 2 Identification of A. *paragallinarum* using a specific PCR. The molecular weight marker of 2000 bp is labeled as M, the negative control is labeled as N. The swabs from wild birds were labeled A to D. A represents the swab from the Golden pheasant, B from Peafowls, C from Temminck's tragopan, and D from Crested ibis; Samples of A, B, and C tested positive for the presence of a 500 bp DNA band

materials from deceased Golden pheasants, also samples of swab from Temminck's tragopan and Peafowl, and DNA were extracted. A specific PCR assay (forward: 5′- TGAGGGTAGTCTTGCACGCGAAT-3′; reverse: 5′- CAAGGTATCGATCGTCTCTCTACT-3′), based on previous studies, was utilized to identify the positive samples (Chen et al. 1996). The PCR identification results showed that *A. paragallinarum* was detected in swabs from all clinically symptomatic birds, while samples from Crested ibis in the region tested negative (Fig. 2). The suspected colonies of *A. paragallinarum* were then inoculated onto a chocolate agar plate (CAP) and incubated in a 5% CO2 incubator at 37 °C for 24–48 h. Total DNA was extracted from the suspected colonies, and PCR of full-length 16S ribosomal RNA was carried out to identify the bacteria (forward: 5′- AGAGTTTGATCCTGG CTCAG -3′; reverse: 5′- TACGGTTACCTTGTTACG ACTT-3′).

Whole gene sequencing

The 16S rRNA sequence suggests that the infection of these birds may have originated from the same source, possibly indicating a common environmental exposure (GenBank: OP355508.1). Then we employed a whole genome shotgun strategy and performed sequencing experiments using the Illumina NovaSeq PE150 system. A library for Illumina sequencing was prepared using the NEBNext®Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) according to the manufacturer's recommendations. The genome assembly was performed using SOAP denovo (version 2.04), and SPAdes, with optimization and gap filling of the initial assembly results using gapclose (Version: 1.12) to obtain the final assembly result. The newly sequenced genome was subjected to gene prediction using Prodigal software (Version 2.6.3). To visualize the annotation results, we generated a circular genome map using the cgview software. The genomes were deposited in GenBank as a Bio-Project with the accession number SAMN33248066.

The final assembly consisted of 99 contigs, with an N50 length of 53,700 bp and an average contig size of 25,849 nt. This assembly yielded an estimated genome size of 2,559,036 bp with a GC content of 42.155%. To visualize the annotated genes, we used the software cgview to create a circular genome map (Fig. 3). A total of 2455 protein-coding genes were predicted, out of which 2410 genes were annotated for NR/NT, COG functional annotation, GO functional annotation, KEGG functional annotation, CARD, and other functional annotations (see supplementary file S1). The Non-Redundant Protein Database (NR) showed the closest match with *Avibacterium paragallinarum* (see supplementary file S2). To examine the genetic relationships of the *A. paragallinarum* isolates, some *Avibacterium* whole genomes were collected from GenBank. The online tool ANItools (https://www.ezbiocloud.net/tools/ani) was used to calculate bacterial genome average nucleotide identity (ANI) (Yoon et al. 2017). LGT2022 had an ANI of 96.76% with GCA_004212415.1 (*Avibacterium paragallinarum* AVPG 221) and an ANI of 95.41% with GCA_020892835.1 (*Avibacterium paragallinarum* M). Based on the results of whole genomes sequencing analysis, the strain identified in this study was confirmed to be *A. paragallinarum*. From the phylogenetic tree (Fig. 4), LGT2022 appeared on two new branches along with AVPG and M and shared 96.5%-98.2% similarity, suggesting that there may be some differences between LGT2022 and the isolates from poultry.

Outcome and follow-up

Table 1 presents a summary of the case records of the infected birds that were collected and isolated for observation and care. Antibiotic sensitivity testing was carried out, and sulfa drugs were administered as treatment. Within three days of treatment, the morbidity rate significantly decreased, and most birds recovered within ten days post-treatment. As of February 2023, the disease has been effectively controlled, and no further occurrences have been reported. However, regular sample collection and pathogen testing are crucial to prevent future outbreaks.

Discussion and conclusions

Avibacterium paragallinarum is a bacterium of the Pasteurellaceae family that is a Gram-negative facultative anaerobe. It is known to cause acute infectious respiratory disease in chickens, with clinical signs resembling

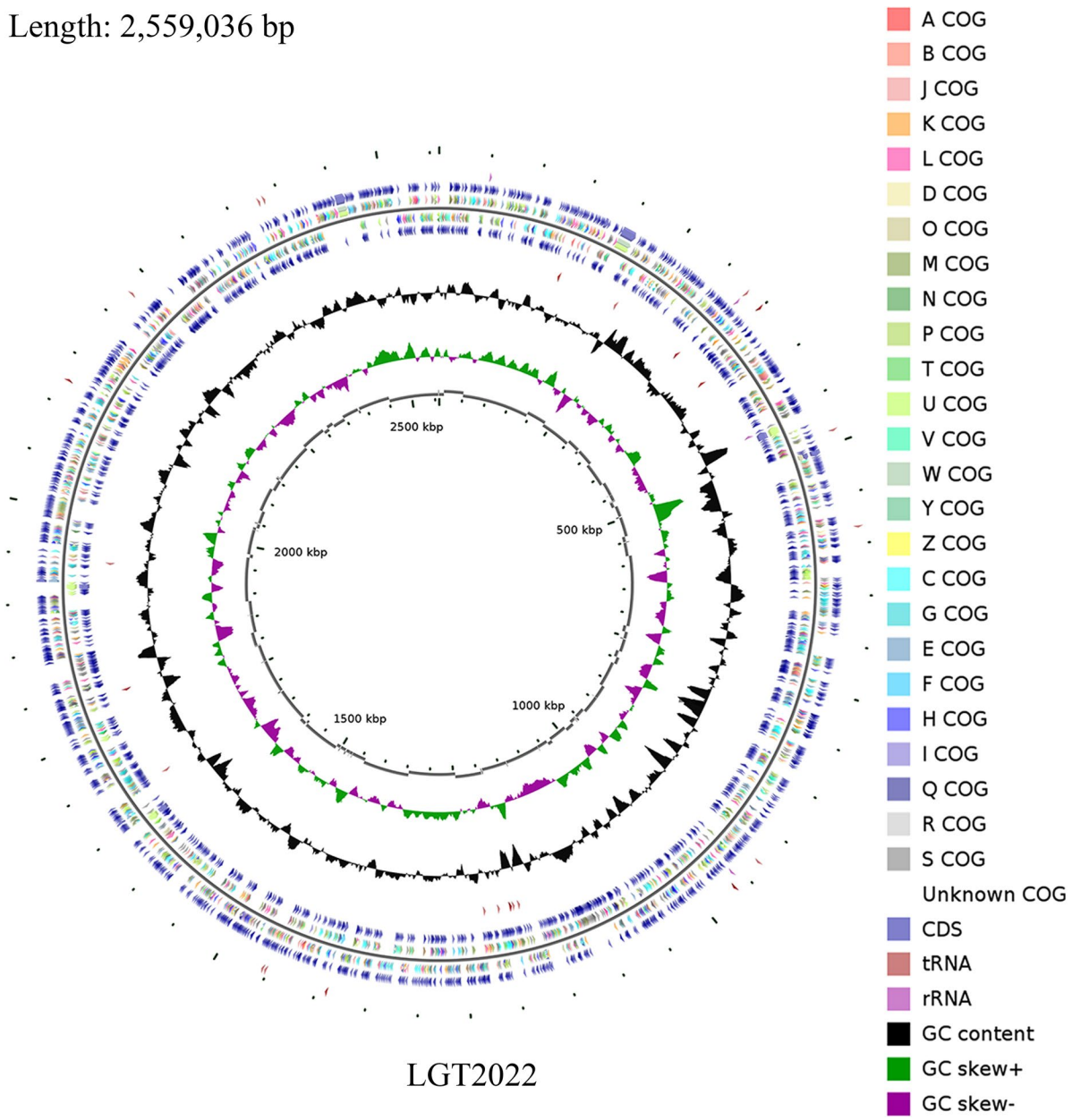

Fig. 3 Circular representation of the *A. paragallinarum* genome. Contigs obtained from whole genome sequencing were used to predict genes and estimate the GC content. The circular plot shows information from the innermost to outermost circle. The first circle represents the GC skew, which is calculated as (G-C)/(G+C) and used to measure the relative abundance of G and C nucleotides. The second circle represents the GC content. The third circle shows the results of tRNA, rRNA, negative strand genes, and gene COG classification. The fourth circle displays the results of gene COG classification, negative strand genes, rRNA, and tRNA

swollen-head syndrome (Blackall et al. 2005).One important feature of *A. paragallinarum* pathogenicity is its ability to adhere to and colonize the nasal mucosa during the early stages of infection. The disease it causes, infectious coryza, typically exhibits high morbidity and low mortality, but its severity can be worsened when other pathogens are present (Mei et al. 2020; Morales-Erasto et al. 2016; Paudel et al. 2017). Most *A. paragallinarum* isolates require nicotinamide adenine dinucleotide (NAD) as a growth factor (Blackall 1988; Blackall and Terzolo 1995). However, NAD-independent isolates and isolates with more atypical growth characteristics have also been described (Feberwee et al. 2019).

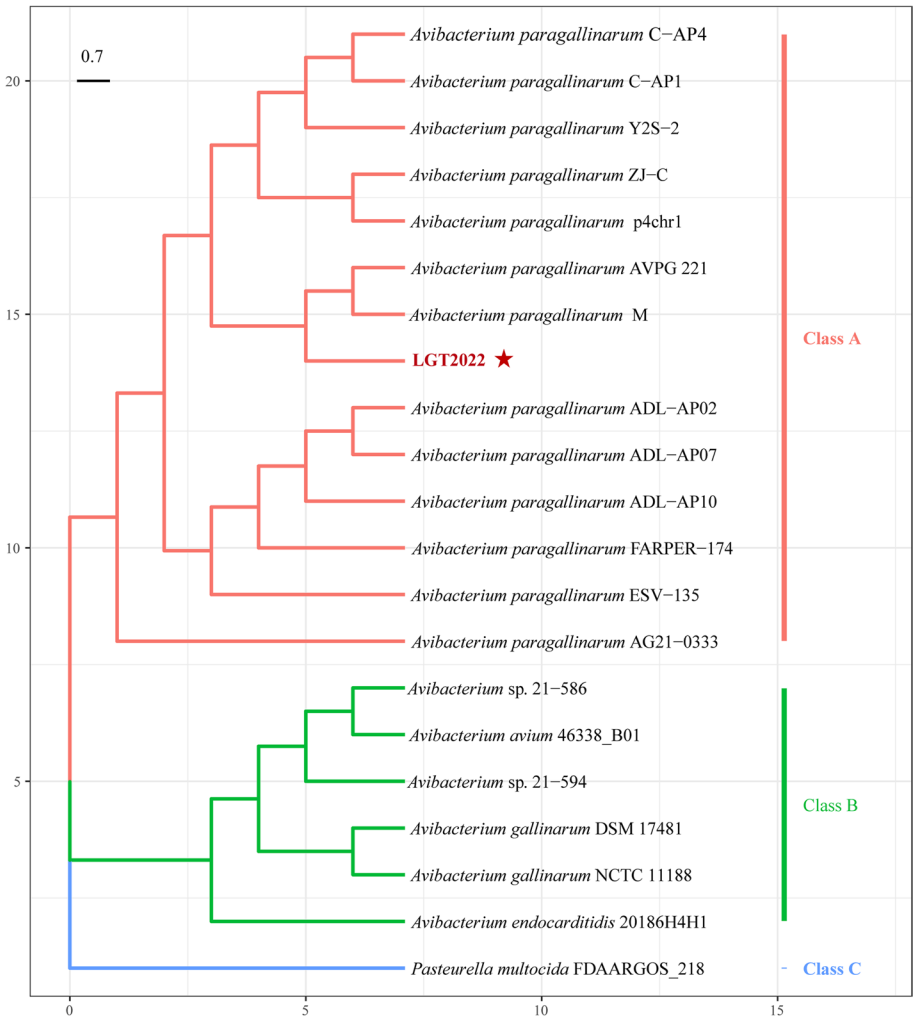

Fig. 4 Phylogenetic tree of the strain LGT2022 was built based on reference genomes from gtdbtk database and constructed using a maximum likelihood by MEGA v11.0.13, with 1000 bootstrap replications. Class A was genus of *Avibacterium paragallinarum*

A gel-based PCR assay was published in 1996 and has since been widely used to detect *A. paragallinarum* in diagnostic samples (Chen et al. 1996) to distinguish the commensal species of *Avibacterium* such as *A. avium, A. endocarditis*, *A. gallinarum*, and *A. volantium*. Currently in China vaccination is the primary method of preventing *A. paragallinarum* infection in poultry farming, the domestic commercial vaccine against chicken IC includes serovar A, serovar B, and serovar C (Blackall and Terzolo 1995; Xu et al. 2019). A study on isolates showed the lowest level of resistance against co-trimoxazole (potentiated sulfonamide) (Nhung et al. 2017). However, antibiotic treatment may reduce clinical signs, but the birds remain carriers for life (Blackall and Argas 2020; Caballero-Garcia et al. 2022).

A. paragallinarum infections in chickens have been reported in many different countries, and cases have also been related to quails and captive grey crowned cranes (Nsengimana et al. 2022; Priya et al. 2012; Wahyuni et al. 2018). In this study, we aim to illustrate the effects of IC on different bird species. This occurrence of IC spreading among various wild bird species is relatively uncommon. The infection caused by *A. paragallinarum* significantly impairs the vision of birds, which can have detrimental effects on their ability to defend against natural predators. In some cases, the infection can even result in death. Our findings suggest that the characteristics of *Avibacterium* infection may differ among different bird species, highlighting the need for further investigation. Additionally, it is crucial to enhance our understanding of how protozoal diseases can jump between host species to mitigate the impact of these infections on birds. Continued monitoring is necessary due to the potential involvement of this *A. paragallinarum* isolate in upper respiratory disease lesions across various bird species.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44149-023-00084-w.

Additional file 1.

Additional file 2.

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Authors' contributions
HX, YM carried out the bacterial isolation and identification, HX, CY and HL participated in the Genome analysis and HX drafted the manuscript. YW, RJ, QZ, GP, QM and KJ were participated in record the diseased birds, treatment and materials collection. XW helped to revision the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
Whole-genome sequencing were deposited in GenBank as a Bio-Project with the accession number SAMN33248066. The gene function annotation were uploaded as supplementary information file.

Declarations

Ethics approval
The bird's autopsy and sample collection were approved by the Animal Care and the Government Office of Forestry and Grassland Administration Shaanxi Province.

Competing interests
No potential conflict of interest was reported by the author(s).

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