Eurasia Specialized Veterinary Publication

International Journal of Veterinary Research and Allied Science

ISSN:3062-357X

2025, Volume 5, Issue 1, Page No: 128-139

Available online at: www.esvpub.com/

Severe Bronchopneumonia and Necrotizing Hepatitis in Post-Hatch Goldfinch Nestlings Due to Concurrent Klebsiella pneumoniae and Pseudomonas aeruginosa Infection

Caruso Antonio[1], Ferrari Beatrice[1]*, Rizzo Pietro[1]

[1]Experimental Zooprophylactic Institute of Lombardia and Emilia-Romagna (IZSLER) "Bruno Ubertini", Modena, Italy.

***E-mail** ✉ beatrice.ferrari1@yahoo.com

ABSTRACT

Bacterial infections are common among pet birds and represent a significant threat, particularly to nestlings and individuals with compromised immune systems, frequently leading to substantial mortality within the initial days post-hatching. This research aimed to characterize the pathological alterations associated with a spontaneous bacterial co-infection involving Klebsiella pneumoniae and Pseudomonas aeruginosa in nestling European goldfinches (Carduelis carduelis), identified as the cause of early post-hatch deaths. Eight chicks housed in a breeding colony exhibited immobility, anorexia, and respiratory distress and were discovered dead between days 1 and 4 of life. Gross lesions revealed diffuse pulmonary edema and hemorrhagic pneumonia, along with occasional hepatic necrosis. Histologically, all examined specimens showed severe, subacute bronchopneumonia and necrotizing hepatitis. Gram-negative bacilli were detected in the parabronchial walls, pulmonary arteries, and surrounding necrotic hepatocellular areas. Bacteriological cultures from lungs, liver, and kidneys yielded two Gram-negative isolates. Species identification was achieved by amplification and sequencing of four housekeeping genes (16S rRNA, rpoB, khe, ecfX). Although K. pneumoniae and P. aeruginosa are well-recognized Gram-negative agents frequently co-isolated in human pneumonia, this combination has not previously been reported in nestling goldfinches. Correct pathogen identification is vital for accurate etiological diagnosis and to inform appropriate therapeutic intervention.

Keywords: European goldfinch, Co-infection, Klebsiella pneumoniae, Pseudomonas aeruginosa, ecfX, Khe, rpoB, 16S rRNA

Received: 05 November 2024
Revised: 29 January 2025
Accepted: 03 February 2025

How to Cite This Article: Antonio C, Beatrice F, Pietro R. Severe Bronchopneumonia and Necrotizing Hepatitis in Post-Hatch Goldfinch Nestlings Due to Concurrent Klebsiella pneumoniae and Pseudomonas aeruginosa Infection. Int J Vet Res Allied Sci. 2025;5(1):128-39. https://doi.org/10.51847/AMitbip0Yj

Introduction

The European goldfinch (Carduelis carduelis) is a small passerine bird belonging to the Fringillidae family, subfamily Carduelinae, commonly bred in captivity for ornamental or selective purposes [1, 2]. Goldfinches are typically kept in groups—either among conspecifics in breeding facilities or with other avian species in mixed aviaries [3]. While such social housing aligns with their natural behavior, it simultaneously facilitates the efficient spread of infectious organisms among individuals [3, 4].

Bacterial agents are widely distributed across One Health systems [5-7], including domestic avian populations, and can pose a substantial hazard to nestlings and immunocompromised birds, with significant morbidity and mortality often occurring within the initial days after hatching [8, 9]. Respiratory and gastrointestinal infections are most frequently observed in chicks and can rapidly develop into bacteremia in severe instances [8]. In many cases, healthy adult birds with intact immunity act as carriers and disseminators of opportunistic or pathogenic

microorganisms in aviaries [10]. These birds may also harbor multidrug-resistant bacteria, posing a potential zoonotic threat to humans and serving as indicators of reverse zoonosis events [3, 11]. Vertical transmission to embryos may occur during egg formation or immediately afterward through shell contamination with maternal cloacal or fecal bacteria, which can invade developing embryos [12, 13]. Additionally, direct transfer post-hatching occurs via feeding, as parents introduce oral microbes to nestlings—an essential process for establishing early gut microbiota [12, 13].

Microbiota play a fundamental role in disease resistance and immune system development in birds [14]. Evidence indicates that captivity alters the composition of both intestinal and respiratory microbiota, while excessive or inappropriate antimicrobial use disrupts this balance and encourages overgrowth of opportunistic bacteria [1, 15-17]. Birds maintained in captivity on limited diets often exhibit an impaired stress response to environmental changes, favoring the proliferation of pathogenic microbes in their gut [17]. Moreover, poor management practices—such as introducing unquarantined individuals—can trigger outbreaks, particularly in nestlings or birds with weakened immunity [4]. Among passerines, the normal bacterial flora is predominantly Gram-positive; an increased presence of Gram-negative bacteria is usually abnormal and associated with disease, especially in vulnerable individuals [1, 18-20].

Gram-negative bacteria are responsible for most systemic infections in passerines, with Klebsiella pneumoniae, Pseudomonas aeruginosa, and Escherichia coli being the most common isolates [8, 21]. K. pneumoniae is a known opportunist frequently recovered from feces and the oropharynx of clinically normal passerines and parrots [22-24]. However, it can cause severe conditions such as pneumonia, sepsis, and urinary tract infections, and has been identified as the etiologic agent of yolk sac infection and dead-in-shell cases among canary chicks [15, 22-26]. The severity and occurrence of K. pneumoniae infection are often influenced by concurrent diseases or stress factors [25]. Similarly, P. aeruginosa is recognized as an opportunistic avian pathogen, frequently acting as a secondary invader [15]. It typically causes localized infections of the upper respiratory tract, though systemic infections, hemorrhagic enteritis, and high mortality events have been documented [15, 27-29].

The present study reports the pathological features of K. pneumoniae infection concurrent with P. aeruginosa in nestling European goldfinches, associated with early-life mortality in the absence of clinical illness among adult birds. To the best of the authors' knowledge, this is the first description of a natural co-infection by these two bacterial species in goldfinch nestlings. Despite bacterial infections being a major cause of mortality in pet birds—particularly in hatchlings—there remains limited information regarding such cases in this species.

Materials and Methods

Animals and clinical description

An aviculturist experienced unusual chick losses (n = 8) among captive European goldfinches (Carduelis carduelis) reared in two separate cages—five in one and three in another—during their first post-hatch week. These mortalities occurred over a four-week interval between April and May 2024. Twelve chicks hatched in total; eight died within 1-4 days after emergence, representing a 66.7% death rate (8/12). The birds originated from a private breeding colony in Messina, Italy, housing approximately one hundred goldfinches maintained in outdoor paired cages exposed to natural daylight and provided with standard commercial feed. Breeding adults were occasionally transported to exhibitions and competitions several times a year.

Within hours after hatching, affected nestlings displayed severe weakness, loss of mobility, absence of appetite, respiratory distress, and abdominal bloating. Death occurred between the first and fourth day of life. No clinical abnormalities or external lesions were noted in adult birds that occupied the same cages. Based on these observations, both bacterial and viral infections were considered as preliminary differential diagnoses.

Postmortem and histological procedures

Four of the eight deceased chicks were submitted to the Department of Chemical, Biological, Pharmaceutical, and Environmental Sciences, University of Messina, for necropsy. Postmortem inspections were performed under a stereomicroscope. Internal organs were collected for microbiological studies as detailed later, preserved by freezing at −80 °C, and fixed in 10% neutral buffered formalin. After fixation, tissues were routinely processed, embedded in paraffin, and sectioned at a thickness of 3 µm. Sections were stained with hematoxylin and eosin (HE) and evaluated by two accredited veterinary pathologists (J.M.A.; G.D.). Gram staining was performed on parallel 3 µm sections to visualize bacteria.

Bacteriological procedures

Aseptic tissue sampling was conducted on lungs, liver, and kidneys from one chick. A sterile scalpel was used to make incisions, and sterile swabs were inserted into each cut. The swabs were streaked onto 5% sheep blood agar (Biolife, Milan, Italy) and incubated at 28 °C under aerobic conditions for 72 hours. Emerging colonies were repeatedly re-streaked on fresh plates of the same medium and incubated for 48 hours at 28 °C until pure, single-colony isolates were obtained. Each isolate was then smeared on a glass slide and Gram-stained for microscopic identification.

Genetic characterization of the bacterial isolates

For molecular testing, two purified bacterial isolates were cultured in liquid medium to reach approximately 2 × 10^6 cells. The suspensions were centrifuged at 5000× g for 10 minutes at 4 °C. DNA was extracted from the resulting pellets using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, Milan, Italy), following the supplier's guidelines.

Four genes—16S rRNA, ecfX, khe, and rpoB—were amplified using primer sets listed in **Table 1** [30-32].

Table 1. Primer sequences used for bacterial species confirmation [30-32].

Gene	Base Pairs	Forward Primer	Reverse Primer
16S rRNA	1500	AGAGTTTGATCMTGGCTCAG	TACGGYTACCTTGTTACGACTT
ecfX	500	ATGGATGAGCGCTTCCGTG	TCATCCTTCGCCTCCCTG
khe	430	TGATTGCATTCGCCACTGG	GGTCAACCCAACGATCCTG
rpoB	1000	GGCGAAATGGCWGAGAACCA	GAGTCTTCGAAGTTGTAACC

PCR reactions were prepared with a total volume of 25 µL consisting of 12.5 µL of GoTaq® Colorless Master Mix (Promega, Madison, WI, USA), 1 µL of each primer (10 µM), and 1 µL of DNA template (<250 ng/µL). Amplification of the 16S rRNA gene followed the parameters previously described by this research group [33]. For the other targets, conditions included: initial denaturation at 95 °C for 5 minutes; 30 cycles of denaturation at 94 °C for 30 seconds (35 cycles at 94 °C for 45 seconds for ecfX); annealing at 58 °C for 45 seconds for ecfX, 68 °C for 40 seconds for khe, 50 °C for 40 seconds for rpoB; followed by extension at 72 °C for 1 minute and a final step at 72 °C for 5 minutes [30-32]. Each batch included a no-template control. PCR amplicons were resolved on 1.5% (w/v) agarose gels and visualized under UV illumination. DNA concentration and purity were assessed using a NanoPhotometer N50 (IMPLEN, Westlake Village, CA, USA).

Sequencing of purified amplicons was carried out by Genechron (Rome, Italy) using the same primer sets. The obtained sequences were aligned using ClustalW (https://www.genome.jp/tools-bin/clustalw; accessed 24 November 2024) and compared to reference sequences in the NCBI database using the BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi; accessed 24 November 2024) to determine species identity and similarity levels.

Detection of viral pathogens by PCR

Sections from all paraffin-embedded organs were placed in individual microcentrifuge tubes and treated with 1 mL of xylene. Tubes were vortexed, kept at room temperature for 30 minutes, and centrifuged at 11, 000× g for 3 minutes. Supernatants were discarded, and pellets were washed twice with 1 mL of 96-100% ethanol, gently inverted, and left open at 37 °C for about 15 minutes to allow complete evaporation of alcohol.

Each sample was then digested in 350 µL of Lysis Buffer T1 (Macherey-Nagel, Düren, Germany) mixed with 50 µL of Proteinase K (Qiagen, Hilden, Germany) and incubated overnight at 56 °C. Viral nucleic acids were extracted using the QIAsymphony DSP Virus/Pathogen Kit on a QIAsymphony SP instrument (Qiagen, Hilden, Germany), following manufacturer recommendations, and eluted in 60 µL of buffer.

Viral genome amplification was carried out using specific primer and probe sets appropriate for each suspected virus through conventional or real-time RT-PCR assays, as summarized in **Table 2**.

Table 2. Primer and TaqMan probe sets were used for RT-PCR and PCR analyses. NDV: Newcastle Disease Virus; BORNA P: Psittaciform 1 orthobornavirus; BORNA C: passerine bornaviruses; CIRCO: Circovirus; VP1 POLY I/II: late gene encoding the VP1 capsid protein of polyomavirus.

Name	Sequence	Final Concentration	Type of PCR Performed
NDV F	GAGCTAATGAACATTCTTTC	0.5 µM	Real-Time PCR
NDV R	AATAGGCGGACCACATCTG	0.5 µM	Real-Time PCR
LPROMGB (PROBE)	FAM-CCAATCAACTTCCC-MGBEQ	0.2 µM	Real-Time PCR
LPROMGB2 (PROBE)	CY5-AATAGTGTATGACAACAC-MGBEQ	0.2 µM	Real-Time PCR
BORNA PCA3 (+) F	CCCGCAGACAGYACGT	0.25 µM	Real-Time PCR
BORNA PCA3 (+) F	GATCCGCAGACAGYACGT	0.25 µM	Real-Time PCR
BORNA PCA6 (-) R	AAGAAYCCNTCCATGATCTC	0.25 µM	Real-Time PCR
BORNAP (PROBE)	FAM-CGAATWCCCAGGGAGGCYCT-BHQ1	0.25 µM	Real-Time PCR
BORNAC (PROBE)	TexasRed-AGATGCATTGACCCARCCRGT-BHQ2	0.25 µM	Real-Time PCR
CIRCO-FOR	TTCACCCTTAAYAAYCCT	0.5 µM	Conventional PCR
CIRCO-REV	CCRTSATATCCATCCCACCA	0.5 µM	Conventional PCR
VP1 POLY I FOR	CCAGACCCAACTARRAATGARAA	0.9 µM	Conventional PCR
VP1 POLY I REV	AACAAGAGACACAAATNTTTCCNCC	0.9 µM	Conventional PCR
VP1 POLY II FOR	ATGAAAATGGGGTTGGCCCNCTNTGYAARG	0.9 µM	Conventional PCR
VP1 POLY II REV	CCCTCATAAACCCGAACYTCYTCHACYTG	0.9 µM	Conventional PCR

Newcastle disease virus

Newcastle disease virus detection was achieved through quantitative RT-PCR, employing the VetMAX-Plus Multiplex One-Step RT-PCR Kit (Applied Biosystems, Foster City, CA, USA). The reaction composition followed the manufacturer's guidelines, with a total volume of 25 µL. The amplification run included an initial incubation at 48 °C for 10 minutes and 95 °C for 10 minutes, succeeded by 40 thermal cycles (denaturation at 95 °C for 15 seconds, annealing and extension at 50 °C for 45 seconds) [34].

Avian bornavirus

Screening for avian bornavirus was performed using real-time reverse transcription PCR, again utilizing the VetMAX-Plus Multiplex One-Step RT-PCR Kit (Applied Biosystems, Foster City, CA, USA). The reaction mix (25 µL) underwent the following program: 50 °C for 20 minutes for reverse transcription, 95 °C for 5 minutes for activation, followed by 40 cycles of denaturation at 95 °C for 15 seconds and annealing/extension at 53 °C for 1 minute [35].

Canary polyomavirus

A nested PCR protocol was carried out to detect canary polyomavirus. Reactions were assembled in 25 µL using GoTaq G2 Hot Start Master Mix 2× (Promega, Madison, WI, USA), according to kit directions. During the first PCR round, the samples were preheated at 95 °C for 5 minutes, processed through 45 repetitions of 94 °C for 30 seconds, 52.2 °C for 1 minute, and 72 °C for 1 minute, and finalized with 5 minutes at 72 °C. The second amplification step used 4 µL of the previous PCR as a template, with an initial denaturation at 95 °C for 5 minutes, then 45 cycles of 94 °C for 30 seconds, 62 °C for 30 seconds, and 72 °C for 30 seconds, and a final extension at 72 °C for 5 minutes [36].

Canary circovirus

Detection of canary circovirus was carried out using a conventional end-point PCR with the GoTaq G2 Hot Start Master Mix 2× (Promega, Madison, WI, USA). The assay was performed in a 25 µL total reaction as instructed by the supplier. The procedure began with denaturation at 94 °C for 2 minutes, continued through 40 thermal cycles at 94 °C for 30 seconds, 52 °C for 30 seconds, and 68 °C for 1 minute, and was completed with a 5-minute extension at 68 °C [37].

The PCR amplicons were loaded onto 2% agarose gels (Tris-acetate-EDTA buffer), stained with GelRed (BIOTIUM), and visualized by UV transillumination. The 100 bp DNA Ladder (Invitrogen, Waltham, MA, USA) served as a molecular size marker.

Results and Discussion

Gross pathology

Necropsy was conducted on four goldfinch nestlings (n = 4), each measuring 7.50-8.20 cm in length and weighing between 2.5 and 3.9 g. All exhibited cachexia and total depletion of body fat. The most consistent macroscopic findings were crop enlargement in two individuals (2/4; 50%) and distension of the coelomic cavity in every specimen (4/4; 100%) **(Figure 1a)**.

a) b)

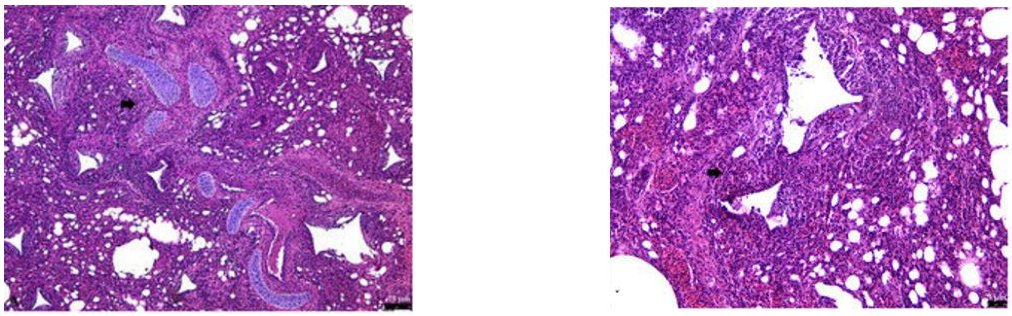
Figure 1. Macroscopic observations. (a) Emaciated body with swollen crop (arrow) and expanded coelomic region. (b) Lungs demonstrating generalized congestion, edema, and multifocal hemorrhage in all examined birds.

The liver appeared unchanged in two nestlings (2/4; 50%), exhibited mild hepatomegaly with rounded borders in one (1/4; 25%), and showed scattered pale necrotic areas in another (1/4; 25%).

Pulmonary lesions were present in every subject (4/4; 100%), characterized by diffuse redness, fluid accumulation, and patchy bleeding **(Figure 1b)**. Additionally, a single bird (1/4; 25%) displayed localized epicardial hemorrhage.*Histopathology*

Respiratory tract

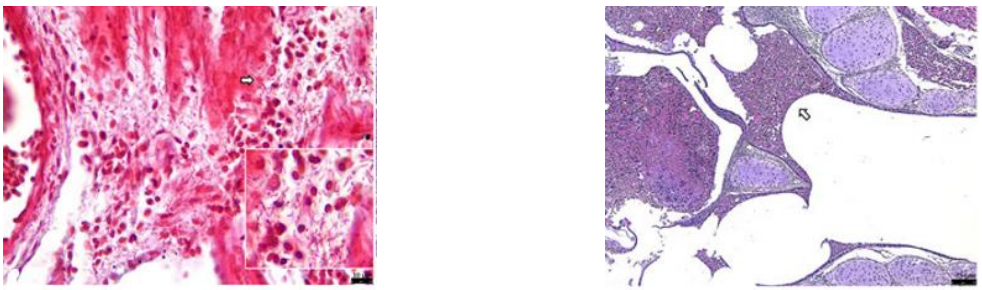
Microscopic lung evaluation revealed extensive inflammatory processes occupying nearly 70-80% of the pulmonary tissue in all four nestlings (n = 4; 100%). The inflammation was irregularly distributed but intense, causing destruction and thickening of parabronchial walls, with frequent occlusion of secondary bronchi and respiratory atria **(Figure 2)**.



a) b)

Figure 2. Lung sections.
Inflammatory infiltrate expanding parabronchial walls and narrowing air passages (arrow). HE stain: (a) ×100; (b) ×200.

The infiltrate contained lymphocytes, with moderate populations of macrophages and plasma cells, alongside heterophils, both viable and degenerating. Eosinophilic fibrin deposits and necrotic epithelial fragments were often present, and hemorrhagic foci were interspersed with hemosiderin-laden macrophages. The bronchial and atrial lumina were filled with mucus, exudate, and necrotic debris, and vascular congestion was prominent.
In every bird, numerous bacillary forms (2-3 µm) were visible within parabronchial septa and arterial lumina, staining as Gram-negative **(Figure 3a)**. These findings correspond to severe, multifocal-to-coalescing, subacute lymphoplasmacytic bronchopneumonia.



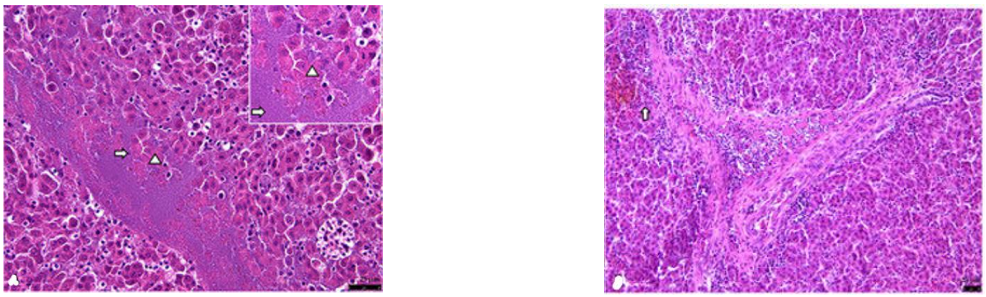
a) b)

Figure 3. (a) Lung: clusters of Gram-negative bacilli (arrow) within parabronchial and vascular regions (inset, magnified). Gram stain, ×630. (b) Syrinx/trachea: lumen containing mucus, necrotic fragments, inflammatory cells, and red blood cells (arrow). HE stain, ×100.

The trachea and syrinx in all samples contained mucous and necrotic material, mixed inflammatory infiltrates, and occasional basophilic bacilli **(Figure 3b)**. The nasal mucosa showed epithelial necrosis and exfoliation, with the lumen obstructed by mucus and inflammatory debris.

Liver

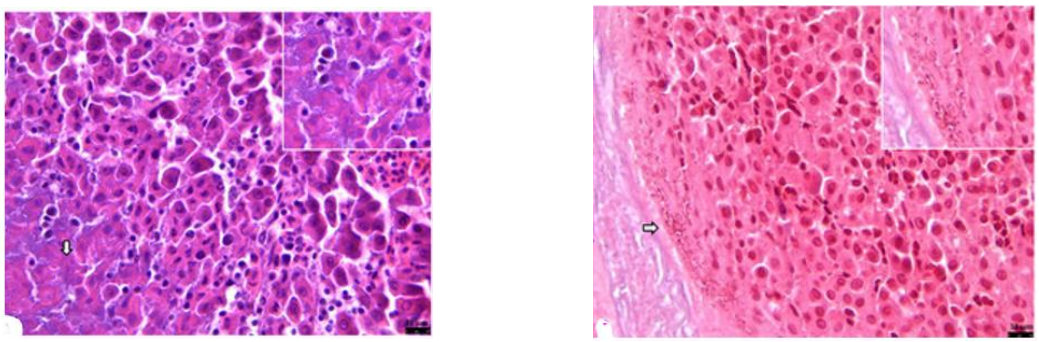
In all examined goldfinch chicks (n = 4; 100%), the liver exhibited multiple, randomly distributed areas of extensive cell death, often containing dense clusters of small basophilic rods (2-3 µm). Roughly 30-40% of the hepatic tissue was affected, showing zones of lytic necrosis with occasional dystrophic calcification, resulting in loss of hepatic cord organization and disruption of the normal parenchymal framework. The portal and periportal spaces were moderately infiltrated by lymphocytes, along with macrophages and a few plasma cells. Sinusoids appeared markedly congested with red blood cells, and focal hemorrhages were evident, sometimes containing macrophages loaded with hemosiderin pigment **(Figure 4)**.



a) b)

Figure 4. Liver histopathology. (a) Hepatic tissue showing destruction of hepatocyte cords, widespread necrosis, mineral deposits (arrow), and clusters of basophilic bacilli (triangle). Inset: mineralization (arrow) and intralesional bacteria (triangle). Hematoxylin-eosin (HE), ×400. (b) Infiltration of inflammatory cells within periportal regions and hemorrhage between necrotic hepatocytes (arrow). HE, ×200.

Dense bacterial aggregates were distributed within and surrounding the necrotic lesions **(Figure 5a)** and were identified as Gram-negative bacilli using Gram staining **(Figure 5b)**.



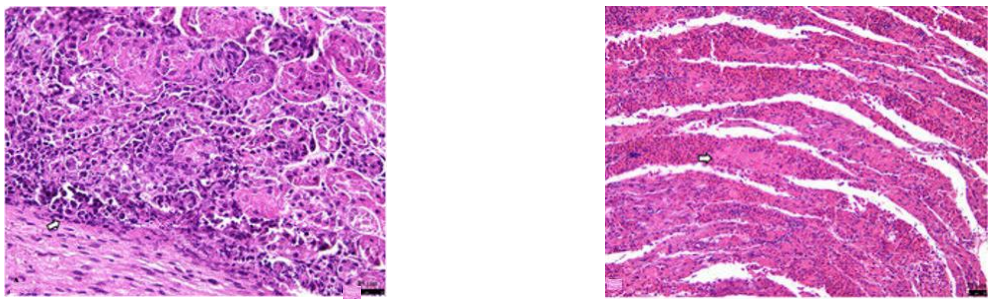
a) b)

Figure 5. Liver: histological and Gram stain views. (a) Clusters of basophilic bacteria (arrow). Inset: bacterial colonies within the liver. HE, ×630. (b) Gram-negative rods (arrow). Inset: necrotic liver regions showing Gram-negative bacilli. Gram stain, ×630.

Other organs

Two of the four chicks showed mild multifocal interstitial nephritis, characterized by small collections of lymphocytes, macrophages, and both intact and degenerating heterophils. The proximal convoluted tubules often contained necrotic epithelial cells, loss of brush borders, and shrunken or fragmented nuclei. These necrotic cells were frequently shed into the tubular lumen, sometimes accompanied by focal dystrophic mineralization **(Figure 6a)**.

In one specimen (1/4; 25%), the myocardium presented a localized hemorrhagic focus, along with segmental necrosis of muscle fibers and infiltration by inflammatory cells **(Figure 6b)**. No noteworthy histological changes were identified in the remaining organs.



a) b)

Figure 6. Histopathology of the kidney and heart. (a) Kidney: interstitial lymphocytic-plasmacytic nephritis (arrow) and necrosis of proximal tubular epithelium. HE, ×400. (b) Heart: myocardial hemorrhage with necrotic cardiomyocytes and inflammatory infiltration (arrow). HE, ×100.

Bacterial identification

Two Gram-negative bacteria were successfully isolated from the lung, liver, and kidney samples. PCR sequencing of the 16S rRNA gene revealed identical nucleotide sequences across biological replicates. BLAST alignment (NCBI) showed 99.22% homology with Klebsiella pneumoniae (Query Cover: 99%, E-value: 0) and 99.81% homology with Pseudomonas aeruginosa (Query Cover: 100%, E-value: 0). These 16S rRNA sequences were submitted to GenBank under accession numbers PP980564 and PP980565 (File S1).

Further identification at the species level was achieved through amplification of ecfX, rpoB, and khe genes, generating products of 500 bp, 1000 bp, and 430 bp, respectively. The ecfX fragment showed 100% sequence identity with P. aeruginosa (Query Cover: 100%, E-value: 0), while the rpoB and khe fragments confirmed K. pneumoniae with 100% similarity to the reference database. The verified sequences were archived in GenBank as PV578954, PV578955, and PV578956.

Molecular detection of viral agents

No viral genomes were detected in any of the specimens tested, indicating that none of the screened viruses were present in the examined tissues.

The present investigation describes the pathological consequences of a natural co-infection with Klebsiella pneumoniae and Pseudomonas aeruginosa, responsible for mortality in nestling European goldfinches during the first post-hatching week. While these two Gram-negative bacteria are recognized pathogens frequently implicated in human bacterial pneumonia [38], no documented cases of K. pneumoniae and P. aeruginosa infections in goldfinches were identified in the literature. The clustering of deaths among nestlings in the same breeding facility, exhibiting similar but nonspecific gross lesions, suggested a contagious etiology. Differential diagnoses initially considered both bacterial and viral causes. Subsequent histopathological, microbiological, and molecular analyses confirmed a mixed bacterial infection driven by two opportunistic Gram-negative organisms. In passerines, K. pneumoniae is generally regarded as a primary pathogen [21], whereas P. aeruginosa usually behaves as a secondary invader, causing disease predominantly in birds weakened by prior infections or nutritional deficits [15, 39].

Previous studies indicate that co-infection with P. aeruginosa can enhance proliferation of K. pneumoniae within pulmonary tissue in mice, leading to bacteremia and increased mortality compared with infection by K. pneumoniae alone [40]. In humans, the simultaneous presence of these two bacteria is a strong independent predictor of 90-day mortality, and their frequent co-isolation from lungs demonstrates their natural tendency to coexist [38, 41]. In environmental and host-associated niches, microorganisms often form multi-species biofilms, where interspecies interactions—competitive or cooperative—govern species distribution, biofilm stability, and overall microbial behavior [42, 43]. Both K. pneumoniae and P. aeruginosa utilize quorum sensing (QS) for coordinating virulence factor expression, biofilm formation, and other pathogenic behaviors [44]. In polymicrobial infections, QS mediates dynamic interactions that can either facilitate cooperation or competition [45]. Specifically, cooperative interactions between K. pneumoniae and P. aeruginosa have been documented [42], supporting the likelihood that, in these nestlings, co-infection exacerbated pneumonia primarily caused by K. pneumoniae, eventually leading to bacteremia and death.

Bacterial infections are prevalent in domestic birds and pose a high risk of early mortality, particularly in immunocompromised or weak chicks during the initial days after hatching [9]. Transmission can occur vertically, with embryos becoming infected during development in the reproductive tract, which can result in death shortly after hatching [12, 13]. Opportunistic bacteria may also colonize the maternal cloaca or feces and gain access to eggs during oviposition or incubation, contaminating the nest environment and infecting newly hatched chicks [12, 13, 46, 47]. Additionally, early feeding behavior enables transfer of microbes from the oral cavity or crop to the chick, which plays a key role in establishing the gut microbiota in passerines [48].

K. pneumoniae is frequently recovered from oropharyngeal and fecal samples of clinically healthy passerines and psittacines, although it is not a normal component of the gut flora in granivorous pet birds, including finches [23, 49, 50]. This bacterium can persist in the environment under favorable conditions, and infection typically requires predisposing factors, even for intestinal or extraintestinal disease in birds [23, 49, 50]. Similarly, P. aeruginosa ranks as the second most commonly isolated Pseudomonadaceae species in wild birds, colonizing the pharynx and cloaca, while in domestic birds, contaminated food and water represent the main sources of infection [21, 51]. In the nestlings of this study, bacterial spread likely occurred horizontally, either via direct contamination of the eggshell during the 10-14 day incubation period of European goldfinches or shortly after hatching through maternal oral or cloacal microbes present in the nest. For Pseudomonas species, the fecal-oral route is generally considered the primary transmission pathway, and similar routes are suspected for K. pneumoniae infections in canaries [21, 26]. Adult goldfinches may have acted as reservoirs for these pathogens, although this was not investigated in the current study and should be addressed in future research. Given the broad host range of both organisms, wild birds could also serve as infection sources, especially since goldfinches were housed in open-air cages [51, 52]. Indeed, K. pneumoniae is commonly isolated from pigeons and other free-living birds, while P. aeruginosa is part of the natural pharyngeal and cloacal microbiota of some wild bird species [51].

Nestling goldfinches in this investigation exhibited nonspecific clinical manifestations, including depression, weakness, lack of appetite, abdominal swelling, and respiratory difficulties, and died within 1-4 days post-hatching. No signs of illness were observed in adult finches sharing the same cages. The severity and presentation of bacterial infections are influenced by factors such as host immune status, genetic background, nutritional condition, and management practices in captive passerines [4]. Consequently, nestlings with immature or compromised immune systems are likely more vulnerable than healthy adults, which can act as asymptomatic carriers of potentially pathogenic bacteria. In addition, adult birds may carry less virulent K. pneumoniae strains that encode fewer virulence determinants compared with the strains associated with clinical disease [23].

The principal pathological lesions in the nestlings examined included severe subacute bronchopneumonia and necrotizing hepatitis, with numerous clusters of Gram-negative bacilli observed in parabronchial walls, pulmonary artery lumina, and around necrotic hepatic foci. K. pneumoniae commonly induces respiratory disease in birds but can also cause gastrointestinal disease, systemic dissemination, and high mortality, especially in chicks under unfavorable conditions [15, 21, 22, 23, 26]. Necrotizing hepatitis with intralesional bacilli has previously been reported in canary chicks naturally infected with K. pneumoniae originating from the gastrointestinal tract, leading to extraintestinal and systemic lesions [26], resembling the hepatic pathology observed here. Similarly, P. aeruginosa is known to cause pneumonia and airsacculitis in passerines and has been implicated in necrotizing hepatitis in pet birds, including cases linked to Pseudomonas fluorescens [21, 53]. Respiratory infections by these bacteria can progress to bacteremia, with the liver being primarily affected [5]. Gastrointestinal infections may also lead to bacteremia, either systemically or through ascending infection via the biliary system [8]. In the nestlings analyzed, given the tissue tropism of the isolated pathogens, it is likely that primary respiratory co-infection led to bacteremia and severe liver involvement.

Because most cases of bacterial pneumonia in domestic birds occur secondary to viral infections or other predisposing factors [8], the presence of viral pathogens was assessed. Based on clinical history and gross pathology—including respiratory lesions, necrotizing hepatitis, and occasional interstitial nephritis—viruses considered in the differential diagnosis included Newcastle Disease virus, Canary Circovirus, Canary Polyomavirus, and Avian Bornavirus [1, 8, 54, 55]. However, no histopathological evidence of viral infection or inclusion bodies was detected, and molecular testing of FFPE tissue samples confirmed the absence of these viruses.

For species-level bacterial identification, four housekeeping genes (16S rRNA, rpoB, khe, and ecfX) were targeted [31, 32]. While 16S rRNA sequencing can differentiate bacterial genera, it is often insufficient for resolving closely related species, such as Klebsiella and Pseudomonas [30, 31, 56]. The khe and rpoB genes were employed for K. pneumoniae identification; khe encodes a unique hemolysin highly conserved among K. pneumoniae strains [32, 57, 58], while rpoB allows for more precise discrimination at the species or subspecies level [32, 57]. For P. aeruginosa, the ecfX gene provides a species-specific, highly sensitive target, encoding an extracytoplasmic sigma factor implicated in heme uptake and virulence [31].

Conclusion

This work describes the pathological consequences of co-infection with K. pneumoniae and P. aeruginosa in nestling goldfinches, resulting in primary respiratory disease with subsequent bacteremia and mortality within the first week post-hatching. Although these Gram-negative pathogens are commonly co-isolated in human bacterial pneumonia, such a co-infection has not previously been reported in goldfinches. Accurate pathogen identification is critical for establishing a definitive etiological diagnosis and for guiding therapy, including antibiotic susceptibility testing, which warrants further investigation. Given the opportunistic nature of these bacteria and their potential carriage by clinically healthy birds, the introduction of new individuals into flocks based solely on clinical evaluation poses a substantial management challenge.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Dorrestein GM. Passerines. In: Tully TN Jr, Dorrestein GM, Jones AK, editors. Handbook of Avian Medicine, 2nd ed. Seneca Falls, NY: Sauders; 2009. p. 169–208.
2. Zuccon D, Prys-Jones R, Rasmussen PC, Ericson PGP. The phylogenetic relationships and generic limits of finches (Fringillidae). Mol Phylogenet Evol. 2012;62(3):581–96.

3. Boseret G, Losson B, Mainil JG, Thiry E, Saegerman C. Zoonoses in pet birds: Review and perspectives. Vet Res. 2013;44(1):36.
4. Joseph V. Infectious and parasitic diseases of captive passerines. Semin Avian Exot Pet Med. 2003;12(1):21–8.
5. Marouf S, Li X, Salem HM, Ahmed ZS, Nader SM, Shaalan M, et al. Molecular detection of multidrug-resistant Pseudomonas aeruginosa of different avian sources with pathogenicity testing and in vitro evaluation of antibacterial efficacy of silver nanoparticles against multidrug-resistant P. aeruginosa. Poult Sci. 2023;102(10):102995.
6. Shoaib M, Tang M, Aqib AI, Zhang X, Wu Z, Wen Y, et al. Dairy farm waste: A potential reservoir of diverse antibiotic resistance and virulence genes in aminoglycoside- and beta-lactam-resistant Escherichia coli in Gansu Province, China. Environ Res. 2024;263:120190.
7. Asokan S, Jacob T, Jacob J, AlSosowaa AA, Cherian T, Peijnenburg WJGM, et al. Klebsiella pneumoniae: A growing threat in the era of antimicrobial resistance. Microbe. 2025;7:100333.
8. Schmidt RE, Reavill DR, Phalen DN. Pathology of pet and aviary birds, 2nd ed. Hoboken, NJ: Wiley Blackwell; 2015. p. 95–125.
9. Nolan LK, Vaillancourt JP, Barbieri NL, Louge CM. Colibacillosis. In: Swayne DE, editor. Diseases of Poultry. Ames, IA: Wiley Online Library; 2020. p. 770–830.
10. Qui Y, Lv C, Chen J, Sun Y, Tang T, Zhang Y, et al. The global distribution and diversity of wild-bird-associated pathogens: An integrated data analysis and modeling study. Med. 2025;6(4):100553.
11. Kobuszewska A, Wysok B. Pathogenic bacteria in free-living birds, and its public health significance. Animals. 2024;14(1):968.
12. Gantois I, Ducatelle R, Pasmans F, Haesebrouck F, Gast R, Humphrey TJ, et al. Mechanisms of egg contamination by Salmonella enteritidis. FEMS Microbiol Rev. 2009;33(4):718–38.
13. Gast RK, Porter RE Jr. Salmonella infections. In: Swayne DE, Boulianne M, Logue SM, editors. Diseases of Poultry, 14th ed. Hoboken, NJ: Wiley; 2020. p. 719–53.
14. Ottinger MA, Mani S, Krüger S, Coverdale B, Willows-Munro S, Combrink L. Microbiomes in birds: A review of links to health and reproduction. Reprod Fertil. 2024;5:e230076.
15. Gerlach H. Bacteria. In: Ritchie BW, Harrison GJ, Harrison LR, editors. Avian Medicine: Principles and Application. Lake Worth, FL: Wingers Publishing, Inc.; 1994. p. 949–83.
16. Zhou Y, Li Y, Zhang L, Wu Z, Huang Y, Yan H, et al. Antibiotic administration routes and oral exposure to antibiotic resistant bacteria as key drivers for gut microbiota disruption and resistome in poultry. Front Microbiol. 2020;11:1319.
17. Sun F, Chen J, Liu K, Tang M, Yang Y. The avian gut microbiota: Diversity, influencing factors, and future directions. Front Microbiol. 2022;13:934272.
18. Benskin CMH, Rhodes G, Pickup RW, Wilson K, Hartley IR. Diversity and temporal stability of bacterial communities in a model passerine bird, the zebra finch. Mol Ecol. 2010;19(24):5531–44.
19. Garcia-Mazcorro JF, Castillo-Carranza SA, Guard B, Gomez-Vazquez JP, Dowd SE, Brightsmith DJ. Comprehensive molecular characterization of bacterial communities in feces of pet birds using 16S marker sequencing. Microb Ecol. 2017;73(2):224–35.
20. Matteucci G, Binanti D. Presence and significance of Gram-negative bacteria in the cloacal microbiota of Psittacine and Passerine. In: Proceedings of the ICARE 2017—International Conference of Avian Herpetological and Exotic Mammal Medicine; 2017 Mar 25–29; Venice, Italy.
21. Coles BH, Krautwald-Junghanns M, Orosz SE, Tully T. Essentials of Avian Medicine and Surgery, 3rd ed. Hoboken, NJ: Wiley-Blackwell; 2007.
22. El Fertas-Aissani R, Messai Y, Alouache S, Bakour R. Virulence profiles and antibiotic susceptibility patterns of Klebsiella pneumoniae strains isolated from different clinical specimens. Pathol Biol. 2013;61(4–5):209–16.
23. Davies YM, Cunha MPV, Oliveira MGX, Oliveira MCV, Philadelpho N, Romero DC, et al. Virulence and antimicrobial resistance of Klebsiella pneumoniae isolated from passerine and psittacine birds. Avian Pathol. 2016;45(2):194–201.
24. Ahmed HA, Awad NFS, Abd El-Hamid MI, Shaker A, Mohamed RE, Elsohaby I. Pet birds as potential reservoir of virulent and antibiotic resistant zoonotic bacteria. Comp Immunol Microbiol Infect Dis. 2021;75:101606.

25. Razmyar J, Zamani AH. An outbreak of yolk sac infection and dead-in-shell mortality in common canary (Serinus canaria) caused by Klebsiella pneumoniae. Iran J Vet Res. 2016;17(2):141–3.
26. Nakhaee P, Zarif Moghadam H, Shokrpoor S, Razmyar J. Klebsiella pneumoniae infection in canaries (Serinus canaria domestica): A case report. Iran J Vet Res. 2022;23(2):280–4.
27. Kock N, Kock R. Disease epidemic in lesser flamingos (Phoeniconauis minor) in Kenya. In: Proceedings of the American Association of Zoo Veterinarians. East Lansing, MI: American Association of Zoo Veterinarians; 1995. p. 115.
28. Momotani E, Kiryu M, Ohshiro M, Murakami Y, Ashida Y, Watanabe S, et al. Granulomatous lesions caused by Pseudomonas aeruginosa in the ostrich (Struthio camelus). J Comp Pathol. 1995;112(3):273–82.
29. Bailey TA, Silvanose CD, Naldo JN, Howlett JH. Pseudomonas aeruginosa infections in kori bustards (Ardeotis kori). Avian Pathol. 2000;29(1):41–4.
30. Diancourt L, Passet V, Verhoef J, Grimont PAD, Brisse S. Multilocus sequence typing of Klebsiella pneumoniae nosocomial isolates. J Clin Microbiol. 2005;43(8):4178–82.
31. Lavenir R, Jocktane D, Laurent F, Nazaret S, Cournoyer B. Improved reliability of Pseudomonas aeruginosa PCR detection by the use of the species-specific ecfX gene target. J Microbiol Methods. 2007;70(1):20–9.
32. He Y, Guo X, Xiang S, Li J, Li X, Xiang H, et al. Comparative analyses of phenotypic methods and 16S rRNA, khe, rpoB genes sequencing for the identification of clinical isolates of Klebsiella pneumoniae. Antonie Van Leeuwenhoek. 2016;109(8):1029–40.
33. Palazzolo S, Gervasi C, Abbate JM, Gjurčević E, Falleti R, Piro MG, et al. Natural bacterial co-infection in farmed European sea bass intended for experimental research in Sicily, southern Italy: Pathological findings. Fishes. 2024;9(6):360.
34. Sutton DA, Allen DP, Fuller CM, Mayers J, Mollett BC, Londt BZ, et al. Development of an avian avulavirus 1 (AAvV-1) L-gene real-time RT-PCR assay using minor groove binding probes for application as a routine diagnostic tool. J Virol Methods. 2019;265:9–14.
35. Sigrist B, Geers J, Albini S, Rubbenstroth D, Wolfrum N. A New Multiplex Real-Time RT-PCR for Simultaneous Detection and Differentiation of Avian Bornaviruses. Viruses. 2021;13(7):1358.
36. Johne R, Enderlein D, Nieper H, Müller H. Novel polyomavirus detected in the feces of a chimpanzee by nested broad-spectrum PCR. J Virol. 2005;79(6):3883–7.
37. Todd D, Weston J, Ball NW, Borghmans BJ, Smyth JA, Gelmini L, et al. Nucleotide sequence-based identification of a novel circovirus of canaries. Avian Pathol. 2001;30(4):321–5.
38. Okada F, Ando Y, Honda K, Nakayama T, Ono A, Tanoue S, et al. Acute Klebsiella pneumoniae pneumonia alone and with concurrent infection: Comparison of clinical and thin-section CT findings. Br J Radiol. 2010;83(992):854–60.
39. Abd El-Ghany WA. Pseudomonas aeruginosa infection of avian origin: Zoonosis and one health implications. Vet World. 2021;14(9):2155–9.
40. Jones-Nelson O, Hilliard JJ, DiGiandomenico A, Warrener P, Alfaro A, Cheng L, et al. The neutrophilic response to Pseudomonas damages the airway barrier, promoting infection by Klebsiella pneumoniae. Am J Respir Cell Mol Biol. 2018;59(6):745–56.
41. Marchaim D, Perez F, Lee J, Bheemreddy S, Hujer AM, Rudin S, et al. "Swimming in resistance": Co-colonization with carbapenem-resistant Enterobacteriaceae and Acinetobacter baumannii or Pseudomonas aeruginosa. Am J Infect Control. 2012;40(9):830–5.
42. Muzaki MZBM, Subramoni S, Summers S, Kjelleberg S, Rice SA. Klebsiella pneumoniae AI-2 transporters mediate interspecies interactions and composition in a three-species biofilm community. NPJ Biofilms Microbiomes. 2024;10(1):91.
43. Elias S, Banin E. Multi-species biofilms: Living with friendly neighbors. FEMS Microbiol Rev. 2012;36(6):990–1004.
44. Castillo-Juárez I, Maeda T, Mandujano-Tinoco EA, Tomás M, Pérez-Eretza B, García-Contreras SJ, et al. Role of quorum sensing in bacterial infections. World J Clin Cases. 2015;3(7):575–98.
45. Cui S, Kim E. Quorum sensing and antibiotic resistance in polymicrobial infections. Commun Integr Biol. 2024;17(1):2415598.
46. Martin-Vivaldi M, Soler JJ, Peralta-Sánchez JM, Arco L, Martín-Platero AM, Martínez-Bueno M, et al. Special structures of hoopoe eggshells enhance the adhesion of symbiont-carrying uropygial secretion that increase hatching success. J Anim Ecol. 2014;83(6):1289–301.

47. Van Veelen HPJ, Salles JF, Tieleman BI. Microbiome assembly of avian eggshells and their potential as transgenerational carriers of maternal microbiota. ISME J. 2018;12(6):1375–88.
48. Chen CY, Chen CK, Chen YY, Fang A, Shaw GTW, Hung CM, Wang D. Maternal gut microbes shape the early-life assembly of gut microbiota in passerine chicks via nests. Microbiome. 2020;8(1):129.
49. Gibbs PS, Kasa R, Newbrey JL, Petermann SR, Wooley RE, Winson HM, Reed W. Identification, antimicrobial resistance profiles and virulence of members from the family Enterobacteriaceae from the feces of yellow-headed blackbirds (Xanthocephalus xanthocephalus) in North Dakota. Avian Dis. 2007;51(3):649–55.
50. Horn RV, Cardoso EM, Lopes ES, Teixeira RSC, Albuquerque AH, Rocha-e-Silva RC, et al. Identification and antimicrobial resistance of members from the Enterobacteriaceae family isolated from canaries (Serinus canaria). Pesq Vet Bras. 2015;35(6):552–6.
51. Stenkat J, Krautwald-Junghanns ME, Schmitz OA, Eilers A, Schmidt V. Aerobic cloacal and pharyngeal bacterial flora in six species of free-living birds. J Appl Microbiol. 2014;117(6):1564–71.
52. Raue R, Schmidt V, Freick M, Reinhardt B, Johne R, Kamphausen L, et al. A disease complex associated with pigeon circovirus infection, young pigeon disease syndrome. Avian Pathol. 2005;34(5):418–25.
53. Jackson NK, Philips SN. Necrotizing hepatitis in pet birds associated with Pseudomonas fluorescens. Avian Dis. 1996;40(3):473–6.
54. Crosta L, Sironi G, Rampin T. Polyomavirus infection in Fringilidae. In: Proceedings of the 4th Conference of the European AAV, London, UK, 19–24 May 1997; European Association of Avian Veterinarians: Madrid, Spain, 1997. p. 128–31.
55. Shivaprasad DL, Hill D, Todd D, Smyth JA. Circovirus infection in a Gouldian finch (Chloebia gouldiae). Avian Pathol. 2004;33(6):525–9.
56. Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pulses, perils, and pitfalls. J Clin Microbiol. 2007;45(9):2761–4.
57. Adekambi T, Raoult D, Drancourt M. The rpoB gene as a tool for clinical microbiologists. Trends Microbiol. 2009;17(1):37–45.
58. Yin-Ching C, Jer-Horng S, Ching-Nan L, Ming-Chung C. Cloning of a gene encoding a unique haemolysin from Klebsiella pneumoniae and its potential use as a species-specific gene probe. Microb Pathog. 2002;33(1):1–6.