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CASE REPORT

Cranial osteomyelitis associated with *Pasteurella canis* in broiler chickens

Leandro Cádiz[1,2], Fernando Navarrete[2], Paulina Torres[2], Héctor Hidalgo[2*]

[1]Núcleo de Investigaciones Aplicadas en Ciencias Veterinarias y Agronómicas, NIAVA. Facultad de Medicina Veterinaria y Agronomía, Universidad de las Américas, Campus Maipú, Santiago, Chile.

[2]Laboratory of Avian Pathology, Department of Animal Pathology, Faculty of Veterinary and Animal Sciences, Universidad de Chile, Santiago 8820808, Chile.

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Corresponding author
*Héctor Hidalgo
hhidalgo@uchile.cl

ABSTRACT. Species of the *Pasteurella* genus are part of the oropharyngeal microbiota of many animal species. In poultry, *Pasteurella multocida* causes fowl cholera, the chronic manifestation of which can include neurological symptoms. *Pasteurella canis* causes osteomyelitis and other infections in humans. To the best of our knowledge, this is the first report of cranial osteomyelitis associated with *Pasteurella canis* in broiler chickens in Chile.

Keywords: poultry, infections, zoonotic pathogen, dogs, *Pasteurella canis*

Cranial osteomyelitis, otitis, swollen head syndrome, and meningoencephalitis in poultry are associated with various infectious agents, including *Avibacterium paragallinarum* (Crispo *et al.*, 2018), *Ornithobacterium rhinotracheale* (Banani *et al.*, 2015; Al-Hasan *et al.*, 2021), and particularly *Pasteurella multocida* (Boulianne *et al.*, 2020). Species of the *Pasteurella* genus are part of the normal oropharyngeal microbiota of many animal species and cause multiple infectious diseases in a wide range of organisms, including humans and poultry (Wilson & Ho, 2013). *Pasteurella multocida* and *P. canis* are the main *Pasteurella* genus species associated with human diseases following septic bite wounds and inflammation at the injury site (Gautier *et al.*, 2005). *Pasteurella spp.*, which belongs to the *Pasteurellaceae* family, are small, non-motile, facultative anaerobic, gram-negative coccobacilli measuring 1–2 µm in length (Boulianne *et al.*, 2020). *Pasteurella multocida* is the causative agent of fowl cholera, a contagious disease affecting domestic and wild birds, and mortality usually ranges from 0% to 20% in naturally infected chickens (Boulianne *et al.*, 2020). The main clinical signs of acute presentation are fever, anorexia, ruffled feathers, mucoid discharge from the mouth, diarrhea, and an increased respiratory rate. Chronic presentation can cause torticollis and opisthotonos due to meningeal infection (Boulianne *et al.*, 2020). *Pasteurella canis* is part of the normal microbiota of healthy companion animals, particularly dogs. On several occasions, *P. canis* infections have been reported in humans and are associated with osteomyelitis and cutaneous abscess in the right digit (Hara *et al.*, 2002), soft tissue infection (Kim *et al.*, 2016), breast implant infection (Hannouille *et al.*, 2019), abdominal infection (Mensah-Glanowska *et al.*, 2020), septic arthritis of the femorotibial joint (Nascimento *et al.*, 2021), and, recently, endophthalmitis (Bathula *et al.*, 2023). *Pasteurella canis* has also been associated with endocarditis in dogs (Kern *et al.*, 2019) and pneumonia in black-tailed marmosets (Da Silva *et al.*, 2020).

To our knowledge, this is the first report of cranial osteomyelitis associated with *P. canis* infection in broiler chickens in Chile, and it is supported by *pre-* and *post-mortem* findings, bacterial culture, biochemical characteristics, and histopathological analysis.

Between September 2022 and August 2023, the Avian Pathology Laboratory at Universidad de Chile received 35 broiler chickens aged 34–45-d-old for diagnostic evaluation following the sudden onset of neurological symptoms. The fowl came from a commercial broiler farm in the Libertador General Bernardo O'Higgins Region, where the condition affected 4% of the birds, showing a slight increase in mortality, which was quickly controlled through treatment with amoxicillin administered in drinking water at 20 mg/kg body weight daily at 12 h intervals for 5 days. All broiler chickens received live attenuated vaccines against infectious bursal disease (IBD) and avian infectious bronchitis (IB) at one day of age.

Prior to necropsy, the chickens presented severe disorientation (n = 31), torticollis (n = 29), opisthotonos (n = 29), and difficulty standing (n = 21) (Figure 1). They were euthanized by cervical dislocation, and the gross post-mortem examination revealed mild nasal discharge (n=32), caseous material, and fragility in the cranial bone (n = 29), mainly in the ventral area close to the ear (Figure 2). No other significant injuries were observed in the outer, middle, or inner ear; upper respiratory system; joints; or internal organs.

Samples of the brain, cranial bone, nasal discharge, and middle ear of the affected birds were obtained aseptically, plated on tryptone soy agar containing 5% blood and

Figure 1.
Broiler chicken with severe disorientation, displaying symptoms of torticollis and opisthotonos, and difficulty standing.

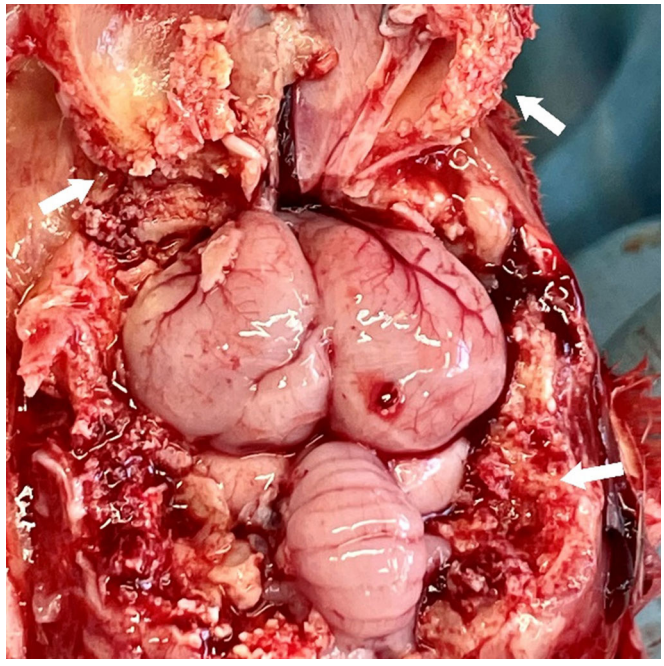

Figure 2.
Macroscopic lesions in affected birds. Cranial osteomyelitis with the presence of caseous material (white arrows).

MacConkey agar, and incubated in 5% CO_2 at 37°C for 24 h. Cranial bone (occipital) sections (n = 5) were collected, decalcified with 3% nitric acid, fixed in 10% neutral-buffered formalin for three days, embedded in paraffin wax, cut into 4 µm sections, stained with hematoxylin and eosin, and examined under a light microscope.

Brain samples were aseptically collected and homogenized in phosphate-buffered saline (PBS; pH 7.2) 10% containing 200 U/mL penicillin and 0.2 mg/mL streptomycin. Homogenates were vortexed for 10 s, subjected to three freeze-thaw cycles, and centrifuged at 3000 × *g* for 20 min at 4 °C. The supernatant was transferred to a sterile tube and preserved at –80 °C to rule out the presence of Newcastle disease virus (NDV) and avian influenza virus (AIV) infection by viral isolation and hemagglutination assays (Alexander, 2000). Nasal discharge samples were collected to exclude the presence of *Mycoplasma synoviae* and *Mycoplasma gallisepticum* by polymerase chain reaction (PCR) under previously reported conditions (Lauerman, 1998).

After 24 h incubation on blood agar plates, cultures from the cranial bone (n = 29), brain (n = 20), and middle ear (n = 10) presented smooth, grayish-white, mucoid, non-hemolytic colonies compatible with *Pasteurella spp*. (Figure 3). No bacterial growth was observed on MacConkey agar plates. No colonies compatible with *Pasteurella* were observed in nasal discharge samples. Pure subcultures were obtained from single colonies on a blood agar plate for Gram staining, catalase and oxidase tests, and biochemical testing using the Vitek® 2 Compact identification system (BioMerieux, Marcy-l'Étoile, France) according to the manufacturer's instructions. The latter identified the species as *Pasteurella canis* with an accuracy of 99 %. The main results of the biochemical testing that identified *Pasteurella canis* isolated in this study, distinguishing it from other species of the *Pasteurella* genus, such as *Pasteurella multocida*, *Pasteurella stomatis*, and *Pasteurella dagmatis* (Christensen & Bisgaard, 2024), are detailed in Table 1. All the isolates were gram-negative.

Histopathological examination of cranial bone samples revealed a chronic inflammatory response and dense infiltration of inflammatory cells, including heterophils that form granular eosinophilic aggregates surrounded by macrophages, along with extensive bone resorption and necrotic bone tissue. Several bacteria were observed in the center of the lesions. These microscopic alterations were consistent with granulomatous osteomyelitis (Figure 4). The NDV and AIV testing by viral isolation and hemagglutination assay were negative, as were PCR analyses for *Mycoplasma synoviae* and *Mycoplasma gallisepticum*. Given the macroscopic and microscopic lesions observed, characteristics of the isolated colonies, Gram staining, and biochemical testing of the isolates obtained from brain, cranial bone, and middle ear samples, we confirmed the diagnosis of cranial osteomyelitis associated with *Pasteurella canis*. Antimicrobial susceptibility testing was performed using the disk diffusion method (Kirby-Bauer) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines to determine the antimicrobial susceptibility of *Pasteurella canis* isolated from the cranial bone (Table 2).

Table 1.
Results of the main biochemical tests on the *Pasteurella canis* strains isolated in this study and other species of the *Pasteurella genus*.

Test	*Pasteurella canis*	*Pasteurella multocida*	*Pasteurella stomatis*	*Pasteurella dagmatis*
Catalase	+	+	+	+
Oxidase	+	+	+	+
Indole production	+	+	+	+
Urease	-	-	-	+
Ornithine decarboxylase	+	+	-	-
Lysine decarboxylase	-	-	-	-
Acid from:				
D-mannitol	-	+	-	-
Dulcitol	-	V	-	-
D-sorbitol	-	V	-	-
Maltose	-	-	-	+
D-arabinose	-	V	-	V

+: Positive; -: Negative; V: Variable

Neurological symptoms such as severe disorientation, torticollis, opisthotonos, and difficulty standing could cause significant losses for the poultry industry owing to poor feed conversion ratios, higher mortality, and increased slaughterhouse condemnation of broilers. Like any infectious disease, it causes immunological stress that drives down food consumption, keeps affected birds from feeding properly, delays growth, and lowers the weight of chickens in slaughterhouses (Boulianne *et al*., 2020).

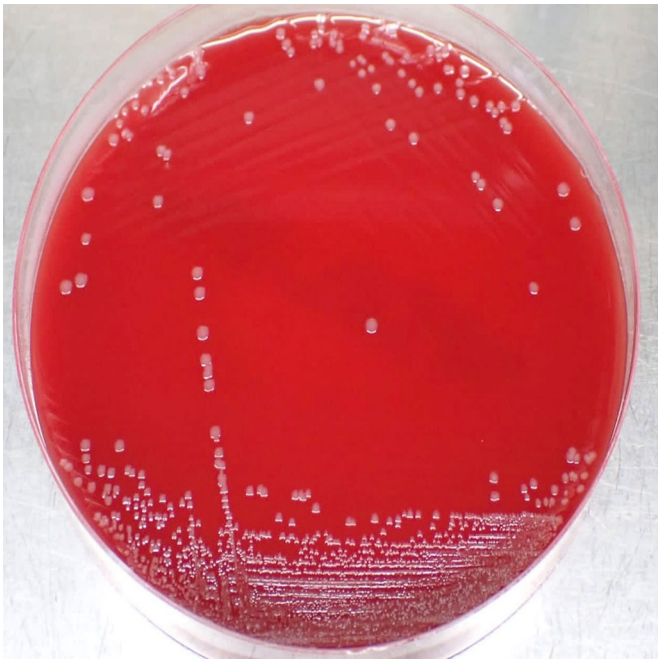

Figure 3.
On 5% blood agar, after 24 h incubation, smooth, grayish-white colonies were observed.

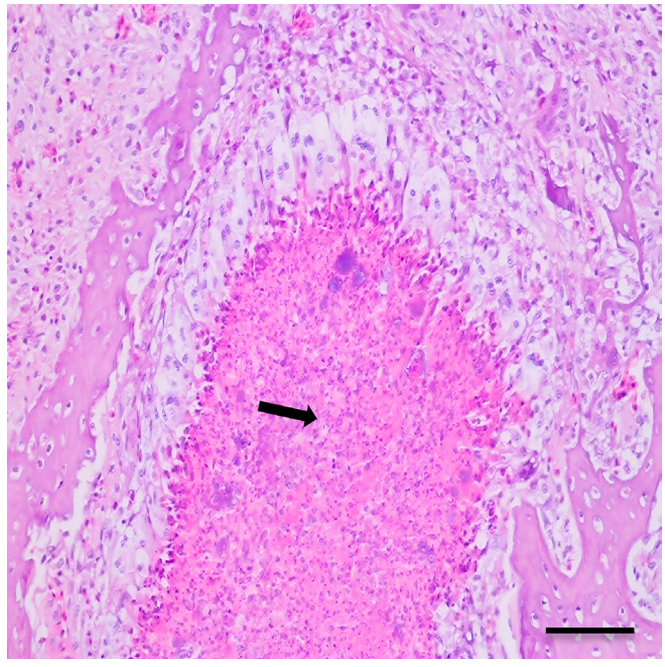

Figure 4.
Histological lesions of affected broiler chickens. Granulomatous osteomyelitis is observed with the presence of inflammatory cells. There is extensive bone resorption and necrotic bone tissue. Several bacteria are observed in the center of the lesion (black arrow). H&E. Bar = 100 µm.

Table 1.
Antimicrobial susceptibility test results for *Pasteurella canis* isolate from cranial bone.

Antimicrobial	Inhibition halo (mm)	Interpretation
Amoxycillin	31	Sensitive
Colistin	15	Sensitive
Enrofloxacin	31	Sensitive
Streptomycin	10	Resistant
Florfenicol	33	Sensitive
Fosfomycin	30	Sensitive
Fosfomycin + Tylosin	25	Sensitive
Lincomycin	0	Resistant
Lincomycin-Spectinomycin	20	Sensitive
Norfloxacin	29	Sensitive
Oxytetracycline	23	Sensitive
Sulfadoxine-Trimethoprim	28	Sensitive
Sulfisomidine	0	Resistant
Tiamulin	15	Intermediate
Tylosin	16	Intermediate

Pasteurella canis, formerly known as *P. multocida* biotype 6, or 'dog type' (Mutters *et al.*, 1985), is part of the normal microbiota of the oral cavity of dogs and cats (Wilson & Ho, 2013). Two biotypes of *P. canis* have been described: biotype 1 is mainly observed in the oral cavities of dogs, whereas biotype 2 is isolated from calves. Biotype 1 is also normally isolated from bite wounds of carrier animals (Gautier *et al.*, 2005). Dogs and cats found near farms are likely sources of infectious agents in poultry, such as *Pasteurella canis*.

Although middle and inner ear infections are uncommon in birds (Shivaprasad *et al.*, 2006), pathogens can migrate from the nasal turbinate, oral cavity, or infraorbital sinus, and extend into the middle ear through the ear canal to colonize the inner ear. Another possibility is that *Pasteurella canis* spreads from the sinuses to the adjacent air-filled skull bones, with subsequent necrosis and the onset of neurological symptoms, producing secondary ear infections (Boulianne *et al.*, 2020). This could explain the absence of any evident inflammation in the inner, middle, or outer ear despite the isolation of *Pasteurella canis* in the middle ear samples. Although the isolated *Pasteurella canis* strain is susceptible to 10 of the 15 antimicrobials tested, its presence in animals intended for human consumption could constitute a serious public health issue. Given its ability to cause infection without a bite or direct inoculation, it can potentially become a zoonotic foodborne pathogen (Hannouille *et al.*, 2019). Furthermore, the use of antimicrobials to control infection increases the risk of antimicrobial resistance owing to the selective pressure of antibiotics and their limited availability for poultry farming. All isolated *Pasteurella canis* strains were sensitive to amoxicillin, consistent with decreased mortality after treatment.

As pathogens can be transmitted directly or indirectly via different routes, the design and implementation of biosecurity measures must consider all possible pathogens and production chain entry routes. In poultry production, biosecurity measures focus primarily on preventing highly transmissible exotic diseases (e.g., avian influenza and Newcastle disease) and foodborne zoonotic diseases caused by bacteria that are part of the normal intestinal microbiota of birds (e.g., *Salmonella* and *Campylobacter*) (Souillard *et al.*, 2024). However, biosecurity measures must also control pathogenic agents with uncommon entry routes, particularly those transmitted by biological vectors, such as dogs and cats living near or inside poultry farming facilities. These vectors include workers' companion animals, wild cats, and guard dogs, which carry pathogens with great zoonotic potential that could emerge through poultry farming, such as *Pasteurella canis*.

DECLARATIONS

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Competing interests

The authors declare that they have no competing interests.

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