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Case report

Bacterial meningoencephalitis in a free Chimango Caracara (*Milvago chimango temucoensis*)

Mauricio Seguel[1*], Manuel Moroni[1], Marcelo Gomez[2], Carlos Hernández[1], Enrique Paredes[1].

[1] Instituto de Patología Animal, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Isla Teja s/n, Valdivia, Chile.
[B] Instituto de Farmacología y Morfofisiología, Facultad de Ciencias Veterinarias,
Universidad Austral de Chile, Isla Teja s/n, Valdivia, ZIP: 5090000, Chile.
***Corresponding Author:** Mauricio Seguel, Instituto de Patología Animal, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Isla Teja s/n, Valdivia, ZIP: 5090000, Chile. Phone + 56-63-221062, e-mail: mseguel@alumnos.uach.cl

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Abstract

A subadult male Chimango Caracara *(Milvago chimango temucoensis)* was found in an agricultural beef cattle field of Southern Chile. The bird was non-responsive to visual or auditory stimulation, and unable to fly. Clinical examination showed moderate body condition, partial blindness and a left wing fracture. The bird was euthanized and a complete necropsy was performed. The most important macroscopic findings were a complete left radius fracture, a subcutaneous hematoma over the occipital bone region and the complete loss of structure of the left pallium and striatum of the telencephalon. Necrotic areas of greenish discoloration in the caudal telencephalon were observed. Histologically, the brain had wide areas of liquefactive necrosis surrounded by abundant inflammatory infiltrate. *Escherichia coli* was isolated from the affected areas of the brain. Although bacterial encephalitis is uncommon among free ranging birds, it should be considered as a candidate diagnosis in wild birds with neurological signs.

Key Words: Encephalitis, *Milvago chimango*, *E. coli*. wild bird.

Introduction

The Chimango Caracara, *Milvago chimango* (Caracarinae, Falconidae; hereafter "Caracara"), is one of the most common and widespread birds of prey in South America. This species inhabits many different ecosystems including agricultural fields, native forests, and urban areas. Caracaras are generalist foragers that can be beneficial animals to farming communities where the species feeds on various small vertebrate and invertebrate agricultural pests (4). However, despite its abundance, wide geographic range, and performance of important ecological services, reports about diseases in this species are scarce (17, 18).

In wild raptors, most reported cases of encephalitis are caused by viral agents, including some agents of zoonotic importance, such as West Nile virus (20, 21). Bacterial encephalitis are rarely reported in wild or aviary birds, although is believed that any bacteria reaching the central nervous system can produce damage to the tissues (3). *Escherichia coli* is mostly reported as a secondary pathogen in birds, producing disease when host response mechanisms have been decreased by noxious agents such as viruses or environmental stress (10). For example, in poultry, *E. coli* has been associated with encephalitis as a consequence of bacteremia where the source of infection is a respiratory disease (sinusitis) (8). This paper reports a case of necrotic meningoencephalitis due to a localized *E. coli* infection in a juvenile Caracara found moribund in a beef cattle farm of southern Chile.

Case Report

A subadult male Caracara (200 g weight approximately), was found in an open beef cattle field in a rural area of Villarrica county, southern Chile (39°16'S, 72°19'W). The bird was found standing with the head at shoulder height, both wings held limply and the wingtips

dragging on the ground. The bird did not respond to visual or auditory stimuli, but moved away when touched. The animal was sedated in the field to perform a complete physical examination which revealed partial blindness, moderate body condition and a partial compound fracture of the left radius. No cerebrospinal fluid analysis or imaging studies were performed. Because of the severity of clinical signs and lesions, the bird was euthanized.

***Pathology*.** A complete postmortem examination was performed immediately after euthanasia. All macroscopic lesions were recorded and samples of brain, heart, lung, kidney, liver, spleen, bursa, pancreas, thyroid glands, skeletal muscle, bone marrow, aorta, trachea, crop, proventriculus, ventriculus, pancreas, small intestine, colon, adrenal gland and eye were collected and stored in 10% buffered formalin. These tissues were processed by standard histological techniques, sectioned at 5 µm, stained with hematoxylin and eosin (H&E) and observed by light microscopy. Selected tissues also were submitted to special stains using Schiff´s periodic-acid (PAS), Ziehl-Neelsen, and Gram (Brown-Brenn method) (19).

***Bacteriology*.** Samples of liver, kidney, lung, spleen, blood and brain were aseptically collected, and subsequently cultured on agars of 5% sheep blood, McConkey and XLD, and incubated at 37°C for 48 hr. Isolates from these agars were identified following standard methods (2).

Antimicrobial susceptibility testing was performed on the *E. coli* isolate, using the disc diffusion method according to the Clinical and Laboratory Standards Institute (6). Antimicrobial drugs tested were amikacin (AMK), amoxicillin-clavulanic acid (AMC), ampicillin (AMP), cefazolin (CFZ), ceftiofur (CFT), ceftizoxime (ZOX), chloramphenicol (CHL), enrofloxacin (ENR), gentamicin (GEN), tetracycline (TET), Cefoperazone (CFP), Cefuroxime (CXM) and trimethoprim-sulfamethoxazole (SXT).

***Virology*.** Tissue samples from liver, kidney, spleen, lung and brain were macerated, suspended in antibiotic medium, and filtered. This solution was inoculated in the allantoic and amniotic cavities of 10- to 11-day-old embryonated specific pathogen free chicken eggs for 48 to 72 h at 35°C to 37°C. A total of three inoculation passages were performed.

Results

Macroscopic observation revealed a partial compound fracture of the left radius with mild signs of hemorrhage and healing of the surrounding muscular and subcutaneous tissues. Moderate atrophy of pectoral musculature and lack of subcutaneous and intracelomic adipose tissues were also observed. The ventriculus had trace amount of food material, and the small intestine presented scarce content. Gastrointestinal parasites were not found. In the subcutaneous tissue over the occipital bone a greenish hematoma measuring 1 cm in diameter was observed, coincident with a small puncture wound (0.5 mm diameter) in the skin. At the opening of skull, meninges were dark red over the caudal portion of the cerebrum. The brain presented an abnormally soft consistency with complete loss of normal structure in the left striatum and pallium of the telencephalon. Additionally, greenish areas with necrotic appearance were observed in the caudal telencephalon (Fig. 1). Histopatology examination of the telencephalon revealed wide areas of liquefactive necrosis extending from the cerebral cortex to the white matter. In these areas, leptomeninges were impossible to identify because of the extensive loss of normal tissue structure. However in zones adjacent to necrotic tissue, leptomeninges were markedly infiltrated by macrophages and heterophils. Surrounding the necrotic cerebral tissue there were abundant inflammatory infiltrations composed of microglial cells, lymphocytes, and few heterophils (Fig. 2). These cells were in several stages of degeneration and necrosis. Large amounts of short, basophilic (H&E), Gram negative (Brown and Brenn) bacilli were observed in the liquefactive areas and inside macrophages (Figures 2 and 3). Heterophils and macrophages were also randomly scattered throughout the cerebral parenchyma (Fig. 4). In these areas some neurons and glial cells presented several stages of degeneration and necrosis, and the neuropil showed edema. In the liver moderate hyperplasia of periportal lymphoid centers was observed. Also, there was moderate infiltrate of heterophils and macrophages. Moderate lymphoid hyperplasia was observed in the bursa and spleen.

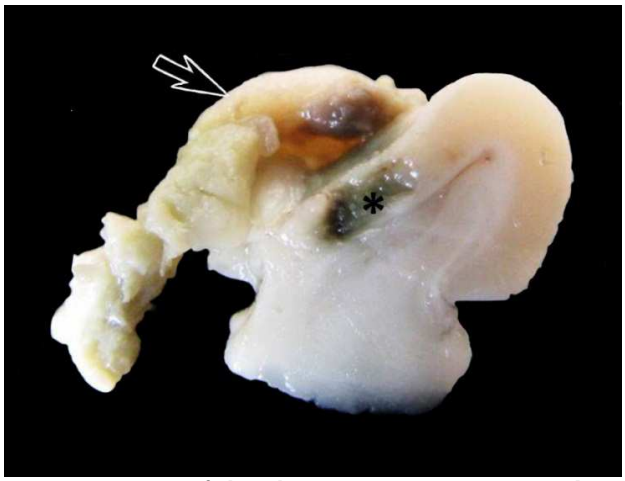

Figure 1 - Brain of the Chimango Caracara. Transversal cut There is a complete loss of normal structure of the left caudal telencephalon hemisphere (Arrow), and a dark greenish area of necrosis in the pallium of the right caudal telencephalon (Asterisk) (Formaline fixed specimen).

No bacteria were isolated from blood, kidney, liver, lung, or spleen. The brain samples cultured on blood agar showed moderate growth of bacterial colonies, about 1-2 mm in diameter, without hemolysis. This strain produced pink lactose-positive colonies in Mc Conkey agar

and yellow xylose-positive colonies in XLD agar. Additional biochemical characteristics included fermentation of indole, sucrose, lactose and glucose but not citrate. This isolated was positive for production of lysine decarboxylase and catalase but negative for production of ornithine decarboxylase, urease and sulfide hydrogen. These reactions were consistent with the identification of the isolated as *Eschericia coli* (Barrow and Feltham 2003). The strain was sensitive to most antibiotics tested, but resistant to amoxicillin-clavulanic acid.

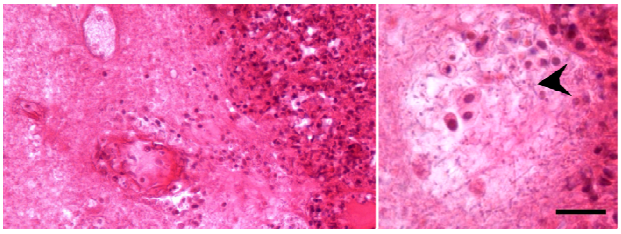

Figure 2 - Brain of the Chimango Caracara . Extensive liquefactive necrosis area (N) surrounded by abundant mononuclear inflammatory infiltrate (I). H&E; bar= 50µm. Inset: Higher magnification from the necrosis-inflamatory infiltrate interphase. Abundant short bacilli (arrowhead) are distributed through the brain parenchyma. H&E; bar= 15µm.

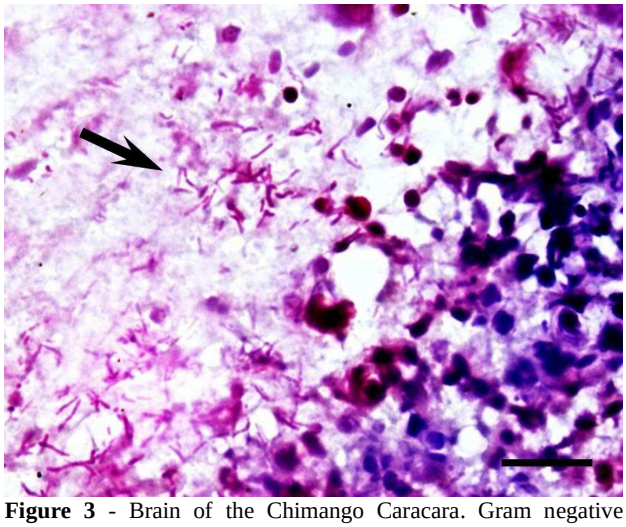

Figure 3 - Brain of the Chimango Caracara. Gram negative bacilli in the necrotic tissue (arrow) surrounded by inflammatory cells. Gram stain (Brown and Brenn); bar= 15µm.

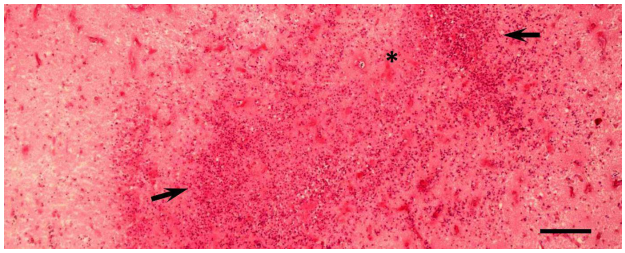

Figure 4 - Brain of the Chimango Caracara. Large amount of inflammatory infiltrate (arrows) scattered throughout the brain parenchyma associated to necrosis (asterisks). H&E; bar = 100 µm.

The virological assessment showed no lesions in embryos after three serial passages.

Discussion

The Caracara described herein had clinical signs such as blindness and auditory impairment. These signs are coincident with a lesion of the pallium and striatum of the caudal telencephalon, because these areas are important pathways for audition and vision in birds. Because of the wing fracture, the neurological signs could have been attributed to recently trauma, however, postmortem examination revealed a severe necrotic meningoencephalitis associated with *Escherichia coli* localized infection. Although difficult to establish accurately, the traumatic lesion of the head seemed older than the fracture of the wing. This suggests that the wing fracture was generated as consequence of the severe neurologic damage to the bird. This is consistent with several reports of the cause of mortality in raptors wherein underlying conditions such as infectious disease or neoplasia lead to fatal trauma (15, 12, 16).

The puncture lesion found on the head was likely produced by intraspecific aggression. Such aggression is typical of several species of Caracaras where juveniles and subadults are subordinate to older and bigger conspecifics (11). The pathological findings also suggest that entry of bacteria to the brain could have occurred through this puncture lesion. The lack of bacteria isolation from blood and major organs, and the absence of any acute or chronic lesions due to bacteria in most tissues support this idea. A single collision event was unlikely to have simultaneously caused both the puncture lesion in the head and the broken wing because healing around the wounds indicated they happened at different times. Although no macroscopic fractures were found on skull, a small fissure could have been overlooked. Head trauma is one of the major causes of acquired meningitis in adult human patients, and is sometimes recorded without any sign of skull fracture (1), thus supporting the hypothesis. In these cases bacteria colonize the cerebrospinal fluid directly at the cerebelomedullary cistern causing acute meningoencephalitis typical of the clinical signs in this case (13). However, in poultry *E. coli* is reported rarely as cause of meningitis and/or encephalitis, and is generally as a consequence of colisepticemia (8).

The spread of resistant bacteria from livestock to wild animals in close proximity to farms has been indicated as possible explanation for the occurrence of resistant bacteria in birds of prey (5, 11). Because of their predatory nature, raptor species may serve as important indicators of environmental contamination with antimicrobial-resistant bacteria (14). Since this bird was found in an agricultural ecosystem where cattle farming were the main activity, some degree of resistance in the bacterium isolated would have been expected. On the another hand, the resistance profile of this bacterium was very low compared with data from wild birds all over the

world (9, 11), even though most pathogenic *E. coli* isolated from bovines in Southern Chile present a multi-resistance profile (Moroni, unpublished data). This suggests that the bacterium that caused the meningoencephalitis described herein could have been part of the bird's normal microbiota rather than an environmental bacterium. This would support the thesis of the inoculation of the bacteria through intraspecific aggression, however to prove the source of the bacterium was not possible in this case.

Differential diagnoses of encephalic neurologic signs in birds include other infectious diseases (i.e. chlamydiosis, aspergillosis, sarcocystosis, Newcastle´s disease, etc.), intoxications by heavy metals (lead, zinc, mercury) and vitamins (E and B-complex) deficiency (7). Additionally, free ranging birds with neurological signs should be examined by carefully looking for diseases of importance to the public health such as botulism, avian influenza or West Nile virus. Although bacterial encephalitis is uncommon among free ranging birds, encephalitis should be considered as a differential diagnosis in wild birds with neurological signs, especially if a history of trauma or other weaknesses are known to exist in an individual bird.

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