SHORT COMMUNICATIONS

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Oropharyngeal Trichomonosis Due to *Trichomonas Gypaetinii* in a Cinereous Vulture (*Aegypius Monachus*) Fledgling in Spain

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ABSTRACT: A juvenile Cinereous Vulture (*Aegypius monachus*) fledgling was found disorientated on the roof of a building in Madrid City, Spain, in October 2016. A veterinary examination revealed multiple plaques distributed throughout the oropharyngeal cavity. Lesions were located under the tongue and at the choanal slit, hard palate, and esophagus opening and ranged from 2 to 7 mm, coalescing in areas up to 2 cm, with a yellowish color of the surface. Motile trichomonad trophozoites were detected in fresh wet mount smears from the lesions. Sequence analysis of the internal transcribed spacer (ITS)1/5.8S/ITS2 and small subunit ribosomal RNA confirmed that *Trichomonas gypaetinii* was the etiologic agent. Microbiologic cultures did not reveal any pathogenic bacteria or fungi. The animal recovered successfully after treatment with metronidazole and trimethoprim-sulfamethoxazole and was later released in a suitable habitat. Avian trichomonosis lesions caused by *T. gypaetinii* have not been reported.

Key words: Avian trichomonosis, Cinereous Vulture, macroscopic lesions, *Trichomonas gypaetinii*.

Trichomonas gallinae is a protozoan parasite found at high prevalence in Columbiformes—the reservoir hosts—in which subclinical effects predominate (Villanúa et al. 2006; Amin et al. 2014; Marx et al. 2017). Clinical signs are most frequent in Accipitriformes, Falconiformes, and Strigiformes and include caseonecrotic granulomas that occupy the lumen of the oropharyngeal cavity, crop, and proximal esophagus; impair the swallowing of food; and could provoke death by starvation. Avian trichomonosis is the most frequent infectious disease of raptors in wildlife recovery centers (Molina-López et al. 2011). Clinical forms of the disease have never been described in vultures.

Avian trichomonosis also has been identified as an emergent parasitic disease among Passerine species with several outbreaks across Europe and North America (Amin et al. 2014). The etiology of the disease is complex, since new genetic variants and species have been recently described, like *Trichomonas stableri* and *Trichomonas gypaetinii* (Girard et al. 2014; Martínez-Díaz et al. 2015; Martínez-Herrero et al. 2017). *Trichomonas gypaetinii* was found only in asymptomatic Egyptian Vultures (*Neophron percnopterus*), a Bearded Vulture (*Gypaetus barbatus*), a Bald Eagle (*Haliaeetus leucocephalus*), and Cinereous Vultures (*Aegypius monachus*).

The Cinereous Vulture is an endangered species in Europe because of direct persecution and poisoning with diclofenac, strychnine, carbofuran, and aldicarb, among other substances (Hernández and Margalida 2008; BirdLife International 2018). Additionally, changes in the law about the management of carcasses left in the fields by farmers because of the bovine spongiform encephalopathy crisis have also affected vulture populations (Regulation [EC] 1774/2002 of the European Parliament and of the Council, subsequently replaced by EC 1069/2009). Several conser-

vation programs, such as the one carried out by Grupo de Rehabilitación de la Fauna Autóctona y su Hábitat (GREFA), are trying to increase the number of individual vultures and to connect the different meta-populations across Europe by assisting in the reintroduction of animals in the Iberian Peninsula, Italy, and the Balkans region.

Here we report a description of oropharyngeal trichomonosis in a Cinereous Vulture fledgling admitted to the GREFA veterinary hospital (Majadahonda, Madrid, Spain). The animal was captured from the roof of a building in Madrid City, Spain, in October 2016. It was emaciated (4.3 kg), weak, and depressed, with 10% dehydration, stress bands on plumage, and infested with mallophages. Its body temperature was 39.6 C, and a poor prognosis was assessed. Values of serum protein profile, blood cell counts, and biochemistries were found to be within the reference range (Seok et al. 2017).

Several lesions ranging from 2 to 7 mm and resembling the membranous type of avian trichomonosis (Samour and Naldo 2003) were observed in the oropharynx. Lesions were located under the tongue and at the choanal slit, hard palate, and esophagus opening, with a yellowish to light brown color of the surface (Fig. 1a, b). Samples were taken with sterile cotton swabs from the upper digestive tract for microbiological cultures and parasitological examination.

Trophozoites with their characteristic size, morphology, and motion were visualized on a direct wet mount smear made from one swab. Another swab, previously moistened in the culture medium, was inoculated into 5 mL of pH 6.5 tryptose-yeast-maltose (TYM) medium (Martínez-Herrero et al. 2014). The culture was immediately incubated at 37 C and visually monitored with an inverted microscope every 24 h. Trophozoites moved slightly but they did not grow in TYM and died within 24 h.

To characterize the trichomonads, DNA was extracted from 1 mL of culture sediment at passage 0 using a commercial kit (DNeasy Blood and Tissue Extraction Kit, QIAGEN, Valencia, California, USA). A PCR and sequence analysis was performed including the ITS1/5.8S/ITS2 region (ITS) and ribosomal RNA small subunit (SSU) gene. Oligonucleotide primers and thermal cycler temperature profiles were used according to Ganas et al. (2014). All reactions were carried out in a GeneAmp 2700 thermal cycler (Applied Biosystems, Foster City, California, USA). Amplified products were analyzed by electrophoresis in 1% agarose gels stained with ethidium bromide. Results were observed under ultraviolet light in a transilluminator (Syngene, Cambridge, UK).

Amplicons were submitted to a laboratory (Sistemas Genómicos SA, Paterna, Valencia, Spain) for Sanger sequencing in a 3730XL DNA Analyzer with the ABI PRISM® BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems). Sequence chromatograms were examined in both directions and assembled with Lasergene SeqMan software version 7.0.0 (DNASTAR, Madison, Wisconsin, USA). The Nucleotide Basic Local Alignment Search Tool (BLAST, version 2.7.0) was employed for the genetic study by the MegaBlast algorithm (optimization for highly similar sequences) and low–complexity region filter (National Center for Biotechnology Information 2017a). Sequences were compared with the GenBank database (National Center for Biotechnology Information 2017b). The length of sequences obtained were 295 nucleotides for the ITS region and 1,433 nucleotides for the SSU region. Both sequences had a 100% BLAST identity with the corresponding sequences of *T. gypaetinii* (KF993707 for the ITS and KM246610 for the SSU).

Bacterial flora of the upper digestive tract was investigated employing Columbia agar with 5% defibrinated sheep blood (Oxoid SA, Madrid, Spain), chocolate agar with IsoVitaleX (Becton-Dickinson, Sparks, Maryland, USA), MacConkey agar (Oxoid SA), and Sabouraud dextrose agar with chloramphenicol (Oxoid SA). Oral samples were tested for the presence of *Candida* spp. in CHROMagar Candida Medium (Becton-Dickinson).

MacConkey agar revealed the presence of *Escherichia coli* in the oropharynx. Addition-

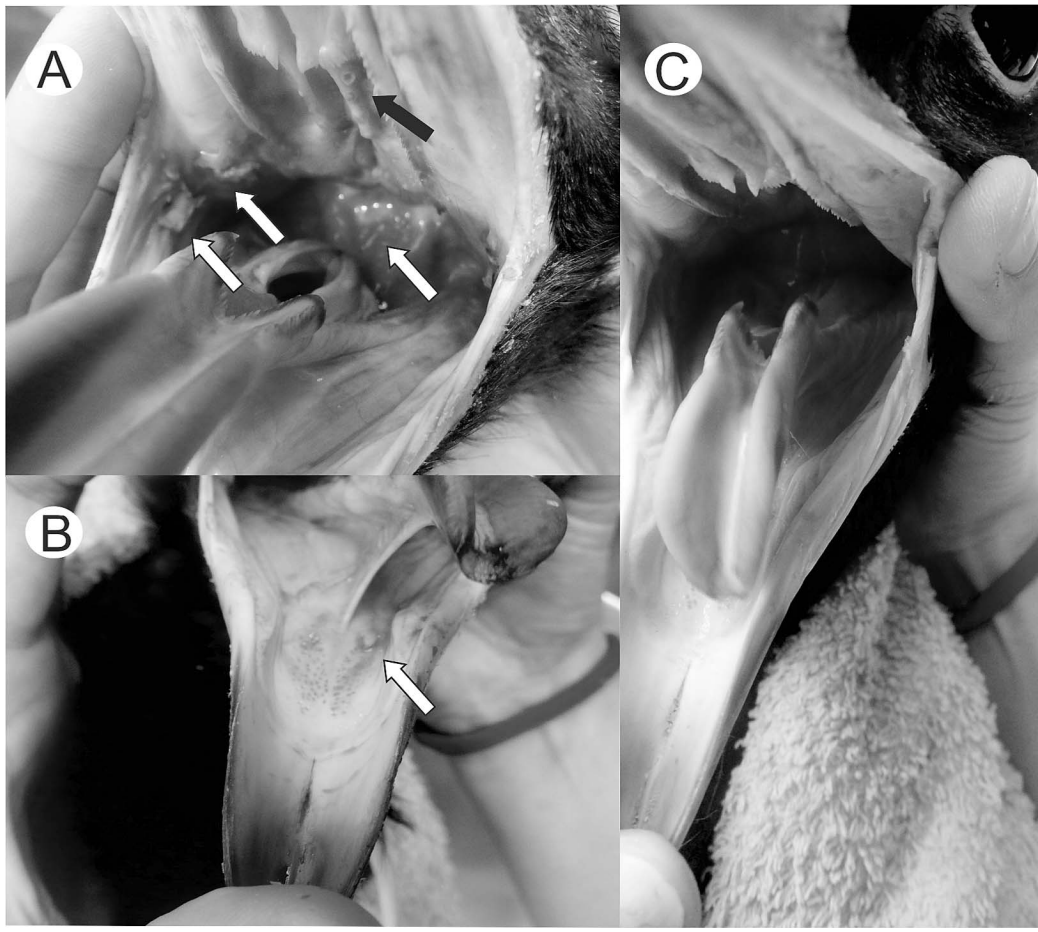

FIGURE 1. Oropharyngeal cavity of a juvenile Cinereous Vulture (*Aegypius monachus*) found ill in Madrid City, Spain, in October 2016 before and after treatment. (a) Several granulomas in the upper oropharyngeal cavity (white arrows) and small ulcer at the choanal slit (black arrow). (b) Granulomas in the lower oropharyngeal cavity. (c) Oropharyngeal cavity of the animal after treatment.

ally, different colonies of nonfermenting gram-negative coccobacilli were isolated onto Columbia agar with 5% defibrinated sheep blood and chocolate agar with IsoVitaleX. No fungi were isolated in Sabouraud dextrose agar. Cultures for *Candida* spp. were also negative.

The vulture received antibiotic treatment with trimethoprim-sulfamethoxazole (Septrin Pediatric Suspension, UCB Pharma, Madrid, Spain) at 48 mg/kg orally for 10 d and metronidazole (Metrobactin, 250 mg, Dechra Pharmaceuticals, Northwich, UK) at 50 mg/kg orally for 5 d. A new plaque was observed in the larynx 10 d after finishing the metronidazole administration, and a second 5-d metronidazole treatment was provided. Supplementary food, progressive rehydration, and general care produced a successful recovery of the lesions after 32 d and a body mass of 6.9 kg (Fig 1c).

Several etiologies need to be considered as possible causes of oropharyngeal lesions in wild birds. With our case, poxvirus and hypovitaminosis A were discarded because of the characteristics of the macroscopic lesions, and aspergillosis and candidiasis were eliminated according to the microbiological analysis results (Deem 2003; Schmidt et al. 2015). Visualization of motile trophozoites in a wet

mount smear from the upper digestive tract and resolution of the lesions after treatment with metronidazole added circumstantial evidence for *T. gypaetinii* as the etiologic agent in the Cinereous Vulture.

Bird trichomonads are able to survive in their host environments in absence of lesions for up to 20 mo (Bunbury et al. 2007). The finding of this flagellate in a weakened vulture might have reflected the opportunistic nature of this protozoan, and the poor nutritional state of the fledgling could have favored the development of lesions. Indeed, opportunistic yeast infections in vulture nestlings have been reported (Pitarch et al. 2017), possibly because of the antibiotic residues from the carrion that is their diet. In the present case, no fungi, yeasts, or bacteria compatible with the observed lesions and signs were detected.

Mixed infections have been documented previously with different *T. gallinae* genotypes, or even different species in the same host (Sansano-Maestre et al. 2009; Grabensteiner et al. 2010; Girard et al. 2014; Martínez-Herrero et al. 2014). In the present case, molecular analysis of the sequence chromatograms for the ITS and SSU regions eliminated the possibility of mixed infections. Besides, *T. gallinae* has a rapid in vitro growth in TYM medium compared with *T. gypaetinii*, which is difficult to grow in the same medium. Wildlife recovery centers must be aware of this emerging pathogenic organism and inclusion of avian trichomonosis in the differential diagnosis of oropharyngeal lesions in vultures is recommended.

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