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Oral mycoses in avian scavengers exposed to antibiotics from livestock farming

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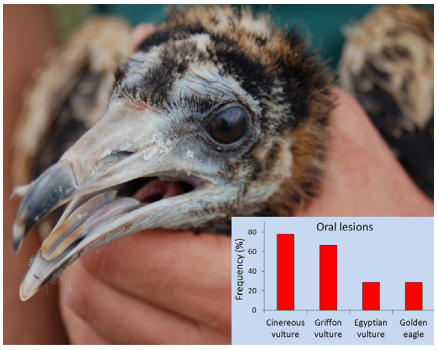
HIGHLIGHTS

- Fungal infections pose a major threat to animals worldwide.
- Thrush-like lesions were recorded in four avian scavenger species exposed to antibiotics.
- 14 yeast species were isolated from the oral lesions in this unprecedented outbreak.
- These finding are alarming due to the delicate conservation status of the affected hosts.

GRAPHICAL ABSTRACT



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ABSTRACT

The exposure to antimicrobial pharmaceuticals as environmental contaminants can exert direct and indirect detrimental effects on health of wildlife. Fungal infections pose a major threat to domestic, captive-housed wild and free-ranging wild animals worldwide. However, little is known about their role in disease in birds in the wild. Here, we evaluated the incidence of thrush-like lesions in the oral cavity of wild nestling cinereous vultures (*Aegypius monachus*), griffon vultures (*Gyps fulvus*), Egyptian vultures (*Neophron percnopterus*) and golden eagles (*Aquila chrysaetos*) exposed to veterinary antibiotics via the consumption of medicated livestock carcasses. Lesions, which varied in number, size and location, were more frequent in the cinereous (77.8%, $n = 9$) and griffon vultures (66.7%, $n = 48$) than in the Egyptian vultures (28.6%, $n = 21$) and golden eagles (28.6%, $n = 7$). In all individuals (100%, $n = 24$) of a subsample of the affected nestlings, yeast species were isolated from thrush-like oral lesions and identified using a well-established system based on their carbohydrate assimilation profiles and other complementary tests. Fourteen yeast species from seven genera (*Candida*, *Meyerozyma*, *Pichia*, *Yarrowia*, *Cryptococcus*, *Rhodotorula* and *Trichosporon*) were isolated from the lesions of the four host species. We found differential infections and effects depending on host age-related exposure or susceptibility to different yeast species across the development of nestling griffon vultures. This unprecedented outbreak of oral mycoses is alarming because of the delicate conservation status of several of the affected species. The role of livestock antibiotics in the transition of yeast species from commensal to opportunistic pathogens should be evaluated in an attempt to avoid the detrimental effects of contamination and disease on host health, as well as on the transmission of fungal emerging pathogens among wildlife populations and species, and their dissemination across livestock and human populations.

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1. Introduction

Fungal infections have recently caused alarming population declines in several wild animals and plants worldwide (Fisher et al., 2012). Diverse yeast-like fungal species, such as some *Candida* spp., are common commensal members of the normal microbiome in the oral cavity and gastrointestinal tract of humans, livestock and wild animals (Velasco, 2000; Cabañes, 2010; Farah et al., 2010; Mukherjee et al., 2015; Neville et al., 2015). However, these and other opportunistic fungi can cause infection when an imbalance is produced in their ecologic niches. This can occur from an impairment of the host's immune system, disruption of the host's natural barriers, or alterations in the host's normal microbiota composition due, for example, to the prolonged use of broad-spectrum antibacterial therapy (Lin et al., 2005; Pitarch et al., 2006; Hebecker et al., 2014). The exposure to antimicrobial pharmaceuticals as environmental contaminants can exert direct detrimental effects on wildlife (Arnold et al., 2014). Indirect effects on health by promoting the alteration of the normal and protective microbiota are also likely but they have been much less investigated.

In birds, opportunistic fungal infections (in particular, candidiasis) have mostly been reported in stressed, immunocompromised and medicated poultry and pets (Mancianti et al., 2002; Vieira and Acqua-Coutinho, 2009; Wu et al., 2012). Their main clinical manifestations include circular and ulcerative milky nodules and plaque-like areas that often form clusters in the upper areas of the digestive tract (Friend and Franson, 1999; Velasco, 2000; Deem, 2003), resembling oral thrush in humans (Farah et al., 2010; Lalla et al., 2013). Other clinical manifestations of these mycoses in poultry and pet birds comprise dysphagia, regurgitation, vomiting, retarded growth, ruffled feathers, depression, emaciation and anorexia (Friend and Franson, 1999; Velasco, 2000; Deem, 2003). In apparently healthy wild individuals, multiple yeast species have been isolated from normal or transient gastrointestinal microbiota (Cafarchia et al., 2006; Brilhante et al., 2012). Oral mycoses have also been described in wild birds that are temporally or permanently housed in captivity and have several predisposing factors, including underlying immunosuppressive or debilitating diseases, prolonged therapy with broad-spectrum antibiotics, malnutrition, unsanitary conditions, and captivity-related stresses (Velasco, 2000; Deem, 2003; Caliendo and Bull, 2011; Brilhante et al., 2012). However, fungal infections have rarely been found causing clinical lesions in free-range wild birds (Friend and Franson, 1999; Redig, 2003).

Because yeasts are cosmopolitan and ubiquitous saprophytes, environments contaminated with refuse, wastewater from urban effluents, and litter and excreta from livestock operations could favor their proliferation and dissemination (Friend and Franson, 1999; Feng et al., 2010; Meissner et al., 2015). These unsanitary conditions, including the accumulation of decomposing carcasses and the fecal material of several livestock species, putrid water, and the spread of unwanted organisms like rats, often concur in feeding stations used as management and conservation tools for vultures and other avian scavengers worldwide (Cortés-Avizanda et al., 2016). In particular, European vultures have increased their dependence on livestock carcasses from these feeding stations and farm surroundings over carcasses of free-ranging livestock (Cortés-Avizanda et al., 2016). These carcasses could pose health risks to avian scavengers due to ingestion of veterinary pharmaceuticals (Blanco et al., 2016, 2017a, 2017b), exposure to parasites (Blanco et al., 2017c) and microbial pathogens that may have developed multi-resistance mechanisms to these drugs (Blanco, 2015). This is mainly because carrion used for this purpose is generally from livestock from factory farming (such as poultry and swine) that are often sick, medicated just before death, and disposed of in feeding stations a short time afterwards without a withdrawal period or residue control (Blanco et al., 2016, 2017a; Cortés-Avizanda et al., 2016).

The ingestion of veterinary drugs present in carcasses of medicated livestock may have direct effects on the health of avian scavengers with eventual catastrophic consequences on their populations, as occurred in the diclofenac crisis in Asia (Prakash et al., 2012). The antimicrobial agents frequently used in livestock farming may potentially have direct negative effects on scavenger's health when an acute exposure occurs from intensively medicated livestock carcasses, but also indirect negative effects. They can depress the ability of the immune system to fight against infection (Walker, 2000; Keeney et al., 2014), alter the host microbiota composition and cause other imbalances that lead to gut dysbiosis and colonization and proliferation of opportunist pathogens (Levy, 2000; Keeney et al., 2014). Antibiotic misuse or overuse can be directly implied in the overgrowth of opportunist fungi, especially *Candida* species (Cunha, 2001; Caliendo and Bull, 2011), able to cause superficial or even invasive mycoses by eliminating or disturbing competing bacteria of the host microbiome (Lin et al., 2005; Pfaller and Diekema, 2007; MacCallum, 2010).

The occurrence of disease, represented by gross lesions compatible with *Candida*-caused fungal infections, has been recorded in the oral cavity of nestling and adult wild vultures exposed to livestock antibiotics in central Spain (López-Rull et al., 2015; Blanco et al., 2017a). These lesions appear as milky, prominent and strongly attached nodules of variable size with a circular-to-elliptic form that often extends to larger and ulcerated plaque-like areas. The prevalence and the number of *Candida*-like yeast lesions are higher in nestlings than in adult vultures, and individuals with more lesions show a weaker hemagglutination reaction than those with a lower intensity infection (López-Rull et al., 2015). Microscopic observation of samples collected from these lesions revealed the presence of abundant yeast-like fungi as the potential etiological agents, but failed to record parasites such as *Trichomonas* sp. or helminths (Blanco et al., 2017a), which can cause similar lesions in the oral cavity of birds (Friend and Franson, 1999; Carter et al., 2008). Despite the widespread array of yeast species that may cause pathogenic effects in avian scavengers exposed to fluoroquinolones and other drugs, no study has yet identified the species involved in the mycoses recorded thus far.

In this work, we evaluated the prevalence, number and location of lesions present in the oral cavity of nestling cinereous vultures (*Aegypius monachus*), griffon vultures (*Gyps fulvus*) and Egyptian vultures (*Neophron percnopterus*), three obligate avian scavengers exposed to antibiotics through livestock carcasses in central Spain (Blanco et al., 2016, 2017a). We also examined nestling golden eagles (*Aquila chrysaetos*), a top predator and facultative scavenger raptor exposed to these drugs (Blanco et al., 2017b). For a subgroup of nestling cinereous and griffon vultures, we further conducted a selective cultivation and characterization of yeast-like fungi isolated from oral lesions to identify the species involved in infection. This information was complemented with the identification of yeast species from the lesions recorded in single nestlings of the other two obligate and facultative scavenger species. In addition, we assessed potential associations between the occurrence of particular yeast species in the oral lesions and host features, including age, sex and stressful conditions during growth in the griffon vulture, for which we could obtain a larger number of samples from nestlings with lesions.

2. Materials and methods

2.1. Study area, study species and fieldwork

Regular and intensive nest monitoring of the populations of obligate and facultative avian scavengers was conducted throughout the breeding season of 2013 and 2014 in the Central Mountains and associated canyons of the Castilian Highlands in central Spain (see details of the study area in Fargallo et al., 1998; Blanco, 2014). This area have one of the highest concentrations of farms devoted to fattening pigs reared under intensive conditions in Spain (Blanco, 2014).

The study species are listed in different categories of conservation, ranging from 'Least Concern' in the case of the griffon vulture and golden eagle, 'Near Threatened' in the cinereous vulture, and globally