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Food safety in scavenger conservation: Diet-associated exposure to livestock pharmaceuticals and opportunist mycoses in threatened Cinereous and Egyptian vultures

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ABSTRACT

Pharmaceuticals from veterinary treatments may enter terrestrial food webs when medicated livestock are available to wildlife in supplementary feeding stations aimed at the conservation of endangered scavengers. Here, we hypothesized that the exposure risk to livestock fluoroquinolones, as indicators of pharmaceutical burden in food, is related to the variable reliance of scavengers on domestic versus wild animal carcasses. Since the misuse of broad-spectrum antibiotics is a major predisposing factor for opportunistic mycoses, we evaluated disease signs potentially associated with diet-dependent drug exposure in nestlings of two threatened vultures. A greater occurrence (100%, n=14) and concentration of fluoroquinolones (mean ± SD=73.0 ± 27.5 μg L^{-1}, range=33.2–132.7), mostly enrofloxacin, were found in Cinereous vultures, *Aegypius monachus*, due to their greater dependence on livestock carcasses than Egyptian vultures, *Neophron percnopterus* (fluoroquinolones occurrence: 44%, n=16, concentration: 37.9 ± 16.6 μg L^{-1}, range=11.5–55.9), which rely much more on carcasses of wild animals (42% of remains vs. 23% in the cinereous vulture). The chaotic, chronic and pulsed ingestion of these drugs throughout nestling development is proposed as one of the most plausible explanations for the high occurrence and intensity of oral *Candida*-like lesions in nestling vultures. The high occurrence of fluoroquinolone residues and disease hindered the probing of a cause-effect relationship between both factors in individual vultures. This relationship could be evaluated through a population-based approach by sampling vultures not exposed to these drugs. The high dependence of vultures on domestic animals today compared to past decades and the growing intensification of livestock farming, imply an expected increase in the impact of pharmaceuticals on scavenger populations. This requires further evaluation due to potential consequences in biodiversity conservation and environmental health. We encourage the prioritization of efforts to promote the use of less medicated free-ranging livestock carcasses left in the countryside, rather than stabled stocks made available in vulture restaurants. Additionally, attention should be paid to the population recovery of wild species that dominated scavenger diets in the past.

1. Introduction

Environmental contamination with pharmaceuticals is a major public health concern (Boxall, 2010; Arnold et al., 2014). The occurrence of anti-inflammatories, antibiotics, analgesics, hormones, etc. has been repeatedly recorded in aquatic environments as a consequence of their continuous release from urban sewage systems and livestock operations (Daughton and Ternes, 1999, Kummerer, 2010; Gothwal and Shashidhar, 2015). Pharmaceuticals, generally designed to be highly specific and potent in their respective therapeutic effects, may be biologically active and toxic to non-target organisms even at very low concentrations (Christen et al., 2010; Corcoran et al., 2010; Brausch et al., 2012). These compounds are prone to bioaccumulation in long-term or chronic exposure conditions and bio-magnification in food chains (Boxall et al., 2010; Lazarus et al., 2015). As a consequence, multiple adverse effects of their residues have been reported in aquatic wildlife (Robinson et al., 2005; Christen et al., 2010; Brausch et al., 2012; Fabbri and Franzellitti, 2016). In terrestrial

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ecosystems, wildlife exposure to pharmaceuticals may arise as a consequence of the use of contaminated effluents into irrigation of agricultural areas or by direct manuring with litter and excreta of medicated livestock (Sharma et al., 2009; Daughton and Ruhoy, 2009; Boxall, 2010). Pharmaceuticals from veterinary treatments may also enter terrestrial food webs when excreta and carcasses of medicated livestock become available for coprophagous and scavenger wildlife (e.g. Floate et al., 2005; Richards et al., 2014; Hammer et al., 2016).

Attempts to reconcile sanitary and environmental policies have addressed the exposure to veterinary pharmaceuticals from livestock carcasses in the management and conservation of avian scavengers in Spain (Donázar et al., 2009; Margalida et al., 2014). Spain is the major stronghold for obligate and facultative scavengers in the Western Palearctic. Several species that were very abundant in the past, especially the Egyptian vulture (*Neophron percnopterus*) and the Red kite (*Milvus milvus*), have suffered severe declines (Blanco and Montoya, 2004; Donázar, 2004; Carrete et al., 2007; Blanco, 2014). In addition, decreases in population growth and breeding success in other species, such as the Eurasian griffon vulture (*Gyps fulvus*) and the Cinereous vulture (*Aegypius monachus*), have been recorded in Spain over the past few decades (Camiña, 2004, Donázar et al., 2002, 2009, authors' unpublished data). The reduction and changes in the nature of food supplies available for scavengers has been repeatedly highlighted as one of the main causes of these declines and the reduction in breeding success (Donázar, 2004; Sánchez, 2004). However, despite great interest and concern in the distribution, accessibility and availability of food for avian scavengers due to the impact of sanitary regulations (Tella, 2001; Donázar et al., 2009), there exists little information on the role of the different livestock species from stabled and extensive farming schemes in scavenger diets (Donázar et al., 2010; Blanco, 2014), contamination with pharmaceuticals (Mateo et al., 2015; Blanco et al., 2016) and exposure to pathogens (Marín et al., 2014; Blanco, 2015).

Livestock carcasses used to feed avian scavengers may include sick and medicated livestock, consumed by scavengers a short time after disposal in carcass dumps and supplementary feeding stations, colloquially termed "vulture restaurants" (Cortés-Avizanda et al., 2016). Therefore, the exposure to livestock pharmaceuticals and pathogens that are potentially harmful to scavengers is likely an unintentional consequence of conservation management of these species (Cortés-Avizanda et al., 2016). This exposure may have direct adverse effects on scavenger populations, exemplified by the crash of vulture populations in Asia due to the anti-inflammatory diclofenac (Watson et al., 2004; Gilbert et al., 2007). Information is also available on pathogen transmission from livestock to wildlife (Friend and Franson, 1999; Thomas et al., 2007). However, the likely indirect negative side-effects of pharmaceuticals via the overgrowth of disease-causing opportunistic pathogens remain largely unexplored.

A previous study recorded lesions caused by *Candida*-like yeast in the oral cavity of griffon vultures in central Spain (López-Rull et al., 2015), where most nestlings of this species showed circulating residues of several fluoroquinolones (Blanco et al., 2016). Opportunistic fungal pathogens may cause infection when a disruption of the host's natural barriers or an impairment of the host's immune system occurs (Lin et al., 2005; Pfaller and Diekema, 2007; Hebecker et al., 2014). The prolonged use of broad-spectrum antibiotics can cause these effects directly or indirectly by altering the host's normal microbiota composition (Patterson,1991; Lin et al., 2005; MacCallum, 2010; Dethlefsen and Relman, 2010; Cho et al., 2012; Keeney et al., 2014). Gut dysbiosis and other microbial imbalances may be especially detrimental in nestling vultures subjected to long-term or chronic exposure to antibiotics and other pharmaceuticals (Blanco et al., 2016), exacerbated by an immature immune system (López-Rull et al., 2015). However, no information is available on exposure to antibiotics in vulture species that include a proportion of wild animals in their diets, thereby being less dependent on livestock carcasses. This lack of information precludes the proper evaluation of the factors determining exposure to pharmaceuticals from different livestock species due to the variable availability and elimination methods of their carcasses, and its implications on the conservation of their populations.

In this study, we evaluated the exposure to livestock pharmaceuticals in nestling cinereous and Egyptian vultures, two threatened scavenger species exploiting carcasses of domestic livestock and wild animals. Egyptian vultures exploit carcasses of a wider variety of wild animals than cinereous vultures, which today rely much more heavily on livestock carrion than in the past (Hiraldo, 1976; Donázar, 1993). Therefore, a greater dependence on domestic versus wild animals in the cinereous vulture was predicted to be reflected in a higher exposure to pharmaceuticals than in Egyptian vultures. This prediction was tested by using fluoroquinolones as indicators of the overall exposure to livestock pharmaceuticals, as these antibiotics are often used in combination with other drugs in multiple treatments (Andriole, 2005; Sharma et al., 2009) and because they are highly stable compared with other pharmaceuticals (Martinez et al., 2006; Okerman et al., 2007). Because the prolonged use of broad-spectrum antibiotics is a major predisposing factor for opportunistic mycoses (Cunha, 2001; Lin et al., 2005; Pfaller and Diekema, 2007), we evaluated signs of disease caused by *Candida*-like yeast. Specifically, we hypothesized potential cause-effect relationships between diet-associated exposure to livestock antibiotics and occurrence of disease. We critically assessed whether the evaluation of these relationships may be obscured by variations in exposure and the concentration of fluoroquinolones, and their effects on disease signs, due to chronic contamination with these drugs throughout the nestling development period (Blanco et al., 2016). We discuss whether the interactions between diet, exposure to pharmaceuticals and disease can be modulated by species-specific life-history traits such as size, metabolism and foraging strategies. Finally, we reviewed information on the occurrence and concentration of pharmaceuticals in avian scavengers, to call attention to the increasing impact of these drugs on scavenger populations and environmental health due to supplementary feeding with medicated livestock.

2. Material and methods

2.1. Study area and species

The study was conducted in central Spain (provinces of Avila and Segovia). This area holds numerically important populations of vultures that are highly dependent on livestock carrion at vulture restaurants and other carcass dumps (see details of the study area and vulture populations in Donázar et al., 2002; Blanco, 2014). In addition to the griffon vulture, which shows a large and increasing population (Martínez et al., 1997; authors' unpublished data), about two hundred pairs of cinereous vultures and about 30 pairs of Egyptian vultures nest in the study area (Donázar et al., 2002; authors' unpublished data).

The cinereous vulture is the largest avian scavenger in the Palearctic (8–12 kg). It is a year-round resident in the study area, where it nests in pines and forages across large open areas around the colonies (Fargallo et al., 1998; Donázar et al., 2002). It searches for carcasses of livestock and wild prey, especially lagomorphs, that may be opportunistically predated when injured or sick (Hiraldo, 1976). Exploited livestock carcasses are mainly pigs and poultry predominantly present in vulture restaurants in the study area (Blanco, 2014). This species is categorized globally as "Near Threatened" and included in category SPEC 1 in Europe, due to the declining population trend over the last century (BirdLife International, 2016). In Spain, the stronghold for the species in Europe, major threats include illegal persecution through poisoning, habitat alteration and reduction of food supplies, especially of wild prey and free-ranging livestock (Sánchez, 2004).

The Egyptian vulture is a small (~1.8 kg) cliff-nesting vulture living