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Livestock farming practices modulate vulture diet-disease interactions

Guillermo Blanco [a], [*], Ainara Cortés-Avizanda [b], [c], Óscar Frías [a], Eneko Arrondo [b], José A. Donázar [b]

[a] Department of Evolutionary Ecology, Museo Nacional de Ciencias Naturales (CSIC), C/. José Gutiérrez Abascal 2, 28006, Madrid, Spain
[b] Animal Demography and Ecology Group, IMEDEA (CSIC)-UIB, C/. Miquel Marqués 21, 07190, Esporles, Illes Balears, Spain
[c] Department of Conservation Estación Biológica de Doñana (CSIC), C/. Americo Vespucio 26, 41092, Sevilla, Spain

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ABSTRACT

Low- and high-intensity farming exert different direct and indirect effects on vulture populations by driving the availability, exploitation and characteristics of carrion. This is especially true for the levels of pharmaceuticals in wild and domestic animal carcasses. However, the impact of farming systems on the diet-related health of avian scavengers remains unclear. Here, we evaluate diet and disease signs in nestlings of three European species of vultures (Cinereous, *Aegypius monachus*, Griffon, *Gyps fulvus*, and Egyptian, *Neophron percnopterus*), living in different regions of Spain under contrasting farming schemes. We test the hypothesis that disease (oral mucosal lesions caused by mixed fungal and bacterial infections) in vultures is influenced by features of food and foraging conditions derived from farming systems, especially due to the expected chronic and irregular ingestion of pharmaceuticals under intensive (factory farms) compared to extensive systems. A large proportion of nestlings of the three vulture species in central Spain (high-intensity farming area) continue to be affected by oral lesions (cinereous: 75%, $n = 16$; griffon: 61%, $n = 28$; Egyptian: 46%, $n = 13$). The same type of lesions, at a much lower frequency, was found in nestling of the three vulture species in each of the selected areas corresponding to low-intensity farming areas in southern and northern Spain (Cinereous: 39%, $n = 13$; Griffon: 7%, $n = 14$; Egyptian: 6%, $n = 17$). As predicted, a positive relationship was found between the proportion of nestlings with lesions and the frequency of intensive livestock from factory farms (swine and poultry) in the diet. The intensive medication in factory farms deserves further research to assess its implications in vulture diet-disease interactions at large geographical scales. Assessing the presence of oral lesions as an indicator of physiological alterations is encouraged along with pharmacovigilance in surveillance programs aimed at evaluating the direct and indirect effects of livestock farming practices on vulture health. Given the increasing exploitation of domestic instead of wild animals by vultures, the growth in high-intensity farming and the medication practices in free-ranging and semi-extensive farming systems, this assessment would help to characterize the risks associated with different farming operations for wildlife. This evaluation is crucial to avoid exacerbating the detrimental consequences of supplementary feeding programs contrary to their primary aim of the conservation of endangered species.

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[*] Corresponding author.
E-mail address: g.blanco@csic.es (G. Blanco).

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1. Introduction

Livestock farming is one of the most prevalent causes of habitat transformation and loss of biological diversity worldwide (Foley et al., 2005; Steinfeld et al., 2006; Ellis, 2011). These impacts vary greatly depending on farming intensity, associated with the prevailing environmental and socio-economics conditions at different scales and geographical regions (Strijker, 2005; Robinson et al., 2014). Pastoral systems have been highlighted as a model of sustainable management of natural environments by simulating the life conditions of wild ungulates in their original ecosystems, including the free movement of livestock at large scales, seasonal migrations (transhumance), low stocking densities, natural pasture feeding, and population dynamics that are little influenced by artificial birth and growth control (Bignal and McCracken, 1996; Gibon, 2005). These systems yield traditional and organic food, and generate impacts generally compatible with environmental conservation (Bignal and McCracken, 1996; Sundrum, 2001; Lebacq et al., 2013). However, these systems are at serious risk of disappearing on a global scale due to the large land extension necessary to make profitable the investment in labor, which requires long periods of demanding work in the countryside (Strijker, 2005; Bernués et al., 2011). In contrast, high-intensity systems such as factory farms are growing worldwide, and often face the challenge of producing competitive products in terms of nutritional and environmental quality (Tilman et al., 2002; Ilea, 2009). Industrial systems of food-producing animals imply intensive medication for dealing with challenging sanitary problems, and generate huge volumes of excreta and meat residues whose elimination entails significant economic and environmental costs (Tilman et al., 2002; Menzi et al., 2010; Arnold et al., 2014). On the contrary, low-intensity farming requires less medication, and its waste can be integrated into ecosystems in a low-impact way and exploited by coprophagous and scavenger wildlife (Bignal and McCracken, 1996; Donázar et al., 2009).

These general differences between low- and high-intensity farming operations have important implications for avian scavengers feeding on livestock carcasses (Donázar et al., 2009; Olea and Mateo-Tomás, 2009; Blanco, 2014a,b; Morales-Reyes et al., 2018). Each of these farming schemes generate different resources in terms of animal-food species, stocks, abundance and carcass predictability, as well as meat characteristics in terms of nutrients, pathogens and pharmaceuticals (Cortés-Avizanda et al., 2016). These traits exert clear influences on avian scavengers, permeating individuals to communities, and including effects on health, density, distribution, guild composition, movements, intra- and interspecific interactions, and impacts on breeding success, survival and ultimately population trends (Cortés-Avizanda et al., 2016). Livestock carcasses from low and high-intensity farming can especially differ in the content of pharmaceuticals, and thus in scavenger exposure to particular compounds and drug cocktails that can cause mortality directly or indirectly (Margalida et al., 2014; Blanco et al., 2017a).

Recently, a high occurrence of oral mucosal lesions have been recorded in vultures from central Spain, which showed chronic exposure to antibiotics from the consumption of medicated livestock carcasses (Blanco et al., 2016, 2017a; Pitarch et al., 2017). The irregular ingestion of these pharmaceuticals in terms of frequency, type, concentration and mixtures may alter the host's normal microbiota, thus facilitating the overgrowth of opportunistic microorganisms able to cause disease (Pfaller and Diekema, 2007; MacCallum, 2010; Keeney et al., 2014). Previous studies revealed the presence of abundant yeast-like fungi of several ubiquitous species causing some of the lesions in all affected individuals, as well as environmental non-yeast fungi and bacteria isolated from the same lesions (Pitarch et al., 2017; J. Diéguez-Uribeondo and G. Blanco unpubl. data). However, the high occurrence of antibiotics and lesions in vultures from central Spain has made it difficult to demonstrate a cause-effect relationship at the individual level (Blanco et al., 2017a; Pitarch et al., 2017). Adequate within-individual testing of this relationship faces additional challenges derived from the fact that lesions in low areas of the digestive tract can pass unnoticed, and because they can persist beyond antibiotic metabolization and excretion (Blanco et al., 2017a). Because no European vulture population relies exclusively on non-domestic animals (Cortés-Avizanda et al., 2016), it has been proposed that the relationship between pharmaceutical ingestion and oral disease could be addressed by evaluating differences in vulture diets between geographical areas, specifically where carcasses from high- and low-intensity farming account for a contrasting proportions of the scavengers' diet (Blanco et al., 2017a).

In this paper, we evaluated diet and disease signs in nestlings of three European species of vultures, Cinereous (*Aegypius monachus*), Griffon (*Gyps fulvus*) and Egyptian (*Neophron percnopterus*), living in different regions of Spain subjected to contrasting farming schemes. We tested the hypothesis that disease in vultures is influenced by the contrasting features of food and foraging conditions derived from low-and high-intensity farming practices. Specifically, it has been argued that the infections behind oral lesions are triggered by the chronic and irregular ingestion of pharmaceuticals that are expected to be more frequently encountered in higher variety and concentrations in livestock carcasses from high-intensity farming than from low-intensity schemes (Blanco et al., 2017a). This hypothesis predicts that vulture populations mostly relying on carcasses from wild animals and free-ranging livestock herds under low-intensity farming should not show lesions or should show them at a lower frequency than those that feed mostly on carcasses of intensively medicated swine and poultry from factory farms. However, even free-ranging livestock from low-intensity schemes may be subjected to some degree of medication depending on stock age, season and particular exploitation (Page and Gautier, 2012), which can introduce ‘noise’ obscuring the ideal hypothesis testing regarding exposure *vs.* lack of exposure to pharmaceuticals. To attempt to overcome this difficulty, we evaluated whether variations in diet composition regarding wild animals and free-ranging livestock with no or low medication, and intensive livestock from factory farms with high medication, can contribute to explaining the occurrence of lesions in each case. We assessed the management and conservation implications derived from prevailing livestock farming operations on the diet and health of avian scavengers, including the influence of carcass elimination practices in supplementary feeding programs for endangered species. We aimed to highlight the impact of different livestock operations on scavenger wildlife within the framework of the debate on environmentally-sustainable farming.

2. Methods

2.1. Study areas

The study was conducted in several regions of Spain (Fig. 1) that are characterized by contrasting farming practices. These regions differ in their livestock species, their management practices in terms of confinement conditions and rearing intensity (factory farms versus free-ranging farming) and in their associated forms of eliminating carcasses and meat by-products that are not intended for human consumption (Cortés-Avizanda et al., 2016).

Segovia province and its close surroundings in Avila province (central Spain, Fig. 1), was selected as a region representative of high-intensity farming areas (hereafter HIA) increasingly based on factory farms of fattening pigs (Blanco, 2014a,b). In fact, this area has one of the highest concentrations and numbers of farms devoted to this kind of swine operation in Spain. Numerically important populations of Griffon, Cinereous and Egyptian vultures are highly dependent on pig carrion, which is available at multiple supplementary feeding stations and at carcass dump sites around farms (Blanco, 2014a,b; 2018; Blanco et al., 2017a). Free-ranging livestock are also present in this area in variable numbers showing contrasting temporal trends over the last few decades, especially decreasing in the case of sheep and goats (Blanco, 2014a,b). More details about supplementary feeding stations and vulture populations in this study area can be found elsewhere (e.g. Fargallo et al., 1998, 2018; Donázar et al., 2002; Blanco, 2014a,b).

In contrast with this HIA farming scheme, we selected three additional study areas representative of less intensive farming practices. These low-intensity farming areas (hereafter, LIA) are mostly based on free-ranging livestock living over extensive pastured zones and/or transhumance regimes. These LIA were selected according to the existence of vulture populations of each study species, and by considering the logistics related to monitoring their populations, specifically for nest access with the aim of evaluating diet and the occurrence of oral lesions in nestlings. Because not all vulture species are present or are easily monitored in the same areas, we selected different LIA for each vulture species.

Sierra Norte Natural Park and its surroundings in the Sevilla province (Andalusia, southern Spain, Fig. 1) was selected as a representative LIA for the cinereous vulture, which breeds in a loose colony occupying scattered Mediterranean woodland patches within large extensions of dehesas and Mediterranean scrublands (Fernández-Bellón et al., 2016). Mountain

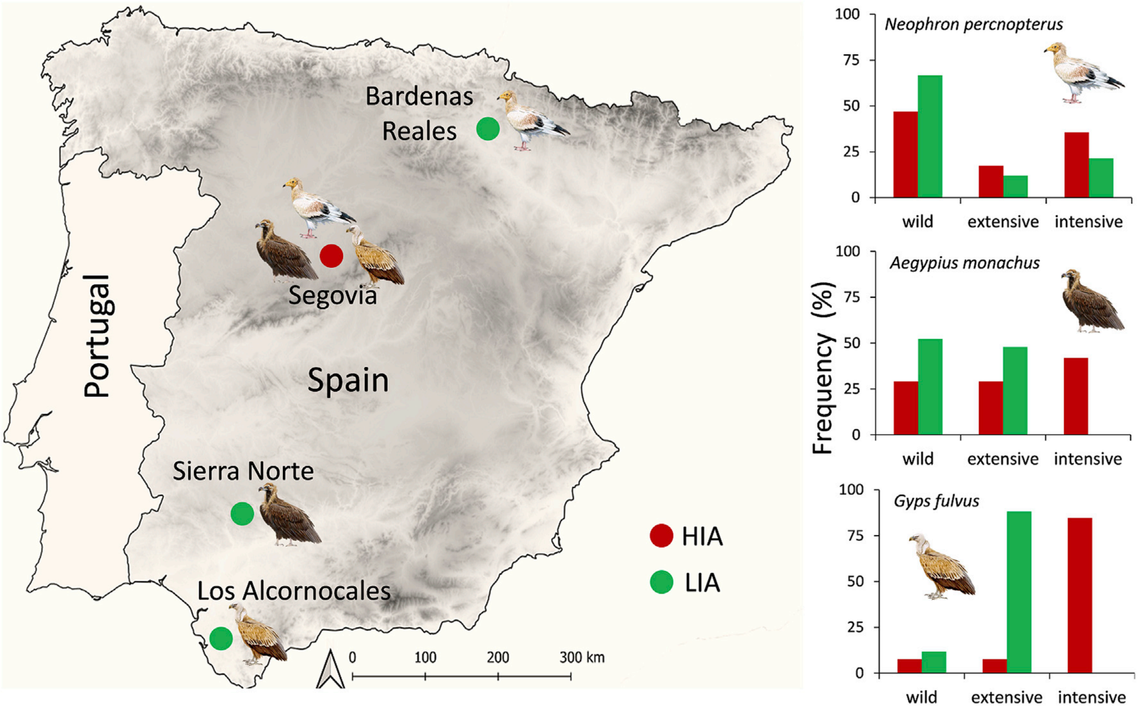

Fig. 1. Frequency (%) of remains corresponding to wild animals, free-range extensive livestock and intensive livestock from factory farms in the diet of nestling cinereous, griffon and Egyptian vultures in a high-intensity farming area (HIA) in central Spain (red point), and several low-intensity farming areas (LIA) in northern and southern Spain (green points). Details on diet composition are shown in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

woodland and scrubland areas are devoted to big game, whereas dehesas and open areas are exploited for semi-intensive grazing (sheep and goat, and less frequently pigs) while intensive livestock farming has almost no presence (García-Barón et al., 2018). Consequently, cinereous vultures feed mainly on wild ungulates, especially Red deer (*Cervus elaphus*) and Wild boar (*Sus scrofa*), sheep, goats and wild European rabbits (*Oryctolagus cuniculus*) (Dobado et al., 2012). There are no supplementary feeding stations in this area.

In the case of the Griffon vulture, we selected the Alcornocales Natural Park and its surroundings in Cádiz province (Andalusia, southern Spain, Fig. 1). This area is dominated by Mediterranean woodlands and extensive dehesas exploited for free-ranging livestock (cows, goats and sheep) pasturing and cork production. The vulture diet in the area includes carcasses of domestic ungulates (mainly cows and goats) exploited *in situ* in the field, and wild ungulates, especially Red deer and Wild boar exploited for hunting. In this area, there is a supplementary feeding station supplied with remains of big game from hunting, livestock from local farming and slaughterhouse remains (Benıtez et al., 2009).

Finally, for the Egyptian vultures, we selected Bardenas Reales Natural Park and its surroundings in Navarra (Ebro Valley, northern Spain, Fig. 1) as a representative LIA. This area encompasses around 50,000 ha of flat areas and small hills with natural vegetation dominated by steppe/badlands and wooded/forested patches without permanent human settlements, where traditional land uses and extensive sheep grazing dominate. Neighboring areas include irrigated cultures and many intensive livestock operations (mainly pigs) (see Cortés-Avizanda et al., 2015b for further details). New sanitary legislations allow the abandonment of carcasses of extensive livestock herds (sheep and goats) under a semi-extensive regimen on which Egyptian vultures mainly rely, although wild animals, especially rabbits, are also an important component of the diet (Donázar et al., 2010). No permanent supplementary feeding stations exist within the limits of the Natural Park, but there are some feeding stations in their surroundings where Egyptian vultures often forage.

2.2. Fieldwork and data analysis

During the breeding season of 2017, nest observations of the three vulture species were conducted following standard protocols (Martínez et al., 1997; Donázar et al., 2002), as part of the long-term monitoring of their populations in the study areas (details in Donázar et al., 2002; Sanz-Aguilar et al., 2017; Fargallo et al., 2018). Nests were accessed to band, measure and weigh a sample of nestlings following standard methods (details in López-Rull et al., 2015; Blanco et al., 2017a).

To assess nestling diet, food remains found in and below the nests of the three vulture species were collected at the time of nestling banding, when they were feathered but prior to the time when they are ready-to-fly. Remains were identified to the species level and quantified assuming the smallest possible number of individuals according to size, age and anatomical position. Pellets found in the nests were also considered in the case of Cinereous vultures because food remains are generally scarce or lacking in a proportion of nests in this species. Bones, hair and feathers were identified to the species level by means of reference collections and standard methods. The occurrence of each animal species was recorded qualitatively in each pellet given the difficulty of quantifying the corresponding number of carcasses (Donázar et al., 2010). Accordingly, to avoid pseudoreplication, the presence of identifiable remains of each species found in pellets from each Cinereous vulture nest was considered only when food remains (bones, fur) of the same food species were not found in the same nests. In the case of Griffon vultures, we attempted to identify the remains vomited by the nestlings during their handling, specifically attending to the presence of identifiable livestock bones, hair and feathers. Cinereous and Egyptian vultures do not vomit during handling.

Food remains were categorized as belonging to (a) wild animals such as reptiles, birds, mammals, etc. obtained by scavenging (hereafter, wild animals), (b) livestock coming primarily from extensive free-ranging operations (sheep, goats, cows and horses), whose carcasses are exploited in the countryside or in supplementary feeding stations depending on the study area (hereafter, extensive livestock), and (c) livestock carcasses from intensive stock farms (swine and poultry) exclusively found in supplementary feeding stations and farm surroundings after being intentionally discarded there, often illegally, for elimination by scavenger species (hereafter, intensive livestock). To analyze differences in the consumption of livestock carrion, the proportions of remains belonging to each food category (wild animals, extensive livestock and intensive livestock) were compared between the corresponding HIA and LIA for each vulture species by means of the Freeman-Halton extension of the Fisher exact probability tests for a 2×3 contingency table. Remains of domestic refuse obtained by the vultures in rubbish dumps, and unidentified remains, were very scarcely represented in the diet, and therefore excluded from the analysis.

The occurrence of disease, represented by gross lesions in the oral cavity, was recorded by conducting visual inspection of nestling vultures as described previously (López-Rull et al., 2015; Blanco et al., 2017a; Pitarch et al., 2017). Briefly, 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 (see pictures in Blanco et al., 2017c; Pitarch et al., 2017; Fig. 2). Overall, we examined a sample of nestling Griffon ($n = 28$), Cinereous ($n = 16$) and Egyptian ($n = 13$) vultures in the HIA in central Spain. In LIAs (northern and southern Spain), we recorded a sample of nestling Griffon ($n = 14$), Cinereous ($n = 13$) and Egyptian vultures ($n = 17$). The lower and upper limits of the 95% confidence interval (CI) for the proportion of nestlings with lesions were calculated with the Wilson procedure with correction for continuity (Newcombe, 1998). Univariate comparisons of the proportion of nestlings with lesions were conducted between the corresponding HIA and LIA for each vulture species by means of Fisher's exact probability tests and odds ratios and 95% CI as a measure of effect size. The relationships between the occurrence of lesions ($0 =$ absence, $1 =$ presence), farming intensity of each study area (under regimen HIA or LIA) and vulture

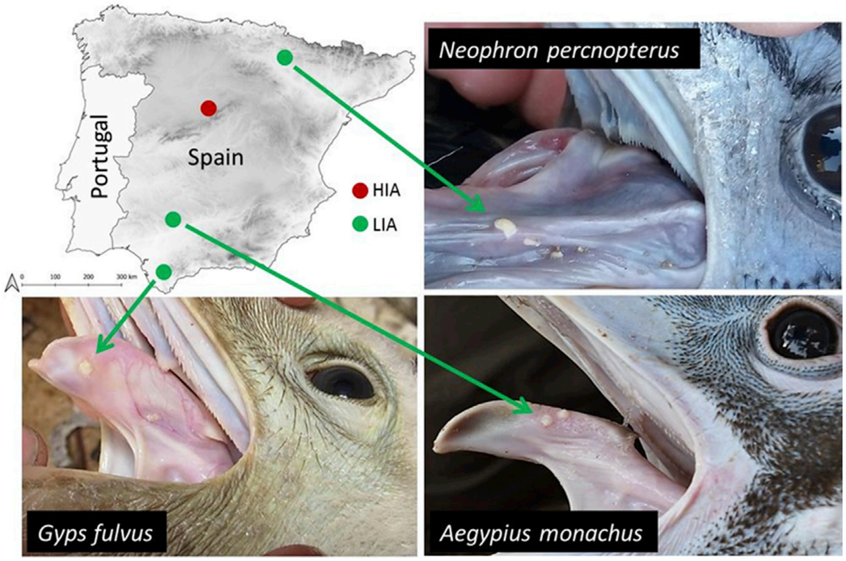

Fig. 2. Examples of mucosal lesions in the oral cavity of nestlings of three vulture species examined in each low-intensity farming area (LIA) in northern and southern Spain (green points and arrows). The location of the high-intensity farming area (HIA) in central Spain is shown (red point); pictures showing lesions in the three vulture species in the HIA are shown in Fig. S1. All photographs were taken in the breeding season of 2017 by M. de la Riva (*A. monachus*), A. Cortés-Avizanda (*N. percnopterus*) and R. Sánchez-Carrión (*G. fulvus*). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

species were evaluated with fully-saturated log-linear analysis. The test was run hierarchically, beginning with the three-order interaction and proceeding backwards until all two-order interactions retained by the model reached significance ($P < 0.05$) according to the χ^2 likelihood ratio. Nonparametric Spearman rank-correlation coefficients were used to test the predictions of our hypothesis, stressing that the proportion of nestlings with lesions should vary negatively with the proportion of wild animals and extensive livestock, and positively with that of intensive livestock in the diet of the six populations (three in HIA and three in LIA) of the three vulture species.

3. Results

Nestling Cinereous vultures in the HIA feed primarily on pig carcasses from factory farms and wild lagomorphs, especially rabbits, while in the LIA, they rely mostly on large game (Red deer, Wild boar and Mouflon, *Ovis orientalis*), sheep and goats (Table 1). The Griffon vulture diet was dominated by swine and poultry in the HIA, and by cattle, sheep, goat and large game (Red deer) in the LIA. The diet of nestling Egyptian vultures was comparatively more diverse, mainly including carcasses of pigs, and less frequently those of free-grazing livestock and a variety of wild animals in the HIA. In the LIA, they mainly feed upon wild animals, especially rabbits, and a balanced combination of intensive and free-grazing livestock (Table 1). Overall, the contribution to diet of each food category (wild animals, extensive livestock and intensive livestock) differed between the corresponding HIA and LIA for each vulture species (Fig. 1; Fisher exact test, Cinereous vulture, $P < 0.001$; Griffon vulture, $P < 0.001$; Egyptian vulture, $P = 0.02$).

We found a high occurrence of lesions in the three vulture species in the HIA (Table 2). Single Griffon and Egyptian vultures, and several Cinereous vultures, showed the same kind of lesions (Fig. 2) at each corresponding LIA but in a lower frequency of occurrence (Table 2); in the case of the Cinereous vulture the difference did not reach statistical significance due to limited sample size (Table 2). A log-linear analysis taking into account simultaneously the relationships between the occurrence of lesions, farming intensity and vulture species showed no significant three-way interactions ($\chi^2 = 1.32$, $P = 0.52$, df = 2), a higher occurrence of lesions in the HIA than in the LIA ($\chi^2 = 22.39$, $P < 0.001$, df = 1, Table 2), and significant differences in the occurrence of lesions between vulture species ($\chi^2 = 7.67$, $P = 0.02$, df = 2, Table 2), with the highest in the Cinereous vulture, intermediate values in the Griffon vulture and the lowest values in the Egyptian vulture (Table 2). The fit of the model was adequate: $\chi^2 = 5.07$, $P = 0.28$, df = 4.

As predicted, the frequency of nestlings with lesions was not correlated with the frequency of wild animals (two-tailed Spearman rank correlation coefficient $r_s = -0.54$, $P = 0.27$, Fig. 3a) and free-ranging extensive livestock ($r_s = -0.20$, $P = 0.70$, Fig. 3b) in the diet, including the three vulture species in both the HIA and LIA. The relationship between the occurrence of lesions and the frequency of consumption of carcasses of intensive livestock from factory farms in the diet was positive but did not reach statistical significance ($r_s = 0.75$, $P = 0.08$, Fig. 3c, see also Fig. S1 for data pooling (a) wild animals and extensive livestock, $r_s = -0.75$, $P = 0.08$, and (b) extensive and intensive livestock, $r_s = 0.54$, $P = 0.27$).

Table 1
Number and percentage of food items in each wild and domestic animal category from remains, pellets and vomit found in the nests of cinereous, griffon and Egyptian vultures. The diet was sampled in a high-intensity farming area (HIA) in central Spain, and each of the low-intensity farming areas (LIA) in northern and southern Spain, respectively (see Fig. 1 for reference). The sampling was conducted in the breeding season of 2017.

Food item	Cinereous vulture		Griffon vulture		Egyptian vulture	
	n (%)		*n* (%)		*n* (%)	
	HIA	LIA	HIA	LIA	HIA	LIA
Wild animals						
Reptiles and amphibians	–	–	–	–	10 (8.2)[a]	10 (10.9)[b]
Lagomorphs[c]	8 (25.8)	2 (8.7)	–	–	11 (9.0)	29 (31.5)
Other mammals	1 (3.2)[d]	10 (43.5)[e]	1 (7.7)[f]	2 (11.8)[g]	17 (13.9)[h]	7 (7.6)[i]
Birds	–	–	–	–	12 (9.8)[j]	10 (10.9)[k]
Fishes	–	–	–	–	4 (3.3)[l]	–
Domestic animals						
Sheep and goats	7 (22.6)	11 (47.8)	1 (7.7)	4 (23.5)	17 (13.9)	10 (10.9)
Cattle and horses	2 (6.5)	–		11 (64.7)	3 (2.5)	
Pigs	10 (32.3)	–	6 (46.2)	–	33 (27.0)	16 (17.4)
Poultry	3 (9.7)	–	5 (38.5)	–	8 (6.6)	2 (2.2)
Others[m]	–	–	–	–	7 (5.7)	8 (8.7)
Total	31 (100)	23 (100)	13 (100)	17 (100)	122 (100)	92 (100)

[a] 4 species.
[b] 4 species.
[c] European wild rabbit (*Oryctolagus cuniculus*) and Iberian hare (*Lepus granatensis*).
[d] European roe deer (*Capreolus capreolus*).
[e] Red deer (*Cervus elaphus*), Wild boar (*Sus scrofa*), European mouflon (*Ovis orientalis musimon*).
[f] *Felis* sp.
[g] Red deer.
[h] 7 species.
[i] 3 species.
[j] 8 species.
[k] 7 species.
[l] Common carp (*Cyprinus carpio*).
[m] Unidentified remains and rubbish.

Table 2
Frequency of vulture nestlings with mucosal lesions in the oral cavity. The nestlings were sampled in a high-intensity farming area (HIA) in central Spain, and several low-intensity farming areas (LIA) in northern and southern Spain (see Fig. 1 for reference). The sampling was conducted in the breeding season of 2017. Comparison of the proportion of nestlings with lesions between the HIA and the corresponding LIA was conducted by means of Fisher's exact probability tests and Odds ratios.

Vulture species	HIA	No. of nestlings	No. with lesions	% positive (95% CI)	LIA	No. of nestlings	No. with lesions	% positive (95% CI)	HIA-LIA comparison	
									Fisher exact test, *P*	Odds ratio (95% CI)
A. monachus	Segovia-Ávila	16	12	75.0 (47.4–91.7)	Sierra Norte	13	5	38.5 (15.1–67.7)	0.066	4.8 (0.98–23.54)
G. fulvus	Segovia-Ávila	28	17	60.7 (40.7–77.9)	Los Alcornocales	14	1	7.1 (0.4–35.8)	0.002	20.09 (2.29–176.10)
N. percnopterus	Segovia-Ávila	13	6	46.2 (20.4–73.9)	Bardenas Reales	17	1	5.9 (0.3–30.8)	0.025	13.71 (1.38–136.22)

4. Discussion

This study suggests that interactions between diet and disease in three vulture species can be modulated by livestock farming schemes at a large geographical scale. Despite intense debate on farming impacts on biodiversity, the implementation of environmentally-sustainable management has rarely considered the influence of livestock operations of different intensity on vulture populations (Donázar et al., 2009; Olea and Mateo-Tomás, 2009; Morales-Reyes et al., 2018). Low- and high-intensity farming can exert contrasting direct and indirect effects on vulture populations by driving the availability and exploitation of wild and domestic animals, and the intrinsic features of food in terms of nutrients, pathogens and pollutants like pharmaceuticals (Cortés-Avizanda et al., 2016). However, the impact of farming on the diet-related health of avian scavengers remains unclear (Blanco et al., 2017a). Disease in wildlife associated to concentrated animal feeding operations (CAFO) have been extensively reported (Schwarz et al., 2004; Miller et al., 2013; Wiethoelter et al., 2015) but, to our knowledge, no specific information on oral lesions has been recorded in avian scavengers in other regions worldwide (Blanco et al., 2017a; Pitrach et al. 2017).

Vultures show a strict scavenging specialization among terrestrial vertebrates, but diet and carcass exploitation strategies greatly differ between species depending on size, feeding guild and geographical region (Donázar, 1993; Hertel, 1994; Cortés-Avizanda et al., 2012; Ballejo et al., 2017). In this study, we found clear differences in diet composition among three vulture species sampled across distant regions representative of high and low farming intensity schemes. Our results agree with

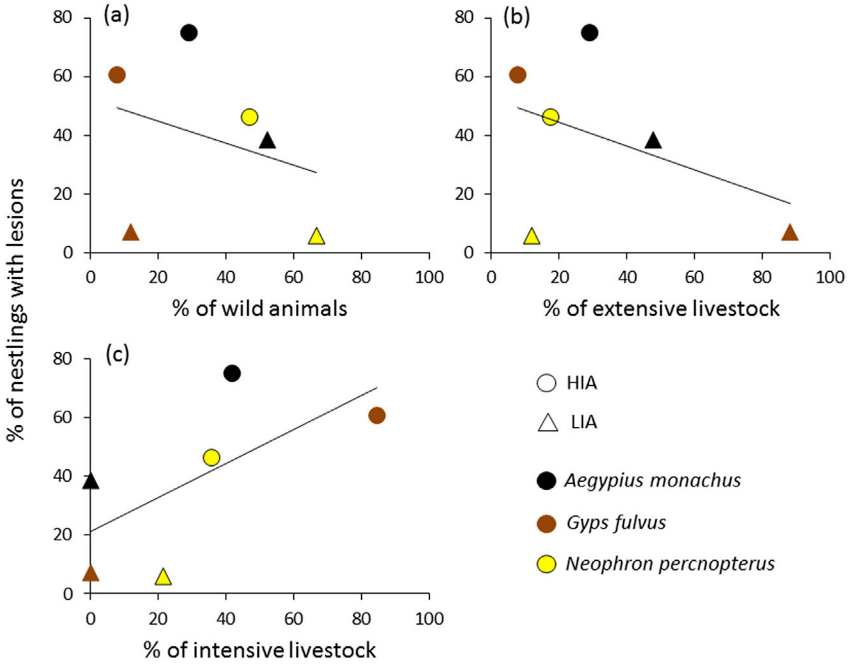

Fig. 3. Relationships between the proportion of nestlings with lesions with the proportion of (a) wild animals, (b) extensive livestock and (c) intensive livestock in the diet of the six populations (three in HIA and three in LIA) of the three study vulture species. Least squares regression lines of the correlations are shown for graphical representation of trends.

general knowledge on the exploitation of different-sized carcasses by Old World vultures (Mundy et al., 1992; Donázar, 1993; Campbell, 2015). Despite including different species of wild and domestic animals as food depending on farming practices and regions, the diet sampling supports the adaptation of Griffon vultures to exploit carcasses of large ungulates, the preference of Cinereous vultures for small and medium-sized mammals, and a high trophic plasticity widening the food spectrum of the Egyptian vulture, including carcasses of variable size from all vertebrate groups, invertebrates and faeces (Donázar, 1993). A general replacement of wild by domestic animals has occurred progressively in the vulture's diet worldwide (Cortés-Avizanda et al., 2016). This trend is expected to continue in the near future due to growing livestock operations and associated global reductions in biological diversity affecting wild animals exploited as food by vultures (Donázar et al., 2010; Milchev et al., 2012; Lambertucci et al., 2018; Henriques et al., 2018). Accordingly, clear differences in diet were found between vulture populations of each study species inhabiting selected regions subjected to farming schemes of contrasting intensity regarding livestock management and their residues. These results support previous studies indicating a dominance of livestock from factory farms exclusively exploited in supplementary feeding stations in the diet of avian scavengers at the HIA in central Spain (Blanco, 2014a,b; 2018; Blanco et al., 2017a). As expected, the vultures' diets were mostly based on free-ranging livestock and wild animals, especially large game species and rabbits, in each corresponding LIA (see also Benıtez et al., 2009; Donázar et al., 2010; Dobado et al., 2012). Therefore, prevailing livestock farming practices exert a strong predictable influence on foraging ecology and the diet of vultures, which have clear implications for the management and conservation of their populations.

The large accumulation of predictable carcasses from factory farms at vulture “restaurants” has been highlighted and attract artificially large numbers of dominant scavenger species, often promoting a diet simplification, negative effects on health derived from the ingestion of pharmaceuticals and pathogen transmission, and the alteration of population dynamics and community-wide processes (Cortés-Avizanda et al., 2016; Blanco et al., 2017a,b). This study confirms that a large proportion of nestling Cinereous, Griffon and Egyptian vultures in central Spain continue to be affected by oral mucosal lesions caused by mixed fungal and bacterial infections (López-Rull et al., 2015; Blanco et al., 2017a; Pitarch et al., 2017). The irregular ingestion in terms of frequency, “dosage” and drug cocktails from factory farms has been argued to alter the normal microbiota and immune systems of nestling vultures (Blanco et al., 2017a; Pitarch et al., 2017). These factors, coupled with the risk of pathogen acquisition from livestock (Blanco, 2018; Blanco and Díaz de Tuesta, 2018; Marin et al., 2018), and from increased intra-and interspecific transmission in the crowding and unsanitary conditions generally encountered in feeding stations (Blanco et al., 2017b), suggest an overall negative health impact on scavengers relying on carcasses from factory farms instead of wild animals and free-ranging livestock (Cortés-Avizanda et al., 2016). Paradoxically, few studies have evaluated the physiological and immunological effects of supplementary feeding on vultures, or the impact of particular diets based on wild animals and livestock from different farming systems on scavenger health (Blanco, 2006, 2014b). Our sampling showed the same kind of oral lesions in nestlings of the three vulture species in each corresponding LIA, although at a much lower frequency than in the HIA. These findings suggest that oral disease in vultures can be influenced by the contrasting features of food and foraging conditions derived from low- and high-intensity farming practices.

The evidence of oral disease in nestlings of the three vulture species in each LIA, although at a low-medium frequency depending on species, is concerning because it expands a health problem across a widespread area, previously detected only in vultures and other scavengers with confirmed circulating antibiotics in central Spain (Blanco et al., 2016, 2017a; Pitarch et al., 2017). These lesions showed the same appearance and location as those previously described in central Spain (see pictures of the lesions in Blanco et al., 2017a; Pitarch et al., 2017). Therefore, we assumed that these lesions have the same etiology and similar detrimental effects on health recorded previously in the HIA (Blanco et al., 2017a; Pitarch et al., 2017). In particular, the single Griffon vulture with lesions in the LIA was the only one that died before fledging among the sampled nestlings (R. Sanchéz-Carrión, pers. com.), as occurred with several Griffon and Egyptian vultures monitored in the HIA (see also Blanco et al., 2017a), although no systematic monitoring was conducted to quantify nestling survival before fledging at the population level in each study area.

As predicted, the proportion of nestlings with lesions increased between LIA and HIA for each vulture species, as did the consumption of carcasses of intensive livestock from factory farms. The occurrence of lesions even in the selected LIAs supports the hypothesis stated here in the case of the Egyptian vulture, because this species included intensive livestock (swine and poultry) in their diet at this area (see also Donázar et al., 2010). Cinereous and Griffon vultures showed a proportion of affected nestlings in the respective LIA even when no remains of intensive livestock were found in the sampled nests. This can be explained by two non-mutually-exclusive factors. First, a small proportion of carcasses from intensive livestock could have passed unnoticed in the diet sampling, owing to the difficulty of identifying partially digested food remains vomited by Griffon vultures, and by the general scarcity of food remains typically found in nests of Cinereous vultures. This is not surprising considering that very large-scale movements performed by breeding vultures (Arrondo et al., 2018) can imply feeding their nestlings with carcasses from intensive livestock exploited outside of the range of each respective LIA. Second, free-ranging livestock may be subjected to varying levels of medication depending on several factors and particular exploitations (Page and Gautier, 2012). In fact, a recent study has shown the presence of multiple antibiotics in different tissues of sheep and goats from extensive farms disposed for feeding Cinereous vultures (Gómez-Ramírez et al., 2018). Therefore, this and other endangered scavengers relying on carcasses of extensive livestock treated with antibiotics are expected to show levels of these and other drugs, and to suffer the negative consequences of this contamination. The argued greater sensitivity of the Cinereous vulture to disease due to a slower size-related drug metabolization and excretion (Blanco et al., 2017a), and other potential factors related to digestive physiology (Blumstein et al., 2017), is supported by the relatively high proportion of nestlings with lesions despite a lower dependence on livestock carrion compared with Griffon vultures in each corresponding LIA.

Alternative hypothesis potentially explaining the higher frequency of lesions in the HIA in central Spain compared with the respective LIA for each species include genetic differences between populations. It assumes that genetic variability should be lower in the LIA than the HIA for the three vulture species, and that it has an influence on host susceptibility to the microorganisms forming the lesions. However, vultures show not clear genetic structure across Spain, at least for the two studied species in this regard (Martínez-Cruz, 2011). Regardless of the content of pharmaceuticals, nutritional features of carcasses from intensive livestock (pigs, poultry) compared with those from extensive schemes (e.g. sheep, goats) and wild animals could contribute to explain the differences in oral disease between HIA and LIA, which deserves further research. Non-food habitat-related factors (e.g. climate) could have some influence in the frequency of lesions through host physiology, but differences regarding temperature and rainfall are small between HIA and LIAs during the breeding season (Bustamante, 2003). In this sense, it is important to note that the frequency of lesions was higher in the HIA than in the three LIA for each vulture species even although these species show clear differences in breeding phenology but not clear differences among geographical areas within species (Donázar, 1993). As alternative hypothesis, the impact of population density on the frequency of lesions could have an influence in colonial species like the Griffon vulture. This alternative assumes a density-dependant probability of infection by the microorganisms involved in the lesions. However, while population density and overcrowding can exert an influence on the probability of acquiring infectious pathogens and parasites affecting avian scavengers (Blanco et al., 2017b; Blanco, 2018), the lesions reported here are caused by ubiquitous microorganisms acting as opportunistic pathogens when disturbing factors alter host homeostasis. Among these microorganisms, yeast as *Candida* spp (Pitarch et al., 2017), filamentous fungi and bacteria (especially *Escherichia coli*) (J. Diéguez-Uribeondo and G. Blanco unpubl. data) form part of the normal microbiota of many vertebrates, including vultures (Brilhante et al., 2012; Mora et al., 2014; Roggenbuck et al., 2014). Ingestion of pharmaceuticals, especially antibiotics, is expected to dramatically alter normal microbiota of the digestive tract, thus promoting that particular microorganisms of the normal microbiota turned to opportunistic pathogens causing the lesions (see Blanco et al., 2017a and references therein), which deserves further research.

5. Concluding remarks and applications

Establishing the level of reliance on carcasses of wild and domestic animals can indicate exposure to livestock pharmaceuticals and its impact on disease, thus contributing to our understanding of the factors limiting vulture populations and to improving the efficacy of conservation management measures. A straightforward approach implies the assessment of the presence of particular pharmaceuticals and drug cocktails in livestock carcasses and scavengers, rather than assuming that drug content depends on livestock species and exploitation schemes. Although costly, this can avoid misleading assumptions with negative implications in conservation. For instance, a recent study found antibiotics in carcasses of extensive livestock disposed for feeding Cinereous vultures in a special protected area of 2000 Nature Network, even when these carcasses were thought to be untreated with pharmaceuticals (Gómez-Ramírez et al., 2018). Given the increasing vulture exploitation of domestic instead of wild animals, the growth in high-intensity farming, and the medication in even free-ranging and semi-extensive farming systems (Page and Gautier, 2012), this assessment should be implemented with the aim to characterize the risks associated with different farming operations on wildlife. It should consider the effect of stock age, season, type of exploitation, geographical region and especially the form of carcass elimination by scavengers. Regarding supplementary feeding programs, this evaluation is crucial to avoid exacerbating detrimental consequences contrary to their primary aim of the conservation of endangered species (Cortés-Avizanda et al., 2016).

Combining pharmacovigilance with pathogen assessment can contribute to the anticipation of direct toxic effects of drugs (Margalida et al., 2014) and, importantly, their health effects by promoting disease (Blanco et al., 2017a). Further research is required to evaluate the magnitude of the oral disease detected in avian scavengers, including the affected species and populations across their distribution ranges, and its impact on survival and population dynamics. Investigating the direct and indirect effects on vultures' normal physiology and microbiology of each pharmaceutical and drug mixture, and other influential factors related to food features, is essential to understanding the processes governing the formation and prognosis of lesions behind oral disease. Additional research should explicitly disentangle the impact of different pathogens forming the lesions, and their specific action form and effects on tissues, as well as the mechanisms of the immune system to fight these infections. The presence of oral lesions can function as prior indicators of a host's physiological alterations that can be easily assessed in surveillance programs. Because nestlings have not fully developed normal protective microbiota and immune systems, they are expected to be more susceptible to the direct impact of pharmaceuticals and disease than adults (López-Rull et al., 2015; Blanco et al., 2017a). Therefore, nestlings should be preferred sentinels of the presence and impact of pharmaceuticals and disease in surveillance programs aimed at proposing adequate conservation management actions. More generally, efforts should be prioritized to rebuild trophic interactions between avian scavengers and carcasses of wild animals exploited in natural environments (Cortés-Avizanda et al., 2015a; Morales-Reyes et al., 2018). The progressive reduction of pharmaceutical treatment of livestock to provide high quality food in sustainable environments is encouraged through specific programs highlighting their benefits for environmental health, wildlife conservation and human well-being.

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Appendix A. Supplementary data

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