

## Review Article

# Implications of bacterial, viral and mycotic microorganisms in vultures for wildlife conservation, ecosystem services and public health

 PABLO I. PLAZA,<sup>1\*</sup>  GUILLERMO BLANCO<sup>2</sup> & SERGIO A. LAMBERTUCCI<sup>1</sup> 
<sup>1</sup>*Grupo de Investigaciones en Biología de la Conservación, Laboratorio Ecotono, INIBIOMA (Universidad Nacional del Comahue – CONICET), Bariloche, Quintral 1250 (R8400FRF), Argentina*
<sup>2</sup>*Department of Evolutionary Ecology, Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain*

The effects that microorganisms (bacteria, viruses and fungi) have on their hosts remain unexplored for most vulture species. This is especially relevant for vultures, as their diet consists of carcasses in various stages of decomposition, which are breeding grounds for potentially pathogenic microorganisms. Here we review current knowledge of bacterial, viral and mycotic microorganisms present in wild vultures. We consider their potential to cause disease in vultures and whether this poses any population-level threats. Furthermore, we address the question of whether vultures may act as disease spreaders or mitigators. We found 76 articles concerning bacterial, viral and mycotic microorganisms present in 13 vulture species, 57 evaluating bacteria, 13 evaluating viruses and six evaluating mycotic microorganisms. These studies come from all continents where vultures are present, but mainly from Europe and North America, and the most studied species was the Griffon Vulture *Gyps fulvus*. We found that vultures are colonized by zoonotic pathogens, and even host-specific human pathogens. Some recorded bacteria showed multi-antibiotic resistance, especially those that can be associated with anthropogenic food subsidies such as supplementary feeding stations. We found evidence that vulture health can be affected by some microorganisms, producing a wide array of clinical alterations that have the potential to influence mortality risk and fitness. We did not find clear scientific evidence that vultures play an epidemiological role spreading microorganisms to humans and other species. However, there is evidence that vultures could prevent the spread of infectious diseases through their removal of decomposing organic material. The evaluation of vulture exposure to microorganisms is of fundamental importance to design better conservation policies for this threatened group, which may serve a key role as ecosystem cleaners.

**Keywords:** antibiotic resistance, epidemiology, food subsidies, microbiome, pathogen, zoonotic disease.

Bacterial, viral and mycotic microorganisms may present contrasting (harmful, beneficial or conditional) interactions with different hosts (Casadevall

& Pirofski 2000). For instance, among other functions, the bacterial intestinal microbiome of vertebrates provides resistance to pathogens, stimulates the immune system and performs nutrient synthesis, resulting in a mutually beneficial interaction (Rosenberg & Zilber-Rosenberg 2013). In contrast, some microorganisms (e.g. bacteria or viruses)

Corresponding author.  
 Email: plazapablo22@gmail.com  
 Twitter: @PlazaPablo22

colonize the host, producing disease (clinical manifestation of tissue damage) and declines in fitness, which in some cases can be persistent (Casadevall & Pirofski 2000), to the point that infectious diseases have been identified as an increased threat to biodiversity (Daszak *et al.* 2000, Dobson & Foufopoulos 2001, Cunningham *et al.* 2017). However, the interplay of beneficial, detrimental and conditional effects of microorganisms has not been investigated in many obligate avian scavengers.

Obligate avian scavengers such as vultures and condors (hereafter, vultures) are exposed to various bacterial, viral and mycotic microorganisms present in the environment. This exposure could be favoured by their gregarious behaviour at carcasses and roost sites (Ferguson-Lees & Christie 2001, Cortés-Avizanda *et al.* 2016). In addition, being obligate scavengers, vultures feed on tissues that are in different stages of decomposition, which especially exposes them to a host of microorganisms associated with decomposing animal matter (Houston & Cooper 1975, Winsor *et al.* 1981, Ducatez *et al.* 2007, Marin *et al.* 2014, Cortés-Avizanda *et al.* 2016, Blanco 2018).

Because of their diet, vultures perform an important ecosystem service by removing decomposing organic material from the environment, which in turn could be important for human health (Markandya *et al.* 2008, Ogada *et al.* 2012a, Moleon *et al.* 2014). In fact, given that vultures and humans have been sympatric for millions of years, this ecosystem service may have been relevant since ancient times (Morelli *et al.* 2015). It has been suggested that vultures have multiple mechanisms that allow them to cope with microorganisms present in the environment (especially in their diet), such as a very acidic stomach pH, immune system adaptations and a stable protective intestinal microbiome (Roggenbuck *et al.* 2014, Blumstein *et al.* 2017). However, although vultures could be considered resistant to certain microorganisms (Blumstein *et al.* 2017), they may be negatively affected by others, depending on multiple host and environmental factors that may disturb homeostasis (Kumar *et al.* 2012, Gomez *et al.* 2014, Pitarch *et al.* 2017). In addition, vultures could act as carriers of microbial pathogens, thus playing a potential role in their spread to other species and even humans (Houston & Cooper 1975).

The occurrence and impact of bacterial, viral and mycotic microorganisms in vultures may have

important conservation, management and health risk implications for these birds (Cortés-Avizanda *et al.* 2016). To date, however, a thorough assessment of the microorganisms present in vulture species is lacking. This is of concern, given the importance of evaluating which microorganisms affect an avian guild that is one of the most threatened around the world (Ogada *et al.* 2012a) and that occurs in four continents (all but Australia and Antarctica) (Ferguson-Lees & Christie 2001). In fact, 16 of the 23 extant vulture species are showing important population declines (especially in Africa and Asia) due to human disturbances such as intentional poisoning, lead contamination and ingestion of veterinary drug residues (Shultz *et al.* 2004, Ogada *et al.* 2012a, 2016, Plaza & Lambertucci 2019, Plaza *et al.* 2019). In addition, due to their highly mobile nature, with migrating species that travel thousands of kilometres every year, and non-migratory species travelling long distances per day (García-Ripollés *et al.* 2010, Lambertucci *et al.* 2014, Alarcón & Lambertucci 2018), vultures could have the potential to spread pathogens over very large distances. Moreover, it is especially relevant to study microorganisms in vultures because some people consider that they are harmful (e.g. spread disease), which leads to hostile attitudes (e.g. lethal control) towards them (Henriques *et al.* 2018, Morales-Reyes *et al.* 2018).

Here, we review current knowledge of bacterial, viral and mycotic microorganisms in wild vulture species. We analyse microorganism-associated disease occurrence in vultures and whether this represents a population-level threat. We then evaluate the available information on whether vultures act as dispersers of pathogenic microorganisms and so may affect other species or human health. Finally, we emphasize the role of vultures in mitigating the spread of infectious diseases by means of scavenging on organic material.

## METHODS

We reviewed the scientific literature using two different search engines: Google Scholar ([www.scholar.google.com](http://www.scholar.google.com)) and Scopus ([www.scopus.com](http://www.scopus.com)). We performed four general searches using the following terms: (1) 'Condor' OR 'Vulture' OR 'Scaveng\* bird' AND 'Bacteria'; (2) 'Condor' OR 'Vulture' OR 'Scaveng\* bird' AND 'Virus'; (3) 'Condor' OR 'Vulture' OR 'Scaveng\* bird' AND 'Mycotic disease'; (4) 'Condor' OR 'Vulture' OR

'Scaveng\* bird' AND 'Fungi'. In addition, we performed other searches using different combinations of the following relevant terms: vultures or condors, and microorganisms, bacteria, virus, mycotic disease, fungi, infectious diseases, disease regulation and ecosystem service. We reviewed the returns of each search until we were sure the results were no longer related to the search criteria (every paper in Scopus and up to a maximum of 1000 returns in Google Scholar). Finally, we examined the references of the articles we reviewed for additional reports not found in our searches. We excluded articles based on vultures in permanent captivity in zoo collections and information originating from the 'grey literature' (e.g. technical reports, theses and posters). To define and employ epidemiological concepts (e.g. infection, colonization, disease) we followed the lexicon of Casadevall and Pirofski (2000).

We focused on the geographical location where the studies were performed, the vulture species studied, and the bacterial, viral and mycotic species isolated or detected in the different vulture species. Based on the information provided by the articles reviewed, we also considered the patterns of resistance of bacteria isolated, and the laboratory techniques used to detect microorganisms. Then, we assessed the possibility that vultures may: (1) suffer disease associated with bacterial, viral and mycotic microorganisms and (2) play an epidemiological role as spreaders of microorganisms producing diseases that affect them, other wild or domestic species and humans. Finally, based on the review of articles following the methods described above, we consider the role of vultures in diminishing the spread of infectious diseases given the ecosystem service they may provide scavenging on organic material.

## RESULTS AND DISCUSSION

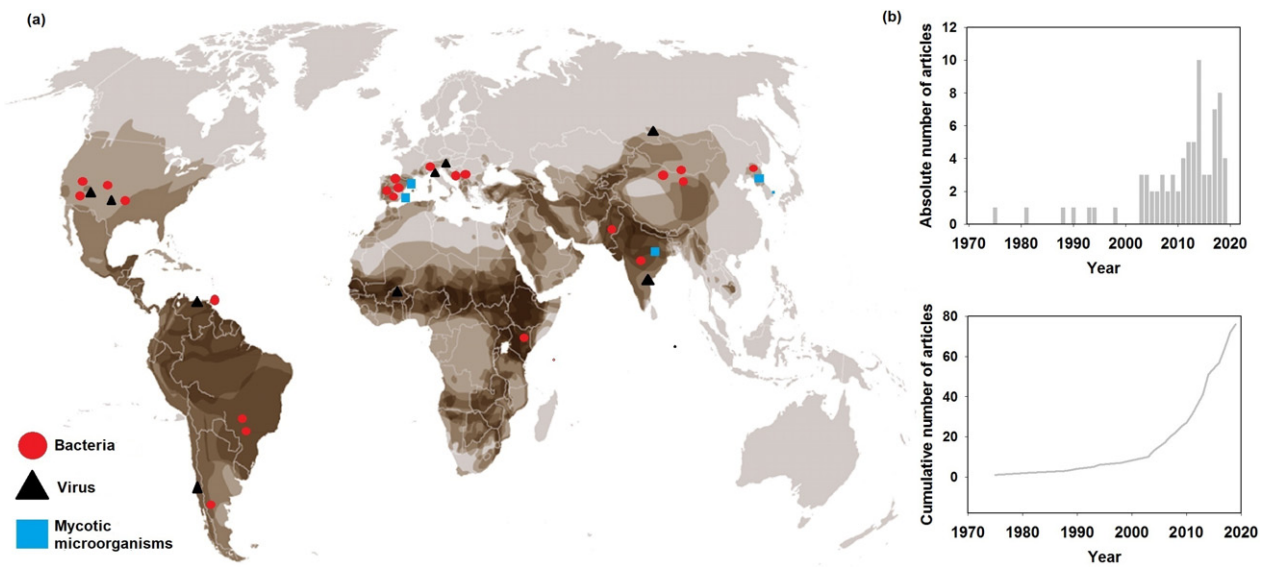
### Geographical and species biases

We found 76 articles concerning microorganisms present in 13 of the 23 extant vulture species: 57 related to bacterial species, 13 related to viruses and six related to mycotic microorganisms (Table S1). The studies were performed between 1975 and 2019, but most (82%, 63/76) were performed between 2005 and 2019 (Fig. 1) and focused mainly on bacteria, suggesting that this is a topic of recent increasing interest (Fig. 1). To

our knowledge, no study has been published on the occurrence of microorganisms for six wild accipitrid vultures (*Gypohierax angolensis*, *Gyps rueppellii*, *Gyps coprotheres*, *Torgos tracheliotos*, *Trigonoceps occipitalis*, *Sarcogyps calvus*) and four wild cathartid vultures (*Cathartes burrovianus*, *Cathartes melambrotus*, *Sarcoramphus papa*, *Vultur gryphus*), despite the fact that several of these species are of high global conservation concern. It is important to note that there is less information available about microorganisms present in vultures than in other avian guilds (see for instance Benskin *et al.* 2009) and this could be related to a general perception that vultures are resistant to pathogenic microorganisms.

The studies concerning bacterial species were performed in both Old and New World vultures (Tables 1 and S1). The most studied species was the Griffon Vulture *Gyps fulvus*, representing almost half of the studies (25/57), followed by the Cinereous Vulture *Aegypius monachus* (12/57 studies), the American Black Vulture *Coragyps atratus* (10/57 studies) and the Turkey Vulture *Cathartes aura* (8/57 studies) (Table S1). Excluding a global review on *Chlamydophila* spp. (Kaleta & Taday 2003) that represented 1.7% of the articles about bacteria on vultures, most studies come from Europe (47.4%, 27 articles), especially Spain (35%, 20), but also from Asia (23%, 13), North America (17.5%, 10), South America (7%, 4), Africa (1.7%, 1) and the Caribbean (1.7%, 1) (Table S1, Fig. 1). This indicates that information on bacteria present in vultures is not equitably distributed geographically but predominates in developed regions of the world, as is the case with scientific information in general (King 2004). The fact that most articles come from Spain is not surprising given that this country represents a stronghold for these species in Europe (Ferguson-Lees & Christie 2001, Margalida *et al.* 2010). Regrettably, little information is available from regions such as South America, Asia and Africa, where vulture species with important conservation problems and critical population declines are found. Given that microorganisms exhibit biogeographical patterns that could modify the relationships with their host (Martiny *et al.* 2006), future research in these areas is crucial to better understand geographical differences in the microorganisms present in vultures.

Compared with bacteria, there is little information about viruses and mycotic microorganisms



**Figure 1.** (a) Map showing the geographical location of the articles published about bacterial, viral and mycotic microorganisms present in vulture species. (b) Absolute and cumulative number of articles published studying bacterial, viral and mycotic microorganisms present in vultures according to the year of publication. The map with the world distribution of vultures was obtained from [www.nation-algeographic.com](http://www.nation-algeographic.com) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

that are present in vultures. In the case of viruses (Table S1), the information available comes from both the Old and the New World vultures, particularly from North America (31%, four studies), Europe (31%, four studies), South America (15.5%, two studies), Africa (15.5%, two studies) and Asia (7%, one study) (Table S1, Fig. 1). The most studied species (3/13 studies) was the California Condor *Gymnogyps californianus* (Table S1). In the case of mycotic microorganisms, studies were performed in Old World vultures and only in Europe (specifically Spain, 50%, three studies), and in Asia (50%, three studies) (Table S1, Fig. 1). The most studied species (3/6 studies) was the Cinereous Vulture. So, for viruses and mycotic microorganisms, there is also an important geographical but also a species bias, which merits further research in most regions of the world where vultures occur.

### Bacteria in vultures

All of the studies we reviewed about bacteria present in vultures were descriptive, presenting a list of bacterial species extant in different types of samples (Table S1), some of them potentially pathogenic for birds and other species, including humans (Table 1). The samples used were mainly faecal, cloacal, oropharyngeal, tracheal and from the skin,

but also from pathological lesions such as those from arthritis and from different internal organs such as the spleen or liver, these being the type of samples generally used for bacteriological studies on raptors (Bildstein & Bird 2007; Table S1). The laboratory techniques used to isolate or detect bacteria were traditional microbiology (culture, biochemical reactions and serotyping) and also genetic techniques (PCR), and combinations of the two.

More than half (61%; 35/57) of the studies we reviewed reported potentially zoonotic bacteria in diverse vulture species, and even the presence of host-specific human pathogens (Table 1). The most important zoonotic bacteria reported include *Salmonella* spp. (Marin *et al.* 2014, 2018, Molina-López *et al.* 2015, Jurado-Tarifa *et al.* 2016, Blanco 2018, Blanco & de Tuesta 2018, Plaza *et al.* 2018), *Campylobacter* spp. (Molina-Lopez *et al.* 2011, Marin *et al.* 2014, Sulzner *et al.* 2014, Jurado-Tarifa *et al.* 2016), *Chlamydia psittaci* (Kaleta & Taday 2003, Plaza *et al.* 2018) and even some *Mycobacterium* species (Cunha *et al.* 2017). Vultures can also be exposed to host-specific human pathogens. For example, Plaza *et al.* (2018) isolated *Salmonella enterica* ser. Typhi and Paratyphi A in American Black Vultures foraging in a rubbish dump in Patagonia, but also in the soil at this site. These pathogens mainly have a human

**Table 1.** Information of vulture exposure to avian pathogens/opportunistic pathogens, potential zoonotic microorganisms, bacteria with antibiotic resistance and disease signs caused by pathogens or opportunistic pathogens. We also included human activities potentially involved in pathogen acquisition, and geographical area where studies were performed for the different vulture species.

Vulture species	Conservation status	Presence of avian pathogens or opportunistic pathogens <sup>a</sup>	Presence of potential zoonotic pathogens	Presence of bacteria with antibiotic resistance	Presence of disease <sup>b</sup>	Impact of human activities <sup>c</sup>	Geographical area
Bearded Vulture <i>Gypaetus barbatus</i>	Near Threatened	Reported (3) <sup>d</sup>	Reported (3) <sup>e</sup> (e.g. West Nile virus, <i>Chlamydia psittaci</i> )	Not reported <sup>f</sup>	Not reported	Not reported	Asia–Europe
Egyptian Vulture <i>Neophron percnopterus</i>	Endangered	Reported (10)	Reported (5) (e.g. <i>Salmonella</i> spp., <i>Chlamydia psittaci</i> )	Reported (5)	Reported (e.g. oral lesions compatible with <i>Candida</i> -yeast species)	Reported (4) (acquired from pig carcasses in vulture restaurants)	Asia–Europe
Cinereous Vulture <i>Aegypius monachus</i>	Near Threatened	Reported (15)	Reported (11) (e.g. <i>Salmonella</i> spp., <i>Chlamydia psittaci</i> , West Nile virus)	Reported (5)	Reported (e.g. oral lesions compatible with <i>Candida</i> -yeast species)	Reported (1) (acquired from rubbish dumps)	Asia–Europe
Griffon Vulture <i>Gyps fulvus</i>	Least concern	Reported (27)	Reported (19) (e.g. <i>Salmonella</i> spp., <i>Mycobacterium</i> spp., <i>Campylobacter</i> spp., <i>Chlamydia psittaci</i> )	Reported (10)	Reported (e.g. oral lesions compatible with <i>Candida</i> -yeast species)	Reported (5) (acquired from livestock carcasses and pig carcasses from vulture restaurants)	Europe
White-backed Vulture <i>Gyps africanus</i>	Critically Endangered	Reported (1)	Reported (1) (e.g. <i>Escherichia coli</i> )	Not reported	Not reported	Not reported	Africa
Himalayan Griffon <i>Gyps himalayensis</i>	Near Threatened	Reported (4)	Reported (2) (e.g. <i>Chlamydia psittaci</i> )	Not reported	Reported (e.g. pulmonary aspergillosis produced by <i>Aspergillus fumigatus</i> )	Not reported	Asia
White-rumped Vulture <i>Gyps bengalensis</i>	Critically Endangered	Reported (3)	Reported (2) (e.g. <i>Escherichia coli</i> , <i>Salmonella enterica</i> )	Reported (1)	Reported (enteritis produced by <i>Escherichia coli</i> )	Not reported	Asia
Long-billed Vulture <i>Gyps indicus</i>	Critically Endangered	Reported (2)	Reported (1) (e.g. <i>Escherichia coli</i> , <i>Salmonella enterica</i> )	Not reported	Not reported	Not reported	Asia
Slender-billed Vulture <i>Gyps tenuirostris</i>	Critically Endangered	Reported (1)	Reported (1) (e.g. <i>Escherichia coli</i> , <i>Salmonella enterica</i> )	Not reported	Not reported	Not reported	Asia

(continued)



Table 1. (continued)

Vulture species	Conservation status	Presence of avian pathogens or opportunistic pathogens <sup>a</sup>	Presence of potential zoonotic pathogens	Presence of bacteria with antibiotic resistance	Presence of disease <sup>b</sup>	Impact of human activities <sup>c</sup>	Geographical area
Hooded Vulture <i>Necrosyrtes monachus</i>	Critically Endangered	Reported (2)	Reported (2) (e.g. avian influenza virus H5N1)	Not reported	Reported (e.g. dyspnoea and neurological signs produced by avian influenza virus H5N1)	Reported (1) (acquired from poultry carcasses)	Africa
Turkey Vulture <i>Cathartes aura</i>	Least Concern	Reported (10)	Reported (8) (e.g. <i>Salmonella</i> spp., <i>Campylobacter</i> spp., <i>Chlamydia psittaci</i> )	Reported (1)	Reported (e.g. dermal lesions produced by poxvirus)	Reported (1) (acquired from livestock carcasses)	North and South America
American Black Vulture <i>Coragyps atratus</i>	Least Concern	Reported (10)	Reported (7) (e.g. <i>Salmonella</i> spp., <i>Chlamydia psittaci</i> , West Nile virus)	Not reported	Reported (e.g. footpad abscess, abscess in joints produced by <i>Mycoplasma</i> spp.)	Reported (2) (acquired from rubbish dumps)	North and South America
California Condor <i>Gymnogyps californianus</i>	Critically Endangered	Reported (3)	Reported (3) (e.g. West Nile virus)	Not reported	Reported (e.g. inflammation and haemorrhages produced by West Nile virus)	Not reported	North America

<sup>a</sup>The classification of a microorganism as pathogens or opportunistic pathogens was performed following the criteria presented in Benskin *et al.* (2009) and Ritchie *et al.* (1997). <sup>b</sup>Refers to the isolation/identification of the reported microorganisms as forming part of macroscopic lesions or disease signs for each vulture species. <sup>c</sup>Refers to the human activities reported influencing the acquisition and potential spread of pathogens by vultures. <sup>d</sup>Number of articles reviewed reporting each topic is given in parentheses. <sup>e</sup>These are the most representative examples. For the complete list, refer to Table S1. The classification of a microorganism as potentially zoonotic was based on Acha and Szyfres (2003). <sup>f</sup>Not reported refers to the absence of articles reporting each topic.

reservoir, and produce a human disease characterized by diarrhoea, fever and high mortality rates (Bhutta 2006). In summary, most studies we found detected zoonotic pathogens in vultures, which can be considered important for public health. However, it is not clear whether vultures are merely colonized by these bacteria or they play an epidemiological role in spreading these pathogens through the ecosystem (see: Vultures as potential disseminators of pathogens).

Nine (16%) of the articles reviewed isolated *Mycoplasma* species from vultures (Panangala *et al.* 1993, Oaks *et al.* 2004, Loria *et al.* 2008, Ruder *et al.* 2009, Lecis *et al.* 2010) (Table S1). Some of

the isolates were found in individuals showing physical signs of disease (Panangala *et al.* 1993, Ruder *et al.* 2009), suggesting that this microorganism can produce health alterations (disease) in vultures (Table 1). For instance, *Mycoplasma vulturni* was detected in American Black Vultures with arthritis (Ruder *et al.* 2009) or a footpad abscess (Panangala *et al.* 1993). Similarly, this microorganism was associated with mild respiratory disease in Griffon Vultures (Poveda *et al.* 1990, Lecis *et al.* 2010). Given that some *Mycoplasma* spp. could produce disease in this avian guild, it is therefore important to consider this microorganism as a potential diagnosis when

vultures arrive at rehabilitation centres with respiratory signs, weakness or joint problems.

Finally, six studies (10%) were designed to study the skin or intestinal microbiome in Old and New World vultures (Table S1). Two of these studies used traditional microbiology (Carvalho *et al.* 2003, Vela *et al.* 2015) and the rest used metagenomics techniques (Roggenbuck *et al.* 2014, Meng *et al.* 2017, Mendoza *et al.* 2018, Crespo *et al.* 2019). These studies showed that the skin microbiome was in general more diverse and had a different microbial composition compared with the intestinal microbiome, which is more specialized and less diverse (Roggenbuck *et al.* 2014, Mendoza *et al.* 2018). In the study of the intestinal microbiome, there were common findings between vulture species. For instance, all studies found that bacteria such as *Clostridium* spp., *Fusobacterium* spp., *Enterococcus* spp. and *Lactobacillus* spp. are common species present in the microbiome of the gastrointestinal tract. The vulture microbiome can therefore be considered unique due to its high specialization, where a few groups of bacteria out-compete others (Roggenbuck *et al.* 2014). Moreover, some of these bacteria, such as *Enterococcus* spp. and *Lactobacillus* spp., produce substances that inhibit the growth of diverse bacterial species (Carvalho *et al.* 2003, Arbulu *et al.* 2016). These results, added to the very acidic stomach pH and the particularities of the immune system functioning (Blumstein *et al.* 2017), suggest that the intestinal microbiome could be considered an important trait that vultures have evolved to cope with microbial pathogens from their food. Given that the intestinal microbiome could vary according to the geographical area (Gupta *et al.* 2017), future research is needed to describe and compare microbiome composition and function in other vulture species in different regions of the world, and under different environmental conditions. In addition, it could be interesting to study whether alterations in the microbiome produced, for instance, by veterinary drugs such as antibiotics, generate less tolerance to microorganisms (Pitarch *et al.* 2017, Blanco *et al.*, 2017a, 2017b).

### Antibiotic resistance reported in bacteria present in vultures

A third of the studies found (33%, 19/57) evaluated bacterial antibiotic resistance using disc diffu-

sion and broth micro-dilution methods, but they also studied resistance genes by PCR (Table 1). These studies reported bacterial species with different degrees of antibiotic resistance, such as *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Staphylococcus* spp., *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. For instance, in faecal material of Griffon Vultures and Egyptian Vultures *Neophron percnopterus* from Spain there were isolates of *Salmonella* serotypes resistant to antibiotics such as amino-penicillins, streptomycin, tetracyclines and sulfamethoxazole-trimethoprim (Molina-Lopez *et al.* 2011, Molina-López *et al.* 2015, Jurado-Tarifa *et al.* 2016, Blanco 2018). Similarly, in Griffon Vultures from Spain there were isolations of *Campylobacter* spp. resistant to gentamicin, ciprofloxacin and tetracycline (Jurado-Tarifa *et al.* 2016). In addition, *E. coli* resistant to amino-penicillins (Mora *et al.* 2014) and extended-spectrum  $\beta$ -lactamase were isolated from faecal samples of Griffon Vultures from Spain (Alcalá *et al.* 2016) and in cloacal samples of Cinereous Vultures from Portugal (Pinto *et al.* 2010) and Mongolia (Guenther *et al.* 2012, 2017). Methicillin-resistant *Staphylococcus aureus* (MRSA), an important pathogenic bacterium for humans that can produce severe disease (Klevens *et al.* 2007), was isolated from the respiratory tract of Griffon Vultures (Porrero *et al.* 2013) and Cinereous Vultures from Spain (Ruiz-Ripa *et al.* 2019). Finally, multidrug-resistant *P. aeruginosa*, *E. coli* and *K. pneumoniae* were isolated from faeces of Egyptian Vultures from India (Sharma *et al.* 2014a, 2014b, 2018). Whether antibiotic resistance is acquired from bacteria present in medicated livestock or is developed *de novo* in vultures ingesting antibiotics from carcasses of medicated livestock (Blanco *et al.* 2016, 2017a, 2017b), and the relevance of these findings, remain unexplored topics despite their potential implications for public health. However, it is important to note that some studies suggest that antibiotic resistance could be spread to humans by facultative scavenger birds such as gulls (Bonnedahl & Järhult 2014 and references therein). It is therefore timely to address the significance and implications of the presence of microorganisms with antibiotic resistance in vulture species, especially in migrant species that could introduce and transfer drug-resistant bacteria to geographically remote areas (Benskin *et al.* 2009, Bonnedahl & Järhult 2014, Dolejska 2020).

## Viruses in vultures

The studies we reviewed show that wild vultures could be affected by different viral pathogens such as West Nile virus, avian influenza virus, poxvirus, papillomavirus and herpesvirus (Table S1). Some of these reported viruses can produce serious health alterations and even death in vultures, but may also produce disease in humans. In fact, 70% (9/13) of the studies reported viruses that are considered zoonotic (Table S1).

There are reports of California Condors in the USA and Bearded Vultures *Gypaetus barbatus* in Austria dying as a consequence of West Nile virus (Rideout *et al.* 2012, Bakonyi *et al.*, 2013). A similar situation was reported in Cinereous Vultures from Russia (Loktev 2004, Ternovoï 2006). West Nile virus is a mosquito-borne flavivirus which affects humans, equids and avian hosts (Campbell *et al.* 2002). Birds are natural reservoirs and even considered amplifying hosts of this virus (Campbell *et al.* 2002). Neurological signs such as ataxia, lethargy and the inability to hold the head upright characterize the disease produced by West Nile virus in birds, ultimately leading to death (Komar *et al.* 2003). In humans this virus can produce sub-clinical infections but also can produce a severe disease characterized by neurological signs and encephalitis, with fatal outcomes in some cases (Campbell *et al.* 2002). The presence of this virus in vultures may therefore have public health and wildlife conservation implications and deserves further research.

Avian influenza virus (specifically strain H5N1) has been detected in Hooded Vultures *Necrosyrtes monachus* from Burkina Faso, with associated neurological signs, ataxia and locomotion alterations (Ducatez *et al.* 2007, Tarnagda *et al.* 2011). Vultures are likely to have acquired this virus via ingestion of infected poultry, which highlights the risk of bird-to-bird infection from carcasses of domestic birds (Ducatez *et al.* 2007, 2008). Given that humans can be infected by this pathogen, this finding is relevant from an epidemiological perspective, especially when vultures live at high abundances in the proximity of cities (Gangoso *et al.* 2013, Barbar *et al.* 2015, Plaza & Lambertucci 2018) and in the areas where they are used for traditional medicine, thus promoting transmission to humans (Ducatez *et al.* 2007).

Finally, we found reports of vultures with detections of poxvirus, papillomavirus and

herpesvirus (Cardoso *et al.* 2005, Gomez *et al.* 2014, Di Francesco *et al.* 2019, Mora-Carreño *et al.* 2019). Poxvirus was detected in cutaneous lesions from Turkey Vultures from Chile and Venezuela (Gomez *et al.* 2014, Mora-Carreño *et al.* 2019). Similarly, a poxvirus together with a papillomavirus were identified in cutaneous lesions from a Griffon Vulture from Italy (Di Francesco *et al.* 2019). According to a study of individuals from zoo collections, other vulture species could be susceptible to this virus (Kim *et al.* 2003). The presence of poxvirus in wild vultures therefore requires particular attention due to its potential threat to endangered species. Finally, a novel herpesvirus was detected in a Long-billed Vulture *Gyps indicus* from India, which may have implications in the production of severe disease in wild birds (Cardoso *et al.* 2005). Future research evaluating the occurrence and impact of viruses affecting vultures is encouraged, especially those potentially acquired from poultry. In addition, it is important to investigate viral disease in vultures found dead of non-apparent causes to establish the importance of viruses as a threat to these species.

## Mycotic microorganisms in vultures

Oral lesions compatible with opportunistic disease by *Candida* spp. and other yeasts have been reported in Griffon, Egyptian and Cinereous Vultures from Spain (López-Rull *et al.* 2015, Pitarch *et al.* 2017, Blanco *et al.*, 2017a, 2017b, 2019). Interestingly, these lesions were found in some individuals with antibiotic residues in their blood (fluoroquinolones), attributed to the consumption, at feeding stations, of medicated pig and poultry carcasses coming from intensive farming operations (Blanco *et al.* 2016, 2017a, 2017b, 2019, Pitarch *et al.* 2017). This suggests that antibiotics could produce alterations in the local microbiome, resulting in opportunistic mycotic diseases (Pitarch *et al.* 2017, Blanco *et al.*, 2017a, 2017b). Consequently, some threats for vultures could act synergistically, producing population effects that may be difficult to predict. While aspergillosis is a common problem in captive individuals (Chege *et al.* 2013), some studies in wild Cinereous and Himalayan *Gyps himalayensis* Vultures have reported cases of pneumonia due to *Aspergillus* spp. probably associated with concomitant pathologies that produce an immune alteration, suggesting that this fungus could be an opportunistic pathogen for



vultures that should be evaluated (Jung *et al.* 2009, Barathidasan *et al.* 2013). Although there is little information about isolations of mycotic microorganisms in wild vultures, the few articles we found suggest that opportunistic mycosis could be a potential threat for vulture populations producing disease, in some cases accompanied by severe lesions such as oral ulcers or pneumonia (Jung *et al.* 2009, Barathidasan *et al.* 2013, Pitarch *et al.* 2017).

### Food subsidies and pathogens

Predictable anthropogenic food subsidies such as organic waste and feeding stations can increase the risk of pathogen infections (Oro *et al.* 2013, Plaza & Lambertucci 2017). We found that approximately 17% (13/76) of the articles we reviewed detected in vultures zoonotic pathogens and even pathogens showing antibiotic resistance that are likely to have their origin in different anthropogenic food subsidies such as supplementary feeding stations (vulture restaurants), carcass dumps, livestock carcasses and rubbish dumps (Table 1). For instance, the presence of zoonotic bacteria with different degrees of antibiotic resistance in vultures from Spain was associated with carcasses discarded in supplementary feeding stations, especially those of pigs (Marin *et al.* 2014, 2018, Blanco 2018). Similarly, American Black Vultures foraging at rubbish dumps were colonized by important zoonotic and even human pathogens (Adesiyun *et al.* 1998, Plaza *et al.* 2018). Egyptian Vultures sampled in a carcass dump from India showed bacterial species such as *P. aeruginosa*, *E. coli* and *K. pneumoniae* with different degrees of antibiotic resistance (Sharma *et al.* 2014a, 2014b, 2018). Finally, Hooded Vultures were infected by avian influenza virus (H5N1) due to the ingestion of infected poultry carcasses that had been improperly disposed of (Ducatez *et al.* 2007). However, it is interesting to note that some microorganisms could be acquired not only from food subsidies but also from congeners and other bird species (e.g. facultative scavenger birds) when they congregate in large abundances at sites where food subsidies are provided (Jurinović *et al.* 2014, Blanco *et al.*, 2017a, 2017b).

It is important to consider that the positive effects of anthropogenic food subsidies can hide potential threats not only to vultures but also to

other wildlife species and humans, if vultures disperse zoonotic pathogens. In the case of feeding stations, it is necessary to avoid the disposal of carcasses with zoonotic-resistant pathogens (Blanco 2018, Marin *et al.* 2018). In the case of rubbish dumps, it is necessary to perform better waste management strategies to reduce the presence of organic material and pathogens at these sites (Plaza & Lambertucci 2017), and carcasses infected with harmful pathogens should be discarded properly to avoid contact with scavengers (Ducatez *et al.* 2007).

### Disease as a threat to vulture populations

The effects that microorganisms produce on the health of vultures have not yet been quantified in depth, as is usual for any diverse taxon in the wild. However, we found evidence suggesting that wild vultures can be negatively affected by some bacterial, viral and mycotic microorganisms that produce disease and variable impacts on their health and fitness (Panangala *et al.* 1993, Ducatez *et al.* 2008, Jung *et al.* 2009, Ruder *et al.* 2009, Kumar *et al.* 2012, Pitarch *et al.* 2017, Di Francesco *et al.* 2019, Mora-Carreño *et al.* 2019) (Table 1). This overlooked threat should therefore be taken into account because it could lead to population declines, especially if associated synergistically with other major threats to which vultures are exposed, such as intentional poisoning or ingestion of veterinary drug residues (e.g. antibiotics) (Jung *et al.* 2009, Blanco *et al.*, 2017a, 2017b, Plaza *et al.* 2019). In this sense, further research should evaluate how microorganisms affect the different age classes of vultures and whether they can be transmitted vertically (through the egg), producing reproductive failure, especially in order to understand effects on the demographic rates and population dynamics of threatened vulture species. However, it is important to take into account that studying infections requires challenging evaluations of individuals and population health (Vicente & VerCauteren 2019), especially given that there is a lack of baseline information for most vulture species. This constraint probably explains the current lack of knowledge of the individual and population effects of microorganisms on vultures. While an experimental approach could be useful in understanding endemic infections and immunological response in these species, it is almost impossible to

perform, as most vultures are threatened and permission to conduct such studies in species of lesser conservation concern may be difficult to obtain for ethical reasons.

Evidence from captive vultures in zoo collections suggests that they could be susceptible to microorganisms not yet reported in wild populations. For instance, vultures can be affected by hepatitis E virus (Li *et al.* 2015) and Newcastle virus (Lublin *et al.* 2001). Future research is therefore needed to expand our knowledge of the different microorganisms that can affect vulture health and may threaten wild vultures due to transmission from captive populations or due to reintroduction programmes.

### **Vultures as potential disseminators of pathogens**

Vultures can be colonized by different zoonotic bacteria and viruses that could potentially affect other animal species (wild and domestic) and even humans (Loktev 2004, Ducatez *et al.* 2007, Marin *et al.* 2014, Blanco 2018, Plaza *et al.* 2018). However, to our knowledge, vulture–human transmission has not been reported, even when these species are used as food and traditional medicine for humans in some regions (McKean *et al.* 2013, Boakye *et al.* 2019). However, vulture acquisition of multiple zoonotic bacteria (e.g. *Salmonella*) has been reported as originating from anthropogenic food subsidies from livestock farming, especially pigs from intensive farming operations (Blanco 2018, Marin *et al.* 2018) or rubbish dumps for urban refuse (Plaza *et al.* 2018).

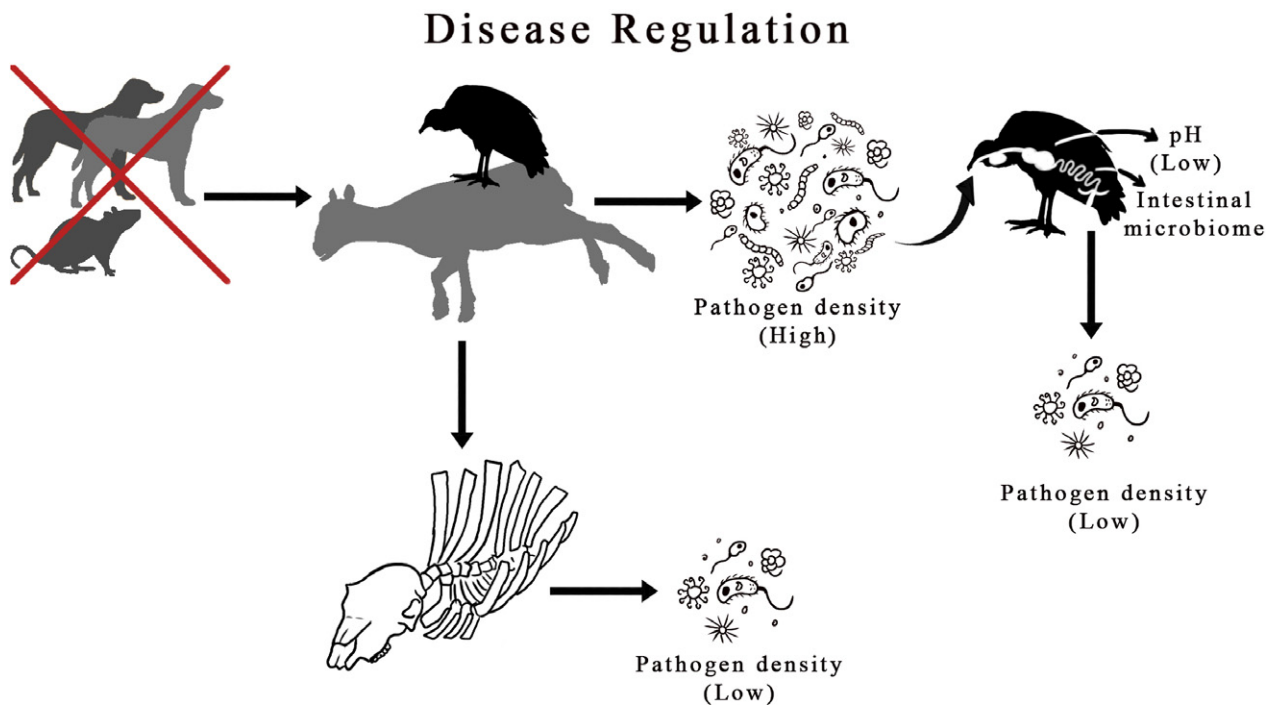
Currently, most emerging pathogens are considered zoonotic (Jones *et al.* 2008). The number of emerging infectious disease events caused by microorganisms originating in wildlife has increased significantly with time (Jones *et al.* 2008). Several wildlife species, such as rodents, birds, monkeys and bats, have caused events of zoonotic disease (Daszak *et al.* 2000) (e.g. the Covid-19 pandemic is thought to have originated from bats, Guo *et al.* 2020). Until now, and to the best of our knowledge, there is no clear scientific evidence showing that vultures can play a role in microorganism spread to other animal species, including humans (see below). However, more research is needed on this topic in order to evaluate the potential of vultures as microorganism spreaders, especially with modern diagnostic methodologies (e.g. genetic tools).

The studies we reviewed only speculate on the potential role of vultures as carriers and spreaders of particular pathogens, without strong scientific evidence. One example is the case of anthrax disease. We did not find any recent articles specifically focused on addressing the presence of *Bacillus anthracis* in vultures. Some publications regarding the general epidemiology of this disease have occasionally reported vultures as potential spreaders of spores (Kehoe 1919, Bullock 1956, Pienaar 1967). In an experimental study, Houston and Cooper (1975) found that spores of *B. anthracis* could survive passage through the gastrointestinal tract of vultures given that they recovered ingested spores in faecal material approximately 5 h after being administered. In addition, spores of anthrax were reported in faecal material of vultures in the vicinity of an infected carcass (Lindeque & Turnbull 1994, Hugh-Jones & De Vos 2002). However, evidence that vultures can disperse this pathogen and affect the health of other species, including humans, is not clear and it is only based on the presence of spores in a proportion of their faeces. In this sense, it is important to note that the capacity of vultures as spreaders of anthrax spores is surely outweighed by their capacity to diminish environmental contamination through rapid consumption of contaminated carcasses (Lindeque & Turnbull 1994). In fact, vulture population declines in India have been potentially associated with increases in the incidence of human anthrax because people are in greater contact with contaminated carcasses that remain in the environment (Mudur 2001) (see next section).

In summary, it is crucial to avoid misconceptions about the role of vultures as microorganism spreaders based on non-scientific, partial and inconclusive information, which could produce hostile attitudes to species of conservation concern (e.g. farmers believing that vultures spread disease). Thus, the potential of vultures as pathogen disseminators requires further research due to the latent implications for the epidemiology of human–livestock–wildlife interfaces (e.g. a migratory species feeding on livestock close to human-habited areas and interacting with other wild species).

### **Vultures as disease mitigators**

It is widely known that vultures perform an important ecosystem service by removing organic material from the environment, and it has been



**Figure 2.** Scheme of the ecosystem service provided by vultures reducing the availability of rotten organic material and potentially the spread of pathogens. The crossed-out rats and dogs refers to the probably lower abundances of these species in sites where vultures are present, compared with sites where vultures are not present. The black arrows represent the expected flux of microorganisms. The density of microorganisms is expected to be higher in the whole carcass than in the skeletal parts. When vultures ingest organic material, microorganism densities decrease when passing gastrointestinal systems, which have an acidic stomach pH and a protective intestinal microbiome. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

suggested that this could reduce the spread of pathogenic microorganisms in the ecosystem (Fig. 2) (Mudur 2001, Markandya *et al.* 2008, Ogada *et al.* 2012b). However, we found very little information about disease regulation by vultures. Research on this topic is therefore needed to elucidate whether vultures effectively regulate infectious diseases, and the mechanisms behind this process. Some indirect evidence comes from geographical areas where vultures have suffered population declines and lost their ecosystem role and where there was then an increment in infectious disease transmission (Mudur 2001, Markandya *et al.* 2008). The decline of vultures would mean that uneaten carcasses stay in the environment longer, and that other facultative scavenger species such as dogs and rats take advantage of them (Ogada *et al.* 2012b), with a potential increase in their population abundances and of zoonotic diseases associated with them. Rabies and leptospirosis are examples of zoonotic diseases that could enhance their transmission when the

abundances of rats and dogs increase (Pain *et al.* 2003, Markandya *et al.* 2008). In addition, if carcasses remain longer in the environment, contact with humans may increase, which in turn represents a risk to human health because these carcasses can host and be a source of different pathogenic microorganisms (Mudur 2001). So, although indirectly, the presence of vultures that remove organic material should influence the spread of microorganisms (Fig. 2).

Less evidence is available about the intrinsic mechanisms involved in pathogenic microorganism reduction associated with vultures. Some vulture characteristics could be at play, including a very acidic stomach pH (Houston & Cooper 1975) and their highly specialized microbiome (Roggenbuck *et al.* 2014) (Fig. 2). However, recent studies on American Black Vultures showed high variability of stomach pH (Graves 2017). It is also important to assess processes in the intestinal microbiome such as secretion of inhibitory substances, which can result in pathogen regulation (Roggenbuck

*et al.* 2014, Arbulu *et al.* 2016). Clearly, more research is needed on this key topic to avoid faulty assumptions about the potential of vultures as disease mitigators, without having strong scientific evidence. This information may help to understand fully the effectiveness of the ecosystem service that vultures provide by consuming decaying carcasses.

Additionally, it could be interesting to compare the physiological mechanisms involved in reduction of pathogenic microorganisms (e.g. intestinal microbiome, stomach pH and immune system characteristics) not only in obligate scavengers such as vultures but also in facultative scavenger birds in order to evaluate potential differences between these two groups. This is especially relevant because obligate and facultative scavengers interact at carcasses (Cortés-Avizanda *et al.* 2010, Blanco 2018), with the possibility of sharing microorganisms between them. In addition, unlike for vultures, scientific evidence suggests that facultative scavenger birds could spread pathogenic microorganisms to the ecosystem (Bonnedahl & Järhult 2014, Toro *et al.* 2016). The spread of microorganisms in the ecosystem could therefore be conditioned by differences in physiological characteristics present in obligate and facultative scavenger birds.

## CONCLUSIONS

We found that vultures may be colonized by different microorganisms. Some of these can be beneficial to them, as occurs within the intestinal microbiome. However, some of the microorganisms reported may impact vulture health, producing disease. Moreover, vultures may carry zoonotic and human-specific pathogens with antibiotic resistance, which can be of concern for public health if vultures disperse them in the environment. Currently, there is no scientific evidence that vultures play a role in the spread of microorganisms and bacterial resistance to other wildlife, livestock and humans. However, this merits further research, especially with genetic methods (e.g. metagenomic studies or sequencing of microorganisms) that allow determination of the potential origin of microorganisms. On the contrary, vultures may act as cleaners of the environment and thus limit the spread of pathogenic microorganisms (Fig. 2), resulting in disease regulation in the ecosystem, although this also requires further scientific

evaluation, as evidence remains limited. Finally, it is necessary to include microorganisms as a potential threat to the conservation of vultures because they can produce disease in individuals, and indeed may act synergistically with other threats, such as contaminants and deliberate poisoning.

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## CONFLICT OF INTEREST

No conflict declared.

## AUTHOR CONTRIBUTION

**Pablo Ignacio Plaza:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Writing-original draft (equal); Writing-review & editing (equal). **Guillermo Blanco:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Resources (equal); Writing-original draft (supporting); Writing-review & editing (supporting). **Sergio A Lambertucci:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Supervision (lead); Writing-original draft (equal); Writing-review & editing (equal).

## ETHICAL STATEMENT

No ethical approval was required, as this is a review article with no original research data.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Vulture species studied in the articles we review and their conservation status, samples used to detect microorganisms, microorganisms present in the different vulture species, bibliographic reference and site where the studies were performed.