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Review Article

West Nile Virus in Vultures From Europe – a Sight Among Other Raptors

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Abstract: The West Nile virus (WNV) is an arbovirus mainly transmitted by *Culex* spp. and the causative agent of a zoonotic disease that is present worldwide. This pathogen is endemically maintained in a life cycle with birds acting as reservoirs, and humans and horses as accidental and dead-end hosts. Sporadic WNV outbreaks have been reported in Europe, and the potential impact of WNV infection on populations of threatened or endangered birds of prey is considerable. Surveillance programs are needed for early detection of this virus. All four species of vultures present in Europe are considered protected species. As scavengers, vultures are at the top of the food chain, and can be susceptible to, and negatively affected by, pathogens like WNV. In a conservation perspective, the impact of WNV in European vultures, alone or concomitantly with other factors, should be addressed. This review of documented cases can be considered a starting point.

Introduction

Vultures are the biggest European bird scavengers, performing an essential role in the environment. Information and awareness-raising are needed to change the paradigm of the bad reputation they are given. Vultures are often seen as sinister and death-dealing, being spotted with their large wings wide spread, gliding in circles. And even in literature, comics or cinema, they usually appear as a forewarning of bad things to come, a sort of bad omen. This negative image may be due to their threatening appearance and the fact that they are scavengers. However, vultures provide an important service to the ecosystem by cleaning up and recycling carcasses of dead animals. They can quickly eliminate large amounts of flesh, in different stages of decomposition, that may potentially host pathogenic microorganisms, thus removing this potential source of infection from the environment. Furthermore, thanks to its extremely acidic gastric pH and stable intestinal microbiome with remarkable anti-microbial activity, they can neutralize pathogens that pass through their gastrointestinal tract, limiting the spread of diseases (1-4). This majestic group of raptors also proves to be of great socioeconomic value to local communities. Bird-watching of scavenging birds has been yielding economic benefits to rural economies within developing regions (5).

Four species of vulture are found in Europe: the bearded vulture (*Gypaetus barbatus*), the cinereous vulture (*Aegypius monachus*), the griffon vulture (*Gyps fulvus*) and the Egyptian vulture (*Neophron percnopterus*). Two hundred years ago, these species of vultures were among the most common breeding bird species in the mountains of central and southern Europe. Yet, the decreasing availability

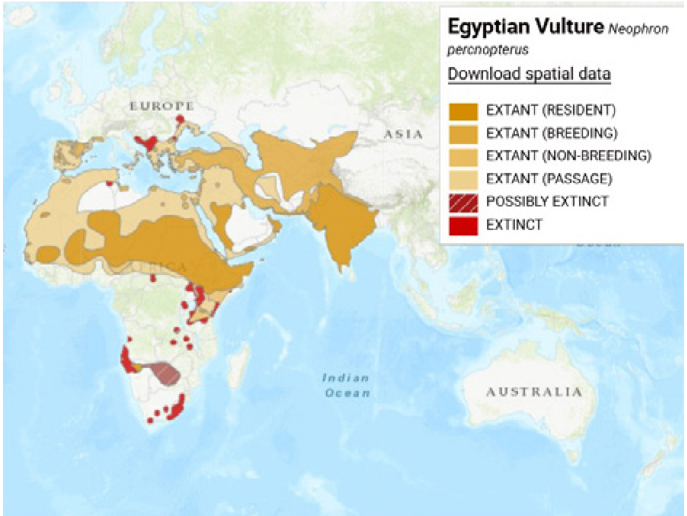

Figure 1: Distribution map of Egyptian vulture (*Neophron percnopterus*). Reproduced with permission from BirdLife International and Handbook of the Birds of the World 2021. *Neophron percnopterus*. The IUCN Red List of Threatened Species. Version 2022-2. (6)

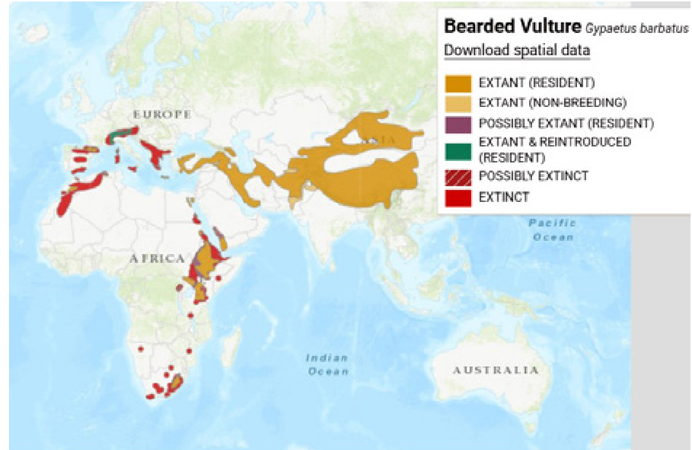

Figure 2: Distribution map of bearded vulture (*Gypaetus barbatus*), Reproduced with permission from BirdLife International and Handbook of the Birds of the World 2021. *Gypaetus barbatus*. The IUCN Red List of Threatened Species. Version 2022-2. (6)

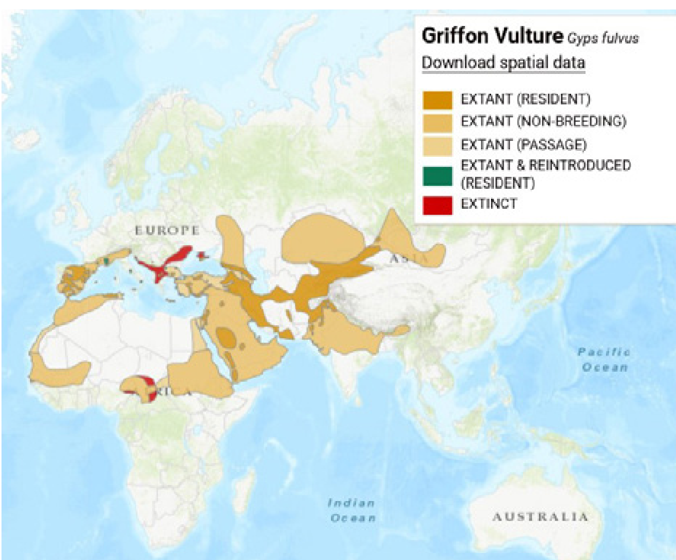

Figure 3: Distribution map of griffon vulture (*Gyps fulvus*), Reproduced with permission from BirdLife International and Handbook of the Birds of the World 2021. *Gyps fulvus*. The IUCN Red List of Threatened Species. Version 2022-2. (6)

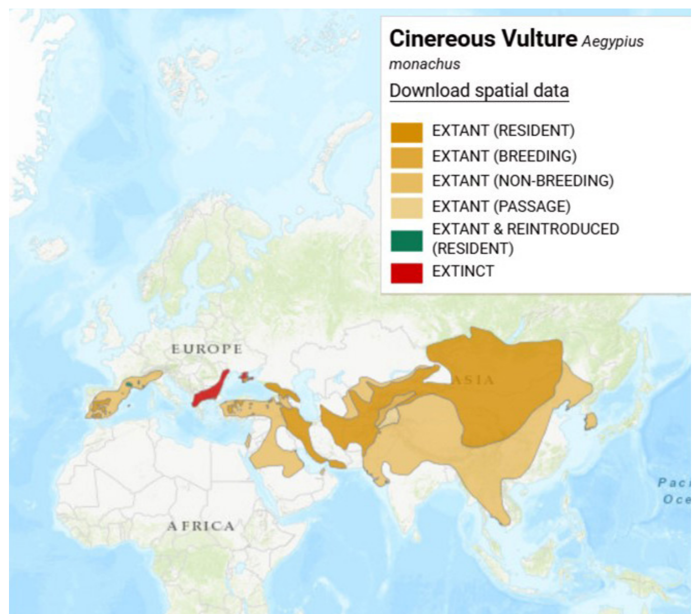

Figure 4: Distribution map of cinereous vulture (*Aegypius monachus)*, Reproduced with permission from BirdLife International and Handbook of the Birds of the World 2021. *Aegypius monachus*. The IUCN Red List of Threatened Species. Version 2022-2. (6)

of food, coupled with habitat loss, persecution and poisoning, made vultures disappear from most of their European range, with populations considerably smaller and increasingly isolated by the 1960s. Many conservation efforts have been and are being made; as a result, European vulture populations are steadily recovering.

In 2007 the Egyptian vulture was declared globally 'Endangered' by the International Union for the Conservation of Nature (IUCN) Red List (6). The majority of its European population is found in the Iberian Peninsula with an estimated 1,300–1,500 couples. This species is a long-distance migratory bird of prey, spending its winters in sub-Saharan Africa and returning in March to reproduce in Europe (Figure 1). It faces many threats, mostly electrocution and poisoning.

The bearded vulture's population drastically decreased at the beginning of the 20th century, and was driven to extinction throughout most of its former range. Nowadays, it is the rarest vulture in Europe, and its presence is limited to the Alps, Pyrenees and Andalusia, with isolated populations in Crete and the Corsica islands (Figure 2). It is considered 'Near Threatened' by IUCN Red List (6). This vulture has a unique feeding habit: its diet consists of a huge percentage of bleached carcass bones (7). In the wild, they rub themselves with ferric oxides, which turns their plumage rusty

Table 1: Reported causes of free-living vulture morbidity and mortality (adapted from the review by Ives *et al.*, 2022).

Species	IUCN global status[a]	Toxic	Trauma	Infectious	Idiopathic	Metabolic	Inflammatory	Total[b]
A. monachus	NT	9 (485)	1 (9)	6 (46)	1 (1)	2 (16)	1 (1)	20 (558)
G. barbatus	NT	8 (61)	7 (80)	1 (3)	2 (2)	0	1 (1)	19 (147)
G. fulvus	LC	18 (615)	12 (978)	5 (73)	1 (5)	1 (51)	0	37 (1722)
N. percnopterus	EN	15 (500)	13 (176)	3 (38)	0	0	0	31 (714)

[a] LC – Least concern; NT – Near threatened; EN – Endangered. [b] Number of studies (number of affected individual vultures reported).

orange. Major threats are changes in farming practices and direct persecution (8-10).

Griffon vultures are the most social of European vultures, with gregarious and competitive habits, breeding in large colonies. They feed in groups and can penetrate animal carcasses to feed on the softer tissues, such as muscles and viscera (11-12). Following a decline in the 20th century, as a result of wildlife poisoning, hunting and decreasing of food supplies (13), in recent years the species has significantly increased in some areas, particularly in France and in the Iberian Peninsula (Figure 3), and has an extremely large range in Europe, being now considered 'Least concern' by IUCN (6,12).

The cinereous vulture is the biggest raptor in Europe, with a wingspan almost reaching 3 meters. Its conservation status is listed as 'Near Threatened' by IUCN's red list and as 'Critically Endangered' in Portugal (6,14). Over the last century, the population of this species has decreased across its distribution area in Europe, and it is now extinct in many European countries (namely in Italy, Austria, Poland, Slovakia and Romania) (Figure 4) (6). Major threats to the viability of the species include habitat destruction by increasing forest fires, illegal use of poisons, limited food availability due to health restrictions and reduced wild herbivore populations, consumption of food resources contaminated by veterinary drugs or lead (from hunting ammunition), human disturbance during the breeding season and death by collision with/electrocution on power lines (14).

The most frequently reported cause of free-living vulture death and disease worldwide (Table 1) was due to toxic agents, with special emphasis on lead and pesticides. Regarding traumatic causes of morbidity and mortality, the second highest prevalence, the most reported are of anthropogenic origin: collision with and electrocution by power lines, gunshot, and direct persecution, among others (15). It is not uncommon for the main cause of vulture admission at wildlife rehabilitation centres and the cause of subsequent death, when it occurs, to remain unknown (16).

Vultures are considered to be resistant to certain microorganisms, such as *Bacillus anthracis* (17) or *Clostridium botulinum*, due to having naturally occurring antibodies against the toxins of these bacteria, coming from previous exposure to these pathogens (18). However, vultures can be susceptible to and negatively affected by other pathogens. The effects of infectious diseases have not been thoroughly investigated for most vulture species, obligate scavengers. Some pathogens should be seen as a potential threat to vulture conservation because they can cause disease in individual birds, and potentially jeopardize vulture health when associated with other menaces, such as contaminants and intentional poisoning (5). Highly virulent avian influenza virus, for instance, has caused a significant impact on griffon vultures in France and may have dramatic effects on the populations around the world, especially in the most critically endangered species (19). Attention should be paid to other viruses.

Far beyond the need for conservation, it makes perfect sense to use vulture and other raptor research and monitoring to a One Health approach, which brings together environmental, human and animal health. Vultures can be used to provide information relevant to public health (20). In this case, the scope can be the detection and monitoring of the spread of a recently emerged viral zoonosis in Europe such as WNV (21). The aim of this study is to bring together all the information on the WNV in vultures and highlight the need for further studies on the expression of the agent in this group of birds. We consider it is a topic highly relevant to animal and public health.

West Nile Virus

West Nile virus (WNV), lying within the genus Flavivirus and family Flaviviridae, is one of the most widespread arboviruses (22-23). The cycle of the WNV is maintained between birds (as reservoirs) and mainly mosquitos of the genus *Culex* (as vectors). Spread occurs when infected mosquitoes bite mammals. Horses and humans can be accidental dead-end hosts, along with other mammals (24-25). WNV has recently emerged as a major public health concern in Europe. The infection numbers have recently increased in European countries, while some remarkable socio-economic and environmental changes were noticed, including an economic crisis (in part due to the COVID-19 pandemic) and the occurrence of very high temperatures (26-27).

WNV strains have been grouped into nine genetic lineages, by phylogenetic analysis, being lineages 1 and 2 the most relevant (28-29). Both lineages 1 and 2 have been shown to cause severe disease in birds, horses and humans (30-32).

In humans, the severity of infections can range from asymptomatic to serious or fatal hemorrhagic fever or neurological disease. Most people (around 80%) with WNV infections remain asymptomatic. Among those who develop symptoms and clinical signs, the most common presentation is febrile illness (fever) accompanied by an acute syndrome, and less than 1% develop encephalitis or other forms of neuroinvasive disease (33-35).

As the key vertebrate hosts in WNV transmission cycle, avian species are the focus of surveillance worldwide (36). The information on the role of the birds of prey as reservoirs, spreaders, or sentinels of WNV is not yet clearly described, and specially the information available on the influence of vulture species on the epidemiology of WNV remains scarce (37-38).

For birds of prey, the pathogen is usually detected in the carcasses of birds found dead or moribund through wildlife surveillance programs or raptors admitted to wild-life rehabilitation centres (39). Three modes of WNV infection may be considered: mosquito-borne transmission, contact transmission and oral transmission (38). Variations in the prevalence of WNV infection between different species and populations of birds of prey can be explained by differences in exposure, based on infection prevalence values of prey species, differences in roosting habitat and exposure to the ornithophilic mosquito (40-42).

WNV infection in raptors appears to act as a multisystemic disease, affecting different organs, depending on the host species and also individuals. The disease can be fatal in most raptors, with an acute onset of illness a few days after infection occurs (43). On the other hand, the infection can also originate a debilitating and chronic disease, predisposing the affected animals to concurrent pathological conditions. In these cases, non-specific signs often occur, like depression, dehydration and severe weight loss (40,44-45). The WNV is neurotropic and, consequently, neurological signs are a very common outcome of infection, such as alterations in the mental status, head tilt, tremors, leg paresis or paralysis, among others. Ocular disorders often occur as well (32,46-48). The most common abnormalities in haematology screening are anaemia and leukocytosis (49), accompanied by splenomegaly, which can be seen in the ventrodorsal and laterolateral radiographic projections (50). Long-term sequelae have been detected in raptors, among which the recurrence of neurologic signs, feather pulp abnormalities and abnormal molt can persist for long periods of time, and may have a negative impact on the longevity of these species (37,43).

WNV infections can be diagnosed by isolating the virus, detecting viral antigens or RNA, or using serological methods. Research on avian immunity has focused on humoral immunity and there is still lack of information on cellular immunity. Humoral immunity against WNV is determined by the presence of antibodies in the blood of animals. This can be measured by a range of serological assays, being the virus neutralization test (VNT) considered the gold standard in flaviviruses serology, which detects serum neutralizing antibodies and more accurately detects protective antibodies, being more specific than other serological techniques (38). The main alternative is the capture enzyme-linked immunoassay (ELISA), which detects antibodies directed against the virus envelope protein. There are class-specific ELISAs for the detection of immunoglobulin (Ig) Y (the avian equivalent of IgG in mammals) or IgM against WNV. In adult birds, antibodies are developed following exposure and infection with WNV (38,51). In juveniles, presence of maternal antibodies can be detected (52). The long-term stability of antibodies confirmed in several species of raptors suggests that humoral immunity to WNV may be long-lasting in most individuals that survive infection (53). That is why positive serological results from ELISA suggest that a bird has been exposed to WNV, but generally do not indicate when the infection occurred (54). It is highly recommended to use VNTs to confirm WNV in all positive and doubtful samples detected by ELISA, in order to increase the accuracy of estimated seroprevalences in wild birds. The use of VNT will be especially important in areas where co-circulation of related flaviviruses exists (51).

The molecular diagnosis of WNV infection can be made using specific RT-PCR. Fresh brain samples, cloacal, and choanal swabs can be tested for this virus, as well as other organs where the virus is commonly found in raptors (heart, spleen, liver, kidney, adrenal gland and intestine). West Nile virus can also be detected in serum samples. Immunohistochemistry (IHC) also can be useful to detect WNV antigens in affected tissues from both living and dead birds (44,46,55-56).

There is still no specific treatment for WNV infection in raptors. In spite of the supportive treatment and anti-inflammatory drugs, to minimize the effects caused by infection, the majority of affected birds end up dying (57-58). There are still no vaccines specifically available for use in birds (38,45,48) or humans (24).

West Nile Virus in vultures Literature review

We have reviewed the scientific literature retrieved from PubMed and ScienceDirect databases, using general searches with the following terms: 'West Nile Virus' AND 'Vultures'. We performed other searches using different combinations of the following relevant terms: 'Scaveng* bird' AND 'flaviviruses'. We filtered our search based on the

Table 2: Diagnosed cases of WNV infection in European vultures

Species	WNV diagnosis (positives/sample size)	Geographical area	Year	Study
G. barbatus	ELISA; VNT (10/12; 2/3)	Austria	NM	Wodak *et al.*, 2011
G. barbatus	RT-PCR (1/1)	Austria	2008	Bakonyi *et al.*, 2013
A. monachus *N. percnopterus*	ELISA (2/8 – wild; 0/1 – captive) (1/9 – wild; 1/17 – captive)	Spain	2006-2009	García-Bocanegra *et al.*, 2011
G. barbatus	ELISA; SNT 14; 2	Spain	2017	Busquets *et al.* 2019
N. percnopterus	ELISA; MNT (2/2; 2/2)	Iran	2017-2018	Bakhshi *et al.*, 2021
A. monachus *G. fulvus*	ELISA; VNT; RT-PCR (19/20; 4/19; 0/0) (45/110; 20/45; 1/5)	Spain	2017-2019	Bravo-Barriga *et al.*, 2021
G. fulvus	RT-PCR 4/25a	Serbia	2018-2022	Marinković *et al.*, 2023

ELISA – enzyme-linked immunosorbent assay; MNT – microneutralization test; NM – not mentioned; RT-PCR – reverse transcription polymerase chain reaction; SNT – serum neutralization test (=VNT); VNT – virus neutralization test. [a]The authors report that 4 of the 25 tested positive for infectious agents, including WNV, among others. It is not mentioned how many of the 4 were in fact positive for WNV.

geographical location where the studies were performed, focusing on Europe and European species of vultures (Table 2). Eight reports were found that reported WNV infections in European vulture species, in Russia, Serbia, Austria and Spain, plus one in Iran.

Between 2008 and 2009 outbreaks of WNV infection occurred in central Europe, with an unexpected spread of a lineage 2 WNV strain. In 2011, a study from Austria described a WNV outbreak in birds of prey in 2008 and 2009 in the eastern part of the country. Samples were collected from birds nearby the location of the first detected infections and from poultry houses at the Research Institute of Wildlife Ecology in Vienna. ELISA positive results were found in 10 bearded vultures among other species of birds. 66% of the positive ELISA reactions could be confirmed with VNT, including two out of three vultures tested (59). One wild bearded vulture was confirmed by RT-PCR and IHC as positive for WNV, also in Austria (60).

In 2011 the first report of flaviviruses circulation in Egyptian vulture was published in Spain. Between 2006 and 2009 serum samples were obtained from several bird species in Andalusia, southern Spain, among free-ranging and captive animals held in rehabilitation centres. Two cinereous vultures and two Egyptian vultures tested positive on competitive enzyme-linked immunosorbent assay (cELISA) for WNV. As the used ELISA kit has been designed to detect anti-bodies directed against the envelope protein (pr-E) of WNV, which contains an epitope common to Japanese encephalitis virus (JEV) antigen, the VNT was further performed as a confirmation and more specific test for the positive samples on ELISA (61).

In 2017, the WNV was detected in one bearded vulture in Lleida, Northeastern Spain. The animal had been admitted to a local wildlife rehabilitation centre, and evidenced neurological signs. Serum samples were analysed and the results were positive on cELISA and negative on VNT. One month later, antibodies against WNV were detected on VNT. Thirteen additional vultures of the same species tested positive for cELISA, from which two were confirmed for VNT (titres between 1/20 and 1/40) (62).

Among samples collected in 2017-2018, two Egyptian vultures tested positive with micro-VNT, after a positive result on ELISA in Iran (63). This species is estival migratory in the European continent, and migrates to the African Sahelian region in the winter (64). Birds from Western Europe usually winter in Western Africa, and birds from Eastern Europe winter in Eastern Africa or the Arabian Peninsula (65). They are the same birds that return to Europe, a reason for mentioning this study, despite the fact that it is based on another continent.

From October 2017 to December 2019, almost 400 wild birds were sampled in the Cáceres and Badajoz provinces, Western Spain, in collaboration with two local rehabilitation centres. Analysis by RT-PCR in sick birds confirmed the presence of WNV line-age 1 RNA in a griffon vulture and specific antibodies against WNV in juvenile birds were detected in specimens of griffon vulture and cinereous vulture (66).

In the period between 2018 and 2022 in Serbia, 25 dead griffon vultures from the wild were submitted to macroscopical and histopathological examination, followed by different diagnostic screening. Infectious agents were detected in

4/25 animals, obtaining PCR confirmation of a WNV infection, with lymphoplasmacytic encephalitis (67).

WNV emerged in the United States of America (USA) in 1999 (68) and has since spread throughout much of the American continent. The WNV was identified as the primary cause of death in one nestling wild California condor (*Gymnogyps californianus*). The infection was diagnosed by IHC on the heart in conjunction with compatible lesions. In the USA, the WNV infection is an emerging mortality factor for young, wild-hatched birds and vaccination is required at nest sites where the WNV is prevalent (69). Other New World vulture species, such as the black vulture (*Coragyps atratus*) and theTurkey vulture (*Cathartes aura*), were confirmed to have WNV infection (49,53,70), as well as the Lappet-faced vulture (*Torgos tracheliotus*), an Old World vulture not present in Europe (57).

Epidemiology of WNV in vulture populations

The probability of a WNV case being reported depends on many factors, which can be divided in two main groups: factors related to the probability of infection itself, and those related to the probability of its detection. As with other birds of prey, the probability of infection in vultures depends on the blood feeding preferences of the main vector species, the animal species they bite, and the intrinsic susceptibility of the host animal species. The probability of detection will be affected by the intensity and specificity of clinical signs exhibited by the host, the location and distance in relation to a wildlife rehabilitation centre, and the size of the population of that species. The larger the population, the more likely the infection will be detected (38). The estimated population of mature Egyptian vultures is around 12,400-36,000, but on a downward trend; the bearded vulture population is between 1,675-6,700 mature individuals, and that of the cinereous vulture is around 16,800-22,800 mature birds. Only the griffon vulture population, counting with 80,000-90,0000 individuals worldwide, is currently tending to increase (71). These circumstances mean that these are not very large populations, making the task of identifying WNV infections more difficult and raising concerns that the WNV could jeopardize the conservation of these species.

One pertinent question is 'how do vultures acquire WNV infection?'. Most of the WNV infections in the Palearctic region have origin in a bird-mosquito cycle and the main WNV transmission mode to birds of prey is through mosquito bites (40). Mosquitoes are considered the main biological vectors of the WNV and, in Europe, two species are pointed out as key to transmission: *Culex pipiens* (Linnaeus), biotypes *pipiens* and *molestus*, and *Culex modestus* (Ficalbi). In fact, it is reported that *C. pipiens* mosquitoes do feed on vultures in the wild. In Switzerland, Egyptian vultures were found to be a host species for blood-fed mosquitoes (72).

In susceptible birds, besides inducing a viremic phase sufficient to infect additional mosquitoes and enhancing its transmission, the WNV can also be shed orally and through the cloaca (73-74), which represent alternative transmission mechanisms beyond vectors (75). Oral transmission by consumption of infected prey or carrion has also been described in birds of prey, and experimentally documented (40,43,74). Infection through feeding on WNV-infected preys may be more common in species like the Northern goshawk (*Accipiter gentilis*), which predates smaller birds (38), or in species that consume small mammals, potential reservoirs of WNV in both cases (40,76), but should not be excluded in vultures.

Vultures of the genus Gyps are obligatory scavengers, usually feeding in large groups on a resource that is random and unpredictable in space and time (77). This feeding behaviour, with several birds huddled together to feed on the same carcass, creates the opportunity of oral transmission of the virus among them, if the carcass has been contaminated by an infected bird. Typically, the griffon vulture's diet is based on carcasses of medium and large ungulates, whereas the Egyptian vulture largely consumes small and medium-sized vertebrates, and garbage, despite also feeding on large carcasses (78). From the year 2000 onwards, following the bovine spongiform encephalopathy crisis, government legislation has been introduced across Europe, imposing the removal and disposal of the carcasses of dead livestock. Farmers have been forbidden to leave carcasses in the field, and the food availability for scavengers dramatically decreased. Further European Union (EU) legislation included the maintenance of some feeding stations (commonly known as 'vulture restaurants'), in the scope of vulture conservation, but food remained scarce. This has triggered considerable changes in the diet composition of the griffon vulture, which started to consume small vertebrates, like rabbits, and to explore garbage dumps (79). The garbage consumption can bring direct consequences, such as ingestion of foreign bodies and poisoning (80), and indirect problems, like immunosuppression. Furthermore, corvids are commonly seen in dumps (81) and, as birds in this family are very susceptible to the WNV infection (40,82-83), they can be an important source of transmission for vultures.

Early detection of pathogens, like the WNV, will allow the establishment of effective measures to prevent or mitigate the effect of the virus on human populations, as well as to protect susceptible endangered species.

Discussion and Conclusion

Zoonotic emerging infectious diseases represent a rising and important threat to public health, and vector-borne diseases were responsible for almost a quarter of the documented emerging infectious diseases events in the first decade of the 21st century (84).

The West Nile virus (WNV) was first reported in Uganda in 1937 (85) and was subsequently isolated from patients, birds, and mosquitoes from the early 1950s (86). The virus became endemic in several parts of the African continent (87) and the first outbreak of WNV in Europe was reported in 1962 in the French Camargue region (88). Since 1996, after a high rate of confirmed cases in Eastern Europe (Romania) (89), several outbreaks have been often reported in the European continent, with a noticeable seasonal pattern, during the warmer weather (July to October) (90). Birds of prey are especially susceptible to the WNV (38). Upon the species and individuals, WNV infection can cause acute death, a fatal outcome several weeks after infection, or birds can eventually survive to chronic infections (43,57-58). Both in Europe and North America, raptors are among the birds described as more frequently infected during WNV outbreaks (50,60). Identifying target species is important for developing an efficient surveillance and monitoring program, a reason why targeting specific raptor species as disease sentinels may be beneficial.

The role of Palearctic-African migratory birds in the introduction of the WNV to Europe has long been the subject of debate (22-23,91). The suspicion that migratory birds could be the main introductory hosts of the WNV in new regions was based on the following: the majority of outbreaks in temperate regions occurred during late summer or early fall, when migratory birds and mosquitoes coexist in a large scale (86,90,92); the principal vectors from which the virus has been isolated are mainly ornithophilic mosquitoes (93-94); circulating antibodies against the WNV have been found in many migratory bird species, and long-distance migrants in Europe (in particular, species wintering south of the Sahara) are exposed during their migratory journeys and/or their winter stay in Africa to higher levels of WNV circulation (or a closely antigenically related Flavivirus), when compared with the levels found in their breeding grounds (95) and, finally, migratory birds have been linked to transporting related viruses in the Western Hemisphere (96). It is difficult to define a population geographically, especially for migratory birds the WNV has been isolated from some actively migrating species of birds (e.g., the White stork (Ciconia ciconia) (97) and the Turtle dove (Streptopelia turtur) (98). Migratory birds play an essential role in the long-distance movement of JEV serocomplex flaviviruses. When migrations between enzootic and areas free of WNV occur, birds that become infected prior to or during migration can actively transport the virus in their blood or tissues and infect mosquitoes and/or their predators (95). The establishment and maintenance of vector populations and the associated threat of vector-borne pathogen transmission in northern latitudes may be facilitated by global environmental change (98-99). Even non-migratory vultures make large dispersal movements and have a large foraging range (100), which explains why they can possibly help spread pathogens around.

The West Nile virus (WNV) poses a threat to endangered species around the world, such as the Iberian imperial eagle (Aquila adalberti) (101). The Egyptian vulture is a long-distance migratory species, as mentioned above (6), and therefore may be even more susceptible to contracting a WNV infection.

A successful introduction of the WNV in destination territories depends on the conditions for local maintenance, such as ecological key factors, which promote the virus maintenance and transmission, like presence and abundance of competent birds (hosts) and mosquitoes (vectors), and favorable abiotic conditions (102-103). Temperature is the most frequently used environmental condition regarding the WNV and/or its vectors, followed by precipitation (102). Mediterranean savannahs, in Spanish known as 'dehesas' and in Portuguese 'montados', are found in regions with mild, rainy winters and very hot, dry summers occupied by an agro-silvopastoral landscape in the Southwest of the Iberian Peninsula. These areas are threatened by climate change and the abandonment of traditional uses, and are therefore protected under the European Habitats Directive (104-105). Mediterranean 'dehesas' or 'montados' hold a very important fraction of the European populations of some endangered avian scavengers such as cinereous and Egyptian vultures, as well as other endemic globally endangered top predators. Foraging griffon vultures from different populations located across Western Europe make long trips into these regions of the Iberian Peninsula, suggesting that these areas have a beneficial added value for griffon vultures and other avian species, also providing the main habitat for wintering bird species that come from Northerly latitudes (104,106). According to Casades-Martí (103), this continental Mediterranean territory, with wildlife–livestock interaction, is favorable to the circulation on the WNV and other flaviviruses. Interactions between wild birds and farm animals are more likely to occur here, resulting in a higher probability of exposure to flaviviruses (107). Although mosquitoes are considered the main vectors of WNV, this agent has occasionally been isolated from other hematophagous arthropods, namely argasid and amblyommine ticks (93). As other soaring bird species, griffon vultures perform long-scale movements (106,108) and are considered a partial migratory species, with juveniles (especially in the first year of live) crossing the Strait of Gibraltar to Africa during fall and returning to Europe in the following years (109).

The abundance of vectors is a relevant parameter for pathogen transmission. Those habitats more suitable to the expansion of Culex mosquitoes during peak times of the WNV transmission represent the highest risk for the potential spillover of WNV into human populations (110).

In spite of some ticks (soft ticks) being considered resident and sedentary (111), that does not mean they have a limited role in the circulation of infectious agents. Pathogens transmitted by these ticks can be spread into new areas taking advantage of the large foraging movements or migration of its hosts, namely griffon vultures (106,108-109). Although ticks can be alternative vectors, a recent study in the Pyrenees (Northeastern Spain), flavivirus was detected in all seven vultures' blood samples by the generic qRT-PCR, but all analyzed ticks were negative for flavivirus detection, which reinforces the potential involvement of other more common arthropod vectors, like mosquitoes, in the transmission of the virus (112-113).

Scavengers are susceptible to be infected by consuming WNV-infected carcasses (40,83). The WNV activity is far more likely to be detected in urban areas than in rural areas, suggesting that human density and associated factors should be considered when interpreting dead wild bird surveillance for the WNV (83). Long-lived avian scavengers are affected by the habitat where they live, anthropized landscapes being considered a more stressful habitat. Birds that live there generally present poor body condition and are more vulnerable to disease. Foraging in more anthropized areas can bring both advantages and disadvantages in terms of energy balance and stress. In these territories, availability and predictability of food is likely higher, but data suggest, on the other hand, that the food quality is not so good, leading to a poor nutritional status (114), which contributes to higher levels of circulating glucocorticoids. Concurrent factors that lead to lower immunity must be taken in consideration, such as the risk of lead accumulation, ingested from hunting ammunition, or severe competition in a high density and challenging environment, especially in highly social species, like griffon vultures, or less capable species, for example Egyptian vultures (115). It is known that the WNV has killed many thousands of birds around the world, but it is difficult to measure the long-term impact of the disease on wildlife populations.

Deforestation, besides being a major cause of biodiversity loss by itself and causing a negative impact on human health (116-117), is linked to the emergence of zoonotic and vector-borne diseases, like the WNV. Deforestation can increase contact between vectors and avian reservoirs. Forest loss may facilitate exchanges between human and zoonotic cycles, as open areas favor the human presence and settlement (116). The relationship between deforestation and the occurrence of zoonotic outbreaks has already been suggested (90,117), and should be further investigated for WNV.

There are relatively few reports of infectious diseases as a direct and primary cause of mortality in avian scavengers, and it has been a neglected topic of research so far. Given the current decline in scavenging bird populations, baseline information on exposure to infectious agents will be helpful for monitoring population health and investigating future disease-related epidemics, thus helping to guide conservation efforts. Although WNV infections have been registered in numerous species of birds of prey in Europe and North America, actual rates of morbidity and mortality associated with the WNV in wild raptors' populations remain unknown. The implementation of a collaborative international, holistic and multi-disciplinary One Health action is crucial to allow a more accurate risk assessment and an early response to the WNV and other emerging zoonotic pathogens. The presence of the WNV in vultures may therefore have public health and wildlife conservation implications and deserves further investigation.

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Virus Zahodnega Nila pri jastrebih iz Evrope – opaznih med drugimi ujedami

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Izvleček: Virus Zahodnega Nila (WNV) je arbovirus, ki ga prenašajo predvsem Culex spp., in je povzročitelj zoonoze, prisotne po vsem svetu. Ta patogen se endemično ohranja v življenjskem ciklu, v katerem so ptice rezervoarji, ljudje in konji pa so naključni in končni gostitelji. V Evropi so poročali o sporadičnih izbruhih WNV, potencialni vpliv okužbe z WNV na populacijo ogroženih ptic ujed pa je lahko zelo velik. Za zgodnje odkrivanje tega virusa so potrebni programi nadzora. Vse štiri vrste jastrebov, ki živijo v Evropi, so zavarovane vrste. Kot mrhovinarji so jastrebi zaradi zasedanja vrha prehranjevalne verige lahko dovzetni za patogene, kot je WNV. Z varstvenega vidika je treba obravnavati neodvisen ali z drugimi dejavniki povezan vpliv WNV na evropske jastrebe. Ta pregled dokumentiranih primerov se lahko šteje kot izhodišče za ta namen.

Ključne besede: epidemiologija; Evropa; mrhovinarji; jastrebi; virus Zahodnega Nila; zoonoza