

Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado em Biologia da Conservação

Dissertação

Factors influencing foraging site selection by the endangered Egyptian vulture in the Douro region of Portugal-Spain.

Tiago de Castro Brandão

Orientador(es) | Rui Lourenço

José Pedro da Fonseca Tavares Louis Phipps

Évora 2021



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Acknowledgements

Agradecimentos

Gostaria de começar por agradecer a todos os agricultores e também veterinários que despenderam um bocadinho do seu tempo de trabalho para participar neste estudo. Sem a vossa colaboração, uma grande parte do trabalho não seria possível.

Gostaria, claro, também de agradecer aos meus orientadores Rui Nascimento, Louis Phipps e José Pedro Tavares, pela vossa orientação ao longo de todo o projeto.

Gostaria também de agradecer à equipa do projeto MAF "Risky business for nature's clean-up crew: does coprophagy exposeEgyptian vultures to toxins?" na qual este projeto se enquadra: Kat Whitehouse-tedd, Ngaio Richards, Sarah Hall e Franziska Lörcher, e de facto, ao Morris animal Fundation, por financiar o projeto.

Um agradecimento especial vai também para a equipa da Palombar, que foi uma grande maisvalia na minha estadia no belíssimo Parque Natural do Douro Internacional.

Quero também agradecer a um certo grupo de veneradores de uma certa ave por serem por partilharem a mesma paixão por aves (quase todos) e por fazerem saídas de campo sempre muito mais interessantes

Preciso também de dar um agradecimento especial à Maria Eduarda Marques, por estar sempre disponível para falar de qualquer coisa, e por ser sempre uma motivação positiva.

Finalmente, não posso deixar de agradecer á minha família. Aos meus pais pelo apoio incondicional que mostraram durante esta jornada toda, pelos baixos e altos, e aos meus irmãos, com o qual posso sempre contar para falar, e cujo as palhaçadas são (quase) sempre uma distração bem necessária.

Resumo

Fatores que influenciam seleção de locais de alimentação pelo Abutredo-egipto na região do Rio Douro de Portugal-Espanha

Uma boa compreensão da ecologia do movimento e do comportamento de alimentação das espécies é essencial para que os gestores da conservação e os decisores políticos tomem decisões informadas. Neste estudo, através da análise de dados GPS de indivíduos marcados, bem como de entrevistas com agricultores locais, pretendemos identificar e caracterizar os locais preferidos, bem como identificar algumas ameaças potenciais a um abutre do velho mundo ameaçado, o Abutre-do-*Egipto Neophron percnopterus* (Linnaeus, 1758), numa região fronteiriça entre Portugal e Espanha (Douro Internacional). Os resultados dos dados do GPS mostram que os indivíduos marcados têm preferência em procurar alimento em Espanha e em locais associados à presença de gado. Conseguimos também estudar o comportamento de coprofagia desta espécie através das entrevistas, e identificámos os riscos associados a este comportamento de alimentação como forma de exposição a fármacos veterinários nocivos à espécie.

Palavras-chave: aves necrófagas; recursos tróficos; comportamento; pecuária; coprofagia.

Abstract

A good understanding of species' movement ecology and feeding behaviour is essential for conservation managers and policy makers to make informed decisions. In this study, through the analysis of GPS data from tagged individuals, as well as interview with local farmers, we aim to identify and characterize the preferred sites, as well as identifying some potential threats to an endangered old-world vulture, the Egyptian vulture *Neophron percnopterus* (Linnaeus, 1758), in a transborder region between Portugal and Spain (Douro Internacional). Results from the GPS data show that the tagged individuals have preference to forage in Spain and in sites associated with livestock presence. We were also able to study the coprophagy behaviour of this species through the interviews, and identified the risks associated with this feeding behaviour as behaviour path of exposure path to harmful veterinary pharmaceuticals.

Key words: Necrophagous birds; Trophic resources; behaviour; livestock farming; coprophagy.

1. Introduction

1.1. Background information

Trophic ecology is one the most important aspects of animal ecology, affecting individual behaviour and survival, population dynamics and distribution, and food availability is often the most limiting factor throughout an animal's life cycle (White, 1978; Costa, 1993; Barbosa and Moreno, 1999; Blanco, 2006; Nunn, 2012; Monsarrat, 2013). Therefore, a good understanding of a species' feeding behaviour and habits and dietary preferences is essential for efficient and effective wildlife conservation management, especially in species that perform long-range movements, because it allows to more effectively protect the areas and resources that are important for these species (Papastamatiou *et al.*, 2010; Ashrafi *et al.*, 2011; Ramírez *et al.*, 2016; Figgener *et al.*, 2019).

Vultures are highly specialized obligate necrophagous birds, that is, they have adapted to feed almost exclusively on carrion, the remains of dead animals (Ruxton and Houston, 2004). By doing so, they are thought to play an essential role in the ecosystems and provide important ecosystem services for humans, as they are able to mitigate the spread of dangerous meat-borne pathogens and also play an important role in nutrient recycling processes in terrestrial ecosystems (Şekercioğlu *et al.*, 2004; Whelan *et al.*, 2008; Ogada *et al.*, 2012; Gangoso *et al.*, 2013; Hill *et al.*, 2018).

Vultures can be divided into Old World Vultures and New World Vultures. Old World Vultures can be found in Europe, Africa, and Asia, and are part of the family Accipitridae (Order Accipitriformes). On the other hand, New World Vultures, which includes condors, are from the family Cathartidae (Order Cathartiformes) and can be found in the American continent. Despite sharing the same ecological niche and many of the same adaptations, vultures are a polyphyletic group, meaning that these similarities exist due to convergent evolution of different ancestors (Wink, 1995).

Carrion is an unpredictable food source, as well as being potential pathogen hotspots. As such, vultures have evolved a set of adaptations that make them uniquely suited for carrion exploitation (Ruxton and Houston, 2004). Vultures are extremely efficient soarers, allowing them to cover large areas with little effort. Additionally, their large size allows them to feed irregularly, and consume larger quantities when they do find food (Ruxton and Houston, 2004; Ogada *et al.*, 2012). Finally, their extremely low pH stomach acid, specialized intestinal flora and high immunocompetence allows vultures to withstand the pathogens that are often found in carrion (Houston and Cooper, 1975; Carvalho et al., 2003; de la Lastra and de la Fuente, 2007).

Humans and scavengers, such as vultures, have had a long history of interdependence. As early as human hunter-gatherer societies, vultures were useful for locating prey, and then as humans started to domesticate ungulates such as cattle, sheep and goats, vultures were useful for disposing of spoiled meat (Moleón *et al.*, 2014; Morelli *et al.*, 2015). In this context, humans provided an important food source for vultures, and in turn, scavenging birds provided an essential service by removing carrion from the landscape (Şekercioğlu *et al.*, 2004; Dupont *et al.*, 2012; Lee, 2018; O'Bryan *et al.*, 2018). This relation has lasted until modern times, and in some parts of the world, plays an even bigger role in human society. Up until recently, it was common for European farmers, such as in Spain and Portugal, to dispose of livestock carcasses that were not fit for human consumption by using sites called "*Muladares*", which functioned as feeding stations for vultures. In some countries, vultures can act as the "clean-up crew" in high density urban areas, removing waste and preventing the spread of infectious diseases (Gangoso *et al.*, 2013). At the same time, foraging in urbanized areas may prove dangerous for vultures (Henriques *et al.*, 2020). The collapse of vulture populations in the Indian subcontinent has been linked to an increase in cases of rabies, though a cause-and-effect relationship

hasn't been proven and stronger evidence is needed (Pain *et al.*, 2003; Markandya *et al.*, 2008; Plaza *et al.*, 2020).

Vultures, especially Old-World Vultures in Africa and Asia, are of great conservation concern, with 11 of the 16 living Old Word Vulture species considered threatened (Botha *et al.*, 2017; McClure *et al.*, 2018). While there are numerous threats to be considered, and while they vary in their level of threat between species and regions, unintentional poisoning seems to be the biggest threats overall (Margalida, 2012; Ogada, 2014; McClure *et al.*, 2018; Plaza *et al.*, 2019; Safford *et al.*, 2019; Henriques *et al.*, 2020). One of the ways in which this can happen is through the consumption of contaminated carcasses or other material with Veterinary Pharmaceuticals (VPs). For example, the collapse of vulture populations in the Indian subcontinent has been attributed to the use of non-steroidal anti-inflammatory drug (NSAID), Diclofenac, in livestock, which can be lethal for vultures if exposed via consumption of contaminated carcasses or other material (Oaks *et al.*, 2004). Another way poisoning can happen is by the intentionally set poison bait in carcasses. In Africa, poisoning of vultures, which can be directly targeted for use of vulture parts in the traditional medicine trade or indirectly as non-target species affected by baits set for large carnivorous mammals, has caused severe declines and are now considered a primary main threat (Ogada *et al.*, 2016; Henriques *et al.*, 2020).

While Europe has recorded large vulture population declines in the past, most populations are now stable or increasing. However, concerns with poisoning and other threats still exist. Despite the catastrophic collapse of vultures due diclofenac in the Indian subcontinent, this NSAID is still allowed in Europe (Margalida *et al.*, 2014). Additionally, European Union sanitary restrictions on livestock carcass management have been at the forefront of vulture conservation discussion in Europe since they were applied (Ogada *at al.*, 2012). During the 1990's, an outbreak of Bovine Spongiform Encephalopathy (BSE) occurred in the European Union (Belay and Schonberger, 2002). This led to stricter regulation over the fate of animal by-products not meant for human consumption, with the aim of preventing the presence of pathogenic agents in the food chain (Regulation CE 1774/2002). This meant that carcasses of livestock that had died naturally could not be left in the field for the vultures, greatly reducing food availability for vultures in Europe, which have become largely dependent on domestic livestock as a food source (Margalida and Colomer, 2012).

New legislation was subsequently changed in response to concern from the scientific and conservation communities, allowing the reconciliation of the newly imposed sanitary regulations and conservation concerns over how these sanitary regulations would affect food availability for vultures (Margalida *et al.*, 2012). However, the application of this new legislation has not happened equally among member-states of the EU (Arrondo *et al.*, 2018). For example, in Spain changes were implemented that made the requirements needed for farmers to be able to get a permit that authorized carcass disposal at official selected sites were made more flexible. In contrast, in neighbouring Portugal, farmers are still required, for the most part, to remove livestock carcasses from the fields, creating imbalances in food availability for vultures in Europe. These changes have been especially impactful in vultures nesting near country borders, as foraging areas may include legislative differences that may create large imbalances in the food availability for vultures (Arrondo *et al.*, 2018).

One of the primary ways in which Veterinary Pharmaceuticals (VPs) may enter the environment is through livestock faeces (Jochman *et al.*, 2016). Depending on the method of administration, a large proportion of a VPs dose may remain unmetabolized and be excreted through in the faeces of the animal (Kaczala and Blum, 2016). Livestock faeces often remain in the fields or can even be stored for later use as fertilizer, and so faeces from medicated animals might be a considerable source of negative environmental impact (Kaczala and Blum, 2016). Contamination of the environment by VPs has been shown to result in loss of biodiversity and increase of antimicrobial

resistance of environmental microbiota (Campagnolo *et al.*, 2002; Kools *et al.*, 2008; Verdú *et al.*, 2018). Coprophagic invertebrates are particularly susceptible. High concentration of certain VPs can result in high mortality for coprophagic invertebrates, and even at low concentrations, chronic exposure can reduce the size and longevity of adults, reduce mating success, and decrease fertility (Jochman *et al.*, 2011).

Vertebrates may also be at risk of VP exposure from faeces. Contaminated faeces used as fertilizer may contaminate groundwater or surface water, potentially affecting whole aquatic systems, including vertebrates (Richards *et al.*, 2014), and coprophagic species, such as the Egyptian vultures, may especially at risk, due to direct exposure. Some of the acute effects of VPs may have on vertebrates are known, such as the previous example of diclofenac and vultures. It has also been recognized the indirect hazard of antimicrobial resistant microbiota and anthelmintic resistant endoparasites due to exposure to antibiotics and anthelmintics, respectively (Coles *et al.*, 2006; Vittecoq *et al.*, 2016; Csivincsik *et al.*, 2017). However, little is known of the consequences of chronic (long-term) exposure of sublethal concentrations to these chemicals in vertebrates (Richards *et al.*, 2014).

<u>1.2 Study species: Egyptian vulture</u>

The Egyptian vulture *Neophron percnopterus* (Linnaeus, 1758) is a medium sized Old-World Vulture. Adults have a distinct appearance, making them easy to identify, with a white body, black primary feathers, a yellow facemask and beak, and a visibly long, cuneiform shaped tail. Juveniles are less conspicuous, having a generally dark colour and grey facemask. They become progressively lighter in colour as they age, reaching sexual maturity at around 5 years of age. There is no sexual dimorphism (Cramp, 1980; Svensson *et al.*, 2017).

The species usually forages in open lowland or montane regions. It usually nests in cliffs, or occasionally trees (Botha *et al.*, 2017). Unlike griffon vultures *Gyps fulvus* (Hablizl, 1783), which are very gregarious and nest in colonies, Egyptian vultures are more solitary and highly territorial during breeding season, defending the area around the nest from other Egyptian vultures or other threats (Cramp, 1980; Botha *et al.* 2017). Despite this, congregations may form at feeding sites or in roosts of non-breeding birds (Ceballos and Donázar, 1990).

The Egyptian vultures has a wide distribution, though it does not occur continuously throughout it, but rather populations appear in patches. The species can be found in Southern Europe, Africa, Middle East, Central Asia and the Indian subcontinent (Botha *et al.*, 2017; BirdlLife International, 2019). It is estimated there are around 3000-4700 breeding pairs worldwide. Populations in the northern part of its range are usually migratory, while other populations are resident (Botha 2017; BirdLife International, 2019). The Iberian Peninsula, Spain in particular, is of particular importance for the species in Europe (BirdlLife international, 2015; Botha *et al.*, 2017). In the Douro International/Arribes del Duero Region, the species has been relatively stable, with a population of around 120 breeding pairs. However, this apparent stability is likely the result of improved census efforts in a population who is experiencing some declines (Monteiro *et al.*, 2018).

The Egyptian vultures is a generalist and opportunistic necrophagous bird, and its diet varies greatly between regions, and mainly depends on what is available in the region (Cortés-Avizanda *et al.*, 2012; Margalida and Colomer, 2012; Dobrev *et al.*, 2016; Margalida *et al.*, 2017). It usually prefers small to medium sized prey species, such as rabbits and turtles (Donázar et al., 2010; Birdlife International, 2019). It may also feed on large ungulate carcasses, but it often gets outcompeted by

more aggressive vultures like the griffon vulture (Moreno-Opo, 2020). In addition to carrion, it may also feed on ungulate faeces, human waste and eggs. Its behaviour of feeding on faeces, known as coprophagy, and more specifically, heterocoprophagy – feeding on the faeces of other species - is especially interesting because it is uncommon among vertebrates. Faeces are poor sources of macronutrients, like protein and fat. However, ungulate faeces contain large amounts of carotenoids, which animals cannot synthesise. Besides being important for the immune system, carotenoids are a group of pigments that Egyptian vultures use to obtain their characteristic yellow head and beak, with possibly a more intense colour signalling more fit individuals (Negro *et al.*, 2002). Despite the importance of this behaviour may have for Egyptian vultures, very little is known about it, and it appears only anecdotally in diet studies of the species (Hidalgo *et al.*, 2005).

The Egyptian vultures is considered Endangered (EN) according to IUCN's red list of threatened species, while in Europe it is considered to be vulnerable (VU), with declining populations throughout most of its range (BirdLife International, 2019; BirdLife International, 2021). Although the reasons for the declines vary between regions, the main threats to the Egyptian vultures are typically electrocution on powerlines, collision with wind turbines, reduced food availability, unintentional or secondary veterinary pharmaceutical (VP) poisoning, often from poison baits meant for mammalian carnivores. Other threats for the species include direct belief-based prosecution, habitat degradation and human disturbance (Botha *et al.*, 2017; Birdlife International, 2019).

In Europe, populations have declined 50-79% over the last 50 years, and many still show signs of decline, with mortality being higher in Eastern Europe compared to Western Europe (Liberatori, 2001; BirdLife International. 2015; Velevski *et al.*, 2015; Buechley *et al.*, 2021). Despite this, the Iberian Peninsula has been able to remain a stronghold for the species, where there is a stable population of about 1500-1700 breeding pairs (Hernandez, 2018). However, some concerns still exist, making the Iberian Peninsula of high conservation priority for the species, along with the other vulture species that breed in Europe (Santangeli *et al.*, 2019). The main threats here are poison baits and collision with wind turbines or electric powerlines (BirdLife International, 2015; Botha *et al.*, 2017). Finally, although there are no reports of diclofenac or other NSAID related deaths in Europe, it is also a looming concern, especially after a recent fatal case of diclofenac poisoning was reported in a cinerous vulture *Aegypius monachus* (Linnaeus, 1766) in Spain (Hernandez, 2018; Herrero-Villar, 2021).

1.3. Study aims and main methods

1.3.1 General goals

With this study, we aim to better understand the use of space by the endangered Egyptian Vulture, with particular attention to the use of livestock farms by the species. Additionally, considering the threat that certain veterinary pharmaceuticals pose to vultures as well as the environment in general, we will focus on characterizing the coprophagy behaviour of this species. We are particularly interested in assessing the risk that this behaviour represents as a route of VP exposure, which, to this date, is completely unknown. To this end, two complementary methodologies will be used, as described below.

1.3.2. Telemetry

Using telemetry to study the movements of animals and their spatial ecology is a field with almost 60 years of history, helping to greatly increase our understanding of animal behaviour (Craighead, 1972, Hebblewhite and Haydon, 2014). This is particularly true for wide-ranging, low-density species, such as vultures, whose behaviour is otherwise hard to study through traditional methods. In the case of vultures, telemetry studies have flourished over the past decade, though this group has been studied using satellite data for over three decades (Alarcón and Lambertucci, 2018). Telemetry data has been widely used to study several aspects of vulture biology, ecology and life history, including foraging behaviour (García-Heras *et al.*, 2013; Fluhr, 2017), social dynamics (Harel *et al.*, 2017), migration patterns (W. L. Phipps et al., 2019), and even their physiology (Duriez et al., 2014).

A grid-cell analysis based on GPS telemetry data will allow us to identify the preferred foraging sites used by tagged individuals, and a key feature analysis will help to better characterize these sites. Since important differences in the how transborder vulture populations forage between Portugal and Spain have been identified (Arrondo *et al.*, 2018), this analysis will focus on the differences in features found in the sites used in each country. Due to different carcass disposal legislation, we expect more sites in Spain compared to Portugal, and that these sites more frequently present features associated with livestock in Spain as well.

1.3.3. Interviews

People in constant contact with their local environment often gain invaluable insight on the environment and habitat in which they live and share with other species (Huntington, 2000). This knowledge, known as Local Ecological Knowledge (LEK), supplement knowledge of ecology and species obtained by "conventional" scientific methods. Traditionally, this approach has not been very popular among scientific circles, but it can and has been an invaluable tool to gain important knowledge that can inform wildlife management and conservation practices and can be especially relevant when those conservation actions also affect the life of local peoples (Gilchrist *et al.*, 2005; Huntington, 2000). Because of this, LEK-based approaches have become an ever more important approach to obtaining ecological data.

Interviews conducted in the study area to local livestock farmers will be used to characterize farms in the study area, including carcass management practices and VP usage, and collect reports about the use of Egyptian vultures of these farms, with focus on feeding behaviours shown by the species. We expect to find that farmers indeed see Egyptian vultures in their feeding activities on their farms, and especially coprophagy is expected be reported. We also expect to find more favourable carcass management practices for vultures in Spain compared to Portugal. Based on previous works, that report extensive livestock management practices (Gigante *et al.*, 2020), we expect fairly low levels of VP usage.

2. Methodology

2.1. Study area

The study was conducted in the International Douro Region, also known as *Arribes del Duero*, in the Northwest of the Iberian Peninsula, where the Douro River, and its tributary, Águeda River, act as the border between Portugal and Spain (Figure 1a). Here, we find a highly complex and unique geological, climatic and human occupation situation, creating conditions for high richness and diversity of habitats and species (ICNF, n.d.). The region features a plateau that has historically been used for traditional agriculture and livestock rearing, still practiced today, which alternates with forest patches and other natural vegetation (ICNF, n.d. b). This mosaic of habitat types is important for both steppe and forest species (ICNF, n.d.). Additionally, erosion by the Douro River and its tributaries have created large river canyons, which are of great importance to riparian and cliff-nesting birds, such as the Egyptian vultures (ICNF, n.d.).

The work was focused on the Portuguese "Parque Natural do Douro International" (hereafter, PNDI) and the Spanish "Parque Natural Arribes del Duero" (hereafter, PNAD; Figure 1b). Both the PNDI and the PNAD consist of narrow strips along the international sections of Douro and Águeda Rivers, where the two protected areas meet, and have a collective area of 192.605 ha (ICNF, n.d.). PNDI and PNAD were created in 1998 and 2002, by the decree law 8/98 of May 11th and royal decree 5/2002 of April 11th, respectively, with the objective of protecting, valuing, and promoting the natural and human heritage of the region. Additionally, due to the importance of the cliffs and surrounding areas for a large number of birds that breed in this region, including species of conservation priority, two Special Protection Areas (SPA) were created here in order to value and protect their habitat, as part of the Natura 2000 Network. These are the SPA Douro Internacional e Vale do Águeda in Portugal (code PTZPE0038; Decree law 384-B/99 of September 23rd), and SPA Arribes del Duero in Spain (code ES0000118; Royal decree 8/1991, of May 8th), which overlap to a great extent with the aforementioned Natural Parks (Figure 1).

All movements of the tagged Egyptian vultures were considered, and due to the species' large foraging range (López-López *et al.*, 2014; Phipps *et al.*, 2019). In administrative terms, the area used by tagged individuals roughly corresponded with the Portuguese municipalities of Miranda do Douro, Vimioso, Mogadouro, Freixo de Espada à Cinta, in the district of Bragança, and Figueira de Castelo Rodrigo, in the district of Guarda; and the Spanish provinces of Salamanca and Zamora, from the Castilla y León autonomous communities (Figure 1a).

As mentioned before, agriculture, including livestock rearing, is an important activity in the area. However, livestock rearing is not conducted at the same scale in both countries (Figure 2). As it can be seen in figure 2, the density of both cattle and sheep, the main livestock species of the region, are much higher in Spain than in Portugal, though cattle are more abundant in the province of Salamanca, and sheep are more abundant in the province of Zamora. In both countries however, there has been an overall reduction in livestock ownership over the last 10 year (Gigante *et al.*, 2020).

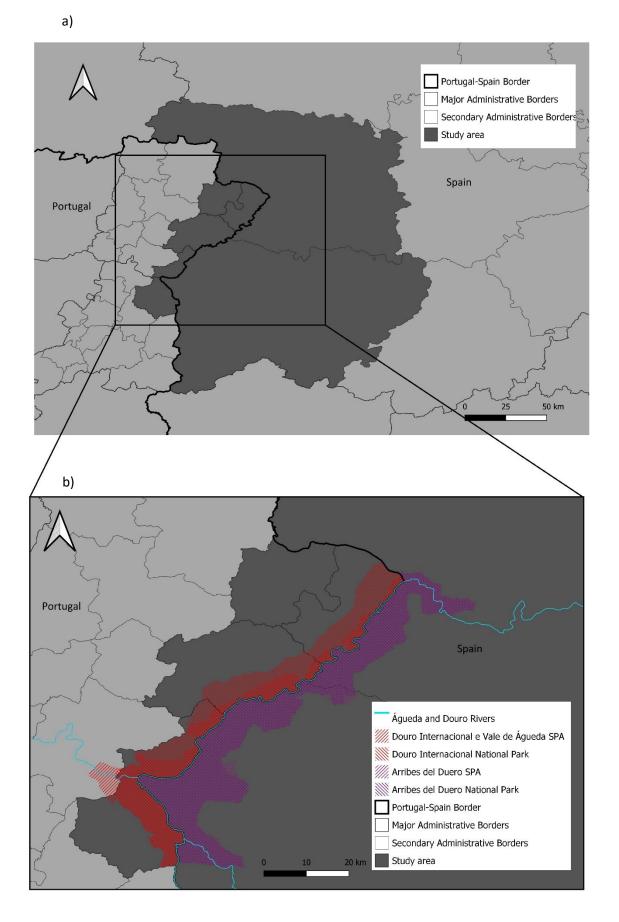
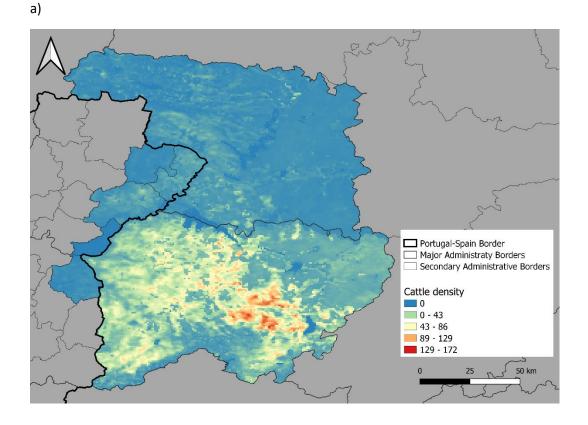


Figure 1 - Map of the a) study area, and with b) focus on the International Douro's protected areas. Egyptian vultures nest where these areas meet the rivers.



b)

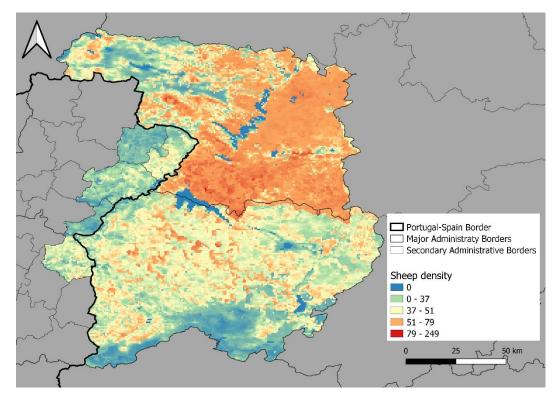


Figure 2 – Livestock density of a) cattle and b) sheep in the study area. Source: https://www.fao.org/home/en/

2.2. Telemetry

2.2.1. Grid cell analysis

2.2.1.1. GPS data collection and filtering

To better understand space use by Egyptian vultures at an individual level in the study area, a grid-cell analysis was used to identify the most used sites by each individual. To this end, data from Egyptian vultures tagged with GPS transmitters in the scope of the LIFE Rupis project and from SALORO S.L.U. team were used in the analysis (table 1).

LIFE Rupis is an EU funded conservation project, that was carried out between 2015 and 2019 in the study area, with the goal of protecting and strengthening the breeding populations of the Egyptian vultures and the Bonelli's eagle *Aquila fasciata* Vieillot, 1822 in the International Douro region (<u>http://www.rupis.pt/pt/</u>). As part of project's goal D6 - "Monitoring of the use of space during breeding season, migration and wintering areas through remote tracking." – a total of eight Egyptian vultures, two juveniles, three immatures and three adults were tagged and released over the course of the project (Table 1a). Additionally, SALORO Exploration S.L.U. funded the deployment of transmitters in four more individuals, two adults and two juveniles, the information of which was kindly provided for the purposes of this study (table 1b), bringing the total number of individuals used in this study to 12.

The data from each individual was categorized by year and analysed separately. The amount of time each bird spent transmitting data in the study area was assessed by visually analysing the data to determine the arrival and departure dates of the birds. For each individual, years with less than 30 days of data in the study area were excluded from the analysis. This resulted in a total of 24 seasons from nine individuals, five adults and four non-breeding (table 1).

Since the bird's foraging and feeding behaviours were the focus of this study, nest-related activities were not important and would introduce noise to the results. As such, when relevant (i.e., for adult birds with known nest site locations), all points less than 1km from the individual's nest were excluded. While this step introduces some probability of excluding important sites found very close to the nest, it is important to help identify the areas that vultures are using for foraging activities. Information on the nest site locations was provided by the Rupis project and Saloro SLU.

2.2.1.2. Data analysis

Following the methodology of Phipps (2019), a grid-cell analysis using 1km² square cell was used to calculate the amount of time spent in a given area. The density of GPS points within a cell were used as proxy for the amount of time the individual spent in that area.

For the purposes of the analysis, cells that contained more than the mean number of points per cell were classified as Intensively Used Areas (IUAs), and the cells that collectively contained the top 20% of GPS points were considered Very Intensively Used Areas (VIUAs; Phipps, 2019).

Table 1 – Information on tagged Egyptian vultures from a) LIFE Rupis project and b) Saloro SLU team, including how they were tagged, age when tagged, number of days spent in the study area in each year, and the birds' current status.

Vulture	GPS tagging	Age when	Transmitter	Days spent in study area					Current Status
ID	method	tagged	type	2016	2017	2018	2019	2020	Current Status
Rupis	Wild-caught	Immature, 3rd year	Ecotone	67	150	174	180	6*	Alive; not transmitting
Faia	Wild-caught	Adult	Ecotone		159	166	178		Alive; likely tag failure
Douro	Wild-caught	Adult	Ecotone		76	186	71		Alive; transmitter lost
Poiares	Rehab-released	Adult	Ecotone		113				Suspected transmitter falure
Bruco	Wild-caught	Immature, 4rd year	Ecotone		48				not transmitting, Unknown
Arribas	Nest tagged	Juvenile	Ortinela				27*		Alive; transmitting data
Sendim	Nest tagged	Juvenile	Ortinela				16*		Confirmed Dead
Fangueiro	Rehab-released	Immature, 2nd year	Ortinela					95	Alive; transmitting data

a)

b)

Vulture	GPS	Age when	Transmitter	Days spent in study area				Current Status	
ID	tagging method	tagged	type	2016	2017	2018	2019	2020	
Batuecas A	Wild-Caught	Adult	Ortinela		90	190	199	199	Alive, transmiting data
Huebra	Wild-Caught	Adult	Ortinela		97	211	46		Suspected transmitter failure
Batuecas P	Tagged in nest	Juvenile	Ortinela		49	175	187	141	Alive; transmitting data
Camaces	Tagged in nest	Juvenile	Ortinela						Possibily dead

*Excluded from the analysis dues to having less than 30 days of tracking

2.2.2. Feature analysis

2.2.2.1 Data collection

This analysis was focused con characterizing the VIUAs. Google Earth Pro was used to check the area of each VIUA individually in order to identify the presence or absence (with one exception) of key features that might be of relevance to the Egyptian vultures. When possible, google street view was used to confirm the presence/absence of the features (Olea and Mateo-Tomás, 2013). In particular, we focused on features that could indicate that the areas were being used for foraging activities, or roosting by vultures, and features that might indicate human disturbance. Note that some features may fit into more than one of these three categories, as describe below for each feature. The features considered were:

Presence of surface water - Usually in the form of water ponds, these are used in farms as a main water source for livestock, which regularly congregate around the ponds, potentially attracting vultures (Meroño and Fernández, 2018). This feature can therefore be used to indicate foraging activities, though it is possible that Egyptian vultures also use these to drink.

Presence of livestock farm buildings - this can be used as a proxy for the presence of livestock in the area and foraging activities by vultures, though it can also indicate some human disturbance.

Livestock - It is sometimes possible to check for the presence of livestock through satellite imagery. Although it is an unreliable feature by itself, it may help to avoid false negatives when proxy features for the presence of livestock are not present.

Cliffs and outcrops - these are used by vultures as roosting sites.

Powerlines - Egyptian vultures often use them for roosting, and in some regions of the world, even build their nests in them. On the other hand, there is also risk of collision or electrocution by vultures, so their presence also relates to human disturbance.

Roads – with the distinction being made between **dirt roads** and **paved roads**. Roads are known to greatly fragment the landscape.

Proximity to human settlements - These were considered to be any group of human housing, like villages.

2.2.2.2 Data analysis

Additionally, the same feature analysis was conducted in 30 randomly selected 1km² sites in each country. Since our goal was to assess the landscape structure of the study area as a whole, and not just sites not selected by the individuals, cells that have been classified as VIUAs already were not excluded from being selected for VIUAs. The comparison of these to VIUAs will allow the assessment which feature vultures prefer or try to avoid, and it will help to understand if differences in VIUAs between countries exist because of differential behaviour by the vultures, or if there are structural differences in the landscapes of each country.

Data analysis was focused on comparing different subsets of samples, specifically focusing on comparisons between countries. Statistical tests were performed to assess differences in the frequency of features found in Portugal and Spain ("VIUA vs VIUA" and "random sample vs random sample"). Additionally, to assess if the frequencies at which features were found in VIUAs were higher, lower, or equal to what was expected, "VIUAs vs random sample" comparisons were made for the study area as whole, for the Portuguese side of the study area and for the Spanish side of the study

area. This analysis will help to better understand which features vultures seem to select for or against, at least when it comes to their most preferred sites.

2.3. Interview

2.3.1. Data collection

In order to assess the Egyptian vultures' behaviour on livestock farms and potential associated risks, individual interviews were conducted to farmers with livestock explorations within or in the immediate vicinity of the protected areas of the study area. The interviews were conducted between May and August of 2021. Due to the large study area, several methods were used in order to most efficiently reach out to famers, which included: approaching farmers that were found working on livestock farms or around herds; using contact networks from local nature conservation associations or veterinarians; and asking in towns and villages, at local cafés or at passer-by, for livestock farmers willing to be interviewed. The only criteria for participants were that they owned or were involved in raising livestock, and that they were familiar with the study species, the Egyptian vultures. If a farmer met those criteria, a structured interview was conducted, either in person, or if necessary, by phone.

Participants were provided with informed consent and made aware of their right to withdraw from the study, even after the interview (Annex 1). Along with the consent form, interviewees were also given a random number ID associated with their interview data. This way, if the interviewee so wished, their data could be removed from the dataset even after anonymization of the data. The interview consisted of fixed and closed-ended questions, complemented with a few open-ended questions. Any additional comments made by farmers were also recorded. The interviews were divided into two sections: questions about the sightings of Egyptian vultures in the interviewee's farm and nearby areas; and questions about the farm itself, including the number and type of livestock owned, use of veterinary pharmaceutical, and farm management (Annex 2).

Farmers were often not able to answer the questions related to veterinary pharmaceutical use. In those cases, it was recorded that the interviewee didn't know this information, and permission was asked to them to speak to the main responsible veterinarian in order to obtain more of this important information.

The interviews received ethical approval from the Nottingham Trent University ethics review panel. Additionally, due to the current pandemic situation, preventative measures recommended General Directorate of Health of Portugal and World Health Organization were taken, including wearing a mask during the interviews and social distancing.

2.3.2 Data analysis

The interview data allowed the assessment of Egyptian vulture visits to the interviewees' farms, as well as the characterization of the farms and the management strategies employed by the famers, with particular focus on veterinary pharmaceutical usage. The data recorded during the interviews was anonymized. Answers given by the farmers were transformed into presence/absence variables, used to determine the frequency of each response for a given question. Exceptions to this were quantitative data, such as the number of animals in the farms. The following descriptive analysis focused on the perceived behaviour of Egyptian vultures reported on the farms by the farmers, characterization of farm management, in particular carcass disposal practices, veterinary pharmaceutical usage, and livestock faeces management.

3. Results

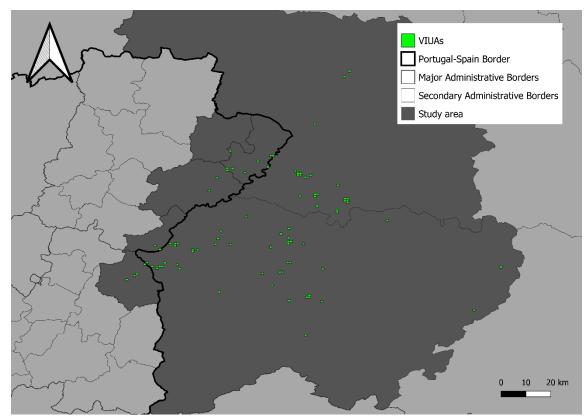
3.1. Telemetry

3.1.1. Grid cell analysis

A total of 85 distinct Very Intensively Used areas (VIUAs) and 570 distinct Intensively Used Areas (IUAs) were identified for the tagged Egyptian vultures in Douro International Region. The distribution of these can be seen in figure 3. Each individual used an average of 4 distinct VIUAs and 44 distinct IUAs per year, with non-breeding birds using more sites on average (7 VIUAs, 85 IUAs) than adult breeding birds (2 VIUAs, 23 IUAs; figure 4b). Among breeding adults, high variation between the average distance travelled between of their nest site and the VIUAs (Figure 5).

Of the 85 distinct VIUAs, 20% were located in Portugal (n=17), while 80% were located in Spain (n=68). Following the same trend, only 12,6% of IUAs are found in Portugal (n=72), while 87,4% were found in Spain (n = 498; Figure 4a). Besides the number of sites used, there is a significant difference in how far from the nest sites Egyptian vultures forage into each country. The average distance of VIUAs located in Spain to the border, where the nest sites are located, was 16.3km, while for VIUAs in Portugal, it was significantly lower, with the average distance to the border being 1.9km (student's t-test, *p*-value < 0.001).

While the important nesting sites are completely protected by the protected areas, the majority of the VIUAs (72%; n=62) and IUAs (74%; n = 422) used by vultures in their scavenging activities are not. However, looking at each country individually, only 19.1% (n=13) of VIUAs in Spain were inside protected areas, while 58.8% (n=10) of VIUAs in Portugal were inside protected areas. The same trend was found in IUAs, with only 19.3% of IUAs in Spain found within the protected areas, while 74,3% of IUAs in Portugal are found within the protected areas. Egyptian vultures foraged more outside of protected areas in Spain than in Portugal, despite the similar areas of the protected areas. High variation between the adult birds on the distance of their nests to the VIUAs, suggesting high variability between individuals (Figure 5).





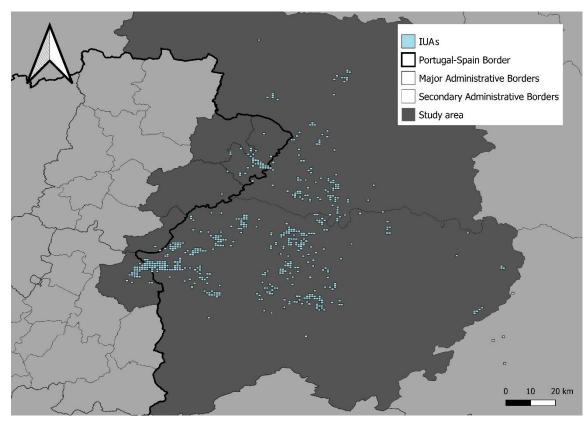


Figure 3 – Distribution of a) VIUAs and b) IUAs in the study area.

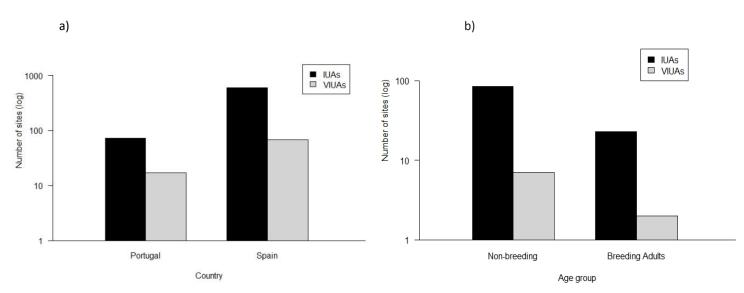


Figure 4 – Comparison of a) average number of Very Intensively Used Areas (VIUAs) and Intensively Used Areas (IUAs) per year between breeding adults and non-breeding individuals (juveniles, immatures, and sub-adults) and b) number of total distinct VIUAs between countries.

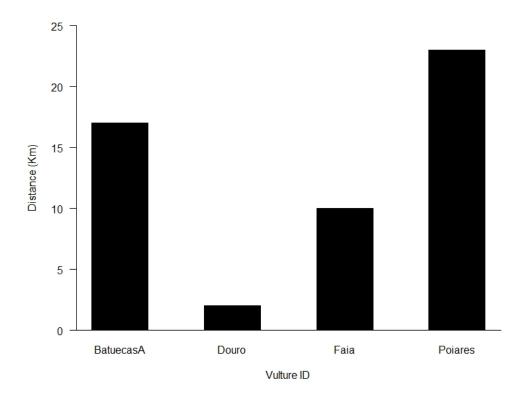


Figure 5 – Average distance, in meters, of between the VIUAs of breeding adults and their respective nests.

3.1.2. Feature Analysis

The results from the VIUA feature analysis found interesting differences and similarities in the features found in the sites, when comparing countries. The results can be found in figures 6 and 7. The most frequent feature in all samples are dirt roads, which were ubiquitous in the study area. The most second most feature found in the in VIUAs was surface water, in the form of ponds ("Charcas"). The presence of surface water is significantly higher in Spain than in Portugal (χ 2 test, p-value < 0.001). Furthermore, presence of surface water in Spain in VIUAs was significantly higher than the random sample in Spain (χ 2 test, p-value = 0.027), but not in Portugal (χ 2 test, p-value = 0.95).

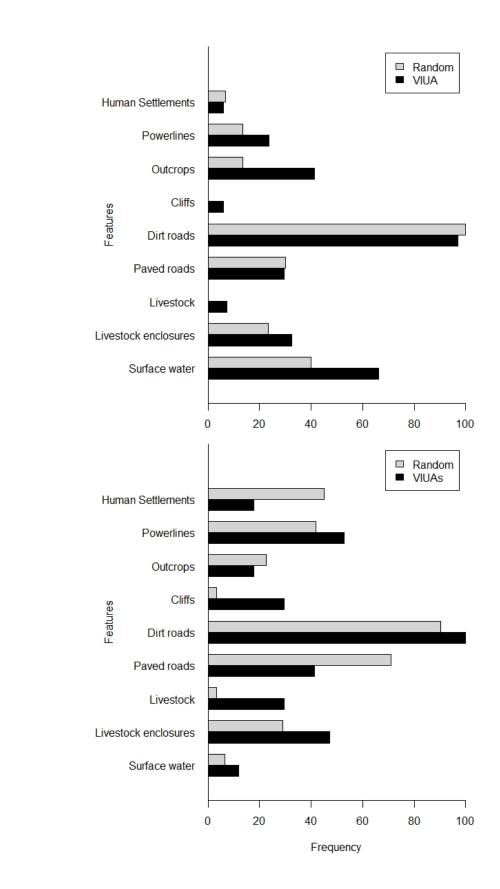
The opposite trend can be found in livestock enclosure presence, though the difference is not significant (χ 2 test, *p*-value = 0.39), and in livestock presence, where the difference is significant (χ 2 test, *p*-value = 0.035). However, as mentioned before, livestock presence, as a feature, needs to be analysed careful, because it is unreliable, and there were no significant differences between VIUAs and random sampling points for this variable in either country. Comparison to the random samples for livestock enclosure presence and livestock presence did not yield any significant differences.

Regarding roads, it seems vultures tend to avoid areas with paved roads them in Portugal, with a significantly lower proportion of VIUAs here featuring paved roads than the random sample (χ^2 test, *p*-value < 0.001), but not in Spain (χ^2 test, *p*-value = 1). Additionally, when using the random samples to compare countries, paved roads were significantly more common in Portugal (χ^2 test, *p*-value = 0.005), but no difference was found between VIUAs (χ^2 test, *p*-value = 0.52). No significant differences were found in any of the comparisons with dirt roads, as they were found in almost all sites analysed.

Egyptian vultures seem to favour rock formations, with significantly more VIUAs featuring outcrops in the whole study area compared to the random sample. A close to significant difference can be found in the presence of cliffs in VIUAs as well (χ 2 test, *p*-value = 0.07). Note that this result for cliffs was despite the exclusion of nest related activities in breeding adults, which nest in cliffs. Comparison of each country's VIUAs and respective random samples suggests that Egyptian vultures preferred outcrops in Spain (χ 2 test, *p*-value = 0.013), but cliffs in Portugal (χ ² test, *p*-value = 0.03), while the opposite comparisons did not show significant differences in either case.

Significant differences were also found in the presence of powerlines between countries, both when comparing VIUAs (χ^2 test, p-value = 0.037) and random samples (χ^2 test, p-value = 0.02). In both cases, powerlines were more common in Portugal. Comparisons between VIUAs and random samples did not yield any significant differences.

In the whole study area, frequency of VIUAs near human settlements was significantly lower than that of the random sample (χ^2 test, *p*-value = 0.005). However, when doing the same comparison in each country separately, there was no difference for Spain (χ^2 test, *p*-value = 1), and it was only close to significant for Portugal (χ^2 test, *p*-value = 0.09). Finally, no significant difference was found comparing VIUAs of each country (χ^2 test, *p*-value = 0.277), but when comparing random samples, human settlements were significantly more common in Portugal (χ^2 test, *p*-value = 0.001).



a)

b)

Figure 6 – Comparison of VIUAs to random sample in a) Spain and b) Portugal

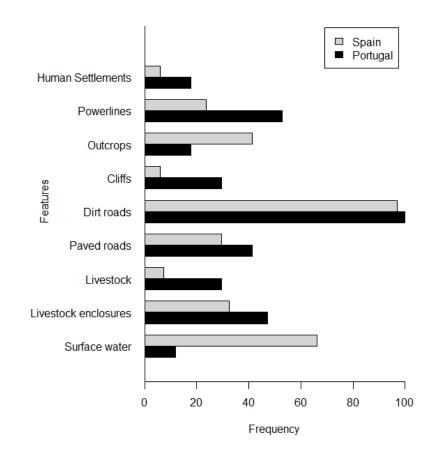


Figure 7 – Frequency of the presence of features in VIUA – comparison between Portugal and Spain.

3.2. Interviews

3.2.1. Farm characterization

The distribution of interview in the study area can be found in figure 8. Most interviewees were exclusively cattle farmers, representing 78.1% of interviewed people (n=25). Another 15.6% were sheep farmers (n=5), and finally, 6.3% had both sheep and cattle (n=2). Farms with cattle had on average 129 (range: 8-1000) animals, and farms with sheep had an average of 354 (70-1200) animals. Farms had on average more animals in Spain for both sheep and cattle. In Portugal, farms had on average 170 heads of sheep and 66 heads of cattle, while in Spain these numbers were 600 and 208, respectively (figure 9).

All farmers did rotation grazing, though the spatial and temporal scale of it was not measured. More than half of farmers (56.63%; n=18) did not remove faeces from the fields, leaving it to decompose on its own in the fields. The rest of farmers collected the faeces, particularly in livestock feeding sites or in barns, and used it as fertilizer. Of these, 42% kept dungheaps (n= 6), where faeces are kept to compost before being used as fertilizer. None of the farmers used methods that completely removed faeces from the landscape.

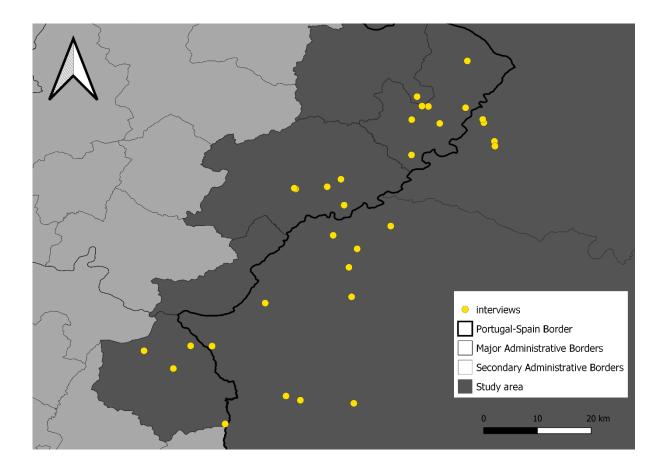


Figure 8 – Location of interviews conducted for the study.

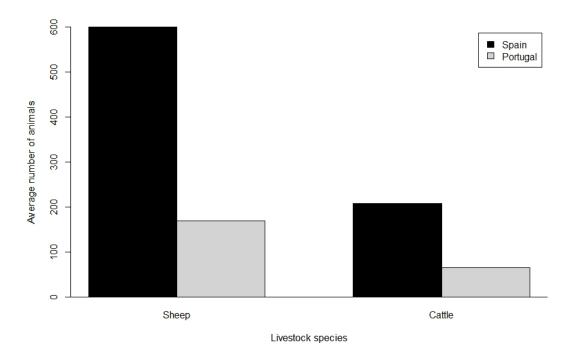
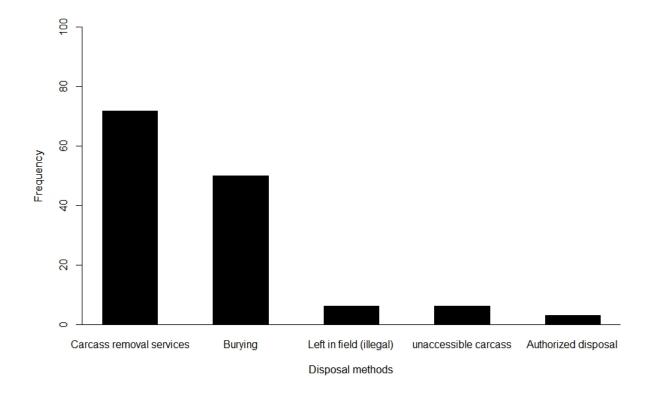
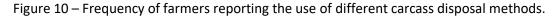


Figure 9 – Farm herd size differences between Portugal and Spain

Most farmers utilized carcass removal services and/or burying for carcass management (90.6%; n = 29). A mixed system, where both methods were used, was also common (31.3%; n = 10). When this mixed system was used, burying was used for younger animals (under 3-4 years old), and carcass removal system was used for older animals. However, among farmers that used at least one of these two methods, two farmers (6.9%) said they were legally allowed to leave carcasses on the field in scenarios where the animal had died in a place inaccessible for the machinery need to move the carcass. The other 9.4% that did not used either of these methods were sheep farmers (n=3). Two of these farmers disclosed to leaving the carcasses on the field illegally. While both farmers knew vultures and other necrophagous animals would "clean" the carcasses, one of the two did it with the clear intent of "feeding the vultures", and noted that, due to the minimal use of veterinary pharmaceuticals, their livestock was safe for vulture consumption. Finally, only one interviewed farmer had the legal requirements that allowed them to leave carcasses on a predetermined site (figure 10.

Most farmers kept their livestock outdoors (93,7%; 30), free to graze within a closed field for the majority of the day. Even after being medicated, the livestock would remain outdoors, unless very ill. A number of farmers also described the livestock's feeding (n = 18). While all farmers relied, to an extent, in fresh feed from the fields, almost all farmers also used hay (94.4%; n = 2) and/or cereal grain (55.5%; n = 10). A smaller percentage of farmers also used commercial feed (11.1%; n = 2).





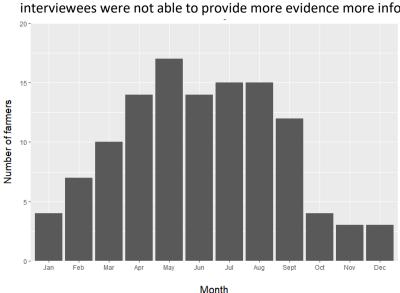
3.2.2. Egyptian vulture's use of farms

Egyptian vultures were most active in the interviewee's farms between April and August, with a small peak in May (figure 11). Interestingly, more than one farmer reported seeing EVs during the winter. Two farmers reported seeing vulture all year around, while one said he saw them in winter only (December, January, and February). In the two cases, the farmers were very familiar with the species, and saw them close to daily, if not necessarily in their farms. Additionally, it is interesting to note that the three farmers who reported seeing Egyptian vultures all year around were located close to each other.

Comparing countries, we find a numerical difference on the frequency of reported farmer visits, with proportionally more farmers in Spain reporting visits by Egyptian vultures at all (figure 12). However, this comparison did not reach statistical significance. In terms of food items that farmers reported Egyptian vultures eating, 25% (n = 8) reported seeing vultures feeding on calf faeces (specifically fresh faeces), 37.5% of farmers reported seeing Egyptian vultures feeding on livestock carcasses (n = 12), and 15.6% of farmers said they saw Egyptian vultures feeding on placenta (n = 5). Figure 13 shows the difference of frequency in these three food items between Portugal and Spain.

One of the questions done during the interview related Because most farmers in our sample raised only one type of livestock, they were rarely able to notice any species preference from the Egyptian vultures in terms of their dietary preferences. However, one farmer that had both species reported only seeing Egyptian vultures showing interest in live cattle, never live sheep. This anecdotal evidence is corroborated by the fact that exclusively sheep farmers saw Egyptian vultures feed only on carcasses.

Some farmers were able to report congregation sites for Egyptian vultures near the farm. The most common site for observed congregations was the "river cliffs" ("arribas"). However, a few other famers knew the exact locations of Egyptian vultures' nests. The few other farmers who knew of vulture congregation sites referred to sites where carcasses were left, official feeding sites or otherwise.



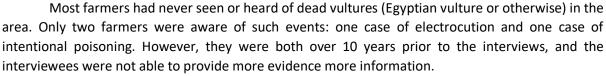


Figure 11 – Temporal distribution of the Egyptian vulture in Douro Internacional – frequency of farmers reporting sightings in their farms

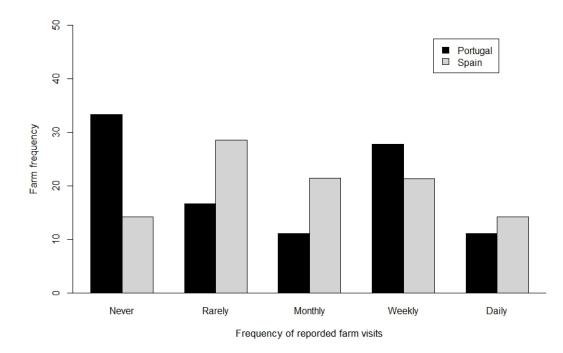


Figure 12 – Frequency of farmers reporting the frequency of Egyptian vulture visits to their farms.

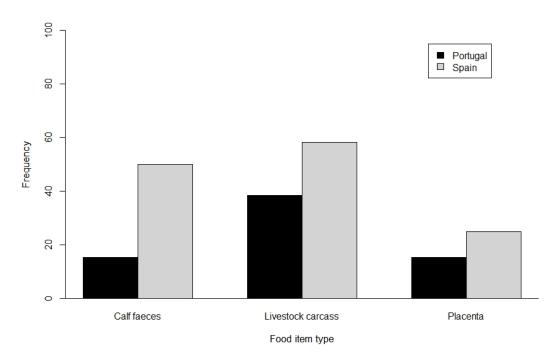


Figure 13 – Frequency of farmers reporting seeing Egyptian vultures feeding on different food items

3.2.3. Veterinary pharmaceuticals' inventory

All farmers used medication in their livestock. However, information on Veterinary Pharmaceutical (VP) usage was collected for 19 of the 32 total interviews (59.4%). The remaining 40.6% of farmers (n = 13) were not able to give specific information on VP usage and were not very comfortable sharing information of their veterinaries. Of the interviews with information about VP usage, 63.2% was given by the farmers themselves (n = 12), and of the remaining 36.8% of interviews (n = 7), the information was provided by veterinaries. The inventory of VPs used is summarized in tables 2 and 3.

During the interviews, it was clear that almost all farmers had the policy of using the least amount of veterinarian pharmaceuticals as possible, because they perceived less VPs leads to a more "natural" – and therefore, better – product, of which they were proud. The VPs that were used were always prescribed, and although some veterinarians left VPs to be used by the farmers as needed, most often it was the veterinarians themselves that administered the VPs to the animals. Anthelmintics were always given to the whole heard at the same time, and was given as preventive treatment, but all other VPs were given as individual animals needed treatment. VPs were always reported to be given at their respective recommended doses.

The main type of veterinary pharmaceutical used were anthelmintics, which most farmers perceived to be "required". Additionally, it was the only type of VP to be used as a preventive treatment by all. Anthelmintics were also the only type of veterinarian pharmaceutical to be used in the whole herd at the same time. Of these, Ivermectin was the most common active principle. The next most common group of VPs are antibiotics, used by 47.4% of the interviewed farmers (n = 9). Only one instance of an NSAID being used was reported, Fluxinin. This VP was prescribed by a veterinarian, and it was used in a farm that was visited by vultures, though they weren't very frequent.

NAME	FREQUENCY (%)	REQUIREMENTS FOR USE
IVOMEC	68.4	prescription needed
TERRAMICINA	26.3	prescription needed
NOROMECTIN POUR-ON	15.8	prescription needed
PANACUR	10.5	can only be administered by a veterinarian
PENDISTREP	10.5	can only be administered by a veterinarian
SEPONVER PLUS	10.5	prescription needed
ACTIONIS	5.3	can only be administered by a veterinarian
CIPERMETRIN	5.3	no prescription needed
DEXABIOPEN	5.3	prescription needed
DINOLYTIC	5.3	prescription needed
DRAXXIN	5.3	prescription needed
FORCYL	5.3	can only be administered by a veterinarian
MEGANYL	5.3	can only be administered by a veterinarian
PENINCILLIN	5.3	prescription needed

Table 2 – Names of veterinarian pharmaceuticals used in livestock and the frequency of farms using them, as well as the legal requirements for their administration.

Table 3 – Frequency of the active principles found in the veterinarian pharmaceuticals used by interviewed farmers.

ACTIVE PRINCIPLE	ТҮРЕ	FREQUENCY (%)
IVERMECTIN	Anthelmintic	68.4
FENBENDAZOL	Anthelmintic	15.8
MEBENDAZOL;	Anthelmintic	15.8
CLOSANTEL SODIUM DIHYDRATE	Antibiotic	15.8
BENZYLPENICILLIN PROCAINE	Antibiotic	15.8
DIHYDROSTREPTOMYCIN	Antibiotic	15.8
DEXAMETHASONE	corticosteroid	10.5
OXITETRACYCLINE	Antibiotic	10.5
MARBOFLOXACIN	Antibiotic	5.3
FLUNIXIN	NSAID	5.3
DINOPROST	Synthetic prostaglandin F2alpha	5.3
CYPERMETHRIN	insecticide	5.3
TULATHROMYCIN	antibiotic	5.3
PENICILLIN	antibiotic	5.3
CEFTIOFUR	antibiotic	5.3

4. Discussion

4.1. Telemetry analysis

4.1.1. Grid-cell analysis

This study yielded some interesting findings. This ecological study of the Egyptian vulture suggest that non-breeding and adult Egyptian vultures use space differently. Non-breeding birds explored a higher number of sites, while breeding adults tended to rely on a lower number of sites (figure 4b), which were likely more reliable for food foraging, that is, with more predictable food availability. Breeding Egyptian vultures are restricted in their movements because they have to carry food from the foraging sites back to the nest to feed their offspring (García-Heras *et al.*, 2013). Furthermore, Egyptian vultures are highly territorial during the breeding season, defending the area around their nest from neighbouring Egyptian vultures' pairs, further limiting available foraging area for breeding adults (García-Heras *et al.*, 2013; van Overdel *et al.*, 2018).

High interindividual variation was found in the average distance between nests and VIUAs of breeding adult Egyptian vultures. For example, Poiares' VIUAs were over five times further away from its nest compared to Douro's (figure 5). The reasons for this variation were not explored further in here, as factors affecting individual foraging decisions by the Egyptian vultures were not the focus of this work. Previous studies have identified a few individual variables, such as sex, age, size, body condition, and dominance status, that might affect foraging behaviour, particularly in the use of predictable and semi-predictable food sources (García-Heras *et al.*, 2013; von Overveld *et al.*, 2018).

One of the most important findings is the difference in space use made by Egyptian vulture of Portuguese and Spanish landscapes. Despite the nesting sites being located along the border, all vultures used more sites intensively in Spain compared to Portugal. Arrondo *et al.* (2018) reached similar results with 60 griffon vultures and 11 cinereous vultures from populations nesting near in Tejo Internacional, another transborder region between Portugal and Spain. In that study, it was concluded that differential legislation regarding the disposal of livestock carcasses was the main factor influencing space use. However, other environmental and landscape differences may be at play for this study's study species and study area (Phipps, 2019).

The greater use of Spanish land and Spanish farms means that the birds forage significantly further from the nesting areas into Spain, and as a result, from the protected areas. This idea is confirmed both by the proportion of Very Intensively Used Areas (VIUAs) and Intensively Used Areas (IUAs) found outside of the protected areas of each country, and by the average distance of VIUAs to the border. The fact that most of the sites used by vultures in the study area are found outside of the protected areas are not very effective in helping to protect a species it is meant to protect because, despite protecting nesting areas, most of the foraging areas used by vultures are outside the protected areas. While these results serve to highlight the faults of the protected areas that exist in Douro Internacional, it is still too little data to inform the decision-making in regard to conservation like the creation of new protected areas or implementing changes to existing ones. This is because 1) only a small sample (n = 5) of breeding adults was tracked, from a population of about 120 breeding pairs of breeding Egyptian vultures found in the study area (Monteiro *et al.*, 2018); and 2) other species of large body and long-range species that nest in the study area were not considered. (Thaxter *et al.*, 2012)

4.1.2. Feature analysis

The most common features among VIUAs were dirt roads, at 97.6%, followed by surface water presence at 55.3%, outcrops at 35.5%, livestock enclosures at 35.3%, paved roads at 31.8%, and powerlines at 29.4%. Being Surface water, in the form of naturally occurring ponds, often serve as hotspots for livestock, that congregate around them to drink (Moreño and Fernández, 2018). As such, surface water presence can be a proxy for livestock presence. It is also possible, though, that vultures themselves use ponds as drinking sites.

Comparing the VIUAs found in each country revealed some interesting differences. The frequency of surface water presence was significantly higher in VIUAs in Spain, while the frequency of VIUAs with cliffs and powerlines were significantly higher in Portugal. As discussed, seeing as often surface water is related to livestock presence, this likely means Egyptian vultures spend proportionally more time and effort foraging in Spain compared to Portugal. Regarding cliffs, this feature was found more frequently in the VIUAs located in Portugal compared to Spain. Cliffs in the study area are predominately found along the border, where the vultures nest.

Comparison between random samples from both countries revealed some important differences between Portugal and Spain. Frequency of surface water presence was significantly higher in Spain, while presence of powerlines, paved roads and human settlements were higher in Portugal, which seem to indicate a more disturbed landscape in Portugal. Notably, Livestock enclosure presence was not significantly different.

Comparison of VIUAs to the random sample seems to suggest preference by the vultures for certain features and avoidance of others. In the study area as whole, surface water presence and outcrops were significantly higher in VIUAs than in the random sample, while cliffs were close to significant, suggesting that Egyptian vultures show a preference for these features. Note that cliffs achieved a close to significant result despite the exclusion of GPS points around the breeding adults' nests, which are located on cliffs. On the other hand, paved roads and human settlements were significantly less frequent in VIUAs compared to the random samples, suggesting avoidance behaviour towards features that are associated with human disturbance and habitat degradation.

However, when comparing VIUAs to the random samples separately for each country, it seems to show that Egyptian vultures behave differently in Portugal and in Spain. While Egyptian vultures still show clear preference for surface water presence and outcrops in Spain, no difference was found in the same comparisons for Portugal. On the other hand, selection against paved roads in Portugal, while the same is not true in Spain, where no significant difference was found. Frequency of human settlements was close to significantly lower compared to the random sample in Portugal, while no difference was found for the same comparison in Spain.

In general, the feature analysis indicates that vultures, when foraging in Portugal, appear to select against features associated with human disturbance (human settlements), and when foraging in Spain, Egyptian vultures seem to be to select for features related with foraging and roosting. The methods used here have significant limitations, because the analysis was only conducted for VIUAs – the most used areas by individuals – which likely excluded other less used but also important sites. As such, while the results are able to determine preference for certain features (water surface), conclusions regarding avoidance behaviour cannot be made. Additionally, contradictory results can be found in literature. Breeding density and nest site selection of Egyptian vultures have been shown to be negatively influenced by human presence and activity in other regions of Spain (Margalida *et al.*, 2007; Zuberogoitia *et al.*, 2008). Conversely, the opposite results have been found in other regions of

the Egyptian vulture's range, namely in Africa. Arkumarev *et al.* (2014) found that in an overwintering population in Afar, Ethiopia, there were slightly more birds roosting near human settlements than further away. Similarly, Gangoso *et al.* (2013) found that the Egyptian vulture population in the Socotra archipelago, Yemen, reach its highest densities within or near human settlements. Similar results have been found in other species, such as the hooded vulture *Necrosyrtes monachus* (Temminck, 1823) in Guinea-Bissau (Henriques *et al.*, 2018), and the black vulture *Coragyps atratus* (Bechstein, 1793), a New World vulture, in Brazil (Novaes and Cintra, 2015).

Other studies have pointed to the location of predictable food sources (vulture restaurants and landfills) and livestock density as predictors of use of space (López-López *et al.*, 2014; Tauler-Ametller *et al.*, 2017). Additionally, studies on the subspecies Canary Egyptian vulture *Neophron percnopterus majorensis* Donázar *et al.*, 2002 found that environmental factors such as foraging habitat quality (food availability in the landscape), as well as individual factors mentioned above, influenced how much the Egyptian vultures used these predictable food sources (García-Heras *et al.*, 2013; von Overveld *et al.*, 2018). All things considered, it seems Egyptian vultures are sensitive to human disturbance; however, the presence of predictable food sources inside urban areas outweighs the negative effects of higher disturbance.

4.2. Interview analysis

4.2.1. Farms Characterization

The sample of interviewed farmers does not represent the reality of the region, because sheep farming is dominant in terms of number of farmers, while in our interviews 75% (n=24) of farmers raised exclusively cattle, 18.7% raised exclusively sheep, and 6.3% raised both cattle and sheep. None of the farmers interviewed raised goats, which are rare in the study area (e.g., Gigante *et al.*, 2020). Overall, interviewed farmers used management practices that could be considered in line with extensive farming, for example by letting the animals graze in open grassland fields and, with the exception of anthelmintics, use of little to no veterinary pharmaceuticals (VPs) as needed.

Most farmers relied on carcass disposal services for carcass management or buried the carcasses (90.6%; n = 29). Despite this, vultures still had access to livestock carcasses in both countries. Due to different reasons, four (12,5%) of the farmers would still leave carcasses available for vultures, despite not having a permit to do so. Additionally, despite more flexible requirements in Spain for allowing carcasses to be left for vultures, only one interviewed Spanish indicated doing so. Other Spanish farmers, when asked about this, most were unaware of this option. It is generally assumed that the main factor for the differential use of space in transborder vulture populations between Portugal and Spain is the result of different legislation regarding carcass management practices in each country (Arrondo et al., 2018). While legislation undoubtedly plays an important role, this idea does not take into consideration how the legislation is applied in practice, considering most farmers, both in Portugal as well as in Spain, still relied on methods that removed carcass availability from the landscape (figure 8). Furthermore, the duration of time between the death of the animal and its disposal was often enough that vultures would still have access to the carcasses, regardless of the country. For example, one Portuguese farmer reported a recent case of an animal having died at the beginning of the weekend (Friday), and subsequently being completely consumed by vultures (Egyptian vultures and griffon vultures) in the interim, because carcass disposal services do not operate on weekends. Therefore, although there are important differences in carcass disposal practices and legislation between Portugal in Spain, in practice there is still livestock carcasses are available to vultures in Portugal. However, as Egyptian vultures prefer predictable food sources (López-López et al. 2014), the more regular and predictable sources of carcasses in Spain, due to higher livestock density, may be preferred.

4.2.2. Egyptian vulture's use of farms

The temporal distributions of Egyptian vultures provided by the frequency of farmers seeing Egyptian vultures in each month followed a curve that is to be expected, given what it is known about the Egyptian vultures' life history. The caveat to this is that three of these farmers reported to seeing Egyptian vultures during the winter months, in November, December and January. These farmers were very familiar with the Egyptian vulture and griffon vulture, as they knew the species by name and were able to easily identify them in the field, so while not impossible, confusion with other species with similar colour patterns, such as the white stork *Ciconia ciconia* (Linnaeus, 1758) or other vulture species is unlikely. Common scientific knowledge is that this population is migratory. In this case, they breed in the study area, and then migrate to overwinter in sub-Saharan Africa at around the end of summer and migrate back to the study area by the end of winter or early spring. However, there are reports of wintering of a few individuals in Sicily, where the population is thought to be migratory otherwise, and one immature individual in this study (Batuecas P) has wintered in Extremadura, less than 200km south of the study area (Vittorio *et al.*, 2016; Phipps, 2019). While most of the study population is migratory, the existence of a few individuals remaining in the breeding grounds might have management implications and should be further investigated.

In principle, the main reason for birds to migrate is to seek better feeding areas or more favourable climatic conditions. However, migration is a very energy consuming and risky endeavour. Therefore, if resources start to become available all year around or winters become milder in the breeding sites - making the risks of migration outweigh the benefits - populations might forego migration (Sanz-Aguilar *et al.*, 2015; Gilbert *et al.*, 2016; Vittorio *et al.*, 2016). While we have too little information to conclude whether these data pertain to one or a few outlier individuals or a population trend, due to the endangered status of the species and the low density at which this species breeds, even if only a few individuals are wintering in the study area, conservation managers should take these individuals into account (Vittorio *et al.*, 2016).

Other than feeding on livestock carcasses, 25% of all farmers reported seeing Egyptian vultures feeding on livestock faeces (n = 8) and 15.6% on placenta (n = 5). However, these feeding behaviours were only seen by cattle farmers, so if we look at just this group, the frequency of farmers seeing these behaviours is 29.6% for faeces and 18.5% for placenta. One farmer who had both cattle and sheep reported that Egyptian vultures had no interest in sheep, only cattle. Egyptian vultures always fed on fresh calf faeces, particularly from the first days after the calf was born. Some farmers noted that these faeces are usually lighter in colour, perhaps an indication of higher content of carotenoids. The consumption of faeces by Egyptian vultures (coprophagia) has been known for a long time and has received some scientific attention (Negro *et al.*, 2002), but close to nothing was known about this behaviour, particularly in a natural setting.

The reason for this lack of information is the difficulty of systematically collecting data on the coprophagic behaviour of wild Egyptian vultures, which have large foraging ranges, making it challenging to trace vultures and observe their natural feeding activities. To make matters more complicated, Egyptian vultures are timid animals, and can easily become wary and fly away if they feel

like they are being observed, something that was experienced while visiting some of the farms of the interviewees. Because of these reasons, Egyptian vulture diet studies have most often focused on identifying food remains or cast pellets in the Egyptian vulture's nest or in communal roosts. However, faeces are difficult to detect through these methods, due to being indistinct, so they are often not mentioned in these studies, and when they are, it is in an anecdotal manner (Grubac, 1989; Hidalgo *et al.*, 2005; Milchev *et al.*, 2012).

The difficulty in studying this coprophagic behaviour may be why it can be said it has been often overlooked when it comes to the conservation of this endangered species. As it will be explored in greater detail below, a large portion of veterinarian pharmaceuticals used in livestock go through the animal almost unchanged, ending up in the faeces, which can have negative impacts once in the environment. The effects of this have been extensively studied on coprophagic invertebrates, such as dung beetles, but the same cannot be said for Egyptian vultures. As a result, coprophagy by this species may represent as yet undetected exposure path for veterinary pharmaceuticals to enter the Egyptian vulture population. While the risk of Veterinary Pharmaceutical (VP) contamination by way of coprophagy in this species has not been assessed, the current MAF project this study is part of is currently attempting to measure this risk. While there is still much to be discovered, such as the real importance of faeces in the Egyptian vulture's diet, the results achieved here have helped shed some light on the characteristics of coprophagy of the Egyptian vulture, and its conservation implications.

4.2.3. Veterinary Pharmaceuticals' use

While explaining the types of questions included in the interview to participants, the fact that certain questions focused on VPs were often met with confusion. Interviewees did not understand how this particular topic could relate to Egyptian vultures, and even after the rationale for these questions was explained, some farmers showed reservations about it. It seems veterinarians, much less farmers, seem generally unaware that VPs may negatively impact the environment. On the other hand, other than anthelmintics, use of VPs was scarce, and for the interviewed farmers in the study area, only used when "strictly necessary", although what exactly this meant in practice was not elaborated upon. Interestingly, interviewed farmers seemed to hold pride in being able to sell a more "natural" product to consumers, which included minimal use of VPs, with associated benefits of this for the environment are incidental.

The most widely used type of drug was anthelmintic, and it was the only type of VP that was indicated to be used preventively. Anthelmintics were always used on the whole herd at the same time, usually once or twice a year, and were administered by veterinarians. Farmers would often mention that deworming is "required". However, this seems to be a misbelief, because although they are not required, they are highly recommended by the competent authorities, and it might happen that veterinarians recommend its use as well in a way that makes farmers think they are necessary.

Among anthelmintics and all veterinarian pharmaceuticals, Ivermectin was the most common active ingredient (Table 5). Ivermectin was the first Avermectin, a family of macrocyclic lactones, to be produced and introduced to the market, in 1980. It had a great impact in eradicating parasitic diseases both in livestock and human populations, and its use remains widespread to this day, due to the wide spectrum of parasites it is effective against (Omura, 2008; Laing, 2017). Ivermectin itself is not a drug of concern for vultures, in fact being used for treatment of a recovering Himalayan griffon *Gyps himalayensis* Hume, 1869 (Khadka, 2018). However, the high use of this and other similar drugs in the study area may confer risk for chronic exposure of vultures to ivermectin and other similar

anthelmintics, the effects of which are largely unknown in vertebrates (but see Lankas *et al.*, 1989). Furthermore, because it is so widely used, concerns over its negative effects on the environment have surged, which led to extensive research on the impacts of this drug, particularly on invertebrates, both in terrestrial and aquatic environments. Up to 89% of the administered dose can survive its journey through cattle's body and be excreted through the faeces (Sommer *et al.*, 1992). If contaminated faeces are left in the fields, as they often are in the study area, ivermectin is able to contaminate the environment for months, and in doses high enough to be lethal for several types of invertebrates (Sands and Noll, 2021). More specifically, the effects of this VP have been extensively researched on dung beetles (Verdú *et al.*, 2015; González-Tokman *et al.*, 2017; Baena-Díaz *et al.*, 2018).

Similarly, up to 75% of antimicrobials can be expelled in the faeces unaltered (Martinez, 2009). Antibiotics in the environment are a concern because they may increase antibiotic resistance in environmental microbiota, thus increasing the likelihood of human and animal pathogens becoming resistant to antimicrobials (Martinez, 2009). Additionally, lesions found in the oral cavities caused by yeast or bacterial infections were found in Spanish Egyptian vulture, cinereous vulture, griffon vulture and golden eagle *Aquila chrysaetos* (Linnaeus, 1758) populations. These lesions have been linked to consumption of antimicrobials from contaminated carcasses, which disrupted the natural microflora of the animal' digestive tract (Pitarch *et al.*, 2017). The specific group of antimicrobials suspected to have caused these lesions, fluoroquinolones, has not been reported in Douro International Valley, and antibiotic use was very low in general (Blanco *et al.*, 2017).

Relevant literature regarding VP use in livestock in agricultural areas is surprisingly scarce, considering the threat that certain VPs pose to vultures. To our knowledge, the only other veterinarian pharmaceutical inventory studies were technical reports prepared for Egyptian vultures New LIFE project, one in Bulgaria and one in Greece. The former focused on the use of Non-Steroidal Inflammatory Drugs (NSAIDs) in the region and was conducted in three regions of Bulgaria: Lomovete, Provadiisko-Royaksko Plateau, and Eastern Rhodopes; and the latter entailed a general survey about VP use in livestock, and was conducted on the regions of Thrace, Central Greece and Epirus. In Bulgaria, a total of 18 NSAID were reported to be used, including Diclofenac. The use of NSAIDs seems to be much lower in Greece, with 6 active principles reported being used. Flunixin, the only NSAID reported in our study area, was among the most common drugs reported in both Bulgaria and Greece. This NSAID is suspected of having caused vultures deaths in Andalucía, Spain (Zorrilla et al., 2014). In Greece, 24 different antibiotics were also reported, with Oxytetracycline and Streptomycin-penicillin being the most common. Use of both Oxytetracycline and penicillin was also reported in our study area. Other antibiotics found both in Greece and in our study area are Benzylpenicillin Procaine, Dihydrostreptomycin, Marbofloxacin and Tulathromycin. Finally, 12 different active principles used for deworming were reported in Greece, with Ivermectin, Fenbendazole and Albendazole being the most used. Other than Ivermectin and Fenbendazole, Cypermethrin was also used both in Greece and our study area (Καψάλης et al., 2019; Kostadinova et al., 2019).

The use of VPs by farmers without prescription or supervision of a veterinarian, and often by farmers with little knowledge about the causes for disease or the effects of VPs, was very common in Bulgaria. Moreover, some of the NSAIDS used did not appear in Bulgaria's National registry, meaning they their use was unauthorized. In contrast, farmers in our study always relied on veterinary oversight or prescription of VPs, and VPs were generally administered by the veterinarian rather than the farmer. In general, less regulation of VP usage seems to exist in practice in Bulgaria compared to our study area, and VPs use was much lower in International Douro as well. Additionally, in Greece, VPs were reported to be used at the first sign of disease, whereas in Bulgaria it was reported that VPs were used even when the animal was close to dying, meaning that VPs would likely remain in the tissues of

the animal, and potentially expose vultures to them ($K\alpha\psi\dot{\alpha}\lambda\eta\varsigma et al.$, 2019; Kostadinova et al., 2019). In our study area, other than anthelmintics, VPs were only used when considered "strictly necessary".

5. Conclusion

This work was able to reach important findings that have conservation implications for the Egyptian vulture, and that at the same time merit further scientific research in other to fully understand this species' ecology.

In accordance to results found in other vultures (griffon vulture and cinerous vulture; Arrondo *et al.*, 2018), the Egyptian vulture shows clear preference for foraging in Spain. Factors that influence this behaviour include habitat differences, livestock density, different carcass disposal policies. However, interview results show that carcass disposal practices may not play as big of a role as previously thought. Firstly, shortcomings of the carcass removal system in practice (in both countries) indicate that vultures may still have access to carcasses they should not otherwise have. Secondly, reports by farmers indicate that availability of other food resources, namely placenta and faeces, also plays an important role in determining the use of space by this species. Considering that these behaviours were reported only by cattle farmers, cattle density - regardless of carcass removal policies - should be considered when analysing foraging behaviour in other parts of its distribution. However, especially considering the species' opportunistic nature and flexible diet, our results do not conclude definitively that it feeds exclusively on cattle faeces. In fact, it is likely that faeces from other domestic ungulates, are consumed in areas where cattle is less abundant or not present.

The findings regarding coprophagy also have direct conservation implications for the species, as it represents a path of exposure to harmful VPs that has not been studied. With this study, we were able to show that Egyptian vultures regularly feed on domestic livestock. While this study could not prove directly that Egyptian vultures are feeding on contaminated faeces, lab testing of faeces samples collected at the interviewer's farms, conducted as part of the MAF project this study is part of, will hope to shed some light in that part of the puzzle as well.

Other results with conservation implications for Egyptian vultures is that the protected areas (Natural Parks and Special Protection Areas - SPAs) are insufficient for protecting this species, as shown by the high proportion of intensively used areas and very intensively used areas that are located outside of the protected areas, especially in Spain. While this study only focused on one species, other long range bird species that nest in Douro International Region, such as the griffon vulture and golden eagle, are likely to be affected by this as well. While the creation of these areas, particularly SPAs, focused and the protection of nest sites cliff nesting birds and immediate surrounding areas, it failed to consider the movement ecology of these species, which is a common issue for many protected areas. As such, it is necessary that the creation of future protected areas or changes made to current ones take in consideration the ecology of species they are meant to protect.

Another important finding is the possibility of an overwintering population of Egyptian vultures in the study areas, where the species is usually thought to migrate and overwinter in Sub-Saharan Africa. Even if it is a small number of animals, further monitoring and research efforts should be dedicated to 1) corroborating these results, that is, the existence of wintering Egyptian vultures in the study area; 2) studying this population's demographic parameters as well as behaviour; 3) determine whether these over-wintering individuals are outliers or a growing population trend, and 4) understand why these individuals choose to winter in the breeding areas.

Finally, it is recommended that future research efforts are invested in inventorying and monitoring VP usage near important vulture populations, as it is helpful for more easily assess the danger that VPs pose to vultures, and the local environment in general.

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7. Annex

Annex 1 - Consent form for interviewed farmers, english version.

Interview Consent Form

Participant ID Number:.....

Researcher: Tiago Brandão Email address: <u>tbrandao@ua.pt</u>

I am a MSc student at Universidade de Évora (Portugal), investigating Egyptian vulture feeding habits on farmlands in Spain/Portugal. I am particularly interested in their feeding on faecal material from livestock. As part of my research, I would like to learn about the farm management strategies you use to protect livestock from disease and the use of veterinary drugs on your farm. I have a few questions I would like to ask you relating to this. This information will form the basis of my MSc study and is part of an international research study with Vulture Conservation Foundation (Franziska Lörcher <u>f.loercher@4vultures.org</u>).

All participation in the study is voluntary. There is no time limit on the interview and it may be as long or short as you wish. Most interviews last up to 15 minutes. All questions are optional, you can choose to stop and withdraw from the study at any time and without reason. You have been given a unique participant number (provided at the top of the page) and if you wish to withdraw from the study please contact me and let me know this number and I will remove your data from the project database. You can withdraw from the study up until 2 months after your interview date, after this time the information that you have provided will have been fully anonymised and it will not be possible to identify your responses in order to remove them from the dataset.

This study will adhere to Data Protection Law. Therefore, if you agree to take part in this study, I will only use your information in the ways needed to conduct and analyse the research study and with your permission. I will record your responses to my questions in writing only (no audio recording). Any information that could identify you and your involvement in this study, i.e. any personal data that you provide such as your name and contact details, will be stored securely and confidentially. Only I will be able to access your personal information. Data will be anonymised for the purposes of analysis and reporting. My findings will be reported in my thesis and other academic forums such as academic journals. All of the data required to verify my findings, will be archived and preserved for at least 10 years and then destroyed. Anonymised data will be shared for use in future ethically approved research. However, confidential information, or any data that might identify you, will not be released.

If you have any questions or concerns at any time during the project, please let me know.

Please read the following statements:

I have read the above information, understand the purpose of this study and have had the opportunity to ask questions about the research. Yes \Box No \Box .

I understand that I can stop and withdraw from this study by following the process outlined above. Yes \Box ~ No $\Box^{{}_\bullet}$

I agree that anonymised data I provide in the interview may be included in material published from this research. Yes \Box No \Box .

I agree that anonymised data can be made available for use in subsequent research studies.

Yes 🗆 🛛 No 🗆 •

Thank you for your time and cooperation with my research project.

Signed..... Date Annex 2 – Interview manuscript

Site name & code:

N:

W:

Interviewee Occupation and role in the farm (owner, manager, etc): <u>Participant ID (as above):</u>

Questions to the interviewee	Answer	Comments
How often do you see Egyptian	Daily / Weekly / Monthly / Rarely / Never	
vultures (EVs) at this site?	never	
During which months do you see EVs at		
this site?		
Please tell us about any observations		
of vulture feeding.		
Are there livestock on this site and	Cattle:	
if so approximately how many?	Sheep:	
(A range is sufficient e.g. 5-10; 11-20; 21-	Goats:	
30 etc)	Horses: Chickens:	
	Pigs:	
Have you seen EVs on this site without	Yes/No	
livestock present?		
Does the presence of EVs appear to be	Yes – please provide details of	
higher or mostly associated with	which livestock they are	
any particular type of livestock on this	associated with	
site? If so, which livestock?	No	
If livestock die on this site, what happens to the carcasses?		
Do you know of any (other) congregation		
sites of EVs in this area?		
Have any dead EVs been found at or near	No / Yes	
to this site? If so how many? Do you know	Number:	
the cause of death? [ask for dates etc]	Cause(s):	
	No / Yes	
at or near this site? If so, how many? Do	Number:	
you know the cause of death? [ask for dates etc]	Cause(s):	
Do you or your vet treat your livestock	Yes/No/Unsure	
with any medicines at any time in the		
year?		
If yes, please provide the following		
details:		
Which drugs do you use? And for	List of names (brand or	
which livestock animals?	drug) (prompt with a list of	
	drugs, e.g. de-wormers, lice or other	
	parasite) treatment, anti-	
	inflammatories, anti-biotics, etc.)	

What are the reasons for using these drugs?	For each drug – provide reason (may need to prompt, e.g. vet prescribed, farmer concerned,	
How often do you administer these drugs?	routine preventative treatment, other)	
What dose do you give for each drug?	For each drug – frequency (daily/weekly/monthly/yearly/only as required (please provide details for the latter))	
How many animals are given this drug at each time?	For each drug – dose per animal	
When was the last time you used each drug?	For each drug – whole herd (please specify size)/single animals/other (please specify)	
	For each drug – provide a specific date (as accurately and precisely as possible but could be number of weeks/months ago instead of actual date)	
If unsure, would you permit us to speak to the veterinarian in charge of your animals in order to ask these questions of them?	Yes/no If yes – please provide contact details for vet	
What happens after the animals are dosed with any of these drugs?	They are kept indoors (please specify for how long after treatment) They are kept outdoors on pasture Other (please specify)	
What sort of pasture management system do you use?	Tilling Removal of manure Rotation grazing Other (please specify)	
How do you manage the livestock manure on your farm?	Dung heap Harrowing Collection and disposal off site (please provide details) Left in the pasture Other (please specify)	
If you use a dung heap, how do you manage it?	Composting Turning Other technique (please specify)	