Non-Steroidal Anti-Inflammatory Drugs (NSAIDS) and their Effect on Old World Vultures: A Scoping Review

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NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS) AND THEIR EFFECT ON OLD WORLD VULTURES: A SCOPING REVIEW

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ABSTRACT.—Diclofenac, a non-steroidal anti-inflammatory (NSAID), was the cause of the rapid vulture population decline in the Indian subcontinent in the 1990s. Since 2013, diclofenac has been approved for veterinary use in Spain. The Iberian Peninsula (Portugal and Spain) hosts more than 95% of the Old World vulture population in Europe, so this situation poses a significant potential threat for European vultures. Other NSAIDs may also pose a risk to vultures, but a thorough review of available evidence has not been conducted. We conducted a scoping review to analyze the published research on the impact of NSAIDs to Old World vultures to ultimately use the synthesized information to inform risk assessments. We implemented a search strategy of four bibliographic databases (1990–2019). Publications were screened in two steps, and two reviewers independently extracted data relevant to the phases of a risk assessment (release, exposure, and consequences). Among the 78 studies included in the review, 41% focused on India, 21.8% studied the White-rumped Vulture (*Gyps bengalensis*), 38.5% evaluated diclofenac, and 12.8% meloxicam. There was substantial evidence that diclofenac can lead to mortality in Old World vultures. There was also some evidence of mortality and clinical signs caused by carprofen, ketoprofen, and flunixin meglumine. Mild clinical signs were reported for phenylbutazone, and nimesulide, and no consequences were reported for meloxicam. However, the effects of all these other NSAIDs to vultures need further research.

KEY WORDS: *diclofenac*; *meloxicam*; *NSAID*; *raptor*; *review*; *scavenger*; *toxicology*; *vulture*.

ANTIINFLAMATORIOS NO ESTEROIDEOS (AINES) Y SU EFECTO EN LOS BUITRES DEL VIEJO MUNDO: UNA REVISIÓN DE AMPLIO ALCANCE

RESUMEN.—El diclofenaco, un antiinflamatorio no esteroideo (AINE), fue la causa del rápido declive de buitres en el continente asiático en la década de 1990. En 2013, el diclofenaco se aprobó para uso veterinario en España. Dado que el 95% de la población de buitres europeos se encuentra en la península Iberica (España y Portugal), esta medida genera una amenaza potencial de importancia para los buitres europeos. Otros AINEs podrian tambien ser un riesgo para los buitres, pero la información disponible al respecto no ha sido aun revisada en profundidad. Hicimos una exhaustiva revisión bibliográfica para analizar la información publicada sobre el impacto de los AINEs en buitres del viejo mundo con el fin de utilizar esta información para evaluación de riesgos. Se estableció una estrategia de búsqueda en cuatro bases de datos bibliográficas, incluyendo el periodo 1990–2019. Las referencias se filtraron en dos pasos, y dos revisores extrajeron de forma independiente los datos pertenecientes a las fases de una evaluación de riesgo (liberación, exposición, y consecuencias). Entre los 78 estudios incluidos, 41% se centraron en India, 21.8% estudiaron *Gyps bengalensis*, 38.5% evaluaron diclofenaco y 12.8% meloxicam. Se encontró evidencia

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concreta de que el diclofenaco puede ocasionar la muerte de los buitres del viejo mundo y evidencia de mortalidad y morbilidad causados por carprofeno, ketoprofeno y megluminato de flunixina. Para fenilbutazona y nimesulida, se reportaron algunos signos clínicos leves, mientras que para el meloxicam no se reportó ninguna consecuencia asociada a su uso. Sin embargo, el efecto de estos AINEs en buitres necesita más investigación.

[Traducción de los autores editada]

INTRODUCTION

Old World vulture (Order Accipitriformes) populations suffered a severe decline in the Indian subcontinent in the 1990s that was associated with diclofenac poisoning (Oaks et al. 2004, Green et al. 2006b). Diclofenac is a non-steroidal anti-inflammatory (NSAID) drug used to treat inflammatory processes and pain (Small 1989). It is primarily used in humans, but it is also approved for veterinary use in several European member states (European Medicines Agency [EMVA/CVMP] 2014), and from the 1990s until 2006 it was also registered for veterinary use to treat inflammatory processes in domesticated ungulates in the Indian subcontinent (Risebrough 2006). Vultures are obligate scavengers that feed on animal carcasses including livestock left in fields or at carcass dumps. Carcasses containing diclofenac residues were the source of vultures becoming intoxicated and dying in south Asia (Watson et al. 2004, Richards et al. 2018). As shown in experiments, vultures start showing clinical signs of intoxication and likely death 24–48 hr after being exposed to a carcass with diclofenac residues (Oaks et al. 2004, Naidoo and Swan 2009a).

The impact on vulture populations in Asia was so dramatic that the manufacture and import of veterinary diclofenac was banned in 2006 by the Governments of India, Nepal, and Pakistan (Prakash et al. 2012). In 2013 however, the Spanish Drug and Health Products Agency authorized two formulations that contained diclofenac for use in livestock (Margalida et al. 2014). The Iberian Peninsula (Portugal and Spain) hosts more than 95% of the Old World vulture population in Europe, so this situation poses a significant potential threat for Old World European vultures.

Since diclofenac was approved in Spain, researchers have intensified the monitoring of diclofenac residues and other NSAIDs in dead vultures. In fact, flunixin meglumine (another NSAID) was the suspected cause of death of a Griffon Vulture (*Gyps fulvus*) in Spain (Zorrilla et al. 2015). This case resulted in renewed concerns of the potential threat from other NSAIDs beyond diclofenac.

To adequately evaluate the risk of not only diclofenac but also of other NSAIDs to Old World vultures in the Iberian Peninsula, a risk assessment is needed. The effects of diclofenac on Old World vultures have been well established (Oaks et al. 2004), and evidence to date suggests that the *Gyps* genus is particularly sensitive to NSAID toxicity (Sumpter et al. 2010). However, data on the risks from all NSAIDs across all Old World vulture species have not been thoroughly compiled and reviewed. Having this information is paramount when conducting a risk assessment.

Evidence-synthesis (e.g., systematic reviews, scoping reviews) is a useful methodology to inform risk assessments because it summarizes findings from the available literature following a rigorous and reproducible process. Scoping reviews in particular are a descriptive evidence-synthesis tool that follows a specified methodology with the goal of charting the available literature on the topic of interest (Sargeant and O'Connor 2020). This type of review has been widely used in human medicine, but is still underused in the fields of animal health and ecology. While they are time- and resource-intensive, they have the advantage (as opposed to a narrative review) of being reproducible, allowing for the possibility of updating the review as more literature on the topic becomes available. The aim of this study was to synthesize the published research on the impacts of NSAIDs to Old World vultures to inform risk assessments.

METHODS

We conducted a scoping review following a modified version of the methodology proposed by Arksey and O'Malley (2005). Our review team comprised the four authors and included expertise on Old World vultures as well as evidence-synthesis methodology. The scoping review steps included: (1) defining the research question, (2) conducting a search strategy, (3) selecting relevant publications through a two-phase screening process, and (4) extracting, collating, and summarizing the data. The specifics of these steps include the following.

Table 1. Search strings for the scoping review of the effects of NSAIDs on vultures. Search strings were used in the search engines CAB International (CAB Abstracts©), Scopus©, Web of Science, and Wildlife and Ecology Studies Worldwide. Note: ".af" indicates all fields of the record.

Search Strings
1. (vulture* and diclofenac).af
2. (vulture* and meloxicam).af
3. (vulture* and aceclofenac).af
4. (vulture* and flunixin).af
5. (vulture* and nimesulide).af
6. (vulture* and ketoprofen).af
7. (vulture* and carprofen).af
8. (vulture* and ibuprofen).af
9. (vulture* and NSAID).af
10. (vulture* and "non steroidal anti inflammatory").af
11. (accipitridae and diclofenac).af
12. (vulture* or accipitridae) and (cattle or livestock) and (nsaid or "non steroidal anti inflammatory").af
13. (vulture* or accipitridae) and (cattle or livestock) and (nsaid or "non steroidal anti inflammatory" or diclofenac).af
14. (vulture* or accipitridae) and (cattle or livestock) and (nsaid or "non steroidal anti inflammatory" or diclofenac) and (kidney or nephrotoxicity).af

Research Question. We defined our review question as the following: "What are the effects of NSAIDs on Old World vultures?"

Search Strategy. We conducted a literature search in May 2019 CAB International (CAB Abstracts©), Scopus©, Web of Science, and Wildlife and Ecology Studies Worldwide (Table 1). There were no language or geographical restrictions on the search, and we included studies published from 1990 through 2019. We chose 1990 as the start date because Old World vulture rapid population declines due to diclofenac on the Indian subcontinent occurred during the 1990s (Oaks et al. 2004, Green et al. 2006b). All publications were imported into Mendeley Desktop reference management software version 1.19.4 for management and duplicate removal.

Screening Process. We screened all publications in two steps. During the first step, we only evaluated the title and abstract of each study. We included studies related to Old World vultures (either in general or to a particular species) and to NSAIDs (either broadly or to a particular NSAID). We excluded those studies where the title and/or abstract did not mention these two criteria. At this stage, we also excluded those publications where the abstract was not available and the title was too inconclusive for us to decide if the study should be included or excluded. During the second step, we evaluated the full text of the studies that passed the first screening step. Publications were included only if they reported original research. We made this decision *post hoc* once we had become more familiar with the literature as suggested by Arksey and O'Malley (2005). At this stage, we also excluded those studies that did not have an available full text through the University of Minnesota Interlibrary Loan and Digital Delivery. We also excluded studies that were not relevant to the research question. Throughout the entire screening process, two researchers assessed all publications independently, and resolved any conflicting opinions during regular meetings.

Data Extraction, Collation, and Synthesis. We divided the final set of included studies equally between two researchers who extracted relevant data into a customized spreadsheet (Microsoft Office Excel 2016® Microsoft Corporation, Redmond, WA, USA). Extracted data consisted of general characteristics of the study (publication year, geographic location, and study design), vulture species, NSAID, and specific parameters (e.g., LD_{50}, half-life, vulture mortality, NSAID residue concentrations). We pretested the customized spreadsheet by extracting data from a sample of publications randomly chosen from the final set of included publications.

For the purpose of data synthesis, we grouped the studies under the main three steps of a risk assessment framework: release, exposure, and consequences (Jakob-Hoff et al. 2014). Briefly, release refers to the likelihood of introduction (release) of a "hazard" into the area of concern. In our review, hazard refers to the NSAIDs that might pose a risk to vultures. For release, we reviewed data available on veterinary sales of NSAIDs and NSAIDs for use in livestock. Exposure refers to the likelihood that the population of interest (Old World vultures) would be exposed to the hazard (NSAIDs). Vultures would most likely be exposed to NSAIDs through the consumption of carcasses contaminated with NSAIDs, and thus we reviewed data about NSAID residues in carcasses, but we also summarized any available data from other potential exposure pathways. The last phase, consequences, refers to the outcome given that release and exposure have occurred. For consequences, we reviewed data on vulture mortality, clinical signs, pathological findings, pharmacokinetics, and pharmacodynamics

data, in addition to data related to vulture population impacts.

RESULTS

Search strings returned 286 studies after deduplication. A total of 217 articles remained after the first screening step, and a total of 78 articles remained after the second screening step (Fig. 1; Supplemental Materials Table S1, S2).

Dates of publication ranged from 2004 to 2019, with the highest number of publications in 2007, 2011, and 2016 ($n = 8$; Fig. 2A). The majority of studies were conducted in India ($n = 32$) followed by South Africa ($n = 16$), and there were five studies that included more than one location (Fig. 2B). Diclofenac was the most frequently studied NSAID ($n = 30$) followed by meloxicam ($n = 10$), and there were studies that reported either findings on more than one NSAID ($n = 8$), or evaluated NSAIDs in general ($n = 2$; Fig. 2C).

The White-rumped Vulture (*Gyps bengalensis*) was the most frequently mentioned Old World vulture species across the studies ($n = 17$; Fig. 2D). There were studies that evaluated Old World vultures in general ($n = 15$), and one study that specifically focused on the *Gyps* genus ($n = 1$). Among other avian species used as a model for Old World vultures, the domestic chicken (*Gallus gallus domesticus*) was the most common ($n = 7$). Regarding study design, 51 were observational, 23 were experimental, and four studies had a combination of both designs. What follows are the results of the 78 studies divided within the risk assessment phases (release, exposure, and consequences; Jakob-Hoff et al. 2014).

Release. Nine papers reported results related to the use, availability, and/or sale of NSAIDs for veterinary purposes. In general, sales of NSAIDS shifted from diclofenac to other NSAIDS over time. All veterinarians and veterinary pharmacies ($n = 74$) that were surveyed in 2002–2003 sold diclofenac in Pakistan (Oaks et al. 2004). Similarly, in Nepal, diclofenac was the only NSAID available to treat livestock based on a survey conducted between 2002 and 2005 (Acharya et al. 2009), but another survey from 2012 found that no veterinary supply stores had diclofenac, and that one agro-shop sold nimesulide (Phuyal et al. 2016). Between 2007 and 2011, meloxicam was the most frequently NSAID sold (70% of pharmacies across 11 states) according to a survey of 250 veterinary and general pharmacies in India, while diclofenac and ketoprofen were sold at 36% and 29%, respectively (Cuthbert et al. 2011a). In Cambodia, there were no NSAIDs (including diclofenac) available for sale in pharmacies in veterinary form based on 74 interviews conducted in 2016 (Loveridge et al. 2019).

Use of NSAIDS varied among locations and over time. Veterinarians interviewed in India in 2004 reported diclofenac as the NSAID of choice due to low cost and efficacy, but there was a perceived decrease of diclofenac use and increase of meloxicam use after 2006 (Khan 2013, Cuthbert et al. 2014). According to interviews with local people in Myanmar between 2006 and 2007, diclofenac was not used as a veterinary drug there (Hla et al. 2011). Veterinarians from zoos and wildlife rehabilitation centers across the world reported observed mortality of birds (including vultures) associated with the use of certain NSAIDs (carprofen, flunixin meglumine, ibuprofen, and phenylbutazone), and no mortalities associated with meloxicam use (Cuthbert et al. 2007b).

Exposure. To summarize the potential exposure of vultures to NSAIDs, we divided these studies into experimental ($n = 4$) and observational ($n = 9$). Among the experimental studies, one evaluated aceclofenac (Galligan et al. 2016), one study examined carprofen (Naidoo et al. 2018), and two studies evaluated diclofenac (Green et al. 2006a, Taggart et al. 2007a). After 2 hr of an aceclofenac bolus at 2 mg/kg in domestic cattle (*Bos taurus*), plasma concentration for diclofenac was 48–318% greater than the concentration of aceclofenac (Galligan et al. 2016). For diclofenac, half-life ($t_{1/2}$) ranged from 6–8 hr (fat, kidney, intestine, and liver) to 15 hr (muscle) in one study (Green et al. 2006a), and 12.2 hr in plasma in another study (Taggart et al. 2007a). For carprofen, the $t_{1/2}$ in an experiment with domestic cattle was 46.9 ± 2.5 (SD) hr, and the apparent clearance was 0 L/kg*h (geometric mean; coefficient of variation = 9.92%; Naidoo et al. 2018).

For the experiments that quantified NSAIDs, diclofenac residues ranged from below the limit of quantification (LOQ) in muscle, to 0.1 mg/kg in kidney and liver in one study using cattle (Green et al. 2006a), whereas in another study using domestic goats (*Capra aegagrus hircus*), diclofenac residues ranged from 0.04 mg/kg in liver to 0.47 mg/kg in muscle (Taggart et al. 2007a). For carprofen residues, tissue concentrations in cattle ranged from 3.7 ± 2.5 (SD) mg/kg in omental fat to 289.1 ± 0.5 (SD) mg/kg in the muscle at the injection site (Naidoo et al. 2018, Table 2).

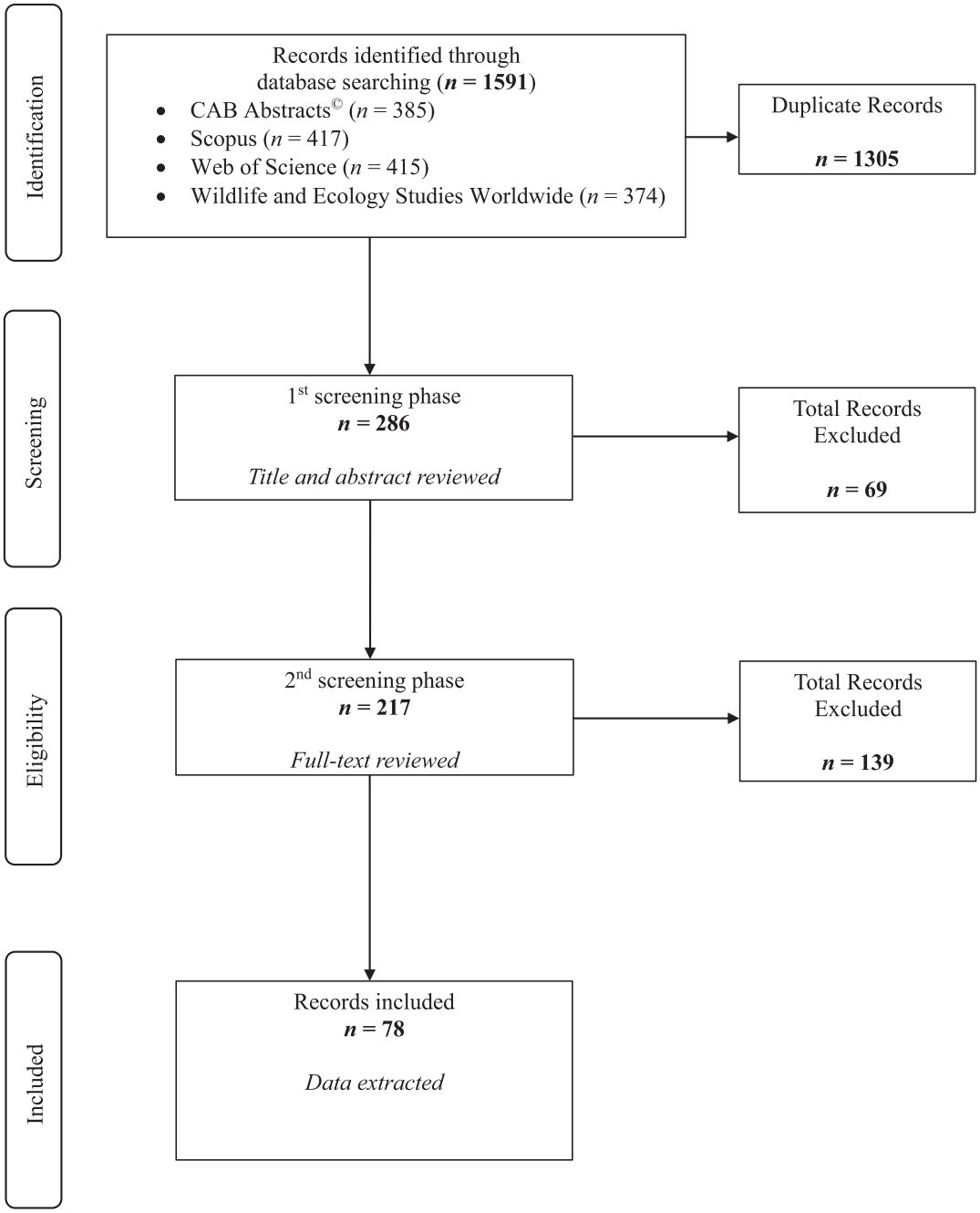

Figure 1. Flow chart of the scoping review process and the number of studies that were included and excluded at each step. Flow diagram adapted from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al. 2009).

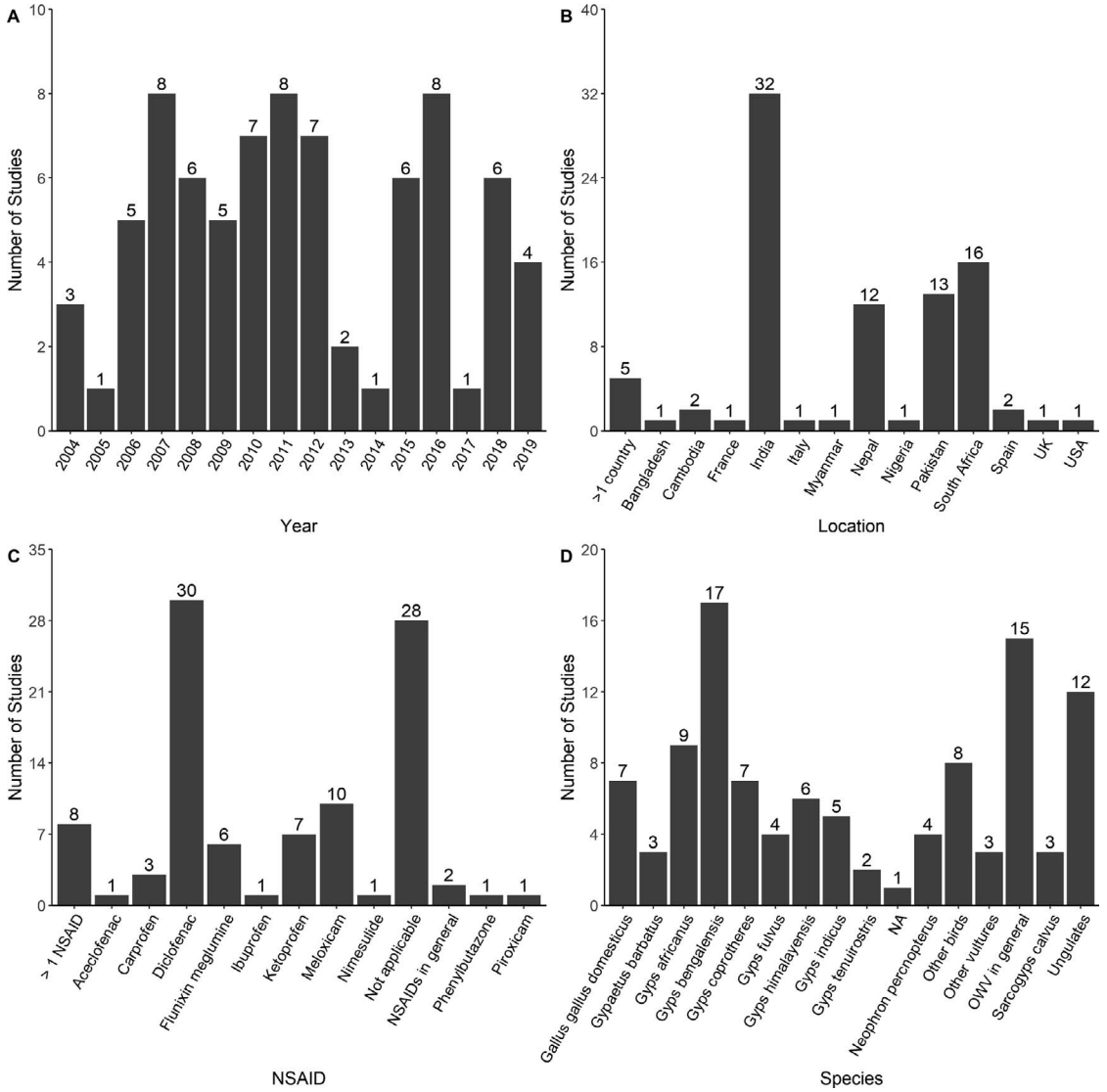

Figure 2. Characteristics for all the studies included in the scoping review ($n = 78$): (A) Year of publication, (B) Location of the study, (C) Non-steroidal anti-inflammatory (NSAID) evaluated, (D) Species. Note: NA = not applicable; OWV = Old World Vultures.

Among the observational studies quantifying NSAIDs in carcasses, diclofenac residues were reported in eight studies (Taggart et al. 2007a, 2007b, 2009; Senacha et al. 2008; Cuthbert et al. 2011b, 2011c, 2014; Saini et al. 2012), meloxicam residues were reported in three studies (Cuthbert et al. 2011b, 2014, Taggart et al. 2009), flunixin in one study (Richards et al. 2011), and ibuprofen and ketoprofen in one study (Taggart et al. 2009). All these studies reported on carcasses sampled at carcass dumps, slaughterhouses, and opportunistically in the field. Diclofenac prevalence and concentrations in liver samples decreased from 2004 to 2006 (prevalence: 10.1% in 2004–2005, 11.1% in 2006, median concentration: 994 μg/kg in 2004–2005, and 874 μg/kg in 2006) and for 2007–2008 (prevalence: 5.6%, median concentration: 569 μg/kg; Cuthbert et al. 2011b, 2011c). For meloxicam,

Table 2. Experimental studies included in the exposure phase of the scoping review of the effects of NSAIDs on vultures.

NSAID	REFERENCE	DOSAGE/ROUTE	SPECIES (n)[a]	RESULTS
Aceclofenac	Galligan et al. 2016	2 mg/kg bolus/2 hr. Blood samples at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 7, 9, 12 hr	Domestic cattle (6)	Plasma concentration: Diclofenac 48–318% > aceclofenac
Carprofen	Naidoo et al. 2018	1.4 mg/kg intramuscular (IM) once. Blood samples at 0.25, 0.75, 1.5, 2, 3, 5, 7, 9, 12, 24, 36, 48, 96, 120 hr	Domestic cattle (4)	$t_{1/2}$: 46.9 ± 2.5 hr; Apparent organ clearance: 0 L/kg*hr; 9.92% CV
		2.8 mg/kg IM once. Slaughtered at 12 hr	Domestic cattle (4)	Residues: 3.7 ± 2.5 mg/kg in omental fat, 289.1 ± 0.5 in muscle (injection site)
Diclofenac	Green et al. 2006a	1 mg/kg IM once. Slaughtered at 21, 46, 71, 167, 334 hr	Zebu (10)	$t_{1/2}$: 6–8 hr for fat, kidney, intestine, liver; 15 hr for muscle
		2.5 mg/kg IM 6 days. Slaughtered at 2-4, 12, 24, 96, 144, 176 hr	Domestic cattle (24)	Residues: 0.1 mg/kg in liver, kidney; < LOQ in muscle
	Taggart et al. 2007a	1 mg/kg IM once. Blood samples at 15, 30 min, 1, 2, 3, 4, 6, 12, 24, 36, 48 hr. Slaughtered at 21, 46, 71, 167, 334 hr	Zebu (4)	$t_{1/2}$: 12.2 hr in plasma
		1 mg/kg IM once. Slaughtered at 4, 26, 73, 167, 334 hr	Domestic goats (2)	Residues: 0.035 mg/kg in liver; 0.465 mg/kg: muscle

[a] Scientific names: Domestic cattle (*Bos taurus*); Zebu (*Bos indicus*); domestic goats (*Capra aegagrus hircus*).

the prevalence in liver samples did not increase from 2006–2008, though median concentration did (2006: prevalence = 4%, median concentration = 80 µg/kg; 2007–2008: prevalence = 5.4%, median concentration = 700 µg/kg; Cuthbert et al. 2011b, 2011c, Taggart et al. 2009; Table 3).

Consequences. We grouped the studies for this section into experimental ($n = 19$), *ex vivo* ($n = 2$), and observational ($n = 14$). We also summarized vulture population studies ($n = 33$).

Experimental studies. Among experimental studies, NSAIDs evaluated included carprofen ($n = 2$), diclofenac ($n = 7$), flunixin ($n = 3$), ketoprofen ($n = 3$), meloxicam ($n = 4$), phenylbutazone ($n = 1$), and piroxicam ($n = 1$). One study evaluated multiple NSAIDs (Fourie et al. 2015). All NSAIDs except for meloxicam resulted in lethargy, depression, and even death in Old World vultures and/or in other avian species used as a model for vultures (Supplemental Materials Table S3).

Ex vivo studies. Two studies evaluated the toxicity of NSAIDs in *ex vivo* tissues (Naidoo and Swan 2009a, Adawaren et al. 2018). They showed that liver slices were not a useful model to investigate NSAID toxicity (Adawaren et al. 2018), but harvested kidneys from domestic chickens and from one White-backed Vulture (*Gyps africanus*) showed that meloxicam was only toxic *in vitro* when exposure times to the drug were extended beyond the drug *in vivo* residence time; in addition, diclofenac depressed transport of uric acid (Naidoo and Swan 2009a).

Observational studies. Observational studies reported vulture mortality associated with NSAIDs. Diclofenac poisoning was the suspected cause of death for 48% (14/29) of White-rumped Vultures and 75% (9/12) of Himalayan Griffons (*Gyps himalayensis*) in India (Nambirajan et al. 2018). In this study, 69% of White-rumped Vultures and 75% of Himalayan Griffons had detectable diclofenac residues (mean ± standard error): 0.13 ± 0.01 mg/kg (kidney) and 0.16 ± 0.01 mg/kg (liver); and 0.21 ± 0.03 mg/kg (kidney) and 0.26 ± 0.03 mg/kg (liver), respectively. In another study, of 62 dead vultures found in India, 33% of Himalayan Griffons (1/3), 53% of Long-billed Vultures (*Gyps indicus*; 9/17), and 73% of White-rumped Vultures (29/40) had visceral gout upon post-mortem examination, and mean concentrations of 0.18 and 0.25 mg/kg in liver and kidney, respectively (Cuthbert et al. 2016). In another study, tissues from 11 White-rumped Vultures, and blood plasma from 12 White-rumped Vultures, four Egyptian Vultures (*Neophron percnopterus*), and two Griffon Vultures were collected between 2005 and 2007 (tissues), and during 2005 (blood plasma) from vultures found dead or dying

Table 3. Observational studies included in the exposure phase of the scoping review of the effects of NSAIDs on vultures.

NSAID	REFERENCE	COUNTRY/DATE/SITE[a]	SAMPLES (*n*)[a]	PREVALENCE [a]	CONCENTRATION (μg/kg)[a]
Diclofenac	Saini et al. 2012	India/2007–2008/carcass dumps	Livers from cow, buffalo, sheep, goat, horse, camel (1251). Analyzed with ELISA and LC-ESI/MS	4.8% by both methods	Ranges: 10–4348 (ELISA) 10–4441 (LC-ESI MS)
	Taggart et al. 2007a	India/not reported/carcass dumps, slaughterhouse, field	Liver and kidney from cow (30) and buffalo (6)	14.0%	5 samples (ranges): Liver: <LOQ–5387 Kidney: 19–6914
	Taggart et al. 2007b	India/2004–2005/carcass dumps	Liver from cow, buffalo, sheep, goat, horse, camel (1848)	10.1%	Range: 11–13723
	Taggart et al. 2009	India/2006/carcass dumps	Livers from cow, buffalo, sheep, goat, horse (1488)	11.1%	Range: 11–10088
	Cuthbert et al. 2011c	India/2004–2005 (T1), 2006 (T2), 2007–2008 (T3)/carcass dumps, slaughterhouses	Liver from cattle, buffalo: T1 (1848), T2 (1488), T3 (1251)	T1: 10.1% T2: 11.1% T3: 5.6%	Median values: T1: 994 T2: 874 T3: 569
	Cuthbert et al. 2011b	India/2004–2005 (S1), 2006 (S2), 2007–2008 (S3)/carcass dumps, field	Liver from cattle, buffalo, sheep, goat, horse, camel: S1 (1445), S2 (1488), S3 (1251)	S1: 10.6% S2: 11.1% S3: 5.6%	Median values: S1: 994 S2: 874 S3: 569
	Cuthbert et al. 2014	India/2005–2009/carcass dumps	Liver from cattle, buffalo, sheep, goat, other mammals (6207)	2005: 12.2% 2009: 6.2%	2005: 868 2009: 735
	Senacha et al. 2008	India/2004–2005/carcass dumps, slaughterhouses	Liver from cattle, buffalo, sheep, camel (1848)	10.1%	Range: 11–13723
Flunixin	Richards et al. 2011	USA/2011/Zoo	Wool from sheep	Flunixin detected qualitatively	Flunixin detected qualitatively
Ibuprofen	Taggart et al. 2009	India/2006/carcass dumps	Livers from cow, sheep, goat, horse (1488)	0.6%	Range: 14–681
Ketoprofen	Taggart et al. 2009	India/2006/carcass dumps	Livers from cow, sheep, goat, horse (1488)	0.5%	Range: 155–5603
Meloxicam	Taggart et al. 2009	India/2006/carcass dumps	Livers from cow, sheep, goat, horse (1488)	4%	Range: 11–1647
	Cuthbert et al. 2014	India/2004–2010/carcass dumps	Liver from cattle, buffalo, sheep, goat, other mammals (6207)	2006: 5.8% 2009: 8.3%	2006: 58 2009: 83
	Cuthbert et al. 2011b	India/2004–2005 (S1), 2006 (S2), 2007-2008 (S3)/carcass dumps, field	Liver from cattle, buffalo, sheep, goat, horse, camel: S1 (1445), S2 (1488), S3 (1251)	S1: NR S2: 4.0% S3: 5.4%	Median values: S1: NR S2: 80 S3: 700

[a] Abbreviations: ELISA (enzyme-linked immunosorbent assay; LC-ESI MS (Liquid Chromatography-Electrospray Ionization-Mass Spectrometry); LOQ (Limit of quantification); T1 (years 2004–2005); T2 (year 2006); T3 (years 2007–2008); S1 (years 2004–2005); S2 (year 2006); S3 (years 2007–2008); NR (Not reported).

in India due to kite-related injuries. On post-mortem exam, one White-rumped Vulture (out of the 11) from 2005 had visceral gout, and diclofenac residues of 1.42 mg/kg in liver, and 1.18 mg/kg in kidney. Although the other White-rumped Vultures from 2005 ($n=4$) did not present visceral gout, diclofenac residues ranged from 0.25 to 1 mg/kg. The other six White-rumped Vultures collected in 2006 and 2007 had no diclofenac residues. Residues were found in plasma samples in 16 out of the 18 vultures collected in 2005 ranging from below detection limit (BDL) to 0.17 mg/kg (Muralidharan et al. 2010). In a separate study, 15 White-rumped Vultures and 13 Long-billed Vultures were found dead or dying due to unknown causes in India and Nepal, and 72% (20/28) had visceral gout (Shultz et al. 2004). One Himalayan Griffon died within 48–56 hr in India after receiving 3.8 mg/kg of diclofenac intramuscular to treat a broken leg (Das et al. 2011); necropsy revealed visceral gout, and diclofenac residues in kidney (0.087 mg/kg) and liver (0.034 mg/kg).

There were also case reports of vulture mortality associated with other NSAIDs. Two Rüppell's Vultures (*Gyps rueppelli*) and one White-backed Vulture died in a zoo in Italy due to contamination of a beef carcass with flunixin meglumine. On post-mortem examination, these vultures had visceral gout, and flunixin residues in kidney, liver (0.016–0.039 mg/kg), and muscle (<0.0055–0.04 mg/kg; Eleni et al. 2019). In Spain, a necropsy on a Griffon Vulture revealed visceral gout, with levels of flunixin meglumine in liver (median: 2.7 mg/kg) and kidney (median: 6.5 mg/kg). Two studies reported qualitative detection of ketoprofen in liver samples (Zorrilla et al. 2015) and in quantities below the LOQ (0.01 mg/kg) in one of three unfertilized eggs of Bearded Vulture (*Gypaetus barbatus*; Zorrilla et al. 2018). This study also reported quantities below the LOQ (0.01 mg/kg) of meloxicam in 2/3 of the unfertilized eggs. Another study reported detection of both meloxicam and nimesulide above the LOQ (0.01 and 0.005 mg/kg, respectively) in liver and kidney samples (Cuthbert et al. 2016).

Five studies conducted simulation models to estimate vulture mortality associated with NSAIDs (Cuthbert et al. 2011b, Green et al. 2004, 2006a, 2007, 2016). Population declines in India, Pakistan, and Nepal could be caused by diclofenac residues at lethal levels in a small proportion of ungulate carcasses available to vultures (between 1:130 and 1:760), and the proportion of excess mortality (i.e., the number of deaths expected under typical conditions) that could be accounted for by diclofenac poisoning was 78–87% for White-rumped Vultures and 71–91% for Long-billed Vultures in India, and 92–100% for White-rumped Vultures in Pakistan (Green et al. 2004). Furthermore, a dose-dependent model estimated that vultures ingesting an average concentration across all tissues with sufficient dose would be enough to kill >10% of birds if the animal treated with diclofenac had died within 33 to 59 hr (Green et al. 2006a). A decline of 80–99% per year for White-rumped Vultures was expected if the level of diclofenac found in field carcasses during 2004 and 2005 remained constant (Green et al. 2007). After the diclofenac ban in India, the rate of decline of White-rumped Vulture populations slowed to 40% of the rate before the ban (Cuthbert et al. 2011b). After diclofenac was approved for veterinary use in Spain in 2013, and using data from the Indian subcontinent, one model estimated between 715–6389 Griffon Vulture deaths and an annual decline rate range of 0.9–7.7% for the Griffon Vulture population in Spain (Green et al. 2016).

Vulture population studies. Thirty-three studies evaluated vulture populations through opportunistic census or through pre-established surveys. All these studies were conducted in the Indian subcontinent, mostly in relation to declines caused by diclofenac. We summarized here the most relevant findings from this group of studies. In Nepal, Himalayan Griffon declines were reported at a rate of 31–33% per year between 2002 and 2005 (Acharya et al. 2009), at a rate of 14% per year between 2002 and 2011 (Chaudhary et al. 2012), and in 2014 the population was estimated to be 25% smaller than in 2002 (Paudel et al. 2016a). An 84% decline in nests was estimated for the period 2002–2005 (Acharya et al. 2009) and the decline continued until 2008 when a slow recovery began (Paudel et al. 2016a). For the Slender-billed Vulture (*Gyps tenuirostris*), the population decline estimate was 19% for the period 2002–2011 (Chaudhary et al. 2012) and for the Bearded Vulture, approximately 89.3% between 2002 and 2014 (Paudel et al. 2016b).

In India, the population of Egyptian Vultures declined by 91% between the 1990s and the mid-2000s, and the population of Red-headed Vultures (*Sarcogyps calvus*) declined by 94% during the same period (Galligan et al. 2014). Decline estimations for the period of 2000 and 2003 for these two species were 35% and 44%, respectively (Cuthbert et al. 2006), with no signs of recovery between 2011 and

2013 (Navaneethan et al. 2015). The population of White-rumped Vultures decreased by 2007, and although still low, the population decline slowed and may have reversed by 2011 both in India and Nepal (Prakash et al. 2012). More recent surveys showed that the population of White-rumped Vultures was stable or possibly slowly increasing, while populations of Long-billed Vultures and Slender-billed Vultures were declining due to unknown causes (Prakash et al. 2019). Nesting success for White-rumped Vultures was higher in a forested area than in a plantation area of the Raigad district, India; authors suggested that adults in the plantation may have been more exposed to contaminated carcasses, potentially bringing such food to the nest (Majgaonkar et al. 2018).

In Pakistan, nesting success of White-rumped Vultures declined between 2000 and 2004 at three survey sites and the adult population decreased 42.7–80.1%; these changes were significantly correlated with mortality due to diclofenac (Gilbert et al. 2006, Siddique and Khan 2016). The number of occupied nests of White-rumped Vultures also declined in other sites in Pakistan, going from 418 in 2000–2001 to none in 2007–2008 (Johnson et al. 2008). Later, from 2011 to 2014, the number of White-rumped Vulture nests tripled (Murn et al. 2015). The relative abundance of Long-billed Vultures declined by an estimated 61% between 2003 and 2004, and increased by 55% by 2007–2008, after the diclofenac ban in 2006 (Chaudhry et al. 2012). In Bangladesh, the population of White-rumped Vultures declined 60% over 4 yr (2008–2012), with overall breeding success ranging from 16 to 26% (Khan 2013). In Myanmar, populations of Slender-billed Vultures, White-rumped Vultures, and Red-headed Vultures declined during the period 2006–2007 compared to historical trends (Hla et al. 2011), while in Cambodia these species also showed a decline in 2016 compared to 2004 (Loveridge et al. 2019).

DISCUSSION

We conducted a scoping review to summarize the available information about the effect of NSAIDs to Old World vultures. The advantage of following an evidence-synthesis methodology (as opposed to a narrative review) is that it is reproducible, and its rigorous systematic process reduces the introduction of potential biases (Sargeant and O'Connor 2020). Our focus was on Old World vultures because of the need to evaluate NSAID risk to this group of birds in the Iberian Peninsula after the licensing of diclofenac for veterinary use in Spain in 2013. Risks from NSAIDs to other avian scavengers were out of the scope of our work, though this is a topic warranting further research. We organized the review into release, exposure, and consequences, so that data are more readily available when conducting a risk assessment on this topic.

For the release phase, there was an observed decrease on diclofenac use and a corresponding increase in meloxicam use after the ban of diclofenac in 2006 across India, Pakistan, and Nepal. Of the studies included in the review, we found no published data on NSAID release (sale and/or use) from locations other than the Indian subcontinent. There have been, however, recent efforts to obtain data in other locations. For example, 82 of 230 official pharmaceutical distributers in Spain acknowledged that they were selling diclofenac (Camiña et al. 2017). Given that diclofenac was approved for veterinary use in Spain, it is now critical to keep collecting data on sales and use of this NSAID.

India was the most common location for the studies included in the review, and White-rumped Vulture the most commonly studied species. This was expected, as the dramatic decline of vulture populations due to diclofenac occurred on the Indian subcontinent and the White-rumped Vulture was the most common vulture species on the continent prior to the decline. Also as expected, diclofenac was the NSAID most frequently studied, followed by meloxicam, which was suggested as a safe alternative (Swarup et al. 2007) after the banning of the manufacture of veterinary formulations of diclofenac in India, Nepal, and Pakistan (Taggart et al. 2009). Following India, South Africa was the second-most frequent location of studies. Although diclofenac was not authorized for veterinary use in South Africa, concerns from conservationists led to a large effort that generated valuable understanding of the effects of not only diclofenac but other NSAIDs on populations of African vultures, especially Cape Vultures (*Gyps coprotheres*) and White-backed Vultures (Anderson et al. 2005).

After livestock have been treated with NSAIDs, a vulture must be exposed to those NSAIDs for it to be at risk. In India, Hindu culture considers cattle sacred (Simoons et al. 1981). When they die, they are left in open fields for scavengers. If they had been treated with diclofenac before dying, diclofenac residues may be present, as was the case for 10–

11% of livestock carcasses collected in the field in south Asia (Taggart et al. 2007b). Other NSAIDs have been detected in carcasses, but more studies are needed to monitor the presence of NSAIDs in livestock carcasses, especially in areas beyond Asia. The concentration of NSAIDs varies depending on the tissue, and may be higher at the injection site (Naidoo et al. 2018). This information, mostly derived from experimental studies, is critical for assessing the potential exposure of vultures to different NSAID concentrations in carcasses.

Consequences to vultures of NSAID exposure vary, based not only on the dose but also on the individual NSAID. Based on available evidence to date, diclofenac is the greatest threat among the NSAIDs, but this may be biased by the higher number of studies evaluating diclofenac compared to other NSAIDs. Furthermore, mortality and signs of toxicity have also been reported in vultures and other avian species after exposure to other NSAIDs such as flunixin and ketoprofen (e.g., Naidoo et al. 2010b, Kapadiya et al. 2015, Zorrilla et al. 2015). Other NSAIDs such as carprofen and nimesulide may also pose a risk to vultures, but there is still a lack of compelling evidence, and some of the currently available data from other avian species cannot be fully extrapolated to vultures given their very different physiology (Cuthbert et al. 2007a, Hassan et al. 2018). Meloxicam appears to be the safest NSAID thus far for vultures, but research is still limited (Adawaren et al. 2019).

Ultimately, diclofenac had a significant impact on vulture populations in South Asia (Oaks et al. 2004, Green et al. 2006b), and diclofenac and other NSAIDs may do the same elsewhere. Thus, it is important to monitor vulture populations over time, including investigations into the causes of mortality of adults and the direct and indirect effects on nestlings, as well as to conduct rigorous simulation models to predict population changes under different scenarios. This has become even more important in the Iberian Peninsula since the approval of diclofenac for veterinary use in Spain (Green et al. 2016).

One limitation of scoping reviews is that they do not assess the robustness of the findings (Arksey and O'Malley 2005, Sargeant and O'Connor 2020). This means that although the quality of the studies we evaluated was not equal, our assessment was not weighted to reflect that. Another limitation of reviews in general is that they become outdated as new literature becomes available, so having a reproducible methodology as published here permits easier updating of the literature. Finally, although we followed the methodology proposed by Arksey and O'Malley 2005, we did not include the last step of expert elicitation (Arksey and O'Malley 2005). Although the search engines we used are supposed to include gray literature, our search strategy may have not captured some relevant reports, such as those from the European Commission LIFE programme (European Commission 2020). Our methodology did not include manual searches of specific websites and technical reports, but this should be pursued in future assessments.

Based on the data we reviewed, we here list the key knowledge gaps and areas that would benefit from further research: (1) NSAIDs sales and use data (not only diclofenac but other NSAIDs as well) by veterinarians in Europe and Africa, and changes of availability and use over time; (2) Pharmacokinetics data of other NSAIDs such as carprofen, flunixin, and ketoprofen in other livestock species including swine (*Sus scrofa domesticus*); (3) NSAID toxicity data for other vulture species beyond the *Gyps* genus, such as the Egyptian Vulture and the Bearded Vulture; (4) NSAID toxicity data for avian scavengers other than vultures; (5) Direct and indirect effects of NSAID adult toxicity on nestlings.

This scoping review highlighted the evidence of NSAID risks to Old World vultures, and areas requiring further research. The results from this study, in combination with information from key gray literature reports not captured here, will be useful in conducting assessments of NSAID risk to vulture species in the Iberian Peninsula and other geographic regions.

Supplemental Material (available online). Table S1: Studies included in the scoping review. Table S2: Studies excluded during the scoping review. Table S3: Experimental studies included in the consequences phase of the scoping review of the effects of NSAIDs on vultures.

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