SHORT TERM SCIENTIFIC MISSION (STSM) SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

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STSM title: Review of the detection of veterinary pharmaceuticals in avian scavengers in Europe
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PURPOSE OF THE STSM:

The main objective was to carry out a classic literature review of research published to date regarding the detection and potential effects of veterinary drugs in avian scavengers, (including NSAIDs, antibiotics, antiparasitics and barbiturates). This review considers key exposure pathways, not least via the disposal of carrion on land. It also compiles existing information regarding product use rates in Europe, typical environmental residues, compound toxicokinetics-toxicodynamics and key toxicity data (where available). Additionally, it considers regulatory issues, risk assessment and avian physiology - as a factor in determining risk in different scavenging species.

Furthermore, as a secondary output, I have created a template for use in recording visceral gout (a key indicator of NSAID related toxicity in certain species – i.e., *Gyps* vultures) for laboratories to use when undertaking post-mortem examinations. This will collect individual and contextual data, alongside providing basic guidance regarding sampling and distinguishing NSAID intoxication. This template will potentially be available through the ERBFacility website so that labs and institutions have open acess to it.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM:

To perform the review I collected published papers regarding four groups of pharmaceuticals: NSAIDs, antibiotics, barbiturates and antiparasitics. The review structure for each group differed slightly given the information available, i.e., given that the greatest body of published data by far regards NSAIDs (and their impact on south Asian vulture populations since the mid-1990's). Firstly, I wrote a brief introduction considering the use of pharmaceuticals in Europe, and, potential routes of exposure and pathways to avian top predators (in both terrestrial and aquatic systems). Then, I undertook a review for each of the 4 key compound groups – as the example of contents given below for NSAIDs:

NSAIDs:

- **Use of products in different EU countries**
- **Residues detected in carcasses available to avian scavengers**; pointing out that available information is largely from India (for carcasses from 2004-2008), noting observed trends pre- and post-bans on diclofenac. We also compiled information regarding detection of meloxicam, ibuprofen, ketoprofen (and mixtures of these/other compounds) in Asia.
- **Toxicokinetic-toxicodynamic parameters in domestic animals and avian scavengers**; compilation of data regarding pharmacokinetic parameters and consideration of how this differs between species. Parameters selected were clearance (Cl), distribution volume (Vz), half-life of elimination (T½) and area under the curve (AUC).
- **Metabolic pathways**; this section considered existing hypotheses for diclofenac/meloxicam pathways, in relation to cytochrome P450 which is directly related to elimination. Also, *in vitro* assays propose that reactive oxygen species and uric acid transporters are key in explaining the vulnerability of *Gyps* to diclofenac (and potentially other NSAIDs).
- **Accumulation, histopathology, clinical parameters**; key indicators of NSAID intoxication (mainly visceral gout). Compilation of information regarding this discovery and description of histological findings and clinical parameters that may be altered (and therefore can be used as biomarkers).
- **Toxicity**; compilation of concentrations found in avian predators in the field and in captive conditions due to NSAID intoxication. Also, results of experimental studies carried out to prove toxicity of other compounds, and, toxicity in other species (summarised in tables).
- **LD_{50} in avian scavengers**; diclofenac is the only NSAID with sufficient volume of information regarding toxicity to reliably define an LD_{50} value - and even then, only for *Gyps bengalensis* (estimated by probit analysis after Oaks et al. 2004).
- **Risk assessment; regulation and effectiveness**

Similar sections were created for the other three target groups – acknowledging that this information was very sparce for scavenging raptors. A broader discussion and conclusions section was then drafted to consider overarching principles and make recommendations for further work, highlight areas where data is lacking, and noting where research priorities should perhaps lie.

The **visceral gout template** created was divided into seven sections:

1. Objective and data regarding the laboratory performing the necropsy.
2. Bird identification and preservation status of the carcass.
3. Necropsy: External and internal examination (affected systems and remarkable tissues).
4. Histology description: Sampling protocols and expected histology findings.
5. Toxicology analysis: Sampling protocols and references to analytical methods.
6. Diagnosis.
7. Summary.

The pilot template furher includes a subsection with basic information: i.e., species affected (based on published data), key indicators (gout: tick boxes for present/not present) and main etiology in wild birds. Flow charts point out presumptive diagnosis considering necropsy findings in a suspected NSAID intoxication and identify target samples. Finally, other important findings (when visceral gout not present) can be recorded.

DESCRIPTION OF THE MAIN RESULTS OBTAINED DURING THE STSM:

The main aim of this review has been to identify gaps in knowledge regarding potential impacts of pharmaceuticals on avian top predators. There is a lack of information regarding the detection and effects of these compounds in Europe on scavenging raptors. Published data is neither largely comparable or of sufficient volume to carry out a useful and detailed metanalysis, or consider trends through time. However, useful data has been collated, this should act as a springboard for future effort and focus. A brief of some of the information collated is given below.

NSAIDs: These have been detected in carcasses available to avian scavengers in S.Asia and have caused an ecological catastrophe recently. Risk modelling showed that only 0.13-0.75% of carcasses available to vultures in India/Pakistan needed to contain a lethal dose of diclofenac to explain rapid population decline. The prevalence of carcasses containing diclofenac residues in India was >10% in the mid-2000's, with concentrations from 11-13723 µg/kg. Whilst not all contained levels high enough to provide a lethal dose, a sufficiently high proportion did. Other NSAIDs detected in Asia include meloxicam, ibuprofen, ketoprofen, and combinations of these.

For diclofenac - pharmacokinetics (for domestic animals) often shows slow clearance, a small distribution volume, and a long half-life of elimination. *Gyps* vultures are particularly vulnerable to diclofenac nephrotoxicity - hypothesized to be provoked by interference with proximal tubule cell function: inhibition of uric acid excretion, cascading to increased ROS (oxygen ions/free radicals) and subsequent damage to mitochondria/cell death. Experimental studies (on vultures) show marked similarities in pharmacokinetics between carprofen, flunixin, ketoprofen, phenylbutazone and diclofenac - suggesting the same pathways may be involved. To date, meloxicam is the only NSAID considered 'vulture safe'. Meloxicam has a rapid half-life of elimination, which is perhaps extremely important (preventing drug accumulation/delayed toxicity).

Visceral gout is the key sign of NSAID toxicity. A diclofenac LD_{50} has been estimated at 0.098-0.225 mg/kg bw for *G.bengalensis.* This is in the same realm as recognized toxicants such as the pesticide aldicarb (2.82 mg/kg) – often used to persecute/poison raptors/predators. Further work is needed to better understand NSAID toxicity in birds, and provide an EU risk assessment approach for pharmacueticals and scavenging wildlife.Thus, flunixin detection has already been reported in one Eurasian griffon from Spain with levels arround 2.70 and 6.50 mg/kg in liver and kidney respectively, and preliminar non published studies suggest a broader NSAID exposure in Spain.

Antibiotics: For antibiotics, there is a paucity of data for scavenging raptors – much of which has been retracted from publication in recent years. One study considers exposure via ungulate carcasses, detecting oxytetracycline, trimethoprim, penicillin-G, sulfadiazine, ciprofloxacin and enrofloxacin. Further, quinolone residues have been detected in plasma from raptor nestlings in Spain: enrofloxacin, ciprofloxacin and marbofloxacin in Golden eagle, Eurassian griffon, Cinereous and Egyptian vultures.

Avian scavengers have also been highlighted as potential carriers and dispersers of antimicrobial resistant bacteria (ARB) because they access treated carcasses. Published data has considered *Salmonella* in Egyptian vulture faeces – and all strains isolated were resistant to tetracyclines, 95.7% to ampicillin, amoxicilin and streptomycin, 82.6% to neomycin and none to quinolones/cephalosporins. A recent study on MRSA (methicillin-resistant *Staphylococcus aureus*) in Eurasian griffon confirmed all isolates (5% of total sample) were resistant to tetracycline, 61.5% to ciprofloxacin, and 38.5% to erythromycin/clindamycin.

Antiparasitics: Only one paper to date has considered external antiparasitic residue exposure - from lambs feet scavenged from a slaughterhouse. This noted diazinon, pirimiphos-methyl, chlorpyrifos, fenthion, permethrin and cypermethrin as residues; and diazinon was further detected in bearded vulture and in a pellet collected from a failed nest. Permethrin was also found in liver from another bearded vulture.

Barbiturates: A recent review has compiled data on the number of individuals and species affected, obtained through database research/personal communication/questionnaire responses. This highlighted incidences involving 66 bald eagles in the US, 46 in Canada; 12 golden eagles (US); 12 Eurasian griffons (France) and 1 red kite (Ireland). In addition, pentobarbital intoxication has been described in Cooper's hawk, Red-shouldered hawk, Red-tailed hawk, Egyptian vulture and Eurasian buzzard.

FUTURE COLLABORATIONS (if applicable)

The visceral gout template is an important output – which will hopefully be a helpful tool to create consistent reporting across the EU (and perhaps beyond) and guide those involved in suspected NSAID intoxication cases. This could be used to register and collate data regarding pharmaceutical intoxication in avian top predators at a pan-European scale via this COST project. For this work we have also reached out and sought guidance from other pathology experts who have worked on the S.Asia vulture population crash, including Dr. Carol Meteyer (USGS). The template is a '*work in progress*' at the moment, but, we should be able to complete this in the coming month or so (by April end 2019).

For the review paper - I intend to complete this with help from both my host and home supervisors (Dr. Mark Taggart and Dr. Rafael Mateo). This review will highlight key gaps in knowledge and summarize available information - providing a springboard for future research in this arena.

In future, I would like to continue working with Dr. Rafael Mateo in the ecotoxicology department at the Institute of Game and Wildlife Research (IREC) and Dr. Mark Taggart from the Environmental Research Institute (ERI), and potentially take this project forward as the beginning of a PhD. One possible future collaboration would be to continue to be involved with this COST project – and help collate the data collected through the visceral gout template and analyse this information.

I would like to focus my PhD in Spain as it holds the biggest vulture populations in Europe and is one of the countries were diclofenac is now authorized. In addition, a wide range of pharmaceuticals are commercially available and regulations may not be as strict as they perhaps need to be. There is very little knowledge regarding the effects of pharmacueticals on individuals or populations. As such, pharmaceuticals could be a real hazard to avian scavengers in Europe and Spain – and work in Spain could be used as a model to provide (and then extrapolate) data more widely for European avian top predator risk assessments.

Given that the biggest problem is currently the lack of information regarding this topic, to provide a good risk assessment in Spain, I would follow these steps:

1. *Collate a list of commercialised compounds for the four categories mentioned*
2. *Undertake research regarding regulation and treatment protocols for the target compounds*
3. *Utilise questionnaries to estimate the use of these compounds in veterinary medicine*
4. *Undertake domestic animal carcass sampling/analysis, considering different species and tissues*
5. *Sample avian top predators: carcasses and/or blood from live birds*
6. *Undertake risk modelling*