Veterinary Research Communications
https://doi.org/10.1007/s11259-023-10093-2

BRIEF REPORT

Pentobarbital intoxication as a potential underlying cause for electrocution in a bearded vulture

M. Herrero-Villar[1] · I Sánchez-Barbudo[1] · T Cardona-Cabrera[1,2] · U Höfle[1] · A Sánchez-Cano[1] · G Baguena[2] · R Mateo[1]

Received: 4 December 2022 / Accepted: 27 February 2023

Abstract

A bearded vulture (*Gypaetus barbatus*) found dead in northern Spain presented external lesions consistent with electrocution as the cause of death. During forensic examination, macroscopic lesions suggested potential comorbidity, so samples were collected for molecular and toxicological analyses. Gastric content and liver were analysed for toxic substances, and pentobarbital (a common pharmaceutical used for euthanasia in domestic animals) was detected at a concentration of 37.3 and 0.05 µg/g, respectively. Other toxicological, viral and endoparasite analyses (avian malaria, avian influenza and flaviviruses) were negative. Thus, although the cause of death was electrocution, pentobarbital intoxication likely impaired the equilibrium and reflexes of the individual, possibly causing the bird to contact energized wires that it would not have otherwise. These results underline the importance of comprehensive analysis of forensic cases of wildlife deaths and reveal barbiturate poisoning as an additional threat for the conservation of the bearded vulture in Europe.

Keywords Forensic · Power line · Spain · Vulture

Veterinary pharmaceuticals can contaminate the environment and impact wildlife (Arnold et al. 2013). Some can cause population-level effects, such as the diclofenac (a non-steroidal anti-inflammatory drug, NSAID) poisoning of Asian and African vultures (Pain et al., 2008). Livestock carcasses, which play a major role in the survival of avian scavengers, are the main route of exposure to veterinary drugs (Shore et al. 2014). One example are barbiturates, which in veterinary medicine, are mainly used for euthanasia. If euthanised animal carcasses are incorrectly disposed, barbiturate residues in their tissues pose a risk to avian scavengers and wildlife that may feed on them (Shore et al., 2014; Viner et al., 2016).

Pentobarbital, the most frequently used euthanasia drug in animals, has been reported in acute intoxication of avian scavengers in the USA and the EU (Russell and Franson 2014; Herrero-Villar et al. 2021). A single pentobarbital-contaminated carcass can cause massive intoxication events. In Spain, the incidence of barbiturate poisoning events in wildlife is increasing, especially in northern Spain and in avian scavengers (Herrero-Villar et al. 2021). As an obligate scavenger, the endangered bearded vulture *(Gypaetus barbatus)* (Antor et al. 2004) is vulnerable to veterinary drug exposure. Here we report pentobarbital intoxication as a potential underlying cause for an electrocution in this endangered species in Spain.

A 3-year-old female bearded vulture marked with a GPS device and patagial tags was found dead in the Asturias region next to a high voltage powerline of northern Spain on February 2nd 2022 (Fig. S1), and submitted for forensic analysis to the Institute for Game and Wildlife Research (IREC). External examination revealed good body condition, with two burn injuries in the digits I and III of the left claw and at the base of the beak and tongue (Fig. 1a, b). Burn marks were present on the GPS transmitter harness (Fig. 1c). No other traumatic lesions were observed in radiographs or during post-mortem examination. The bird had abundant visceral fat deposits and well-developed muscular tissue. The lungs showed haemorrhage and oedema, and the liver, pancreas, spleen, kidneys, and intestines were

✉ M. Herrero-Villar
marta.herrero@uclm.es

1 Instituto de Investigación en Recursos Cinegéticos-IREC, CSIC-UCLM-JCCM, Ronda de Toledo 12, Ciudad Real 13005, Spain

2 Fundación para la Conservación del Quebrantahuesos, FCQ, LIFE Pro BV, Plaza San Pedro Nolasco, 1, Zaragoza 5001, Spain

Published online: 05 May 2023

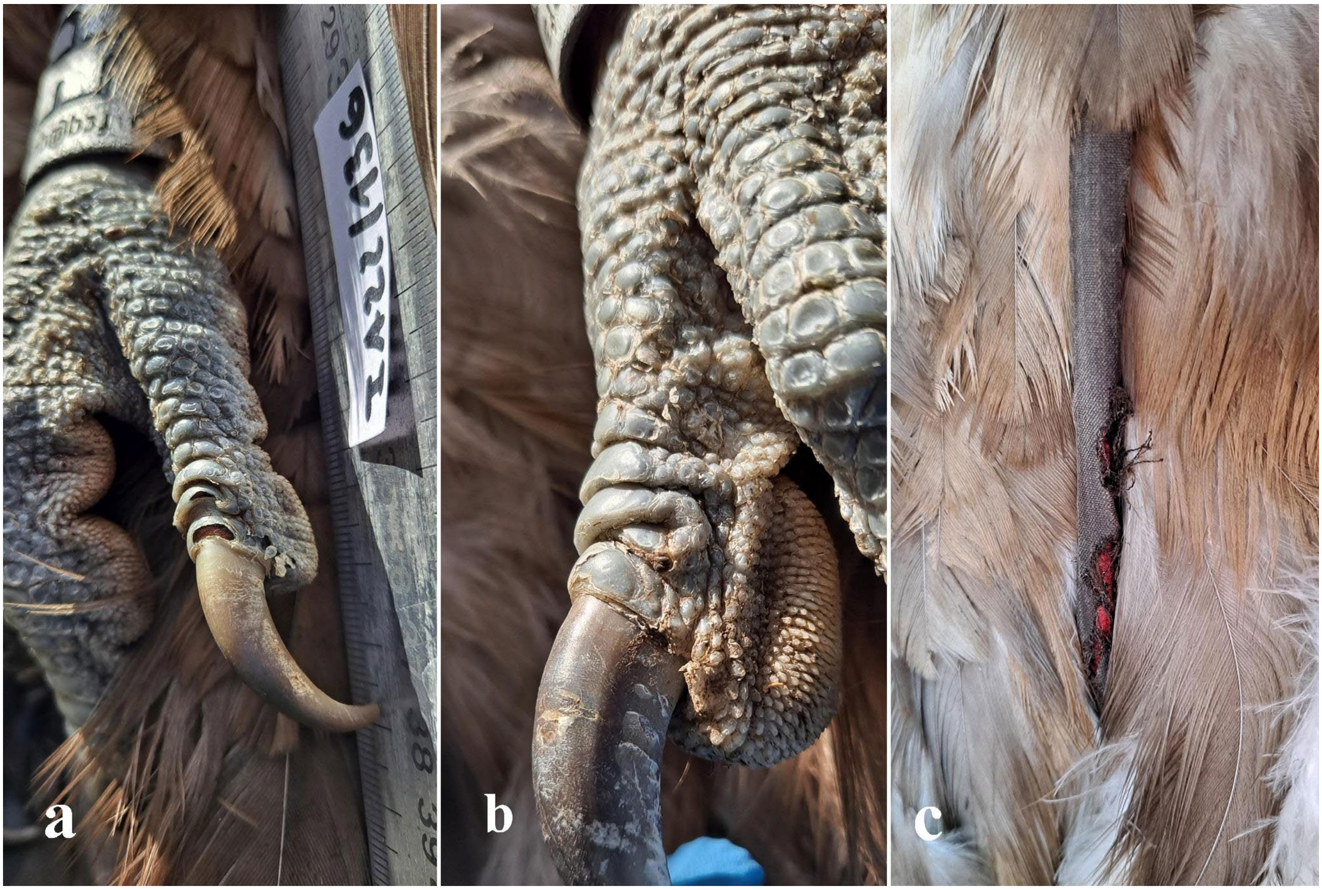

Fig. 1 Macroscopic lesions related to electrocution. Left claw with focal burn lesion at the base of digit I (a) and III (b). Signs of burning observed on teflon harness, compatible with contact with an ignition source (c)

highly congestive (Fig. 2a, d). Both the liver and kidneys were markedly enlarged. Central nervous system examination revealed intracranial haemorrhage, subdural haemorrhage in the dorsal aspect of the cerebellum, and oedema in the brain.

Samples from liver, kidney, gastrointestinal tract, lungs, central nervous system, and spleen were fixed in 10% neutral buffered formalin for histopathological analyses and frozen at -80º C for pathogen detection. Total RNA was extracted from a vascular feather and from liver, spleen, kidney, brain, lung, and intestine using Tri-Reagent (Sigma Adrich, Merck, Madrid, Spain). The samples were analysed via real time RT-PCR to detect the presence of avian influenza and flaviviruses (Munster et al. 2007; Moureau et al. 2007) and nested PCR for detection of avian malaria parasites (Waldenström et al. 2004). Samples of liver and gastric content were collected and analysed following the method described in Herrero-Villar et al. (2021) for chemicals commonly involved in animal intoxications, including barbiturates, using gas chromatography coupled to a single quadrupole mass detector with electron impact ionization (GC-EI-MS) in full-scan mode. Formalin-fixed samples were processed to obtain haematoxylin-eosin-stained slides and examined using a light microscope.

While samples were negative for the tested viruses and malaria parasites, toxicological analyses of both gastric content and liver were positive for pentobarbital at concentrations of 37.3 µg/g and 0.05 µg/g, respectively. These levels were within or below the range of previous findings in avian scavengers in Spain, where minimum-maximum values were 0.12–344 µg/g in gastric content and 0.2–164 µg/g in liver (Herrero-Villar et al. 2021). No other toxic substances (i.e., carbamates, organophosphates, anticoagulant rodenticides, NSAIDs, strychnine, etc.) or elements (i.e., metals) were detected.

Histopathology revealed severe congestion in most tissues, but especially erythrocytolysis, congestion, hemosiderosis, and cytoplasmic vacuolation of hepatocytes in the liver and tubular epithelial cell vacuolation and eosinophilic deposits in glomeruli in the kidney (Fig. 2). Generalized congestion reported here has already been described in barbiturate intoxication in avian scavengers in Spain (Herrero-Villar et al. 2021).

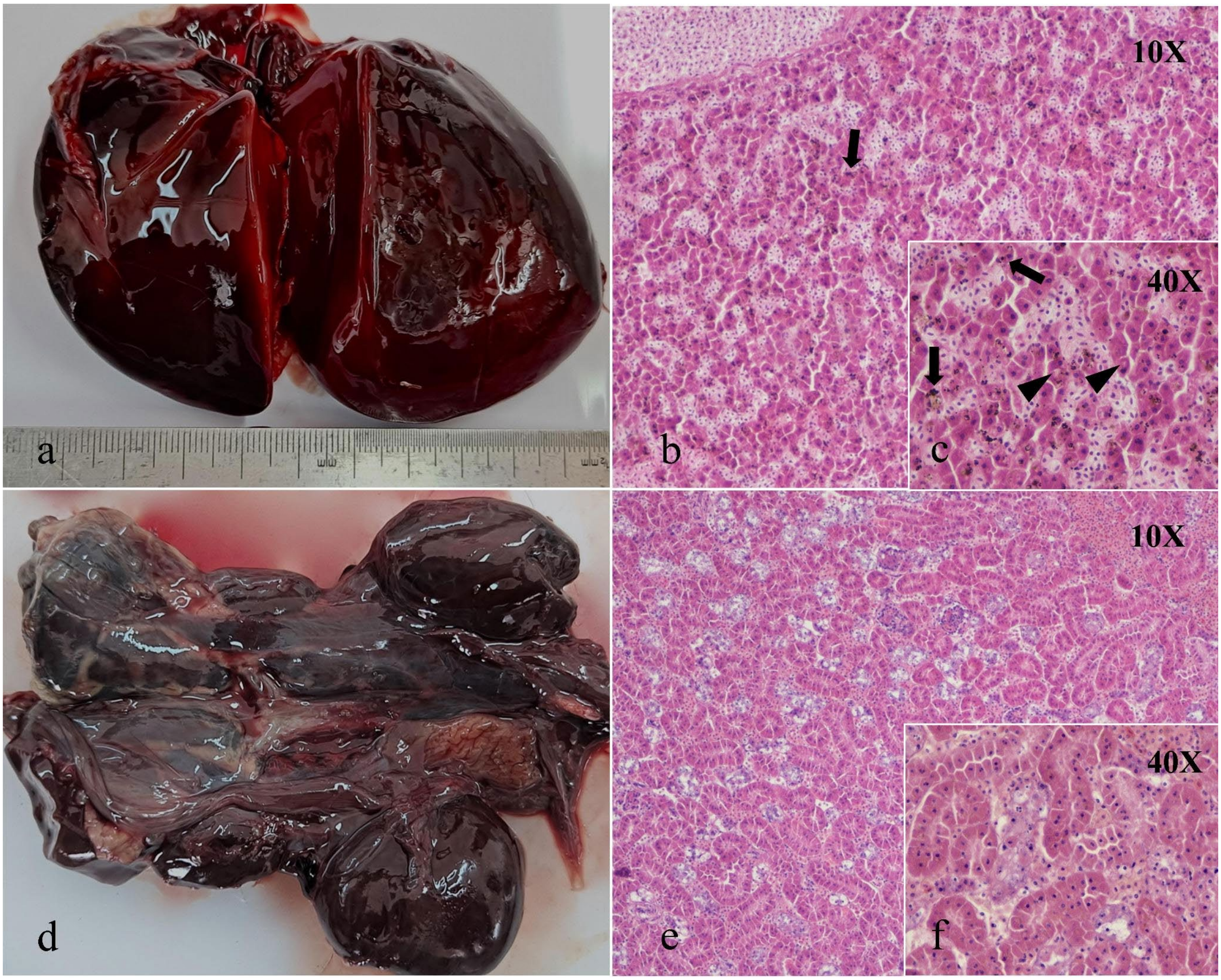

Fig. 2 Bearded vulture, pentobarbital intoxication. Markedly enlarged and congested liver (a) and kidney (d). Microscopically (haematoxylin and eosin) in the liver (b) severe congestion and hemosiderosis (arrows) can be observed (c) in addition to vacuolization of the cytoplasm of hepatocytes (arrowheads inset). Similarly in the kidney (e) congestion, erythrocitolysis, and vacuolization of epithelial cells, especially in glomeruli is evident, in addition to deposits of eosinophilic material in tubuli (f)

The pentobarbital exposure may not have been lethal if electrocution had not occurred. The estimated dose ingested by the bearded vulture was 3.2 mg/kg b.w. (based on the concentration detected in the gastric content (37.3 mg/kg), a food intake of 0.492 kg/day and a body mass of 5.79 kg (see Mateo et al., 2015)), which is below the LD50 of pentobarbital in birds (75 mg/kg b.w.; see Herrero-Villar et al., 2021), but it is within the order of doses that have sedative effects in chicken (2.5–20 mg/kg b.w.; Feltenstein et al., 2004). This suggests that electrocution in this bird may have occurred as a consequence of pentobarbital intoxication due to the sedative properties of the compound due to their depression effect on the CNS and consequent loss of consciousness (AVMA, 2020). To the best of the authors knowledge, this study is the first to report pentobarbital intoxication in a bearded vulture. Even though this specie is listed as near-threatened worldwide (Birdlife International, 2023), this case is particularly concerning due to the potential implications to conservation of the bearded vulture, which is endangered in Europe (Antor et al. 2004). Spain remains a stronghold for the species with at least 252 individuals in 2018, primarily in the NE of Spain (Margalida and Martínez 2020); thus 55.7% of the species in Europe (excluding Armenia, Azerbaijan, Russia, Turkey).

Barbiturate intoxication in avian scavengers is an emerging problem in Spain, with an increase in prevalence from 0.54% to 2012 to 3.4% in 2020 and a significantly higher prevalence in some northern regions (Navarra, 34.5%; Herrero-Villar et al., 2021). In that study, pentobarbital was the most detected barbiturate. The compound was also responsible for 5.9% of the deaths of the griffon vultures (*Gyps fulvus*) (Herrero-Villar et al. 2021), which matches data for bald eagles (*Haliaeetus leucocephalus*) and golden eagles (*Aquila chrysaetos*) from the USA (4.3% and 2.6%, respectively; Russell and Franson, 2014).

Despite feeding on ungulate bones (Margalida et al. 2009), bearded vultures are at risk of exposure to veterinary pharmaceuticals with a high distribution volume like barbiturates. In euthanised animals, pentobarbital can be detected in the bone marrow at similar concentrations to blood or other tissues (Winek et al. 1985; Cartiser et al. 2011), which could be the route of exposure for bearded vultures.

In summary, this study describes pentobarbital as a toxic threat for bearded vultures in northern Spain. This intoxication highlights the need of implementation of mitigation and control strategies to avoid veterinary pharmaceuticals from entering the avian scavenger's trophic chain and becoming an additional risk for endangered wildlife.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11259-023-10093-2.

Acknowledgements Authors acknowledge Life Pro BV LIFE 20 NAT/ES/001363 financed by European Union LIFE program. Preliminary results were presented as an Abstract at the 24th Spanish Toxicology Congress, Córdoba, 9–11 November 2022. Authors acknowledge José Carlos González Ruiz and Javier Gil Vaquero from the FCQ for providing the pictures included in supplementary information and graphical abstract.

Author contribution Marta Herrero-Villar: Performed research, Analyzed data, Writing - Original Draft. Inés S. Sánchez-Barbudo: Performed research, Analyzed data. Teresa Cardona-Cabrera: Performed research, Analyzed data, Writing - Review and Editing. Ursula Höfle: Analyzed data, Conceived study, Writing - Review and Editing. Alberto Sánchez-Cano: Performed research, Analyzed data. Gerardo Baguena: Performed research, Project administration, Funding acquisition. Rafael Mateo: Analyzed data, Conceived study, Writing - Review and Editing. All the authors reviewed the manuscript.

Funding European Union LIFE program, Life Pro BV LIFE 20 NAT/ES/001363.

Data availability Not applicable.

Declarations

Ethical approval Not applicable.

Competing interests None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

References

American Veterinary Medical Association (2020) AVMA Guidelines for Euthanasia of Animals. https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf

Antor RJ, Margalida A, Heredia R (2004) Quebrantahuesos, *Gypaetus barbatus*. In: Madroño A, González C, Atienza JC (eds) Libro rojo de las Aves de España. Dirección General para la Biodiversidad-SEO/BirdLife, Madrid, p 124

Arnold KE, Boxall ABA, Brown AR, Cuthbert RJ, Gaw S, Hutchinson TH, Jobling S, Madde JC, Metclafe CD, Naidoo V, Shore RF, Smits JE, Taggart MA, Thompson HM (2013) Assessing the exposure risk and impacts of pharmaceuticals in the environment on individuals and ecosystems. Biol Lett 9:1–4. https://doi.org/10.1098/rsbl.2013.0492

BirdLife International (2023) Species factsheet: Gypaetus barbatus. http://www.birdlife.orgon16/01/2023

Cartiser N, Bévalot F, Fanton L, Gaillard Y, Guitton J (2011) State-of-the-art of bone marrow analysis in forensic toxicology: a review. Int J Legal Med 125:181–198. https://doi.org/10.1007/s00414-010-0525-6

Feltenstein MW, Warnick JE, Guth AN, Sufka KJ (2004) The chick separation stress paradigm: a validation study. Pharmacol Biochem Behav 77:221–226. https://doi.org/10.1016/j.pbb.2003.10.019

Herrero-Villar M, Sánchez-Barbudo IS, Camarero PR, Taggart MA, Mateo R (2021) Increasing incidence of barbiturate intoxication in avian scavengers and mammals in Spain. Environ Pollut 284:117452. https://doi.org/10.1016/j.envpol.2021.117452

Margalida A, Bertran J, Heredia R (2009) Diet and food preferences of the endangered bearded vulture *Gypaetus barbatus*: a basis for their conservation. Ibis 151:235–243. https://doi.org/10.1111/j.1474-919X.2008.00904.x

Margalida A, Martínez JM (2020) Resultados generales. In: Margalida A, Martínez JM (eds) El quebrantahuesos en España, población reproductora en 2018 y método de censo. Instituto de Investigación en Recursos Cinegéticos (CSIC-UCLM-JCCM). Ciudad Real, pp 20–21

Mateo R, Sánchez-Barbudo IS, Camarero PR, Martínez JM (2015) Risk assessment of bearded vulture (*Gypaetus barbatus*) exposure to topical antiparasitics used in livestock within an ecotoxicovigilance framework. Sci Tot Environ 536:704–712. https://doi.org/10.1016/j.scitotenv.2015.07.109

Moureau G, Temman S, Conzalez PJ, Charrel RN, Grard G, Lamballerie X (2007) A real- time RT-PCR method for the universal detection and identification of flaviviruses. Vector Borne Zoonotic Dis 7:467–477. https://doi.org/10.1089/vbz.2007.0206

Munster JV, Baas C, Lexmond P, Waldenström J, Wallensten A, Fransson T, Rimmelzwaan GF, Beyer WEP, Schutten M, Olsen B, Osterhaus ADM, Fouchier RAM (2007) Spatial, temporal, and species variation in prevalence of influenza a viruses in wild migratory birds. PLoS Pathog 3:e61. https://doi.org/10.1371/journal.ppat.0030061

Pain DJ, Bowden CG, Cunningham AA, Cuthbert R, Das D, Gilbert M, Jakati RD, Jhala Y, Khan AA, Naidoo V, Oaks JL (2008) The race to prevent the extinction of South Asian vultures. Bird Conserv Int 8(S1):S30–S48. https://doi.org/10.1017/S0959270908000324

Russell RE, Franson JC (2014) Causes of mortality in eagles submitted to the national wildlife health center 1975–2013. Wildl Soc Bull 38:697–704. https://doi.org/10.1002/wsb.469

Shore RF, Taggart MA, Smits J, Mateo R, Richards NL, Fryday S (2014) Detection and drivers of exposure and effects of pharmaceuticals in higher vertebrates. Philos Trans R Soc B 369:1–10. https://doi.org/10.1098/rstb.2013.0570

Viner TC, Hamlin BC, McClure PJ, Yates BC (2016) Integrating the Forensic Sciences in Wildlife Case Investigations: a Case Report of Pentobarbital and Phenytoin Toxicosis in a Bald Eagle (Haliaeetus leucocephalus). Vet Pathol 53:1103–1106. https://doi.org/10.1177/03009858166411

Waldenström J, Bensch S, Hasselquist D, Ostman O (2004) A new nested polymerase chain reaction method very efficient in detecting Plasmodium and Haemoproteus infections from avian blood. J Parasitol 90:191–194. https://doi.org/10.1645/GE-3221RN

Winek CL, Costantino AG, Wahba WW, Collom W (1985) Blood versus bone marrow pentobarbital concentrations. Forensic Sci Int 27:15–24. https://doi.org/10.1016/0379-0738(85)90100-8