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Bulgarian society for the protection of birds

Screening vet medicines within LIFE Re-Vultures

International Workshop on Vultures & Veterinary Drugs, February 19th 2019, Dadia, Greece
FOCUS:
Study the health status of large vultures with focus on lead poisoning and veterinary drugs as threats for the Black and Griffon vultures in Bulgaria and Greece

- Pesticides, antibiotics, heavy metals, inbreeding and diseases are factors that negatively influence the health of vulture populations worldwide (Lemus et al., 2008)

- The problem is widely discussed in numerous publications
Sample collection (BSPB)

Blood samples, throat and cloacal swabs of European griffons (*Gyps fulvus*) (N=33)

Samples collection period: 2016 - 2017
Sample proceduring

Standart protocols, received by VCF
Sample collection (The Dadia-Lefkimi-Soufli Forest National Park)

Blood samples, throat and cloacal swabs of Cinereous vultures (Aegypius monachus) (N=45)
Sample proceduring

Samples collection period: 2016 – 2017

Standart protocols, received by VCF
All analyses performed by

Institute for Game and Wildlife Research, Ciudad Real, Spain
Type of analyses:

- Analysis of organochlorine compounds in plasma samples (mainly p,p’-DDE (dichlorodiphenyldicloroethylene) and polychlorinated biphenyls (mainly PCBs 138, 153, 180);

- Analysis of heavy metals in blood samples;

- Analysis of antibiotics and anti-inflammatory drugs;

- West Nile virus;

- Chlamydia;
Methods used:

(p,p′-DDE) and polychlorinated biphenyls (mainly PCBs 138, 153, 180) - an extraction with hexane, a clean-up with sulfuric acid and an analysis by gas chromatography coupled to an electron capture detector (Mateo et al. 2012).

Heavy metals in blood samples by atomic absorbance spectroscopy after the digestion of the sample with nitric acid and hydrogen peroxide (Ortiz-Santaliestra et al. 2015).

The analysis of antibiotics and anti-inflammatory drugs were performed by liquid chromatography coupled to a Time of Flight mass spectrometer (LC/MS-QTOF) (modified from Taggart et al., 2009).

West Nile virus genome was performed by Real Time RT PCR after extraction of RNA and DNA (for detection of Chlamydia) using a commercial kit Macherey-Nagel.

Chlamydia by Conventional PCR targeting the OMP Gene and restriction enzyme analysis and posterior visualization by agarose gel electrophoresis for species differentiation.

ELISA (bELISA 10.WNV.K3 INGEZIM West Nile COMPAC®, Ingenasa, Madrid, Spain) was employed for the detection of West Nile virus and cross-reacting Flavivirus antibodies, after inactivation of plasma (30 min at 56° C).
Results

Organochlorine compounds were present in blood samples at very low levels, and only ppDDE and PCB180 were detected and quantified.

ppDDE levels showed an increase with the age of the bird as can be expected for this bioaccumulative contaminant.

Eurasian griffons had higher levels of ppDDE than cinereous vultures.
Antibiotics and NSAIDs were not detected in none of the analysed blood samples.

Cadmium levels in blood were low. Lead levels were elevated in some individuals (6.7% of cinereous vultures and 24.2% of Eurasian griffons), but these levels were below those compatible with clinical poisoning in birds (>500 ng/g; Franson and Pain 2011).

Blood lead levels were higher in the Eurasian griffon than in the Cinereous vulture. This result is in agreement with other studies performed with Eurasian vultures in Spain, where this species has very elevated Pb levels (Mateo-Tomás et al. 2016).
None of the vultures tested positive for the presence of West Nile virus or other Flaviviruses genome.

All swab samples tested negative for the presence of Chlamydia. 10 out of 79 vultures (12.66%) had antibodies against West Nile virus or cross-reacting Flaviviruses.

The results are in line with previous results in Spain that did not find antibodies against West Nile virus or cross-reacting Flaviviruses in Griffon vultures (Jurado Tarifa et al., 2016) but did so in cinereous vultures (Garcia-Bocanegra et al., 2011; Camacho et al., in preparation).
Thank you kindly!