SOME INTERESTING FINDINGS IN WILDLIFE BIRDS IMPORTANT IN FORENSIC VETERINARY PATHOLOGY

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INTRODUCTION

Over the period 2010 – 2019, a pathomorphological survey of the causes of death of various birds species was done at the Laboratory of Pathology, Croatian Veterinary Institute, Zagreb, Croatia. A total 6,638 birds were examined. In 335 (5,05%) wild bids, the primary cause of death was established. The present cases showed that the greatest threats to health and survival as well as the most common causes of sudden death or prolonged suffering with exitus in wild birds were: (un)intentional poisoning; gunshot wounds; drowning; collision; electrocution; wounds sustained by another animal or man, and (sporadically) disease.

In most cases processed so far, some points might be interesting from the forensic point of view.

MATERIAL AND METHODS

X-ray Examination. The initial X-rays were obtained with limited postmortem imaging (orthogonal lateral and dorsoventral views).

Pathomorphology and Histopathology Analyses. At necropsy, tissues were taken for additional diagnostic examinations. For histopathology analysis, organ samples embedded in paraffin and cut into 4 µm-thick sections, were stained with hematoxylin and eosin (H&E).

Bacteriology and Mycology Analyses. *Salmonella* was isolated from the organs following the instructions for the standard EN ISO 6579-1:2017 and OIE methods. For the isolation of the genera *Streptococcus, Staphylococcus, Escherichia coli* and *Clostridium,* blood agar, Columbia agar, MacConkey agar, and TBX agar were used. For the *Clostridium* spp. isolation, the organs were incubated in an anaerobic environment, Sabouraud glucose agar was used for the isolation of fungi and molds.

Virological Examination. One-step reverse transcription followed by real-time PCR was used to test the samples for the presence of West Nile virus, avian influenza virus and Newcastle disease class I and class II viruses.

Toxicological Analysis. *Carbofuran detection.* All samples were analysed using gas chromatography-mass spectrometry Shimadzu QP 2010 Ultra/SE, Japan. *Lead detection in tissue sample.* The level of lead in tissue samples was determined using ICP-MS (7800 Agilent, 2017, USA). For validation experiments, lead standards as well as reference materials, CRM DORMA 4 Fish protein (NRC Canada) were used.

Parasitological Examinaton. Detection and determination of parasite development stages and their adult forms in the organs were done using standard parasitology methods based on morphometric properties of parasites.

Entomological Investigation. *Postmortem interval (PMI).* To estimate the PMI, the stage of development of the oldest *Caliphora vicina* larvae found on the carcass and the average environmental temperature of the crime scene while the body was in situ were used. The development rate was taken from the literature data. For the identification, unfrosted fly specimens were used. Morphological identification of immature and adult blow fly specimens was done using the identification keys. The developmental stages were identified and the length of individuals of the most advanced stages was measured.*Molecular identification.* Barcode approach using general invertebrate primers LCO-1490 (5' - GGTCAACAAATCATAAAGATATTGG - 3') and HCO-2198 (5' - TAAACTTCAGGGTGACCAAAAAATCA - 3') targeting the mitochondrial cytochrome oxidase I (COI) gene were utilized to identify the larvae in the first larval development stage that were abound in the sample. The DNA was extracted from two individuals (one from the cloaca, and the other from the eyes of the carcass) using Chelex 100 (Sigma, USA), and amplified by PCR generating a 710-bp fragment of the COI.

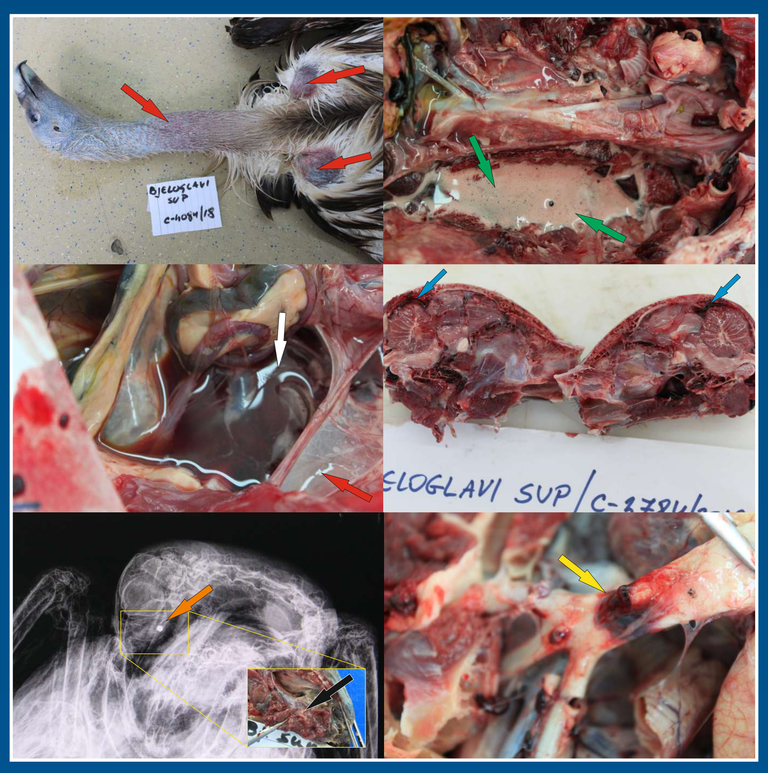
Electrocution

Electrocution and collision with power lines have caused significant levels of wild bird mortality. A crucial mistake in diagnosing electrocution as the cause of casualties and death is to base the diagnosis on the history without necropsy.

Female griffon vulture (Gyps fulvus). Usual posture of a bird killed by electrocution (orange arrow). Burns to the foot skin (green arrows). Burned feathers in mandibular area (yellow arrow). An electrocution-like damage of a wing feather in electrocuted bird (red arrow). Electrocution entry wound in the abdominal wall (white arrow). Subperiosteal haemorrhages in the ribs (blue arrows).

Drowning

The findings of watery fluid in air sacs (particularly in the abdominal air sac) and a marked congestion of the occipital sinus (and other dural venous sinuses) observed in drowned birds are not normally seen in birds dying of other causes (Simpson and Fisher, 2017).



Male griffon vulture (Gyps fulvus). Subcutaneous bruising around the base of the neck and over the shoulders are common findings in drowned birds (red arrows). The intensely congested and oedematous lungs with a white and frothy fluid exuded from cut surfaces (green arrows). The watery fluid in the abdominal air sacs (white arrow). Marked congestion of the occipital sinus (sagittal section of the head; blue arrows). Lead shot pellet in the caudal dorsal concha (X-ray image, a lateral view /orange arrow/). Calcified lead shot pellet in the caudal dorsal concha (small picture in lower right corner; black arrow) (accompanying finding in the drowned bird). Truncus brachiocephalicus in one of the drowned birds (see the rupture with hemorrhage in the vessel wall; yellow arrow) (accompanying finding in the drowned bird).

Poisoning

Different courses of poisoning by different types of poisons in different bird species and different environments showed that birds are an important and visible part of our environment serving as sentinels of general environmental health. Therefore, toxicological investigation of sick and dead wild birds is not dependent on the primary diagnosis /cause of death: in order to increase the degree of protection of the entire ecosystem, it is always NECESSARY.

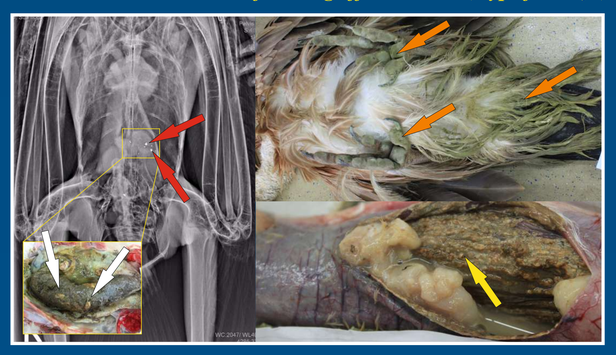
Intentional (acute) poisoning in common buzzard (Buteo buteo) and in pigeon (Columba livia domestica) (forensic cases)

(Per)acute poisoning with methomyl in a common buzzard (Buteo buteo). The beak and the oesophageal cavities were completely filled with raw meat pieces (red arrow). Massive haemorrhages in the gizzard (white arrow).

Acute carbofuran poisoning in common buzzard (Buteo buteo). Spasmodic contraction of the fingers and soles (orange arrows). Fresh meat content with dark bluish-gray powder in the gizzard cavity (blue arrow). Chicken as a bait (see huge skin lesion with dark bluish-gray carbofuran powder) (green arrow).

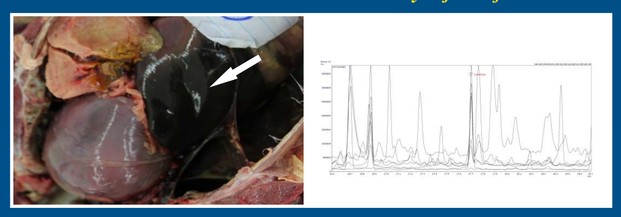
Acute poisoning with methiocarb in a pigeon (Columba livia domestica). Crop is fully filled with red stained corn seeds (black arrow). See hyperaemia and heamorrhages in the subcutaneous tisue (the neck area) (white arrow). Haemorrhages in the lungs (yellow arrows).

Acute lead intoxication in female griffon vulture (Gyps fulvus) (lead concentration in the liver measured 34.5 mg/kg)

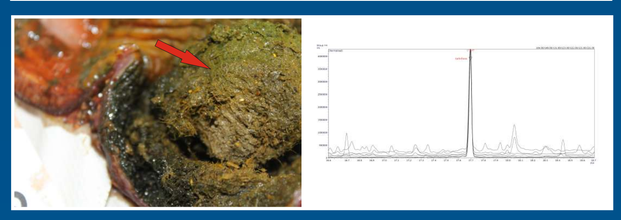


Multiple metal fragments in the caudal part of the coelomic cavity (see the anatomic region of the stomach and distally into the bowel) (red arrows). X-ray image, dorsoventral view. Green stained feathers in the pericloacal area and the sole skin (orange arrows). Content of a distended glandular stomach with ceramic coating lead particles (small picture in lower left corner; white arrows). An extremely distended oesophagus completely filled with raw meat pieces. Note massive mucosal necrosis (yellow arrow).

Chronic carbofuran intoxication in a drowned male griffon vulture (Gyps fulvus) and a traumatically injured male white stork (Ciconia ciconia), and chronic lead intoxication in a traumatically injured female white-tailed eagle (Haliaeetus albicilla), respectively (unexpected cases)



A drowned male griffon vulture (*Gyps fulvus*). The abdominal part of the thoracic-abdominal cavity (organs in situ /liver, white arrow). Chromatogram (liver sample).



A traumatically injured male white stork (*Ciconia ciconia*). A greenish content in the gizzard cavity. See a dark grey gizzard cuticula (red arrow) and an equally coloured content in this area. Chromatogram (gizzard content).

A traumatically injured white-tailed eagle (*Haliaeetus albicilla*) (the lead concentration in the cervical vertebra measured 5.72 mg/kg).

The decline in avian population shows a collapsing ecosystem (US FWS, 2002).