Acute Lead Poisoning in a Griffon Vulture (*Gyps fulvus*) in Israel

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ABSTRACT
A free-ranging Griffon Vulture (*Gyps fulvus*) found lying in a feeding station in the Golan Heights region of Israel was admitted to the Israeli Wildlife Hospital in Ramat Gan, Israel. The adult female vulture presented weak, cachectic, and standing on her tarsometatarsi. She had green diarrhea on her feathers and around her cloaca, a distended crop, and dropped head. X-ray images showed a circular radio-opaque object in the proventriculus and gastric lavage resulted in the regurgitation of a 9 mm lead bullet. The vulture was diagnosed with suspected lead toxicity, which was later confirmed with a blood lead level of 804.8 µg/dL. On day 7, a blood assay showed a lead level of 341.7 µg/dL, an overall marked decrease though still extremely toxic. Crop stasis and distension persisted despite chelation therapy and supportive care and the vulture died 7 days after hospital admission, prior to ingluviotomy. To the best knowledge of the authors, this is the most toxic case of lead poisoning discovered and published to date of a Griffon Vulture in the Palearctic Zone.

Keywords: Griffon Vulture, *Gyps fulvus*, Crop Stasis, Lead Toxicity, Gastric Lavage.

INTRODUCTION
The Israeli Wildlife Hospital (IWH), established in 2005, treats over 2,400 injured and sick birds brought in by civilians and Israel’s Nature and Park Authority (NPA) rangers every year. Finding birds that are still alive with lead poisoning is extremely rare in Israel (1); the IWH typically receives only about two of such cases per year. Toxic blood lead levels are most often attributed to digestion of gunshot pellets in the stomach, where the toxic metal is broken down by the strong acidity and further absorbed into the blood stream (2). The bird, however, may not be a direct target of the gunshot, but rather suffer from lead poisoning after ingesting bullets remaining in carcasses left in hunted animals (2, 3, 4).

General clinical symptoms of lead toxicity include weakness, lethargy, and weight loss. Gastrointestinal signs often present as dark green or black diarrhea, ileus of the upper gastrointestinal tract, and crop stasis with resulting distension and regurgitation. Neurological signs include head tilt and tremors, paresis, and paralysis (4, 5). Hematological signs include erythrocyte fragility and anemia due to the adhesion of lead to erythrocytes in the blood stream (3).

Because of a dearth of literature regarding the toxic blood lead levels of different bird species, the clinical standards of toxicity commonly used are those established for other raptors such as those reported on the Bald Eagle (*Haliaeetus leucocephalus*) population by Redig (1984) at the University of Minnesota (6, 7, 8, 9). According to these thresholds, there are four stages of lead exposure measured in the blood: background (<20 µg/dL), subclinical exposure (20-59 µg/dL), clinical exposure (60-99 µg/dL), and acute exposure (>100...
The blood lead level of the 8 year old Griffon Vulture upon admission to the Israeli Wildlife Hospital was 804.8 µg/dL, more than eight times the threshold for toxic lead exposure.

**CASE HISTORY**

On February 17, 2014 (day 0), an adult female Griffon Vulture (*Gyps fulvus*) was found unresponsive by NPA rangers in a feeding station in Gamla, in the Golan Heights region of Israel. Known to the NPA from previous population monitoring captures, she presented extremely weak and cachectic at 5.3 kilograms, down 2.2 kilograms from her weight recorded in September 2007 (7.5 kg). She was immediately transported to a local veterinary clinic for examination. Because of recent episodes in organophosphate-related deaths of local wildlife scavenging on tainted livestock carcasses, she was administered an undetermined amount of atropine for treatment for suspected organophosphate intoxication. The vulture was then transported 180 kilometers to the IWH by NPA rangers for further diagnosis and treatment.

Upon arrival to the IWH, the vulture showed signs of cachexia, green diarrhea, and dry third eyelids. She was moderately weak with her head dropping forward and balance maintained on her tarsometatarsi, but did occasionally resist handling. Her crop was completely full and distended and simple palpation elicited vomiting. Temperature, pulse, and respiration were all within normal limits. A 20G catheter (Delta Ven 2, Delta Med Medical Devices, Viadana, Italy) was inserted into the metatarsal vein, whole blood was collected in heparinized, serum, and EDTA blood collection tubes (Greiner Bio-One GmbH, Austria) for external laboratory diagnostics (PathoVet, Ltd., Rehovot, Israel and Tel Hashomer Hospital Toxicology Laboratory, Ramat Gan, Israel), and parenteral fluids (Lactated Ringer’s solution with 5% Dextrose, 30ml/hr, Teva Medical Ltd., Ashdod, Israel) were administered intravenously. An in-house PCV/TS test showed a packed cell volume of 40%, just under the normal range determined for Griffon Vultures in captivity (43 ± 2.1%), and total solids of 4 g/dL, which was within the normal range of total protein for Griffon Vultures in captivity (4.0 ± 0.21) (10). Dorsoventral and lateral radiographic images revealed a 9 mm round radio-opaque object in the proventriculus (Figure 1). According to the presenting signs and radiology, IWH veterinarians diagnosed the vulture with suspected lead toxicity.

Hematology and biochemical results are presented in Table 1. Hematology indicated moderate leukocytosis with a relative heterophilia, left shift, and slight to moderate monocytosis with no apparent toxicity of the heterophils.

**Figure 1:** a) A radiograph of a Griffon Vulture with suspected lead poisoning shows a 9mm radio-opaque object in the proventriculus (indicated with an arrow), assumed to be the source of intoxication. The second radio-opaque object near the right wing is a wing tag pin. b) Gastric lavage led to the expulsion of gastric content containing a 9mm lead bullet.
Hemoglobin levels were significantly decreased (11), indicating severe anemia. Blood films revealed abundant polychromasia (~15%) and occasional rubricytes. All biochemical measurements were unremarkable.

These findings indicated an inflammatory leukogram, which could be associated with an infectious agent or non-infectious condition (i.e. toxicity) or in response to environmental stress (stress leukogram). Immature erythrocytes (i.e. rubricytes) coupled with polychromasia in the blood of an anemic bird indicate a marked erythrocyte response. In addition, these immature erythrocytes could be a sign of premature release from hematopoietic tissue due to toxicity (i.e. lead poisoning) (12). Lead also directly interferes with the enzymes involved in the biosynthesis of the heme group, contributing to the fragility of red blood cells and a decrease in hemoglobin levels (3, 13). The effect of lead in both processes supports the differential diagnosis of lead poisoning in the Griffon Vulture.

Atomic absorption spectroscopy at the Tel Hashomer Hospital Toxicology and Pharmacology Laboratory (Ramat Gan, Israel) later confirmed the lead poisoning diagnosis with a blood lead level of 804.8 µg/dL (normal blood lead level <20 µg/dL, subclinical exposure level 20-59 µg/dL). This value is more than eight times the threshold established by Redig (1984) for acute lead exposure in raptors.

**Gastric Lavage**

In preparation for removal of the radio-opaque object by gastric lavage and treatment of lead toxicity, the vulture was administered butorphanol (Torbugesic 10 mg/ml, 0.5 mg/kg IM; Fort Dodge Animal Health, Fort Dodge, IO, USA) for analgesia and midazolam (Midazolam 5 mg/ml, 0.5 mg/kg IM; Rafa Laboratories Ltd., Jerusalem, Israel) for pre-anesthesia sedation. Thirty minutes after administration of butorphanol and midazolam, the vulture was anesthetized with an isoflurane mask (Terrell, Piramal Critical Care, Bethlehem, PA, USA), intubated with a 7 mm endotracheal tube (Hi-Contour, Mallinckrodt Medical, Athlone, Ireland), and maintained under anesthesia with isoflurane via intubation.

With the vulture positioned sternally on the examination table, a custom-made 1.2 cm PVC orogastric tube was measured to the proventriculus, lubricated with a water-based lubricant (K-Y Lubricating Jelly, Johnson & Johnson, Sézanne, France), and inserted into the proventriculus (Figure 2). The proximal end of the body was lifted and 200 ml of LRS was inserted through the orogastric tube into the proventriculus. The distal end of the table was then lifted to a 75° angle, directing the vulture’s head down towards the floor. Fluid and gastric content were expelled into a bucket, but an X-ray of these contents did not show a radio-opaque object. The gastric lavage procedure was repeated once again, and this second attempt resulted in the expulsion of a 9 mm lead bullet amid the remaining gastric content (Figure 1).

**Follow-up Care**

Upon completion of the 20 minute gastric lavage procedure, the vulture was removed from isoflurane, placed in an ICU unit (1.35 m x 2.15 m), and continued on fluids and heating. She was started on meloxicam (Meloxicam 5mg/ml, 0.5 mg/kg IM SID for 2 days; Vetmarket, Segula, Israel), cefazolin (Pan-Cefazolin 1g, 50 mg/kg IV SID for 8 days; Panpharma, USA) for treatment of lead toxicity.

**Table 1: Hematology results**

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 6</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed Cell Volume (%)</td>
<td>40</td>
<td>28</td>
<td>43 ± 2.1*</td>
</tr>
<tr>
<td>Total Solids (g/dL)</td>
<td>4</td>
<td>3.6</td>
<td>4.0 ± 0.21*</td>
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<tr>
<td>Leukocytes (estimated count 10³/µL***)</td>
<td>15.75</td>
<td>14.00</td>
<td>-</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>11.3</td>
<td>9.4</td>
<td>15.1 ± 1.9**</td>
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<tr>
<td>Heterophils (%)</td>
<td>91</td>
<td>96</td>
<td>57.83 ± 9.87**</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>2</td>
<td>1</td>
<td>40.67 ± 10.52**</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>7</td>
<td>2</td>
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</tr>
<tr>
<td>Eosinophils (%)</td>
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<td>1</td>
<td>0.86 ± 1.57**</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0</td>
<td>0</td>
<td>0.00**</td>
</tr>
</tbody>
</table>

* Ferrer, M. et al., 1987, references provided are for PCV and total protein.
** Polo, F. et al., 1992.
*** Method described in Campbell, T. and Ellis, C., 2007.

Figure 2: Gastric lavage preparation for a Griffon Vulture with lead poisoning; the insertion of endotracheal and orogastric tubes.
France), vitamins B<sub>1</sub> and B<sub>12</sub> (Dodicile Veterinary, 30 mg/kg SQ SID for 2 days; FATRO S.p.A, Bologna, Italy), and chelating agent CaEDTA (200mg/ml, 30 mg/kg IM SID for 5 days on, 3 days off; Hebrew University of Jerusalem Veterinary Hospital, Rehovot, Israel) (3, 5). Three hours later, she was vomiting fluids and digested meat and attempted to stand up unsuccessfully due to poor balance.

On day 1 after the procedure, there was no change in the vulture’s clinical appearance. She was quiet, weak, not standing, and continued to drop her head forward, though vital signs remained normal. She was started on preventative metronidazole (Metronidazole 10%, 30 mg/kg SQ SID for 2 days; Vetmarket, Segula, Israel). On day 2, she was administered a gastric stimulator metoclopramide HCl (Pramin Tablets 10mg, 0.3 mg/kg IM SID for 6 days; Rafa Laboratories Ltd., Jerusalem, Israel). Similar to previously reported symptoms of crop stasis, the vulture had a strong appetite and continued to eat gazelle carcass, liver, and small mice as normal. Though there was the impression that a portion of the food was passing through to the stomach, the majority of her food was lodged in the crop. Palpation induced vomiting, and vomiting continued for the next few days even after the removal of food. On day 3, she was dosed preventative itraconazole (Sporonox Capsules 100mg dissolved, 10 mg/kg PO SID for 5 days; Janssen Cilag, Italy) and weighed 6.7 kg with a distended, full crop.

On day 6, the vulture continued to vomit with every handling, remained weak with her head dropped, and presented clutched talons and hypothermia (37.5°, normal ~40°). In-house PCV/TS results decreased to 28% and 3.6 g/dL, respectively, and her weight reduced to 5.2 kg after excessive vomiting. Multivitamins (Dumphalyte, 1 mg/kg IM; Fort Dodge Veterinaria, S.A., Girona, Spain) and hydroxyethyl starch (HAES-steril 10%, 1 ml/kg/hour IV; Fresenius Kabi, Germany) were added to her IV fluids and another blood sample was sent to external laboratory facilities for a complete blood count and lead assay. Aside from a decrease in hemoglobin levels from 11.3 g/dL to 9.4 g/dL (Table 1), hematology results showed findings similar to those of day 1.

As her condition deteriorated on day 7, the veterinary staff at the IWH started preparations for insertion of an ingluviostomy tube on the morning of day 8, a procedure which has previously been successfully implemented on California Condors (Gymnogyps californianus) with severe lead poisoning. Ingluviostomy tube placement has been proven to effectively bypass crop stasis and circumvent gastrointestinal complications induced by lead toxicity (14, 15).

On the morning of day 8 just prior to the procedure, the Griffon Vulture was found dead. The cause of death was attributed to complications of severe lead toxicity. Though an exact cause remains unknown, death can most likely be attributed to organ failure of the known targets of lead toxicity (13).

The lead test results returned from blood drawn on day 6 showed a blood lead level of 341.7 µg/dL, a marked decrease though still in the acute exposure threshold (6).

**Post mortem and histological results**

Post-mortem analysis found very dark, almost black food content in the proventriculus and ventriculus. This finding, along with a stable weight over the course of the week (at day 7, 5.2 kg with empty crop), confirmed the earlier suspicion that some food was passing through the crop. On gross pathology, the vulture’s kidneys were mildly enlarged. No other significant pathological or histological abnormalities were found in the known target organs of lead poisoning (13, 16). This finding is in agreement with an experimental lead poisoning study in Turkey Vultures (Cathartes aura), in which no gross pathological or microscopic pathognomonic lesions for lead intoxication were found (16). The blood levels of lead were clear indications of the ongoing toxicity in this case and it appears that the pathology may not be suitable to make a definitive diagnosis of lead poisoning.

**DISCUSSION**

Though Griffon Vultures (Gyps fulvus) are currently listed on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species as of Least Concern (17), the local population in Israel is experiencing a rapid state of decline. Dropping from 1000 nesting pairs in the 1940’s to only 39 in 2011, only 10 chicks were successfully reared in the wild during the 2011 breeding season (1). As part of the larger program to reinstate the declining population, the IWH aims to act as quickly as possible on the treatment of injured or sick Griffon Vultures arriving at our facility. Of the 148 Griffon Vultures treated at the IWH from 2001 to 2011, 61 were suf-
fering from various kinds of poisonings or suspected poisonings, only 5 of which were determined to be from lead (1).

The amount of time required to confirm a diagnosis of lead toxicity and begin treatment is often the crucial factor between survival and mortality. Due to the indicative clinical presentation of lead toxicity similar to those reported in numerous publications across the world (2, 3, 14, 15, 16), initial radiographic diagnostics and presenting signs were adequate for IWH veterinarians to make a provisional diagnosis of lead toxicity and to appropriately treat this 8 year-old female Griffon Vulture. Gastric lavage and chelation therapy were administered before suspected lead poisoning was confirmed by the blood lead level laboratory diagnostics; the removal of the source of poisoning remarkably reduced blood lead levels by 463.1 µg/dL (804.8 µg/dl down to 341.7 µg/dL) in only 7 days.

The IWH maintains a full stock of diagnostic tools for incoming lead toxicity cases. The life or death of animals suffering from lead poisoning depends on the availability of these diagnostic and treatment options, which can be utilized prior to laboratory confirmation of toxicity and minimize the time gap between arrival and diagnosis confirmation.

Veterinarians treating suspected lead intoxication cases should not always depend upon physical evidence for a differential diagnosis—data estimates that only 15% of birds suffering from lead poisoning actually contain a lead pellet in their gut upon arrival to an animal clinic (18). In such cases, presentation of some or all of the lead-induced clinical signs (weight loss, lack of appetite, anemia, difficulty in standing, crop stasis, diarrhea, mild head tremors, head drooping, depression, nistagmus), can indicate lead as a possible source of intoxication (2, 3, 4, 5).

Should lead particles be present in the gastrointestinal tract, gastric lavage and chelation therapy are clearly demonstrated in these findings to be an effective treatment that both remove the source of toxicity and greatly lower the existing toxic components in the blood stream. Should crop stasis and distension persist after initial chelation therapy, ingluviotomy placement can be considered as an additional treatment.

The challenge in such cases is deciding when treatment should be administered. Research in two previous publications reports that ingluviotomy tubes were inserted after 8 to 12 days after initiation of treatment, but not all cases resulted in successful recovery (14, 15). Lead toxicity in any avian species clearly poses a treatment challenge because of the need to address the damage to many organ systems affected by the consumption of lead (13).

To date, this is the most severe case of lead poisoning that has been observed in the State of Israel and, though the vulture died of lead toxicosis-related complications, serves as an example off of which to build a systematic treatment plan. A significant amount of data has been published regarding lead toxicity in vultures across the world, but to the best knowledge of the authors, this is the most severe case of lead poisoning of a Griffon Vulture in the Palearctic Zone.

ACKNOWLEDGMENTS
The authors would like to extend a special thanks to the skilled staff and volunteers who treat and manage all of the animals admitted to our facility with passion and dedication.

REFERENCES

**Correction**

Investigation of Swine Influenza Sub-Types H1N1, H3N2, H1N2 in Pigs Population in Israel (2002-2009).


In the heading of the article published the designation of the virus types were printed incorrectly in the heading of the article although in the abstract and body of the article the correct names were given throughout the article.

The virus designations have been corrected from H32 to H3N2 and N1N2 has been corrected to H1N2.

The incorrect naming of the swine influenza strains in the heading does not reflect any changes in the article itself or in the conclusions made by the authors. The error made in the heading was a typographical error only.

The correct title reads: *Investigation of Swine Influenza Sub-Types H1N1, H3N2, H1N2 in Pigs Population in Israel (2002-2009).*

The online website (www.ijvm.org.il) has been corrected both in the table of contents and in the heading of the article itself.