

Evaluation of capacity for necropsy and toxicological analysis on the island and development of a network of existing labs and expertise to support effective diagnostic (Action A6.3)

LIFE WITH VULTURES: SAVING GRIFFON VULTURES IN CYPRUS
THROUGH CONCRETE CONSERVATION ACTIONS
(LIFE18 NAT/CY/001018)

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**Project Partners:** 

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### **About the project**

LIFE with Vultures is a targeted conservation project for the protection of the Griffon Vulture in Cyprus. In this four-year endeavor (2019-2023), BirdLife Cyprus, the Game and Fauna Service, Terra Cypria – The Cyprus Conservation Foundation and the Vulture Conservation Foundation have joined forces to tackle the main threats facing the Griffon Vulture and prevent Cyprus' most threatened bird of prey from going extinct. The project has a €1,375,861 budget and is co-funded (60%) by the EU's LIFE programme. Find out more at: www.lifewithvultures.eu













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#### 1. Background

Despite that the use of poisoned baits in Cyprus and the consequent poisoning and killing of wildlife is illegal according to national law, this problem continues to exist and is recorded throughout the island. The poisoning of wildlife through the use of poisoned baits, including wild birds and especially birds of prey (e.g. vultures) and other predators, constitutes unlawful conduct, which is criminalized under the Law on the Protection and Management of Wild Birds and Prey of 2003 (No. 152(I)/2003) as amended, and by the Law on Environmental Protection through the Criminal Law of 2012 (22 (I) / 2012).

In particular, the use of poisoned baits is the main cause of the dramatic decrease in the population of Griffon Vulture in Cyprus from the 1950s until today. Moreover, the use of poisoned baits is a method of mass and non-selective killing, as it can cause mass killing of different species in a short time and without any possibility of quantitative control. Therefore, it poses a critical threat to the Griffon Vulture and affects other birds of prey such as the Bonelli's Eagle (*Aquila fasciata*), the Long-legged Buzzard (*Buteo rufinus*), the Black Kite (*Milvus migrans*), the Northern Goshawk (*Accipiter gentilis*), Marsh Harrier (*Circus aeruginosus*) and more raptorial birds that may visit the island.

Poisoned baits are mostly used to control animals that are considered damaging (e.g. crows, foxes, stray dogs), protect hunting areas as well as a means of resolving human conflicts (e.g. hunting in some areas). Although not often a target, vultures are the most likely victims of poisoning, as they are scavengers and feed in large groups, which presupposes that many vultures can be poisoned simultaneously by a single poisoned carcass. For example, according to the Department of Forests a mass poisoning incident occurred in 1996 in the area of Pigenia, near the community of Pano Pyrgos, where an entire colony of 36 vultures was decimated due to the use of goat carcasses laced with poison by shepherds, with the aim of killing foxes threatening their herds. In another more recent mass poisoning incident, 8 vultures were found dead from November 2015 until December 2017 near Sterakovou village in Limassol district. This incident hindered the restocking efforts of the Cyprus Griffon Vulture population under the Cross-Border Project "GYPAS" between Cyprus and Greece (2011-2014). For this incident, liver and stomach samples from six dead vultures had to be sent to Israel to test for toxic substances and to investigate the cause of death, since the State's General Lab was lacking the appropriate equipment for toxicological analyses at that time.

However, it is not only the lack in capacity for toxicological analyses that hinders the efforts of tackling the poison threat. It is highly essential to have a standard procedure for responding and investigating poisoning incidents that involves the relevant law enforcement authorities to ensure the chain of custody and achieve identification of the perpetrator and













conviction. Until today, none of the poisoning incidents that occurred on the island has ever reached the court.

Nevertheless, the problem of lack of appropriate laboratory equipment was resolved at the end of 2017, when the General State Laboratory acquired the necessary high-tech equipment that is needed to conduct toxicological analyses in cases of poisoning of wild birds and other species of wildlife. The appropriate equipment was funded by the Cyprus Police and the Ministry of Transport, Communications and Works for the purpose of conducting Narcotics' test. Since then, the State's General Lab has been conducting the toxicological analyses for poisoning incidents that involved wild birds as victims.

This report aims to identify any shortcomings, needs and possibilities of the State General Lab and Veterinary Services in Cyprus but also their current capacity in relation to the efforts of dealing with poisoned baits within the project "LIFE with Vultures CY". By identifying gaps that could cause a drawback for efforts to tackle the poisoning threat the project team will be in the position to assess which of these gaps can be covered through the LIFE with Vultures CY project. In addition, the foreseen preparation of protocols and trainings can be adjusted to fill in identified gaps.

## 2. Evaluation of Current Capacity

#### 2.1. Current Capacity of the State Laboratory

The Unit of Forensic Chemistry and Toxicology of the State General Laboratory (SGL) is primarily specialised in the detection of drugs and medicines in items of the Police and Hospital samples, in cases related to forensic investigation of unnatural deaths, driving under the influence of substances (narcotic or alcoholic), and emergencies from hospitals for which poisoning is suspected. The SGL is the primary agent that deals with toxicological analyses in cases where poisoned baits have been used due to the absence of a National Wildlife Forensic Lab. The cases that demand toxicological analyses for animal and wild bird poisoning account for 5 - 10% of all cases examined by the SGL annually. Between 2015 and 2020, the SGL has detected and identified 7 different toxic substances from analysis in 68 samples.













**Table 1:** Showing the toxic substances that have been detected in poisoning incidents by the State General Laboratory from 2017 until 2020, categorized in 5 different groups.

Group of toxic substances	Toxic substances
Pesticides	Aldicarb, Chlorpyrifos ethyl/methyl, Carbofuran
	Dimethoate Cypermethrin Cyflurthrin Endosulfan
Insecticides	Methomyl
Rodenticide	Bromadiolone Broadifacoum Flocoumafen
Fungicides	Penconazole
Other	Warfarin

In 2016, samples (stomach content) from two Griffon Vultures found dead, likely due to poisoning, were sent to a laboratory in Israel where Carbofuran, Tebuconazole and Propamocarb were detected.

A common poisoning method in neighboring Greece is the use of cyanide. However, in Cyprus a toxicological test for cyanide has never been carried out. Currently there is no standardised methodology within the SGL for the detection of cyanide and in most cases the state of the dead animals that arrive in the necropsy lab is not suitable to detect this substance. Given its widespread use in neighboring Greece, there is a suspicion that there is use of this group of substances in Cyprus. Therefore, it is important that we are in a position to test and detect cyanide in order to better understand its usage in Cyprus and act against it.

**Table 2:** Capacity on toxicological tests

Information needed on toxicological analysis	Current capacity	
Substances that can be traced with toxicological analyses	Pesticides: Aldicarb, Chlorpyrifos ethyl/methyl, Carbofuran Dimethoate Cypermethrin Cyflurthrin Endosulfan	
	Insecticides: Methomyl	
	Rodenticides: Bromadiolone Broadifacoum Flocoumafen	













From what type of samples can each of these substances be detected, i.e. stomach, liver, palate?	Stomach Liver Urine
Potential of quantitative detection of such substances	Quantitative examination is not performed in stomach /urine samples. Interpretation of a quantitative results in the above samples could not offer additional information rather than the detection of poison in animal samples
List of heavy metals that can be detected	Not applicable in the SGL
Does a report of toxicological analysis results has the form of a legal report? Please describe	Report format is legal, since is under the authorization of the QA system and according to the ISO 17025.

**Table 3:** Methods for pesticide quantification that follow the Directive 2002/657/CE (22) as practiced in other LIFE projects (e.g. LIFE+ project "The Return of the Neophron"; LIFE10 NAT/BG/000152, www.LifeNeophron.eu).

Methods used to quantify pesticide concentration in blood (in alive animals)	SGL (Yes/No)
Gas chromatography coupled to tandem mass spectrometry (GC-MS/MS)	Yes
Liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS)	Yes

Toxicological analyses of samples taken from dead or alive animals or other samples (pieces of meat) with suspected poisoning are crucial for identifying the toxic substances used. The accurate determination of what substance was used provides information on whether the substance is legal or illegal, its toxicity and side effects, and sources from which it can be purchased/provided.

The laboratory staff in Cyprus is essential to have a network of collaborating labs which can fill gaps, transfer knowledge and broaden the existing expertise and know-how.

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**Table 4:** A list of collaborating labs that could either perform specialised toxicological tests or share expert knowledge.

LAB Name	Location
CAD (Centre for Analysis and Diagnosis of Wildlife)	Malaga, Spain
Laboratory in Israel through contact in Nature and Parks	Israel
Authority	
CVIA (Center of Veterinary Institutes of Athens)	Greece
Private Laboratory in Cyprus (Foodlab) for heavy metals	Cyprus
and lead	

#### 2.2. Current Capacity of the Veterinary Services

The existing laboratories under the Veterinary Services are differentiated to the Animal based Food Laboratory and the Animal Health Laboratory. The Animal Health Laboratory includes five departments: Bacteriology-Serology, Encephalopathies, Virology, Histopathology and Pathological Anatomy-Bacteriology-Parasitology. It is in the latter that forensic necropsies take place.

Currently, there are no gaps in the capacity of the Veterinary Service's laboratory regarding the examination of a poisoning wild bird incident. The main issues that affect the identification of cause of death and collection of evidence is the advanced state of decomposition in which wild birds usually arrive and the lack of description and information that might be relevant from the site that the carcass or affected animal is found. The operation of the Anti-poison Dog Units and the preparation of poison response protocols aim to address these issues and improve ability to identify cause of death and collect evidence for further analysis.

Records show that during the last 16 years (2005-2020) the Veterinary Services have conducted forensic necropsies for samples taken from around 65 different poisoning incidents that included dead/affected, domestic or wild animals, and/or just poisoned baits. Forensic necropsy for such evidence is an essential part of the investigation of suspected poisoning incidents. It can reveal cause of death (e.g. poisoning, shooting, disease), the state of decomposition of baits or carcasses which subsequently gives indication of about when a poisoning incident took place, and extra anatomical or pathological information that may give light in such cases. For instance, in cases where alive poisoned animals are found, the Veterinary Services can determine the factor leading to their state based on the symptoms observed.

In cases where dead animals are found at an advanced stage of decomposition and thus, no samples can be retrieved for toxicological analyses. For instance, a recovered dead vulture found













on 25/7/2019 at an advanced stage of decomposition in the sea in Episkopi/ west Tunnel Beach area was transferred to the Veterinary Services. The necropsy and X-ray examinations showed lead pellets in the stomach of the juvenile bird which suggested that the cause of death was poisoning from lead pellets.

The Veterinary Services do not conduct X-ray examinations and samples are taken to a private veterinarian.

Currently, in a possible poisoning incident with wildlife species as victims, the Game and Fauna Service collects the dead animals and forwards these to the Veterinary Services. The veterinarian who conducts the necropsy collects the samples and forwards these to the State General Laboratory for toxicological tests. The results are then reported back to the Game and Fauna Service. However, this procedure does not ensure protection of the chain of custody, and this is a significant gap for Cyprus and for further criminal investigation.

However, a standard procedure is established for poisoning of domestic animals. In these incidents, the Cyprus Police is involved by attending the scene, collecting samples and dead animals and then transfer of samples to the Veterinary Services and then to the SGL.

## 3. Building capacity

In the course of the project "LIFE with Vultures CY" (LIFE18 NAT/CY/001018) after the legal framework and competences of each Cypriot public agency have been clarified in the chain of custody for poisoning incidents' management, and with the protocols in hand (with the completion of Actions A1.1, and A6), practical and effective training will be offered. This training will be offered to relevant staff from the State General Laboratory, the Veterinary Services, the Game and Fauna Service, Cyprus Police and BirdLife Cyprus. The first four are institutions/authorities that will be involved in the investigation of wildlife poisoning incidents in order to secure an adequate response and law enforcement.

Once a case of wild bird poisoning has been well documented in the field and adequate samples and evidence have been collected from the field authorities, the investigation will continue in laboratories of the Veterinary Services and the State General Laboratory. Therefore, project toxicologists and veterinarians will have the opportunity to receive knowledge and develop skills in forensic pathology and toxicology which are the most important components when it comes to managing illegal wildlife poisoning incidents. The anti-poisoning training aims to raise operational capacities of staff from the Game and Fauna Service, the State General Laboratory, the Veterinary Services and the Cyprus Police rendering their response to future poisoning incidents more effective. Expert staff from Spain, primarily from the Junta de Andalucía, will teach attendees all about investigation, forensic pathology and toxicology.













Competent authorities from Cyprus will participate in a series of official anti-poison training courses within the Wildlife Crime Academy based in Spain, building capacities to effectively investigate, manage and tackle illegal wildlife poisoning incidents. As part of BalkanDetox LIFE, the Vulture Conservation Foundation will coordinate the Wildlife Crime Academy, which relies on the support of the Regional Government of Andalusia (Junta de Andalucía) and the Spanish Ministry for the Ecological Transition and the Demographic Challenge (Ministerio para la Transición Ecológica y el Reto Demográfico).

In addition, a training will be organized in Cyprus by Spanish experts that will target among other agencies, the laboratories responsible for wildlife forensics and the veterinary services. This training will be co-organized with the Game and Fauna Service and BirdLife Cyprus and co-facilitated by the Cypriot delegation who received the training in Spain.

All these training programmes fall under Action E1.1 ("Training on innovative toxicological analysis techniques and criminal investigation of poisoning incidents for relevant Cypriot institutions and staff") and will extend the current capacity of relevant staff in Cyprus.

In addition to these trainings, operational protocols and standard response procedures will improve coordination between involved entities and will enhance capacity in relation to appropriate collection of evidence and transport to the relevant laboratories.

Finally, the operation of the Anti-poison Dog Units that will be conducting frequent patrols in the countryside are expected to achieve early detection of affected poisoned wild animals thus allowing reliable necropsies and identification of cause of death as well as collection of samples and further toxicological analysis by the State General Laboratory.











