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**CAUSES OF MORBIDITY AND MORTALITY IN DIURNAL AND NOCTURNAL
BIRDS OF PREY IN HUNGARY**

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**CAUSES OF MORBIDITY AND MORTALITY IN DIURNAL AND NOCTURNAL
BIRDS OF PREY IN HUNGARY**

Értekezés doktori (PhD) fokozat elnyerése érdekében
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Abstract

**CAUSES OF MORBIDITY AND MORTALITY IN DIURNAL AND NOCTURNAL
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The aim of our work was to establish the cause of morbidity and mortality of free-ranging birds of prey. Such an in-depth, broad scale pathological study has not been performed on birds of prey in Hungary to date. Following necropsy, histopathology, microbiology and analysis of trace elements from liver and bone tissue samples, we found non-infectious causes were described in 78% of the 85 cases (17 species) and 22% had an infectious etiology. Electrocutation was found to be the most frequent non-infectious cause, and respiratory mycosis (*Aspergillus* sp.) cases were most numerous on the infectious front. We identified the first case of poxvirus in Common kestrel, and avian tuberculosis in Common buzzard in Hungary. Trace elements concentrations were deemed low, though some exceptions were noted. In the Common kestrel, levels of various metals were significantly higher in bone tissue as compared to liver.

Kivonat

RAGADOZÓMADARAK EGÉSZSÉGGÁROSODÁSÁNAK VIZSGÁLATA MAGYARORSZÁGON

A tanulmány során ragadozómadarak egészségkárosodását vizsgáltuk; a célunk az volt, hogy a Fővárosi Állat- és Növénykert Természetvédelmi Mentőközpontjába bekerült, majd elhullott vagy végleg elaltatott egyedeknél 2015-2017 között feltárjuk a leggyakrabban előforduló tényezőket, kórokozókat. Munkánkba retrospektív módon az Állatorvostudományi Egyetem Patológia Tanszékén 2000 és 2013 közötti években iktatott, patológiai vizsgálatra érkezett tetemeiket is bevontuk. A hazai szakirodalmat tekintve ilyen mintaszámon végzett részletes patológiai elemzést még nem végeztek. A kórboncolás során a kiegészítő vizsgálatokhoz való mintavétel is történt. Kórszövettani vizsgálatához, virológiára és nehézfém kimutatásához különböző szervekből vettünk szövetmintákat, míg a bakteriológiai vizsgálatra kloákatamponthoz gyűjtöttünk.

Összesen a két intézményben a vizsgálatba vont fajok száma 17 volt, melyek közül 12 nappali, míg 5 éjjeli ragadozómadár; összesen 85 ragadozómadár teteme került patológiai vizsgálatra a munka során.

Az elemzés során kiderült, hogy a nem fertőző elhullási okok domináltak (78,0%) a fertőző eredetűekkel szemben (22,0%). Szinte minden ragadozómadár fajnál lehetett elektromos áram okozta égési sérülésekkel találkozni, mely elváltozások a minták felében voltak kimutathatóak. Az elhullási okok között gyakoriságban 12,0%-kal a májdystrophia állt a második helyen. A gyakoriság tekintetében a harmadik elhullási ok a testüregbe vagy a szervekbe történő elvérzés volt a vizsgálatunk során. Ezt mintegy 11,0%-ban lehetett kimutatni, zömében a nappali ragadozó fajokban.

Eredményeink alapján a fertőző betegségek közül a leggyakoribbként (47,0%-os gyakoriság a fertőző betegségeken belül) az *Aspergillus* sp. okozta tüdő- és légzsákmikózist sikerült igazolni. Mindamellett több fertőző betegség első előfordulását is sikerült megállapítanunk hazai, vadonélő ragadozómadarakban (poxvírus fertőzést vörös vércsében és *Mycobacterium avium* okozta madárgümőkórt egerészölyvben). A nehézfémek esetében a tanulmány során egyes szervek (így a csont és a máj) mintáiban összesen 12 különböző nehézfém értékét mértük meg. Megállapítottuk, hogy a legtöbb esetben ezek szintje nem volt különösebben emelkedett a szakirodalomban fellelhető adatokhoz képest. Itt több elemnél egyes egyedeknél találtunk magasabb értékeket, amit annak tudtunk be, hogy hazánk egyes területein előfordulhatnak nehézfémek magasabb mennyiségben. Kapacitás hiányában nem tudtuk feltárni, hogy mely területek lehettek érintettek, de mivel a ragadozómadarak amúgy is

képesek nagy területeket bejárni a táplálékszerzés során, ezért egyáltalán nem törvényszerű, hogy ezen adatok értékelhetőek lettek volna-e. Ezen felül bizonyos nehézfémek a csontokban mért szintje szignifikánsan magasabb volt a májban észlelthez képest. Ennek a kutatásnak a további folytatása azért is lenne üdvözítő, mert újabb információkat nyerhetnénk ezekről a karizmatikus madarokról, trendeket állapíthatnánk meg a bekerülés okairól, illetve egyes betegségek monitoringja is javulna, hogy a morbiditás és mortalitás minimalizálható legyen a mentőmunka során.

1. Introduction

Birds of prey occur in great diversity and numbers in Hungary especially compared to the relative size of the country. 18 species of raptor and nine owl species breed on a regular basis, whilst a further 19 and three diurnal and nocturnal predatory birds respectively, have either bred sporadically, overwinter, pass through or are vagrants in our country. Because our country is located in the central part of Europe, it is the western-most breeding range of certain species, such as the Saker falcon (*Falco cherrug*) and the Eastern imperial eagle (*Aquila heliaca*), in the Western Palearctic. Moreover, despite being a relatively small country, the variation in habitat, from the mountainous northern regions to the great plains, provides variable territory for birds of prey eliciting many different lifestyles.

Such variable and unique avian fauna face the same risks as any free-living birds do, and natural and anthropogenic factors alike, pose a threat to all birds of prey today. The Budapest Zoo and Botanical Garden, through its Wildlife Rescue Center, has been committed to the care of all protected and strictly protected species of Hungarian fauna for more than three decades. With between 2,000-2,500 individuals admitted on an annual basis over the past five years, the aim is to rehabilitate and release as many individuals as is possible back to the wild following diligent, comprehensive and current veterinary care. Admittance to a rescue center can be the consequence of many factors, however any free-living avian individual, falling into human hands implies injuries that prevent them from flying, and thus, surviving in the wild. Moreover, severe lesions incurred from injury often lead to the death of an individual or humane euthanasia for animal welfare reasons, and as is consistent with wildlife rescue centers across the globe, complete rehabilitation can be seen in merely 30-40% of the cases.

Our research was prompted by the idea, that in order for free-ranging birds of prey to be admitted to a wildlife rescue facility, their health must be compromised. Moreover, we stipulated that in these cases, there would be underlying factors, of non-infections or infectious origin, hindering the ability of these birds to survive in the wild. The hypothesis of this study was that the causes of morbidity and mortality of free-ranging birds of prey admitted to the wildlife rescue center were affected by infectious or non-infectious diseases obtained in the wild. Additionally, we aimed to establish the aspects and diseases with the highest incidence overall and for the most frequently occurring species.

The methodology to be employed in this study involved solely *post mortem* examinations performed on birds of prey origination from the wild in Hungary. The portion of the study performed at the Wildlife Rescue Center at the Budapest Zoo and Botanical Garden involved only those birds of prey that died or were humanely euthanized during the two-year

study period. Necropsy was performed in order to establish the cause of death, and whether or not the lesions observed indicated disease of infectious or non-infectious origin. Supplementary examinations were implemented to identify histopathological alterations typical of various ailments and screening for various infectious agents according to scientifically accepted methodology. Moreover, samples were taken for both qualitative and quantitative analysis of 12 different trace elements (including heavy metals) to determine whether or not these birds had accumulation of these elements and biometric data was recorded for all individuals included.

The goal of our research was to determine the infectious and non-infectious factors that pose the greatest threat to predatory birds in the wild in Hungary, the frequency of their occurrence and where possible, provide a possible explanation for preventive measures to be implemented to avoid their occurrence in the future.

2. Literature Review

2.1. Birds of prey represented in this study

Altogether there are 37 species of diurnal and 12 nocturnal birds of prey that are either resident to Hungary, overwinter or pass through during migration or as vagrants, or have been documented in our country on one of more occasion. Listed below are the species relevant to this study with the IUCN (International Union for Conservation of Nature) Red List of Threatened Species categorization (IUCN, 2020) as well as their status and theoretical conservation value according to the second appendix of the 13/2001(V.9) KöM decree in Hungarian Nature Conservation legislature.

Raptors

European honey buzzard (*Pernis apivorus*)

IUCN least concern, Hungarian strictly protected species, theoretical conservation value: 100,000 HUF.

As the European honey buzzard has been considered a species preferring forested areas, it has been known to utilize wooded areas for nesting in both the mountainous regions and on the plains of Hungary as well (HARASZTHY, 2019). For nesting, this species appears to prefer oak (*Quercus* sp.) forests, in which they build their nest high up in the canopy (BERECZKY, 2017), though on occasion, beech (*Fagus sylvatica*) forests may also serve as nesting sites. Courtship rituals begin in the month of May and subsequently, two eggs are laid generally by mid-June, which are incubated for 30-35 days. Fledging of chicks occurs around six weeks of age (HARASZTHY, 2019). The European honey buzzard, as its name suggests, feeds on bees, their larvae and pupae, however they also have been recognized to consume various reptiles and avian species as part of their diet (SCHWARTZ, 2017b). Owing to their relatively late breeding season and the shy nature, finding all nesting sites can be challenging (BERECZKY, 2017). After raising their young, the Hungarian Honey buzzard population, approximated at 750-775 pairs in 2018 (MME RAGADOZÓMADÁR-VÉDELMI SZAKOSZTÁLY [RMVVSZ], 2020), begin their migration to their overwintering grounds in late August to early September (AGOSTINI et al., 2007) and travel as far as the southern part of Africa (SZITTA, 2009). Though impending threats are numerous to the breeding success these species, and include habitat destruction and deforestation (SCHWARTZ, 2017b), more recently the negative effect of pesticides used in agriculture on bee populations has also been questioned (BYHOLM et al., 2018), indicating a direct threat to the feed-source of these raptors.

White-tailed eagle (*Haliaeetus albicilla*)

IUCN least concern, Hungarian strictly protected species, theoretical conservation value: 1,000,000 HUF.

Nesting across Asia Minor and Central Europe, the White-tailed eagle is a regular breeding species in Hungary (SVENSSON et al., 2018). As confirmed through studies using satellite transmitters, this species does not migrate, rather, individuals roam long distances regionally before reaching sexual maturity (VÁCZI, 2020). With nesting pairs to be found across the country (HARASZTHY, 2015), the population was believed to be 308-339 pairs in 2018 (MME RMvSZ, 2020). Each breeding pair will hold their territory year-round with the greatest number of birds occurring in regions near either the Danube and Tisza rivers, or fishpond systems surrounded by wooded areas. Building nests in the canopy of old trees in the vicinity of water, they begin their nesting season at the end of the winter and usually lay two eggs (rarely one or three). Though both parents take part in the 36-38-day incubation period of their eggs, the female spends more time on the nest. After hatching, the pair care for their chicks for three months before fledging ensues (HARASZTHY, 2019). Various species of fish and waterfowl are their primary source of prey, though observations have been made of their capturing larger bodied birds, such as herons (HARASZTHY, 2015). Moreover, ornithologists have described White-tailed eagles catching fish weighing up to 200-400 grams (TUVI & VÁLI, 2007). They are also scavengers and carrion eaters, and as a result, face the risk of ingestion possibly toxic substances accumulated in their prey (SÓS et al., 2013; DEÁK et al., 2020) as well as lead (KRONE, 2009; NADJAFZADEH et al., 2012).

Northern goshawk (*Accipiter gentilis*)

IUCN least concern, Hungarian protected species, theoretical conservation value: 50,000 HUF.

The Northern goshawk can be found across the globe and in all regions of Hungary. Primarily nesting in forested areas, urbanization of the species has become increasingly frequent (HARASZTHY, 2019). Though northern populations migrate, Hungarian specimens are largely resident in their breeding area, with juvenile individuals roaming regionally (BAGYURA, 2009). The breeding season commencing as early as February-March, when 2-6 egg clutches are laid and incubation lasts 33-38 days, with male chicks fledging after 35-36 days and females at 40-42 days of age (HARASZTHY, 2019). Between 2014-2018, there were an estimated 1,100-1,300 pairs in our country (MME RMvSZ, 2020). KÖHALMY (1994) indicates that in Hungary, the Ring-necked pheasant (*Phasianus colchicus*), the Hungarian grey partridge (*Perdix perdix*) and the European brown hare (*Lepus europaeus*) rank high on the list of preferred prey, thus

creating a connection between this species and the game farming industry. FARAGÓ (1997) goes so far as to say that the Goshawk actually has a considerable negative impact on Grey partridge population in Hungary. Resultant of their speed and agility during flight, the Goshawk is able to prey on Turdidae, Corvidae and Columbidae species, in addition to the pheasant and the partridge, subsequently, posing a threat to the game farming industry, particularly the rearing of pheasant chicks (FARAGÓ, 2002). Threats on this species are multifactorial, including destruction to the forest habitats crucial for their nests and food availability (REYNOLDS et al., 2006), however urban populations specialized in feeding on Feral pigeons (*Columba livia domestica*) run the risk of contracting various infectious diseases from their prey (KRONE et al., 2005). Moreover, Northern goshawks appear to have a higher sensitivity to West Nile virus (ERDÉLYI et al., 2007; WODAK et al., 2011; HUBÁLEK et al., 2018; BUSQUETS et al., 2019; SÓS-KOROKNAI et al., 2019) and can perhaps serve as an indicator species for this viral disease.

Sparrowhawk (*Accipiter nisus*)

IUCN least concern, Hungarian protected species, theoretical conservation value: 50,000 HUF.

The distribution of the Sparrowhawk extends from Europe through the vast majority of Asia, including all regions of Hungary (SVENSSON et al., 2018), where it allegedly prefers patchy pine (*Pinus* sp.) forests to build its nest. The species is present year-round in our country and urbanization has increased over the past decades. They begin their breeding season in the month of May by building their nests in the canopy of trees, close to the trunk. In the majority of instances, they lay 4-6 eggs, which they incubate for a total of 39-42 days, and fledging ensues around 29-30 days of life (HARASZTHY, 2019). The Hungarian population was estimated at 3,600-5,900 breeding pairs between 2017-2018 based solely on the assessment of sample monitoring sites (MME RMvSZ, 2020). Though the primary source of prey has been described to be small passerines, species of small mammals, reptiles, amphibians and even invertebrates have been described as part of their diet (HEINTZELMAN, 1964). Although the Sparrowhawk is a resilient species able to adapt to many different habitats, a decline has been described in the reproductive success of these birds, which has primarily been attributed to changes in the composition of trees in nesting areas. Moreover, increases in species competing for similar prey also pose a substantial threat to these birds (BÉRCES, 2017), though as generalist predators, Sparrowhawks are able to adapt the composition of their diet to the ecological changes whilst maintaining a preference to specific feed items (MILLON et al., 2009).

Common buzzard (*Buteo buteo*)

IUCN least concern, Hungarian protected species, theoretical conservation value:

25,000 HUF.

The Common buzzard is found widely across Europe and Hungary. Traditionally, the species was found in the mountainous and vast forested regions of the country, however today, they nest in essentially any wooded area (TÓTH, 2009; HARASZTHY, 2019), and even on smaller trees (TÓTH, 2009) and bushes in undisturbed areas (HARASZTHY, 2019). Considered to be one of the most common raptor species in Hungary (DEMETER et al., 2019), the population does not migrate, but members of the species roam regionally in the winter months. Studies demonstrate that the most ideal nesting grounds for Common buzzards are mosaic patterned agricultural expanses, as these habitats provide them with ample prey (TÓTH, 2009). They build their nests in the month of March, laying between 1-5 eggs, incubating them for 33-35 days and their chicks fledge approximately 50-55 days after hatching (HARASZTHY, 2019). FARAGÓ (2002) indicated that in Hungary, the Common vole (*Microtus arvalis*) was the most predominate source of prey for this species during the nesting period, while in Norway, SELÅS et al. (2007) found the prey of Common buzzards to primarily be comprised of small birds. However, this species shows a remarkably wide range of potential prey from reptiles, including small mammals, passerines, columbids and in some instances, even remains of *Strigiformes* were found in nests of buzzards (BERECZKY et al., 2018). Due to their widespread distribution and vast population, this species faces not only naturally occurring threats, but must live amidst the imminent danger of bird-crime (DEÁK et al., 2020) and electrocution (TÓTH, 2018), and has indeed been shown to be one of the most frequent species admitted to rescue centers in Hungary (SÓS-KOROKNAI et al., 2020a).

Rough-legged buzzard (*Buteo lagopus*)

IUCN least concern, Hungarian protected species, theoretical conservation value:

50,000 HUF.

Building their nests in the tundra regions of our planet and described as a circumpolar breeder (POKROVSKY et al., 2014), the Rough-legged buzzard is a winter visitor in our country spending time on Hungarian soil between October and March (FINTHA & KALOTÁS, 1988). During this time, they are found in the greatest abundance east of the Tisza river (SVENSSON et al., 2018). Nesting in boreal forest and on the tundra, nests are constructed on rock faces, cliffs or in trees. Beginning their breeding season in April, typically 2-3 eggs are laid, which are incubated for 28-31 days. However, in years when Lemmings (*Lemmus* sp.) are abundant, Rough-legged buzzards can lay up to 5-7 eggs. Chicks fledge 40-41 days post hatching (FINTHA

& KALOTÁS, 1988). Considered to be a small rodent specialist, observations have been made to indicate that this species does show interest in other sources of prey in times when small mammals are scarce (POKROVSKY et al., 2014), including small birds, reptiles, amphibians and insects (FINTHA & KALOTÁS, 1988) and hunt with a typical hovering technique. Preferring areas of agriculture, these buzzards are often seen perched on the ground, particularly regions with low vegetation on its wintering grounds (WUCZYNSKI, 2005).

Lesser spotted eagle (*Clanga pomarina*)

IUCN least concern, Hungarian strictly protected species, theoretical conservation value: 1,000,000 HUF.

Generally, the Lesser spotted eagle nests in Eastern Europe, with Hungarian population showing a preference to areas near water (HARASZTHY, 2015). Wintering in Southern and Central Africa, breeding individuals arrive in Hungary between March-April (HARASZTHY & SZITTA, 2009) and build their nests in the canopy of large trees, often nesting adjacent to the trunk (HARASZTHY, 2019). Typically, they lay two eggs and incubate them for 38-43 days. Post hatching, aggression and siblicide has been observed in this species (MEYBURG, 2001), where the stronger chick will often kill its weaker sibling, a behaviour elicited solely while the chicks have their downy, white plumage (HARASZTHY, 2015). The Hungarian population was recorded as 42 breeding pairs in 2018 (MME RMV SZ, 2020). A substantial proportion of their diet is composed of amphibians, reptiles and small mammals, however DRAVECKY et al. (2008) showed that in Slovakia, fish and birds are also a source of nutrition. Utilizing predominantly areas of agriculture for hunting, Lesser spotted eagle population has been experiencing a slow decline in Hungary with the major threats to our nesting birds occurring in the form of habitat loss and human disturbance (PONGRÁCZ, 2020). Furthermore, owing to the low fecundity of the species and their elusive nature, authors have noted that increased efforts are required to further promote conservation (HORVÁTH, 2002).

Eastern imperial eagle (*Aquila heliaca*)

IUCN vulnerable, Hungarian strictly protected species, theoretical conservation value: 1,000,000 HUF.

Following the decimation of their population in the last third of the 20th century, the Eastern imperial eagle nested solely in the north-eastern mountainous regions of Hungary (HARASZTHY, 2019). Over the past decades however, nesting pairs are becoming increasingly numerous on the central plains (HORVÁTH et al., 2018). The adults will principally remain in the vicinity of their breeding grounds year-round, while subadult birds stray south in the winter months. Initiating the breeding season in late March to early April, they build their nests at the

top of trees where they subsequently lay 1-3 eggs. The incubation period lasts 43 days and the chicks fledge after 65-80 days. Between 2017-2019, breeding success of the species was 1.4-1.5 in Hungary (HORVÁTH et al., 2020) and the population was estimated at 260-280 pairs in 2018 (MME RMV SZ, 2020). Traditionally, the European souslik (*Spermophilus citellus*) and the European hamster (*Cricetus cricetus*) were considered their primary source of prey (HARASZTHY, 2015), however, work by HORVÁTH et al. (2010) recognized the European hare and the Ring-necked pheasant to compose a considerable part of their diet. Owing to the latter, owners of captive populations of these game animals place baits for the intentional poisoning of birds of prey, which has led to intoxication becoming the leading cause of mortality in this species in Hungary. However, because of their occurrence across the plains of our country, the threat of collisions and electrocution caused by powerlines also rank high on the list of factors harmful to these birds (DEÁK et al., 2020).

Common kestrel (*Falco tinnunculus*)

IUCN least concern, Hungarian protected species, theoretical conservation value: 50,000 HUF.

One of the most frequently occurring raptors in Hungary (DEMETER et al., 2019), according to HARASZTHY (2019), the Common kestrel is found in virtually every region of the country and is prone to urbanization. A portion of the Hungarian population will migrate south for the winter (departing in October-November and returning in March), however, older birds will generally remain throughout the winter months (HARASZTHY & BAGYURA, 2009a). Nesting on cliffs, quarries and tree canopies, some individuals exploit even largest cities in our country. With the onset of the breeding season from late March, kestrels lay 3-7 eggs, which they incubate for 27-32 days and their chicks will fledge after 27-32 days in the nest (HARASZTHY, 2019). Kestrels enjoy a great variability in diet extending from small rodents, to reptiles, insects and even small birds on occasion (PROMMER et al., 2019). CARRILLO & GONZÁLEZ-DEVILA (2009) emphasize the key importance of sufficient prey to the breeding success of the Common kestrel. As these birds of prey utilize farmland, as well as the plains of our country (PROMMER et al., 2019), they are subject to the dangers of these surroundings, which include electrocution, responsible for a high morbidity rate in this species (SÓS-KOROKNAI et al., 2020b). Moreover, urbanized populations must cope with the constant danger of human disturbance, which has led to a decrease in breeding numbers in Budapest (MORANDINI, 2017).

Eurasian hobby (*Falco subbuteo*)

IUCN least concern, Hungarian protected species, theoretical conservation value:

50,000 HUF.

The Eurasian hobby is present in Hungary between April and September, nesting primarily on the plains, though hilly regions of the country may also be utilized by this species (HARASZTHY, 2019). Occurring across the Palearctic region, individuals migrate in groups and travel as far as the southern part of Africa for the winter months (HARASZTHY & BAGYURA, 2009b). Though CLEMENTS & EVERETT (2012) showed that 68% of the hobbies nest in woodlands, poplar trees (*Populus* sp.) in the vegetation separating agricultural fields are more characteristic nesting site for this species in Hungary (HARASZTHY, 2019). However, artificial nest boxes on powerlines have been utilized readily in recent years (BAGYURA et al., 2019b). They lay between 1-4 eggs, hatching after 28-31 days and their chicks fledge 28-34 days thereafter (HARASZTHY, 2019). Between 2014-2018, the Hungarian population was estimated to be 2,600-2,900 pairs (MME RMV Sz, 2020). Small songbirds make up the largest proportion of their diet (SERGIO & BOGLIANI, 1999; BAGYURA et al., 2019b), though remains of swifts (SERGIO & BOGLIANI, 1999), swallows and even various species of bats have been found in their nests during the breeding season (BAGYURA et al., 2019b). Though the Eurasian hobby has been described as a versatile species, able to adapt to fragmentation caused by agriculture, human disturbance and the cutting down of potential nesting trees does impact their fecundity (SERGIO & BOGLIANI, 1999).

Saker falcon (*Falco cherrug*)

IUCN endangered, Hungarian strictly protected species, theoretical conservation value:

1,000,000 HUF.

With a distribution across Eastern Europe and Asia, the Saker falcon was found primarily on the southern slopes of mountainous regions of Hungary after the population was decimated through hunting in the mid 1900s (HARASZTHY, 2019). Today, nesting pairs are present in the jurisdiction of all ten national parks, with the highest numbers residing in the eastern Hortobágy National Park and the south-western Körös-Maros National Park (BAGYURA et al., 2019a), and solely on the plains of our country, very often in artificial nests on electrical pylons (HARASZTHY, 2019). In years when the temperature is milder in the winter months, the Hungarian Saker falcon population will migrate slightly south of the border and merely as far as Northern Africa and return to their breeding grounds by February (BAGYURA et al., 2019a). 165-180 pairs of Sakers were recorded in 2018 (MME RMV Sz, 2020). In general, the species feeds on small mammals, such as the European souslik and European hamster, but on occasion,

observations have been made indicating that they prey on small birds as well. Owing to a drastic decline of small mammals in Hungary, the European siskin only comprised 1% of the Saker's diet in a study done by BAGYURA et al. (2019a), whilst the feral pigeon has become increasingly frequent prey for this species. In addition to the aforementioned decline in preferred prey, habitat loss, various forms of bird-crime (i.e. intoxication, illegal hunting), electrocution and capture for use in falconry are aspects hindering the Saker falcon populations of Hungary (BAGYURA et al., 2019a).

Peregrine falcon (*Falco peregrinus*)

IUCN least concern, Hungarian strictly protected species, theoretical conservation value: 500,000 HUF.

Present across the globe, the Peregrine falcon had a well-established nesting population in Hungary until the 1960s when the species all but disappeared from our country not to return until the late 1990s (HARASZTHY, 2019). Hungarian individuals are resident and do not migrate, however specimens from northern regions in Europe do come to overwinter (PROMMER & BAGYURA, 2009). Preferring to nest on rock faces, breeding pairs have also been observed occupying trees and sites on high buildings, though northern populations may build their nests on the ground. Initiating their breeding season in April, Peregrine falcons lay 2-6 eggs, which they incubate for 30-32 days and their chicks will fledge around 35-42 days of age (HARASZTHY, 2019). In 2018, 84-90 pairs were recorded in Hungary (MME RMV SZ, 2020). Being the most rapid birds in flight, the Peregrine falcon prey predominantly on birds whilst soaring through the air (BAGYURA, 2019). Species such as pigeons that ascend to high altitudes are at risk of being predated by the stealth of these falcons (BAGYURA & PROMMER, 2019). Many aspects affecting other species of raptors may also cause morbidity and mortality of Peregrine falcons, however, owing to their choice of prey, disputes with pigeon fanciers involve the persecution of this species directly (LÓPEZ-LÓPEZ et al., 2009).

Nocturnal predatory birds

Eurasian scops owl (*Otus scops*)

IUCN least concern, Hungarian strictly protected species, theoretical conservation value: 100,000 HUF.

The distribution of the Eurasian scops owl extends from Africa throughout the southern part of Eurasia (BAJOR, 2019) and is not only considered a long-distance migrant (KALOTÁS, 2009), it is also the only migratory resident owl species in Hungary (BAJOR, 2019). Arriving in the month of April, this relatively small owl occupies burrows of trees on the perimeter of the mountainous regions of the country, though over the past few decades, they have become

increasingly numerous in forested areas and near marshes on the plains. Laying 3-7 eggs, the female will subsequently incubate for 24-25 days and after hatching, the chicks fledge at 20-32 days of age (HARASZTHY, 2019). Between 2017-2018 the estimated population in Hungary was 1,600-4,300 breeding pairs (MME RMvSZ, 2020). MORI et al. (2016) disclose that in Italy, the greatest component of the analyzed pellets was insects (Coleoptera), however in some cases, birds, rodents and geckos were also found to be a part of their diet. Fragmentation of habitat and decline in prey due to extensive use of pesticides are the primary factors threatening this species, though the risk of hunting and predation along its migratory route also impact the population of this small-bodied owl (LACZIK, 2017).

Eurasian eagle-owl (*Bubo bubo*)

IUCN least concern, Hungarian strictly protected species, theoretical conservation value: 500,000 HUF.

The Eurasian eagle-owl is a largest-bodied resident owl species occurring across the Eurasian continent and nesting in Hungary (PETROVICS & FIRMÁNSZKY, 2009a; BAJOR, 2019). Residing in the northern mountainous regions of the country and forested areas on the plains in the vicinity of rivers, they remain near their nesting ground throughout the year (HARASZTHY, 2019). In Hungary, the vast majority nest in quarries, however cliff faces also provide suitable sites for them, and on occasion, trees, and the nests of other birds are also utilized by these owls (SCHWARTZ, 2019). They start reproducing late February to early March, and lay 1-5 eggs, which the female incubates for 31-36 days. Chicks will fledge after 55-70 days after hatching (HARASZTHY, 2019). Between 2015-2108, 76-86 pairs were recorded in Hungary (MME RMvSZ, 2020). With a variable pallet, Eagle-owls feed on a variety of songbirds, small mammals and insects (SCHWARTZ, 2017a), though *Leporidae*, household poultry (SERRANO, 2000), lizards and amphibians (SÁNDOR & IONESCU, 2009), and the preferred brown rat (*Rattus norvegicus*) (DARÓCZI, 2017b; SCHWARTZ, 2017; BAJOR, 2019) have also been found as part of their diet through pellet analyses. Major risk factors affecting these nocturnal hunters include various forms of trauma elicited by collisions, though electrocution also poses a considerable risk (DARÓCZI, 2017b). Additionally, secondary poisoning through the prey they consume has been deemed a potential cause of morbidity and mortality (DARÓCZI, 2017b; DEÁK et al., 2020).

Tawny owl (*Strix aluco*)

IUCN least concern, Hungarian protected species, theoretical conservation value: 50,000 HUF.

The range of the Tawny owl extends across Eurasia and Northern Africa, and therefore includes Hungary, where it is a resident species (PETROVICS & FIRMÁNSZKY, 2009b; BAJOR,

2019). Traditionally, these birds nested primarily in the northern mountainous regions, however today, they are readily found in forested areas on the plains and even in larger urban parks. Occupying nest holes in large tree, their breeding season can begin as early as January when they lay 3-5 eggs, which are incubated for 28-30 days. Fledging occurs around 32-37 days of age (HARASZTHY, 2019) and the current population in Hungary was estimated at 5,000-8,000 pairs between 2013-2018 (MME RMvSZ, 2020). GALEOTTI & PAVAN (1991) analysed the pellets of urbanized Tawny owls and reported that in the winter months, half of their prey is composed of mammal species, whilst the other half is that of birds. Many human-related threats are worth mentioning in the case of the Tawny owl, including those of electrocution and collision, however ingestion of poisoned rodent prey, and habitat and nesting site loss are also key factors. Moreover, the Northern goshawk, the Eurasian eagle-owl, Beech martin (*Martes foina*) and the European pine martin (*Martes martes*) are notorious for predated their eggs, chicks as well as smaller-sized individuals (TÓTH, 2017).

Ural owl (*Strix uralensis*)

IUCN least concern, Hungarian strictly protected species, theoretical conservation value: 100,000 HUF.

The Ural owl can be found throughout Eurasia and is a resident species of the Hungarian fauna (BAJOR, 2019). Nesting predominantly in forested areas of the mountainous regions of our country (HARASZTHY, 2019), in colder winters, individuals have also been sighted on the plains (BAJOR, 2019). Predominantly occupying nest holes in beech trees, they have been observed to occupy nests of Black storks (*Ciconia nigra*), Common buzzards and Northern goshawks as well. Laying 2-6 eggs in March, the female will incubate them for 28-29 days and chicks fledge when they reach 34-35 days of age (HARASZTHY, 2019). 80 pairs of Ural owls were recorded in 2018 (MME RMvSZ, 2020). Living predominantly in forested habitats, studies reveal, that the species preys principally on small mammals, displaying a particular preference for Field mice (*Apodemus* sp.), the distribution of which enables the dispersal of this owl species (HARASZTHY, 2019). Deforestation, loss of nesting habitat and the decline of their food source are factors primary influencing the population dynamics of the Ural owl (DARÓCZI, 2017a).

Long-eared owl (*Asio otus*)

IUCN least concern, Hungarian protected species, theoretical conservation value: 50,000 HUF.

Scattered across the northern hemisphere, the Long-eared owl is a well-known resident and breeding species in Hungary (BAJOR, 2019). Dispersed throughout the country for most of the year, individuals often form groups in the winter months as birds from northern regions of

the species range migrate south for the winter (LACZIK & SEBE, 2009). Correlation can be seen between the number of birds in these winter congregations, decreased ambient temperature and increased precipitation in a given season (VÉGVÁRI & KONYHÁS, 2003). Frequently occupying the nest of corvids and choosing nesting sites in wooded areas adjacent to agricultural fields, Long-eared owls lay 2-7 eggs in March, after which, the female incubates them for 25-30 days. Chicks will fledge 21-24 days after hatching (HARASZTHY, 2019). The current population is estimated at 10,000-12,000 pairs (BAJOR, 2019). The Common vole has been documented to be the predominate prey-source during the breeding season in Hungary (HARASZTHY, 2019), though a study in Israel indicates, that in this region, pellets were primarily composed of House sparrow (*Passer domesticus*) remains (KIAT et al., 2008). As their chicks leave the nest, still incapable of fending for themselves, they are at an increased risk of predation (CSIPÁK, 2017) and are indeed often collected by the general public and admitted to wildlife rescue centers in high numbers (CSIPÁK, 2017; SÓS-KOROKNAI et al., 2020a). Moreover, intensive agriculture and the use of pesticides and rodenticides pose great threats to the Hungarian Long-eared owl population, as well as electrocution and increased vehicular traffic across the country (CSIPÁK, 2017).

2.2. Special anatomic characteristics of raptors and owls

Raptors possess physical attributes that allow them the ability to hunt through their stealth and speed in flight, whilst owls have the ability to fly nearly soundlessly. The feathers of diurnal predatory birds lie close to their bodies enabling them to remain aerodynamic as they glide through the air to capture prey (GÁL, 2006). Their tail, primary and secondary flight feathers are moulted continuously to prevent them from losing their ability to fly (SVENSSON et al., 2018) as opposed to anseriform species which change these feathers all at one time (GÁL, 2006; SVENSSON et al., 2018). Owls possess the ability to soar through the air in silence because their feathers have fringes and a velvet-like dorsal aspect that could explain this phenomenon (BACHMANN et al., 2007). Some species, particularly those living in colder climates, have a specialized feather-covering of their talons (GÁL, 2006). The talons of all predatory avian species are spear-like and well specialized for the task of grasping and killing their prey, which is particularly important to those species that snatch their prey out of water (GÁL & MAROSÁN, 2002).

The skeletal system of birds is light, strong and comprised partially of pneumatic bones. The pneumatic cavities in the humerus and tibiotarsus are connected to the air sacs, allowing these bones to remain light and therefore aid flight (GÁL, 2006). Raptors have very well

developed superficial and deep pectoral muscles (*musculus pectorales superficiales et profundus*), which are inserted onto a curved sternal crest (GÁL & MAROSÁN, 2002).

All species of diurnal and nocturnal birds of prey have curved, hook-like beaks. In raptors, the upper portion of the beak is used to eviscerate their prey (GÁL & MAROSÁN, 2002), whilst carrion eaters have particularly strong and developed upper beaks enabling them to strip the flesh off the bones of cadavers (GÁL, 2006). The beaks of owls are primarily used in the snatching and killing prey as they tend to swallow victim whole. The tongue of owls is cone-shaped and their choanal fissure, connecting the oral and nasal cavities, has a well-developed row of papillae, even though the cleft itself is relatively narrow (SHAWKI et al., 2016). The esophagus of owls is very versatile, allowing them to consume relatively large-sized prey. As for raptors, they have a spindle-shaped crop (FORD, 2010) and subsequently, a sizable and expansive glandular stomach located at the base of their heart, which produces a portion of the essential digestive enzymes. FORD (2010) states that the pH level of the raptors stomach is 1.6-1.7, and 2.4 in owls, which is subsequently buffered by the pancreatic enzymes in their small intestine. The gizzard of owls is thin, sack-like with a muscular wall lacking gastroliths (grit) and it is typical for them to regurgitate undigested remains in the form of pellets (FOWLER, 1986). Pellets are compact, oval structures composed of the undigestible remnants of their prey, including fur, teeth, claws and pieces of larger bones. In insectivorous species, chitin shells can also be detected in pellets. Analysis of pellets is a method widely used to determine the composition of the diet of a given species (GÁL & MAROSÁN, 2002).

The gastrointestinal tract of raptors is relatively short compared to other taxa, with non-functional, underdeveloped appendices (FOWLER, 1986; GÁL, 2006; FORD, 2010). The colorectum in diurnal predators is short, straight and strewn with mucosal villi (FORD, 2010). The excrement of these species is loose, with a chalk-white uric acid portion and a fecal portion brownish-black in colour.

The lungs of predatory birds are interconnected with a network of air sacs as in other avian species. The syrinx of these species is membranous and responsible for vocalization, while the paranasal sinuses are not as well developed (GÁL & MAROSÁN, 2002).

The excretory system enables these birds to remove by-products such as uric acid from their circulation. This does not differ significantly from that of other avian species (GÁL & MAROSÁN, 2002).

The vision of raptor species is highly developed and is their most important sensory organ. These species possess the highest visual acuity, which is attributed to the tubular shape of their eye, their large pupils and a high density of photoreceptors in their retina (GONZÁLEZ-

MARTÍN-MORO et al., 2017). The orbit, wherein the globe sits, is composed entirely of bony structures and is the site of attachment of the muscles moving the eye (GÁL & MAROSÁN, 2002). Over the years, work has been done to establish the ocular parameters of raptors and owls (BECKWITH-COHEN et al., 2005) and reference values have been formulated for Schirmer tear tests and intraocular pressure in Great grey owls (*Strix nebulosa*) and Snowy owls (*Bubo scandiacus*) (WILLS et al., 2016). Nocturnal predators are able to hear and sense even the most subtle noise made by their prey owing to their exceptionally sensitive auditory system. Moreover, the ears of owls are positioned asymmetrically in the skull (KNUDSEN, 1981).

2.3. Body dimensions

Biometric data of birds of prey emphasizes the measurement of body length in various species, though wingspan is also recorded by various authors (**Table 1**).

Table 1. Body length and wingspan of a number of predatory bird species included in a study by HUME (2003).

Scientific name	Body length (cm)	Wingspan (cm)
<i>Strix aluco</i>	37-39	94-104
<i>Otus scops</i>	19-21	47-54
<i>Asio otus</i>	35-37	84-95
<i>Haliaeetus albicilla</i>	70-92	200-245
<i>Buteo buteo</i>	50-57	113-128
<i>Buteo lagopus</i>	50-60	120-150
<i>Pernis apivorus</i>	52-60	135-150
<i>Accipiter nisus</i>	28-40	60-80
<i>Accipiter gentilis</i>	48-61	95-125
<i>Falco tinnunculus</i>	34-40	65-80
<i>Falco subbuteo</i>	28-35	70-84
<i>Falco peregrinus</i>	39-50	95-110

Detailed biometric data is accessible for a number of predatory avian species present in Hungary. FARAGÓ (2002; 2015) examined the wing length, tail length, beak length and the tarsometatarsal length in those raptor species which have an impact on the game farming industry (**Table 2**).

Table 2. Biometric data of various raptor species relevant to our study (FARAGÓ, 2002).

Species (sex)	Wing length (mm)	Tail length (mm)	Beak length (mm)	Tarsometatarsal length (mm)
<i>Accipiter gentilis</i> (male)	300-325	210-235	20.5-24.0	72.5-78.0
<i>Accipiter gentilis</i> (female)	341-365	243-270	23.0-27.5	82.0-87.5
<i>Buteo buteo</i> (male)	380-415	193-222	20.0-23.6	72.5-82.0
<i>Buteo buteo</i> (female)	401-440	210-235	21.7-25.5	70.0-81.0

Studies of body dimensions show the correlation between the wing growth and the age of the individual, as was seen in the Barn owls (*Tyto alba*) in the study by TAYLOR (1993), hence making detailed biometric measurements useful in determining the age and sex of birds.

2.4. Non-infectious diseases of birds of prey

The following section outlines causes of morbidity and mortality elicited by non-infectious diseases in various organ systems.

Damages to the plumage or missing feathers of raptors, especially the primary flight feathers or the tail, can lead to impaired ability to maneuver in the air and can thus hinder hunting capability in affected individuals (GABRISCH & ZWART, 1987; GÁL, 2006).

Factors damaging the integrity of the integument can mainly be attributed to mechanical trauma. These occur regularly during collisions or abrasion with various objects, eliciting injuries that harm all layers of the skin almost without exception. Other underlying structures, such as the musculature or the skeletal system are often damaged in conjunction with the skin. The loss of integumentary integrity can serve as a site of entry for pathogens and may result in local cellulitis. Anthropogenic factors can also damage the skin and are often induced by foreign bodies such as bullets or shrapnel. In owl species, the above-mentioned skin injuries appear more significant due to their soft and loose plumage (GÁL, 2006).

Unfortunately, many predatory birds are presented with burn wounds caused by collisions with powerlines, an estimated 0.9-11.6 million birds are believed to succumb as a result of electrocution annually in the United States (KAGAN, 2016). Aside from shock inflicted from impact with the powerline, second- and third-degree burns can be seen on the skin. Two points of contact are typical and especially seen on the contralateral wings and talons. Lesions are initially edematous, followed by locally occurring coagulation necrosis leading to parchment-like integument in the affected area (KAGAN, 2016). Overall, the prognosis is poor to grave in the case of such injuries, can result in neurological or circulatory failure (GÁL, 2006) and KAGAN (2016) states that electrocution can lead to hemopericardium and damages to the vasculature walls in conjunction with the burn wounds. Some authors go so far as to say collision with powerlines is the number one cause of mortality in birds of prey (GUIL et al., 2011; MELERO et al., 2013; KAGAN, 2016) and even vulnerable species, such as the Steller's sea eagle (*Haliaeetus pelagicus*) have become victims of this ailment (SAITO et al., 2019). In Hungary, previous studies indicate that the Common buzzard, the Long-eared owl (TÓTH, 2018) and the Common kestrel (SÓS-KOROKNAI et al., 2020b) are frequently victims of electrocution, however over the past few decades, 13% of mortality of Eastern imperial eagles was also attributed to this ailment (DEÁK et al., 2020).

Subcutaneous hematomas can be a result of colliding with a static or mobile object during flight. Additionally, substantial impact may also lead to the rupture of internal visceral

organs (most commonly the liver) and consequently hemorrhage into the celomic cavity (GÁL, 2006).

Tumors have also been described in predatory avian species of which papillomatosis is considered to be the most documented. However, SWAYNE & WEISBRODE (1990) did published a case of a mastocytoma in a Great horned owl (*Bubo virginianus*), whilst GREENLEE et al. (2011) reported a bronchial carcinoma in a Red-shouldered hawk (*Buteo lineatus*) and VISSER et al. (2018) record a B-cell lymphoma in the central nervous system of a Bald eagle (*Haliaeetus leucocephalus*) resulting in nystagmus and an inability to fly. Furthermore, FORBES et al. (2000) compiled a list of 122 neoplasms across 44 species of birds in an extensive review indicating that though rarely, but neoplasms are to be considered in raptor medicine.

Pododermatitis is an ailment primarily affecting the sole of captive raptors and owls. Individuals undergoing rehabilitation, particularly eagles and hawks, are considered to be at an increased risk of developing such lesions (RODRIGUEZ-LAINZ et al., 1997). Thus, care must be taken in wildlife rescue centers, to ensure that birds residing at the facility for extensive period of time maintain adequate circulation to their feet and are housed on suitable flooring. If blood circulation in the talons is impaired, necrosis of affected region can follow, resulting in a potential entry site for pathogens (JONES, 2006). Bacteria can invade and multiply in the underlying tissues and infection can spread to the joints and tendons (GÁL, 2006) and consequently, osteomyelitis (SANDER et al., 2013) or endocarditis may occur (WILLETTE et al., 2009). BEAUFRÈRE et al. (2016) account of a case of pododermatitis with subsequent tetanus infection in a Gyrfalcon (*Falco rusticolus*), indicating just how high a risk a secondary infection can pose.

Injuries pertaining to the musculature of raptors are perceived as tears or other damages ensuing from mechanical trauma. Extensive hemorrhage can be seen in the muscle layers subsequent to serious injury (GÁL, 2006).

The skeletal systems alterations are primarily those attributed to mechanical trauma and are typically fractures. Wings are the most frequently localizations of such injuries leading to hindrance in flight, and therefore survival. The integument overtop these fractures can either remain intact, or in some instances, in regions where the bone is merely covered by a thin overlying soft tissue layer, the skin can be pierced leading to open fractures. Such alteration can often be accompanied by injury to the adjacent joints, tendons and muscles as well (GÁL, 2006).

BAUDVIN (1997) notes in his work done by analyzing the cause of mortality along highways in France, that the majority of predatory birds suffered from various forms of trauma.

Such injuries accounted for 1,598 deaths in the study timeframe pertaining to the species listed in **Table 3**.

Table 3. Species involved in road traffic accidents in a study done by BAUDVIN (1997).

Diurnal predatory species		Nocturnal predatory species	
Species	Number of cadavers	Species	Number of cadavers
<i>Buteo buteo</i>	213	<i>Tyto alba</i>	674
<i>Falco tinnunculus</i>	48	<i>Asio otus</i>	300
<i>Falco columbarius</i>	1	<i>Strix aluco</i>	53
<i>Milvus milvus</i>	7	<i>Athene noctua</i>	1
<i>Milvus migrans</i>	2		
<i>Accipiter gentilis</i>	1		
<i>Accipiter nisus</i>	1		

BESTON et al. (2016) considers the strength of the wind and air turbulence to play a key role in altering flight pattern of birds and their ability to change direction even to the point of being responsible for their collision with objects if circumstances are hostile.

In owls, a study done by NEWTON et al. (1997) states that in the examined region, the past 30 years yielded mechanical trauma to be responsible for injuring and killing a majority of Barn owls. Other publications demonstrate a similarly high incidence of traumatic injuries seen in diurnal and nocturnal predatory avian species (WENDELL et al., 2002; KOMNENOU et al., 2005; MOLINA-LÓPEZ et al., 2011; MONTESDEOCA et al., 2016).

Though extremely rare in free ranging birds, captive raised raptors have been observed to develop rickets, a disorder in ossification. The background of this condition is an incorrect calcium to phosphorous ratio in the feed leading to D-hypovitaminosis and subsequent disturbances in ossification of the developing bones of the individual. Long bones are most discernibly affected and will not be sufficiently ossified, leading to bowed or splayed limbs directly influencing the movement of these birds (FOWLER, 1986; GÁL, 2006).

The so-called “angel wing” is a regularly seen pathological finding in duck and goose species (GÁL, 2006), occurring when the developing wings of young birds become dislocated. This abnormality causes the wing to lie in an utterly unphysiological position, giving the impression that the wing has been turned upside down. Seen rarely in predatory birds, ZSIVANOVITS & MONK (2006) account for an example of a Northern goshawk with bilateral “angel wing”.

Other alterations affecting the bones are rare in birds of prey, however, there is a single account of chondro-osseous metaplasia consistent with synovial chondromatosis in a Great horned owl (XIE et al., 2014).

Ailments causing changes to the beak of predatory birds are also seldom documented. Nonetheless, GÁL (2006) account of a developmental malformation of the lower portion of the

beak in a Common buzzard leading to the inability to take up food and subsequently, emaciation. Furthermore, work done by the same author documents fusion of the upper and lower portions of a beak in a Northern goshawk elicited by a fibroma (GÁL, 2006). Moreover, in a review of orthopedic cases involving the head of raptors, WHEELER (2002) reports that the vast majority beak alterations occur consequently to trauma.

Zootrichobezoars occur under physiological circumstances in the glandular portion of the stomach and the gizzard of diurnal and nocturnal predators. These bezoars are composed of the indigestible parts of the prey (hair, teeth, bones and claws) and are termed pellets. These are regurgitated by the animal at the end of their digestive process. Pellets are widely used in scientific studies to determine the prey of various species and populations.

As an ailment of the alimentary tract, ileus can occur in birds of prey particularly in raptors according to FORD (2010), when prey items are insufficiently digested, and bones become lodged in the intestinal tract further distally. Colonic cloacal prolapse leading to the protrusion of the cloaca is well documented in predatory avian species (DUTTON et al., 2016) generally requiring surgical attention. Furthermore, intussusception has been recorded in a Tawny eagle (*Aquila rapax*) where the ileo-ceco-rectal alteration required surgical attention and anastomosis of the intestine (SABATER et al., 2015).

In raptors, the most frequent non-infectious hepatic pathology documented is that of liver rupture associated with mechanical trauma. In such cases, damage to the capsule is followed by internal bleeding and death in the majority of birds (GÁL, 2006). As far as liver pathology is concerned, SHRADER et al. (2014) recorded a case myelocytomatosis in the liver and spleen of an Eastern screech owl (*Megascops asio*), while WERNERY et al. (2004) and HAMPEL et al. (2009) detected hepatic amyloidosis in numerous falcon species where hepatomegaly, and a brownish green coloured liver was observed on necropsy. In such instances, the liver is firm and rubber-like on palpation during necropsy owing to the pathological protein accumulation therein (DOBOS-KOVÁCS, 2014).

Asphyxiation may also arise in predatory avian species when the upper respiratory tract becomes blocked. BERKÉNYI (2012) documents a case in a Merlin (*Falco columbarius*) where a fibrin particle, originating from an oral fungal inflammatory process, obturated the upper respiratory tract and led to the suffocation of the individual. Moreover, WEECH et al. (2003) found asphyxiation to be attributed to trauma in Bald eagles.

GÁL et al. (2003) conducted a study on the examination of mortality in 21 raptors and found visceral gout to be the cause of death in 5% of these birds, with the Northern goshawk being the most frequently affected species. With gout, one must always consider renal damage

as the causative factor, which can have both an infectious and a non-infectious origin. Of the non-infectious contributing factors GÁL (2006) deems lack of drinking water (unlikely in raptors) and the uptake of nephrotoxic material (lead, cadmium and fungal toxins, such as ochratoxin) to be the most significant. Additionally, nonsteroidal anti-inflammatory drugs (NSAIDs) such as flunixin (ZORRILLA et al., 2015; ELENI et al., 2019) and diclofenac (SHULTZ et al., 2004; METEYER et al., 2005) are known etiologies of visceral gout in predatory birds, with vultures showing particular sensitivity to these agents. Uric acid build-up on the visceral organs of raptors with renal failure can be found on gross necropsy as the predominant alterations (WERNERY et al., 2004; METEYER et al., 2005).

Of the ailments to the thyroid gland published in raptors, unilateral hyperplasia in an adult female Saker falcon is noteworthy (RAHIM et al., 2013). According to the authors in this instance, this abnormally enlarged thyroid gland was not of a neoplastic nature. However, BRANDAO et al. (2012) did record malignancy of this organ through a follicular thyroid carcinoma in an adult Barred owl (*Strix varia*) with an inability to fly and identified pancytokeratin and thyroglobulin reactivity on immunohistochemistry. Additionally, SAMOUR et al. (2001) diagnosed a thyroid cystadenocarcinoma in a Saker falcon, whereas BATES et al. (1999) discovered a thyroid adenocarcinoma in a Bald eagle.

Widely known in to cause mortality in human medicine, atherosclerosis is also known in avian medicine with a pathogenesis similar to that in people. Though this disorder has been identified on several occasions in the major vessels around the heart in parrots (BEAUFRÈRE, 2013), work by GARNER & RAYMOND (2003) attributed cause of death to be due to atherosclerosis in 4.2% of Falconiformes and 2.9% of Strigiformes. However, GRINER (1983) found 10.8% of the predatory birds to be affected by this ailment 20 years earlier, whilst NEMETH et al. (2016) considers this ailment to be a common in raptors. POTIER (2013) suggested a link between the feeding of polyunsaturated fatty acids and predisposition in certain species such as the Brahminy kite (*Haliastur indus*) (POTIER, 2013) and Black kite (*Milvus migrans*) (FACON et al., 2014) in captivity. Furthermore, GÁL (2006) determined hypertrophic cardiomyopathy in conjunction with atherosclerosis in a Saker falcon and implies that the disorder led to regressive changes in the vessel walls and their subsequent weakening, rupture and death of the individual. Additionally, BEAUFRÈRE (2013) asserts atherosclerosis to occur subsequently to *Chlamydia psittaci* infection in predatory birds linking non-infectious diseases to their infectious counterparts.

Through their study and examination into the cause of mortality in raptors, GÁL et al. (2003) stipulates the frequency of shock, followed by circulatory failure in birds with traumatic

injuries. Furthermore, KAGAN (2016) describes hemopericardium as well as the rupture of larger vessels to be consequences of electrocution affecting the circulatory system.

Numerous alterations can affect the eyes of raptors, including bilateral cataracts subsequent to electrocution, as seen in a juvenile female Great horned owl by DEES & MACLAREN (2013). LAU et al. (2016) account of unilateral corneal dryness in the same species where the pathology caused disruption to the function of the nictitating membrane with a definitive diagnosis documented to be keratoglobus. Moreover, signs of vitamin-A hypovitaminosis allegedly led to swelling of the eyelids in Golden eagles (*Aquila chrysaetos*) occurring in conjunction with metaplasia of the propria of the esophagus in this species (FOWLER, 1986).

Multiple conditions can lead to central nervous signs (CNS) in predatory birds and though in most cases the etiology is an infectious agent (see below), vitamin deficiencies can also provoke such symptoms. FOWLER (1986) describe a case of vitamin-B₁ deficiency in a Prairie falcon (*Falco mexicanum*) eliciting CNS signs. Moreover, B₂-hypovitaminosis can elicit paralysis of the muscles in the limbs in such animals. SAMOUR et al. (2016) also describe neurologic symptoms to vitamin-B₆ toxicity in various falcon species and states a 5 mg/kg i.m. to be highest non-lethal dosage.

Though rare, developmental abnormalities have also been published in predatory avian species. COOPER (1984) accounts of cases of supernumerary talons in Peregrine falcon and Merlin and GÁL (2006) reports a case of a twin embryo in a Saker falcon egg, which died in the second developmental phase.

2.5. Intoxication and trace element accumulation

Top predatory avian species are at great risk for the uptake of fatally toxic poisonous toxins. Substances that are harmful to these charismatic birds are often insecticides and pesticides used to treat plants in the agriculture industry. Many of these products are used illegally or are intended for poisoning pest carnivores (SÓS et al., 2013) and vermin (CHRISTENSEN et al., 2012).

Anticoagulant intoxication (AR) was established to be the cause of morbidity in raptors at a Wildlife Clinic in Massachusetts in the United State of America (MURRAY, 2011), while WALKER et al. (2016) examined the impact of these poisons (intended for the extermination rodents) on the Red kite (*Milvus milvus*) population in England over a 5-year period. The latter state that the most harmful substances to mammals and birds alike, are the second-generation ARs. In this study, levels of difenacoum, bromadiolone and brodifacoum were measured in hepatic tissues of deceased birds of prey and concluded, that these materials can cause

secondary toxicosis, particularly to those species consuming rodents. At the Budapest Zoo and Botanical Garden Wildlife Rescue Center brodifacoum poisoning was identified to be the cause of death in a rescued Eurasian eagle-owl breeding pair (unpubl. data) and indeed intoxication by these substances is becoming a greater hazard to birds of prey in Hungary (DEÁK et al., 2020). NEWTON et al. (1997) also confirms the existence of second-generation rodenticide intoxication in Barn owls in the United Kingdom, whereas SARAVANAN & KANAKASABAI (2004) and HUANG et al. (2016) describe similar findings in this species in Canada and India, respectively. Furthermore, ALBERT et al. (2010) mention that bromadiolone and brodifacoum pose the utmost danger of the ARs to a number of owl species in a retrospective study done in Canada. Other authors have similarly implicated various rodenticide agents to be the cause of morbidity in various species of nocturnal and diurnal predatory avian species (THOMAS et al., 2011; STANSLEY et al., 2014). ELLIOT et al. (2014) discovered rodenticide metabolites in owl pellets in the vicinity of agriculture, however, a significant link was not confirmed. RUIZ-SUÁREZ et al. (2014) identified AR residues to be more frequently detected in mammal-eating birds of prey compared to those that feed on avian species.

Through a study examining contaminants in birds of prey, ERIKSSON et al. (2016) found polyfluorinated alkyl acid in some individuals, and polychlorinated dibenzofuran and polychlorinated dibenzo-dioxin contaminants were also discovered in Great horned owls. Additionally, ARRONA-RIVERA et al. (2016) determined organochlorine contamination in the Ferruginous pygmy-owl (*Glaucidium brasilianum*) through the investigation of blood and feather samples, and this very same chemical was observed in Tawny owls in Norway by YOCCOZ et al. (2009). The latter stated that eggshell thinning was notable in individuals that ingested the substance, thus implicating organochlorine contaminating in having a negative correlation with breeding success.

Multiple studies have examined the accumulation of various heavy metals in predatory birds. Lead load of raptors is a subject that has drawn the attention of many professionals working with wildlife over the past decades. KRONE (2018) states that aside from areas of volcanic activity, lead levels in birds of prey should be insignificant under ideal circumstances and that the presence of this heavy metal in these species always has an anthropogenic etiology (i.e. previous leaded gasoline and lead-containing chemicals and paints, and hunting ammunition). In a study by FRANSON et al. (1983) on the American kestrel (*Falco sparverius*), elevated levels of lead were measured in their circulation altering the function of delta-aminolaevulinic acid dehydratase (ALAD) and significant correlation was determined. Therefore, by measuring ALAD activity, the amount of lead in an individual can be quantified.

Similarly, the detection of this enzyme was used to identify lead levels in a study by ESPÍN et al. (2015) in Griffon vulture (*Gyps fulvus*) and Eagle owl. PAIN et al. (2007) and KRONE (2009) provide explanation for two ways that predatory birds can become exposed to lead in nature. Firstly, if individuals are shot, the lead from the bullets can penetrate their bodies and become lodged in their muscles, however, lead intoxication can also occur if the prey source contains particles of this heavy metal. Toxicosis occurring from the former has a chronic course, whereas intoxication through ingestion is often more devastating with a rapidly progressing clinical picture and death if left untreated particularly in instances with a high lead load. The presence of bullets or shrapnel in the prey of raptors is important to consider in rescued specimens and very well plays a key role in whether a patient can be rehabilitated or not (GÁL, 2006). Consequently, the aforementioned author emphasizes the importance of lead load analysis *in vivo*, which is most frequently and often routinely performed by LeadCare® I or II analyzers (FALLON et al. 2017; YAW et al. 2017).

Mercury accumulation has been studied in predatory birds and KALISINSKA et al. (2014) considers this heavy metal to affect raptors based on their analysis of soft tissue samples originating from White-tailed eagles and Osprey (*Pandion haliaetus*) in Poland. GRUZ et al. (2019) assessed the presence of mercury as a contaminant in Hungarian birds of prey without significant results, however, in this case, feathers were used as opposed to soft tissue samples. Interestingly, DIETZ et al. (2006) stipulated that through the examination of feathers, the age of the individual could be determined based on the degree of mercury accumulation.

OROSZ et al. (2001) conducted a detailed study on heavy metal accumulation in Hungarian raptors and owls by analysis of cadmium, lead, mercury and chromium in liver, kidney and bone tissue samples. The authors included 12 Barn owls, one Little owl (*Athene noctua*), one Eastern eagle owl on the nocturnal predator front and one Short-toed snake eagle (*Circaetus gallicus*), one Peregrine falcon, one Eurasian hobby, one Red-footed falcon (*Falco vespertinus*), one Common buzzard and one Hen harrier (*Circus cyaneus*) as raptors in this investigation. Overall, all metals were found in a lower concentration in nocturnal predators with the Barn owl showing the highest values of cadmium in the liver (0.37 mg/kg) and kidney (1.17 mg/kg) in this group. Levels of lead were not determined to be elevated significantly on a whole, except in Little owl bone tissue where the concentration was 10.9 mg/kg. Mercury was present in higher quantity in the liver (23.3 mg/kg) and kidney (17.6 mg/kg) of the Peregrine falcon, but chromium values were below the threshold (0.15 mg/kg) in all cases the liver and kidney were sampled. Highest values of zinc were measured in the liver (461.0 mg/kg) of the Barn owl in the scope of this study.

Work done with similar methodology by KIM & OH (2016) in Korea determined elevated levels of lead (6-30 mg/kg) in 17 examined individuals. These authors determined in an additional study that lead levels were at the highest in the liver and bone samples originating from Eagle owls, whilst cadmium was recorded at the greatest concentration in the liver and kidney of this species, suggesting that the former tissue samples are recommended for such studies (KIM & OH, 2012). Studies performed by HORAI et al. (2007), PÉREZ-LÓPEZ et al. (2008), PAGEL et al. (2012) and KITOWSKI et al. (2017) also measured concentrations of a number of different trace elements and heavy metals from various tissue samples of predatory birds in various locations across the globe. Other authors (DEBÉN et al., 2012; ANSARA-ROSS et al., 2013; ROQUE et al., 2016; GRUZ et al., 2019) however, have conducted similar work to the aforementioned biomonitoring studies of trace elements using feather samples in diurnal and nocturnal predatory birds.

As heavy metals have been documented to accumulate the higher up you go in the food chain and top predators, for example owls, are at a higher risk of becoming intoxicated (DUXBURY & HOLROYD, 1997). PÉREZ-LÓPEZ et al. (2008) examined sublethal accumulation of various heavy metals in predatory birds in Spain and concluded that this phenomenon has a negative association with the survival of these birds. KITOWSKI et al. (2017) goes so far as to say that immature Common buzzards have been found to have a higher lead load level, as these birds often are inexperienced hunters and scavenge on carrion and wounded prey, which have an increased probability of containing bullets. Moreover, ESPÍN et al. (2014), through an examination of owls, deems even low concentrations to elicit negative impact on the health of these birds because of the oxidative stress generated by these heavy metals. SHEFFIELD (1997) states that as top predators, owls are not only very sensitive to accumulated heavy metals in their prey, but the metabolites of pesticides in their food source can lead to their morbidity as well.

Carbofuran, a cholinesterase inhibitor, is an insecticide used widely in agriculture and implicated in deliberate poisoning worldwide (GUPTA, 1994). Banned in the European Union in 2008, baits are often used to poison mammalian carnivores (NTEMIRI et al., 2018) and predatory birds are not always the intended victims (SÓS et al., 2013). As these organophosphates inhibit cholinesterase activity in the brain (FLEISCHLI et al., 2004), neurological signs, such as tremors, vomiting and clenched talons are seen in clinical cases, whereas the latter can also be observed *post mortem* (SÓS et al., 2013). Atropine can be administered as an antidote to alleviate the symptoms of carbofuran toxicity (GUPTA, 1994; SÓS et al., 2013) and *in vivo* diagnostics can be performed by determining cholinesterase activity

using the Ellman's method (SHIMSHONI et al. 2012). Intoxication has been widely documented in eagles in Hungary (SÓS et al., 2013) though current studies have shown the highest incidence of deliberate poisoning to be in the Common buzzards (DEÁK et al., 2017; DEÁK & HORVÁTH, 2018). Deliberate poisoning is considered to be bird crime and the discussion and implementation of anti-poisoning strategies (SÓS et al., 2013; NTEMIRI et al., 2018) are of vital importance to decrease incidence in the future.

2.6. Infectious diseases of birds of prey

2.6.1. Viral diseases

Strigid herpesvirus-1: Herpesviral infection affecting owl species across the globe is caused by the Strigid herpesvirus 1 (StHV-1). In Hungary, this illness has been found in Long-eared owl, Snowy owl and Eurasian eagle owl, and common symptoms seen in affected individuals are lethargy and inappetence (GÁL, 2006). Otherwise called inclusion body hepatitis (RAGHAV & SAMOUR, 2019), it is not surprising that on necropsy, necrotic foci are visible in the liver, in addition to the spleen and the bone marrow (GÁL, 2006). Tracheitis can also be seen (GÁL, 2006) and alterations to the eye (chronic superficial keratitis and proliferative conjunctivitis) have been recorded (GLEESON et al., 2019). In their work with StHV-1, RAGHAV & SAMOUR (2019) conclude that this virus is in fact one and the same as Columbid HV-1 (CoHV-1) affecting pigeons, and Falconid HV-1 of Falconiformes.

Falcon herpesvirus-1: Falconid herpesvirus-1 (FaHV-1) is the causative agent of this highly lethal ailment in raptors. First described by MARÉ & GRAHAM (1973), and also known as inclusion body hepatitis of falcons (WERNERY et al., 2004), this pathogen leads to whitish-grey coloured necrotic foci in the liver and spleen (KOCAN et al., 1977; GÁL, 2006). Clinically, nonspecific signs are noted, including generalized weakness, inappetence and lethargy after a short incubation period (GRAHAM et al., 1975).

Columbid Herpesvirus-1: Affection principally pigeons, CoHV-1 has been implicated in causing illness in Cooper's hawks (*Accipiter cooperii*) in North America (PINKERTON et al., 2008) and WOZNIAKOWSKI et al. (2013) identified clinical signs in a Peregrine falcon to be caused by this virus as well.

Adenovirus: Described in 1996 in the United States following an outbreak in falcons, the disease is widespread in the Midwest region (SCHRENZEL et al., 2005). OAKS et al. (2005) report the prevalence of adenovirus inclusion body hepatitis in various Falconid species, but state that though seropositivity can reach up to 80-100% in examined birds, subsequent clinical disease is rare. Nonetheless, certain species, such as the Taita falcon (*Falco fasciinucha*) (DEAN et al., 2006), Northern aplomado (*Falco femoralis septentrionalis*), Peregrine falcon (OAKS et al.,

2005) and American kestrel (TOMASZEWSKI & PHALEN, 2007) in the United States, Common buzzards (FRÖLICH et al., 2002) in Germany have been seen to elicit symptoms of disease from the infection. Moreover, recent work done by MOHAMED et al. (2018) indicates cross-species transmission between falcons and chickens. Furthermore, siadenovirus has previously been identified in predatory birds in Hungary (KOVÁCS & BENKO, 2009; KOVÁCS & BENKO, 2011). **Marek's diseases:** Caused by an avian herpesvirus, this pathogenic agent causes inflammation of the nerves leading to alterations in movement of effected specimens (DOBOS-KOVÁCS, 2014). HALLIWELL (1971) accounts of a single Great horned owl to have been diagnosed with this ailment.

Avipoxvirus: Avian species have shown an increased susceptibility to poxviruses (FOWLER, 1986; GÁL, 2006). Falconpox (FAPV) has been implicated in causing integumentary lesions in raptors (WERNERY et al., 2004). Brown-coloured classical pox alterations can be perceived on the eyelids (COOPER, 2002), in the periocular region (SAITO et al., 2019) hindering their ability to navigate through the air (COOPER, 2002) and on the talons (COOPER, 2002; SAITO et al., 2009). Moreover, ÖZMEN & DORRESTEIN (2002) record a case of generalized pox in a Common buzzard. KRONE et al. (2004) describe Peregrine falcons with widespread integumental involvement in a German wildlife rescue center, though cases with severe lesions have also been documented in White-tailed eagles (SAITO et al., 2009) and more recently in Griffon vultures (DI FRANCESCO et al., 2019). Pathologies cause by this virus can also lead to secondary bacterial infection as was noted by SCHOMAKER et al. (1998) who found *Staphylococcus aureus* infection in a young, male Sparrowhawk following a poxvirus event. Furthermore, SHRUBSOLE-COCKWILL et al. (2010) document a disseminated, fatal secondary *Candida albicans* case in a juvenile Golden eagle after being affected by the aforementioned viral pathogen.

Newcastle disease: Illness caused by this Paramyxoviral pathogen is widely seen, not only in domesticated avian species, but also in their free-living counterparts. Newcastle disease is caused by the avian paramyxovirus-1 and strains of variable virulence are recognized (GÁL, 2006). Birds living in nature take the pathogen up orally and, depending on the virulence of the strain and the individual susceptibility of the bird, variable clinical signs can be observed ranging from respiratory and gastrointestinal symptoms, to severe neurological alterations (GÁL, 2006). Found in free-ranging and captive predatory birds alike (WERNERY et al., 1992; COOPER, 2002; HADDAS et al., 2014), the virus is likely transmitted to these birds from infected pigeons or poultry. Moreover, according to SAMOUR (2014) both neurotropic and viscerotropic forms have been identified in falcon species. VAN BORM et al. (2016) identified the viral genome from various owl species through PCR taken by cloacal swabs and disease has been confirmed in

Eurasian scops owl (CHOI et al., 2008), Barn owl, Tawny owl (SCHETTLER et al., 2003) and Little owl (HADDAS et al., 2014). Raptors, including the Common buzzard, Osprey, Marsh harrier (SCHETTLER et al., 2001) and European kestrel (SCHETTLER et al., 2003) have similarly been victims of this ailment in Europe.

Avian influenza: Belonging to the Orthomyxoviridae family, all avian species are susceptible to the HA and NA recombinant influenza viruses. Asymptomatic carriers have been identified in wild bird populations, however, clinical illness (respiratory, gastrointestinal and neurological symptoms) are recorded following oral infection and can lead to fatalities (GÁL, 2006). REDIG & GOYAL (2012) recorded cases of avian influenza-A in the United States through ELISA testing in Peregrine falcons, Cooper's hawks and Great horned owls. However, highly pathogenic strains of avian influenza have been recorded in multiple free-ranging species. H5N8 caused massive losses in White-tailed eagles in Germany (KRONE et al., 2018), and hawks and falcons in the Netherlands (KLEYHEEG et al., 2017). Multiple species of falcons used for hunting in Dubai (NAGUIB et al., 2015), Peregrine falcons in Slovakia and Germany (NAGY et al., 2009; VAN DEN BRAND et al., 2015), Eurasian eagle owl in Korea (CHOI et al., 2013), Common buzzards in Germany (VAN DEN BRAND et al., 2015) and in Bulgaria (MARINOVA-PETKOVA et al., 2012) and a Mountain hawk-eagle (*Nisaetus nipalensis*) in Japan (SHIVAKOTI et al., 2010) have all incurred losses to H5N1 over the past decade. KOHLS et al. (2011) state in their work that this devastating viral pathogen poses serious threats to the falconry industry owing to the high susceptibility of the birds used. In Hungary, cases were reported of H5N8 HPAI (highly pathogenic avian influenza) in a free living Saker falcon and a Peregrine falcon used in falconry during the avian influenza outbreak in 2017 (ERDÉLYI et al., 2019).

Usutu virus: This viral pathogen, found in both free-ranging and captive predatory birds (MANAROLLA et al., 2010; STEINMETZ et al., 2011), is transmitted by mosquitos. Eliciting signs of depression, incoordination followed by seizures and death, marked splenomegaly, hepatomegaly and pulmonary hyperemia are discernible on necropsy (STEINMETZ et al., 2011). RIJKS et al. (2016) and ZIEGLER et al. (2016) confirmed the presence of this virus in captive Great grey owls in the Netherlands and Germany respectively, following *post mortem* examination and RT-PCR, whereas STEINMETZ et al. (2011) also implemented USUV-specific immunohistochemistry to obtain a diagnosis in various Strigiformes.

Avian hepatitis-E virus: Manifested most commonly in layer hens (*Gallus domesticus*) in the poultry industry, this viral disease elicits signs of hepatitis and cirrhosis on necropsy (DOBOS-KOVÁCS, 2014). More recently, avian hepatitis-E virus (HEV) has been identified in the Little owl and Common buzzard (ZHANG et al., 2017), whilst REUTER et al. (2016) reported a

divergent HEV in Common kestrels and Red-footed falcons in Hungary. Based on their work, ZHANG et al. (2017) states that cross-species transmission could result from chickens to wild birds.

West Nile Encephalitis: Cause by a Flavivirus and transmitted by mosquitoes, West Nile (WN) virus is known to cause CNS signs in wild birds (GANGOSO et al., 2010) and in captive individuals as well (GANCZ et al., 2004; QUAGLIA et al., 2014). Eliciting symptoms of incoordination and ataxia, clinical signs often develop in three stages: first unresponsiveness and apathy despite being upright and alert, secondly progressive CNS signs (opisthotonus, anisocoria, tremors and nystagmus) and finally paralysis and death (SÓS-KOROKNAI et al., 2019). Raptors are sensitive to WN virus infection (ANGENVOORT et al., 2014) and the ailment has been documented in American kestrel (MEDICA et al., 2007), Cooper's hawk (ANDERSON et al., 1999), Golden eagle (WÜNSCHMANN et al., 2017), Red-tailed hawk, (WÜNSCHMANN et al., 2017) and frequently in Bald eagles in the United States (HARRIS & SLEEMAN, 2007; WÜNSCHMANN et al., 2017). In Europe, many predatory avian species have been found to elicit seropositivity (WODAK et al., 2011; QUAGLIA et al., 2014; JURADO-TARIFA et al., 2016; ANA et al., 2017) and some species, such as the Northern goshawk appear to have a higher sensitivity (ERDÉLYI et al., 2007; WODAK et al., 2011; HUBÁLEK et al., 2018; BUSQUETS et al., 2019; SÓS-KOROKNAI et al., 2019) and can perhaps serve as an indicator species (HUBÁLEK et al., 2018). Rare and endangered species, such as the Spanish imperial eagle (*Aquila adalberti*) have also succumbed to this ailment (HÖFLE et al., 2008). Owl species elicit considerable sensitivity to WN virus (GANCZ et al., 2004; LOPES et al., 2007) and in the Great horned owl, a study shows that this species is more likely to develop neurological signs than others (NEMETH et al., 2009). In Europe, FISCHER et al. (2019) write that almost one third of captive Grey owls in Germany succumb to Flaviviruses such as WN virus. The presence of WN virus in Hungary was first determined by ERDÉLYI et al. in 2007 from a Sparrowhawk and several Northern goshawks and has since been identified in a number of species in our country. Vaccines intended for other taxa are implemented against WN virus by a number of authors (JOHNSON, 2005; REDIG et al., 2012; ANGENVOORT et al., 2014; FISCHER et al., 2015) with variable results.

2.6.2. Bacterial diseases

Gram positive cocci: *Staphylococcus* sp. and *Streptococcus* sp. are associated with clinical disease in variable ages of birds (GÁL, 2006). Moreover, these organisms have been implicated in the development of bumblefoot in raptors (COOPER, 2002; VIDAL et al., 2017) and MAIER et al. (2015) account of a case of vertebral osteomyelitis and septic arthritis in a Peregrine falcon attributed to these organisms. Additionally, various authors discovered a significant number of

conjunctival swabs to be infected with *Staphylococcus* in raptors (SALA et al., 2016; KIM et al., 2017; VIDAL et al., 2017). SCHOMAKER et al. (1998) describe a case of a juvenile, male Northern goshawk with secondary *Staphylococcus aureus* dermatitis following a pox infection. Furthermore, in recent years, multidrug resistant strains of *Staphylococcus* have been identified from multiple predatory avian species in Europe (SALA et al., 2016; SOUSA et al., 2016).

Escherichia coli: *E. coli* is a bacterium with the ability to cause a wide variety of pathologies in free-living avian species. The severity of disease depends on the pathogenicity of the bacteria, in addition to the age, and susceptibility of the host (GÁL, 2006). COOPER (2002) identified acute septicaemia induced by *E. coli* in raptor species, whereas ALCALÁ et al. (2016) recognized cefatoxim antibiotic resistance strains of *E. coli* in 16% of the cloacal swabs taken from 100 wild birds. Antibiotic resistant strains were discovered in Golden eagles and Northern goshawks in Slovakia (HANDROVA & KMET, 2019), whereas HATHCOCK et al. (2019) and RADHOUANI et al. (2012) described the presence of multidrug-resistant *E. coli* in a number of raptors in the United States and Portugal respectively.

Pasteurella multocida: This pathogen has the ability to cause disease in all avian species including predatory birds. Typically, acute septicemia is observed in affected individuals, however a chronic form can also be identified in the form of avian cholera (GÁL, 2006; DOBOS-KOVÁCS, 2014). Both WILSON et al. (1995) and COOPER (2002) have described the occurrence of this pathogen in raptor species, whilst MORISHITA et al. (1997) account of cases in nocturnal predators. Aside from the typically occurring septicemia, MORISHITA et al. (1997) also identified esophageal abscesses in multiple hawk species.

Erysipelothrix rhusiopathiae: Causing predominantly hemorrhagic lesions on the serous membranes and sometimes leading to septicemia in avian species (GÁL, 2006; DOBOS-KOVÁCS, 2014). *Erysipelothrix rhusiopathiae* has been documented to cause disease in Hen harrier and Sparrowhawk (FOWLER, 1986), and moreover, it was isolated from free-ranging Griffon vultures (VELA et al., 2015).

Salmonellosis: Numerous *Salmonella* serotypes are capable of causing morbidity and mortality from embryos and day-old birds, to adult individuals (GÁL, 2006). Very often, free-living (MOLINA-LÓPEZ et al., 2015) and captive (WERNERY et al., 1998) birds of prey are asymptomatic carriers of this pathogen (MOLINA-LÓPEZ et al., 2015), with the route of infection predominantly found to be through their prey (COOPER, 2002; WERNERY et al., 2004). BATTISTI et al. (1998) record multiple cases of embryonic and neonatal death caused by salmonellosis in a number of both diurnal and nocturnal predators, whereas KOCABIYIK et al. (2006) document fatal disease inflicted by *Salmonella enteritidis* in an Eagle owl. PLAZA et al. (2019) recently

found carrion eating species, such as the Black vulture (*Coragyps atratus*), to be at a higher risk of contracting salmonellosis and could serve as reservoirs and therefore play a key role in the dispersal of these pathogens.

Corynebacterium sp.: In domesticated and exotic birds, this pathogen induces the development of necrotic foci in the liver and the spleen (GÁL, 2006; DOBOS-KOVÁCS, 2014). KATUSKAWA et al. (2016) identified *Corynebacterium ulcerans* in a wild Ural owl and examined the zoonotic potential of this bacterium. Moreover, *Corynebacterium* has also been isolated from various eagle species (FERNÁNDEZ-GARAYZÁBAL et al., 2003) and from Griffon vultures (VELA et al., 2015).

Listeriosis: Not considered a very common disease of birds, this pathogen is implicated in inducing septicemia and hepatic necrosis in affected individuals (DOBOS-KOVÁCS, 2014). BAKER (1967) accounts of a fatal case in a Merlin, whereas DHAMA et al. (2013) describes the ailment in eagle species and in Snowy owl.

Pseudomonas aeruginosa: This bacterium has been documented through the literature to cause clinical disease in birds of prey, largely manifested in the form of septicemia or respiratory disease (VIDAL et al., 2017). Moreover, SAMOUR (2000) describes a case of stomatitis in a Saker falcon, whereas other authors discuss its relevance in the gastrointestinal tract (FORD, 2010).

Chlamydia psittaci: The causative agent of a disease leading predominantly to respiratory signs and enteritis also has zoonotic potential (GÁL, 2006; DOBOS-KOVÁCS, 2014). COOPER (2002) and WERNERY et al. (2004) describe cases in raptors and discuss the importance of consumption of infected prey in the spread of disease. FOWLER et al. (1991) document disease in Red-tailed hawks and BLOMQUIST et al. (2012) identified this ailment in 1.3% of all raptors during the course of their study. SCHETTLER et al. (2003) found *C. psittaci* to occur commonly in various species of diurnal and nocturnal predators in Germany and even Galapagos hawks (*Buteo galapagoensis*) were identified to be seropositive (DEEM et al., 2012).

Ornithobacterium rhinotracheale: Causing inflammation in the airways of affected individuals, this pathogen is only considered to elicit clinical diseases in the presence of predisposing factors (DOBOS-KOVÁCS, 2014). HAFEZ & LIERZ (2010) report this bacterium to have caused an outbreak of airsacculitis in a falcon breeding facility.

Mycoplasmosis: With a widespread occurrence, these bacteria are known to colonize the mucous membranes of the respiratory tract of birds, but predisposing facts are necessary to provoke the initiation of toxin production. This process in turn activated the goblet cells in the mucous membrane allowing secondary bacteria, such as *E. coli*, to replicated leading to fibrinous inflammation (DOBOS-KOVÁCS, 2014). Based on PCR examinations, LIERZ et al.

(2008b) were able to identify *M. buteonis*, *M. falconis*, *M. gypis* and *M. corogypis* from raptors, and furthermore, LIERZ et al. (2008c) were the first to describe *Mycoplasma* infection in the Marsh harrier, Eurasian hobby and in the Barn owl in Germany, as well as in the Lesser Kestrel (*Falco naumanni*) (LIERZ et al., 2008d). ERDÉLYI et al. (1999) also isolated *M. buteonis* associated with perosis from a young Saker falcon in Hungary. Documentation exists of *M. gallinarum* in Griffon vultures in Italy (LORIA et al., 2008) and *Mycoplasma* was also isolated from a Black vulture in the United States (RUDER et al., 2009). Moreover, MORISHITA et al. (1998) identified *M. synoviae* and *M. gallisepticum* in chicks of various predatory avian species. Clinically, a case documents disease elicited by *M. buteonis* in American kestrel, manifested as blepharodema and sinusitis (BEZIJAN & KOLLIAS, 2014). COOPER (2002) also mention sinusitis as a result of mycoplasmosis in conjunction with *Trichomonas* infection.

Avian tuberculosis: Birds appear to elicit a high sensitivity to tuberculosis caused by *Mycobacterium avium* complex (DOBOS-KOVÁCS, 2014). Infection occurs predominantly via the oral route and the pathogen is usually contracted from affected prey animals (GÁL, 2006). Primary lesions develop in the intestine, and following generalization, granulomatous lesions can be found in the liver, spleen, lungs, and on occasion in the bone marrow (SHIVAPRASAD & PALMIERI, 2012). Granulomas may be seen in the air sacs (HEATLEY et al., 2007; SADAR et al., 2015), subcutaneously (HEATLEY et al., 2007) and even associated with the vertebral column (SADAR et al., 2015). COOPER (2002) and WERNERY et al. (2004) both identified and recorded lesions caused by avian tuberculosis (including nodules firm on palpation in the liver) in various species of raptors. KRIZ et al. (2013) isolated *Mycobacterium avium* subsp. *avium* from captive Bald eagle, Eurasian eagle owl, Barn owl and Little owl, identifying the source of infecting to be poultry in these instances. MILLÁN et al. (2010) conducted a study over a 3-year period where 589 predatory bird cadavers were examined for the presence of tuberculosis. The authors found, that 4% of raptors and 7% of owls yielded positive results for the presence of this pathogen. Additionally, SMIT et al. (1987) found the highest prevalence of this ailment to be in Falconiformes in the Netherlands following an extensive study and MULLER et al. (2010) account of secondary infections to occur concomitantly to avian tuberculosis in falcons.

Botulism: Caused by toxins produced by these bacteria and the pathogen directly, this ailment could potentially cause illness in predatory birds, though is more frequently described in waterfowl (SILVA et al., 2017). KURTDEDE & SANCAK (2002) document a case of botulism in a Long-legged buzzard (*Buteo rufinus*).

2.6.3. Fungal diseases

Avian species are highly sensitive and susceptible to disease caused by fungal spores. TARELLO (2011) and LATIF et al. (2015) claim that aspergillosis is the most frequent causative agent of disease in falcons and infections occurs through the inhalation of spores leading to infection in the air sacs and lungs. Subsequently, granulomatous lesions develop in the pulmonary tissue and air sacs become thickened. The surface of the air sacs becomes lined with the sporocarp of the organism, as oxygenation is adequate for sporulation to occur at these sites (GÁL, 2006). According to COOPER (2002) and WERNERY et al. (2004), predatory birds contract fungal spores as prey is torn apart, leading to the aforementioned alterations. REDIG et al. (1980) diagnosed significant *Aspergillus fumigatus* infection in Northern goshawk, though cases have also been reported in Black vulture (JUNG et al., 2009) and Red-tailed hawk (GENTRY et al., 2014). KISER et al. (2018) identified severe *Aspergillosis niger* infection in a Eurasian eagle owl. Diagnosis of pulmonary and air sac mycosis can be difficult, as many illnesses can elicit respiratory signs (COOPER, 2002). VORBRÜGGEN et al. (2013) mentions the use of radiology as a diagnostic tool, however fungal granulomas can be difficult to confirm through this imaging technique, as the lesions do not calcify and thus, identifying them on x-rays can be challenging. Hematological tests and computed tomography can also be employed to obtain a diagnosis and endoscopy can be a useful tool to visualize lesions caused by this ailment in avian species (FISCHER & LIERZ, 2015).

Aside from the afore mentioned organisms, DEEM (2003) account of candida mycosis in the oral cavity of predatory birds and FOWLER (1986) described this pathogen in Prairie falcons. Moreover, SHRUBSOLE-COCKWILL et al. (2010) implicated *Candida albicans* to be the causative agent of encephalitis in a Golden eagle, however the authors state that such an opportunistic fungal agent can be found secondary in the presence of an existing infection and/or immunosuppression. Furthermore, candida mycosis has been noted to occur subsequently to exposure to antibiotics (PITARCH et al., 2017)

Respiratory symptoms elicited by fungal disease are manifested as severe respiratory stridor and laboured breathing often accompanied by weight loss and an impaired ability to fly and hunt (GABRISCH & ZWART, 1987; GÁL, 2006). Dermatophyton has also been implicated in causing stomatitis in a Merlin, leading to suffocation in this individual (BERKÉNYI, 2012).

2.6.4. Parasitic diseases

Infection by hemoparasites, such as *Leucocytozoon* sp. have frequently been identified in diurnal predators (WALTER et al., 2016). HUNTER et al. (1997) mention *Leucocytozoon* sp. in owls and though it was found to be subclinical in the majority of cases, hindrances in

reproduction were reported. However, NIEDRINGHAUS et al. (2018) diagnosed fatal disease in Great horned owl elicited by the aforementioned pathogen.

Plasmodium sp. often cause infection without the presence of clinical signs (GÁL, 2006) and KINGSTON et al. (1976) recognised this hemoparasite in Gyrfalcon and Peregrine falcon individuals, while SALAKIJ et al. (2012) diagnosed *Plasmodium circumflexum* in a Shikra (*Accipiter badius*). GUTIÉRREZ-LÓPEZ et al. (2015) emphasized the importance vectors play in the spread of these organisms within avian populations and northern species, such as the Snowy owl have been found to be more sensitive to this ailment (BAKER et al., 2018).

Another study found that 20% of Prairie falcons in a studied area of the United States were infected by *Babesia moshkovskii* (CROFT & KINGSTON, 1975) and SAMOUR & PEIRCE (1996) diagnosed *Babesia shortti* in Saker falcons.

LIERZ et al. (2008a) demonstrated *Haemoproteus tinnunculi* infection of 5.3% of falcons in the United Arab Emirates, 0.9% caused by *H. brachiatus*, and a 0.9% prevalence of *Leucocytozoon toddi*. KARADJIAN et al. (2013) confirmed the presence of *Haemoproteus syrnii* in a Tawny owl in France, and in another study, KARADJIAN et al. (2014) also identified *H. ilanpapernai* in Spotted wood-owl (*Strix seloputo*). Furthermore, BUKAUSKAITE et al. (2015) confirmed that in owl species, *H. noctuae* and *H. syrnii* are transmitted by bloodsucking insects (*Culicoides nubeculosus*, *C. impunctatus*) wherein sporogonia occurs.

Moreover, feces of Lanner falcons (*Falco biarmicus*) were examined in work by ALYOUSIF et al. (2011) in Saudi Arabia and alimentary single-celled parasites were identified. *Caryospora biarmicusis* oocysts were diagnosed, and in another study, *Eimeria biarmicus* were found, with the latter showing a 5% prevalence out of the studied birds.

Trypanosoma avium hemoparasite infection was noted in 11.3% of 56 clinically healthy birds in Kuwait in work performed by TARELLO (2005).

Living on the mucous membranes of the upper alimentary tract, predominately the oral cavity, actively moving, single-celled *Trichomonas* sp. are notable in falcon species (SAMOUR et al., 1995). URBAN & MANNAN (2014) suggest a link between the pH of the oral cavity and *Trichomonas* susceptibility as they perceived in Cooper's hawks. ROGERS et al. (2016) identified *Trichomonas gallinae* in Spotted owl (*Strix occidentalis*) and QUILLFELDT et al. (2018) goes so far as to say these organisms show a high prevalence in various owl species in Germany.

Cryptosporidium sp. invade the mucous membranes lining the respiratory- and gastrointestinal tracts and the bursa-Fabricii in birds (DOBOS-KOVÁCS, 2014). Various species have been described in predatory birds; *Cryptosporidium baileyi* has been identified in Eurasian

scops owl (MOLINA-LÓPEZ et al., 2010), Saker falcon (BOUGIOUKLIS et al., 2013) and Snowy owl (NAKAGUN et al., 2017), causing respiratory symptoms, otitis media and proventriculitis respectively. Moreover, *Cryptosporidium parvum* infection was established in hybrid falcons in a study performed by AZMANIS et al. (2018).

LOVE et al. (2016) established the presence of seropositivity to *Toxoplasma* in work performed on various raptors in the United States. According to the authors, the presence of antibodies is indicative of correlation with the level of infection in free-living small mammals and the feral cat populations in the area. CABEZÓN et al. (2011) also accounts for seropositivity to *Toxoplasma gondii* in a number of diurnal and nocturnal predatory birds, though DUBEY (2002) states that clinical disease caused by this parasite is manifested in pigeon and canary species, but fatal myocarditis has been confirmed in a Bald eagle (SZABO et al., 2004). Furthermore, severe hepatitis caused by the afore mentioned pathogen was deemed the cause of death in a Barred owl by MIKAELIAN et al. (1997) suggesting incidence of clinical disease in birds of prey as well.

Many studies are accessible in the literature about endoparasites found in predatory avian species. Owing to the large number of parasite species, uncertainties about their pathogenicity and the fact that the parasite-host relationship of a number of these are yet unknown, only a few such publications are mentioned below.

KOMOROVÁ et al. (2016) described the presence of 12 parasite species in nocturnal and diurnal birds of prey, while KUTZER et al. (1980) established infection by four protozoal, one trematode, two cestode, two acantocephala and 13 nematode species in raptors. In examining 285 birds of prey, SANMARTIN et al. (2004) identified 15 parasite species to be affecting Spanish populations of these species.

CHRISTENSEN et al. (2015) determined a 76% prevalence of *Eucoleus contortus* in Gyrfalcons and a further 6 parasites that were present, though in lower numbers. HURNIKOVÁ et al. (2014) diagnosed *Trichinella pseudospiralis* from various species of raptors and owls, whereas BILQEES & KHAN (2006) described *Opisthorchis jonesae* infection in Black kites. SILVA et al. (2014) identified *Pelecitus* sp. in the body cavity, air sacs and the connective tissue underlying the lung fields in a Burrowing owl (*Athene cunicularia*) in Brazil. As parasites can occur almost anywhere in their hosts, a noteworthy case reports the presence of *Angiostrongylus cantonensis* infection in a Pygmy falcon (*Polihierax semitorquatus*) where worms were found in the ventricles of the brain (BURNS et al., 2014).

3. Materials and Methods

3.1. Sample collection method and timeframe

Samples collected over the course of the study originated from patients admitted to the Wildlife Rescue Center at the Budapest Zoo and Botanical Garden. These birds of prey were brought into the facility from all regions of Hungary with various ailments leading to their inability to survive in the wild. During the time spent at the rescue facility, all individuals were kept in separate compartments segregated from one another. These enclosures had wooden walls, a chicken wire covering on the top for ventilation and were fitted with removable glass doors to enable visualization of the patients. Owing to the size differences between species, crates with variable dimensions were available to cater to the individual needs of our patients. The birds included in our work either died at the rescue center or were humanely euthanized resultant to their injuries if the welfare of the individual was in question. Euthanasia was performed under general anesthesia (isoflurane; Foran®, Abbot Laboratories, Switzerland) and analgesia (butorphanol; Butomidor®, Richter Pharma AG, Austria) followed by intramuscular embutarmid (T61®, Intervet International B.V. Boxmeer, Holland) administration. Sample collection ensued between September 9th, 2015 and September 25th, 2017.

The second portion of our work pertained to a retrospectively analyze of data from pathological examinations performed at the University of Veterinary Science in Budapest between January 1st, 2000 and December 31st, 2013. Data collected was in the form of reports containing information about various cases (species, age, sex and cause of death) from the archives of the Department of Pathology.

3.2. Identification of species and recording of biometric data

Data obtained during necropsy was recorded onto individual gross pathology sheets implemented by the Budapest Zoo and Botanical Garden (see Appendix). Date of necropsy and date of death were written on the sheet, in addition to the data about the individual, and all personnel present at the time of *post mortem* examination. All specimens were assigned an identification number in chronological order, and in the case of most patients, the Wildlife Rescue Center ID was also included.

Species name of nocturnal and diurnal birds of prey, and their age and sex (in those species where these features are recognizable based on external physical characteristics and plumage), were identified based on the characteristics described by SVENNSON et al. (2018).

During necropsy, weight of predatory birds was measured using a standard kitchen scale and recorded to one decimal point. Body length, wing length, tail length, tarsometatarsal length and beak length were measured using a ruler cut off at the zero mark, or a tape measure in the

case of the latter. Body length was measured from the top of the head (with the beak facing forward) to the distal portion of the tail feathers. The wing length was measured from the outermost point of the radius/ulna, in a flexed position, to the tip of the longest primary feather (**Figure 1**). The tail was measured from the base to the tip of the longest tail feather. The tarsometatarsal bone was measured along its entire length (**Figure 2**) and the beak (culmen) was measured from the tip of the beak to the set point (**Figure 3**).



Figure 1. Measurement of the wing length in a Common buzzard (*Buteo buteo*) with the points of reference marked by arrows.

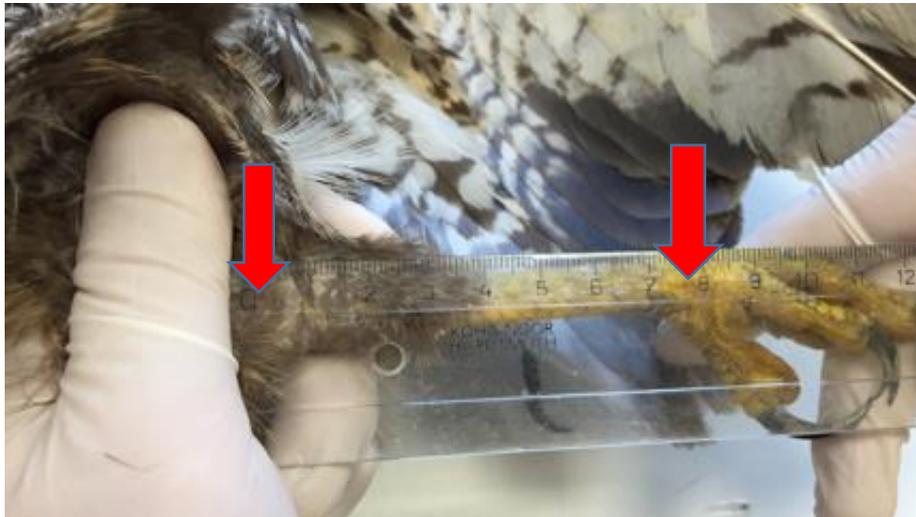


Figure 2. Measurement of the tarsometatarsal bone in a Common buzzard (*Buteo buteo*) with the points of reference marked by arrows.



Figure 3. Measurement of culmen (beak) in a Common buzzard (*Buteo buteo*) with the points of reference marked by arrows.

3.3. Pathological investigation

Necropsy was performed in all cases according to the method described for domestic poultry by VETÉSI & MÉSZÁROS (1998).

Firstly, the plumage of the cadavers was examined, as well as the talons for the presence of external lesions. Additionally, body orifices (oral cavity, nasal sinuses, cloaca) were assessed for the presence of anomalies, and the colour and integrity of mucous membranes was recorded.

In the next step, the birds were placed in dorsal recumbency and the skin was removed from the region overlying the pectoral musculature. The extent of subcutaneous connective tissue was assessed and the *m. pectoralis superficialis* and *profundus* were evaluated to determine the body condition score of the individual. Body condition was determined subjectively as “good” (3), “moderate” (2) or “poor” (1) depending on the extent of the pectoral musculature and the visibility of the sternal crest (**Figure 4**). Cut surfaces of all muscle layers were examined and abnormalities in colour, consistency, fluid content and tearability were documented.



Figure 4. Assessing the body condition by examination of pectoral musculature in a Common buzzard (*Buteo buteo*) in dorsal recumbency.

While opening the celomic cavity, the walls of the air sacs were examined, and the positioning of the internal organs was observed to rule-out abnormalities (**Figure 5**). Subsequently, the visceral organs were removed by severing the alimentary tract between the crop and the gizzard, and at the distal end of the digestive tract. The spleen was then assessed and any alterations in size, colour, shape and features of its cut surface were recorded. Next, the liver was inspected for any pathological changes to its size, shape, colour, edges and the cut surface. The glandular stomach and the gizzard, followed by the length of the intestinal tract, were opened and their mucosal surface and contents were assessed. Any pathological changes were noted.



Figure 5. Assessment of the physiological position of the organs in the celomic cavity in a Common buzzard (*Buteo buteo*).

Organs lodged within the skeletal system (lungs and kidney) were also examined based on the afore mentioned parameters and alteration were recorded.

The reproductive tract was subsequently evaluated, and the sex was determined (in those cases where this was not possible based on external features and plumage) through the presence of the testes in males and the left ovary in females. The activity of the sexual organs was also recorded.

The heart, bone marrow and the brain were assessed thereafter according to the afore mentioned methodology.

Radiographs were performed prior to dissection in instances where it was indicated based on the history and/or clinical signs, but especially in cases of traumatic origin. Two views were taken, latero-lateral and dorso-ventral using the a MEDMOBIL 002-004 x-ray machine and Orex Kodak PcCR 1417 digital plates and developer at the veterinary hospital of the Budapest Zoo and Botanical Garden.

3.4. Histopathological examination

Samples taken during necropsy from various organs were fixed in 8% formaldehyde for histopathological examination.

The fixed samples were cut into small pieces and embedded in paraffin. Once solidified, these blocks were sliced into pieces 3-4 μm thick and placed onto slides with the help of lukewarm water. Deparaffinization was performed on the slides using 80% alcohol prior to staining. Hematoxylin-eosin (H. E.) staining was performed using the following protocol:

1. Xilol bath – three rinses, alternating for 5-5 minutes each
2. Alcohol (Patosol) – two rinses, alternating for 3-3 minutes and a third for 5 minutes
3. Tap water – two rinses, alternating for 1-1 minute
4. Hematoxylin (Hematoxylin solution modified acc to GILL II. Merck) – 2 minutes
5. Tap water – three rinses, alternating for 3 minutes and twice for 5 minutes
6. Alcohol eosin (Eosin Y alcoholic 0.2% - VWR) – 30 seconds
7. Alcohol (Patosol) – three rinses, alternating for 3-3 minutes and then for 5 minutes
8. Xilol bath – two rinses, alternating for 5-5 minutes
9. Covering – specialized material used (DPX Mountant for histology)

In cases where the presence of *Mycobacterium* sp. was suspected based on the lesions found on gross necropsy, Ziehl-Neelsen (acid fast) staining was implemented according to the following procedure:

1. 30 minutes in freshly mixed carbolfuchsin solution (1 ml carbolfuchsin, 20ml distilled water)
2. Two rinses in water
3. Differentiation with hydrochloric acid for 1 minute
4. Two rinses in water
5. Place in 0.5 g/dl calcium permanganate solution for 2 minutes for oxidation
6. Two rinses in water
7. Treatment in 5 g/dl of oxalic acid for 2 minutes
8. Two rinses in water
9. Contra staining for methylene-blue solution for 2 minutes
10. Rinse with alcohol, treatment with xylol and covering

The acid-fast *Mycobacteria* will stain red following this staining methodology whereas the surroundings will be a blue colour.

If lesions typical of poxvirus were observed, histopathological slides were stained using Oil Red O method according to:

1. Slides are rested in propylene glycol for 2 minutes
2. Staining for 5 minutes in 100 ml of 0.5 g Oil Red O diluted in propylene glycol
3. Slides are placed in 85 ml propylene glycol with 15 ml of water for 3 minutes
4. Rinse in distilled water twice
5. Staining of nuclei with alum hematoxylin for a few seconds
6. Rinse with distilled water and then with tap water

The nuclei stain blue with this method, while the cytoplasm and lipids in vacuoles stain red. Following staining, all slides were fitted with the slide cover and were examined by light microscopy.

3.5. Bacteriological examination

For bacteriological investigations, cloacal swabs were taken according to the method described by SOLT & ERDÉLYI (2001) and were placed in transport media. These samples were marked and transferred to the University of Veterinary Medicine, Department of Exotic and Wildlife Medicine in Budapest where they were stored at 4°C until processed.

Swab samples were applied to blood and Drigalszki agar in order to differentiate enterobacteria. Subsequently, they were kept at under aerobic conditions, at 37°C, for 24 hours

in a thermostat incubator. Bacteria were identified based on their growth patterns, biochemical parameters and staining. Gram staining was used according to the protocol below:

1. Add homogenized material for one drop of water and fix above a flame
2. Cover in carbol water with gentian violet stain for 2 minutes
3. Place in Lugol solution without rinsing and stain for 1 minute
4. Tilt slide on a 45° angle and rinse with absolute alcohol, rinse and dry
5. Gram-positive bacteria will stain blue.

3.6. Virological examination

Samples were taken at necropsy from the small intestine, the liver, the spleen and the lungs, and stored at -18°C until examinations could be performed. Unfortunately, owing to a power outage and subsequent malfunctioning freezer, all samples were thawed and deemed useless before broad-scale virology examinations could be performed. Subsequently, viral examinations were performed in those cases where a viral etiology was suspected during necropsy, as part of retrospective portion of our work and from material processed immediately for determining the presence of West Nile virus.

3.7. Mycological examination

Swab samples were taken from lesions suspected to be of mycological origin during necropsy and were applied to blood, Drigalski or conventional agar. Plates were incubated for 24-72 hours, under aerobic conditions at 37°C. Fungi were identified based on their growth patterns, biochemical parameters and staining.

3.8. Trace element accumulation analysis

Liver and bone samples were attained at necropsy and stored at -18°C to be used for determining the presence of various trace elements in the birds in this study.

Chemicals used: Deionized water was produced by a Purite Select Fusion 160 BP water purification system. The conductivity of the ultrapure water was less than 0.1 µS. Concentrated nitric acid (69 m/m%) (HNO₃) and hydrogen peroxide (30% m/m%) (H₂O₂) were obtained from Aristar and Normapur. Both of them were for trace analysis quality. For sample digestion, 0.5 g of samples was decomposed by nitric acid and hydrogen peroxide, by a microwave digestion system. All the laboratory glassware and plastic tools were cleaned with 0.15 M hydrochloric acid (37 m/m%) (HCl) solution obtained from Aristar, then rinsed with deionized water.

Analytical standards inductively coupled plasma (ICP) measurements: ICP multi- and mono-element standards used for quantitative ICP measurement were obtained from Perkin Elmer and Prolabo. 4.6 purity argon gas was from Messer. Quality control (QC) standards were prepared from standard bovine liver (NIST-1577C).

Sample preparation: 0.5 g from each sample was weighted into a CEM MARS6 MARSXPreSS Teflon vessel. 5 ml nitric acid and 5 ml hydrogen peroxide were added, and the decomposition method was initiated. Ramp: 35 min; Temperature: 200°C; hold: 50 min; E: 1700 W. The sample was filled up to 25 ml and analysed by ICP-OES after a double dilution by deionized water using 1 mg/l Y solution as internal standard and 0.25 mg/l Au for the stabilization of the mercury content.

Blank and the QC samples were prepared from NIST-1577C standard by the same method.

Analytical measurements: Determination of heavy metals was carried out by a Perkin Elmer Optima 8300 DV ICP-OES instrument among the following measurement parameters:

RF generator: 40 MHz solid state, free running, flat plate plasma technology

RF power: 1300 W

Nebuliser type: (BURGENER PEEK MIRA MIST)

Plasma gas flow rate: 12 dm³·min⁻¹

Auxiliary gas flow rate: 0.2 dm³·min⁻¹

Nebuliser gas flow rate: 0.7 dm³·min⁻¹

Observation height: 15 mm

Internal standard: 1 mg/l Y

Mercury stabilization standard: 0.25 mg/l Au

Calibration range was between 0 and 200 mg/kg. Lower level of detection (LOD) was the following for the heavy metals analysed (**Table 4**):

Table 4. Atomic number and lower level of detection (LOD) in mg/kg of arsenic (As), cadmium (Cd), mercury (Hg), barium (Ba), cobalt (Co) and lead (Pb), chromium (Cr), copper (Cu), manganese (Mn), molybdenum (Mo), nickel (Ni) and zinc (Zn).

Metals	As	Cd	Hg	Ba	Co	Pb	Cr	Cu	Mn	Mo	Ni	Zn
Atomic number	33	48	80	56	27	82	24	29	25	42	28	30
Unit	mg/kg											
LOD	0.5	0.05	0.5	0.5	0.5	0.2	0.05	0.05	0.05	0.5	0.2	0.05

Internal quality control of the measurements was carried out via measurement of QC samples of known heavy metal concentration a minimum of 10 times. After discarding the extremes, the standard deviation of data (*s*) was established, which must have remained within the ±15% of the nominal concentration value in order to accept the QC measurement. The certified Hg content of the QC standard was less than the LOD (0.00536 mg/kg). The Hg content of all the samples were also less than the LOD.

3.9. Molecular methods for identification of pathogens

Organ samples (small intestine, liver, kidney and lung) taken from the birds of prey included in this study were frozen at -18°C until PCR (polymerase chain reaction) examinations were performed to identify pathogenic agents.

PCR tests were performed from fresh samples for *Chlamydia psittaci* and West Nile Virus Encephalitis using the QIAGEN OneStep RT-PCR Kit protocol. Under sterile circumstances, 10 µl 5x QIAGEN OneStep RT-PCR buffer, 2 µl dNTP Mix, 1-1 µl forward and reverse primers (40 µM), 2 µl QIAGEN OneStep RT-PCR Enzyme Mix, 0.5 µl RNáz inhibitor (10 units) and 7.5 µl template were mixed together. The PCR tubes containing the reactive material were placed into the PCR machine and set to the following program: 30 minutes at 50°C, 15 minutes at 95°C, then 35 cycles at 30mp 9°C, 30mp 5°C, one minute at 72°C, and finally 10 minutes at 72°C followed by cooling to 4°C.

PCR reactions were run on 1.2-1.8% agarose gel in 1X TAE buffer solution and subsequently to electrophoresis, identification could be made according the difference in size of the segments.

3.10. Statistical analysis of results

Statistical analysis was performed through the use column and pie charts in Microsoft Excel to visualize the data in this study. Moreover, following a normality test (Shapiro-Wilk test) of the data gained from the trace element analysis portion of the study, it was established that portions of our data were not suitable for t-tests and therefore, a Mann-Whitney U test was implemented following a Spearman's rank correlation test. A *p* value less than 0.05 was considered to be statistically significant. Moreover, mean and standard deviation values were calculated using AVERAGE and STDEV functions respectively in Microsoft Excel.

4. Results

4.1. Species examined in the scope of the study

Over the course of the study period, necropsy was performed on 70 individuals of 16 species at the Budapest Zoo and Botanical Garden. 85% of these individuals had been at the rescue center for less than seven days (35% less than one day, 27% between 1-3 days and 23% between 3-7 days), whereas 15% of the patients had been on the premises for more than seven days. Birds in the latter category were almost exclusively those, that had traumatic injuries and attempts had been made to rehabilitate them. The majority of the birds in our study pertained to a diurnal lifestyle, however, one quarter of them were owl species. Of the 16 species, 12 were raptors (European honey buzzard, White-tailed eagle, Northern goshawk, Sparrowhawk, Common buzzard, Rough-legged buzzard, Lesser spotted eagle, Eastern imperial eagle, Common kestrel, Eurasian hobby, Saker falcon and Peregrine falcon) and four were nocturnal birds of prey (Eurasian scops owl, Eurasian eagle owl, Tawny owl, Ural owl and Long-eared owl). (**Figure 6**).

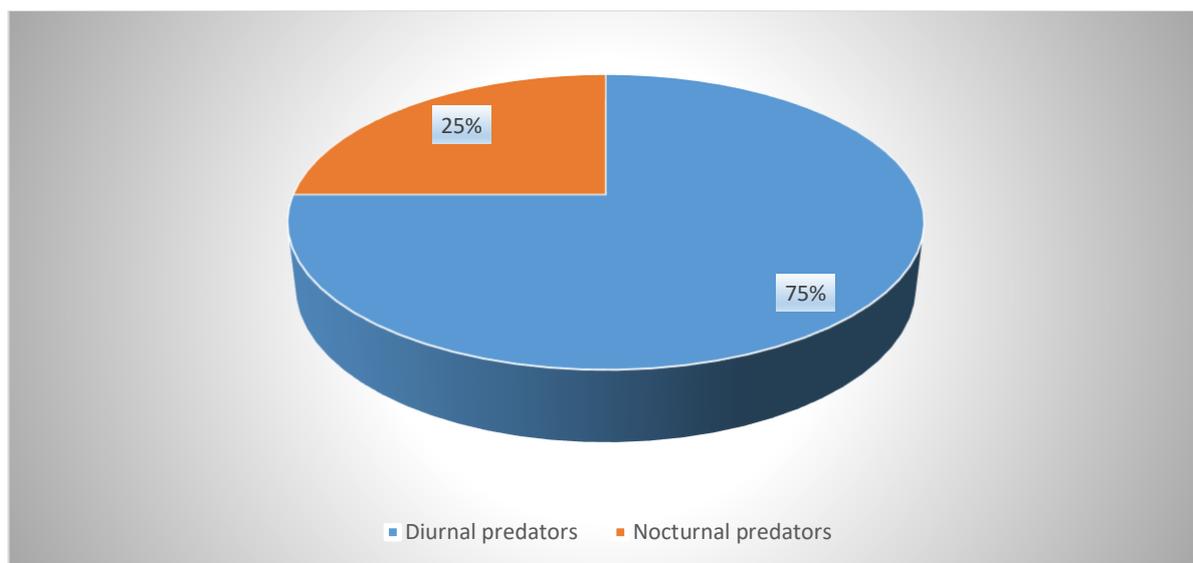


Figure 6. Lifestyle of the birds included in the study (n = 85).

Of the 16 species included, the Common kestrels were the most numerous with 28 individuals represented. Second most frequent were the Common buzzard with 15 individuals and third was the Sparrowhawk with seven cases.

Post mortem examinations performed at the University of Veterinary Medicine included eight species of predatory birds, of which only two were nocturnal predators, whereas the other six were their diurnal counterparts.

Between the two portions of our study 17 species were represented, with 12 eliciting a diurnal lifestyle and five a nocturnal one. Altogether, 85 individuals were examined in the scope of our work (**Figure 7 and 8**).

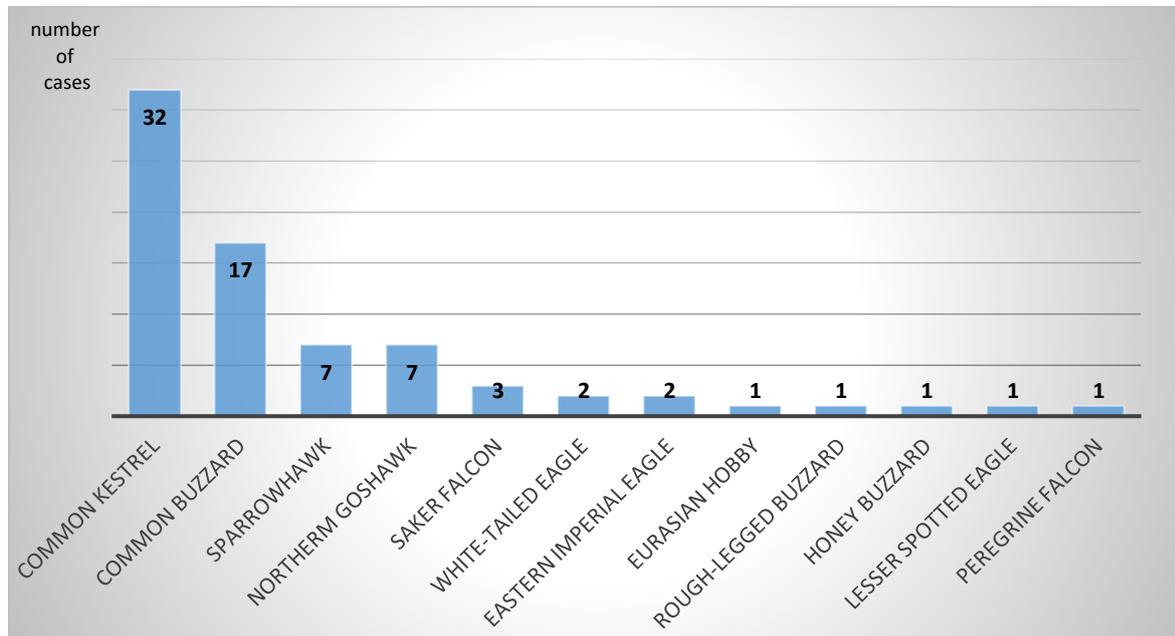


Figure 7. Raptor species examined in the scope of this study (n = 75).

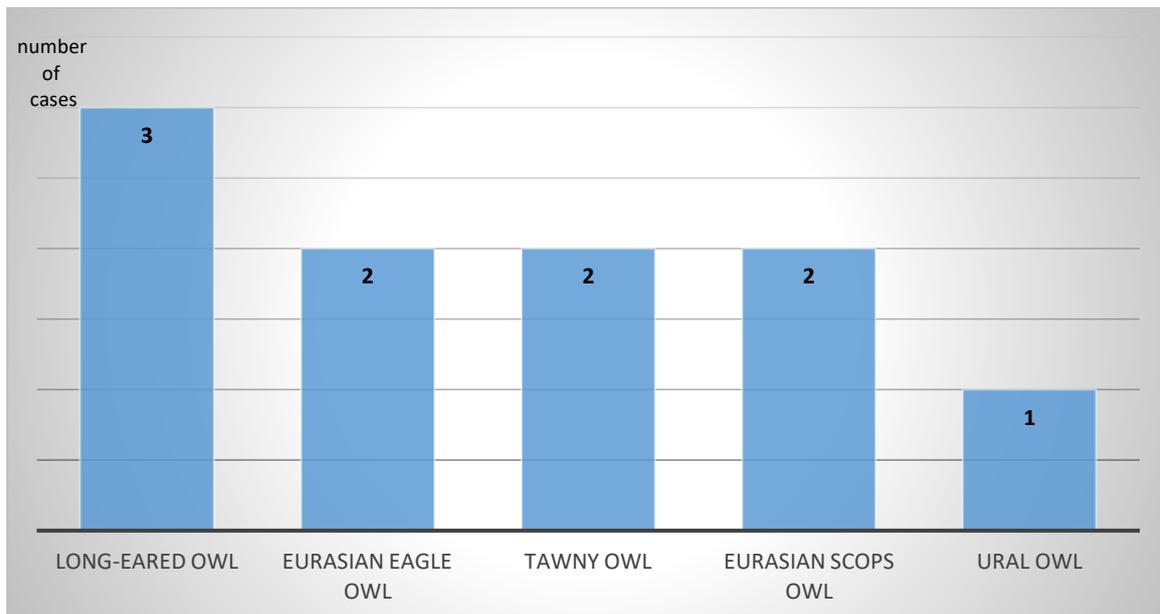


Figure 8. Owl species examined in the scope of this study (n = 10).

Of the frequently examined species, sex ratios were determined, and percentages were recorded. Of the Common kestrels 56.0% were male, while 44.0% were female, 43.7% of Common buzzards were female and 56.3% were male, Sparrowhawks had a higher incidence of females with 57.2%, while 42.8% were male. The greatest difference in sex was seen the case of Northern goshawks where 85.7% were female and only 14.3% were male. Sex ratios in the other species were not determined owing to the small sample size.

Of the individuals examined, 53.0% of diurnal predators were male and 47.0% of them were female, while in nocturnal birds of prey, 67.0% were male and merely 33.0% were female.

Analysis was also performed with regards to the ages of the predatory birds. 52.0% of the individuals were juvenile, based on their plumage, and 48.0% were adults. In owl species, a more significant difference was noted, with merely 11.0% being juvenile, and 89.0% adults.

Of the four most frequently examined species, in Common kestrels 62.0% had their juvenile plumage, while 38.0% were adults, 56.0% of Common buzzards 56.0% were young individuals, whereas 44.0% were adults. Only 29.0% of Sparrowhawks were juvenile and 71.0% were adults, and in Northern goshawks, more adults were examined with 57.0% as compared to 43.0% juvenile birds.

4.2. Biometric data of various species

Four species of the 17 were represented in sufficient numbers in order to be able to compile datasets with regards to biometrics. Common kestrels (**Table 5**), Common buzzards (**Table 6**), Sparrowhawks (**Table 7**) and Northern goshawks (**Table 8**) were the species included. Other species were not present in adequate numbers to obtain a relevant and unbiased dataset.

Table 5. Biometric data (average values) of Common kestrels (*Falco tinnunculus*).

<i>Falco tinnunculus</i> (n=25)	Body weight (g)	Wing length (cm)	Tail length (cm)	Tarso- metatarsal length (cm)	Beak length (cm)	Body length (cm)
Adult female (n=2)	170.0	24.0	18.1	4.4	2.1	33.4
Adult male (n=4)	149.5	25.3	16.6	4.7	1.8	32.5
Juvenile female (n=8)	150.5	25.2	18.4	4.5	1.8	32.5
Juvenile male (n=11)	129.7	23.6	17.3	4.3	1.7	31.6

Table 6. Biometric data (average values) of Common buzzards (*Buteo buteo*).

<i>Buteo buteo</i> (n=14)	Body weight (g)	Wing length (cm)	Tail length (cm)	Tarso- metatarsal length (cm)	Beak length (cm)	Body length (cm)
Adult female (n=3)	649.0	41.1	24.7	8.6	3.7	49.6
Adult male (n=2)	514.0	39.4	24.0	8.7	3.7	47.2
Juvenile female (n=4)	553.0	36.9	24.1	8.4	2.9	49.6
Juvenile male (n=5)	535.6	38.2	23.9	7.9	3.0	46.5

Table 7. Biometric data (average values) of Sparrowhawks (*Accipiter nisus*).

<i>Accipiter nisus</i> (n=6)	Body weight (g)	Wing length (cm)	Tail length (cm)	Tarso- metatarsal length (cm)	Beak length (cm)	Body length (cm)
Adult female (n=3)	209.3	24.2	21.5	6.5	2.1	36.9
Adult male (n=1)	133.0	19.6	16.3	6.0	2.3	30.5
Juvenile female (n=1)	152.0	21.0	13.5	7.0	2.4	29.5
Juvenile male (n=1)	77.0	20.9	17.5	6.0	1.5	31.5

Table 8. Biometric data (average values) of Northern goshawks (*Accipiter gentilis*).

<i>Accipiter gentilis</i> (n=2)	Body weight (g)	Wing length (cm)	Tail length (cm)	Tarso- metatarsal length (cm)	Beak length (cm)	Body length (cm)
Juvenile female (n=2)	521.0	32.8	26.8	7.6	3.0	51.5

It is important to note the discrepancy existing between the afore described cases and the total number of individuals examined for a given species. Certain specimens were excluded, either to not skew the results (i.e. juvenile birds of a very young age with a much smaller body size) or if all measurements were not feasible (i.e. those individuals where both wings were fractured).

4.3. Non-infectious causes of morbidity and mortality

Through the implemented diagnostic methods, cause of death and often the circumstances leading up to it were identifiable. We were able to categorize whether the factor(s) leading to morbidity and mortality were of infectious, or of a non-infectious origin. Cause of death was further classified based on the *post mortem* lesions and histopathological alterations.

Our findings indicate non-infectious factors to account for the majority (78%, n = 66) of the cases, whereas 22% (n = 19) were attributed to infectious diseases. In this portion of the analysis, nocturnal and diurnal predators are grouped together. These causative factors and percent affected are shown in **Figure 9**. In two instances, the cadavers were autolyzed at the time of *post mortem* examination and were therefore not included in this portion of the study tallying the total number of cases at 64.

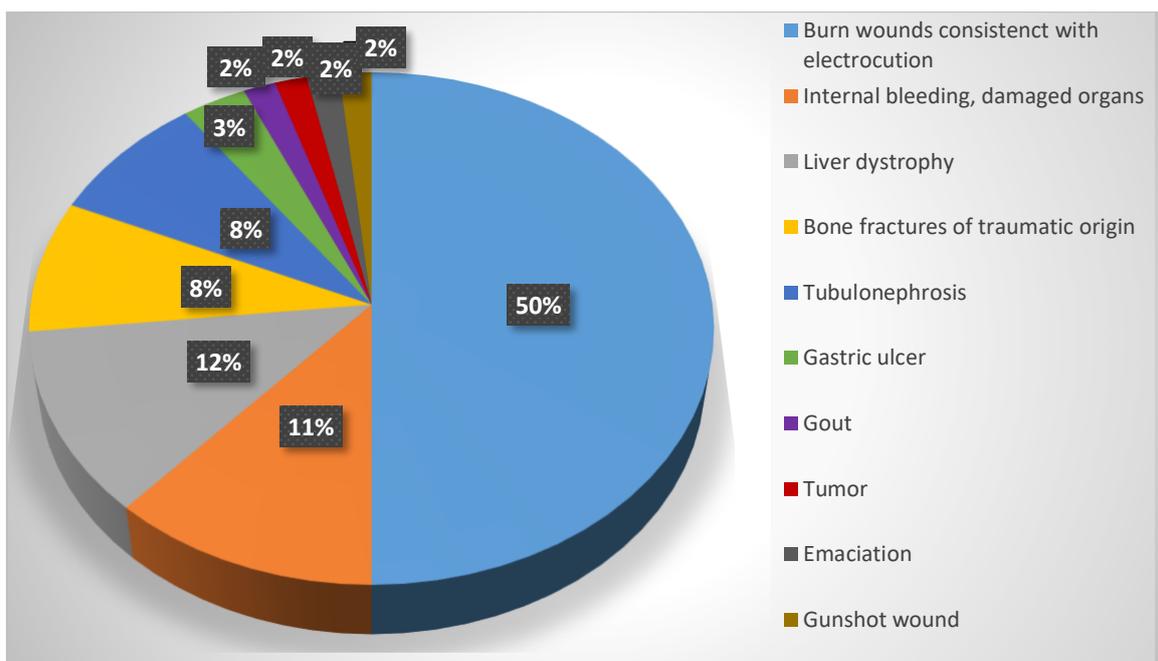


Figure 9. Non-infectious causes of morbidity and mortality (n = 64).

As seen in **Figure 9**, in 50% (n = 33) of the cases such lesions were visible, and in all instances, at least one wing or talon was affected by the typical second- and third-degree burns seen upon collision with powerlines. Injuries caused by electrocution could be noted in both juvenile and adult individuals. Typically, the alterations on the talons affected more than one claw, and in advanced disease, necrosis in the tarsal region was observed with a dry, parchment-like texture (**Figures 10-11**). All joints in the vicinity of such extensive necrosis were rigid. Typically, burn wounds were noted on the distal parts of the wings and frequently, burn lesions were visible on the adjacent feathers (**Figure 12-13**). The underlying skin, though covered by feathers, was also parchment-like on palpation and dark brown in colour, indicative of necrosis.



Figure 10. Parchment-like necrotic skin on the talons of a Common kestrel (*Falco tinnunculus*).



Figure 11. Burn lesions caused by electrocution on the talon of a Common buzzard (*Buteo buteo*).



Figure 12. Parchment-like necrotic skin (arrow) and damaged feathers on the wing of a Common kestrel (*Falco tinnunculus*) with chronic lesions typically seen with electrocution.

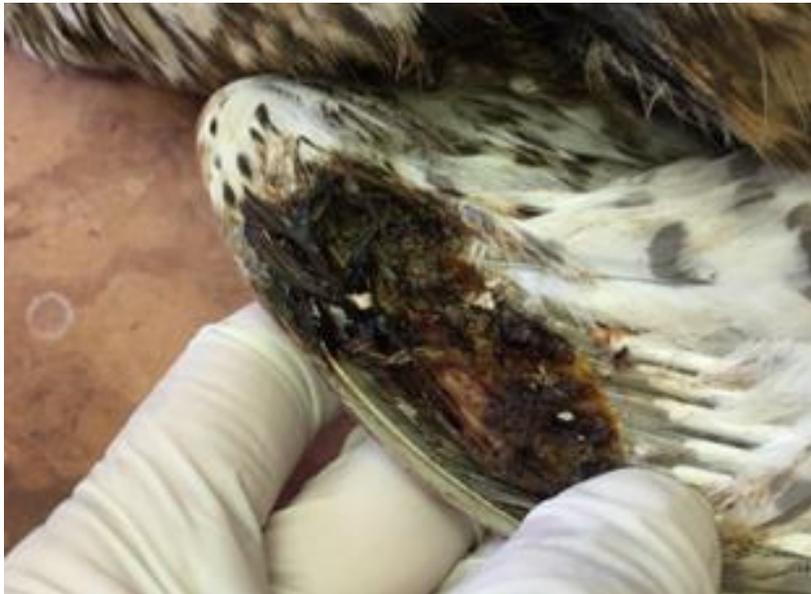


Figure 13. Severe, chronic burn lesions elicited by electrocution on the wing of a Common buzzard (*Buteo buteo*).

Dehydration was frequently observed alongside lesions elicited by electrocution, manifesting primarily as sunken eyes in affected individuals as well as altered skin turgor (Figure 14).



Figure 14. Indication of dehydration seen as sunken eyes in a Common kestrel (*Falco tinnunculus*).

As stated above, hepatic dystrophy was the second most frequently occurring ailment and accounted for morbidity in 12% ($n = 8$) of the birds of prey in this study. Seen primarily in raptors, a few examples pertained to owl species. On gross necropsy, the liver was smaller and was a yellow-brown colour (**Figure 15**), with a very fragile consistency. Moreover, pinpoint petechial hemorrhages were discernable under the surface of the capsule on macroscopic examination in some cases.

In these cases, pathological lipid infiltration was identified in the hepatocytes on histopathology (**Figure 16**). Regressive changes as rhexis and/or pyknosis were also visible in the nuclei of affected hepatocytes. Furthermore, multiple irregularly shaped and heterogenic vacuoles were present in the cytoplasm of these cells. A number of hepatocytes were apoptotic, stained homogenously with eosin, and had lost their integrity to become rounded, Councilman bodies. Dilated biliary vessels with distended lumens filled with mahogany brown-coloured bile were scattered throughout the liver were as a result of biliary stasis.



Figure 15. Yellow-brown liver typical of liver dystrophy in a Eurasian eagle owl (*Bubo bubo*).

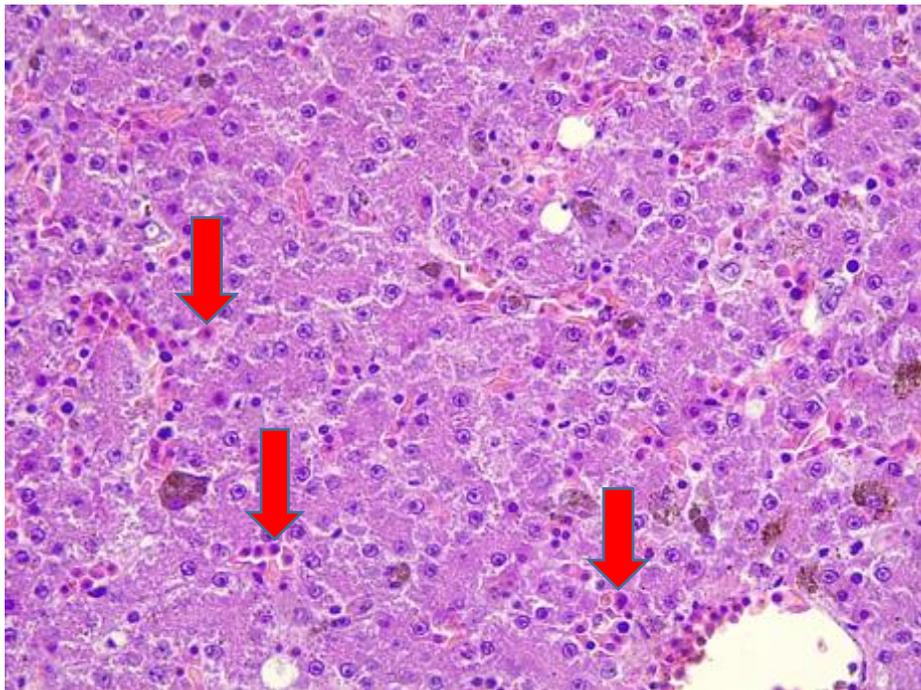


Figure 16. Histopathological lesions associated by hepatic dystrophy seen in **Figure 15**, Councilman bodies are marked by arrows (Hematoxylin-Eosin staining, 120X magnification).

The third most common cause of death in our study was bleeding out into the celomic cavity following internal organ rupture. 11% ($n = 7$) of the cases elicited lesions consistent with the afore mentioned alterations, with the majority of these being juvenile diurnal predators. In such instances, a powerful, blunt traumatic event of mechanical origin was suspected to be the causative factor, leading rupture, and subsequent extravasation and hemorrhage of the fragile

visceral organs (**Figure 17**). The most frequently affected organ was determined to be the liver and the site rupture could be identified under the blood clots.



Figure 17. Ruptured liver, blood clot on the capsule of the liver and subsequent hemorrhage into the celomic cavity in a Long-eared owl (*Asio otus*).

Signs of significant mechanical trauma not affecting the body cavity was observed in a number of the predatory birds (8%, $n = 5$). Fractures of the wings and/or the limbs were confirmed either on gross necropsy or through *post mortem* radiology where the type, extent and affected bones could be visualized. Fractures to one or more bones were recorded and **Figure 18** depicts the radiographic findings in a Sparrowhawk.

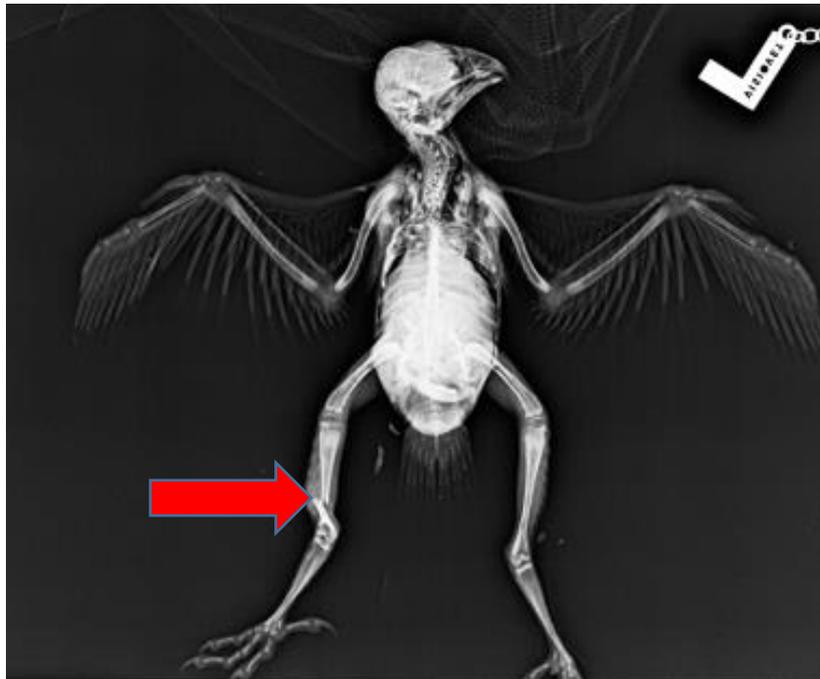


Figure 18. Radiographic finding of a tibiotarsus fracture (arrow) in a Sparrowhawk (*Accipiter nisus*), ventro-dorsal positioning.

In two cases, open fractures (where the fracture of the bone led to a tear in the overlying skin and adjacent soft tissue and the protrusion of the bone) became infested by fly larvae and myiasis was on gross necropsy (**Figure 19**).



Figure 19. Myiasis in the site of an open fracture on the wing of a Common kestrel (*Falco tinnunculus*).

Through diagnostic necropsy, 8% (n = 8) had enlarged, light brown, streaked kidneys indicative of tubulonephrosis. Moreover, pinpoint, grey-white coloured patterns were discernable on the surface of affected kidneys (**Figure 20**). The afore mentioned lesions are typical of gross lesion found in cases of kidney failure. On histopathology, the inner lining of the tubules of the kidney were found to be sloughed into the lumen, nuclei were not stained as strongly as under physiological conditions and pyknosis could be detected in some, whilst vacuolization of the cytoplasm was in other cells (**Figure 21**).



Figure 20. Macroscopic image of acute tubulonephrosis in the kidney of a Northern goshawk (*Accipiter gentilis*).

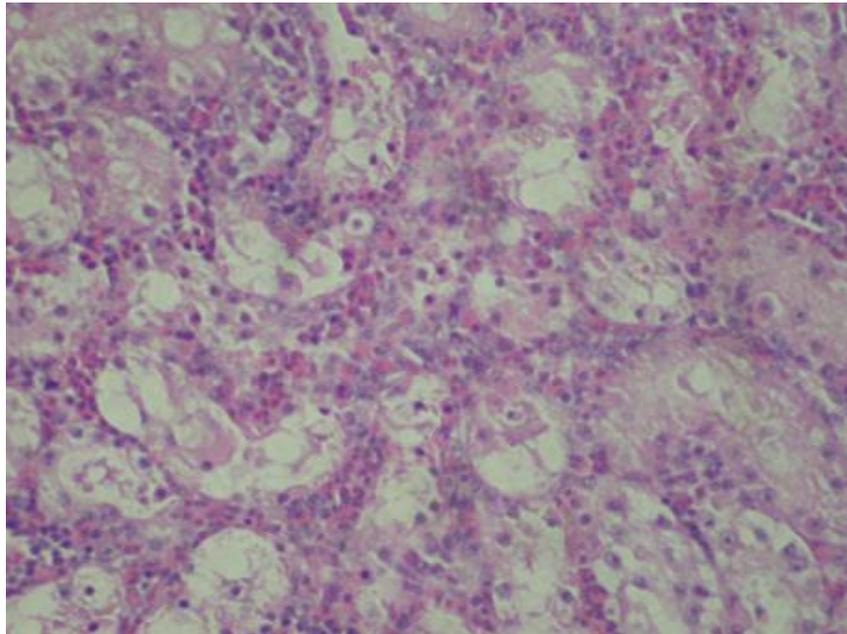


Figure 21. Histopathological lesions associated with tubulonephrosis seen in **Figure 20** (Hematoxylin-Eosin staining, 120X magnification).

Accounting for merely 3% (n =2) of the cases, erosions of the mucous membrane layer of the glandular stomach were recorded. These alterations led to bleeding into the gastric lumen, with considerable blood-loss, and subsequent fatal acute post-hemorrhagic shock. On necropsy, the mucous membranes of the glandular stomach was a chocolate-brown colour, and a large amount of blackish-red, cream-like contents (digested blood) were observed in the small intestinal loops (**Figure 22**). The mucous membranes of the body orifices were porcelain-white in colour.



Figure 22. Digested blood in the glandular stomach and the small intestine of a Common kestrel (*Falco tinnunculus*).

Visceral gout, consequent to chronic renal failure, was observed in just 2% (n = 1) of the cases. A whitish-grey precipitate (uric acid deposition) was noted covering the affected visceral organs during *post mortem* examination. Such precipitates were recorded in the pericardium (**Figure 23**), the capsule of the liver, on the serous membranes of the celomic cavity, and the air sacs adjacent to the afore mentioned organs. The kidneys were smaller than normal, and of a pale, grey-brown colour macroscopically.

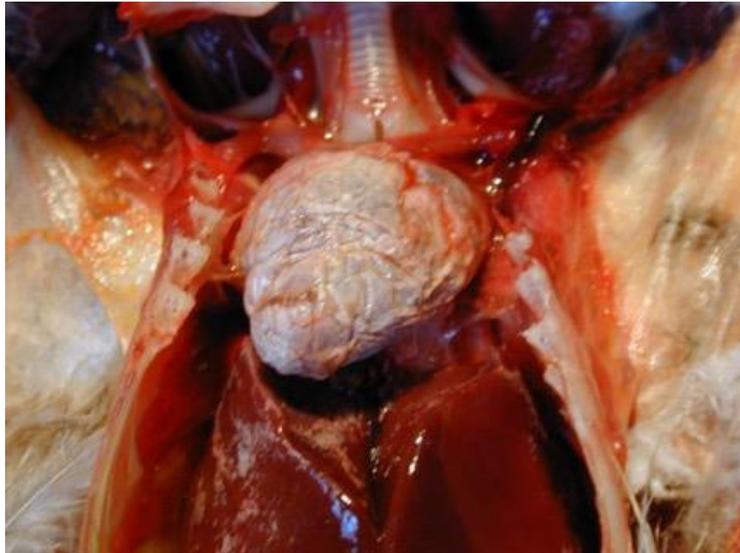


Figure 23. Uric acid deposition in the pericardium of a European honey buzzard (*Pernis apivorus*).

Insufficient nutrition leading to emaciation was also attributed to be the cause of death in 2% (n = 1) of the birds of prey examined. Extremely poor body condition (BCS <1) was perceived and the musculature (*musculus pectoralis*) inserting on the sternal crest (*crista sternii*) was extremely minimal and the crest itself was not covered (**Figure 24**). However, it is important to mention, pectoral musculature can also deteriorate to a certain extent if the individual is unable to utilize these muscles through an inability to fly. Literally no fat tissue was seen in the celomic cavity. Around the coronary groove of the heart a straw-yellow, jelly-like substance was noticeable, a typical alteration observed in emaciated birds (**Figure 25**).



Figure 24. Extremely poor body condition (BCS <1) in a Common kestrel (*Falco tinnunculus*).

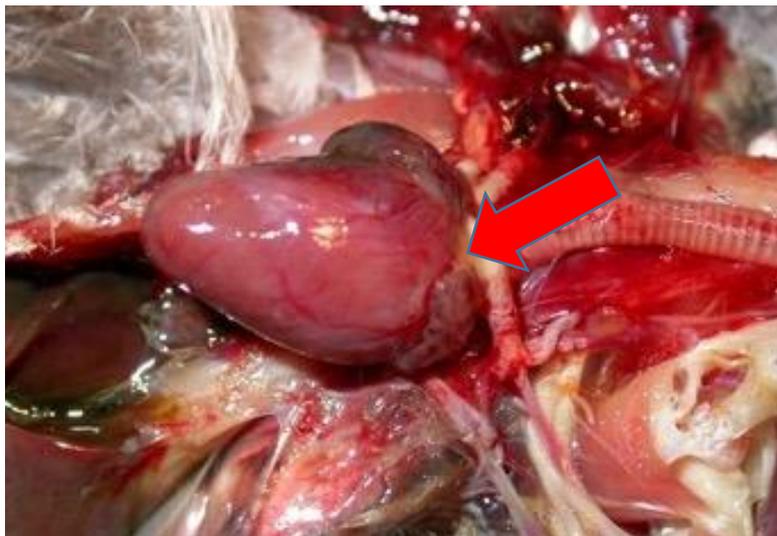


Figure 25. Straw-yellow, jelly-like substance in the coronary groove of an emaciated Common kestrel (*Falco tinnunculus*).

Two percent of the cases (n = 1) accounted for the cause of death to be resultant from a piece of shrapnel piercing the body wall, damaging one or more of the internal organs or large vessels, leading to internal bleeding. The causative bullet was not seen in the celomic cavity of the affected individual during the *post mortem* examination, as it would have passed through the body at the time the injury occurred.

A single neoplasm was recorded (2%) in the retrospective portion. The tumour was identified under the beak of a Saker falcon and was a fibrosarcoma with a homogenous cut surface (**Figure 26**).



Figure 26. Fibrosarcoma in the soft tissue under the beak of Saker falcon (*Falco cherrug*).

Owing to a larger sample size, non-infectious causes of Common kestrels were summarized to allow comparison within cases for this species (**Figure 27**).

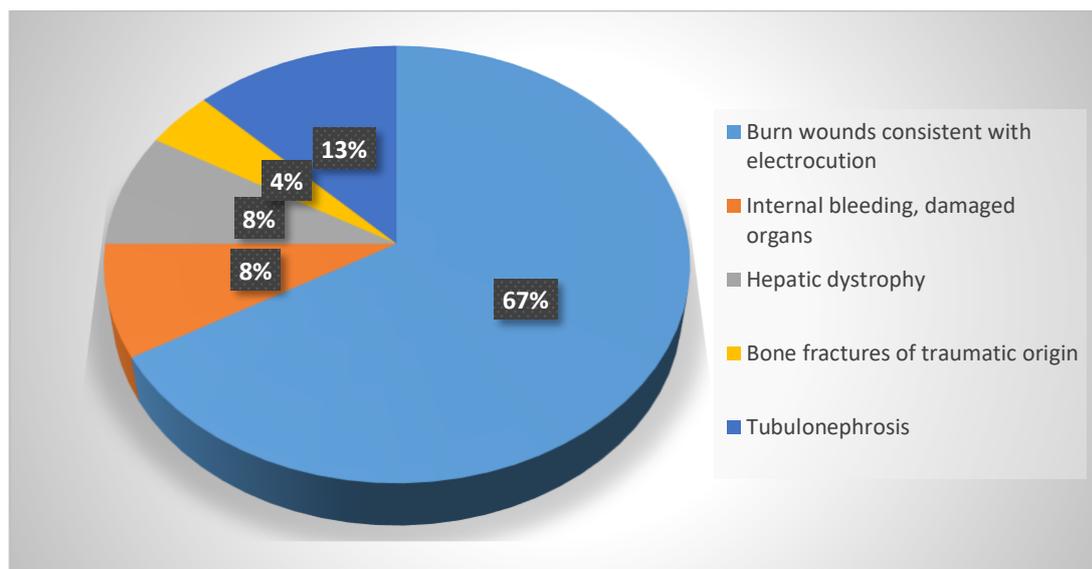


Figure 27. Non-infectious causes of mortality in Common kestrels (*Falco tinnunculus*) (n = 24).

A high proportion of these birds (67%, n = 16) succumbed to or were euthanized because of electrocution. In these instances, second- and third-degree burns were observed on the talons and frequently the contralateral wing. In 13% (n = 3), acute renal failure was implicated to be the cause of death, with the second highest incidence. Hepatic dystrophy and internal bleeding were present in 8% (n = 2) of cases apiece. Merely 4% (n = 1) of cases accounted for fractured bones in the case of the Common kestrels. Of the 67% where cause of death was in connection with electrocution, 71% of the birds were found to be juvenile and 29% were adult birds.

Second most numerous cases in raptors were those pertaining to Common buzzards where the non-infectious causes of mortality are summarized in **Figure 28**.

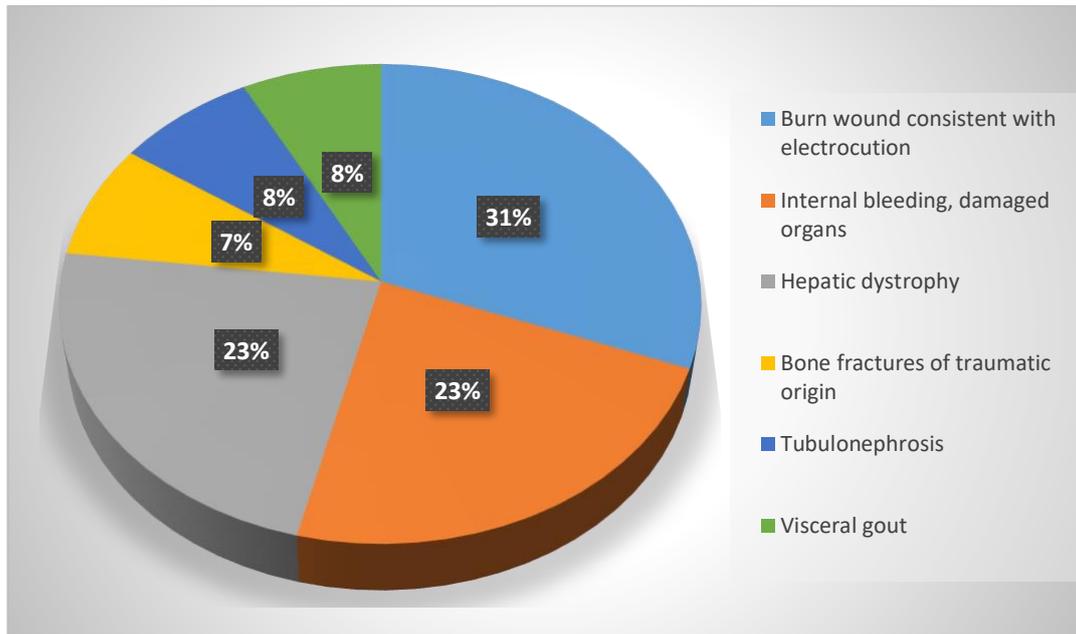


Figure 28. Non-infectious causes of mortality in Common buzzard (*Buteo buteo*) (n = 13).

In the Common buzzards, 31% (n = 4) were victims of electrocution and the second most frequently occurring cause of death was attributed to internal bleeding and hepatic dystrophy at 23% (n = 3) respectively. 8%-8% (n = 1) of these birds suffered from either acute kidney failure in the form of tubulonephrosis or visceral gout, and 7% (n = 1) of *post mortem* examinations yielded traumatic injuries to have led to the death of the individuals.

Though not a pathological finding per say, one of the female Sparrowhawks in the study was found to have bilateral inactive ovaries (**Figure 29**) of gross necropsy.



Figure 29. Bilateral, inactive ovaries in a Sparrowhawk (*Accipiter nisus*).

4.4. Trace element accumulation

Though considered a factor leading to non-infectious morbidity and mortality based on etiology, examination of trace elements from tissue samples was considered a separate part of the study and therefore warranted its own chapter. In order to determine the accumulation of trace elements in birds of prey, liver and bone (tarsometatarsal bone) samples were utilized. Resultant data from bone samples is presented in **Table 8** including average and standard deviation values and the LOD (the lowest level of a given trace element detected by the analyzer machine in mg/kg).

Table 8. Trace element values from bone samples (according to registration number of the sample), mean, standard deviation and LOD (n = 28).

	As	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Zn
Species (in alphabetical order)	mg/kg											
<i>Accipiter nisus</i>	<0.5	3.62	0.119	<0.05	1.19	1.84	1.28	2.35	<0.5	<0.2	<0.2	186
<i>Accipiter nisus</i>	<0.5	3.91	0.142	0.119	3.55	1.23	2.67	3.69	<0.5	1.29	0.53	195
<i>Accipiter nisus</i>	<0.5	1.52	0.055	0.072	2.00	0.98	0.57	2.18	<0.5	0.58	1.27	140
<i>Asio otus</i>	<0.5	1.95	0.118	0.054	1.25	1.29	4.27	3.00	<0.5	<0.2	1.00	154
<i>Buteo buteo</i>	<0.5	3.18	0.160	0.078	1.57	1.11	<0.5	2.54	<0.5	<0.2	0.83	181
<i>Falco subbuteo</i>	<0.5	6.19	0.092	<0.05	1.11	1.25	1.17	2.90	<0.5	<0.2	0.41	130
<i>Falco tinnunculus</i>	<0.5	2.22	<0.05	<0.05	1.08	1.62	<0.5	1.43	<0.5	<0.2	4.24	168
<i>Falco tinnunculus</i>	<0.5	8.57	<0.05	0.078	1.26	2.03	1.10	2.01	<0.5	0.32	3.07	163
<i>Falco tinnunculus</i>	<0.5	4.71	<0.05	0.073	1.49	1.35	1.08	3.67	<0.5	<0.2	0.92	145
<i>Falco tinnunculus</i>	<0.5	3.07	0.073	0.101	1.43	3.25	<0.5	3.70	<0.5	0.30	2.32	140
<i>Falco tinnunculus</i>	<0.5	14.0	0.127	<0.05	1.19	1.67	<0.5	1.97	<0.5	0.35	0.81	129
<i>Falco tinnunculus</i>	<0.5	14.5	0.111	0.302	2.24	4.18	0.91	13.41	<0.5	0.88	3.79	133
<i>Falco tinnunculus</i>	<0.5	2.70	0.115	0.020	1.36	1.87	0.90	2.41	<0.5	<0.2	<0.2	133
<i>Falco tinnunculus</i>	<0.5	2.82	0.082	0.096	2.71	1.56	<0.5	2.70	<0.5	1.39	1.50	128
<i>Falco tinnunculus</i>	<0.5	3.23	<0.05	0.056	1.18	1.68	<0.5	3.22	<0.5	<0.2	1.24	135
<i>Falco tinnunculus</i>	<0.5	4.47	0.104	0.156	5.29	1.77	0.51	3.10	<0.5	2.72	12.98	157
<i>Falco tinnunculus</i>	<0.5	15.4	0.080	0.175	2.32	1.48	<0.5	5.34	<0.5	0.88	0.83	140
<i>Falco tinnunculus</i>	<0.5	8.25	<0.05	0.076	1.44	1.18	<0.5	3.09	<0.5	0.43	1.45	125
<i>Falco tinnunculus</i>	<0.5	15.0	0.135	0.220	2.28	2.14	0.67	6.83	<0.5	0.64	0.86	136
<i>Falco tinnunculus</i>	<0.5	4.46	0.051	0.089	1.63	0.94	0.61	2.30	<0.5	0.37	1.82	127

Table 8. Trace element values from bone samples (according to registration number of the sample), mean, standard deviation and LOD (n = 28) continued.

	As	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Zn
Species (in alphabetical order)	mg/kg											
<i>Falco tinnunculus</i>	<0.5	3.64	<0.05	<0.05	2.10	2.89	<0.5	2.20	<0.5	0.54	4.50	132
<i>Falco tinnunculus</i>	<0.5	1.59	<0.05	<0.05	1.21	2.29	<0.5	1.35	<0.5	0.42	0.97	146
<i>Falco tinnunculus</i>	<0.5	12.8	0.100	<0.05	1.02	2.27	1.10	2.33	<0.5	0.20	1.96	131
<i>Falco tinnunculus</i>	<0.5	3.69	0.083	<0.05	1.16	1.81	0.93	2.84	<0.5	0.27	1.29	132
<i>Falco tinnunculus</i>	<0.5	3.08	<0.05	<0.05	1.16	2.66	<0.5	1.51	<0.5	0.26	3.12	133
<i>Falco tinnunculus</i>	<0.5	2.24	0.058	<0.05	1.01	1.74	1.13	1.18	<0.5	0.37	2.23	176
<i>Falco tinnunculus</i>	<0.5	15.9	0.136	<0.05	1.29	1.28	4.12	2.79	<0.5	<0.2	3.89	135
<i>Strix aluco</i>	<0.5	5.14	0.128	0.088	1.31	2.05	0.74	2.37	<0.5	<0.2	3.52	119
LOD	0.5	0.5	0.05	0.05	0.05	0.05	0.5	0.05	0.5	0.2	0.2	0.05
Mean	<0.5	6.14	0.10	0.11	1.71	1.84	1.40	3.16	<0.5	0.68	2.36	145
Standard deviation	n/a	4.83	0.03	0.07	0.92	0.72	1.16	2.32	n/a	0.62	2.50	20.3

Bone samples originating from all examined individuals and the trace element values measured in mg/kg unit are shown in **Figure 30**.

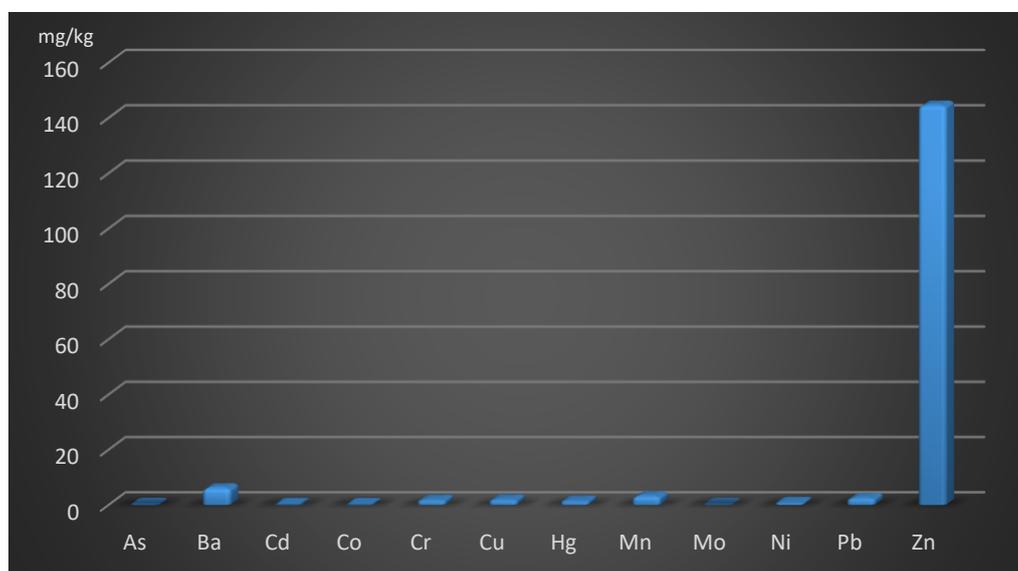


Figure 30. Examined bone samples from all birds of prey in the study (n = 28) with the average trace element values depicted.

Levels of arsenic and molybdenum were below the LOD in all samples. Bone tissue measurements from Common kestrels (n = 22) and the ensuing average trace element values are summarized in **Figure 31**. Aside from bone samples, analysis for the presence of trace element and heavy metal accumulation was also performed from liver tissues and the resultant data is shown in **Table 9** and **Figure 32**.

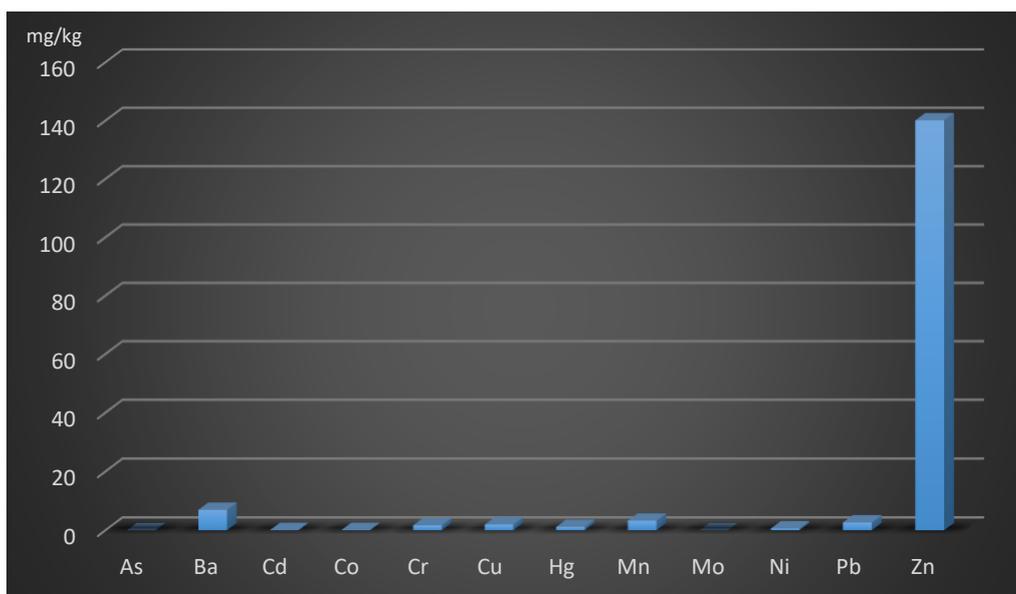


Figure 31. Examined bone samples from Common kestrels (*Falco tinnunculus*) in the study (n = 21) and the average trace element values measured.

Table 9. Trace element values from liver tissue samples in various birds of prey (in order according to registration number of the sample), mean, standard deviation and LOD (n = 42).

	As	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Zn
Species (in alphabetical order)	mg/kg											
<i>Accipiter nisus</i>	<0.5	<0.5	0.099	<0.05	<0.05	6.84	<0.5	4.09	0.814	<0.2	0.33	21.1
<i>Accipiter nisus</i>	<0.5	<0.5	0.125	<0.05	<0.05	11.8	1.72	4.82	1.03	<0.2	<0.2	49.8
<i>Accipiter nisus</i>	<0.5	<0.5	1.393	<0.05	<0.05	6.23	0.568	5.23	0.722	<0.2	<0.2	32.8
<i>Accipiter nisus</i>	<0.5	<0.5	0.561	<0.05	<0.05	3.26	<0.5	1.12	0.660	<0.2	2.24	19.7
<i>Asio otus</i>	<0.5	<0.5	0.090	<0.05	<0.05	3.84	1.36	2.63	0.858	<0.2	<0.2	21.5
<i>Buteo buteo</i>	<0.5	<0.5	0.336	<0.05	<0.05	6.63	<0.5	3.10	<0.5	<0.2	0.34	30.3
<i>Buteo buteo</i>	<0.5	<0.5	<0.05	<0.05	<0.05	3.22	<0.5	1.30	0.729	<0.2	<0.2	14.8
<i>Buteo buteo</i>	<0.5	<0.5	<0.05	<0.05	<0.05	4.09	<0.5	4.99	0.559	<0.2	<0.2	30.9
<i>Buteo buteo</i>	<0.5	<0.5	0.201	<0.05	<0.05	7.62	0.677	5.71	<0.5	<0.2	<0.2	54.7
<i>Buteo buteo</i>	<0.5	<0.5	0.750	<0.05	<0.05	4.43	<0.5	2.80	0.724	<0.2	<0.2	29.5
<i>Buteo buteo</i>	<0.5	<0.5	0.504	0.05	<0.05	11.9	2.28	10.2	0.936	<0.2	0.27	108
<i>Buteo buteo</i>	<0.5	<0.5	0.725	0.07	<0.05	10.19	<0.5	3.41	0.755	<0.2	0.43	51.8
<i>Buteo buteo</i>	<0.5	<0.5	0.701	<0.05	<0.05	5.71	0.743	1.95	0.651	<0.2	0.43	53.9
<i>Buteo buteo</i>	<0.5	<0.5	0.965	<0.05	<0.05	9.61	<0.5	1.28	<0.5	<0.2	<0.2	89.8
<i>Buteo buteo</i>	<0.5	<0.5	0.159	<0.05	<0.05	2.69	0.620	2.80	<0.5	<0.2	0.24	24.3
<i>Buteo buteo</i>	<0.5	<0.5	<0.05	0.05	<0.05	3.50	<0.5	4.42	<0.5	<0.2	<0.2	55.8
<i>Buteo buteo</i>	<0.5	<0.5	0.244	<0.05	<0.05	3.37	0.605	5.07	<0.5	<0.2	<0.2	76.0
<i>Clanga pomarina</i>	<0.5	<0.5	0.059	<0.05	<0.05	7.95	<0.5	2.25	<0.5	<0.2	<0.2	21.9
<i>Falco peregrinus</i>	<0.5	<0.5	0.200	0.09	<0.05	4.86	1.04	5.24	<0.5	<0.2	<0.2	43.2
<i>Falco subbuteo</i>	<0.5	<0.5	0.054	<0.05	<0.05	3.77	1.31	3.00	0.510	<0.2	<0.2	23.5
<i>Falco tinnunculus</i>	<0.5	0.98	<0.05	<0.05	0.15	4.36	<0.5	3.44	<0.5	<0.2	1.17	19.8

Table 9. Heavy metal values from liver tissue samples in various birds of prey (in order according to registration number of the sample), LOD, mean and standard deviation continued.

	As	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Zn
Species (in alphabetical order)	mg/kg											
<i>Falco tinnunculus</i>	<0.5	<0.5	<0.05	<0.05	<0.05	4.06	<0.5	3.91	<0.5	<0.2	0.3	19.1
<i>Falco tinnunculus</i>	<0.5	<0.5	0.106	<0.05	<0.05	13.4	0.522	7.39	0.622	<0.2	0.56	44.2
<i>Falco tinnunculus</i>	<0.5	<0.5	<0.05	<0.05	<0.05	6.05	<0.5	5.37	<0.5	<0.2	0.46	29.9
<i>Falco tinnunculus</i>	<0.5	<0.5	<0.05	<0.05	<0.05	4.88	<0.5	2.30	0.586	<0.2	0.30	17.1
<i>Falco tinnunculus</i>	<0.5	<0.5	<0.05	<0.05	<0.05	5.88	<0.5	7.62	0.673	<0.2	0.63	46.8
<i>Falco tinnunculus</i>	<0.5	<0.5	<0.05	<0.05	0.17	7.95	<0.5	5.04	0.535	<0.2	0.300	26.8
<i>Falco tinnunculus</i>	<0.5	<0.5	<0.05	0.10	<0.05	14.2	<0.5	5.47	0.886	<0.2	<0.2	55.0
<i>Falco tinnunculus</i>	<0.5	<0.5	<0.05	0.11	<0.05	9.82	<0.5	3.99	0.567	<0.2	0.39	27.0
<i>Falco tinnunculus</i>	<0.5	<0.5	0.076	<0.05	<0.05	4.06	0.735	5.20	0.523	<0.2	<0.2	27.9
<i>Falco tinnunculus</i>	<0.5	<0.5	<0.05	<0.05	<0.05	4.46	<0.5	4.98	<0.5	<0.2	<0.2	35.4
<i>Falco tinnunculus</i>	<0.5	<0.5	0.106	<0.05	<0.05	5.78	<0.5	6.08	0.856	<0.2	<0.2	30.1
<i>Falco tinnunculus</i>	<0.5	<0.5	0.086	0.08	<0.05	6.05	0.529	5.23	0.605	<0.2	<0.2	51.7
<i>Falco tinnunculus</i>	<0.5	<0.5	0.127	0.08	<0.05	5.60	<0.5	9.14	1.03	<0.2	<0.2	79.3
<i>Falco tinnunculus</i>	<0.5	<0.5	0.080	<0.05	<0.05	12.1	0.580	4.28	<0.5	<0.2	0.37	133
<i>Falco tinnunculus</i>	<0.5	<0.5	<0.05	<0.05	<0.05	26.1	<0.5	5.86	0.558	<0.2	<0.2	48.4
<i>Falco tinnunculus</i>	<0.5	<0.5	<0.05	<0.05	<0.05	3.97	<0.5	3.42	<0.5	<0.2	<0.2	19.3
<i>Falco tinnunculus</i>	<0.5	<0.5	<0.05	<0.05	<0.05	4.77	<0.5	4.02	<0.5	<0.2	<0.2	31.4
<i>Falco tinnunculus</i>	<0.5	<0.5	0.050	0.07	<0.05	5.23	<0.5	8.75	0.835	<0.2	1.34	42.4
<i>Falco tinnunculus</i>	<0.5	<0.5	<0.05	<0.05	<0.05	3.85	<0.5	3.99	0.895	<0.2	<0.2	39.2
<i>Falco tinnunculus</i>	<0.5	<0.5	0.156	0.06	<0.05	10.4	2.77	6.07	0.997	<0.2	<0.2	82.0
<i>Pernis apivorus</i>	<0.5	<0.5	0.132	0.11	<0.05	22.8	<0.5	11.2	1.04	<0.2	0.41	48.7
<i>Strix aluco</i>	<0.5	<0.5	0.113	0.11	<0.05	20.39	1.05	8.68	0.764	<0.2	<0.2	69.6
LOD	0.5	0.5	0.05	0.05	0.05	0.05	0.5	0.05	0.5	0.2	0.2	0.05
Mean	<0.5	n/a	0.67	0.08	0.16	7.80	1.01	5.00	0.75	<0.2	0.58	44.5
Standard deviation	n/a	n/a	0.34	0.02	0.02	5.40	0.65	2.70	0.16	n/a	0.51	26.0

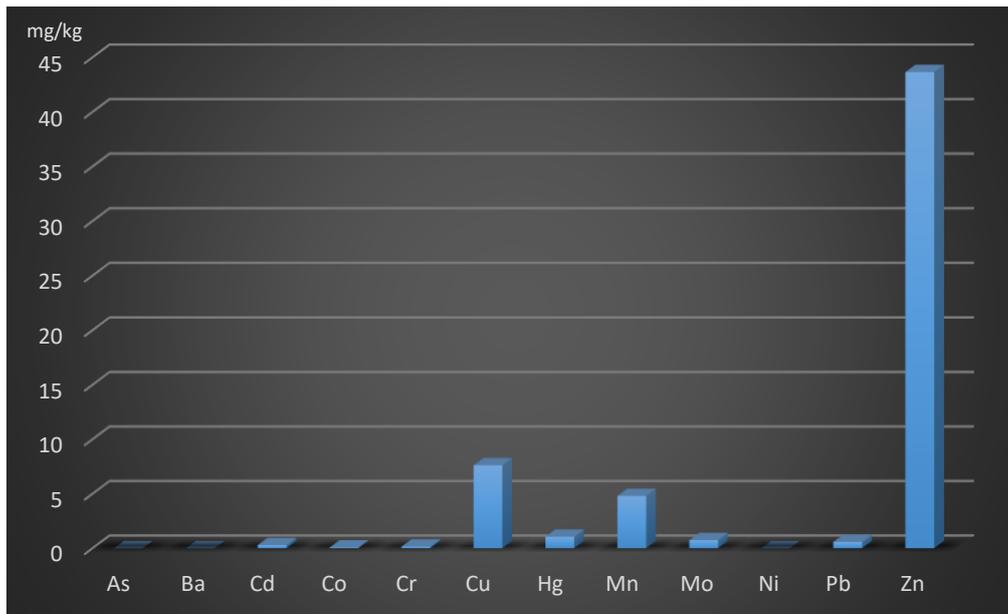


Figure 32. Examined liver samples from all birds of prey in the study (n = 42) and the average trace element values measured.

Trace element values from samples of the liver tissue of Common kestrels (n = 20) are summarized in **Figure 33**, whereas those pertaining to Sparrowhawks can be viewed in **Figure 34**, and Common buzzard results in **Figure 35**.

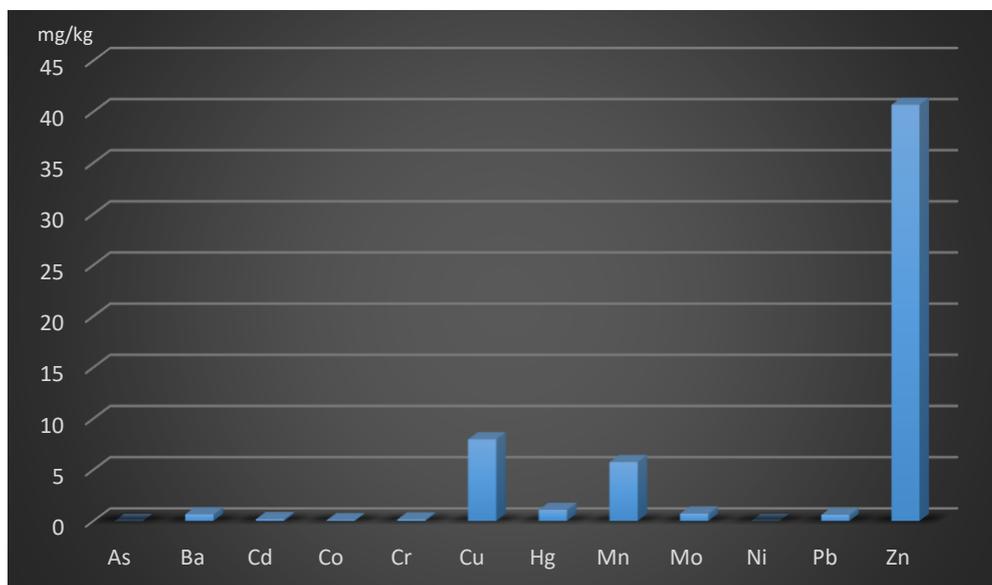


Figure 33. Trace elements measured from liver tissue samples, including those exceeding the LOD, in Common kestrels (*Falco tinnunculus*) (n = 20).

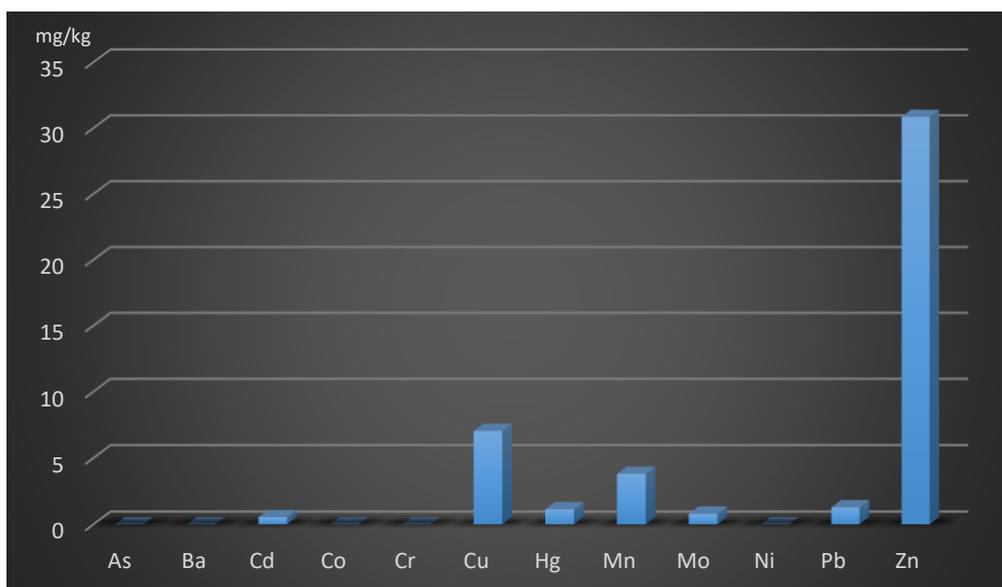


Figure 34. Trace elements measured from liver tissue samples, including those exceeding the LOD, in Sparrowhawk (*Accipiter nisus*) (n = 4).

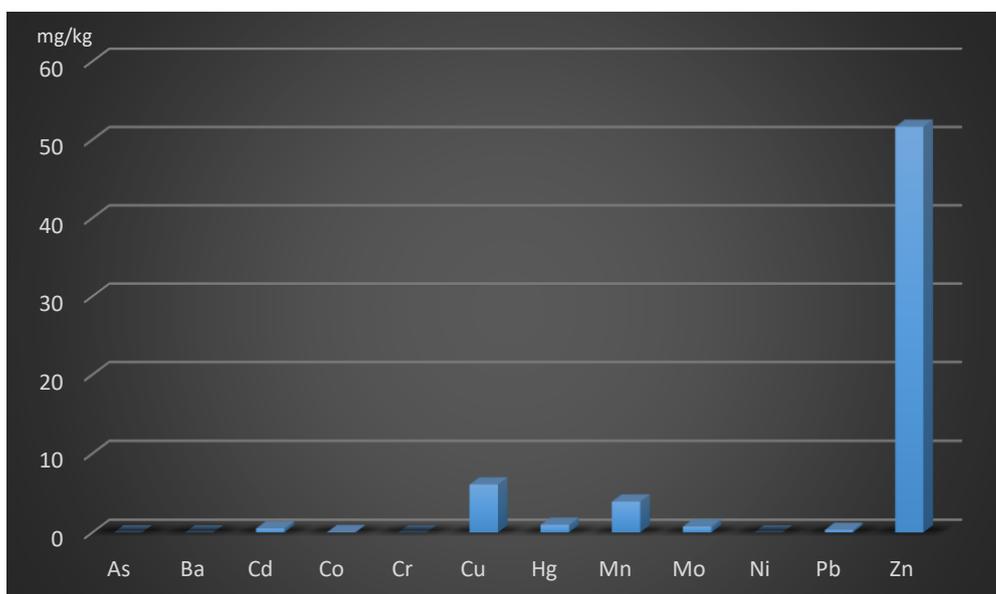


Figure 35. Trace elements measured from liver tissue samples, including those exceeding the LOD, in Common buzzards (*Buteo buteo*) (n = 12).

As sample sizes was sufficient to warrant further investigation, statistical examination to determine mean and standard deviation was performed from those samples originating from Common kestrels. Moreover, following the assessment of normalization, it was evident that paired t-tests could not be performed on the dataset and therefore, a Mann-Whitney U test was implemented to determine statistical significance between levels of the trace elements originating from bone and liver tissues (**Table 10**).

Table 10. Mean and standard deviation values obtained from various trace elements levels measured from liver and bone samples of Common kestrels (*Falco tinnunculus*).

Trace element	Liver		Bone	
	mean (mg/kg)	standard deviation (mg/kg)	mean (mg/kg)	standard deviation (mg/kg)
Arsenic	<0.5	n/a	<0.5	n/a
Barium	0.66	0.28	6.78	5.27*
Cadmium	0.19	0.25	0.10	0.03
Cobalt	0.08	0.02	0.12	0.08
Chromium	0.16	0.02	1.71	0.96*
Copper	7.98	5.58	1.98	0.76*
Mercury	1.09	0.95	1.19	0.99
Manganese	5.76	2.51	3.30	2.67*
Molybdenum	0.73	0.18	<0.5	n/a
Nickel	<0.2	n/a	0.65	0.63*
Lead	0.61	0.39	2.69	2.71*
Zinc	40.70	20.11	140.00	14.35*

*values indicating statistically significant ($p < 0.05$) difference between the values measured in the liver and bone tissue using a Mann-Whitney U test.

Moreover, mean hepatic concentrations of Ba (**Table 10**; Mann-Whitney U test: $Z = 2.75$, $n_1 = 21$, $n_2 = 3$, $p = 0.0059$), Cr (**Table 10**; Mann-Whitney U test: $Z = 2.29$, $n_1 = 21$, $n_2 = 2$, $p = 2.19E-02$), Cu (**Table 10**; Mann-Whitney U-test: $Z = 5.45$, $n_1 = 21$, $n_2 = 21$, $p = 5.15E-08$), Mn (**Table 10**; Mann-Whitney U test: $Z = 4.01$, $n_1 = 21$, $n_2 = 21$, $p = 6.01E-05$), Ni (**Table 10**; Mann-Whitney U test: $Z = 2.35$, $n_1 = 16$, $n_2 = 3$, $p = 0.019$), Pb (**Table 10**; Mann-Whitney U test: $Z = 3.68$, $n_1 = 20$, $n_2 = 9$, $p = 0.00023$) and Zn (**Table 10**; Mann-Whitney U test: $Z = 5.55$, $n_1 = 21$, $n_2 = 21$, $p = 2.91E-08$) were significantly greater than those measured from bone tissue samples in Common kestrels.

The origin of individuals was either geographically vague (i.e. city/town closest to where an individual was found was listed as the site of origin) or not recorded and therefore comparisons were not made as part of this study.

4.5. Infectious causes of morbidity and mortality

Infectious cases of morbidity and mortality of the predatory birds comprised 22% (n = 19) of our patients included in this study and are summarized in **Figure 36**.

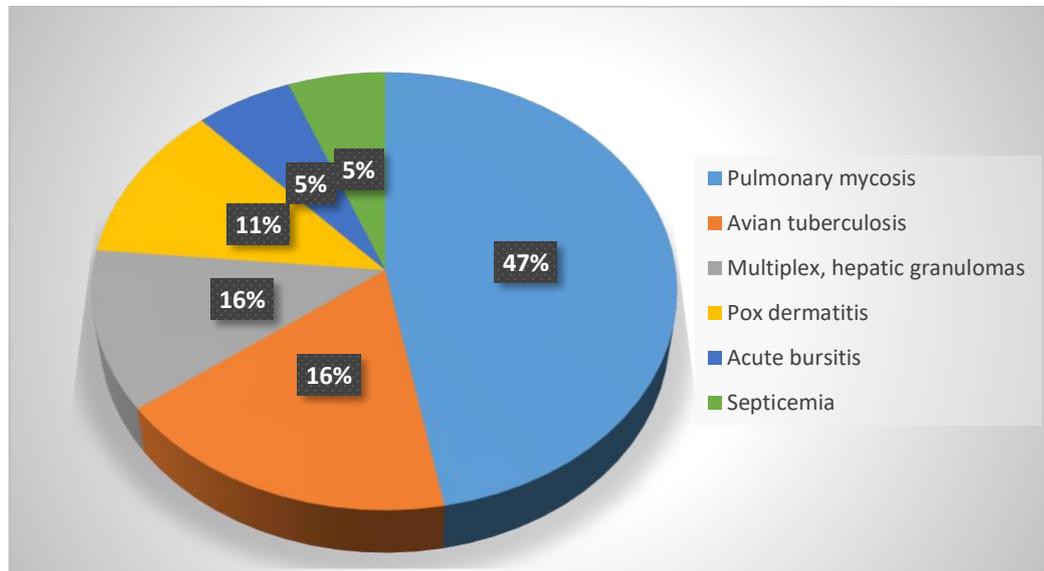


Figure 36. Infectious causes of morbidity and mortality (n = 19).

Infectious causes of illness and death were subsequent to viral infections (i.e. pox dermatitis), those caused by bacteria (i.e. avian tuberculosis) and fungi leading to pulmonary mycosis.

Pulmonary mycosis was the most frequent infectious cause of mortality in the scope of this study (47%, n = 9). In all cases, non-transparent, thickened air sac walls were observed on gross necropsy covered by a film of whitish-grey mould. However, when removed, a yellowish-grey layer was visible underneath, adhered firmly to the air sac itself. Though the pulmonary tissue was intact, physiological shaped, and brick red in colour, the parenchyma was strewn with miliary yellowish-white, firm granulomas. The cut surface of these masses was brittle, and contents were gritty (**Figure 37-38**).

On histopathology, a connective tissue capsule could be visualized with the presence of fungal hyphae within (**Figure 39**). Similarly, fungi were also discernible on the film present on air sac walls and the thickening was attributed to connective tissue proliferation.

Swabs taken from the fungal colonies on necropsy were cultured, and after 48 hours of incubation, greyish-green mould colonies could be visualized. Based on colony morphology, and their growth, these were classified as *Aspergillus* sp. (**Figure 40**).



Figure 37. Mycosis of the air sacs in a Eurasian eagle owl (*Bubo bubo*).

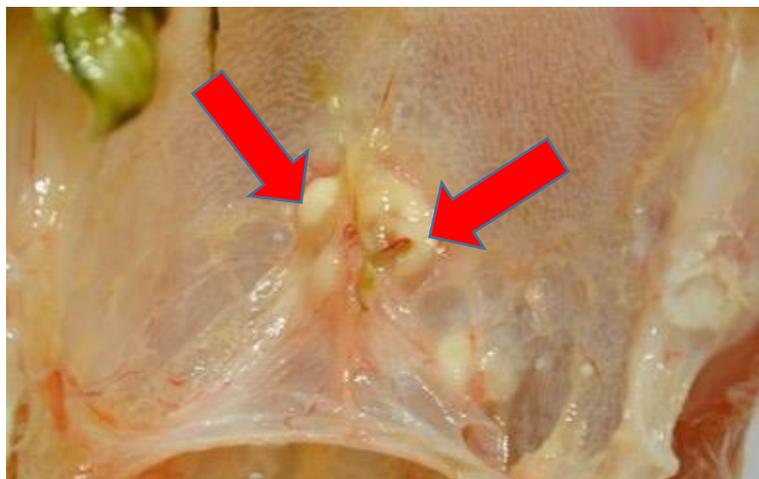


Figure 38. Fungal granulomas (arrows) in a Northern goshawk (*Accipiter gentilis*).

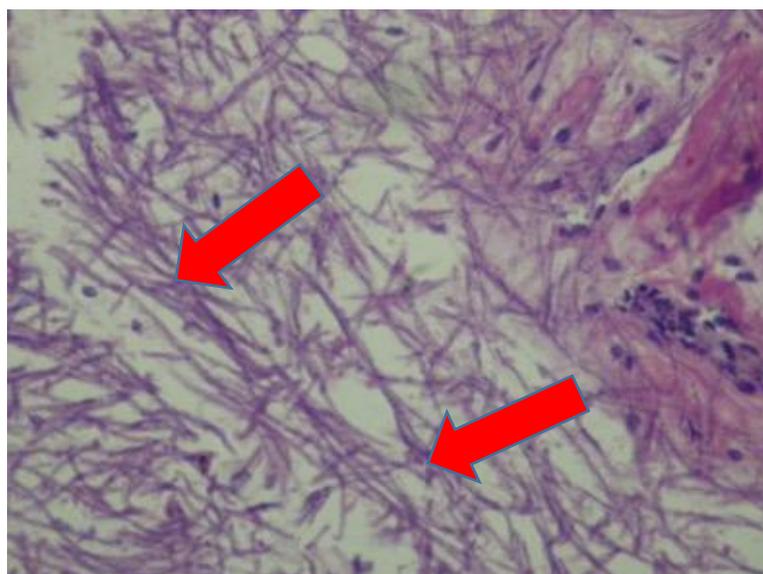


Figure 39. Histopathological image of air sac mycosis, fungal hyphae are marked by arrows (Hematoxylin-Eosin staining, 120X magnification).

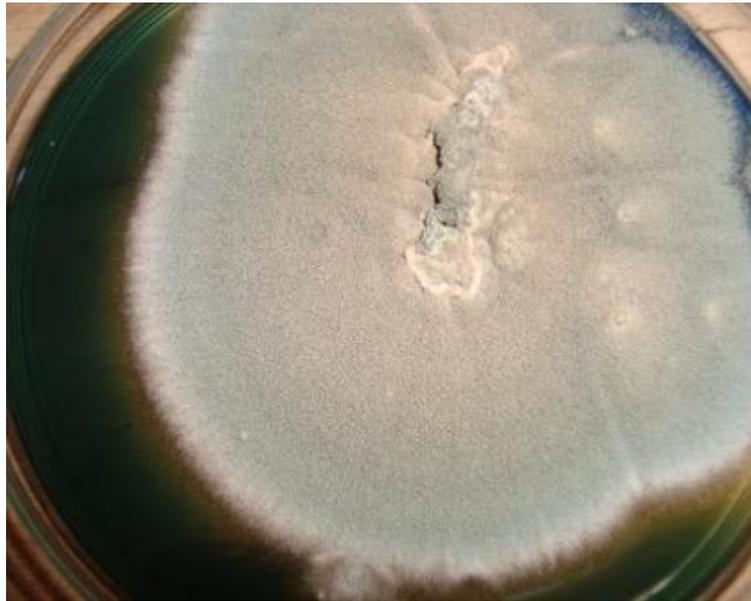


Figure 40. *Aspergillus* sp. colony after culture on Drigalski agar.

The second most frequently diagnosed infectious agent, in 16% (n = 3) of cases, was avian tuberculosis. On gross necropsy, yellowish-brown granulomas (primary lesions) were visible on the serosal aspect of the intestinal tract (**Figure 41**). These were firm on palpation and a few millimetres in diameter. The cut section revealed a granular substance therein. Similar granulomatous lesions were perceived in the lungs (**Figure 42**), liver (**Figure 41**) and the spleen.

The structure of these granulomas on histopathology was as follows. Adjacent to a lympho-histiocytic zone, surrounding an inner caseous region, multinucleated, foreign-body type giant cells were visible microscopically. In the cytoplasm of these giant cells, as well as between cells, carmine-red stained rods were perceived following Ziehl-Neelsen staining (**Figure 43**). This method of acid-fast staining is the gold-standard for the detection of the causative agent of avian tuberculosis; *Mycobacterium avium*.



Figure 41. Granulomatous lesions typical of avian tuberculosis in the intestinal tract (arrow on the left) and in the liver (arrow on the right) of a Common buzzard (*Buteo buteo*).



Figure 42. Granulomatous lesions typical of avian tuberculosis in the pulmonary tissue (arrow) of a Common buzzard (*Buteo buteo*).

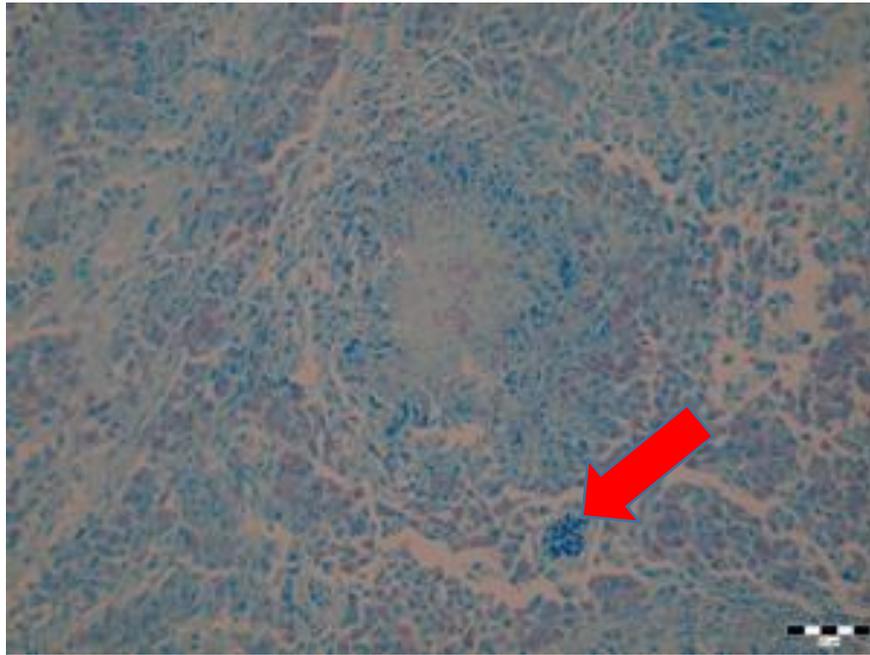


Figure 43. Histopathological image of a granulomatous lesions typical of avian tuberculosis in a Common buzzard (*Buteo buteo*), a foreign-body type giant cell marked by the arrow (Ziehl-Neelsen staining, 120X magnification).

Bacteria causing multiplex granulomatous, necrotic hepatitis in diurnal and nocturnal predators also accounted for 16% (n = 3) of the cases. On *post mortem* examination, all birds had a yellowish-brown liver with demarcated necrotic regions therein and marked hepatomegaly. Bacterial culture from these alterations yielded *Pseudomonas aeruginosa* and *Escherichia coli*, based on the growth pattern, biochemical parameters, and the colouration of the colonies on blood and Drigalski agar.

In a few instances (11%, n = 2), poxvirus infections was also identified through the typical “pock” lesions elicited by this DNA virus. Affected individuals had yellowish-brown granulomas of variable size with an oozing and vascularized base on their eyelids, in the vicinity of their beak, and occasionally along their tarsus. Microscopically, the integument layer showed signs of hyper- and parakeratosis and in places, balloning was also noted on the edges of the pock lesions.

In a Sparrowhawk there was one confirmed case (5%) of infection of the bursa of Fabricii, where necrotic content was observed therein. Histopathologically, the structure of the wall of the bursa was entirely disintegrated. Unfortunately, despite attempts, the causative agent was not confirmed.

With a 5% (n = 1) frequency, signs of acute septicemia was notable on necropsy in a Northern goshawk. The spleen was not enlarged; however, pinpoint petechial hemorrhages were seen in large numbers on the pericardium (**Figure 44**). The positioning of these

hemorrhages was primarily along the fat deposits of the coronary groove. Marked hepatomegaly was observed, and the liver was dark, brownish-red in colour on gross necropsy. The small intestinal loops were filled with mucoid contents.

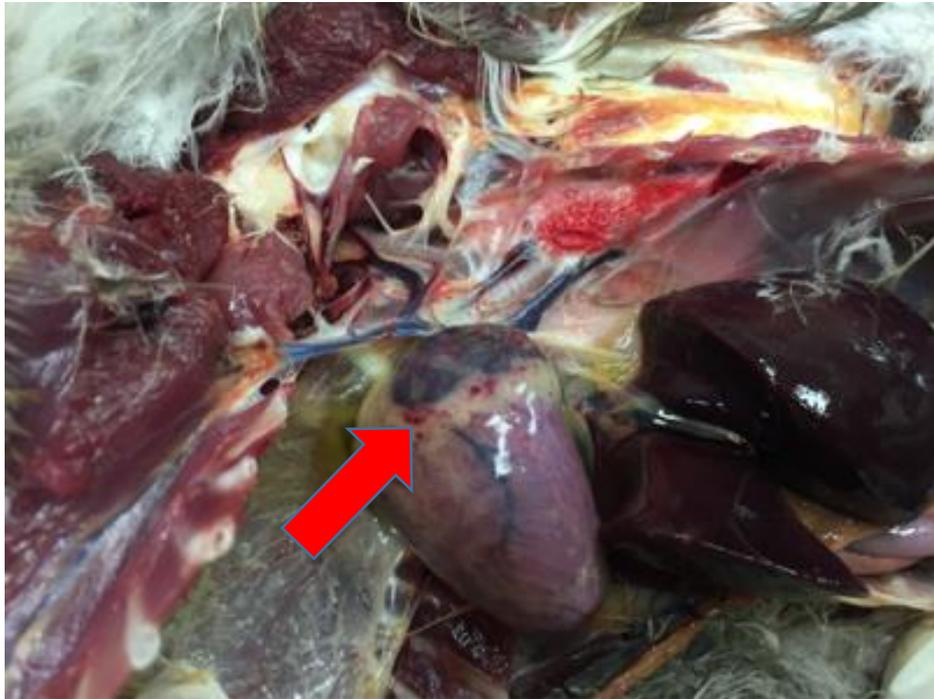


Figure 44. Petechial hemorrhages (arrow) along the coronary groove of the heart of a Sparrowhawk (*Accipiter nisus*).

Swab samples taken from the cardiac chamber yielded the presence of beta-hemolyzing bacterial following inoculation onto blood and Drigalski agar and 24 hours of incubation. Colonies were small, greyish-white, and their growth and biochemical parameters were consistent with those of *Pastuerella multocida*.

As part of our work, cloacal swabs were taken from each individual on necropsy in the portion of the study performed at the Budapest Zoo and Botanical Garden. These samples were subsequently cultured and yielded the presence of various bacteria based on the growth and biochemical parameters of the colonies. *Escherichia coli* was observed in 76% of instances, whereas *Pseudomonas* was cultured in 24%. *Salmonella* sp. were not ascertained from any of the cloacal swab samples cultured.

Moreover, investigations into the presence of active *Chlamydia psittaci* and West Nile virus infection of the birds in the scope of this project were all yielded negative results subsequent to RT-PCR examinations.

5. Discussion

5.1. Species examined in the scope of the study

Seventy specimens, pertaining to 16 species of nocturnal and diurnal birds of prey were examined at the Budapest Zoo and Botanical Garden, and 15 individuals of eight species at the University of Veterinary Medicine in Budapest. As the aim of the Wildlife Rescue Center at the Budapest Zoo and Botanical Garden is to return as many of its inhabitants to nature as is possible, aside from those that died at the facility, only birds with a poor or grave prognosis were humanely euthanized (according to the rigorous animal welfare protocols of the Budapest Zoo and Botanical Garden Veterinary and Conservation Directorate) and examined thereafter to be included in the study. On a whole, 85 birds of prey, belonging to 17 species were analysed for causes of morbidity and mortality. A pathological examination of such scale has not yet been performed in Hungary in predatory avian species. GÁL et al. (2003) performed a similar investigation, however, it included 21 merely specimens and only raptor species. There are no further publications of detailed, large-scale *post mortem* investigations from any of the Hungarian wildlife rescue centers in the literature. Outside our country, accounts of larger scale *post mortem* studies, as done by BAUDVIN (1997) and SMITH et al. (2018), where 1,598 (over a four-year period) and 1,448 birds (over a 13-year period) of prey were assessed respectively through necropsy for cause of morbidity and mortality. However, of the two afore mentioned studies, the latter was performed at a wildlife rescue facility (SMITH et al., 2018), though birds were roadkill collected along highways in the former (BAUDVIN, 1997).

Of the 12 species included in the study, 11 are nesting species of Hungary were represented as per HARASZTHY (2019): White-tailed eagle, Eastern imperial eagle, Lesser spotted eagle, Common buzzard, European honey buzzard, Sparrowhawk, Northern goshawk, Common kestrel, Eurasian hobby, Peregrine falcon and Saker falcon. The only winter visitor was the Rough-legged buzzard. Of the birds of prey eliciting a nocturnal lifestyle, all five species are established breeders in Hungary according to HARASZTHY (2019) and were the Eurasian eagle owl, Ural owl, Long-eared owl, Tawny owl and Eurasian scops owl.

5.2. Biometric data of various species

Though biometric data was recorded in the majority of cases before necropsy, only data from the four most commonly represented species were included in the analysis, as the other species had insufficient data to preclude non-biased results. Therefore, measurements of the body weight, wing length, tail length, body length and tarsometatarsal length of Common kestrels, Common buzzards, Sparrowhawks and Northern goshawks were evaluated.

The trends seen in our results are largely consistent with data published by FARAGÓ (2015) on these very species, except for the beak length, which could not be compared to the afore mentioned study owing to the different measurement methodology implemented in our study. With regards to body weight, we can see a slight deviation from published data, however, this can be attributed to a number of factors. Firstly, as all the individuals in our study originated from the wild and arrived to the rescue facility alive, sufficient time may have elapsed for their gastrointestinal tract to have emptied and it was unknown to us when the individual was able to capture prey for the last time in nature. Owing to the emaciated nature of some birds, it is speculated that consequent to their injuries, some individuals may not have eaten for days before being admitted to the rescue facility. Moreover, *post mortem* dehydration could also have been a contributing factor to the afore mentioned findings.

5.3. Non-infectious causes of morbidity and mortality

Seventy-eight percent of the causes of morbidity and mortality in the predatory birds examined on necropsy had a non-infectious background. The most frequent cause of morbidity in the entire study was attributed to electrocution induced by contact with powerlines, and the subsequent shock, and the second- and third degree burn incurred. Implicated to be the number one cause of death in free-living birds of prey by some authors (GUIL et al., 2011; MELERO et al., 2013, KAGAN, 2016) and leading to severe losses in Falconiformes in particular (HAGER, 2009), burn wounds were observed on two points in a large percent of cases, either both talons or one talon and the contralateral wing (though instances occurred where both wings were affected). GÁL et al. (2003) accounts for similar findings to the afore mentioned. However, cause of death in these cases was attributed to acute circulatory failure and shock and many individuals with such lesions were humanely euthanized upon admittance to the rescue center. Though rupture of large internal vessels and hemopericardium (accumulation of blood in the pericardium) are a frequent finding in traumatized birds suffering from electrocution (KAGAN, 2016), these were not among alterations recorded during necropsy. We can state that the vast majority of the birds affected by electrocution were extremely dehydrated (sunken eyes), which could be attributed to an inability to feed and hydrate *ante mortem* owing to the injuries procured and their inability to fly. In such instances, the injuries might have occurred up to 1-2 weeks prior to their being admitted to the rescue center. Moreover, GÁL (2006) discusses the vital importance of a fast metabolism in smaller sized birds and their significant fluid loss through respiration, and this could have contributed to the state of dehydration seen in some individuals during necropsy.

The occurrence of electrocution also varied among species. In the Common kestrels examined, 67% were affected, whilst merely 31% of the Common buzzards showed lesions consistent with this ailment. In the former, 71% of the individuals were juvenile, a factor most likely attributed to inexperience in flight and the lack of knowledge of the danger electrical pylons pose. Moreover, this high proportion of affected kestrels is reflected in *ante mortem* examinations performed at the Wildlife Rescue Center at the Budapest Zoo and Botanical Garden on living individuals (SÓS-KOROKNAI et al., 2020b), though other studies in Hungary describe Common buzzard and Long-eared owl populations to be most frequently affected by this ailment (TÓTH, 2018).

Twelve percent of the instances of non-infectious morbidity were found to have been hepatic dystrophy. According to GÁL (2006), this ailment is the resultant of aberrant lipid metabolism leading to regressive changes (karyopyknosis, karyolysis etc.) in the hepatocytes and/or hemorrhages in various visceral organs. Such alterations were noted in all cases in the cytoplasm of affected hepatocytes. Moreover, it is noteworthy, that owing to non-physiological metabolism of lipids, irregular and significantly heterogenous vacuolization was also seen in the hepatocytes often displacing the nuclei to the periphery of the cells. Councilman bodies (apoptosed multiplex, solitary cells in the liver) appeared in variable numbers in the liver tissues of examined individuals. Some authors implicate pesticides to elicit hepatotoxic effects (COEFIELD et al., 2010; ERIKSSON et al., 2016) and even meloxicam in extremely high dosages in kestrels (SUMMA et al., 2017), however, confirmation of a specific link has yet to be demonstrated. Additionally, GÁL (2006) discusses toxin-induced (i.e. fungal toxins or certain pesticides) hepatic dystrophy and implicates various deficiencies and infectious diseases to contribute to the etiology of this ailment. Unfortunately, it was not in the scope of this study to perform detailed toxicological examinations, though administration of extremely high levels of NSAIDs can be ruled out.

The third most numerous ailment pertained to internal bleeding of traumatic origin at 11%. Injury of healthy, intact vessel walls in avian species can generally have two pathophysiological bases; rupture, through a strong mechanical force, or perforation by a foreign body (i.e. a metal particle, such as a bullet). Nonetheless, accounts also exist of the rupture of seemingly healthy blood vessels owing to a pathology in the background. GÁL (2006) and BEAUFRÈRE (2013) describe instances where dystrophy of a section of the vasculature led to its rupture following moderate, blunt mechanical trauma. However, upon examination of the major vessel at the sites of the damaged organs through histopathology, no such discoveries were made. GRANNER & RAYMOND (2003) published data on the occurrence of the well-known

human ailment atherosclerosis in predatory birds and found the occurrence to be 4.2% in captive raptors and 2.9% in captive owls. However, no such alterations were recorded through our work and we speculate, that the active and free-ranging lifestyle, with a variable food-source impeded the birds included in this study from developing such an ailment. Blunt mechanical trauma (i.e. collision with a static object) can be the contributing factor to rupture of largest-sized parenchymal organs (i.e. liver) in the celomic cavity of birds leading to massive internal bleeding. Numerous authors (THOMAS et al., 2011; STANSLEY et al., 2014; WALKER et al., 2016) account of hemophilia to be the cause of death in birds resultant of anticoagulant rodenticide intoxication, but it was not in the scope of our work to investigate tissue samples for the presence of these chemicals, though an incident of AR toxicity was determined at our wildlife rescue facility some years ago (unpubl. data). However, limiting the use of such toxic materials in agriculture is key to preserving the naturally occurring fauna of our country.

Previously mentioned strong mechanical forces can also lead to fracture of the skeletal system subsequent to collisions. Such alterations were observed in 8% of the individuals, and in the majority of cases affected the long bones. BESTON et al. (2016) implicates air currents as causative factors for the manifestation of such collisions. In owls, NEWTON et al. (1997) denotes trauma to be number one cause of death in Barn owls whereas WENDELL et al. (2002), KOMNENOU et al. (2005), GOTTDENKER et al. (2008), MOLINA-LÓPEZ et al. (2011) and SMITH et al. (2018) found trauma to have caused morbidity in a large number of predatory birds admitted to wildlife rescue facilities. Although our results do not reflect these trends, it is important to note, that the above-mentioned studies, except the work of GOTTDENKER et al. (2008) and SMITH et al. (2018), were not based exclusively on pathological findings. A possible explanation for such a low proportion of trauma cases in our work could be, that only those individuals were included in our work that either died or were euthanized subsequent to their condition, and therefore, these number do not reflect the cases of our facility on a whole. GÁL et al. (2003) mentions a 38% incidence of trauma in a previous study on raptor morbidity and mortality, however, there are many factors that could have led to different results. Furthermore, the presence of wound myiasis on necropsy was observed in several cases, though only GOTTDENKER et al. (2008) account for similar discoveries in rescue center on the Galapagos Islands, though in this particular study, the authors listed this alteration as an infectious cause of mortality.

Many chemical agents (i.e. fungal toxins and medications, such as sulphonamides), vitamin deficiencies (i.e. A-hypovitaminosis), as well as dehydration, can lead to renal damage through tubulonephrosis in birds (DOBOS-KOVÁCS, 2014). Alterations consistent with this

ailment, necrosis and consequent sloughing of the endothelial layer of the renal tubules, was confirmed 8% of the birds examined. Kidneys were enlarged, light brownish-red in colour, and demarcations caused by uric acid crystals was visible under their capsules, however insight into the background of the pathological findings could not be determined. As previously mentioned, toxicological examinations could not be performed due to financial constraints. It is important to mention, that GÁL (2006) declares acute renal failure to often have a multifactorial etiology with an obscured cause even if the findings are straightforward on both necropsy and histopathology.

Through our research we were able to attain a 3% frequency of the occurrence of ulcers in the glandular stomach and post hemorrhagic anemia. According to GÁL (2006), the background of this ailment is in conjunction with stress, consumption of water of an extremely low (acidic) pH or the ingestion of mycotoxins. SUMMA et al. (2017) found extremely high dosages of meloxicam in American kestrels to elicit such lesions, however, this could be ruled out in our study. Furthermore, DOBOS-KOVÁCS (2014) mentions a correlation in domesticated species between the occurrence of stomach ulcers leading to hemorrhage into the gastrointestinal tract and eating fishmeal. Such a link was not established through our work, however, feed ingested by the individuals in our study before their admittance was, of course, unknown.

Visceral gout, occurring from the insufficient removal of uric acid from the bloodstream by the kidneys and leading to precipitations was merely found in 2% of the birds of prey as a result of chronic renal failure. The pathophysiology of gout is such, that when uric acid in the circulation reaches a threshold value (this can be variable in different species), it extravasates from the vessels, precipitates on the serous membranes of the celomic cavity and often in the pericardium. Such alterations were observed through our work and coincide with lesions described in birds of prey (WERNERY et al., 2004; METEYER et al., 2005). Uric acid can also be deposited in the joints in articular gout. GÁL et al. (2003) found visceral gout in 5% of the 21 raptors in their study and WERNERY et al. (2004) also diagnosed this ailment in a large proportion of predatory birds. As these birds originated from nature, identifying the cause of chronic renal failure would be purely speculation.

In 2% of individuals, cause of death was confirmed to be due to a projectile foreign body (bullets or shrapnel) and therefore anthropogenic in origin. Traumatic injuries were seen in this bird on gross necropsy; however, it is worth mentioning that bullets can also enter birds of prey (particularly those eating carrion) passively if they consume cadavers that have been shot. Though in such cases, the metal particles would be found in the alimentary tract and under

ideal circumstances, be regurgitated in the next pellet. Gunshot wounds have been implicated in causing morbidity and mortality by a number of authors (JAGER et al., 1996; RICHARDS et al., 2005; MOLINA-LÓPEZ et al., 2011) and perhaps initiatives such as decreasing the use of lead pellets can aid the survival of these predatory birds in the future.

Emaciation can be present in many cases in predatory birds admitted to wildlife rescue facilities and in our work, it was the sole pathological finding in a single case. As poor body condition can be multifactorial and therefore, this term was used exclusively in the one instance, where no macroscopic or microscopic pathological lesions were observed, and the infectious agents were not isolated. Emaciation, concomitant with other alterations, were recorded in a number of cases. Though some authors will categorize such cases as “other” (WENDELL et al., 2002; MONTESDEOCA et al., 2016) or “starvation” (KOMNENOU et al., 2005), similar studies in the literature denote mortality to be due to emaciation (SMITH et al., 2018) with such findings.

Neoplasms are a relatively rare occurrence in predatory birds, though accounts are noted in the literature in a number of species of nocturnal and diurnal predatory species (FORBES et al., 2000). In our study, a single case was observed as a fibrosarcoma under the beak of a Saker falcon, though it bears little significance in the scope of the study.

Deemed solely a side-finding, and with no implication on the goal of our work, bilateral ovaries in a female Sparrowhawk is a rare occurrence, and therefore noteworthy. KRAUTWALD-JUNGHANNS et al. (2011) and RODLER et al. (2015) and account of cases of persistent right ovaries in this vary species along with others, whereas FITZPATRICK (1930) and KRAUTWALD-JUNGHANNS et al. (2011) report this phenomenon in Cooper’s hawk and Goshawk respectively.

5.4. Trace element accumulation

Through our work, we were able to analyze the presence of 12 different trace elements from liver and bone tissue samples. In bone samples, all As and Mo values were below the threshold (LOD 0.5 mg/kg and 0.2 mg/kg respectively), and in the liver, As, Ba and Ni (LOD 0.5 mg/kg, 0.5 mg/kg and 0.2 mg/kg respectively) values were lower than the level detected by the analyzer machine.

Barium levels in bone samples showed trends similar to those found by PAGEL et al. (2012) in the United States, though a number of samples were measured at the higher end of the spectrum (14.0-15.9 mg/kg) suggesting isolated elevation of this metal in the regions the affected individuals originated from.

As the largest sample size originated from the Common kestrels, followed by Common buzzards and Sparrowhawks, trends could be established from both liver and bone material in these species. In the kestrels, bone Cd was 0.1 ± 0.03 mg/kg on average and similar (0.19 ± 0.25

mg/kg) in hepatic tissue, but the Sparrowhawk yielded much higher mean liver values with 0.55 mg/kg, suggesting that the Sparrowhawks had a higher accumulation of this heavy metal. CARNIERO et al. (2014) found similar results as those seen in kestrel after analysis of liver samples from numerous birds of prey. Work done by OROSZ et al. (2001), KITOWSKI et al. (2016) and KANSTRUP et al. (2019) presented mean values of 0.72 mg/kg, 0.28-0.78 mg/kg and 0.22 mg/kg for cadmium from hepatic material respectively. KIM & OH (2016) found elevated levels of Cd in urbanized Common kestrels in their work (mean values of 1.53 mg/kg) and though a single sample had a concentration of 1.39 mg/kg in a Sparrowhawk in our work, on a whole, our values did not indicate elevation of this heavy metal. Establishing levels of these heavy metals in avian species can provide insight into the cause of morbidity in these birds.

Chromium concentrations measured from bone samples in Common kestrels weighed in at 1.71 ± 0.96 mg/kg, however, liver samples were below the threshold value deemed to be 0.15 mg/kg through the work of OROSZ et al. (2001). KITOWSKI et al. (2016) also found chromium levels from hepatic samples below the afore mentioned threshold, whereas ZACCARONI et al. (2003) determined a mean value of 2.97 mg/kg from Little owls in Italy and HORAI et al. (2007) also established values far greater than the ones presented in our work (0.26-0.43 mg/kg in various birds of prey), therefore confirming that our values were not elevated as compared to the literature.

Moreover, the levels of mercury presented in our work did not exceed measurements described in the literature. OROSZ et al. (2001) established liver levels of mercury as high as 23.3 mg/kg in Peregrine falcons, whilst hepatic tissue analyzed in our study showed merely 1.09 ± 0.95 mg/kg, 1.14 mg/kg and 1.07 mg/kg in Common kestrel, Sparrowhawk and Common buzzard respectively and 1.40 mg/kg in bone. Results of a large-scale study by CARNIERO et al. (2014) found values of mercury in liver samples to be similar to ours (1.38 mg/kg) as was also the case in work done by HORAI et al. (2007), KITOWSKI et al. (2017) and PAGEL et al. (2012). Nonetheless, a few individuals had higher than average levels of this heavy metal including liver samples from a Common kestrel (2.77 mg/kg) and a Common buzzard (2.28 mg/kg), and bone samples from the afore mentioned kestrel (4.12 mg/kg) and a Long-eared owl (4.27 mg/kg) suggesting once again, a higher prevalence of this element at the site of origin of these birds.

It is common knowledge, that lead, as a heavy metal, has an impact on the environment and causes illness in birds of prey either through ingestion of bullets or subsequent to gunshot injuries (KRONE, 2009, 2018). The highest liver levels recorded in our work were those of

0.61±0.39 mg/kg from Common kestrels, 1.28 mg/kg from Sparrowhawks and 0.36 mg/kg from Common buzzards. OROSZ et al. (2001) found a 10.9 mg/kg lead level in a Little owl, KANSTRUP et al. (2019) identified liver values to be 0.11 mg/kg, KITOWSKI et al. (2016) measured levels between 0.073-0.404 mg/kg, whereas CARNIERO et al. (2014) published 0.541 mg/kg from hepatic samples. Therefore, we can see that aside from the lead level measured from Sparrowhawks, our values did not show considerable variation from values seen throughout the literature and in the case of Common kestrels, significantly lower levels were measured in the bone as compared to the liver. However, we must keep in mind, that according to KRONE (2018), though the presence of a certain amount of lead in the system of raptors may be tolerated, its presence is not physiological, and its introduction must have an anthropogenic cause. Today, birds of prey that ingest carrion and prey wounded by gunshot wounds are most susceptible, though initiatives have been taken globally to limit the use of lead-based ammunition, particularly for the hunting of waterfowl (KRONE, 2018). Such legislation has also been implemented in Hungary through the ban of lead-based ammunition in the hunting of waterfowl in August 2005 according to the 79/2004 (V.4) FVM decree.

Nickel and molybdenum measurements from bone samples, and Co levels were not comparable to values in the literature owing to lack of information.

Aside from the afore mentioned elements, mean barium, copper and manganese levels were significantly higher in bone tissues in Common kestrels than those measured from liver tissues.

The essential elements, Cu, Mn and Zn assessed in this study were recorded at higher concentrations in liver tissue, and Zn was also measured in a greater quantity in bone samples, as compared to the other metals in the study. However, though levels were seemingly elevated, these were within normal range as compared to the literature (HORAI et al., 2007; PÉREZ-LÓPEZ et al., 2008; KIM & OH, 2016). Copper values from the Lesser-spotted eagle (22.8 mg/kg), a Common kestrel (26.1 mg/kg) and a Tawny owl (20.4 mg/kg) far exceeded average values in liver samples and bone levels of this element were also greater in another Common kestrel (4.18 mg/kg), though values did not exceed levels published by KIM & OH (2016) in case of the Common kestrel and HORAI et al. (2007) for the Tawny owl. Data was not available for the comparison on Cu levels in the Lesser-spotted eagle, therefore, it could have been considered elevated. Furthermore, Mn concentrations were also more nearly double of average values (6.07 mg/kg) in liver samples from the afore mentioned Lesser-spotted eagle (11.2 mg/kg), a Common buzzard (10.2 mg/kg) and a Common kestrel (9.14 mg/kg), and bone samples from another Common kestrel (13.41 mg/kg), however these concentrations did not vary

considerable from the literature (HORAI et al., 2007; KIM & OH, 2016). Higher than average values were noted in Zn levels in liver tissue in a Common kestrel (133.0 mg/kg) and a Common buzzard (108.0 mg/kg), however these did not exceed values deemed to be normal by KIM & OH (2016) for Common buzzards, though it was increased in the kestrel when compared to the value (95.8 mg/kg) obtained in the formerly mentioned article (KIM & OH, 2016).

The sites of origin of individuals included in our study were not compared for a number of reasons, even though based on the results, it is evident that environmental load of various metals is greater in certain regions of country (or areas in the vicinity of its border). Firstly, information about the origin of various birds was not recorded through GIS data and in many cases, the city/town closest to where the specimen was found was the only recorded information available or data was inaccessible altogether. Uptake during migration or roaming, in species where this applicable cannot be ruled out. Additionally, as birds of prey hunt across vast areas and it is impossible to predict the site at which uptake of various heavy metals took place. However, if future studies are to be performed, valuable information can be obtained regarding the environmental load of specific areas if birds of prey and substrate are analyzed concomitantly as done by HORAI et al. (2007).

5.5. Infectious causes of morbidity and mortality

As seen through the results, 47% of infectious causes of morbidity and mortality were attributed to alterations caused by pulmonary and air sac mycosis. According to DOBOS-KOVÁCS (2014), infection occurs through the inhalation of fungal spores and several authors implicate the tearing apart of prey to lead to infection (REIDIG et al., 1980; COOPER, 2002; BERKÉNYI, 2012). Subsequently to the inhalation of *Aspergillus* sp. spores, acute inflammatory response is elicited, however, when these inflammatory cells congregate around spores, the process can be deemed a chronic one (LATIF et al., 2015). Termed granulomas, these typical yellowish-white coloured nodules are observed on gross pathology in such instances (FOWLER, 1986; GÁL, 2006) as was also recorded in our work. Within the air sac walls conditions are prime for the survival of fungi and certain moulds will cover the walls along their entirety creating a grey-green lining (GÁL, 2006; DOBOS-KOVÁCS, 2014). Similarly to the findings of LATIF et al. (2015), *Aspergillus* sp. were also the fungi implicated in being the causative agent of disease in the birds of prey in our study, but we speculate on the route of infection. Often, immunosuppression plays a crucial role in the development of fungal disease such as aspergillosis.

Diurnal and nocturnal predatory birds are susceptible to *Mycobacterium avium* complex similarly to other avian taxa (GÁL, 2006; DOBOS-KOVÁCS, 2014). This being said, MILLÁN et

al. (2010) states that owl species appear to elicit a higher sensitivity, though in our study, only Common buzzard were affected and none of the nocturnal predators were. The incidence of mycobacteriosis was found to be similar (4%) to our work in a study performed by MILLÁN et al. (2010), though in this instance, the sample size was greater than our study. Infection of the birds affected by tuberculosis were most likely attributed to the consumption of the organisms through their prey. Thus, *per os* is the principal route of infection and is therefore linked with the development of the primary lesions in the intestinal tract (COOPER, 2002; WERNERY et al., 2004), which was consistent with our findings. As is typical of tuberculosis, the bacteria from these primary lesions disseminate through generalization in waves, but ultimately when the individual is immunocompromised. In generalized cases of mycobacteriosis, granulomas can be found in the liver, the spleen, the lungs, the kidney and the bone marrow (GÁL, 2006; DOBOS-KOVÁCS, 2014).

Infection from avian pox virus was found in our work in Common kestrel. Typical of this ailment are the presence of skin lesions on those regions not covered by feathers (GÁL, 2006; DOBOS-KOVÁCS, 2014), leading to subsequent viremia (GÁL 2006). WERNERY et al. (2004) accounts of cases of Falconpox virus in various diurnal predatory species, whereas ÖZMEN & DORRESTEIN (2002) describe of cases of pox in Common buzzard, KRONE et al. (2004) diagnosed this ailment in Peregrine falcon and SCHOMAKER et al. (1998) attributed infection to poxvirus in Northern goshawk. Though all these species were represented in our study, the only species with pox dermatitis lesions was the Common kestrel. According to GÁL (2006) and DOBOS-KOVÁCS (2014), four forms of this ailment of the integument exist in poultry; pox lesions on the skin and mucous membrane pox, mixed, and viremic forms. Our work only demonstrated the skin pox lesions similar to those described by (SAITO et al., 2009).

Aside from pox, no other viruses found to have affected birds of prey in our work. Unfortunately, though the intention had been to perform full-scale analysis from all dissected birds, the samples were lost due to a technical error outside our control.

Though a necrotic bursitis case was documented through our study where the etiology was not confirmed, lesions were similar to those found with infectious bursal disease (IBD) in poultry (DOBOS-KOVÁCS, 2014), possibly suggesting a change in host for this pathogen. However, this statement is merely speculation, further work in the field is necessary to produce evidence to argue this point.

Acute septicemia leading to death through the effect of toxins produced by the causative agent are caused by pathogenic organisms. Therefore, these bacteria cause acute disease and have been described to affect all species and the majority of age groups (GÁL, 2006; DOBOS-

KOVÁCS, 2014). A Northern goshawk presented with an enlarged spleen and hemorrhages throughout the celomic cavity, with pinpoint necrotic foci seen in the liver. Similar alterations are described in birds of prey eliciting signs of septicemia by other authors in the literature (MORISHITA et al., 1997; COOPER, 2002). The causative agent was determined to be *Pastuerella multocida* in our case, an infamous pathogen of a number of avian species (GÁL, 2006; DOBOS-KOVÁCS, 2014). Moreover, *Pseudomonas aeruginosa* and *Escherichia coli* bacteria were cultured from cloacal swabs taken from a number of the birds (belonging to different taxa) in our study, though in these cases, lesions did not support the afore mentioned pathological picture and it was speculated, that these birds were asymptomatic carriers. Work has been performed to establish the role of *P. aeruginosa* in the alimentary tract of raptors, however, *E. coli* has been documented to be part of the normal flora of the lower intestinal tract in some species (FORD, 2010), though clinical disease can also be elicited (COOPER, 2002). Such individuals lacking symptoms can pose a threat to their conspecifics as they actively spread these pathogens in the population and if birds are housed at higher densities, such as a wildlife rescue centers, these threats can become intensified. Therefore, monitoring activities, such as those performed through our work are cardinal in handling and preventive medical protocols of facilities housing birds originating from the wild.

6. Summary

Upon examining the causes of morbidity and mortality of predatory avian species at Wildlife Rescues Center at the Budapest Zoo and Botanical Garden we discovered various non-infectious and infectious causes to occur with variable frequency. Non-infectious ailments occurred far more common than their infectious counterparts, and electrocution caused by contact with powerlines was found to be the most frequent alteration in free-living birds of prey to be admitted to the wildlife rescue center. Moreover, acute kidney failure, hepatic dystrophy and various forms of traumatic injuries, affecting both the appendicular skeleton and the internal organs were also observed with a high incidence. Though acute kidney failure and hepatic dystrophy can be considered natural occurring ailments, electrocution and trauma pertain to an anthropogenic etiology, and as these alterations were observed in relatively high numbers, negative human impact on free-living raptor populations must be declared based on our results.

Through our work, we were able to identify various infectious agents for the first time in Hungary from certain species (*Mycobacterium avium* in Common buzzard and poxvirus infection in Common kestrel) and attained results indicating that pulmonary and air sac mycosis caused by *Aspergillus* sp. presented with the highest frequency in the studied individuals. Certain ailments postulated to be present in free-living raptor populations (i.e. WN virus, *Chlamydia psittaci* and *Salmonella* sp.) however, active infection was not diagnosed in any of the specimens examined.

Furthermore, upon analysis of liver and bone tissue samples for 12 different trace elements, we were able to confirm that the values obtained in our study were not elevated compared to data available in the literature. Certain individuals had higher concentration of various elements compared to average values, with the possible explanation being that the regions of origin of these birds had higher environmental load of certain metals. Unfortunately, it was not in the scope of this study to perform measurements in the sites of origin of our subjects. Some metals were measured in significantly higher levels in bone tissues as compared to those originating from the liver in the Common kestrel, which had the greatest sample size and therefore warranted detailed statistical analysis.

Though such detailed assessment to determine the cause of morbidity and mortality of free-living birds of prey in Hungary has not been performed to date, more information would still be required in order to extrapolate our findings onto the entire raptor population of our country. Therefore, continuation of this work would be beneficial, to gain further information about these charismatic species, to confirm the trends in the reasons for admittance, as well as for disease monitoring purposes. Reducing anthropogenic causes of morbidity and mortality of

these birds would be the ultimate aim, however, whether or not this is feasible is questionable in the increasingly urbanized world of the 21st century. Instead, we must look to how the results of this study can assist in the conservations of these species locally through the identification of those factors that play a key role in the morbidity of these species, and how these hindrances can be improved to aid free-living populations of these birds of prey.

7. New scientific findings

1. To the best of our knowledge, we are the first to have performed such a detailed and broad-scale *post mortem* examination of free-living birds of prey admitted to a wildlife facility in Hungary and to identify the leading cause of morbidity of predatory avian species to be electrocution caused by powerlines with particular significance in Common kestrels.
2. In the scope of our work, we found acute renal failure (8%) to occur more commonly as a cause of morbidity and mortality in birds of prey than gout or chronic renal failure (2%). Whether or not this was due to chance we feel that owing to the size of the dataset and the broad number of species affected, we deemed it to be a significant finding.
3. Our work is the first record of the analysis of 12 different types of trace elements (including heavy metals) from liver and bone samples origination from 42 individuals of seven species of diurnal and two nocturnal birds of prey. Through this study, it was evident that several elements were below the lower limit of detection in all samples analyzed (Mo and As in bone, and As, Ni and Ba in liver) suggesting that in presumed levels of environmental contamination by these elements did not affect the birds included in this study. As various heavy metals were elevated in certain specimens, implications can be drawn about higher levels of these elements to be present in certain regions of our country (or the specific areas from where individuals originate), but the trends show that levels were not elevated everywhere. Levels of essential elements, such as Cu, Mo and Zn were present at elevated levels (particularly Zn) as compared to the other metals, however, values were within normal range as compared to data in the literature.
4. Identification of pulmonary and air sac mycosis caused by *Aspergillus* sp. was found to be the most frequently occurring infectious disease in the studied individuals and as these lesions lead to respiratory impairment, this finding can truly implicate this ailment to be a major cause of morbidity and indeed mortality in free-ranging birds of prey.
5. Several instances of septicemia and hepatic granulomas were recorded as part of this study suggesting that generalized bacterial infection caused by *Pasteurella multocida* can also pose a threat to free-living predatory birds of Hungary and lead to mortality in multiple species.
6. This was the first account of poxvirus infection in a Common kestrel (*Falco tinnunculus*) in Hungary.

7. Our cases reveal the first instance of avian mycobacteriosis in Common buzzard (*Buteo buteo*) in Hungary.
8. Finding that all our samples were negative for both WN and *Chlamydia psittaci* through RT-PCR and *Salmonella* sp. by culture, can also be significant, as the presumption would have been to find active infection caused by these pathogens (even if only subclinically) in a number of the free-living population.
9. Through our work, we were able to establish that 76% of the birds in this study were carriers of *Escherichia coli* and 24% of *Pseudomonas* sp. indicating the presence and moderate prevalence of potentially pathogenic bacteria in the predatory avian populations of Hungary.

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13/2001. (V. 9.) *KöM rendelet* a védett és a fokozottan védett növény- és állatfajokról, a fokozottan védett barlangok köréről, valamint az Európai Közösségben természetvédelmi szempontból jelentős növény- és állatfajok közzétételéről.

79/2004. (V. 4.) *FVM rendelet* a vad védelméről, a vadgazdálkodásról, valamint a vadásatról szóló 1996. évi LV. törvény végrehajtásának szabályairól.

Appendix

Fővárosi Állat- és Növénykert / *Budapest Zoo and Botanical Garden*
KÓRBONCTANI VIZSGÁLATI LAP / GROSS PATHOLOGY SHEET

Állatfaj / Species:	Dátum / Date:	Sorszám / Nr.:	
	Elhullás ideje / Time of death:	Azonosító / Identification:	
gyűjtemény collection	karantén quarantine	mentett rescued	Állatház / Enclosure:
Testtömeg / Weight:	Ivar / Gender:	Kor / Age:	
Kórelőzmény / Anamnesis:	Makroszkópos lelet / Gross pathology findings:		
Kórszövetten / Histopathology: máj / <i>liver</i> lép / <i>spleen</i> vese / <i>kidney</i> tüdő / <i>lung</i> szív / <i>heart</i> gyomor / <i>stomach</i> bél / <i>intestine</i> gonád / <i>gonad</i>	Bakteriológia / Bacteriology: máj / <i>liver</i> lép / <i>spleen</i> vese / <i>kidney</i> tüdő / <i>lung</i> bél / <i>intestine</i>	Virologia / Virology: máj / <i>liver</i> tüdő / <i>lung</i> bél / <i>intestine</i>	Egyéb minták / Further samples:
Boncolást végzi / GP performed by: SE KV HM	Fotó / Photo:	Hulla / Carcass: fagyasztás kutatásra / <i>freezing for research</i> fagyasztás preparálásra / <i>freezing for taxidermy</i> megsemmisítés / <i>disposal</i>	