The 3rd decade of the 21st century is a time of various challenges to humankind. Authorities all over the world already pay special attention to the protection of biodiversity. For example, one of the priorities of the European Green Deal is to protect nature and try to reverse the degradation of ecosystems in the European Union (24). This objective can be achieved by taking different actions, such as enhancing the protection of endangered animal species, which includes proper wildlife management. A key element of wildlife management is constant health surveillance – both on the individual and herd levels. In some cases, clinical observation is not sufficient to assess the health status of the animal, since many diseases are subclinical. Besides, wildlife species tend to mask or conceal signs of sickness (7). It is of particular importance in the case of infectious diseases, since an asymptomatic individual may transmit pathogens to the environment and/or other herd members (45), thus becoming a hazard for other animals. It is important to note that some infectious diseases may affect humans, which poses a serious threat to public health (45).

One possible solution taken from human and livestock medicine is to determine the serum concentration of acute phase proteins (APPs). APPs are a group of proteins synthesized mainly in the liver (21). Based on the high degree of phylogenetic conservatism of APPs, it is hypothesized that the well-studied APPs in domestic species could also serve as indicators of infection and inflammation in wildlife species (7). A brief summary of APP-related studies in selected wildlife species is given in Table 1.

**Acute phase proteins**

The main biological function of APPs is to restore homeostasis. The secretion of APPs is associated with the acute phase reaction (APR). APR is an innate response of the body to a disturbance of homeostasis, which may be due to various factors, including infection, damage of tissues, neoplastic hyperplasia, immunological disorders, and others (21). The role of APR is to restore homeostasis and eliminate the causative factor. It consists of numerous hormonal, metabolic...
Tab. 1. A brief summary of APPs-related studies on selected wildlife species

| Animal species | Status | APP investigated | Key findings of the study | Reference |
|----------------|--------|------------------|---------------------------|-----------|
| **Artiodactyla** |        |                  |                           |           |
| European bison (*Bison bonasus*) | NT     | Hp, SAA, AGP | Hp and SAA levels were higher in diseased animals | (52) |
| Alpine ibex (*Capra ibex*) | LC     | Hp, SAA, AGP Cp | Hp, SAA, AGP, and Cp concentrations were higher in animals with sarcoptic mange | (56) |
| Red deer (*Cervus elaphus*) | LC     | Hp | Hp level was higher in animals with tuberculosis | (69) |
| African buffalo (*Syncerus caffer*) | NT     | Hp | Hp increased in response to infections and remained elevated for almost a month | (29) |
| **Perissodactyla** |        |                  |                           |           |
| Przewalski’s horse (*Equus ferus przewalskii*) | EN     | SAA | More exacerbated clinical signs of pneumonia induced a higher SAA concentration | (60) |
| Grant’s zebra (*Equus burchellii*) | NT     | Hp, SAA | In clinically abnormal zebras the Hp concentration doubled, while SAA increased up to 220-fold | (12) |
| Southern white rhino (*Ceratotherium simum simum*) | NT   | SAA, Fb, Hp, Ab | Injured animals had higher concentrations of SAA, Fb, and Hp; the level of Ab was lower | (38) |
| **Carnivora** |        |                  |                           |           |
| River otter (*Lutra canadensis*) | LC     | Hp | Hp increased in animals from polluted areas | (20) |
| Brown bear (*Ursus arctos*) | LC     | Hp | Seasonal changes in Hp: lowest in spring, increased in autumn, highest in winter | (48) |
| Giant panda (*Ailuropoda melanoleuca*) | VU     | Cp | The Cp urine concentration was higher in pregnant females | (71) |
| Cheetah (*Acinonyx jubatus*) | VU     | Hp, SAA | Increased concentrations of SAA and Hp in unhealthy individuals | (18) |
| Steller sea lion (*Eumetopias jubatus*) | NT     | Hp | Hp concentrations increased 3.2-fold in free-range animals that were temporarily captive | (66) |
| Steller sea lion (*Phoca vitulina*) | NT     | Hp | Hp was significantly higher in animals infected with Phocine distemper virus | (42) |
| **Sirenia** |        |                  |                           |           |
| American manatee (*Trichechus manatus latirostris*) | VU     | SAA | Elevated SAA serum concentrations in injured animals | (33) |
| **Proboscidea** |        |                  |                           |           |
| Asian elephant (*Elephas maximus*) | EN     | SAA | The serum SAA concentration was significantly higher in herpesvirus-positive animals | (65) |
| **Rodents and lagomorphs** |        |                  |                           |           |
| Capybara (*Hydrochoerus hydrochaeris*) | LC     | Hp, AGP, Ab | Hp and AGP increased and Ab was lower in animals with inflammation | (6) |
| **Chiroptera** |        |                  |                           |           |
| Egyptian fruit bats (*Rousettus aegyptiacus*) | LC     | Hp | Hp significantly increased after injection with LPS | (49) |
| **Marsupials** |        |                  |                           |           |
| Grey short-tailed opossum (*Monodelphis domestica*) | LC     | Hp | After experimental injection with LPS, the Hp concentration increased almost 6 times | (57) |
| Eastern grey kangaroo (*Macropus giganteus*) | LC     | Hp | Hp was 3 times higher in unhealthy individuals | (31) |
| **Primates** |        |                  |                           |           |
| Rhesus monkey (*Macaca mulatta*) | LC     | SAA, Hp, CRP | Moderately higher levels of SAA and Hp in diseased animals; CRP increased 200-fold | (44) |
| Chimpanzee (*Pan troglodytes*) | EN     | Ab | Lower serum Ab concentrations in animals with cardiorenal-like syndrome | (10) |
| Western gorilla (*Gorilla gorilla gorilla*) | CR     | CRP | In injured and stressed animals, CRP increased up to 15-fold | (27) |

Explanations: 1 Endangerment status according to The International Union for Conservation of Nature’s Red List: CR – critically endangered, EN – endangered, LC – least concern, NT – near threatened, VU – vulnerable; Ab – albumins; AGP – α-1-acid glycoprotein; APPs – acute phase proteins; Cp – ceruloplasmin; CRP – C-reactive protein; Fb – fibrinogen; Hp – haptoglobin; SAA – serum amyloid A
and neurological changes, which occur within a short period of time after the injury, at the beginning of infection or inflammation (14). A schematic illustration of APR is given in Figure 1. The most important APPs described in domestic animals are serum amyloid-A (SAA), haptoglobin (Hp), alpha-acid-1-glycoprotein (AGP), C-reactive protein (CRP), fibrinogen (Fb), albumins (Ab), and ceruloplasmin (Cp). Generally, APPs may be classified as positive or negative. This division is based on whether their synthesis is up- or down-regulated in the case of inflammation or infection. They might further be classified as major, moderate, or minor, depending on changes in their concentration (21). Major APPs are those that increase 10- to 100-fold, moderate proteins increase 2- to 10-fold, and minor proteins increase only slightly (9).

The synthesis of certain members of the SAA protein family is significantly increased during inflammation (46). SAA proteins can be considered as apolipoproteins because they associate with plasma lipoproteins mainly within a high density range. The physiological role of SAA in the immune response during inflammation is not well understood, but various effects have been described. These include, e.g., inhibition of lymphocyte proliferation, detoxification of endotoxin, inhibition of platelet aggregation, inhibition of thrombocytes aggregation, and inhibition of oxidative reaction in neutrophils (46). The diagnostic usefulness of SAA has been described in cases of mastitis (15), lameness in cows (3), and respiratory tract diseases in pigs (53).

The major function of Hp is to bind haemoglobin in an equimolar ratio with a very high affinity to prevent haemoglobin-mediated renal parenchymal injury and loss of iron following intravascular haemolysis (68). The level of Hp in cattle increases significantly in the course of APR (from nearly zero to about 2 g/l within 48 h from stimulation).

CRP is an acute inflammatory protein that increases up to 1,000-fold at sites of infection or inflammation. There is now growing evidence that CRP plays important roles in inflammatory processes and host responses to infection, including the complement pathway, apoptosis, phagocytosis, nitric oxide (NO) release, and the production of cytokines, particularly interleukin-6 and tumor necrosis factor-α (64). CRP is regarded as an important biomarker of respiratory tract infections in pigs (53). On the other hand, a high concentration of CRP (> 100 mg/l) in dogs was associated mainly with a severe systemic disease of various etiologies with a guarded prognosis (37).

Ab is a major negative acute phase protein. During APR, the demand for amino acids for the synthesis of positive APPs is significantly increased (67). Consequently, it necessitates reprioritization of hepatic protein synthesis: albumin synthesis is down-regulated, and amino acids are shunted into synthesis of positive APPs (67). Ab is responsible for about 75% of the osmotic pressure of plasma and is the main source of amino acids that can be utilized by the animal’s body when necessary (67).

Fb, a precursor of fibrin, is also an APP that has been used for many years to evaluate inflammatory and traumatic diseases in cattle, and it is characterized by markedly increased synthesis in response to infection (68). Fibrinogen is composed of 3 polypeptide chains linked by disulfide bridges and a glycoprotein (68). It is involved in homeostasis, providing a substrate for fibrin formation, and in tissue repair, providing a matrix for the migration of inflammatory-related cells (67).

Cp is an APP synthesized in hepatocytes that is involved in iron metabolism (oxidation) and transport. In addition, 95% of plasma copper is bound to the Cp (35). Elevated concentrations of Cp occur in acute infection, pregnancy, and inflammatory disease (30). Cp has a key function in iron balance and plays an important role in the reduction of oxidative stress (2).

In many cases, the concentration of one particular APP is not sufficient to assess health status, since APR is more complex. The profile or index of APPs, including the most significant APPs in a given species, can provide more information (54). Some APPs
may also help distinguish between acute and chronic inflammation. For example, SAA is an indicator of acute inflammation, whereas an elevated concentration of Hp rather indicates chronic inflammation, as was described in cows (40).

It is important to note that concentrations of APPs and their diagnostic usefulness differ between species. Interestingly, there are similarities in the concentration pattern of APPs between wild species and their domestic relatives. For example, SAA and Hp serum concentrations are significantly higher in diseased European bison and likewise in cattle (52). Therefore the concentration pattern of APPs will be referred to and compared to that of domestic species, where applicable. This review will focus mainly on free-living animal species in which APPs have been most studied. These baseline data should provide an important basis for future studies of the application of APP determination in monitoring the health and welfare of wildlife species.

**Even-toed ungulates (Artiodactyla)**

Over 190 species have been classified into the order Artiodactyla, which is divided into ten families. This group includes important domestic animal species, such as pigs (Sus domesticus), cattle (Bos taurus), sheep (Ovis aries), and goats (Capra hircus) (59). In domestic ruminants, the most significant APPs are SAA, Hp, AGP, and Fb, whereas CRP is also an important APP in pigs. Elevated levels of these markers have been described in mastitis, lameness, and respiratory diseases in cattle (3, 15), fasciolosis and scabies in sheep (17, 70), and mycoplasma infection in goats (23). With regard to wildlife species, APP concentrations have been described in European bison (Bison bonasus) (52), white-tailed deer (Odocoileus virginianus) and red deer (Cervus elaphus) (13, 69), bongos (Tragelaphus eurycerus) (5), Alpine ibexes (Capra ibex) (56), African buffalos (Syncerus caffer) (29), wild boars (Sus scrofa) (62), and semi-domesticated reindeers (Rangifer tarandus) (50).

In European bison, SAA and Hp concentrations were significantly higher in individuals eliminated due to poor health status compared to healthy animals, which suggests that the concentration of these APPs may potentially be a supportive tool in selecting animals to be eliminated (52). The median concentration of SAA was approximately 4 times as high in diseased animals as it was in healthy ones (70.81 µg/ml and 18.95 µg/ml, respectively). The interquartile range (IQR) for SAA equaled 12.38-30.11 µg/ml in healthy animals and 50.50-112.46 µg/ml in diseased individuals. The median Hp concentration in diseased animals was over twice as high as that in healthy European bison (0.305 mg/ml and 0.176 mg/ml, respectively). The IQR for Hp was 0.1-0.214 mg/ml in healthy animals and 0.270-0.592 mg/ml in diseased individuals. Four APPs were examined in Alpine ibexes (Capra ibex), both healthy ones and those with Sarcoptes scabiei mange. All APPs were elevated in animals showing sarcoptic mange. SAA and AGP serum concentrations were up to 10-fold higher in infected Alpine ibexes (56), similarly as in closely related goats with coccidiosis (34). In white-tailed deer, Cray et al. (13) described a higher concentration of SAA in neonatal fawns (0.1-26 mg/l), compared to adults (0.1-5 mg/l), as well as an increased concentration of SAA in clinically abnormal animals (9-74 mg/l). This suggests that the SAA concentration not only depends on clinical health status, but also varies with age. In red deer (Cervus elaphus), an increased concentration of Hp was recorded in tuberculosis-positive animals (69). In four individuals with a negative cervical skin test, post-mortem macroscopic lesions and culture confirmed tuberculosis. Interestingly, Hp levels in these animals were significantly elevated (69). This suggests that the Hp concentration may be regarded as a supportive tool in diagnosing of tuberculosis in cervids. On the other hand, the concentration of Fb in clinically abnormal bongos (Tragelaphus eurycerus) was significantly higher than it was in clinically healthy animals (525 mg/dl and 342 mg/dl, respectively), while the concentration of Hp was only numerically higher (1.10 mg/ml and 0.65 mg/ml) (5). In the African buffalo, Hp showed the strongest potential as a surveillance marker among investigated biomarkers: its concentration quickly and consistently reached high levels in response to experimental infection and remained elevated for almost a month (29). Moreover, elevated Hp was indicative of recent exposure to such respiratory pathogens as parainfluenza virus-3 (PI-3) and Mycoplasma bovis (29). On the other hand, Smitka et al. (62) reported that concentrations of CRP and Hp were higher in healthy wild boar piglets (280.71 µg/ml and 3.05 mg/ml, respectively) than they were in healthy swine piglets (94.83 µg/ml and 1.11 mg/ml). However, no differences were found in CRP and Hp concentrations between clinically healthy wild boar piglets and diseased piglets from swine (317.22 µg/ml and 2.67 mg/ml, respectively). This suggests that, despite the close genetic relationship, the patterns of APPs in young swine and wild boars are different.

In reindeer, which are regarded as a semi-domesticated ruminant species, concentrations of SAA increased (2- to 29-fold) after challenge with Escherichia coli endotoxin (51). In parallel, the Hp concentration was only numerically higher. In that experiment, SAA appeared to be a more sensitive indicator of APR than Hp during bacterial infection in reindeer (51). In reindeer calves infected with Giardia and Cryptosporidium, a positive correlation was observed between confirmed early infection and SAA concentrations (50). Furthermore, in a detailed health survey of
boreal caribou (*Rangifer tarandus caribou*) conducted in several herds in Canada, the SAA concentration differed between herds (median concentration ranged from 26.7 µg/mL to 94.7 µg/mL), which could have been due to different exposure to various pathogens (8).

Some researchers also investigated the APPs concentration in clinically healthy animals, possibly as a preliminary study to establish the reference values of APPs in wildlife species. Such studies have been conducted on mouflon (*Ovis musimon*) (63), Arabian oryx (*Oryx leucoryx*), impala (*Aepyceros melampus*), and blackbuck (*Antilope cervicapra*) (4).

**Odd-toed ungulates (Perissodactyla)**

Perissodactyla is a mammalian order including five superfamilies: Equoidea (containing the Equidae family), Tapiroidea (containing the Tapiridae family), Rhinocerotidea (containing the Rhinocerotidae family), and two extinct ones, Brontotheriidae and Chalicotheres (39, 47, 55). In the horse (*Equus caballus*), the most significant APPs include SAA, Hp, and Fb. Their elevated levels have been described in the course of bacterial and viral infections (infecctious arthritis, strangles, pneumonia, sepsis, enteritis, herpes, and influenza virus infection), parasite infestations, abscesses, laminitis, colic, grass sickness, and after surgery or parturition (41). In wild equids, the concentration of SAA reflected accurately the severity of pneumonia in a 20-year-old Przewalski’s horse (*Equus ferus przewalskii*) (60). In that study, the clinical observation continued for two months, and every deterioration in physical status was accompanied by an increase in the SAA concentration (up to 1000-fold in the terminal stage). In Grant’s zebra (*Equus burchelli*), the proposed reference intervals are 1.8-31.4 mg/L for SAA and 0.37-1.58 mg/ml Hp. In the same study, elevated serum concentrations of SAA and Hp were observed in clinically abnormal zebras – the increases were up to 85-fold and 1.5-fold, respectively (12). In addition, Seeber et al. (61) found that captivity influences immune functions, including concentrations of Hp and SAA in zebras. Wild zebras had lower concentrations of Hp and SAA (1.92 mg/ml and 2595 ng/ml) compared to those in captive zebras (6.18 mg/ml and 265,506 ng/ml), which suggests that zebras in human care have a poorer health status or a higher stress level.

There are few reports on non-equine odd-toed ungulates. In a study conducted on Malayan tapir calves, the Hp concentration was not found useful as a biomarker of inflammation (36). Hooijberg et al. (38) reported higher concentrations of SAA, Fb, and Hp as well as a decreased level of Ab in an injured southern white rhinoceros (*Cerotherium simum simum*) compared to healthy animals. For example, the SAA concentration > 20 mg/ml had a specificity of 100% in predicting the clinical status of the rhinoceros (healthy vs. injured).

**Carnivora**

The order Carnivora includes 286 recognized species classified into 11 families. It is one of the most diverse groups of animals (22). The domestic species in this order include dogs (*Canis familiaris*), cats (*Felis catus*), ferrets (*Mustela furo*) and fur animals, e.g. minks (*Mustela lutreola*). The concentrations of APPs have been described in brown bears (*Ursus arctos*) (48), giant pandas (*Ailuropoda melanoleuca*) (71), sea lions (*Zalophus californianus*) (11) Steller sea lions (*Eumetopias jubatus*) (66), river otters (*Lutra canadensis*) (20), and harbour seals (*Phoca vitulina*) (25). In brown bears, the serum Hp concentration showed significant seasonal changes: it was lowest in spring (2.29 ± 0.25 mg/ml), increased in autumn, and was highest in winter (4.53 ± 0.72 mg/ml) (48). In river otters, levels of Hp in animals from an oiled area (due to environmental disaster) (3.61 mg/ml) were higher than they were in animals from a non-oiled area (3.06 mg/ml). This suggests that APPs may also be considered as a supportive tool to determine environmental pollution of animal habitats (20). Interestingly, the Cp concentration in urine was proven to be a promising pregnancy marker in giant pandas, in which it increases up to 140-fold during pregnancy (71). In this species, infertility, including pregnancy loss, is of special veterinary importance. Ceruloplasmin concentration did not differ between cheetahs (*Acinonyx jubatus*) fed either beef or whole rabbit carcasses, but the concentration was above the reference range for felids, which may indicate inflammatory processes (19). Another study performed on cheetahs revealed increased concentrations of SAA and Hp (1.5-fold and 2.5-fold increases, respectively) in individuals suffering from renal and gastrointestinal pathologies (18). These results, however, should be interpreted with caution, since the SAA serum concentration is strongly affected by the cheetah genotype (26). As far as marine carnivore mammals are concerned, Hp concentrations increased 3.2-fold in free-range Steller sea lions (*Eumetopias jubatus*) that were temporarily captivated (66), which suggests that stress may significantly influence APR in this species. On the other hand, significant individual differences in the Hp concentration between newborn seal pups were observed during their rehabilitation in a seal center, which suggests that several other factors may influence the secretion of APPs (25). Kakuschke et al. (42) investigated the Hp physiological concentration in a study on 123 seals (mean concentration of 0.51 mg/ml and median of 0.38 mg/ml with the range of 0.01 to 2.17 mg/ml) and revealed that Hp was significantly higher in that population during the phocine distemper virus epidemic in 2002. It was therefore concluded that the Hp concentration may be used as a diagnostic tool to monitor the health status of harbor seals. Later, the same main author investigated the CRP concentration...
in harbor seals and found no significant differences due to sex, season or geographical location, and the most important finding was a higher CRP concentration in juveniles compared to adults (median concentrations of 39 and 31 ug/mL, respectively) (43). Besides, SAA was suggested to be involved in the pathogenesis of amyloidosis, which is an important pathology with unclear aetiology in wildlife species. Apart from amyloid deposition in different organs, the concentration of serum SAA increased approximately 1000-fold in California sea lions (Zalophus californianus) (11). Cases of amyloidosis have also been described in red foxes (Vulpes vulpes) (58) and island foxes (Urocycittus littoralis) (28).

Rodents and lagomorphs (Rodentia and Lagomorpha)

Nearly half of living mammals are classed in the order Rodentia (16). The most common species include squirrels, rats, guinea pigs, and mice. The order Lagomorpha comprises 91 living species, whose representatives include rabbits, hares, and pikas. After experimentally induced inflammation (injection of turpentine) in grass-cutters (Thryonomys swinderianus), concentrations of Hp and Fb increased to the same extent (3.6-3.7 fold) by day 7 or 5 post-injection, respectively (1). The dynamics of serum concentrations of these APPs were compared to those in rabbits and rats, and smaller increases were observed in grass-cutters (1). Another comparative study involved an experimentally-induced Toxoplasma gondii infection in mountain hares (Lepus timidus) and domestic rabbits (Oryctolagus cuniculus). One week post-infection, Hp increased significantly in hares (up to 9-fold), but not in rabbits (less than 2-fold on average), probably reflecting severe tissue damage (32). A study conducted on capybaras (Hydrochorus hydrochaeris) revealed that Hp levels in animals treated with turpentine increased 2.7-fold, while AGP increased 1.75-fold and Ab decreased 0.87-fold (6). In capybaras affected by sarcotic scabies, Hp and APG increased (4.98-fold and 3.18-fold, respectively) whereas Ab decreased (0.87-fold) compared to healthy animals (6).

Summary

Due to numerous factors, studies on wildlife species require great effort and dedication. Therefore knowledge regarding APPs in non-domesticated species is modest compared with that in livestock or companion animals. Concentrations of APPs in wildlife not only reflect health status, but have also been shown to vary depending on age, genotype, type of housing, and physiological status (pregnancy, hibernation). As this knowledge grows, the determination of APP concentrations may become an important tool in laboratory diagnostics of wildlife animals.

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