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HEALTH STATUS MONITORING OF THE EUROPEAN BROWN HARE (*LEPUS EUROPAEUS*) FOR THE PURPOSE OF ASSESSEMENT OF ITS POPULATION SIZE IN SERBIA

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Abstract: In the last couple of decades the case of declining of the European brown hare (*Lepus europaeus*) populations was noted in whole Europe including Serbia. Numerous causes for this can be referred to anthropogenic impacts - landscape changes (agricultural intensification, the use of natural hare habitats by livestock for the purpose of pastures), use of pesticides, machinery and traffic, as well as home range size. Other important causes are inadequate reproduction and the level of survival due to predation (incline in golden jackal population), hunting (especially illegal hunting), variations in climate and diseases, as one of the most important cause of European brown hare population declining. As in other animals, they are etiologically divided to: viral, bacterial, fungal, parasitic and other diseases. During hunting season in 2017 (October and November), a study on 46 hares (whole carcasses or eviscerated organs) from the four locations near Belgrade was conveyed. A post-mortem examination and histopathological investigations were performed and, additionally, routine bacteriology and parasitology of the gastrointestinal system were carried out. The results point to the significance of continuous health status monitoring and discovering the causes and frequency of certain conditions in free-ranging European brown hare populations. In this review paper, some health problems in hares are discussed in more details, taking into account the findings obtained in this preliminary study.

Keywords: health status, *Lepus europaeus*, monitoring

Introduction

The European brown hare (*Lepus europaeus*) is considered as one of the most important game animal species in Europe. Unfortunately, in the last couple of decades the case of declining of the European brown hare (*Lepus europaeus*) populations was noted in whole Europe including Serbia, and this problem is affecting all regions of Europe simultaneously (Frölich et al., 2003; Strandgaard and Asferg, 1980; Tapper and Parsons, 1984; Hutchings and Harris, 1996; Marboutin et al., 2003). Although there are numerous hypothesized causal factors for this decline, it can be referred to health status, or the presence of anthropogenic impacts, different diseases, and the predation (Olesen et al., 2006; Marinković et al., 2018).

During hunting season in 2017 (October and November), a study on 46 hares (whole carcasses or eviscerated organs) from the four locations near Belgrade was conveyed. A post-mortem examination and histopathological investigations were performed and, additionally, routine bacteriology

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and parasitology of the gastrointestinal system were carried out. The results of this recently conveyed preliminary study point to the significance of continuous health status monitoring and discovering the causes and frequency of certain conditions in free-ranging European brown hare populations (Marinković et al., 2018). The aim of this paper is to review possible causes of the decline of the European brown hare (*Lepus europaeus*) population, as well to emphasize the importance of the health status monitoring, especially pathomorphological examination of the hunted animals, and animals found dead in nature.

Factors affecting decline of the European brown hare population

Numerous factors can be referred to the anthropogenic impacts - landscape changes, use of pesticides, machinery and traffic, as well as home range size. One of the important anthropogenic factor are the landscape changes which can inflict the population number of hares. This especially refers to agricultural intensification and use of natural hare habitats as pastures for livestock or for crop production. European brown hare, if available, prefers weeds and wild grasses. However, in areas of agricultural intensification these foods are reduced and crop species, especially monocultures, are increasingly used as a food source. Together with intensive crop production goes the intensive use of pesticides, which are very dangerous and can harm the European brown hare population. The effect of pesticides can be dual. On one side incidents of poisoning have occurred (Chlewski, 1976; Rimkus and Wolf, 1987; Edwards et al., 2000), but on the other side, the use of herbicides is reducing the abundance of weed in cereal fields which decrease the quality of summer diet for the hares. Also, after summer harvest and ploughing fields remain without vegetation, and this is potential lack of summer food for the hares at the time of main breeding and lactating season.

Besides mentioned facts, machinery used for crop harvesting operation is a source of mortality in leverets. Also, "road kills" can additionally decrease the number of hares in the areas where the network of traffic roads is present (Olesen et al., 2006; Posautz et al., 2015; Kornaś et al., 2014).

Other important causes are inadequate reproduction and the level of survival due to predation (incline in golden jackal population), hunting (especially poaching), variations in climate and diseases, as one of the most important cause of European brown hare population declining. The red fox (*Vulpes vulpes*) is considered to be the most important predator for the European brown hare. Oral vaccination of foxes against rabies led to rising of its population in Europe which could be one of the important factors for the declining of hare population (Posautz et al., 2015; Olesen et al., 2006; Reynolds and Tapper, 1995; Panek, 2009; Goszczynski and Waseilewski, 1992). Other predators than the red fox, golden jackal (whose population is in constant incline during past decade), crows, ravens, common buzzards, goshawks, owls, domestic dog and cat also prey on hares, especially leverets (Olesen et al., 2006; Penezić and Ćirović, 2015; Markov and Lanszki, 2012).

Heavy hunting pressure and poaching can jeopardize hare population. Besides that climate influence, particularly climate change can have negative impact on the hare population especially on the leverets (Olesen et al., 2006).

Diseases are considered as one of the natural factors of hare mortality and have greatly influenced the decline of this species in past few decades. As in other animals, diseases in European brown hare can be divided etiologically to: viral, bacterial, fungal, parasitic and other diseases (Frölich et al., 2003; Marinković et al., 2018; Wibbelt and Frölich, 2005).

Among the viral diseases, virus which is the causative agent of European brown hare syndrome (EBHS) is one of the most important. It is classified as a *Calicivirus*, a small (30 to 35 nm) icosahedral, non-enveloped virus (EBHSV) (Ohlinger and Thiel; 1991; Gavier-Widen, 2012). This disease is reported in many European countries (Frölich et al., 2001; Frölich and Lavazza, 2008; Wibbelt and Frölich, 2005) and it is characterized by acute hepatitis and hemorrhages of various internal organs (Poli et al., 1991; Fuchs and Weissenböck, 1992). Interesting fact is that high incidence of degenerative and inflammatory hepatic and renal lesions were noted in liver and kidney tissues originating from the hares from Serbia. So, further investigations are necessary to establish the etiology of these changes (Marinković et al., 2018).

Also, a group of *Leporipoxvirus* are considered as important etiological agents for the hare population, and these two related strains myxoma and fibroma virus can cause fibromatosis as a rare incident in hares or myxomatosis. Fibromatosis is a benign, self-limiting disease with localized fibroblastic nodules within the subcutis (Wibbelt and Frölich, 2005; Grilli et al., 2003; Fenner, 1994). Although myxomatosis is a disease commonly observed in European wild rabbits (*Oryctolagus cuniculus*), hares can also be infected and disease is characterized by multiple firm subcutaneous nodular masses at the head, back and limbs. Virus is transmitted via hematophagous insects or by close contact between hares and affected rabbits or their excretions (Wibbelt and Frölich, 2005; Barlow et al., 2014). Hares are anecdotally infected with papilloma virus usually through cutaneous lesions or by hematophagous insects when focal pedunculated nodular masses – warts can develop (Wibbelt and Frölich, 2005).

Among bacterial diseases, several are important for the health status of the European brown hares: pseudotuberculosis, pasteurellosis, brucellosis, tularemia and staphylococcosis (Wibbelt and Frölich, 2005). Pasteurellosis is considered to be the most important hare disease and sometimes up to 80% of a population might be killed by this infection which is the most common during the winter season, and early spring when sudden, widespread epidemics can occur. This disease is caused by small non-spore forming, gram-negative coccobacillus *Pasteurella multocida* subsp. *multocida*, commonly present in the upper respiratory system. Stress, usually, sudden drop of temperature, especially during the night, inadequate immunological status, food shortage, etc. can induce the multiplication of these bacteria, and the development of disease. The disease can occur either in peracute septicemic haemorrhagic form or acute to chronic with fibrino-purulent pleuro-pneumonia and fibrinous pericarditis.

Other important bacterial disease of lagomorphs and rodents is pseudotuberculosis caused by *Yersinia pseudotuberculosis*, a small rod-shaped gram-negative bacterium, and it is considered to be of one of the most important lethal infections in hare with population losses of up to 50%. This disease can also occur in birds and other animals as well as in humans. Rodents and wild birds are considered to act as a natural reservoir for this microorganism. The disease is common during cold and humid part of the year, but beside weather factors, stress, inadequate food, opportunistic infections are also important factors for the onset of this disease. Common clinical manifestations are dyspnoea and diarrhea, and characteristic morphological lesions are multifocal caeseous granulomatous nodules within spleen, liver, intestine and mesenteric lymph nodes (Wibbelt and Frölich, 2005; Fratini et al., 2017; Frölich et al., 2003).

Staphylococcosis in hares is caused by *Staphylococcus aureus* and characterized by multiple abscessation of the skin, subcutis, and sometimes internal organs and joints, in rare cases as an acute septicaemia which can cause the exitus lethalis without specific morphological changes (Wibbelt and Frölich, 2005).

Two important bacteria can cause disease in hares which also have zoonotic potential: *Francisella tularensis* and *Brucella sp.*, although previously mentioned *Yersinia pseudotuberculosis* also have that kind of potential. Brucellosis is caused by *Brucella suis biovar 2*, gram-negative, non-sporing, small rods. It is believed that wild boars and hares are reservoir species for this agent, but this bacteria is also capable of infecting domestic pigs and other wild or domestic animals and as mentioned humans as well. The most likely source of infection are aborted fetuses. Infection can occur orally, via the reproductive system, the conjunctiva and percutaneously, but the venereal transmission seems to be the dominant way of infection in hares. The course of the disease in hares can be acute or chronic, and the infection in hares is either latent or involves the development of granulomatous nodules or abscesses in the reproductive system – uterus and testes, regional lymph nodes, liver, spleen, lung and other tissues (Thorne, 2001; Gyuranecz et al., 2011; Frölich et al., 2003)

Tularaemia is caused by *Francisella tularensis*, a highly infective gram-negative, non-motile, non-sporing, aerobic coccobacillus with zoonotic potential which occurs naturally in lagomorphs and rodents, with ticks and other arthropods the main vectors, but infections of many other mammals and birds. Infection occurs directly through close contact or indirectly via contaminated food or insect vectors. In the European brown hare in most cases, clinical signs appears as short apathy followed by fatal septicemia. Numerous necrosis are found within liver, spleen and lymph nodes (Gyuranecz et al., 2010; Hestvik et al., 2017; Tomaso et al., 2017; Frölich et al., 2003).

Several other microorganisms can also play important role for the health status of European brown hare, and some of them are also zoonosis and also found in Serbia as well: *Bacillus anthracis*, *Borrelia burgdorferi* (Talleklint and Jaenson, 1994; Jaenson and Talleklint, 1996), *Campylobacter sp.*, *Leptospira sp.*, *Listeria monocytogenes* (Mišić and Marinković, 2002) and *Salmonella sp.*

The most diseases, especially parasitic diseases are density dependent. These diseases can play important role in the health status or can cause mortality in the hare population and in that manner have negative influence on the abundance of this species in nature. Generally, parasitic diseases can be caused by protozoa, nematodes, cestodes, trematodes and acarines and insects.

Coccidiosis is one of the most important protozoal infections in hares. It can occur in the intestinal and hepatic form. Intestinal coccidiosis is caused by variety of species of *Eimeria* such as *E. europea*, *E. hungarica*, *E. robertsoni*, *E. semisculpta*, *E. septentrionalis*, *E. stefanskii* and *E. townsendii*. These coccidia invade cells within epithelial lining of the intestines causing severe catarrhal enteritis and gaseous distension of the gut. Sometimes Often small yellowish nodules within the mucous membranes can be noted. Hepatic coccidiosis is caused by *E. stiedai* which invade epithelial cells of the bile ducts causing hyperplastic lesions of the bile ducts. As in rabbits transmission is feco-oral, and up to 60-100% of the animals in population can be infected with coccidia (Kornaš et al., 2014; Chroust, 1984).

Toxoplasma gondii is protozoal organism causing Toxoplasmosis, disease which in most cases is acute fatal disease in hares. Infection occurs by ingestion of food or water contaminated with oocysts from cat feces. Common pathomorphological changes are interstitial pneumonia, multifocal areas of hepatocellular necrosis, encephalitis, and necrosis of lymphoid follicles of the lymph nodes (Jokelainen et al., 2011; Sedlak et al, 2000, Frölich et al., 2003).

Hares can be infected with both trematodes - *Fasciola hepatica* and *Dicrocoelium dendriticum* and these parasites can cause cholangitis with extensive bile duct hyperplasia with proliferation of mucous glands, and sometimes extensive hepatic cirrhosis (Cuervo et al., 2015; Diakou et al., 2014; Chroust et al., 2012; Sergi et al., 2018). These parasites were also noted in the hare population in Serbia (Marinković et al., 2018).

Gastro-intestinal nematodes can be important due to its pathogenic influence on the process of digestion, and also as a cause of serious anaemia in hares. Several nematode species are important for hares - *Graphidium strigosum*, gastric parasite, *Trichostrongylus retortaeformis*, small intestine parasite, causing catarrhal enteritis and *Trichuris leporis* commonly found on the caecal mucosa, causing necrotic lesions within the gut wall due to its toxic metabolites. *Passalurus ambiguus* can be found both in small and large intestine. The juvenile stages are rather found in the mucosa of the small intestine and the cecum, while the adult worms are located in the anterior part of the cecum and the large intestine (Diakou et al., 2014; Chroust et al., 2012; Sergi et al., 2018; Dubinský et al., 2010).

Lungworms *Protostrongylus sp.* can cause severe infection followed by dyspnoea and seromucosal nasal discharge due to catarrhal pneumonia and pleuritis. This parasitic infection has negative influence to the immunity of the animal, and as a consequence of parasitism hares are more prone to secondary bacterial infection in contrast to clinically healthy hares (Diakou et al., 2014; Chroust et al., 2012; Sergi et al., 2018; Dubinský et al., 2010; Frölich et al., 2003).

Hares also can be hosts for numerous Cestodes - *Paranoplocephala wimerosa*, *Andrya cuniculi*, *Andrya rhopalocephala*, *Cittotaenia denticulata*, *Mosgovoyia pectinata*, *Mosgovoyia ctenoides*. Most of them can cause catarrhal enteritis with malabsorption, degenerative changes on the liver (*Taenia pisiformis* larvae), and some of them are important zoonosis - *Ecchinococcus granulosus* and *Ecchinococcus multilocularis* (Diakou et al., 2014; Chroust et al., 2012; Sergi et al., 2018; Dubinský et al., 2010; Chaignat et al., 2015). Besides mentioned parasites hares can be infected with numerous other parasites such as lice (*Haemodipsus setoni*), *Sarcoptes scabiei* (Restani et al. 1985), *Sarcocystis sp.* and many other.

Conclusion

There is a multicausal etiology for the decline of the population of the European brown hares (*Lepus europeus*), and different diseases have a great importance in this declining. Constant health status monitoring is important for the assessment of the population size and it is necessary to intensify and to facilitate its implementation in Serbia. The pathomorphological examination of the hare carcasses, followed with microbiological and parasitological examinations, plays a crucial role in this process.

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