

## HISTOPATHOLOGICAL CHANGES AND BACTERIOLOGICAL SURVEY OF INTERNAL ORGANS IN THE ASPECT OF THE INDIVIDUAL CONDITION OF A HARE (*LEPUS EUROPAEUS* PALL.)

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**Abstract.** The analysis of bacteriological changes in hare internal organs has been performed in the paper. The assessment was made with regards to the individual condition of animals. Anatomical and pathological changes were found in 80% of the hare from the obtained sample. These occurred regardless of the age and gender of animals and were mostly inflammatory infiltrates and necrosis in the testes of males as well as inflammatory infiltrates of the liver and emphysema found in both males and females. There was also an increase in flora of two bacterial strains in hares internal organs. The *Nocardia* strain was found in the testes of two individuals, whereas the *Providencia rustigianii* strain was found, at various severity levels, in the liver, kidneys, lungs and heart of males and females, regardless of their age. Isolated strain of *Providencia rustigianii* proved to be resistant to three antibiotics, while *Nocardia* strain to one of the antibiotics used. The analyses carried out indicate that in addition to population and ecological factors resulting from environmental changes, a number of pathogenic agents, including the presence of bacteria that have not been described in this animal species, are a significant factor limiting the reproductive potential of the population. Therefore, disturbances of physiological processes having a diverse etiological background can lead to numerous anatomical and pathological changes, and thus increase susceptibility to diseases and falls of animals, and at the same time, deepen the ongoing regress of this species population. Not without significance is the fact that the described changes were found regardless of the gender and age of animals.

**Keywords:** *brown hare, body mass, population structure, bacterial diseases, diseases changes, Lublin Upland*

### Introduction

Almost since the end of the 1970s, both in Poland and many European countries, a downward trend of the hare population has been observed (Burel and Baudry, 1990; Flis, 2009, 2016; Frylestam, 1980; Schmidt et al., 2004; Wibbelt and Frölich, 2005; Nasiadka and Dziedzic, 2014). In our country, in many cases, the level of spring density is currently lower compared to the volume of acquisition in the 1960s and 70s of the twentieth century. In the 1970s, the average population density in our country remained at the level of about 50 individuals per 100 ha of the field areas of hunting grounds (Pielowski, 1976, 1979). At the beginning of the 21st century, the average level of hare density in the country varied in individual regions and was within the range of 5-9 individuals/100 ha (Dziedzic et al., 2002; Wasilewski, 2007; Flis, 2009, 2016). However, there are still areas where density of the population is so high that the species

is still regularly hunted (Dziedzic et al., 2002; Flis, 2009, 2015, 2016; Nasiadka and Dziedzic, 2014).

Despite the fact that there is no possibility of indicating a clear cause of the persistent population trend, usually the most frequent changes are those in agrocenoses constituting the basic areas of this species. Intensification of agriculture significantly influences the simplification of agrocenosis structures, which in turn leads to a significant decrease in the heterogeneity of these environments. This entails the shrinking of optimal hare habitats (Lewandowski and Nowakowski, 1993; Panek and Kamieniarz, 1999; Schmidt et al., 2004; Kryński et al., 2007; Petrovan, 2011; Kamieniarz et al., 2013; Schai-Braun et al., 2015; Panek, 2018). Another, not less important, element that directly affects the dynamics of the number of hares is predation. The use of oral treatments for immunization of free-living foxes eliminated one of the basic factors limiting the population, which in turn increased the impact of this species on small animals (Demirbas, 2015; Gołdyn, et al., 2003; Goszczyński and Wasilewski, 1992; Panek et al., 2006; Wasilewski, 2007; Flis et al., 2017, 2018). Hušek et al. (2015) a high rate of fox predation targeting hares depending on the habitat and reported that hare males tend to fall as a prey. Therefore, an extremely important element in hare population management as a monomorphic species is knowledge of the individual condition and population structures that have a direct impact on the reproductive potential of the population (Méres et al., 2016; Misiorowska et al., 2014; Pintur et al., 2006). The impact of hare population, and thus local density indices, on the impact of synanthropic predation is also significant (Flis, 2013; Nasiadka and Dziedzic, 2014).

Another elements that directly affect survival, and thus the size of the population are numerous disease entities found in this species. Coccidiosis and other parasitic diseases are the basic mention. Nevertheless, bacterial and viral diseases, such as brucellosis and European Brown Hare Syndrome – EBHS – and many other slightly less common ones also exert quite considerable impact (Gavier-Widén and Mörner, 1991; Duff et al., 1994; Pikula et al., 2004; Wibbelt and Frölich, 2005; Frölich and Lavazza, 2008; Dubinský et al., 2010; Decors et al., 2011; Chroust et al., 2012; Kornaś et al., 2014; Flis et al., 2016).

The aim of the study was the assessment of histopathological changes and bacteriological analysis of internal organs as well as individual condition in the population of European hare originating from the region where hunting acquiring is still taking place.

## Materials and methods

### *Characteristics of the research area*

The research was conducted in two hunting districts with a total area of 13,600 ha, the forest cover of which is 25.2%. They are located in the western part of the Lublin region, in the mesoregion of Kotlina Chodelska and the northern part of Wzniesienia Urzędowskie, in Poland (*Fig. 1*). This region is characterized by the presence of fertile soils of the chernozem type on the loess ground (Kondracki, 2000). Despite fairly fertile soils, it is distinguished by significant fragmentation of agricultural crops, and thus large heterogeneity of field environments. Field crops are dominated by plants with high soil requirements, with particular reference to orchards and plantations of perennial soft fruits, mainly raspberries and currants. In the agricultural landscape, there are numerous

wastelands as well as small wooded enclaves and forest complexes (Witek, 1991). Heterogeneity evaluation of the natural environment of agrocenoses in the area of research, performed according to the method proposed by Schrödel (1991) showed an average size of individual fields at the level of 0.95 ha, while the average distance between landscape elements ranged from 0.5 to 0.7 km. In turn, the relative length of contour lines in agrocenoses having significant impact on the functioning of small game population, despite the variation in individual regions of the study area, was on average at 0.1–0.2 km/km<sup>2</sup>. All described elements determine quite significant mosaicism of field environments, creating almost optimal conditions for living and functioning of the hare population (Pielowski, 1979; Nasiadka and Dziedzic, 2014).



*Figure 1. Location of the research area*

### ***Material for research***

As part of the research, hares obtained in collective hunts during the hunting season 2017/18 have been weighed and their gender and age have been determined. The whole material consisted of 20 hares obtained first during two hunts, which is representative of the sample. Due to minimizing the amount of shooting in recent years and the fact that in accordance with the law, holder of the hunting district is the owner of hunting grounds, it was not possible to collect larger number of hare for study. In total, 9 males and 11 females were assessed, of which the share of young and adult hares was half.

### ***Methods and measurements***

All shot-off hares were weighed directly in the field. Weighing was carried out to the nearest 0.1 kg. Also in the field, their gender and age were assessed. Gender assessments were made based on the appearance of secondary sexual characteristics.

The age of hare was also determined directly in the field on the basis of the palpation assessment of the occurrence or disappearance of the Stroh mark (cartilaginous elbow base cap). Such an assessment allows the division of hares into young (up to 1 year old) and adult (over 1 year old) (Stroh, 1931; Pielowski, 1979).

### ***Histopathological and bacteriological material***

Lung, liver, kidneys and testes sections in males were taken from all acquired hares for histopathological and bacteriological examinations. Sectional examination performed under the field conditions and material collection for bacteriological examination required the use of a transport set containing rods to collect swabs from pre-isolated organs. Material for histopathological analysis was fixed in 4% formalin, and then dehydrated and embedded in paraffin. The 4 µm thick sections were stained with hematoxylin and eosin (HE). A bacteriological analysis of the collected samples was also carried out.

### ***Laboratory preparations***

Different propagating and differentiating media were used to identify individual species of microorganisms. The solid substrate was agar with the addition of 5% sheep blood to breed aerobic microorganisms and possible determination of hemolysis. Sugar broth was used as a liquid substrate to reproduce aerobic bacteria. Other substrates were also used, including Chapman, Mac Conkey and Sabouard medium to identify any organ mycoses that occur. The study also applied a medium with cetrimid to identify microorganisms of the *Pseudomonas* genus and D-coccosel medium to identify fecal bacteria. Further identification of individual bacteria types was carried out with the BioMerieux bio-chemical tests (Analytical Profile Index). The control of substrates fertility was carried out using reference strains. After determining the bacterial strain, the growth rate of bacterial flora was assessed using the criterion of low (+), intensive (++) and very intense (+++) growth. Although it is a simplified method, it allowed preliminary assessment of the health status of the hare, and most of all it revealed the type of bacterial environmental threats. An analysis of the sensitivity of isolated bacterial strains to 8 commonly used antibiotics was also carried out applying the criterion of subdivision into sensitive, medium sensitive and resistant ones. The following antibiotics were used for the analyzes in doses: Enrofloxacin ENR – 5 µg, Amoxicillin/clavulanic acid AMC – 30 µg, Gentamycin CN – 10 µg, Penicillin G P – 10 µg, Oxytetracycline OT – 30 µg, Trimethoprim/sulphamethoxazole SXT – 25 µg (trimetoprim- 1.25 µg+sulfametoksazof 23.75 µg), Marbofloksacin MAR – 5 µg, Tylozyna TY – 30 µg.

## **Results**

### ***Characteristics of the structure and individual quality of the population***

The conducted research shows that the average body weight of three young males was 3.9 kg, and 7 young females 3.7 kg (*Table 1*). In adults, the body weight was uniform. The average value of this feature of 6 males and 4 females was at the level of 4.7 kg. The overall value of the gender index was at 1:1.2 in favor of females. The reproductive success rate defined as the ratio of the number of young hares that survived till the hunting season per 1 adult female was high and amounted to 2.5.

**Table 1.** Body mass and results of bacteriological analysis as well as histopathological changes in internal organs of acquired hares

No.	Individuals characterization			Organs covered by the analysis				
	Gender	Age	Body weight (kg)	Testes	Liver	Kidneys	Lungs	Heart
1	♀	Young	4.0	x	Small inflammatory infiltrations	x	x	x
2	♀	Adult	4.6	x	x	x	Emphysema	x
3	♂	Adult	4.7	Small inflammatory infiltrations	x	x	x	x
4	♂	Young	3.5	x	x	x	x	x
5	♀	Young	3.9	x	x	x	x	x
6	♀	Adult	4.7	x	Medium inflammatory infiltrations	x	x	x
7	♂	Adult	4.5	x	x	Low (+) growth of <i>Providencia rustigianii</i>	Intensive (++) growth of <i>Providencia rustigianii</i>	Intensive (++) growth of <i>Providencia rustigianii</i>
8	♂	Adult	4.4	Necrosis Very intense (+++) growth of <i>Nocardia</i>	Intensive inflammatory infiltrations	Glomerulonephritis low (+) growth of <i>Providencia rustigianii</i>	Intensive (++) growth of <i>Providencia rustigianii</i>	Intensive (++) growth of <i>Providencia rustigianii</i>
9	♀	Adult	4.7	x	Small inflammatory infiltrations	x	Emphysema	x
10	♀	Young	4.0	x	Low (+) growth of <i>Providencia rustigianii</i>	x	x	x
11	♂	Young	4.0	x	x	x	Low (+) growth of <i>Providencia rustigianii</i>	x
12	♀	Adult	4.8	x	x	Glomerulonephritis low (+) growth of <i>Providencia rustigianii</i>	x	x
13	♂	Adult	4.6	Necrosis Intensive inflammatory infiltrations	x	x	x	x
14	♂	Adult	4.9	x	x	x	x	x
15	♂	Young	4.2	Medium inflammatory infiltrations Low (+) growth of <i>Nocardia</i>	x	Low (+) growth of <i>Providencia rustigianii</i>	Emphysema	x
16	♀	Young	3.2	x	x	x	Emphysema low (+) growth of <i>Providencia rustigianii</i>	x
17	♀	Young	3.6	x	Small inflammatory infiltrations	x	x	x
18	♂	Adult	5.1	Necrosis	x	Low (+) growth of <i>Providencia rustigianii</i>	x	x
19	♀	Young	3.7	x	x	x	x	x
20	♀	Young	3.6	x	Small inflammatory infiltrations	x	x	x

### **Anatomical and pathological analysis**

Performed anatomical-pathological analyses of testicles showed that in the examined sample, extensive areas of necrosis were found in three adult males. In the central parts of the necrotic areas, there were numerous foci indicating the progressive process of calcification (*Fig. 2*). In addition, in the testes of two young and one adult males, inflammatory infiltrates of varying intensity were found. Numerous purulent infiltrates

contained neutrophil granulocytes, giant cells, fibroblasts and histiocytes. Glandular epithelial atrophy and progressive fibrosis around the glandular tissue were found, which was caused by an ongoing inflammatory process with irreversible effects. Anatomical-pathological changes of liver were found in 6 individuals, which were manifested by a partial lack of individual cells in a radiative manner within some of the hepatic lobes (Fig. 3). There were numerous focal inflammatory infiltrates between the hepatic lobes. Small inflammatory infiltrates occurred in 3 young females and one adult. One adult female showed medium inflammatory infiltrates, while in one adult male - intense inflammatory infiltrates. Analysis of kidneys from shot hares showed numerous inflammatory infiltrations within the glomerulus of the vessels with partial or complete closure of the kidney tubules (Fig. 4). This indicates the occurrence of glomerulonephritis. This condition was found in one adult male and one young female. Among the analyzed sample, one young and two adult females as well as one young male were diagnosed with emphysema with characteristic foci of congestion and inter-vesicular tissue hyperplasia (Fig. 5). Analysis of samples taken from myocardial muscles did not show any anatomical-pathological changes.

### **Bacteriological analysis**

The results of bacteriological analysis of the testes revealed two cases of *Nocardia* bacteria. One young male showed a fairly low (+) growth, and in one adult male a very intense (+++) growth of this bacterium. In turn, in the liver of 1 young female, quite low growth (+) of *Providencia rustigianii* was found. Analysis of kidneys of the acquired individuals showed that 5 of them revealed quite low (+) growth of *Providencia rustigianii*. This condition occurred in one young and three adult males and one adult female. In samples taken from lungs, 2 adult males revealed very intense (++) growth, while in a young male and adult female, quite low growth (+) of *Providencia rustigianii*. In the collected heart tissue samples, two adult males showed a significant very intense growth (++) of *Providencia rustigianii*.

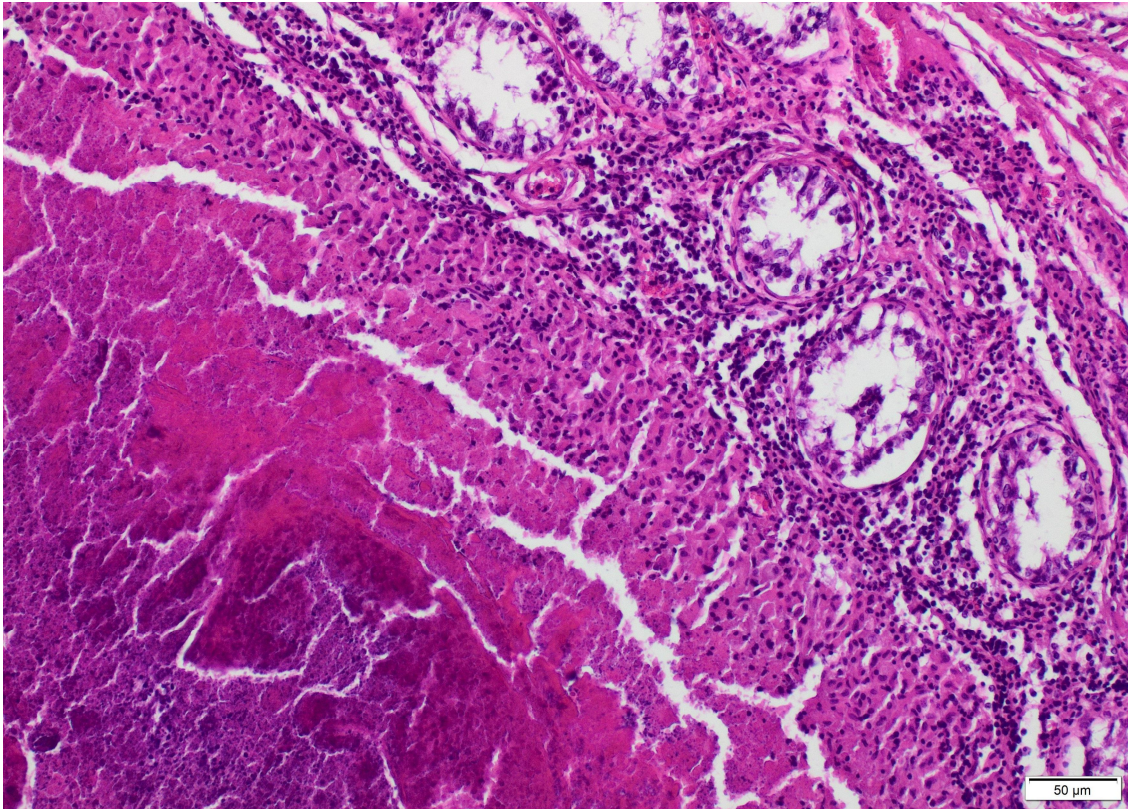
### **Resistance to antibiotics**

As part of the study, the *in vitro* sensitivity assessment of isolated bacterial strains to antibiotics was performed (Table 2). The isolated strain of *Providencia rustigianii* turned out to be resistant to three antibiotics used, moderately sensitive to one and sensitive to another four antibiotics. In turn, the isolated *Nocardia* strain was sensitive to seven of the eight antibiotics used and resistant only to one of them.

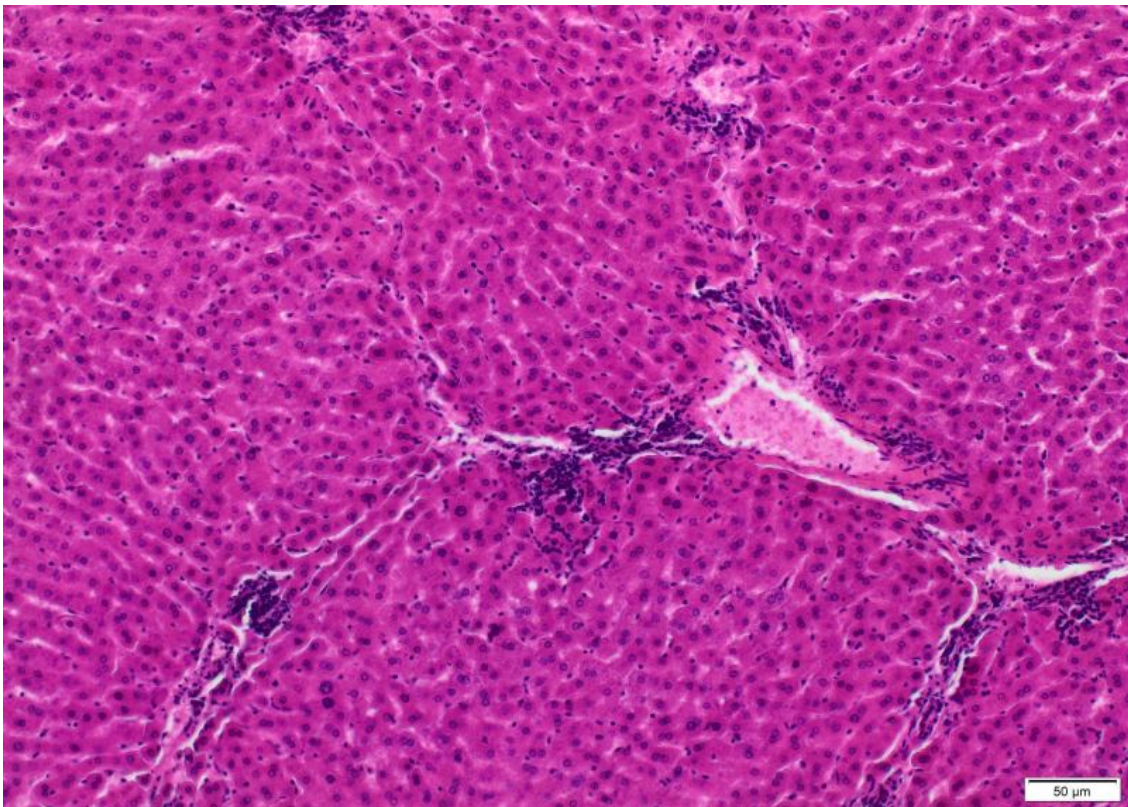
**Table 2.** Sensitivity of isolated bacterial strains to antibiotics

Antibiotic used	Type of bacterial strain	
	<i>Providencia rustigianii</i>	<i>Nocardia</i>
Tylozyna <b>TY30</b>	O	W
Amoxicillin/clavulanic acid <b>AMC 30</b>	O	W
Gentamycin <b>CN10</b>	W	W
Penicillin G <b>P10</b>	O	W
Sulphamethoxazol/Trimethoprim <b>SXT25</b>	ŚW	O
Marbofloksacin <b>MAR5</b>	W	W
Oxytetracycline <b>OT3</b>	W	W
Enrofloxacin <b>ENR5</b>	W	W

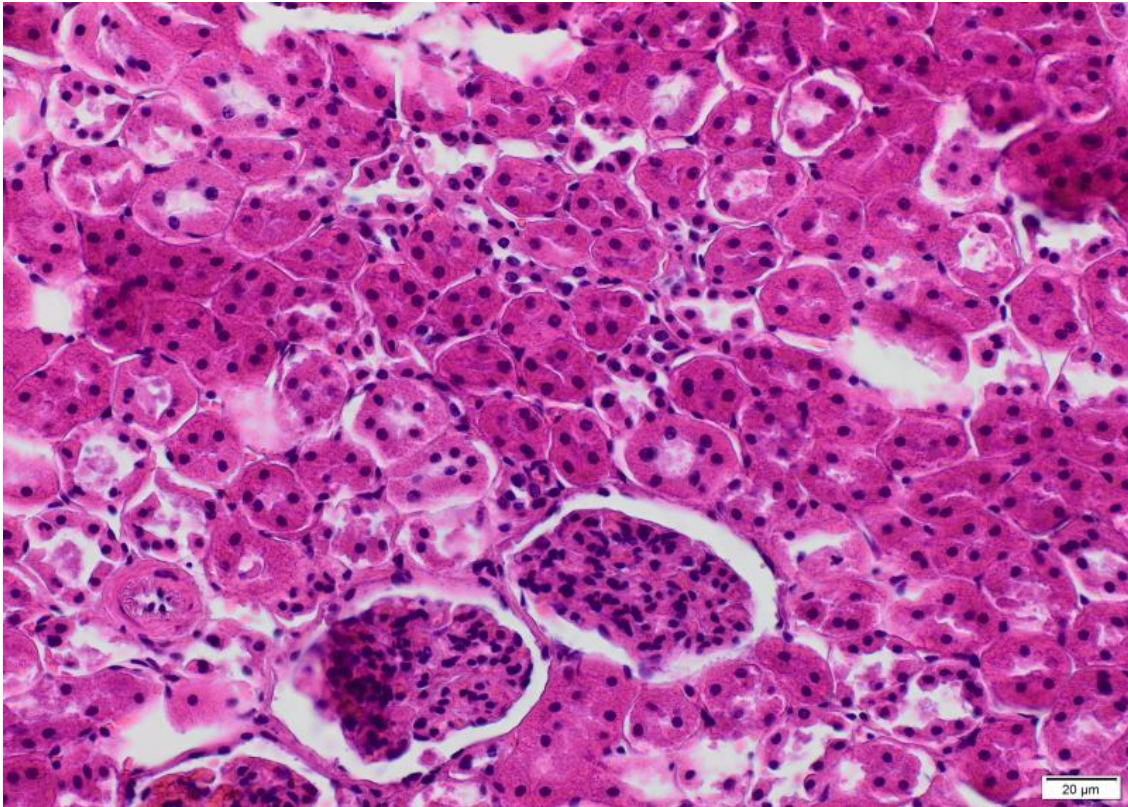
W - sensitive; ŚW - moderately sensitive; O - resistant



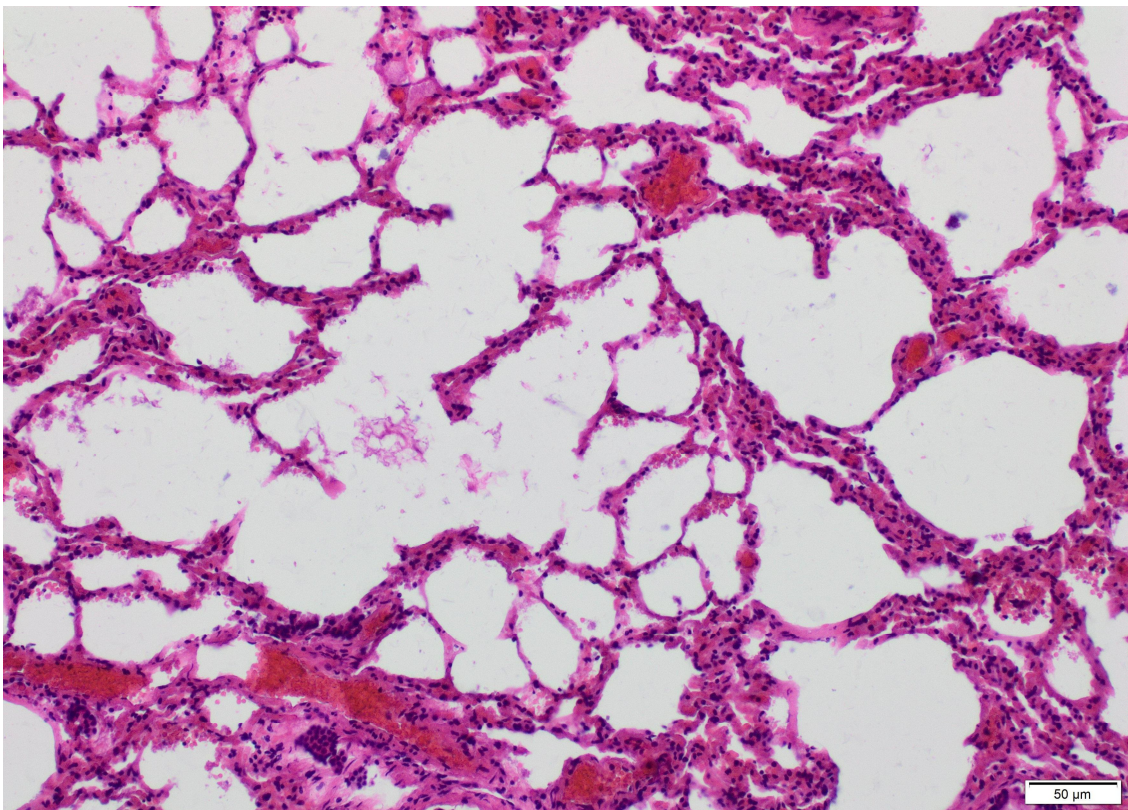
**Figure 2.** Numerous necrotic areas in the testes of males



**Figure 3.** Focal inflammatory infiltrates between the hepatic lobes



**Figure 4.** *Glomerulonephritis with partial or complete renal tubular closure*



**Figure 5.** *Numerous foci of congestion and inter-follicular tissue growth, indicating*



## Discussion

The analysis of population parameters based on a sample of 20 individuals indicates that the values of body weight, being an expression of the individual condition, in significant way do not differ from those reported by Flis (2015). Obtained body mass values slightly exceed those given by Dziejczak et al. (1998) from Lublin Upland and Podlasie region in the mid-1990s. These results are higher than those from research conducted in areas of the highest population size of this species in our country during the hunting season 2009/2010 (Misiorska et al., 2014). The presented results are characterized by much higher body weight values than those in Croatia in the 2004/2005 hunting season, reported by Pintura et al. (2006), as well as in Hungary in both age and gender groups (Farkas et al., 2016). In turn, the obtained gender structure is similar to both the research conducted by Flis (2015), and that reported by Misiorska et al. (2014).

The reservoir of bacteria from the genus *Providencia* are insects, and most of all, houseflies, from which *Providencia alcalifaciens*, *Providencia rettgerii* and *Providencia stuarti* were isolated. The species of *Providencia rettgerii* was isolated from *Helaemyia petrolei* fly larvae found in oil-bearing areas. Bacteria of the genus *Providencia rettgerii* are found as an indispensable element of natural flora of crocodiles, frogs, vipers, as well as snakes, and in particular of python and boa snakes. In addition, the occurrence of *Providencia rettgerii* was found in roundworms, sea lions and seals. Quite rarely, this species is isolated from birds (falcons, bustards). As a rule, they were found in dogs, cats, guinea pigs, as well as in cattle and sheep as part of the natural flora of the gastrointestinal tract and quite often they are referred to as intestinal bacteria. It should be mentioned that the bacteria of *Providencia* genus also cause many diseases. Attention is being paid to *Providencia alcalifaciens* during dog diarrhea and *Providencia rettgerii* causing generalized infections and meningitis, especially in crocodiles up to the age of 3 years. There have also been reports of this type of oral inflammation cases in seals and snakes. These bacteria have never aroused so much interest among the bacteria of the *Enterobacteriaceae* family. This was mainly due to their reduced pathogenicity. Currently, they are beginning to play an increasingly important role as pathogens that cause urinary and digestive tract infections. Up to date, there have been no reports of their presence in hare; however, it is possible that their strong uropathogenic properties confirmed in histopathological studies in mice and monkeys may also have an impact on hare mortality (Janda and Abbot, 2006; O'Hara Mohr et al., 2000).

*Nocardia* belong to aerobic bacteria. Their natural reservoir is water, soil, vegetation and sewage systems. Infection in animals is quite rare, mainly in tropical countries. These bacteria can be transmitted by castor bean tick. Among animals, they were found in cattle, and the symptoms are dermatitis, pneumonia, mastitis and diarrhea. They were also found in dogs and cats, where lesions were found mainly in lungs and pleural cavity. Infection with these bacteria occurs mainly by inhalation or also by damaged skin. In the skin form, inflammation of regional lymph nodes and nodular lymphangitis are mainly observed. Inhaled form affects the lung and pleura and takes the form of inflammatory granulomas, very similar to those caused by closely related *Actinomyces* bacteria. Due to the fact that so far there have been no reports of their presence on hare, it is difficult to indicate their impact on the health status of this species population. Nevertheless, their numerous occurrences in the testes of two males indicate that they

can affect the reproductive processes (Tropiło et al., 1996; Gliński and Chełmiński, 2014; Katkiewicz, 2017; Wiciński et al., 2018).

## Conclusions

1. The individual quality expressed as body weight in both young and adult hares is high, and the obtained values of this feature definitely exceed those from other parts of the country, as well as in some European countries. In turn, the balanced gender structure, with a slight majority of females, indicates that this population has developing characteristics, which should favorably affect the dynamics of its size in subsequent years.
2. In the examined sample, only 20% of hares did not reveal any lesions, and this concerned 3 young and one adult. In the rest of the sample, anatomical-pathological changes were found. They occurred regardless of the age and gender of animals. These were inflammatory infiltrates and necrosis in the testes of males as well as inflammatory infiltrates of the liver and emphysema found in both males and females.
3. There was also a growth in bacterial flora of two strains in the hare's internal organs. The *Nocardia* strain was found in the testes of two individuals, whereas the strain of *Providencia rustigianii* was found to vary in severity in the liver, kidneys, lungs and heart of males and females, regardless of their age. These strains have not yet been described in this species. Therefore, it is advisable to carry out further research in this respect, because described bacteria affecting the reproduction processes may be one of the factors contributing to the ongoing regression of this species population.
4. Performed antibiograms indicate that the isolated strain of *Providencia rustigianii* proved to be resistant to three antibiotics, while *Nocardia* strain to one of the antibiotics used.
5. The analyses carried out indicate that in addition to population and ecological factors resulting from environmental changes, numerous pathogenic factors are significant in limiting the reproductive potential of the population. Thus, disturbances of physiological processes having a diverse etiological background can lead to numerous anatomical and pathological changes, and thus increase susceptibility to diseases and falls of animals.
6. Considering the fact that the individual condition and parameters of the population covered by the research are high, it is necessary to conduct further comprehensive research, mainly in the direction of diseases of diverse etiological background. Research should cover larger areas with different densities, and in particular, if possible, concentrate on low-density areas. Such a comprehensive assessment should help to identify environmental hazards for the hare population and to develop directions for actions to reduce the adverse downward trend.

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