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PROFILE OF WILD HARE CARCASSES FROM SOUTHERN ROMANIA: A COMPREHENSIVE INVESTIGATION OF MICROBIOTA

PROFILUL CARCASELOR DE IEPURI SALBATICI DIN SUD-ESTUL ROMANIEI: UN STUDIU GENERAL ASUPRA MICROFLOREI

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ABSTRACT | REZUMAT

The European hare (*Lepus europaeus*) is an important game animal both nationally and internationally, that has experienced a decline in recent years, with the exact cause remaining unidentified. To understand this phenomenon in southern and southeast Romania, a study over a period of 5 years was conducted. The study aimed at the qualitative and quantitative determination of the microbial composition of more than 3000 European hare carcasses. Classical techniques were used for the diagnosis of isolated bacteria; modern genetics was used for species identification. The analytical statistic of the obtained results shows a significant total number of germs (from 2.1 x 10^7 to 1.7 x 10^8 CFU/g sample). The majority of bacteria were sporulated (3.8 x 10^5) and just a few were unsporulated (4.8 x 10^2). Isolation and identification examinations revealed the presence of pathogenic or conditionally pathogenic bacterial species, some with a relatively high frequency of isolation: *Salmonella sp*. – 5.53 %; *Escherichia coli* – 6.24 %; *Staphylococcus sp*. – 7.81 %; *Proteus sp.* – 2.82 %. Many of the isolated germs found in wild carcasses are pathogens or conditioned pathogens and represent a threat to both domestic animals and humans' beings.

Keywords: hare, microbiota, *Salmonella sp*., *Escherichia coli, Staphylococcus sp*.

Iepurele european (*Lepus europaeus*) este un important animal de vânat atât la nivel național, cât și internațional, care a cunoscut un declin în ultimii ani, cauza exactă rămânând neidentificată. Pentru a înțelege acest fenomen în sudul și sud-estul României a fost realizat un studiu pe o perioadă de 5 ani. Studiul a vizat determinarea calitativă și cantitativă a microbiotei a peste 3000 de carcase de iepuri europene. Pentru izolarea bacteriilor au fost folosite tehnicile clasice, iar pentru identificarea speciilor au fost folosite tehnici moderne de biologie moleculară. Analiza statistică a rezultatelor obținute a evidențiat un număr total de germeni semnificativ (de la 2,1 x 10^7 la 1,7 x 10^8 UFC/g probă). Majoritatea bacteriilor au fost sporulate (3,8 x 10^5) și doar câteva au fost nesporulate (4,8 x 10^2). Examinarea speciilor izolate și identificate au evidențiat prezența unor specii bacteriene patogene sau condiționat patogene, unele cu o frecvență relativ mare de izolare: *Salmonella sp*. – 5,53 %; *Escherichia coli* – 6,24 %; *Staphylococcus sp*. – 7,81 %; *Proteus sp*. – 2,82 %. Mulți dintre germenii izolați găsiți în carcasele evaluate sunt patogeni sau condiționat patogeni și reprezintă focar atât pentru animalele domestice, cât și pentru consumatori.

Cuvinte cheie: Iepure, microbiotă, *Salmonella sp*. *Escherichia coli, Staphylococcus sp*.

European hare populations (*Lepus europaeus*), even with an assessment of Least Concern (LC), have been experiencing declines in many areas across their geographic range in Europe and are under a current population trend of decreasing (12), with potential factors contributing to this decline encompassing agricultural intensification, shifts in climate patterns, and variations in predator populations, but there is no definitive agreement on the specific significance of each of these factors (17). In Romania, there is a lack of quantified data regarding the magnitude of this decline, the present population levels, or any reference point to track potential future changes in population. Only a few scientific studies have focused on this species its pathology (2-5, 8, 10), and this study aims to improve knowledge in this domain.

The European hare represents one of the most important games in both Romanian and European hunting sectors, with more than 5 million specimens harvested annually just in Western Europe (6). In Romania, according to the Ministry of Environment, Waters, and Forests, 79,625 European hares were harvested in 2023 (22), making them one of the most hunting and commercially interesting species.

Currently, there is an increase in the sales market for game meat products, associated with the diversifi-

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cation of assortments. The hygienic quality of game meat, as well as the products and preparations obtained from it, is influenced by numerous factors. An important aspect is the frequent contamination with microorganisms, due to the various sources of contamination to which the game is exposed from the moment of shooting. A series of important elements must be taken into account that converge in increasing the bacterial load of game carcasses: the impossibility of clinical examination of the animal prior to shooting, the chronic evolution of some morbid entities (bacteriosis, viruses, and parasites), the generation of gunshot wounds that constitute deep contamination factors, incomplete bleeding, late evisceration (sometimes much after 30 minutes after death) and defective (most often evisceration on the ground), late cooling of carcasses, etc. Many times, the shot in the abdominal area causes intestinal ruptures and the overflow of the digestive contents into the peritoneum, thus causing an important dissemination for saprophytic and conditionally pathogenic bacteria.

We approached this work from the perspective of the surface and depth microflora of the carcasses, considering the fact that many wild rabbit carcasses end up in processing and there is a risk of obtaining preparations with a bacterial load that is dangerous for the health of the consumer (both qualitatively and quantitatively). In particular, processing in family systems leads to obtaining culinary preparations consumed after insufficient heat treatments or traditionally preserved (homemade sausages, rabbit pastrami, traditional steaks, etc.).

Another important motivation for this work was the frequency with which rabbit carcasses are introduced to advanced ripening, which provides favourable conditions for the bacterial flora to develop abundantly and provides the possibility for some bacteria to synthesise toxins responsible for the production of food poisoning. Of course, a strong argument for the purpose of the paper was also to know the situation of the microbial load in the context of quite poor literature data for this subject.

MATERIALS AND METHODS

To determine the total load of bacteria (NTG), yeasts and moulds (NTF), as well as the types of bacteria present on the internal surface of wild rabbit carcasses or in the depth of the muscles, sanitation swabs and muscle samples were taken from different anatomical regions. Sampling was performed from rabbit carcasses immediately after evisceration to prevent further contamination due to improper transport and incomplete processing of carcasses (carcasses left on the ground, unventilated, slowly cooled to room temperature, unskinned).

The area included in the study was represented by hunting grounds (authorized hunting grounds) in the south and southeast of the country. The collection of samples was mostly carried out by the authors, but considering the restrictive character of the hunters, a number of samples were taken directly from the hunted parts provided by the hunters, who were previously advised on the technical conditions necessary for the correct collection. The samples were taken between 2019-2023 and totaled a total of 3,135 samples. The number of samples processed annually and their presentation method are described in Table 1.

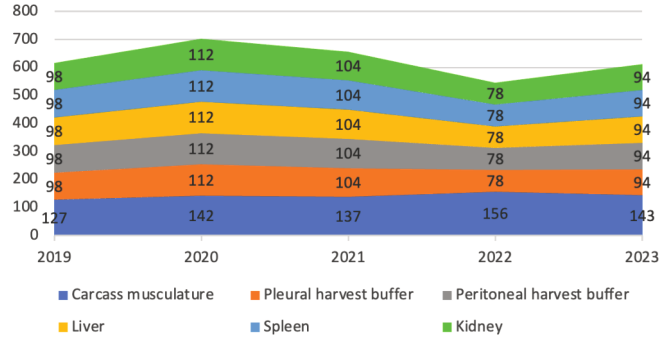

Fig. 1. Distribution of annual harvested types of samples

Sample processing was performed using classic method and standardised ISO methods for the diag-

Table 1

Identifying the types of samples harvested annually

Samples	2019	2020	2021	2022	2023	Entire
Carcass	127	142	137	156	143	**705**
Pleural harvest	98	112	104	78	94	**486**
Peritoneal harvest	98	112	104	78	94	**486**
Liver	98	112	104	78	94	**486**
Spleen	98	112	104	78	94	**486**
Kidney	98	112	104	78	94	**486**
Total samples collected	**617**	**702**	**657**	**546**	**613**	**3,135**

Table 2

The analysed microbiological parameters from sanitation buffer and muscle samples

The year of Report	Microbiological parameter investigated (expression / g sample)				
	NTG	Unsporulated	Sporulated	Total No. of	Total No. of
2019	1.9×10^7	1.0×10^2	2.0×10^5	6.0×10^1	3.5×10^2
2020	2.5×10^7	1.0×10^3	1.5×10^4	4.0×10^1	3.0×10^2
2021	1.5×10^8	7.0×10^2	2.4×10^5	4.5×10^2	2.5×10^3
2022	2.1×10^7	1.0×10^2	1.4×10^6	8.0×10^1	5.6×10^2
2023	1.7×10^8	5.0×10^2	5.4×10^4	5.5×10^2	2.5×10^3
Entire	**7.7×10^7**	**4.8×10^2**	**3.8×10^5**	**2.36×10^2**	**1.25×10^3**

Table 3

The isolation frequency of unsporulated bacterial species from hare case samples

Bacterial genus (species)	Isolation frequency per period (%)					
	2019	**2020**	**2021**	**2022**	**2023**	**Entire period**
Salmonella spp.	6.37	4.81	5.99	5.63	4.91	**5.53**
Escherichia coli	6.48	7.02	4.25	7.57	5.92	**6.24**
Staphylococcus spp.	7.28	7.01	7.56	8.24	8.92	**7.81**
Listeria	1.95	1.44	1.34	2.56	1.64	**1.78**
Pseudomonas spp.	0.24	1.82	2.08	1.76	1.21	**1.43**
Campylobacter spp.	1.49	1.52	0.97	1.08	0.87	**1.18**
Yersinia spp.	0	0.24	0	0	0.45	**0.15**
Shigella spp.	0	0.22	1.02	0.78	0.4	**0.47**
Proteus spp.	2.17	2.98	3.08	2.54	3.22	**2.82**

nosis of isolated bacterial species (23). Classical methods of pre-enrichment, enrichment, growth, isolation, and identification on culture media were used, and API galleries were used to identify and confirm the species isolated from some samples. For some samples, RT-PCR confirmations were also made to establish the species. In general, it was desired to identify the genus in order to ascertain the level of microbiological pollution.

RESULTS AND DISCUSSIONS

Statistical analysis of laboratory studies performed on a total of 3,135 samples demonstrated the presence of a very high total bacterial load (TBC) (Table 2), a very high number of sporulated or non-sporulated bacteria, as well as a particularly high yeast load and mice. Knowing the particular method of evisceration, most of the microorganisms certainly come from the soil due to the processing of game obtained in improper conditions (19-21).

Among the pathogenic or conditionally pathogenic bacteria, species belonging to the following genera were identified: *Salmonella spp*., *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter spp*., *Staphylococcus spp*. (coagulase-positive), *Shigella spp*., *Yersinia spp*., *Proteus spp*., *Pseudomonas spp*., *Bacillus spp*., and *Clostridium perfringens*. Their annual isolation frequency is shown in Tables 3 and 4.

To date, studies describing the gastrointestinal microbiota have focused mainly on the European hare (*Lepus europaeus*) (18). Being an economical game species, both fur and meat can be valued (7). In addition to the revenue generated directly from the animals, a significant amount of money flows back into the economy through hunting fees and expenses on hunting equipment, firearms, and ammunition (1, 9). In recent years, while fur demand has decreased, scientists have discovered that hare meat is nutritionally valuable for human consumption, offering nutritional benefits superior to the meat of farmed or other wild species of birds and mammals (11-16).

The obtained results demonstrate a very high total bacterial load and the presence of some very dangerous bacterial species for human health. The use of game carcasses in the preparation of culinary preparations must consider the use of those processes that involve high temperatures and have sufficient duration of action to effectively destroy the bacteria present both on the surface and in the depth of the product. Based on the same considerations, advanced ripening must be carried out for limited periods of time and at ambient temperatures that do not allow the excessive multiplication of pathogenic and conditionally pathoge-

Table 4

The isolation frequency of sporulated bacterial species and fungi from hare case samples

Microorganism group	Isolation frequency per period (%)					
	2019	2020	2021	2022	2023	Entire
***Bacilus* spp.**	2.57	2.72	1.98	2.78	3.24	**2.67**
***Clostridium* spp.**	5.78	5.17	1.84	4.94	6.28	**4.82**
***Penicillium* spp.**	18.78	17.49	16.54	18.24	15.28	**17.24**
***Aspegillus* spp.**	14.67	16.98	15.78	17.58	12.94	**15.57**
***Mucor* spp.**	2.54	3.78	1.29	2.47	4.58	**2.94**
***Alternaria* spp.**	1.24	2.01	1.79	1.16	2.01	**1.65**
***Rhizopus* spp.**	2.06	1.97	2.12	2.46	1.19	**1.95**
***Candida* spp.**	6.24	6.02	4.58	7.28	5.47	**5.93**

nic bacteria. Before being consumed, portions of cured meat must be prepared by culinary procedures that include thermal treatments at temperatures and for periods of time that ensure the destruction of bacteria in the greatest possible proportion (19, 20).

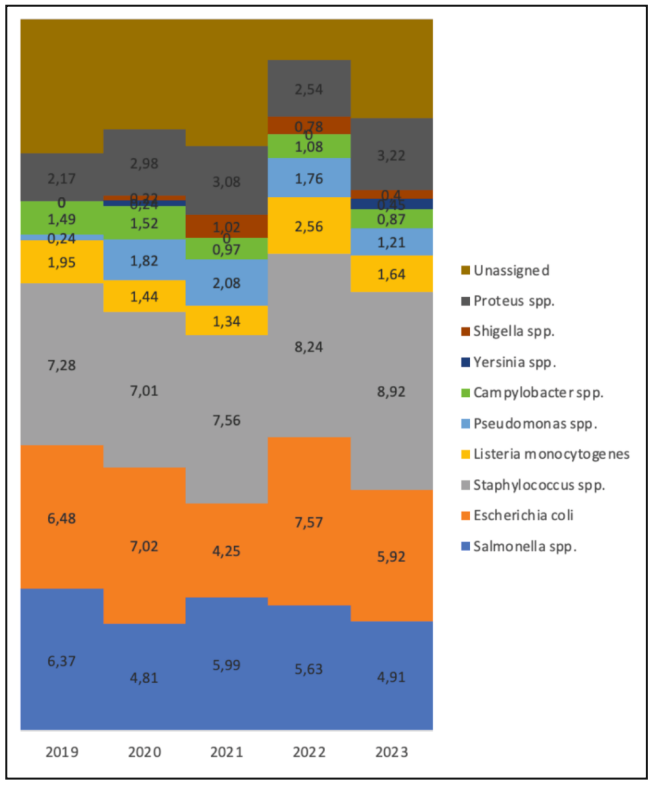

Fig. 2. Isolation frequency period

CONCLUSIONS

Microbiological analysis of the samples taken from the carcasses of wild rabbits showed a very high total bacterial load (TNG): from 2.1 x 107 to 1.7 x 108 CFU / g sample. Of the total number of bacteria, the majority were sporulated bacteria (3.8 x 105) and a smaller number of non-sporulated bacteria (4.8 x 102). Following the isolation and identification examinations, the presence of some pathogenic or conditionally pathogenic bacterial species was found, some having a relatively high isolation frequency: *Salmonella sp*. – 5.53%; *Escherichia coli* – 6.24%; *Staphylococcus sp.* – 7.81%; and *Proteus sp*. – 2.82%. Some bacterial species, although they were isolated in a relatively small proportion (*Pseudomonas sp.* – 1.43%; *Listeria monocytogenes* – 1.78%; *Campylobacter sp.* – 1.18%; *Shigella sp.* – 0.47%; *Yersinia sp.* – 0.15%), are of major importance due to their transmissibility to humans and the increased frequency of food illness. Moulds and yeasts were isolated in a large proportion, through the high isolation frequency of some pathogenic species, significantly increasing the hygienic risk posed by game meat obtained, transported, and stored in improper conditions.

REFERENCES

1. *Arnett E.B., Southwick R.,* (2015), Economic and social benefits of hunting in North America. International Journal of Environmental Studies, 72(5): 734-745
2. *Bărzoi D.,* (1985), Microbiologia produselor alimentare de origine animală [Microbiology of food products of animal origin] (in Romanian), (Ed.) Ceres, Bucharest, Romania
3. *Bărzoi D., Meica S.,Neguţ M.,* (2022), Toxiinfecţiile alimentare [Food poisoning] (in Romanian), (Ed.) Diacon Coresi, Bucharest, Romania
4. *Beatrice A.M., Bogdan S.C., Tiana F., Ionela H., Gheorghe D.,* (2022), Endoparasites found in lepus europaeus hunted in western Romania. International Multidisciplinary Scientific GeoConference Surveying Geology and Mining Ecology Management, SGEM, 22(6.2):691–697
5. *Ciocârlie N., Tudor L., Ceauşi C.,* (2002), Controlul calităţii cărnii [Meat quality control] (in Roma-

nian), (Ed.) Printech, Bucharest, Romania
6. *Chapman J.A., Flux J.E.C.*, (1990), Rabbits, Hares and Pikas Status Survey and Action Plan, (Ed.) IUCN, Gland, Switzerland
7. *Cotta V.,* (1956), Economia vanatului si salmonicultura, (Ed.) Agro-Silvică de Stat, Bucharest, Romania
8. *Cotuțiu V.D., Mihalca A.D., Hołówka K.A., Ionică A.M., Cazan C.D., Gherman C.M.,* (2022), European Hares, *Lepus europaeus*, Represent a Reservoir Host for *Thelazia callipaeda* in Romania. Pathogens, 11(11):1225
9. *Dickson B., Hutton J., Adams W.M.,* (2008), Recreational Hunting, Conservation and Rural Livelihoods: Science and Practice. Recreational Hunting, Conservation and Rural Livelihoods: Science and Practice, (Ed.) Blackwell Publishing Ltd, Hoboken, New Jersey, USA
10. *Dronca D., Păcală N., Bencsik I., Popa R., Ivancea M., Dumitrescu G., Matiuti M., Marcu A., Cean A., Boca L.,* (2011), Estimation of correlation between the number of individuals and biogenic capacity of the hunting terrain 56, forest district Lunca Timişului, for 13 hunting species in 2006-2010 period. Scientific Papers: Animal Science and Biotechnologies, 1:44
11. *Frunză G., Murariu O.C., Ciobanu M.M., Radu-Rusu R.M., Simeanu D., Boișteanu P.C.,* (2023), Meat quality in rabbit (*Oryctolagus cuniculus*) and Hare (*Lepus europaeus* Pallas)—a nutritional and technological perspective. Agriculture, 13(1):126
12. *Hacklander K., Schai-Braun S.,* (2019), *Lepus europaeus* (European Hare). The IUCN Red List of Threatened Species 2019: E.T41280A45187424, (Ed.) IUCN, Gland, Switzerland
13. *Mertin D., Slamečka J., Ondruška Ľ., Zaujec K., Jurčík R., Gašparík J.,* (2012), Comparison of meat quality between European brown hare and domestic rabbit. Slovak Journal of Animal Science, 45 (3):89-95
14. *Nesterov V.,* (1984), Bolile vânatului [Diseases of game animals] (in Romanian), (Ed.) Ceres, Bucharest, Romania
15. *Popa G., Stănescu V.,* (1981), Controlul sanitar veterinar al produselor de origine animală, (Ed.) Didactică şi pedagogică, Bucharest, Romania
16. *Popescu N., Meica S.*, (1995), Bazele controlului sanitar veterinar al produselor de origine animală, (Ed.) Diacon Coresi, Bucharest, Romania
17. *Smith R.K., Jennings N.V., Harris S.,* (2005), A quantitative analysis of the abundance and demography of European hares *Lepus europaeus* in relation to habitat type, intensity of agriculture and climate. Mammal Review, 35(1):1-24
18. *Stalder G.L., Pinior B., Zwirzitz B., Loncaric I., Jakupović D., Vetter S.G., Smith S., Posautz A., Hoelzl F., Wagner M., Hoffmann D., Kübber-Heiss A., Mann E.,* (2019), Gut microbiota of the European Brown Hare (*Lepus europaeus*). Scientific Reports 9(1):1-15
19. *Tudor L.,* (2005), Controlul sanitar veterinar şi tehnologia produselor de origine animală, (Ed.) Printech, Bucharest, Romania
20. *Tudor, L., Ciocarlie N.,* (2022), Inspecţia şi controlul produselor alimentare de origine animală [Inspection and control of food products of animal origin] (in Romanian), (Ed.) Carol Davila, Bucharest, Romania
21. *Tudor L., Ciocirlie N., Tudor A.L., Mitranescu E.,* (2015), The prevalence of *Yersinia* species in slaughtered pigs, Journal of Biotechnology, 208 (Suppl):S75-S75
22. ***, (2023), Efective, Ministerul Mediului, Available at: https://www.mmediu.ro/categorie/efective/292 (Accesed: February 13, 2024)
23. ***, (1995), ISO. Recommendation of the Meeting of the Subcommittee, International Organization for Standardization on Meat and Meat Products. ISO/TC-36/SC 6.10-18. Geneva, Switzerland.