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TULAREMIA IN EUROPEAN BROWN HARE IN THE WESTERN PART OF FYROM MACEDONIA

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ABSTRACT

Tularemia is a communicable bacterial disease which affects both humans and animals. Recently, there are reported severe human cases in Kosovo, Montenegro, the Northern part of Albania and Macedonia. The European brown hare (Lepus europaeus) plays an important role as reservoirs of Francisella tularensis infection. In order to study the prevalence of F. tularensis infection we designed a longitudinal study in hares based on both agglutination test and histopathological results. In this study, we report the preliminary results based on tested samples submitted during 2014-2015 and Spring 2016. Samples are taken from different villages of Fyrom Macedonia such as: Debresh, Nerove, Allbance, Presille, Bellushine, Haracine, Tearce etc..

The serologic test by using F. tularensis antigen was performed at the infectious & wildlife diseases laboratories, Faculty of Veterinary Medicine of Tirana. In addition, we described gross lesions in suspected cases. 14 out of 127 samples were positive in agglutination test Presence of granulomatous inflammation was used as an indicator of F. tularensis infection. Further samples are being analyzed and advanced tests will be used in order to identify the most proper and affordable screening and confirmatory tests for monitoring the epidemiological situation of F. tularensis infection. Despite the serologic test, postmortem examination, Histological examination were also realized. We are in the process of realizing histological examination and bacteria isolation.

INTRODUCTION

It is well known that, Francisella tularensis, the cause of the life-threatening zoonotic disease tularemia, has been common since 1912, when McCoy and Chapin identified it during an outbreak of a ‘‘plague-like’’ disease of ground squirrels in Tulare County, California. There are three subspecies of F tularensis that we recognize: the highly virulent F tularensis ssp tularensis and the moderately virulent tularensis ssp holarctica and F tularensis ssp mediasiatica. F tularensis ssp tularensis has been almost found in North America; however, several isolates of this subspecies were obtained in the 1980s from ticks in Slovakia. F tularensisssp holarctica is found throughout the northern hemisphere, whereas the distribution of F tularensis ssp mediasiatica is restricted to Central Asia8. The broad host range of F tularensis comprises primarily mammalian species and, to a lesser extent, birds, amphibians, and fish and even invertebrates. Lagomorphs, along with rodents, are generally known as the main reservoirs of F tularensis, whereas hematophagous arthropods have a substantial role in disease transmission1. In the New World, the cottontail rabbit (Sylvilagus spp), the black-tailed jackrabbit (Lepus californicus), and the snowshoe hare (Lepus americanus) are important in the ecology of tularemia. The European brown hare (Lepus europaeus), the mountain hare (Lepus timidus), and the Japanese hare (Lepus brachyurus) are the lagomorphs associated with tularemia in the Old World. The European wild rabbit (Oryctolagus cuniculus) and, thus, the domestic rabbit are both relatively resistant to tularemia. Several causes in which human infections appear are [1]. From injuries during the skinning of infected lagomorphs (European brown

hare) and [2]. Bites from infected ticks[2]. An individual may also become infected after inhaling the agent, eating inadequately cooked contaminated meat, or drinking water from public water supplies contaminated by *F tularensis* originating from infected carcasses. *F tularensis* is a highly infectious agent; as few as 10 bacteria can infect animals and humans; and it has been placed on the list of class A biothreat agents[7]

The European brown hare is reported to be a common reservoir of tularemia, and its public health importance is accentuated by being one of the most significant European game species. Despite the significant role of these lagomorphs in the ecology of tularemia and its importance as a human infection source, no report has been published so far about the pathological and microbiological examination of naturally infected European brown hares. The aim of the present study was to identify the gross and histological lesions characteristic for *F tularensis* infection of European brown hares9. This essential information can make it easy for both the detection of tularemia in brown hares by hunters and the recognition and identification of this dangerous zoonotic disease by diagnosticians[4].

MATERIAL AND METHOD

Samples: Postmortem Examination

European brown hares, shot at different locations in FYROM Macedonia during several hunting events over two winter hunting seasons (2014–2015 and 2015–2016), were screened by the slide agglutination test using stained bacteria (Bioveta Inc,) and whole blood. 14 animals were found to be seropositive and were thus used for laboratory examinations.

Carcasses of 2 dead seropositive adult male hares submitted for diagnostic examination were included in the study, so 14 seropositive animals were examined in total. Tissue samples of 113 seronegative hares were collected and thus served as negative controls. All carcasses were necropsied under appropriate biosafety conditions at the Lab of Infection Diseases and Lab Wildlife Diseases in Faculty of Veterinary Medicine were categorized as same-year juveniles and older, based on the so-called Stroh mark.5 The body condition was estimated with a simplified categorical (good, moderate, weak) version of the kidney fat index.5 Tissue samples were collected for histologyand fixed in 10% buffered formalin: The same tissue samples of 113 seronegative hares served as negative controls. Fourmicron- thick sections of formalin-fixed, paraffin-embedded tissue samples were stained with hematoxylin and eosin and examined by light microscopy.

Histological Examination

The foci identified by gross pathological examination corresponded to focal or coalescing granulomatous inflammation, which completely replaced the normal tissue structure of the affected organs. These foci were distributed in the organs, and serosal membranes were frequently involved. Macrophages were the dominant constituent cell type, but other cells were found occasionally, including lymphocytes, heterophil, granulocytes, multinucleated giant cells, and fibrocytes (Fig. 1). Focal or multifocal necrosis was often observed in the center of these lesions (Fig. 1).

RESULTS AND DISCUSSIONS

The serologic test by using F. tularensis antigen was performed at the infectious & wildlife diseases laboratories, Faculty of Veterinary Medicine of Tirana. In addition, we

described gross lesions in suspected cases. 14 out of 127 samples were positive in agglutination test

Table 1

Period of samples collected and results.

Nr	Period of samples collected	Nr of Samples	Negative samples	Positive samples
1	2014-2015 year (April –June)	34	32	2
2	2014-2015(September- October)	53	50	3
3	2016 spring	40	31	9
3	**Total**	**127**	**113**	**14**

All carcasses were necropsied under appropriate biosafety conditions at the Lab of Infection Diseases and Lab Wildlife Diseases in Faculty of Veterinary Medicine were categorized as same-year juveniles and older, based on the so-called Stroh mark.5 for more information, check the results presented in the table. From two out of Fourteen positive samples we estimated gross pathological lesions for the organs: lungs, pericardia, kidneys, bone marrow.

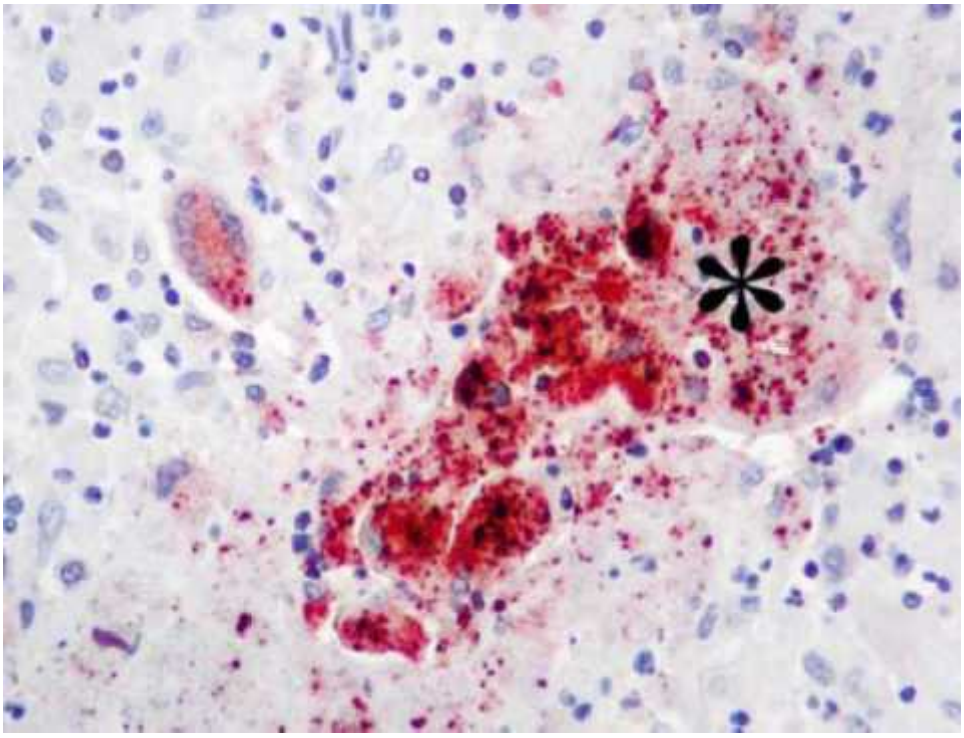
Table 2

Cases With Gross Pathological Lesions in the Organs of Seropositive European Brown Hares.

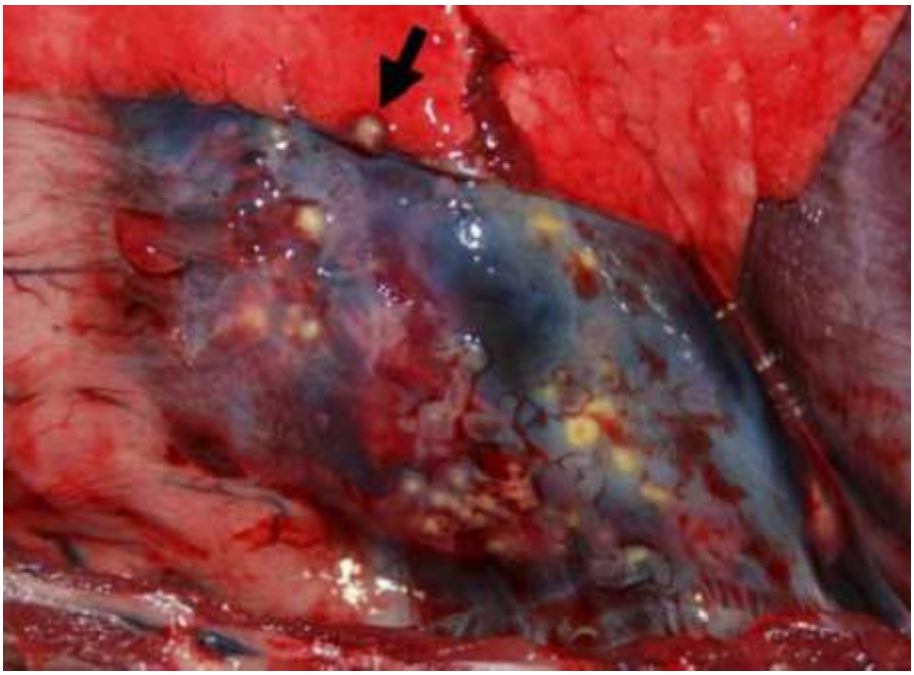
No	Organ	Number of case with gross pathological	Number of case with no gross pathological
1	Lung	8	6
2	Pericardia	10	4
3	Kidneys	12	2
4	Testicles	7	7
5	Bone marrow	4	10
6	Liver	8	6
	Total		**14 (127)**

Histological Examination

The foci identified by pathological examination corresponded to focal or coalescing granulomatous inflammation, which completely replaced the normal tissue structure of the affected organs. After that, the foci were randomly distributed in the organs, and serosal membranes were frequently involved. Macrophages were the dominant constituent cell type, but other cells were found occasionally, including lymphocytes, heterophil granulocytes, multinucleated giant cells, and fibrocytes Figure 1 Granulomatous inflammation was found with microscopic examination but not with gross pathological examination in the mediastinal lymph nodes7. In several cases, the coalescing granulomatous inflammation in the lungs contained no or only minor necrotic areas. Foci of granulomatous inflammation with central necrosis were found in the liver, bone marrow, mammary gland, spleen, and mediastinal lymph nodes.



Lung; European brown hare.



Pericardium and lung.

CONCLUSIONS

This study shows for the first time results related to the presence of Tularemia in European Brown hares in FYROM-Macedonia. This study certifies (through serological method) Tularemia presence in the population of European brown hare in FYROM- Macedonia. The study is quickly continuing and will also apply the methods of Immunohistochemical Examination and Isolation and Identification of Bacteria. Serological methods are used for the diagnosis of tularemia in humans, and they can be applied in animals. Serology has

limited use in highly susceptible species of animals, which usually die before specific antibodies develop6. The study serves as a good basis to realize other achievements as well. It covers the areas near the border of Kosovo, in which Tularemia has been present years ago. There are also plenty of cases in humans. The study can also be used as a good basis in order to run other studies in the whole population of lagomorphs.

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