RESEARCH ARTICLE **(Open Access)**

The identification of Tularemia agent in tissues of wild hare flesh with application of immunohistochemical avidin-biotin complex method (IMHC-ABC)

BESNIK ELEZI[1*], KASTRIOT KORRO[2]

[1] PhD candidate, Faculty of Veterinary Medicine, Agricultural University of Tirana Agricultural University of Tirana

[2] Faculty of Veterinary Medicine, Agricultural University of Tirana Agricultural University of Tirana

*Corresponding author; E-mail: besnikelezi1972@gmail.com

Abstract

The study presents the results achieved from the application of the immunohistochemical avidin-biotin complex method (IMHC-ABC) or the assessment of the risk of the presence of the Tularemia antigen in the flesh of hares haunted by the hunters in some of the regions of Macedonia, the ex-republic of Yugoslavia. The study indicates the method applied in 70 samples taken from the hares killed by the hunters, as well as some samples taken from the restaurants which offer in their menu. The study results with the identification of 4 positive cases with Tularemia from the samples submitted to this check. The check is significant, as it presents a method of assessment of the risk of the meat of the hares with the presence of this zoonotic disease, as well as it exposes a method which has not been applied before for this disease in the region.

Keywords: Immunohistochemical, avidin-biotin method, tularemia, FYROM-Macedonia, IMHC.

1. Introduction

Tularemia is a cyclic infection which affects the animals and the humans [2, 3, 4, 5]. The people get the infection from the animals with bacteremia and toxicemia which is often developed in very severe form. The infection is caused by Francisella tularensis. In the year 1999, in Kosovo were observed about 250 cases with tularemia in humans [1]. The infection was diagnosed in the Institute of Veterinary Research "Bilal Golemi" in Tiranë through the serological test [1, 3, 9, 10] . This was the first case of infection diagnosis in our country in humans. During this period, in our country studies were made in humans who were suspected for its presence. From these checks the infection was diagnosed only in Hasi region. Since from the year 1999, in Albania are not observed suspicious cases of infection either in humans or in wild rodents [3, 7, 8]. From our study during the years 2015-2016 for the diagnosis of Tularemia in wild hares of the western part of Macedonia, we have identified 47 positive cases for the presence of tularemia b means of the serological method [3, 4, 5]. Tularemia is found at least 110 different kinds of animals, 25 kinds of birds and a few kinds of fish. It is found in hard-bodied ticks of the genus *Ixodes*, *Dermacentor*, *Haemophysalis* species, etc, it is also seen in arthropods, in mites and in the flies [1, 10]. They find shelter in the mites, and fleas and they get transported. Rodents, such as the water voles (*Arvicola terrestris*), hares etc, as well as some carnivore insects, are very sensitive towards the infection and they are severely infected [3, 4, 9]. Biovar A is met at lagomorphs, whereas holoarctic biovar B is met mostly at small rodents. Furthermore sensitive but less affected are the field rats (*Microbus arvalis*, *Apodemus-Arten*), hamsters, hares, eagles, etc and from the domesticated animals domestic sheep and hares. Perissodactyls as well as carnivores are less sensitive, but they get affected more than minks and foxes [3, 8]. The animals with very high sensitivity are the 1st grade reservoirs and epizootic before all, contagious for the humans and they create infective foci [1, 5, 7].

2. Material and Methods

For the realization of this study were taken 70 samples from liver, lymph nodes and the lung of the postmortem hares [3, 7].

2.1. The immunohistochemical staining Avidin-Biotin Complex (IHC- ABC) method

The immunohistochemical avidin-biotin complex method (IMHC-ABC), was applied for the demonstration of the lipopolysaccharide antigen of *F. tularensis* in tissue sections. Immediately after the antigen deparafinisation and extraction (in an microwave oven in 750 W for 20 minutes in citrate tampon, pH 6,0), the lesions were incubated in solution 3% H_2O_2 for 10 minutes in pH 6,0 and then in a solution of 2% skimmed milk for 20 minutes[3, 7]. The samples were incubated during the night in 370 C, with a proportion 1: 6000 of the dilution of F tularensis [3, 4, 7, 8]. Lipopolysaccharide with the monoclonal-specific antibodies produced in rats (clones FB11 and T14,MAB8267, Chemicon International Inc, Southhampton, UK) the fusion of antibodies were detected by means of peroxidase reaction and use of specific substrate, a reaction which is exposed with dark brown colour (EnVisionb Kit, Dako, Glostrup, Denmark) [3, 4, 6, 7]. A series of lesions were incubated with phosphate buffer solution and it will be used as a negative control [3, 7]. We used the immunohistochemical avidin-biotin complex method (IMHC-ABC) which was realized on the formalin-fixed tissues respecting the association protocol of the kit [2, 6].

3. Results and Discussion

During our research 70 samples were collected from the tissues of the hares killed by the hunters of Macedonia in the regions of Debresh, Nerove, Allbance, Presille, Bellushine, Haracine, Tearce etc., as well as from the shops and the restaurants which served the hare meat in their menu [1, 3, 10]. The number of collected samples was considerable considering that the population of the above areas does not prefer the consummation of wild hare meat [1, 3]. The collected samples were sent in the Lab of the Wildlife Disease at the Faculty of Veterinary Medicine, in which were realized 70 formalin-fixed lesions and we followed the protocol associating the kit [1, 3, 9, 10].

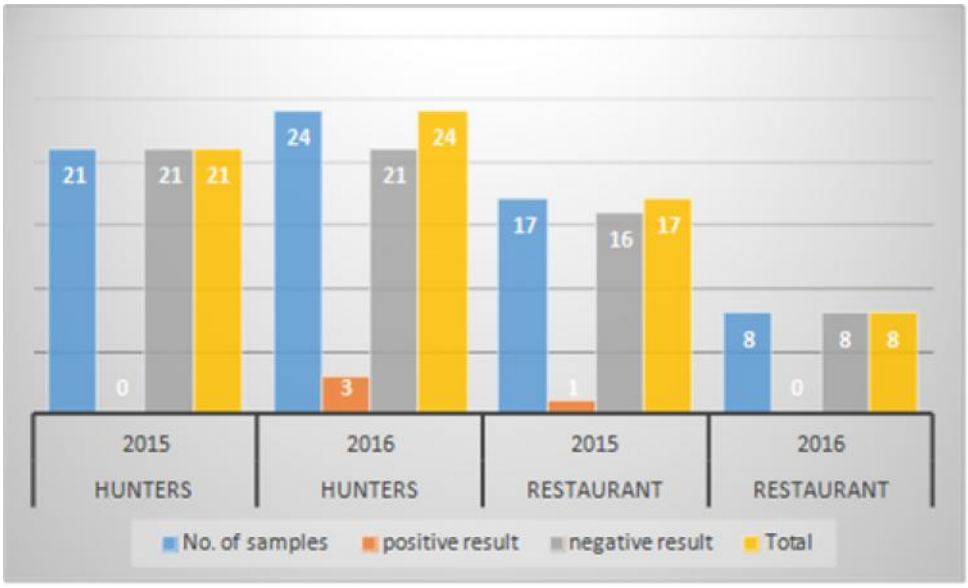

Figure 1. The results acquired from this study.

Figure 2. Region of samples collected.

4. Conclusions

1. Application of the (IHC- ABC) method I discover cases with presence of Tularemia antigen in the liver (1 case), in lymph node 2 cases and in the lung 1 case.
2. Application of the (IHC- ABC) method increases the detection, diagnostic sensitivity and the level of assurance for the consumer concerning the meat of the hare with origin from the wild populations.
3. The results of the method encourage the researchers to apply this method also in other studies on this disease, as an important part in the confirmation of Tularemia antigen.

5. Acknowledgements

We want to thank Associate of Hunters of FYROM Macedonia and staff of Wildlife lab in Faculty of Veterinary Medicine, Agricultural University of Tirana.

6. References

1. Berxholi K: **Zoonozat Bakteriale, Virale dhe parazitare**: Tirane; 2015.
2. DSMZ: **Bacterial Nomenclature Up-to-Date** (Approved List,Validation List). http://www.dsmz.de/microorganisms/bacterial_ nomenclature.php. Accessed September 2009.
3. Elezi B.: **Tularemia under Histological and Immunohistochemistry Examinations**. Anglisticum Jurnal Mk., In press.
4. Friend M: **Tularemia: Circular 1297**. U.S. Geological Survey, Reston, VA, 2006.
5. Gill V, Cunha, BA.: **Tularemia pneumonia.** Semin Respir Infect 1997, **12**:61–67.
6. Greco D, Allegrini G, Tizzi T, Ninu E, Lamanna A, Luzi S: **A waterborne tularemia outbreak.** Eur J Epidemiol **3**:35–38, 1987.

7. Gyuranecz M, Szeredi L, L. Makrai L, Fodor A, Meszaros R, Szepe B, Fuleki M, and. Erdelyi K: **Tularemia of European Brown Hare (Lepus europaeus): A Pathological, Histopathological, and Immunohistochemical Study**. Veterinary Pathology **47**(5) 958-963 http://vet.sagepub.com

8. Gyuranecz M, Fodor L, Makrai L, Szoke I, Janosi K, Krisztalovics K, Erdelyi K**: Generalized tularemia in a vervet monkey (Chlorocebus aethiops) and a patas monkey (Erythrocebus patas) in a zoo.** J Vet Diagn Invest **21**:384–387, 2009.

9. Koleci Xh: **Semundjet infektive**: Tirane; 2009.

10. Korro K, Keci R, Berxholi K: **Semundjet e kafsheve te Egra**: Tirane; 2013.