TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2024, vol. 9, No 1(16): 3–8

SEROPREVALENCE AGAINST MYXOMA VIRUS IN *LEPUS EUROPAEUS* FROM BULGARIA

Iliyan Manev, Krasimira Genova

University of Forestry, Faculty of Veterinary Medicine, Sofia, Bulgaria
E-mail: doc_man08@abv.bg
ORCID: 0000-0001-7733-4840 I.M. 0000-0002-5599-7931 K.G.
(Submitted: 4 May 2023; Accepted: 9 November 2023; Published: 25 June 2024)

ABSTRACT

Myxoma virus (MYXV) is a poxvirus which causes a systemic infection in wild and farm rabbits (Oryctolagus cuniculus) with high morbidity and mortality, called myxomatosis. European hare (Lepus europaeus) was considered to be resistant to the virus. However in the recent years a cross-species jump of MYXV in hare species from different parts of Europe was recognized. The aim of the present survey was to determine the antibody prevalence against myxoma virus in brown hare populations from different parts of Bulgaria with the emphasis to establish the possible circulation of myxomatosis in these communities. From all tested sera (via cELISA) 7.14% (6/84) were positive for specific antibodies against MYXV. All of them were sampled from Kameno, Burgas region (South Bulgaria). The positive cohort represented 10.0% (6/60). No positive cases from the other sampled area were determined. Based on this we could conclude that the studied brown hare populations were severely resistant to myxomatosis and no evidence for significant MYXV circulation was determined. However the lack of specific antibodies cannot exclude emphatically myxomatosis morbidity in brown hare.

Key words: *Lepus europaeus*, myxomatosis, seroprevalence, Bulgaria.

Introduction

Myxomatosis is an infection caused by the myxoma virus (MYXV), belonging to genus *Leporipoxvirus*, subfamily *Chordopoxvirinae* and family *Poxviridae* (Barrett and McFadden, 2007; OIE, 2018). The MYXV leads to development of a global pandemic with massive mortality in rabbit populations *(Oryctolagus cuniculus)*, especially in Australia, France and United Kingdom (Wilkinson, 2001; Kerr et al., 2012; Kerr et al., 2015). However, a rapid evolution of genetic resistance to the disease was achieved (Alves et al., 2019). The disease is characterized by multiple firm subcutaneous nodular masses at the head, back and limbs (Marinković et al., 2018). Since its intentional introduction in Europe in 1952, myxomatosis became endemic in European rabbit populations (Villafuerte et al., 2017). The natural hosts of the virus are some lagomorphs from South and North America (*Sylvilagus brasiliensis* and *Sylvilagus bachmani*) (Regnery, 1971). Hare species were considered to be more resistant to myxomatosis (Wibbelt and Frölich, 2005). Recently, a new cross-species jump of MYXV has been suggested in both Great Britain and Spain, where European brown hare (*Lepus europaeus*) and Iberian hare individuals (*Lepus granatensis*) were found dead with lesions consistent with those observed in myxomatosis (Kerr et al., 2015; Agueda-Pinto et al., 2019). Myxomatosis was also sporadically reported in mountain hare (*Lepus timidus)* (Saari et al., 2005).

European hare (*Lepus europaeus, Pallas, 1778*) is the most representative wild lagomorphs in terms of abundance and hunting interest in Bulgaria. However considerable decline trend in hare population density was established which was attributed to multiply factors (Zhelev et al., 2013; Zhelev, 2015).

The lack of serological surveillance induces uncertainty about the possible MYXV circulation in hare populations, eventual subclinical course of the disease and carrier state. Based on this the current study was the first attempt to investigate the antibody prevalence against MYXV in brown hare populations from Bulgaria.

Materials and Methods

Animals and sampling area

Different studied areas were chosen based on field observations of the hare population density – Kameno, Burgas region (South Bulgaria) (n=60), Iskra, Silistra region (North Bulgaria) (n=24), total number of 84. The studied regions were selected based on the available data for higher hare population density (5 to 8 animals/100 ha) (Zhelev, 2015).

All tested hares were without clinical and pathological signs of myxomatosis.

Sample collection and handling

Blood samples were obtained by heart tap immediately after shooting. Blood was left to clot, stored at 4–6°C in thermal bag and transported to the laboratory. The blood serum was separated using centrifugation at 3000 rpm for 20 minutes, transferred in different tubes and stored at -20 °C until analysis.

Hares were hunted according to the Bulgarian and European legislation. Thus, no animal was sacrificed for the purpose of this study.

Serological testing

Antibodies specific for MYXV were detected by a commercial competitive enzyme-linked immunosorbent assay (cELISA) developed in Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Brescia, Italy (Lavazza et al., 2004). This method is based on the use of a monoclonal antiboby (mAb) (1E5) that recognises the myxoma virus immunodominant envelope protein (M071L). The test antigen was myxoma virus strain California grown on RK13 cell culture. A serum is considered **negative** when the absorbance value A492 of the first dilution (1/10) decreases by less than 15% of the reference value (negative serum 1/10), while it is **positive** when decreases by more than 25%. The sera were tested at only one dilution (1/10 – negative or positive result).

Results and Discussion

The summarized results from the studied hare population were presented in Fig.1. From the total 84 hare sera 6 samples (7.14%) were positive for specific antibodies against MYXV. All of them were sampled from Kameno (South Bulgaria). The positive cohort represented 10.0% (6/60) of all tested animals from this region.

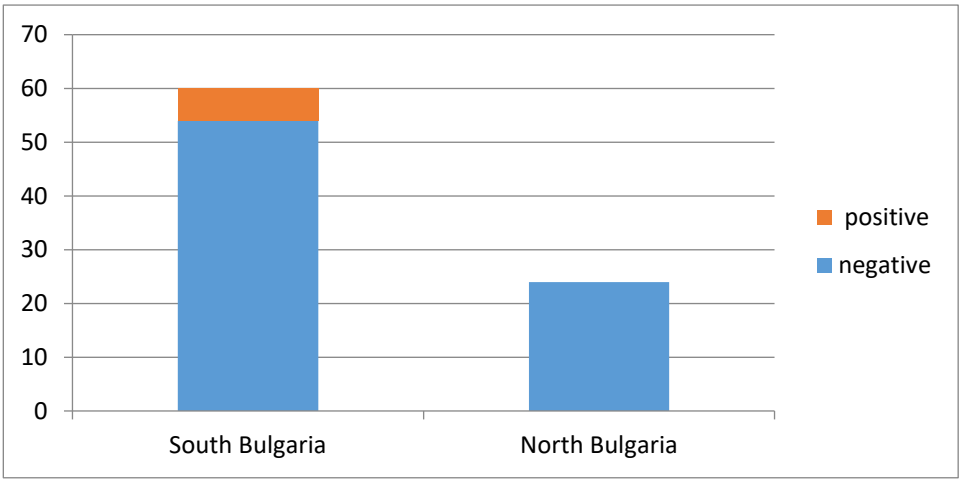

Figure 1: Serological results of hare sera for anti-MYXV antibodies from the tested regions

The obtained results could indicate a possible myxoma virus circulation in this area. However based on the low number of positive individuals in the current population and lack of seropravalence in the other tested hare community could be assumed that the myxomatosis was not a significant infectious threat.

According to Espinosa et al. (2020) the main causes for mortality in wild rabbits and hare were parasitic conditions (n = 65; 24.34%), bacterial diseases (n = 56; 20.97%), nutritional and metabolic disorders (n= 48; 17.97%), viral infections (n = 31; 11.61%), miscellaneous causes (n = 31; 11.61%), neoplasms (n = 12; 4.49%), toxicoses (n = 11; 4.11%), trauma-related injuries (n = 9; 3.37%) and congenital diseases (n = 4; 1.49%). The cited statistics illustrated the significant impact of viral diseases in hare pathology. However similar statistics for lepus populations in Bulgaria is not currently available. To our knowledge no epidemiological information from the investigated area for detection and circulation of virus infections in Lepus spp. was published.

Worldwide, the confirmed cases of hare myxomatosis have been few in number and usually sporadic. The first attempt for experimental inoculation in 1937 of wild-caught brown hares in Australia using virulent myxoma virus from rabbits failed to elicit disease (Bull and Dickinson, 1937). Myxomatosis has rarely been reported in the European brown hare. Few cases have been sporadically reported in France (Lucas et al., 1953; Jacotot et al., 1954), Ireland (Collins, 1955; Whitty, 1955). Some of them were somewhat speculative and unclear in their conclusions and finally the case report from UK was confirmed by PCR (Barlow et al., 2014).

Iberian hare (*Lepus granatensis*), which is comparable as a biology and immunology to Lepus europaeus, was long considered to be resistant to myxomatosis as no prior cases were reported. However in 2018 myxomatosis outbreaks in Spain and in 2020 in Portugal were confirmed (Abade dos Santos et al., 2020b; Carvalho et al., 2020; García-Bocanegra et al., 2020). Furthermore the tested sera samples from the years 1994-1999 and 2017-2019, demonstrated the circulation of MYXV in the Iberian hare for at least 20 years before this severe virus outbreak. Antibodies were detected in 12.6% (57/451) of the hares tested, of which 40.3% (23/57) were also qPCR positive. These conclusions were in accordance with Garcia-Bocanegra et al. (2019) which also reported the presence of MYXV antibodies in Iberian hare population and postulated a theory of enduring circu-

lation of MYXV, or a virus antigenically similar to MYXV, in this host species. The genome sequence showed a new MYXV strain (called Toledo strain) which encodes three disrupted genes (M009L, M036L and M152R). It was hypothesized that the new strain was able to more effectively counteract the host defenses in hares and start an infectious process in this new host (Agueda-Pinto et al., 2019). The reported results clearly demonstrated the potential for continuously subclinical or asymptomatic circulation of MYXV in a naturally resistant hare species. In certain conditions, such as immunosuppression induced by pathogens, as the recently described gammaherpes virus (LeHV-5), hares may eventually become more susceptible to rabbit MYXV (Abade dos Santos et al., 2020a).

Although the present results were not representative enough for overall conclusions because of their number and spatiotemporal limitations, it could be assumed a considerable level of natural resistance to myxomatosis in *Lepus europaeus*. An alternative interpretation of the obtained data could be that the virus does not circulate widely in the studied communities. Furthermore low hare population density required for the natural transmission and maintenance of the infection may serve to explain the observed results. Nevertheless the dynamic nature of myxoma virus infection, as well as the possibility of the natural emergence of new virus strains, does not exclude the possibility of revising the current cogitations.

Conclusion

Based on the current results could be concluded that MYXV circulation was not a widely distributed amongst the tested European brown hare populations from different parts of Bulgaria. However the small number of studied animals as well as the limited geographical areas does not allow postulation of some general conclusions. Further serological and also pathological investigations regarding myxomatosis spread should be considered in order to estimate the potential impact of the disease for the detrimental decline in brawn hare populations in Bulgaria.

Acknowledgements

The authors are grateful to Eng. Chavdar Zhelev, PhD for his logistic and scientific support.

References

1. Abade dos Santos F.A., Monteiro M., Pinto A., Carvalho C.L., Peleteiro M.C., Carvalho P., Mendonca P., Carvalho T., Duarte M.D. (2020a). *First description of a herpesvirus infection in genus Lepus.* PLoS One, 14, 1–20.
2. Abade dos Santos F.A., Santos N., Carvalho C., Martinez M., Gortazar C., Garcia-Bocanegra I., Duarte M., Alves P. (2020b). *Serology unveils decades-long contact of the Iberian hare, Lepus granatensis, with myxoma or antigenically-related virus.* Authorea. August 23, DOI: 10.22541/au.159818365.55937034.
3. Agueda-Pinto A., Lemos de Matos A., Abrantes M., Kraberger S., Risalde M.A., Gortázar C., McFadden G., Varsani A., Esteves P.J. (2019). *Genetic Characterization of a Recombinant Myxoma Virus in the Iberian Hare (Lepus granatensis).* Viruses., Jun 7;11(6):530.
4. Alves J.M., Carneiro M., Cheng J.Y., Lemos de Matos A., Rahman M.M., Loog L., Campos P.F., Wales N., Eriksson A., Manica A., Strive T., Graham S.C., Afonso S., Bell D.J., Belmont L., Day J.P., Fuller S.J., Marchandeau S., Palmer W.J., Queney G., Surridge A.K., Vieira F.G., McFadden G., Nielsen R., Gilbert M.T.P., Esteves P.J., Ferrand N., Jiggins F.M. (2019). *Parallel adaptation of rabbit populations to myxoma virus.* Science 363 (6433), 1319–1326.

5. Barlow A., Lawrence K., Everest D., Dastjerdi A., Finnegan C., Stenbach F. (2014). *Confirmation of myxomatosis in a European brown hare in Great Britain.* Veterinary Record, 175: 75–76.
6. Barrett J. W., McFadden G. (2007). *Genus Leporipoxvirus.* In Poxviruses: Mercer A.A., Schmidt A., Weber O., Eds. Birkhäuser Basel: Basel, pp 183–201.
7. Bull L. B., Dickinson C. G. (1937). *The specificity of the virus rabbit myxomatosis.* Journal of the Council for Scientific and Industrial Research 10, 291–294.
8. Carvalho C.L., dos Santos F.A, Monteiro M., Carvalho P., Mendonca P., Duarte M.D. (2020). *First cases of myxomatosis in Iberian hares (Lepus granatensis) in Portugal.* Vet. Rec. Case Reports 8.
9. Collins J. J. (1955). *Myxomatosis in the common hare.* Irish Veterinary Journal 9, 268.
10. Espinosa J., Ferreras M.C., Benavides J., Cuesta N., Pérez C., García Iglesias M.J., García Marín J.F., Pérez V. (2020). *Causes of Mortality and Disease in Rabbits and Hares: A Retrospective Study.* Animals, 10, 158.
11. García-Bocanegra I., Camacho-Sillero L., Caballero-Gómez J., Agüero M., Gómez-Guillamón F., Ruiz-Casas J.M., Díaz-Cao J.M., García E., Ruano M.J., de la Haza R. (2020). *Monitoring of emerging myxoma virus epidemics in Iberian hares (Lepus granatensis) in Spain, 2018–2020.* Transbound Emerg Dis. 00:1–8.
12. Garcia-Bocanegra I., Camacho-Sillero L., Risalde M.A., Dalton K., Caballero-Gomez J., Aguero M., Zorrila I., Gomez-Guillamon F. (2019). *First outbreak of myxomatosis in Iberian hares (Lepus granatensis).*Transbound. Emerg. Dis. 1–5.
13. Jacotot H., Vallee A., Virat B. (1954). *Sur un cas de myxomatose chez le lievre.* Annales de l'Institut Pasteur 86, 105–107. (in French)
14. Kerr P. (2012). Myxomatosis in Australia and Europe: A model for emerging infectious diseases. Antiviral Research, 93, (3), 387–415.
15. Kerr P., Liu J., Cattadori I., Ghedin E., Read A., Holmes E. (2015). *Myxoma virus and the leporipoxviruses: an evolutionary paradigm.* Viruses, 7, (3), 1020–1061.
16. Lavazza A., Graziani M., Tranquillo V.M., Botti G., Palotta C., Cerioli M., Capucci L. (2004). *Serorological evaluation of the immunity induced in commercial rabbits by vaccination for Myxomatosis and RHD.* In Proc.: 8th World Rabbit Congress, September 7–10, Puebla, Mexico, 569–575.
17. Lucas A., Bouley G., Quinchon C., Tocas L. (1953). *La myxomatose du lievre.* Bulletin de l'Office International des Epizooties 39, 770-776. (in French)
18. Marinković D., Popović Z., Aničić M., Beuković M., Beuković D., Relić R. (2018). Health status monitoring of the European Brown Hare (Lepus Europaeus) for the purpose of assessement of its population size in Serbia. (2018). International Symposium on Animal Science (ISAS), 75–82.
19. OIE Terrestrial Manual. (2018). *Myxomatosis*, pp. 1371–1388.
20. Regnery D. C. (1971). The epidemic potential of Brazilian myxoma virus (Lausanne strain) for three species of North American cottontails. American Journal of Epidemiology 1971, 94, 448 (5), 514–519.
21. Saari S.A., Rudbäck E., Niskanen M., Syrjälä P., Nylund M., Anttila M. (2005). *Contagious mucocutaneous dermatitis of the mountain hare (Lepus timidus): pathology and cause.* J Wildl Dis. Oct; 41(4):775–82.
22. Villafuerte R., Castro F., Ramirez E., Cotilla I., Parra F., Delibes-Mateos M., Recuerda P., Rouco C. (2017). Large-scale assessment of myxomatosis prevalence in European wild rabbits (Oryctolagus cuniculus) 60 years after first outbreak in Spain. Res. Vet. Sci.114, 281–286.
23. Whitty B. T. (1955). *Myxomatosis in the common hare.* Irish Veterinary Journal 9, 267.

24. Wibbelt G., Frölich K. (2005). *Infectious Diseases in European Brown Hare (Lepus europaeus).* Wildl Biol Pract, 1 (1): 86–93.

25. Wilkinson L. (2001). Biological control of vertebrate pests: the history of myxomatosis, an experiment in evolution. Med. Hist., 45, (1), 139–140.

26. Zhelev Ch. (2015). Status and influence of some ecological factors on the stocks of the rabbit (Lepus capensis Linnaeus, 1758) in the plain habitats in our country, PhD Thesis, University of Forestry, Sofia, Bulgaria. (in Bulg.)

27. Zhelev Ch., Ninov N., Mihaylov H., Gruychev G., Stoyanov S., Mirchev R. (2013). *Density of brown hare (Lepus europaeus, Pallas, 1778) in the plain habitats of Bulgaria.* Second International Symposium on Hunting, "Modern aspects of sustainable management of game populations" Novi Sad, Serbia, 17-20 October 2013, In: Proceedings of University of Novi Sad, Faculty of Agriculture Novi Sad, Serbia, 54–59.