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Population density, roads and altitude influences on spatial distribution of hares positive to EBHSV

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Abstract

Understanding of the ecology of infected animals facilitates disease risk assessment and is also crucial for wildlife conservation. Relatively little is known about the spatial distribution of infected wild mammals in relation to environmental factors. In neighboring Mediterranean ecosystems 250 European brown hares (*Lepus europaeus*) were collected and examined with RT-PCR to detect European Brown Hare Syndrome Virus (EBHSV). Multivariate statistics and Geographical Information System (GIS) analysis were applied to estimate spatial patterns of biotic and abiotic factors and human activities as determinants of EBHSV positivity. Hare population abundance was estimated using faeces counts and belt drive censuses. The study showed that EBHSV infected hares had widespread distribution even in isolated areas. However, EBHSV infection prevalence was higher in areas with higher hare abundance, closer to paved road networks and at lower altitudes. The risk map revealed the potential distribution of EBHSV-infected hares. This study shows that host abundance and landscape influence the ecology of the disease, a finding that should be taken into account in future studies. The management of harvest and restocking of hares is also discussed for population conservation.

Keywords: Wildlife; Lagomorphs; Disease ecology; Mammals; Refuges

Introduction

The importance of pathogens as a factor influencing wildlife populations is becoming increasingly recognized, as there are multiple examples of pathogens being involved in the decline or extinction of wildlife species. The inclusion of diseases in wildlife management is a perspective that can assist mitigating risks to populations (Delahay, Smith,

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& Hutchings 2009; Thirgood 2009; Joseph et al. 2013). *Lagovirus* is a genus of the *Caliciviridae* which includes European Brown Hare Syndrome Virus (EBHSV), Rabbit Hemorrhagic Disease Virus (RHDV), and the RHD related virus (RHDV2) that affects both rabbits and hares. All these pathogens cause lethal hepatitis (Frölich & Lavazza 2008; Puggioni et al. 2013).

EBHS affects wild and farmed hares (*Lepus spp.*) and is transmitted directly or indirectly by faecal, oral and respiratory routes. It has been also reported that humans, insects, and vertebrates can act as passive vectors (Frölich & Lavazza 2008; Chiari et al. 2016). However, no reservoir hosts have yet been identified (Chiari et al. 2016). An oral infection route by ingestion of vegetation carrying virus excreted from infected animals, or from the droppings of predators that have consumed infected hares, has been proposed (Frölich & Lavazza 2008). Adult animals are more susceptible, whereas the disease has not been observed in leverets younger than two or three months. According to the literature, EBHS is lethal to 35–60% of infected hares (Duff, Chasey, Munro, & Wooldridge 1994; Zanni et al. 1995; Drews et al. 2011). There is evidence that EBHSV might have occurred in European hare populations many decades before pathological signs of EBHS were identified. An ancestor of the present European EBHSV strain may have been apathogenic (Frölich et al. 2003).

The impact of the disease on hare populations varies depending on the case considered. In fact, following introduction of EBHSV into a naïve population, spread of the disease to contiguous areas is inevitable and results in epidemics, affecting a large proportion of hares, while in the following years the disease occurrence is influenced by the population size and distribution of hares and mortality decreases depending on the immune status at population level (Frölich & Lavazza 2008). According to Lavazza et al. (1999), when hare density is low (<8 hares/km^2), the virus transmission is reduced and most juveniles remain seronegative. On the other hand, when hare density is higher (about 15 hares/km^2), mortality may be reduced due to the development of protective immunity as a consequence of a previous infection at ages of less than two to three months. In North Central Italy, low seroprevalence was correlated to low hare density whereas seroprevalence was high in areas with high and medium population density (Cammi, Capucci, Bernini, & Lavazza 2003; Paci, Lavazza, Ferretti, Santilli, & Bagliacca 2011; Chiari et al. 2014). In Germany, a study of hare populations varying between 6 and 145 hares/100 ha (only three areas were below 15 hares/km^2) revealed no relation between seroprevalence rate and population density (Eskens et al. 2000). In Britain, 90% of dead hares were found in areas with more than 8 hares/km^2 (Duff, Whitwell, & Chasey 1997). These findings reflect the impact of EBHSV introduction into a susceptible naïve population which is characterized by high mortality that is independent from hare population density. Moreover, the impact of factors other than population density, such as environmental parameters or human activities, may also influence the prevalence of the disease (Pfeiffer 2010).

Investigation of the spatial distribution of EBHSV is challenging due to difficulties that include sample collection in large areas, determination of hare abundance, and the description of relevant environmental factors and human activities (Wobeser 1994; Ostfeld, Glass, & Keesing 2005, Clements & Pfeiffer 2009). GIS has contributed to these studies and also to the development of spatial risk models for infectious diseases (Clements & Pfeiffer 2009). This knowledge will likely enable us to predict the distribution of the virus and assist in disease management (Ostfeld et al. 2005; Berry et al. 2015).

The aim of this study was to understand the relationships between EBHSV and hare habitat parameters and to predict the distribution of infected hares within different, neighboring ecosystems. The Mediterranean region is suitable for this investigation due to its high variability of biotic and abiotic parameters. Specifically, the study focused on the detection of EBHSV infected hares and its relation with: (1) biotic parameters, such as density of hare population, presence of livestock and vegetation, (2) abiotic parameters, such as topographic and climatic conditions, and (3) human constructions and activities such as the road network and hunting management. The main hypothesis is the higher the population density the higher the infection rate (Wobeser 2006), but maybe this is not adequately explaining because more factors influence infection.

Materials and methods

Study area

The study was conducted in Macedonia, in the Prefectures of Chalkidiki and Thessaloniki, and on the island of Thasos (Fig. 1), a total surface area of 6981 km^2. An outbreak of EBHS occurred in the region from 1999 to 2003 and during this time numerous dead hares were found by hunters (Billinis et al. 2005). In the years following the epidemic, about 1–2 hares found dead by EBHS annually.

The study area comprises a wide variety of ecosystems (irrigated crops, olive groves, maquis, pine and deciduous forests, see Sokos, Birtsas et al. 2016; Sokos, Giannakopoulos et al. 2016 for full description). Altitudes range from sea level to 1140 m a.s.l. Barriers of hare movements such as sea and closed fence highway roads also exist. The climate is Mediterranean in character with hot, dry, summers, and average summer temperatures between 23 and 34 °C. Mean winter temperatures range from 4 to 19 °C during winter, however, winters are mild closer to the sea and colder inland, where the mean minimum temperature of the coldest month ranges from below 0 to 3 °C. Thirty-seven (37) "Hunting Prohibited Areas" (refuges) and 44 "Hunting Dog Training Areas" are located within the study area. In the latter, their surface area is usually 5–10 km^2, the hunting is permitted (15/9–10/1) and also dog training all year round. Usually