

Research Article

Relationship between Anti-European Brown Hare Syndrome Serological Titers and Brown Hare (*Lepus europaeus* Pallas) Densities

Gisella Paci,¹ Antonio Lavazza,² Marco Ferretti,¹ Francesco Santilli,³ and Marco Bagliacca¹

¹ Department of Animal Production, Faculty of Veterinary Medicine, University of Pisa, Avenue of Piagge 2, 56100 Pisa, Italy

² Virology Department, Experimental Zooprofilactic Institute of Lombardy and Emilia Romagna "B. Ubertini", Street Bianchi 9, 25125 Brescia, Italy

³ Wildlife Technician, Hunting District Livorno (ATC19), Street F. Dini 3, 57021 Campiglia, Italy

Correspondence should be addressed to Gisella Paci, gpaci@vet.unipi.it

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Thirty-three protected wild game reproduction areas, located in the province of Florence (Central Italy), were monitored for habitat characteristics and hare census over a period of 2 years. A total of 172 hares was captured, checked for sex, and age, and blood samples were taken. Serum samples were analyzed by competitive ELISA test for detection and titration of anti-European brown hare syndrome virus (EBHSV) antibodies. Results showed that EBHSV seropositive hares from areas with high and medium population densities had higher antibody titers than those coming from low-density areas and that adults showed lower values than young animals. Anti-EBHSV antibody levels were inversely related to the distances between protected areas and private hunting areas while a high density of protected areas was not associated with any similarity in the values or prevalence of EBHSV.

1. Introduction

European brown hare syndrome (EBHS) is a highly contagious, acute and fatal disease of the European brown hare (*Lepus europaeus*) and mountain hare (*Lepus timidus*). EBHS is caused by a calicivirus belonging to the genus lagovirus of the Caliciviridae family [1, 2]. The disease was first described in Sweden [3], but epidemics have been reported all over Europe: Germany [4], Belgium [5], United Kingdom [6], Croatia [7], Sweden [8], Finland [9], Austria [10], Spain [11], Poland [12], Switzerland [13], and Slovakia [14]. The infection has become endemic in Italy, following the initial epidemic peak that occurred in the late 1980s [15, 16].

In Italy, the outlander territory is divided into (a) public hunting areas (named ATC) where hunting is allowed, (b) private hunting areas (named AATV and AFV), and (c) protected areas (named natural parks, oasis of protection, ZRC, ZRV, and others) where hunting is not allowed. In some of these protected areas, that is, ZRC and ZRV, nonfenced

areas, the resident hare populations are usually managed by translocation.

Because hare populations, like other wild animal populations, are known to undergo cyclic density changes, the regular translocation (i.e., capture in protected areas, transport, and release in public hunting areas) is often used to increase the presence of this specie in the low density hunting areas. This is regularly applied in most hunting areas immediately after the hunting season. However, if capture for translocation and monitoring is not carried out properly, it may induce physical stress, which can suppress the immune response and consequently reduce the resistance to disease, so animals may develop clinical symptoms and spread the infective agent [17–21].

Moreover, it is known that EBHSV circulation and therefore, its chance to infect and/or kill hares is directly linked to hare population densities in areas of limited extension, located within regions where the disease is endemic [22]. The disease has not been observed in hares younger than

approximately 40–50 days, and although those of 2–3 months of age may contract infection, they do not usually develop clinical disease. The reasons of such innate resistance are unknown and just some hypotheses have been put forward. Therefore, as observed in other experiences [22] when hare density is low (<8 hares/km²), the spread of the virus is reduced and most juveniles become adults without ever entering in contact with the virus remaining seronegative. When these animals become eventually infected with EBHSV, they develop clinical signs and die. On the contrary, when hare density is about 15 adults/km², mortality can be reduced due to the rapid transmission of virus, between young hares during their refractory period, that is, till 2–3 months of age. In these areas, the juveniles that are exposed to EBHSV become subclinically infected, do not develop clinical signs, and show long-lasting protective immunity. The high environmental resistance of the virus—it retains infectivity for several months in the open field—may help the spread of infection among young hares. Therefore, in high-density areas, the seroprevalence for anti-EBHSV antibodies in hares could be as high as 95% [16, 23, 24]. Thus, specific monitoring plans, based on the determination of serological anti-EBHSV titers, have been implemented in most Italian provinces during capture operations. Based on these programs, we analyzed the results obtained in the province of Florence in order to improve the epidemiological knowledge on this disease and to find useful indications for a correct game management.

2. Material and Methods

The study was carried out on hares captured in 33 wild-game reproduction protected areas (non fenced areas, average surfaces of 606 Ha \pm 227 std.d.), located in the province of Florence (Central Italy) over a two-year period. In this province, identified as follows at north 44°13' N, 11°25' E, at west 43°37' N, 10°49' E, at south 43°27' N, 10°56' E, and at east 43°52' N, 11°42' E, there are also 57 private hunting areas (no fenced areas, average surfaces of 428 Ha \pm 205 std.d.). All these areas were monitored to characterize the habitat traits and hare densities. The methods used and the specific data concerning these parameters are reported in detail in a previous research [25]. After the census, about 30% of the hares were captured. Each captured animal was checked for sex, age, and bled ($n = 172$) before being transferred to other areas [25].

The blood samples (~ 0.5 mL, serum) were analyzed by competition ELISA test, using the method described by Capucci and Lavazza [26] for presence and titer of antibodies towards EBHSV. The serum titer corresponds to the dilution giving an absorbance value equal to 50% (± 10) of the value of the negative serum at dilution 1/160 (reference value). The average titers are mean values of the titers of the tested sera and are expressed as dilution (1/...).

Relationships between anti-EBHSV antibody serum levels (log linear transformed), sex, age, density, and their interactions were analyzed by ANOVA. Since only interaction sex * age was significant, in the final model only this interaction was considered [27]. Minimum statistical differences

between means were studied using Bonferroni confidence intervals.

The percentages of seropositive hares and antibody serum levels (log linear transformed) were then submitted to linear regressions in relationship either with the number or density of the neighboring private hunting areas. To calculate the density of private hunting areas around each protected area, the average geographic distances between the private areas and each protected area were divided by the number of the formers. Private hunting areas farther than 3 km were not considered, since the average home range of the brown hare is always less than 30 ha [28] and the observed maximum range of hares in Italy (night range) is less than 300 ha [29, 30]. Differences between antiserum levels and number of seropositive hares (absolute values) were analyzed by linear regressions also in relation to the average geographic distances between protected areas [27]. Each habitat distance was calculated by ArchView GIS 3.1 on the digitalized maps of the official wildlife management plan of Tuscany (Central Italy).

3. Results

The distribution of hares with anti-EBHSV antibodies was significantly different in relation to the density of hares; values were higher in the areas characterized by high and medium density than in areas characterized by low density (70.0% and 78.4% versus 25.5%). Percentage of seropositive hares also differed between males and females, within young animals (young males 82.5%, young females 58.5%). The anti-EBHSV antibody titers were significantly affected by the age. Generally, the adults showed lower average values (mean titer 1/39 versus 1/176).

Since the interaction sex * age was significant, a different trend within the ages was observed between the two sexes. In the females, positivity increases along with the age while in the males it decreases. The titers confirm the trend observed for positivity; even if the titers decrease in both sexes, in the adult females they decrease less than in the adult males (Table 1).

Significant positive regression was observed between the anti-EBHSV antibody titers and the density of the neighboring hunting-areas (Table 2).

The distribution of EBHSV seropositive hares did not show any significant relationship with the number of bordering hunting-areas and no relationship was observed between the differences of anti-EBHSV antibody titers or seropositivity and the distances between the protected areas (Table 2).

4. Discussion

Regarding seropositivity to EBHSV, as it was expected, the lower percentage of seropositive animals was directly correlated to low densities whereas the seroprevalence was higher in areas with high and medium densities.

These serological data provide further support for the deterministic model of EBHSV suggested by Guberti and Lavazza to explain the natural diffusion of EBHS in endemic

TABLE 1: Anti-EBHSV antibodies positivity and serological titers in hares in relation to sex, age, sex * age and density.

	Interaction sex * age				Main effect sex		Main effect age		Main effect density (<i>n</i> /100 ha)		
	Adult females	Young females	Adult males	Young males	females	males	Adult	Young	>60	High	20–40 Middling
Anti EBHSV* %	82.3 ^{ab}	58.5 ^b	75.5 ^a	82.5 ^a	69.3	78.8	78.5	70.4	70.0 ^a	78.4 ^a	25.5 ^b
N° pos/N° tot [§]	28/34	24/41	34/45	33/40	52/75	67/85	62/79	57/81	21/30	87/111	41/161
Anti-EBHSV [^]	1/55 ^{ab}	1/173 ^a	1/23 ^b	1/179 ^a	1/114	1/101	1/39 ^b	1/176 ^a	1/80	1/195	1/48
Standard error	1/57.4	1/57.8	1/50.1	1/51.9	1/44.0	1/39.3	1/41.7	1/41.8	1/57.9	1/28.9	1/77.4

Note: means bearing different letters differ for $P < .05$.

*Anti-EBHSV %: percentage of animals positive for antibodies against European brown hare syndrome virus.

[§]Number of seropositive hares out of total hares tested

[^]Anti-EBHSV: mean titers for anti-EBHSV antibodies.

TABLE 2: Serological data in relation to some geographical data.

Parameter Y	Effect X	<i>b</i>	d.f.	R^2	<i>P</i>
Anti-EBHSV* %	Number of bordering hunting areas ($n = 1 - 8$)	+9.4	30	0.11	.64 ns
Anti-EBHSV [^]	Concentration of private hunting areas around the protected area (expressed as $\Sigma 1/\text{distances}$ in km)	+1/47.0	32	0.19	.02
Difference between Anti-EBHSV* %	Difference between localization of habitats (distances between protected areas in km) (0–3000 m)	+5.7	527	0.09	.26 ns
Difference between Anti-EBHSV [^]	Difference between localization of habitats (distances between protected areas in km) (0–3000 m)	+1/10.0	527	0.045	.64 ns

* Anti-EBHSV (%): percentage of animals positive for antibodies against European brown hare syndrome virus

[^]Anti-EBHSV: mean titers for anti-EBHSV antibodies.

areas [22, 31]. Indirectly, these serological results offer evidence that in areas of high density of hares, the EBHS virus persists and circulates without overt disease. In low-density areas, the slow spread of the virus leads to the infection of subadult animals (that have exceeded the refractory “age” period), thus exerting some degree of mortality. The critical density value is between 8–15 hares/100 ha; below this value, mortality due to infection with EBHSV is very high; above 15 hares/100 ha a negligible mortality is observed and almost all the animals are seropositive. This model could also be used to explain the lower anti-EBHSV antibody titers found in adults compared to those found in younger hares, since it can be assumed the latter have had more recent contact with the virus. Once again, as shown in previous surveys [22], these data acquire great importance especially in relation to hare management. In fact, a correct intervention, which could reduce the impact of EBHSV at the population level, is to keep the densities at a proper level in the protected areas where the habitat characteristics are particularly suitable for wild hare reproduction (at least 15 hares/100 ha). EBHSV, in fact, is one of the principle causes of death in hares in Europe [24, 32] and was found to be the most important cause of death in fenced areas.

The existence of a significant effect related to the proximity to neighboring private hunting areas, where hares of different origins (captive farmed and imported) are commonly and repeatedly released, is clearly evident. Some reared hares, survived to the hunting activity in the private

hunting areas, probably succeed to trespass also the strip of public hunting areas (always localized between the protected areas and the private hunting areas), to reach the protected areas and to come into contact with the indigenous resident hares.

Various explanations can be suggested to justify the lack of any correlation among serum titers and distance between protected areas. Each protected area could be considered as an independent spot since the home-range of the hares living there has no contact with the home-range of hares living in other protected areas. This is due to the constant presence of sufficiently large strips of public hunting areas. Neither it is likely that the newborn/juvenile hares form one protected area, in the spring, when the wildlife capacity of that area reaches its maximum, would move to reach and colonize other protected areas. In fact, the periodical capture that is carried out in winter during the prereproductive period reduces the numbers inside the protected areas, and when the remaining newborn hares move by radiation from their birthplace, they easily remain in the strip of public hunting areas where hare density is close to zero, due to the previous hunting season.

To minimize the impact of this endemic disease, it is, therefore important to keep the hares’ density at a very good level in the protected areas (at least 15 heads/100 ha) and to reduce the number of the bordering private hunting areas and/or also control the quality of animals released in the private hunting areas.

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