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Treponemal Infection in Free-ranging European Brown Hares (*Lepus europaeus*) in Central Italy: Serology and Epidemiology

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ABSTRACT: In the winters of 2008 and 2009, treponemal infections in 154 free-ranging wild European brown hares (*Lepus europaeus*) from Central Italy were diagnosed by serologic tests for human syphilis. Antibodies were found in 51/154 samples (33%). Antibody prevalence was positively correlated with age and with population density. There were no significant differences in prevalence between sexes.

Key words: Epidemiology, European brown hare, *Lepus europaeus*, serology, syphilis, *Treponema*.

The European brown hare (*Lepus europaeus* Pallas) population in Europe has declined (Smith et al., 2004) over the last 50 yr. In Italy, the population has increased due to protection in natural areas and restocking with hares mostly from Eastern Europe. Higher population densities could negatively impact the species due to increased transmission of pathogens. Venereal spirochetosis could effectively contribute to a population decline in hares, even if the disease has been only sporadically reported. Clinical manifestations are similar to those seen in rabbit syphilis, including proliferative crusty skin lesions and facial, buccal, mucosal, and genital ulcerations. Treponematosis in hares first was reported by Jakšić (1957), who detected spirochetes in approximately 20% of trapped male hares with purulent skin lesions. *Treponema* sp. infection was serologically documented in Hungary in 27% of 202 hares with external genital lesions, selected from 15,000 trapped hares (Horvath et al., 1980). In another study, treponemal infections in hares were diagnosed using the *Treponema pallidum* hemagglutination Assay (TPHA) and by detection of spirochetes using the Bosma-Steiner silver methodology (Lumeij et al., 1994). Finally, widespread treponemal infections were discovered in several Dutch provinces through histochemical and serologic analysis (Lumeij, 2011). Those authors proposed a new classification of the uncultured infectious agent responsible for hare syphilis and suggested the name *Treponema paraluisleporis*.

We measured the prevalence of treponematosis in wild European brown hares in central Italy using serologic methods. We also investigated the influence of land topography, population density, sex, and age of hares on the epidemiology of the disease.

In January and February of 2008 and 2009, we captured 154 wild hares and collected blood samples via saphenous vein venipuncture prior to their release. Hare age was determined by palpation of the growing cartilage of the radius and ulna bones (Soveri and Valtonen, 1983) and sampled hares were divided into juveniles (<8–9 mo) and adults (≥8–9 mo). Seven protected areas, located in either plains ($n=3$) or hilly regions ($n=4$) were investigated. Scarselli and colleagues (unpubl. data) reported population densities of 2–16 hares per 100 ha. Our study areas were divided into two groups according to population density: low population density (<10 hares per 100 ha) and high population density (≥10 hares per 100 ha). Sex and age of the hares and the population density and land topography are presented in Table 1. Serologic analysis was performed on the samples using a commercial TPHA (Paramedical, Salerno, Italy) according to the manufacturer's instructions. Positive and negative controls were included in each test. TPHA has sensitivity >95% and a specificity of 99% in humans (Wiwanitkit, 2009). TPHA testing has been used for the

TABLE 1. Number of archived serum sampled from European Brown hares (*Lepus europaeus*) collected in seven protected areas of the Province of Pisa, Tuscany (Italy). 2008–2009, and tested for antibodies against *Treponema* sp.

Sampled areas	Land topography	Hare population density[a]	Female (n=81)		Male (n=73)		Total	Positive sera	%
			Juvenile	Adult	Juvenile	Adult			
Asciano (43°45′17″N, 10°27′53″E)	Plains	High	16	23	13	19	71	24	34
Capannoli-Terricciola (43°35′24″N, 10°40′11″E)	Hills	Low	2	3	2	2	9	4	44
Cenaia (43°36′0″N, 10°33′0″E)	Plains	High	6	3	3	10	22	10	45
Collebrunacchi (43°38′27N, 10°51′58″E)	Hills	High	1	3	3	3	10	0	0
Crespina (43°35′0″N, 10°34′0″E)	Hills	Low	2	3	3	2	10	0	0
Lajatico (43°28′20″N, 10°43′46″E)	Hills	Low	4	5	1	1	11	0	0
Navacchio (43°40′48″N, 10°30′1″E)	Plains	High	4	6	4	7	21	13	62
Total			35	46	29	44	154	51	33

[a] Protected areas with low population density (<10 hares/100 ha) and with high population density (≥10 hares/100 ha).

diagnosis of *Treponema* sp. infections in general, and in particular with *T. paraluiscuniculi* in rabbits (Baker-Zander and Lukehart, 1984) and *T. paraluisleporis* in hares (Lumeij, 2011) due to the production of cross-reacting antibodies with *T. pallidum* (Baker-Zander and Lukehart, 1984).

We used chi-square testing with Yates correction to evaluate relationships between antibody prevalence and age, gender, land topography, and population density. Multiple testing for independent parameters also was performed. Statistical significance was based on an alpha of 0.05. Analyses were performed using SPSS Advanced Statistics 13.0 (SPSS Inc., Chicago, Illinois, USA).

Fifty-one of the 154 sera (33%) had detectable antibodies to *Treponema* sp. with a high prevalence in four of seven areas sampled (Table 1). All antibody-positive hares presented no typical lesions of syphilis. We detected a significantly higher prevalence (P<0.025) in areas with high population densities (Table 2). Although the plains areas had higher prevalence than the hilly areas, this correlation appears coincidental because there was a significantly higher population density in the plains areas (Table 1). There was a slightly greater but not statistically significant antibody prevalence in males than females (36% vs. 31%). Adult hares had a higher prevalence (48%) than did juveniles (12%; P<0.001).

The high antibody prevalence in hare blood samples demonstrated the natural occurrence of hare treponemiasis in wild hares from the province of Pisa (central Italy). These results are consistent with previous investigations in other European countries with antibody prevalences ranging from 29% to 60% (Horvath et al., 1980; Lumeij, 2011). Over the past 30 yr, massive restocking of hares to hunting areas has occurred from Eastern Europe to Italy. Although it is not possible to assess the true importance of this activity, the high prevalence of treponemal disease in the hare population throughout Europe suggests that this disease might be spread

TABLE 2. Prevalence of antibodies to *Treponema* sp. infection in hare populations of the Province of Pisa, Tuscany (Italy) subdivided on the basis of population density, sex, and age class.

	Population density			Sex			Age class		
Antibody	Subjects living in low density areas (n=30)	Subjects living in high density areas (n=124)	P	Female (n=81)	Male (n=73)	P	Juvenile (n=64)	Adult (n=90)	P
Positive	4	47	<0.025	25	26	>0.5	8	43	<0.001
Negative	26	77		56	47		56	47	

by movement of animals (Jakšić, 1957; Horvath et al., 1980; Lumejj et al., 1994; Lumeij, 2011). Our data corroborate other studies (Wiwanitkit, 2009) supporting the use of TPHA to detect treponemal antibodies in lagomorphs. Currently, TPHA is considered a confirmatory test in humans, and nonpathogenic treponemes will not cause false positive reactions (Wiwanitkit, 2009). Because of antigenic crossreactivity, this test can also be used for the diagnosis of *T. paraluiscuniculi* infection in rabbits and *T. paraluisleporis* in hares (Lumeij, 2011). Despite differences in host spectra and varying degrees of pathogenicity, it is estimated that the genome sequence similarity between treponemal species is approximately 99% (Strouhal et al., 2002).

There are no reports on prevalence of antibody to *T. paraluisleporis* in hares in relation to age. It has been proposed that this agent spreads mainly by horizontal transmission during reproductive activity and, as expected, the antibody prevalence in adult hares was higher than in younger hares (<8–9 mo). Although the small sample size and the lack of in-depth knowledge about the disease and its transmission make discussions tentative, the results of our study could relate to the reproductive physiology and reproductive behavior of hares. Hares are polyestrous and induced ovulators. In the field, hare parturitions occur from mid-January to mid-October (Lincoln, 1974), with young hares reaching full adult body size by 4 mo of age, usually May to December. The hares born prior to mid-March become sexually mature in August at the end of the reproductive season, whereas hares born during the second half of the breeding season might not reach maturity until the beginning of the next reproductive season (Lincoln, 1974). Therefore, part of the young hares sampled during January and February, in our study would have already reached maturity, mated, and come into contact with *Treponema*-infected hares. The similar antibody prevalence between sexes is probably related to the horizontal transmission of the pathogen, as well as the specific characteristics of the breeding season. Female hares begin breeding in January with marked synchrony in their first ovulation of the season. Mating precedes ovulation and the first pregnancy often results in a single fetus, although pregnancy failure often occurs during the first ovulatory cycle of the season. By March and April, reproductive activity peaks, and 100% of females are pregnant, with most carrying three or more fetuses (Lincoln, 1974). During this period, depletion of sperm reserves occurs in males (Lincoln, 1974).This heightened period of polygynous reproductive activity in hares could therefore be a cause of the diffuse spread of treponemal infection, because transmission would be positively correlated with the number of copulations. The presence of this infection in areas of high population density also could be explained by this high degree of contact between male and female hares. In our study, none of the antibody-positive hares exhibited gross lesions common to treponemal infections. It also has been reported by other authors (Horvath et al., 1980; Lumeij et al., 1994; Lumeij, 2011) that

clinical signs of hare syphilis are rare compared to the frequency of seroconversion. Superficial ulcerations, with or without hemorrhagic crusts, were seen on the genital skin of only 1% of 1,400 trapped hares (Horvath et al., 1979).

Because of the lack of tissue samples, the accuracy of our serologic results cannot be completely assessed. Confirmation of infection in antibody-positive hares only can be achieved by means of histologic and molecular biologic investigations (Lumeij 2011). The absence of visible clinical manifestations means that the true impact of the disease on the hare population is still to be demonstrated. Nevertheless it would be prudent to ensure responsible and well-planned restocking practices. Further serologic and pathologic studies are necessary to clarify the pathogenicity of *T. paraluisleporis* infection in hares.

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