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SHORT COMMUNICATION

Widespread treponemal infections of hare populations (*Lepus europaeus*) in the Netherlands

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Abstract Further to the first description of treponemal infection in hares (*Lepus europaeus*) in one province of the Netherlands (Flevoland), this report provides further evidence for the widespread occurrence of hare syphilis in this country. Hunter-bagged hares which had the typical lesions of the disease were serologically examined using the *Treponema pallidum* hemagglutination assay and the fluorescent treponemal antibody absorption test, and tissue samples were stained with the Bosma–Steiner modified silver stain. Nine out of 12 suspected hares from five different provinces from the Netherlands showed serological and histological evidence of syphilis. Hare populations from at least half of the provinces of the Netherlands are infected with this agent.

Keywords Syphilis · "*Candidatus* Treponema paraluisleporis" · Bosma–Steiner silver stain · Hares

Introduction

Treponemal infections in European brown hares (*Lepus europaeus*) have been sporadically reported. The first evidence for infections of hares with *Treponema* spp. was provided by Jakšić (1957). He demonstrated spirochaetes with India ink stain in histological preparations of purulent genital skin lesions in male hares from Serbia. Horvath et al. (1979) provided further evidence of the disease in Hungary. Using dark-field microscopic examination, they found spirochaetes in five out of 15 hares with genital lesions, which were selected from a total of 1,400 trapped hares. They were able to artificially infect rabbits with material from diseased hares. They further reported on serological evidence of the disease in 27% of 202 clinical suspect animals which were selected from a group of 15,000 trapped hares (Horvath et al. 1980). Lumeij et al. (1994) reported on two clinical cases of treponemal infection in hares, and seroconversion in 60% of the hare population in the province Flevoland from the Netherlands (52°26′N; 5°30′E). Artifical infection of rabbits with a treponemal suspension harvested from preputial lesions of naturally infected hares resulted in orchitis and scrotal dermatitis in which the organisms could be identified.

Two more anecdotal reports of hare syphilis could be traced. From the period 1986–1998, Mörner (1999) reported syphilis in 11 out of 118 brown hares and 25 out of 760 mountain hares (*Lepus timidus*) in Sweden. The University of Cambridge Department of Clinical Veterinary Medicine (2000) in the United Kingdom reported syphilitic lesions in three out of 12 hares.

The most common clinical manifestation in hares resembles that of rabbit syphilis and includes lesions on the mucocutaneous junctions of the face and genital area. Although the external genital organs can be affected in a variety of ways, effects on reproduction are poorly documented. Considering the high prevalence of antibodies in areas with large hare populations, effects on the population seem limited (Wuthe and Blew 1996), but firm epidemiological data are lacking.

In analogy with the nomenclature of *Treponema paraluiscuniculi* in rabbits with syphilis, the species was preliminary identified as '*Treponema paraluisleporis*'

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(Lumeij et al. 1994). According to the recommendations of the "Ad Hoc Committee for the re-evaluation of the species definition in bacteriology" (Stackebrand et al. 2002), the name of this as yet uncultured organism should be "*Candidatus* Treponema paraluisleporis", pending further characterization (Euzéby 1997).

Based on serosurveys which thus far have been performed in three different hare populations in Europe (Horvath et al. 1980; Lumeij et al. 1994; Wuthe and Blew 1996), the conclusion seems to be justified that clinical signs of hare syphilis are rare compared to seroconversion. Lumeij et al. (1994) found 60% seroconversion in a sample of 100 hares randomly shot in the province Flevoland in the Netherlands. Of the shot animals only three had genital lesions which were confirmed histologically to be caused by treponemal infection (Lumeij, unpublished observations). Hares with external lesions seem to represent the tip of the iceberg.

The first reported cases of hare syphilis in The Netherlands were shot in the province Flevoland and subsequent serological examination revealed that infection was widespread in this province (Lumeij et al. 1994). It was unknown whether the disease had been introduced recently, and was a local phenomenon, or whether the disease occurred throughout the whole country, but had never been detected. The principal purpose of the present study therefore was to investigate whether the disease occurred also in other provinces of the Netherlands.

Materials and methods

Hunters from the Netherlands were approached by means of a request for cooperation in the national hunting magazine 1 week before the closure of the hare hunting season in December (Lumeij 1994). Hunters were requested to inspect bagged hares for the characteristic external lesions of hare syphilis, like superficial ulcerations or crusts on the mucocutaneous junctions around mouth, nose, or in the anogenital region. In the 2 weeks following publication, nine hunters who had observed 12 hares with the lesions described, contacted us. Nine serum samples were considered appropriate for serological investigations and 12 tissue samples were appropriate for histological examination (Table 1). Serology was performed by using *Treponema pallidum* haemagglutination assay (TPHA; Fujirebio, Tokyo, Japan) and Fluorescent Treponemal Antibody Absorption (FTA-Abs test; U.S. Public Health Service 1969).

TPHA reagents are used to detect serum antibody to *T. pallidum* by an indirect haemagglutination method. Preserved erythrocytes are coated with antigenic components of pathogenic *T. pallidum* (Nichol's strain). These erythrocytes agglutinate in the presence of specific antibodies to *T. pallidum.* Any nonspecific reactions occurring are detected using erythrocytes not coated with *T. pallidum* antigens.

In the FTA-Abs test, serum antibodies against *T. pallidum* were detected with a fluorescein isothiocyanate-labeled horse anti-rabbit immunoglobulin (diluted 1:400; National Institute for Public Health and Environmental Hygiene, Bilthoven, The Netherlands).

Tissue sections were stained with the Bosma–Steiner silver stain as reported originally for detecting of spirochaetes in patients with Lyme disease (De Koning et al. 1987), and used in a previous study for demonstration of *T. paraluisleporis* in hares (Lumeij et al. 1994).

Results

Seven out of nine serum samples revealed a positive treponemal serology and nine out of 12 tissue samples revealed positive histology (Table 1). All serological results

Table 1 Locations of origin of hares which were reported with clinical signs of treponemal infection and serological and histological findings

Location	Coordinates	TPHA	FTA-Abs	Bosma–Steiner stain
Aalten	51°55′N; 6°35 E	++	++	+
Alphen-1	51°28′N; 4°58′E	–	–	–
Alphen-2	51°28′N; 4°58′E	–	–	–
Bedum	53°18′N; 6°36′E	nd	nd	+
Lochem	52°09′N; 6°25′E	nd	nd	+
Nieuw Weerdinge-1	52°51′N; 7°00′E	1:10240	++	+
Nieuw Weerdinge-2	52°51′N; 7°00′E	1:5120	+	+
Nieuw Weerdinge-3	52°51′N; 7°00′E	1:10240	++	+
Nieuwleusen	52°35′N; 6°17′E	nd	nd	+
Otterloo	52°06′N; 5°47′E	1:160[a]	Nonspecific agglutinins	–
Weerselo	52°21′N; 6°51′E	1:5120	++	+
Wezep	52°28′N; 6°00′E	1:40960	++	+

nd, not done

[a] Nonspecific reaction, see Results

were confirmed by histology (except for the location Otterloo in which the TPHA was considered nonspecific because unsensitized erythrocytes reacted with a titre of 1:320, while the specific reaction with sensitized erythrocytes resulted in a titre of 1:160. In this case, histology was found to be negative by an independent observer. The cause of the lesions was not further investigated). In cases which were histologically positive, the Bosma–Steiner stain clearly showed the black spirochaetes against a yellow background as reported previously (Figs. 1 and 2; Lumeij et al. 1994).

Discussion

The results from this study show that apart from the province Flevoland, clinical hare syphilis occurs also in at least five other provinces (Brabant, Gelderland, Overijssel, Drenthe, and Groningen). Furthermore, the serological studies and anecdotal reports published thus far (see Introduction) seem to indicate that the disease is endemic throughout Europe. The limited data available suggest that the disease does not affect hares at the population level. Interestingly, no cases from the province Flevoland were revealed in the present study, while a previous study has shown widespread occurrence of hare syphilis in this province. This supports the statement that (reported) clinical cases represent only the tip of the iceberg and the results of the present study are only a very conservative estimate of the prevalence.

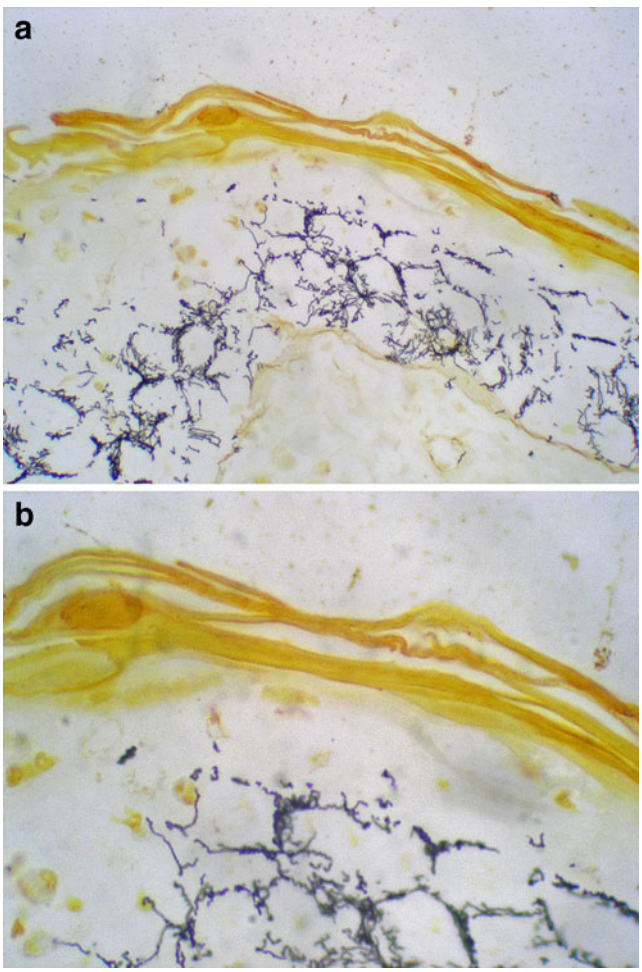

Fig. 1 **a** Section of hare prepuce with epidermis on the top side. Bosma–Steiner staining, ×500; **b** Detail of **a**. Note the presence of many clearly distinguishable *black* spirochaetes on a *yellow* background. Bosma–Steiner staining, ×1000

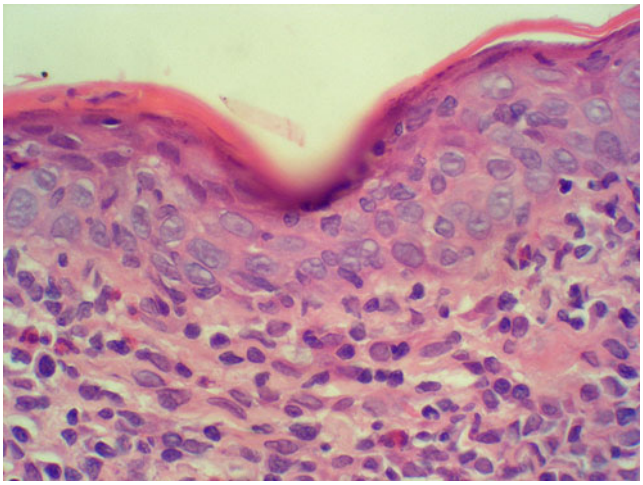

Fig. 2 A section of hare prepuce as in Fig. 1. Note that many inflammatory cells can be seen, but the spirochaetes are not visible with this routine staining method (H & E, ×500)

The reason why hare syphilis in the Netherlands has not been recognized in the past is probably because the level of clinical suspicion is low. Even when suspected animals would have been examined by routine procedures, a specific diagnosis would have been unlikely, because it is difficult to demonstrate the causative organism in histological sections of tissues using routine stains, and serological confirmation of an infection can only be made if specific serology is performed.

Currently, the TPHA and the FTA-Abs test are considered confirmatory test in humans. The sensitivity and specificity of the TPHA test are >95 and >99%, respectively (Wiwanitkit 2009). The FTA-Abs test has a sensitivity of 84% for detecting primary syphilis infection and almost 100% for other stages, and a specificity of 96% (Golden et al. 2003). Nonpathogenic treponemes will not cause false-positive reactions with these tests. Because of the antigenic cross reactivity these tests can also be used for diagnosis of infections with *T. paraluiscuniculi* in rabbits (Baker-Zander and Lukehart 1984). The different subspecies of *T. pallidum*, and *T. paraluiscuniculi*, have various degrees of pathogenicity and different host spectra, but cannot be distinguished morphologically or by routine serological methods. It has

been estimated that the genome sequence similarity between *T. pallidum* subps. *pallidum* from humans and *T. paraluis-cuniculi* from rabbits is about 99% (Strouhal et al. 2007), and it is most likely that the "*Candidatus* Treponema paraluisleporis" from hares is nearly identical.

Only 12 hares were examined in this study to conclude that the disease occurred in wild hare populations in at least five other provinces. Including the province Flevoland which was shown to be positive in a previous study, this means that at least half of the provinces of the Netherlands is infected.

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Conflict of interest The author declares that he has no conflict of interest.

References

Baker-Zander SA, Lukehart SA (1984) Antigenic cross-reactivity between Treponema pallidum and other pathogenic members of the family Spirochaetaceae. Infect Immun 46:116–121

De Koning J, Bosma RB, Hoogkamp-Korstanje JAA (1987) Demonstration of spirochaetes in patients with Lyme disease with a modified silver stain. J Med Microbiol 23:261–267

Euzéby JP (1997) List of bacterial names with standing in nomenclature: a folder available on the internet. Int J Syst Bacteriol 47:590–592, List of Prokaryotic names with Standing in Nomenclature. http://www.bacterio.cict.fr/—Accessed on August 3 rd, 2010

Golden MR, Mara CM, Holmes KK (2003) Update on syphilis: resurgence of an old problem. JAMA 290:1510–1514

Horvath I, Kemenes F, Molnar L (1979) Isolation of pathogenic treponemes from hare. Experientia 34:320–321

Horvath I, Kemmenes F, Molnar L, Szeky A, Racz I (1980) Experimental syphilis and serological examination of treponematosis in hares. Infect Immun 27:231–234

Jakšić BL (1957) Brucellose et syphilis du lievre. Vet Glas 11:423–425

Lumeij JT (1994) Oproep voor het inzenden van materiaal van hazen met syphilis. De Nederlandse Jager 1994(24)

Lumeij JT, De Koning J, Bosma RB, Van der Sluijs JJ, Schellekens JFP (1994) Treponemal infections in hares from the Netherlands. J Clin Microbiol 32:543–546

Mörner T (1999) Monitoring disease in wildlife—a review of diseases in the orders lagomorpha and rodentia in Sweden. Verh ber Erkrg Zootiere 39:255–262

Stackebrand FW, Garrity GM, Grimont PAD, Kämpfer P, Maiden MCJ, Nesme X, Rossello-Mora R, Swings J, Trüper HG, Vauterin L, Ward AC, Whitman WB (2002) Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. Int J Syst Evol Microbiol 52:1043–1047

Strouhal M, Smasj D, Matejkova P, Sodergren E, Amin AG, Howell JK, Norris SJ, Weinstock GM (2007) Genome differences between *Treponema pallidum* subspecies *pallidum* Strain Nichols and *T. paraluiscuniculi* Strain Cunicul A. Infect Immun 75:5859–5866

University of Cambridge Department of Clinical Veterinary Medicine (2000) Postmortem reports on 12 hares killed at coursing meetings in March 2000. http://www.huntinginquiry.gov.uk/mainsections/research/hares.htm (downloaded on 24 February 2010, 16.10)

U.S. Public Health Service (1969) Manual test for syphilis. Publication No. 411. U.S. Governement Printing Office, Washington, D.C., p 15–43

Wiwanitkit V (2009) A cost-utility analysis of *Treponema pallidum* haemagglutination (TPHA) testing for syphilis screening of blood donors: is the TPHA test useful for syphilis screening in a blood center. Blood Transfus 7:65–66

Wuthe H-H, Blew J (1996) Antikörper gegen *Treponema* spec. sind beim Feldhasen (*Lepus europaeus* Pallas) sehr häufig. Z Jagdwiss 42:284–288