Treponemal Infections in Hares in The Netherlands

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Treponemal infections in wild European brown hares (Lepus europaeus) from The Netherlands were diagnosed by means of serological tests for human syphilis and histological demonstration of spirochetes by the Bosma-Steiner silver impregnation method in histological sections of skin lesions. The treponeme should probably be classified as “Treponema paralysedepos.”

Treponemal infections in wild European brown hares (Lepus europaeus) are rarely reported. The existence of natural spirochitosis in hares was suggested over 100 years ago (3) on the basis of granulomatous lesions on the genital organs, but the etiological agent was not demonstrated. Treponemes have been demonstrated in about 20% of trapped male hares with purulent skin lesions by India ink background relief staining (10). Serological evidence for treponemal infections was found in 27% of 202 trapped hares, which were selected from a total of 15,000 trapped hares on the basis of lesions on external genital organs or testicular atrophy, or both (9). Treponemes, however, were not demonstrated by dark-field microscopic examination of extracts of atrophic testes. In another study (8), superficial ulcerations or hemorrhagic crusts, or both, were seen on the genital skin in about 1% of 1,400 trapped hares, and treponemes similar to Treponema pallidum were demonstrated by dark-field microscopy in one-third of the affected animals. An attempt to transmit the disease by injecting tissue fluid from genital lesions of dark-field microscopy-positive hares into the scrotum of New Zealand White rabbits resulted in a superficial scrotal lesion of about 5 cm in diameter containing treponemes after 110 days in one rabbit. The same (German) name has been used (Hasensyphilis, Knotenseuche) to describe brucellosis and spirochetal infections in hares (11, 12, 16).

This report presents both serological and histological evidence for natural spirochitosis in hares.

In two male hares which were shot in The Netherlands, a dermatitis was observed on the oral skin and the prepuce (Fig. 1); the dermatitis was similar to the dermatitis seen in rabbits with syphilis. This observation prompted investigations into the possible involvement of Treponema spp. The hares were shot in an area with a low hare population.

Specimens from the lips and preputial area were fixed in 10% buffered formalin. The tissues were then embedded in paraffin, and sections were cut to 6 μm in thickness. Histological examination was performed on sections stained with hematoxylin and eosin, periodic acid-Schiff, and Giemsa and Grocott’s stains. Additionally, sections were stained for spirochetes by the Bosma-Steiner method (4–6). In this modified technique, a short-time, high-concentration silver impregnation is used after treatment with amylose.

Serology was performed by using the T. pallidum hemagglutination assay (TPHA; Fujirebio, Tokyo, Japan), Veneral Disease Research Laboratory (VDRL) test (Wellcome Diagnostics, Dartford, England), the fluorescent treponemal antibody absorption (FTA-ABS) test (15), and an immobilization test with Percoll-purified treponemes of the Nichols strain of T. pallidum that was propagated in rabbits (7). In the FTA-ABS test, serum antibodies directed against T. pallidum were detected with a fluorescein isothiocyanate-labeled horse anti-rabbit immunoglobulin (diluted 1:400; Rijksinstitut voor Volkgezondheid en Milieuhygiène, Bilthoven, The Netherlands). The immobilization assay was performed as reported previously (7). The sera from the two infected hares and from four TPHA-, VDRL-, and FTA-ABS test-negative hares were used in this assay at a final content of 10% (wt/vol). The percentage of mobile treponemes was determined in wet mounts after 0, 1, 2, 3.5, and 5.5 h by observing at least 100 treponemes in randomly selected microscopic dark fields.

In both hares, an inflammatory reaction was seen in the prepuce. The reaction consisted of plasma cells, lymphocytes, and segmented neutrophilic leukocytes. The Bosma-Steiner-stained sections showed a light yellow background with many black spirochetes. The spirochetes, which were morphologically similar to T. pallidum and Treponema pallidum, were demonstrated in the prepuce, especially in the superficial epithelial layer (Fig. 2A and B), but in some places, the microorganisms were observed beneath the inflammatory reaction, sometimes invading the walls of blood vessels (Fig. 3A and B). Serology of the two hares revealed TPHA titers of 1:20,480 and 1:40,960, respectively, while the VDRL test titers were 1:64 and 1:8, respectively. Both sera were positive (2+) in the FTA-ABS test. In the immobilization assay, the two hares showed a significantly lower prevalence of surviving treponemes in comparison with the prevalence in negative control hares (50 versus 70% after 5.5 h; P < 0.01, one-sided Student t test for unpaired observations).

This report provides the first histological evidence of treponemal infections in hares. The morphology of the etiological agent is similar to those of T. pallidum and T. paralysedepos. Furthermore, this is the first time that a treponemal infection has been reported in wild hares in The...
Netherlands. Failure to demonstrate the causative agent in suspected cases of hare syphilis is common (3, 8-10). The main difficulty in staining spirochetes with silver stains is background interference, especially in tissues with much reticulin and collagen fibers, and the formation of mucopolysaccharides which surround the bacteria and interfere with the uptake of the silver stain. The Bosma-Steiner silver staining method used in the present study is considered an important improvement for the detection of Treponema spp. in lagomorphs. Digestion of mucoid substances by amylase treatment and use of short-time, high-concentration silver impregnation results in better visualization of spirochetes in formalin-fixed tissue sections (4-6). The histologically observed combination of an inflammatory reaction with plasma cells and spirochetes in the epidermis and around blood vessels is suggestive of a treponemal infection. Indeed, the serologies of both hares revealed high antibody titers to the obligate human pathogen T. pallidum (in TPHA). Experimental or natural infection with different pathogenic treponemal species (e.g., T. paraluiscuniculi) in rabbits is followed by production of antibodies that cross-react with T. pallidum (2). Likewise, in humans as well as rabbits, antibody binding to the nontreponemal cardiolipin antigen applied in the VDRL test is associated with the activity of a treponemal infection (2). Therefore, it is concluded that the positivity of the TPHA test in these hares indicates treponemal infection but does not further specify the treponemal species involved, while the positivity of the VDRL test in both hares indicates the actual activity of a treponemal infection. In the immobilization assay, a significant reduction in the number of surviving treponemes was observed in
FIG. 3. (A) Section of hare prepuce with blood vessel infiltrated with spirochetes. Bosma-Steiner staining was used. Original magnification, ×400. (B) Detail of panel A. Note the spirochetes in the vessel wall, which has been indicated with three symbols (◇). Bosma-Steiner staining was used. Original magnification, ×600.

comparison with the number in negative control hares. The percentage of surviving treponemes, however, was higher than would be expected on the basis of the results of studies involving human antibodies directed against *T. pallidum* (7). This might be due to a higher degree of specificity of the immobilization assay compared with those of the other tests used. Thus, at present it is not clear whether this organism is identical to *T. paraluiscuniculi* from rabbits or whether it is a species-specific pathogen in hares. Since the treponeme has been isolated from infected hares and serological evidence exists for a natural treponemal infection in wild hare populations (9), the treponeme should probably be classified as "*T. paraluisleporis*," in analogy with *T. paraluiscuniculi* from rabbits. Infection of wild hares with *T. paraluiscuniculi* from rabbits does not seem likely. Claims as to the occurrence of sexual intercourse between rabbits (*Oryctolagus cuniculus*) and hares that results in naturally occurring hybrids, so-called leporides (13), are anecdotal and have not been substantiated by artificial insemination experiments of rabbit does with hare semen or vice versa (1, 14). Further studies are in progress by artificially infecting both rabbits and hares (using *T. paraluiscuniculi* and "*T. paraluisleporis*" both intratesticularly and intrapreputially after scarification of the prepuce) and characterizing 16S rRNA from *T. pallidum* subsp. *pallidum*, *T. pallidum* subsp. *pertenue*, *T. paraluiscuniculi*, and "*T. paraluisleporis*" to try to elucidate the taxonomy of "*T. paraluisleporis*." Antigenic cross-reactivity between these treponemes will be examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting (immunoblotting) techniques.

The localization of the lesions and the low number of hares present in the area suggest that the infection may interfere with reproduction, as is also known to occur among rabbits with syphilis. Serological studies are in progress to investigate the spread of this disease in the area involved.

Preliminary results indicate that the infection is wide-
spread, since about 60% of a representative sample of the population (n = 100) of an area of 100,000 ha was seropositive in the TPHA. Intratesticular injection of two rabbits with a treponemal suspension harvested from preputial lesions of naturally infected hares resulted in orchitis and seroconversion in both rabbits. Furthermore, one of the rabbits developed a scrotal dermatitis at the injection site 6 weeks after inoculation.

REFERENCES