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Short communication

Is there a difference between hare syphilis and rabbit syphilis? Cross infection experiments between rabbits and hares

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ABSTRACT

Cross infection of rabbits and hares with *Treponema paraluiscuniculi* from rabbits and the related microorganism from hares, which was provisionally named "*Treponema paraluisleporis*", revealed that *T. paraluiscuniculi* affects rabbits clinically, but only causes seroconversion in hares without causing clinical disease, while "*T. paraluisleporis*" induces disease in both rabbits and hares. The 16S rRNA gene of "*T. paraluisleporis*" was sequenced (GenBank acc. no. JX899416) and compared to the sequence of *T. paraluiscuniculi* strain Cuniculi A. A phylogenetic tree based on the sequence alignment of 2002 bp taken from several treponemal strains was constructed. Both "*T. paraluisleporis*" and *T. paraluiscuniculi* are clustered together indicating their common origin. The close phylogenetic relatedness of both representatives supports the conclusion that subspecies or ecovar status should be given to these strains rather than species status. A more appropriate species name might be *Treponema paraluisleporidarum*. The genitive refers to the nominative *Leporidae* (family of rabbits and hares). The naturally occurring strain in rabbits would than be *T. paraluisleporidarum* ecovar Cuniculus and the strain from hares *T. paraluisleporidarum* ecovar Lepus. Since the former seems to have fewer physiological hosts, ecovar Lepus may represent an evolutionary ancestor of ecovar Cuniculus.

1. Introduction

The isolation of treponemes from hares (Lumeij et al., 1994; Lumeij, 2011) prompted the question whether this spirochaete was the same as *Treponema paraluiscuniculi*, causing syphilis in rabbits, or whether it was a strain adapted to the European brown hare, *Lepus europaeus*. The aim of the present study was to investigate the susceptibility of hares to *T. paraluiscuniculi* from naturally infected rabbits and, reciprocally, the susceptibility of rabbits to "*Treponema paraluisleporis*" from naturally infected hares. A further aim was to induce hare syphilis in seronegative hares with "*T. paraluisleporis*" which had been passed through rabbits by intratesticular injection. Another part of the study aimed at sequencing the 16S ribosomal DNA of "*T. paraluisleporis*" for comparison with the 16S rDNA of *T. paraluiscuniculi* strain Cuniculi A from rabbits. Experiments were approved by the Animal Experimentation Committee of the Faculty of Veterinary Medicine at Utrecht University.

2. Materials and methods

Genital lesions from a hunter killed seropositive hare with a naturally acquired syphilis infection (Z27 A77/78) were externally disinfected and a tissue sample was partially used for histology and partially grinded to a tissue suspension under aseptic conditions. The histologically confirmed infected tissue suspension was injected intratesticularly in a clinically healthy and seronegative rabbit (#167). After development of typical lesions in rabbit #167

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and serological and histological confirmation of syphilis, the same procedure was repeated and infective tissue suspension from this animal was inoculated through praeputial scarifications in a seronegative hare (AE71) and another seronegative rabbit (#168). Following the same protocol infected tissue suspension from rabbit 093.0575.P naturally infected with *T. paraluiscuniculi* was injected intratesticularly or applied in scarifications in the vulva or praeputium in three seronegative hares (C21B, F21DC, and 927D, respectively).

Serology in inoculated animals was performed by *Treponema pallidum* haemagglutination assay (TPHA, Fujirebio, Tokyo, Japan) and Fluorescent Treponemal Antibody Absorption (FTA-Abs) test, and the Venereal Disease Research Laboratory (VDRL) test (Wellcome Diagnostics, Dartford, England) (Egglestone and Turner, 2000). The latter is nonspecific and detects antibodies to cardiolipin. Tissue sections were stained with the Bosma-Steiner silver stain as used in a previous study for demonstration of "*T. paraluisleporis*" in hares (Lumeij, 2011).

A tissue sample (designated Hare Netherlands 13/12/2010; ~500 μl) taken from another naturally infected hare from the same population as Z27 A77/78 was minced with 500 μl of PBS buffer. In order to separate treponemes from the eukaryotic cells, the sample was briefly centrifuged at 400 × *g* for 5 min and the supernatant containing treponemes was transferred into a clean tube. This process was repeated three times to remove the majority of eukaryotic cells. To sediment the treponemes, the final solution was centrifuged at 14,100 × *g* for 10 min. The DNA from treponemal cells was amplified using a QIAGEN Whole Genome Amplification REPLI-g Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Amplified DNA was purified with a QIAEX II Gel Extraction Kit (Qiagen) according to the recommended protocol. Pathogenic non-cultivable treponemes contain two rRNA operons (*rrn1* and *rrn2*). Both rRNA operons consist of 16S-23S-5S genes and differ by the presence of tRNA-Ile or tRNA-Ala genes in the intergenic spacer region between 16S and 23S rRNA genes (Čejková et al., 2013).

A nested PCR protocol was used for amplification of both treponemal 16S rRNA regions. The PCR reaction (20 μl) contained 0.4 μl of 10 mM dNTP mix, 2.0 μl of 10× ThermoPol Reaction buffer, 0.1 μl of each primer (100 pmol/μl), 0.1 μl of *Taq* polymerase (5000 U/ml, New England BioLabs, Frankfurt am Main, Germany), 1 μl of DNA and 16.3 μl of PCR-grade water. PCR amplification was completed under the following cycling conditions: 94 °C (5 min); 94 °C (60 s), 72 °C (20 s), 72 °C (150 s), 40 cycles; 72 °C (10 min). The second step of nested PCR reaction was done under the same conditions with the exception of the lower primer annealing temperature (67 °C). In the first step of the nested PCR amplification of 16S rDNA region 1 and 2, a combination of primers were used; RNA1Fb (5′-CCTTGTAGACGTGGTTTTACC-TAATTCCGTGAAGGAAAT-3′) and RNA1-SD-tRNA-Ile5 (5′-GATTAGAGTTGAACTAATGACCCCTTCCTTATCAGA-GAAGTGCTCTAACCAAC-3′) and RNA2Fc (5′-CCGTGAAG-GAAATTGGATCTGGGGC-3′) and RNA2-SD-tRNA-Ala5 (5′-CCCTGGAGATAAGGGGACTCGAACCCCTGACCTACGACCTG-CAAAGCCGTCGCTCTAGCCAGT-3′), respectively (Fig. 1). The lengths of corresponding PCR products were 2.2 kb in both amplifications. In the second PCR step, the amplified DNA from the first step was used as template DNA. The first 16S rDNA region was amplified as two overlapping DNA regions, in two separate amplification reactions, containing RNA1Fc (5′-CCGTGAAG-GAAATTGGATCTGGGGC-3′) and TP0225-6BR (5′-GGGTAAGGTTCCTCGCGTAT-3′) – and – TP0225LF (5′-GCGAACAGGATTAGATACCC-3′) and RNA1-SD-tRNA-Ile1 (5′-ACTGAGCTACAAGCCCCTTT-3′) primers, respectively

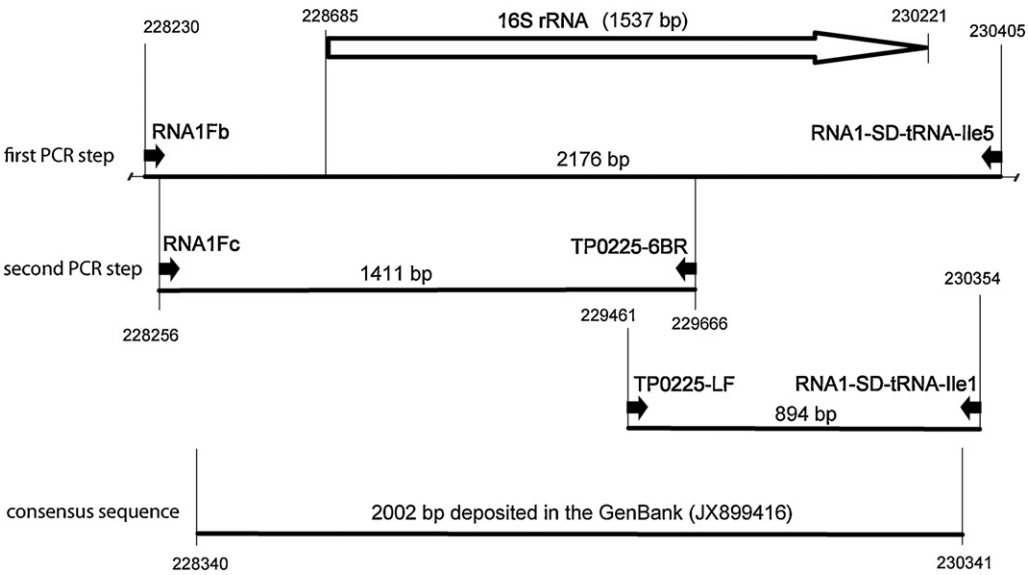

Fig. 1. A schematic representation of nested PCR amplification of the *rrn1* region (containing 16S rRNA gene) used in this study. Primers used in the first and in the second PCR step are shown as arrows. Positions of the 16S rRNA gene and the consensus sequence deposited in the GenBank are indicated. Primers are not shown in scale. The *rrn2* region was amplified similarly to *rrn1* region (see text for details).