

# Susceptibility of rabbits venereally infected with *Treponema paraluis-cuniculi* to superinfections with *Treponema pallidum*

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**SUMMARY** Three female rabbits, venereally infected with *Treponema paraluis-cuniculi* between five and eight months previously, had treponeme-containing genital lesions and positive results to serological tests for syphilis. These rabbits and four normal female rabbits were challenged with *Treponema pallidum* intradermally on the shaved back with triplicate doses of  $10^4$ ,  $10^3$ ,  $10^2$ , and  $10^1$  treponemes (all at different sites). Significantly fewer syphilitic lesions developed in the rabbits previously infected with *T paraluis-cuniculi* (4/36) compared with the control rabbits (37/48), and the mean size of the lesions (diameter of induration) was significantly less (4 mm compared with 10 mm). It appears that previous venereal infection of female rabbits with *T paraluis-cuniculi* induced a level of cross-immunity against infection with *T pallidum*. Protection was however not complete.

## Introduction

*Treponema paraluis-cuniculi* and *Treponema pallidum*, which cause rabbit venereal spirochaetosis and human syphilis respectively, are closely related morphologically<sup>1</sup> and antigenically.<sup>2</sup> Previous studies<sup>2-4</sup> have shown an appreciable level of immunity to *T pallidum* in rabbits inoculated intratesticularly with *T paraluis-cuniculi* between six and eight months previously.

In the experiment now reported venereally infected female rabbits (the natural route of infection with *T paraluis-cuniculi*) were challenged with low serial 10-fold doses of *T pallidum* ( $10^4$ ,  $10^3$ ,  $10^2$ , and  $10^1$ ) intradermally on the shaved back, and the degree of syphilitic infection was measured by the percentage of inoculation sites at which lesions developed and their size. A comparison was made with normal female rabbits challenged with *T pallidum* to ascertain the degree of protection against *T pallidum* offered by the previous venereal infection with *T paraluis-cuniculi*.

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## Materials and methods

### INFECTION WITH *T PARALUIS-CUNICULI*

A male rabbit (rabbit No 82673) was experimentally infected with *T paraluis-cuniculi* and placed in a pen with four normal female rabbits, as described.<sup>5</sup> The four female rabbits became venereally infected, as shown by the development of treponeme-containing lesions and seroconversion about five months after cohabitation with the infected male rabbit.<sup>5</sup> Six months afterwards, when lesions were still present in three of the four female rabbits, they were challenged with *T pallidum*.

### CHALLENGE WITH *T PALLIDUM* (NICHOLS STRAIN)

*T pallidum* was obtained from the testes of a rabbit inoculated intratesticularly with  $5 \times 10^7$  *T pallidum* about 10 days previously. Details of procedure and elution medium used were as published.<sup>6</sup> The same anaerobic medium was used to dilute the *T pallidum* to  $10^5$ /ml and thence in serial 10-fold dilutions to  $10^2$ /ml (under anaerobic conditions).

The four control and four *T paraluis-cuniculi*-infected rabbits had their backs shaved and marked into a  $3 \times 4$  grid, each of the 12 squares being approximately 15 mm in length. Into each square 0.1 ml of *T pallidum* suspension was inoculated

intradermally, so that each rabbit received triplicate inoculations of  $10^4$ ,  $10^3$ ,  $10^2$ , and  $10^1$  *T pallidum*.

The rabbits were examined twice a week for five weeks and the development of indurated syphilitic lesions on the backs was recorded. The rabbits' backs were kept shaved during this period.

#### SEROLOGICAL MONITORING

The rapid plasma reagin (RPR) test was carried out according to the manufacturer's instructions (Commonwealth Serum Laboratories, Melbourne, Australia). Results were recorded as strongly positive, weakly positive, or negative. The *Treponema pallidum* haemagglutination assay (TPHA) was performed using the kit produced by Fujizoki Pharmaceutical Company Ltd. The manufacturer's instructions were followed. Only titres greater than 1/80 were considered positive and therefore indicative of past or current treponemal infection. Known positive and negative control sera were included in each test series. Previous studies have shown that results to these two tests become positive in venereal rabbit spirochaetosis.<sup>5</sup>

#### Results

One of the *T paraluis-cuniculi*-infected rabbits was excluded from the experimental analysis because it had been badly attacked by the male rabbit and had numerous mating bites and infected claw injuries on its back, making detection of classical indurated *T pallidum* lesions at the site of inoculation very difficult.

As shown in the table, the rabbits which had previously been infected venereally with *T paraluis-cuniculi* had developed some degree of protection against challenge with *T pallidum*, as shown by the smaller number of lesions which developed at the inoculation sites and their smaller size, even though the protection was not complete.

After venereal infection of the three female rabbits with *T paraluis-cuniculi* they developed positive

results to the RPR test and TPHA. The uninfected control rabbits had negative results. After challenge with *T pallidum* the test rabbits continued to have positive results and the results for the control rabbits became positive in both serological tests.

#### Discussion

Turner and McLeod<sup>4</sup> and Turner *et al*<sup>3</sup> carried out studies on the susceptibility of rabbits experimentally infected intratesticularly with *T paraluis-cuniculi* to challenge with *T pallidum* (Nichols strain and strain CJ). They found considerable, but not complete, protection. As they comment, their experiments were a severe test of the immune system of the *T paraluis-cuniculi*-infected rabbits, as their intradermal challenge inocula were approximately  $2 \times 10^6$  *T pallidum*. Nevertheless some protection was evident by the absence, or slow development, or smaller size of the lesions after challenge with *T pallidum*. Their results were sufficiently impressive for them to write, "While no (syphilis) vaccine is in sight, the problem (human syphilis) is sufficiently important to warrant the exploration of all avenues of approach, including the potentialities of *T cuniculi* as an immunising agent."

Subsequent work, reported by Turner and Hollander<sup>2</sup> involved inoculating rabbits intratesticularly with *T paraluis-cuniculi* (strain A) and eight months thereafter challenging with a low dose (500 treponemes) of *T pallidum* (Nichols) intradermally on the shaved back. As in the earlier experiments partial protection was obtained.

The purpose of the experiment reported in this paper was to determine whether or not rabbits naturally infected by the venereal route (as distinct from experimental intratesticular inoculation) with *T paraluis-cuniculi* were resistant to low challenge doses of *T pallidum* (Nichols) given intradermally. From the results it appears that significant protection did occur. Low challenges ( $10^1$  and  $10^2$ ) of *T pallidum* failed to cause any observable lesions in the

TABLE Development of syphilitic lesions in normal rabbits and in rabbits previously infected with *Treponema paraluis-cuniculi* on intradermal (id) challenge with four different doses of *Treponema pallidum* (Nichols strain)

| <i>T pallidum</i> challenge doses (id) | Normal female rabbits*         |                                      | <i>T paraluis-cuniculi</i> -infected female rabbits† |                                      |
|--|--------------------------------|--------------------------------------|--|--------------------------------------|
|  | No of lesions/sites inoculated | Diameter of lesions (mm) (mean ± SD) | No of lesions/sites inoculated                       | Diameter of lesions (mm) (mean ± SD) |
| $10^4$                                 | 10/12                          | 11.0 ± 6.9                           | 3/9  | 4.0 ± 3.6                            |
| $10^3$                                 | 10/12                          | 11.1 ± 3.5                           | 1/9  | 4.0                                  |
| $10^2$                                 | 11/12                          | 7.8 ± 5.7                            | 0/9  | NL                                   |
| $10^1$                                 | 6/12                           | 8.3 ± 3.6                            | 0/9  | NL                                   |
| Total                                  | 37/48                          |                                      | 4/36   |                                      |

\*Four rabbits

†Three rabbits

NL = no lesion

immunised rabbits and higher challenges ( $10^3$  and  $10^4$ ) caused significantly fewer and significantly smaller lesions in the immunised rabbits (table).

It appears that *T paraluis-cuniculi* does offer some protection to rabbits against low challenge doses of *T pallidum*. Would it do the same for humans, and could it be a potential vaccine against syphilis? Further studies, in both rabbits and humans, are required to clarify this important question.

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