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Lack of serological evidence for venereal spirochaetosis in wild Victorian rabbits and the susceptibility of laboratory rabbits to Treponema paraluis-cuniculi

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SUMMARY Sera from 608 wild rabbits were examined using serological tests for syphilis as an indicator of infection with *Treponema paraluis-cuniculi.* Only eight sera gave positive or weakly positive results in the rapid plasma reagin (RPR) test, and none of these eight sera gave positive results in the *Treponema pallidum* haemagglutination assay (TPHA). Thus, it appears that wild rabbit populations in Victoria, Australia, are not naturally infected with *T paraluis-cuniculi.*

Normal Australian laboratory rabbits however were readily infected with *T paraluis-cuniculi,* either by intradermal or intratesticular inoculation or by the venereal route. In the latter case, treponeme-containing lesions developed after about five months' cohabitation with infected mates. The disease was successfully transmitted from male to female and from female to male rabbits by the venereal route. In most cases infected rabbits became RPR-positive (17/19 rabbits) and in all cases TPHA-positive (19/19), indicating that serological tests for syphilis can be used to screen rabbits for this disease.

Introduction

Rabbit venereal spirochaetosis is a sexually transmitted bacterial disease caused by *Treponema paraluis-cuniculi.* It occurs in European wild and laboratory rabbits, *Oryctolagus cuniculus* (L), in laboratory rabbits in North America, and probably in European hares,[1] *Lepus europaeus* (Pallus), but it has not been reported in Australia.

Rabbits are not native to Australia. They were introduced in the late eighteenth and nineteenth century as a result of European colonisation of the continent and became well established as a feral animal in Victoria during the 1860s. Although the predominant progenitors were wild-type rabbits, domestic rabbits contributed to the gene pool in several locations.[2]

T paraluis-cuniculi is closely related, morphologically and antigenically, to *Treponema pallidum,* the causative agent of syphilis in man.[3] Rabbits infected with *T paraluis-cuniculi* develop antibodies that can be detected using standard serological tests for syphilis.[4] In this study sera taken from wild rabbits from various parts of Victoria were tested for treponemal antibodies using both a non-specific test for syphilis, the rapid plasma reagin (RPR) test, and a highly specific test for treponemal infection, the *T pallidum* haemagglutination assay (TPHA).

Australian laboratory rabbits were also tested for their susceptibility to *T paraluis-cuniculi* by intradermal, intratesticular, intraurethral, and venereal transmission, and the infections were followed clinically, bacteriologically, and serologically.

Materials and methods

Wild rabbits were collected from 19 areas* of Victoria.[5] Blood was taken via the marginal ear vein from live rabbits and from the jugular vein or heart of shot rabbits. The serum was collected and stored frozen until required.

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*Details of the areas surveyed can be obtained from the authors on request.

SEROLOGICAL TESTS

The 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 TPHA was performed at Fairfield Hospital, Melbourne, or at Monash University, 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.

INFECTION WITH *T PARALUIS-CUNICULI* (LABORATORY RABBITS)

Intradermal inoculation

T paraluis-cuniculi (strain 8816) was obtained from the Center for Disease Control, Atlanta, Georgia, USA, by courtesy of Dr A Balows. It was sent to Australia in the frozen orchitic testis of a rabbit that had been infected intratesticularly. It has subsequently been maintained by intratesticular inoculation in rabbits or stored frozen (−70°C) in an equal mixture of 30% glycerol in isotonic saline (pre-reduced) and *T pallidum* maintenance medium.[6] Male rabbits inoculated intradermally on the shaved back or intratesticularly were kept in individual cages at 16-19°C with antibiotic-free food and water in unlimited quantities.

Venereal transmission

In the venereal transmission experiments rabbits were kept in spacious open pens (completely surrounded with wire-mesh) at the Keith Turnbull Research Institute, Frankston, Victoria. They were fed antibiotic-free food and water and occasionally grass.

In the experiment to test male-to-female venereal transmission of *T paraluis-cuniculi* one experimentally infected male rabbit (No 82673) was enclosed with four normal female rabbits. The male rabbit had been infected intratesticularly, intraurethrally, and under the prepuce (Table I) with a concentrated (approximately 10^7/ml) preparation of *T paraluis-cuniculi* obtained from frozen stocks in glycerol/medium. It was not possible to place more than one male rabbit in the pen with the females because of fighting between the males.

In the experiment to test female-to-male venereal transmission of *T paraluis-cuniculi* one normal male rabbit (No 83324) was enclosed with the four female rabbits above after they had all become infected, as shown by the presence of treponeme-containing genital lesions. The previous male rabbit (No 82673) was removed at this stage.

EXAMINATION FOR LESIONS

Rabbits were examined at regular intervals for genital lesions. Genital scrapings were taken by gently abrading the surface of the lesion with a scalpel blade and adding drops of sterile saline (approximately 0·5 ml) from a Pasteur pipette. The exudate-saline mixture was collected in the same Pasteur pipette and examined by darkfield microscopy for the presence of treponemes. Blood samples were taken from the marginal ear vein and the serum examined for antibodies by the RPR test and the TPHA.

Results

WILD RABBITS

Of the 608 rabbit sera examined, only eight gave positive results in the RPR test, and of these four were weak reactions. These eight sera all gave negative results to the TPHA.

TABLE I *Treponema paraluis-cuniculi infection in male laboratory rabbits with site of inoculation*

	Rabbit No and site of inoculation				
	580	*581*	*582*	*607*	*82673*
Response to infection	*Testes*	*Testes*	*Prepuce/ urethra**	*Testes/ prepuce/ urethra*	*Testes/ prepuce/ urethra*
Orchitis	Yes	Yes	No	Yes	No
Transient granular surface lesions on testes (treponemes present)	No	Yes	No	No	No
Erythematous lesions on prepuce and external genitalia (treponemes present)	No	Yes	Yes	No	Yes
Spontaneous healing of lesions	†	Yes	Yes	Yes	Yes
RPR test (days after infection)	†	+ + (75)	+ (195)	+ (35)	± (48)
TPHA titre (days after infection)	†	1/640 (137)	1/640 (138)	1/1280 (70)	1/1280 (120)

*Surface inoculation only; skin not broken
†Rabbit killed at height of orchitis to transfer bacterium
+ + Very strongly positive; + strongly positive; ± Weakly positive

One of us (RCHS), having examined thousands of wild rabbits throughout Victoria, has never seen genital lesions resembling those of *T paraluis-cuniculi* as manifested in the venereally infected laboratory rabbits in this study. We therefore conclude that this disease is not present in wild Victorian rabbits.

INFECTION WITH *T PARALUIS-CUNICULI* (LABORATORY RABBITS)

Intradermal inoculation

Ten male rabbits, each inoculated intradermally at one site on their shaved backs with 2×10^7 viable treponemes, usually developed lesions by day 6 after inoculation, although in one (No 17) they took 55 days to develop (table II). The early lesions appeared as small areas of erythema and induration in the skin, which gradually enlarged. These early lesions were very similar to early *T pallidum* lesions in rabbits.[3] They reached their maximum size (usually 5-9 mm diameter induration) at various times between 10 and 136 days after inoculation (table II) and then regressed at varying rates. In four of the rabbits the lesions disappeared between 55 and 70 days after inoculation; in another two at about 218 days and in another three the lesions were still present 365 days after inoculation, at the end of the observation period.

Both the RPR test and TPHA gave positive results 84 days after inoculation, indicating that the infection had stimulated both a specific and non-specific antibody response in the rabbits.

As the dermal lesions faded they became less indurated with a smaller zone of erythema. In most cases the lesion surface became granular and crumbled on gentle abrasion. In three rabbits (Nos 14, 21, and 23) secondary (or satellite) lesions formed around the original lesion 202, 149, and 246 days after inoculation. In all cases they were considerably smaller than the original lesion. These three rabbits still had their original lesion 365 days after inoculation and the development of secondary lesions probably represented a poor or slow development of immunity. In one rabbit the four satellite lesions healed before the end of the observation period whereas in the other two they were still present.

Other sites of inoculation

In this study two rabbits were inoculated intratesticularly, two intratesticularly, intraurethrally, and under the prepuce, and one intraurethrally and under the prepuce, care being taken not to pierce the skin in the latter case.

All five rabbits became infected and the four rabbits tested by the RPR test and TPHA developed antibodies (table I). The location of the lesions varied depending partly on the site of infection (table I), but in all cases the lesions healed spontaneously. Rabbits Nos 607 and 82673 were capable of producing normal offspring. The other three rabbits were not tested.

Male-to-female venereal transmission

Male rabbit No 82673 developed treponeme-positive genital lesions after intratesticular, intraurethral, and sub-preputial inoculation with viable *T paraluis-cuniculi* from frozen material. Immediately after inoculation he was placed in a pen with four normal serologically negative female rabbits (Nos 658, 91326, 91249, and 21271) to ascertain whether or not he would transmit the infection to the female rabbits. All rabbits were examined at monthly intervals.

TABLE II *Treponema paraluis-cuniculi infection in male laboratory rabbits infected intradermally with* 2×10^7 *treponemes on the shaved back*

	Rabbit No									
Response to infection	*104*	*23*	*15*	*14*	*939*	*940*	*21*	*22*	*17*	*16*
Latent period of infection (days)*	6	6	6	6	6	20	55	8	10	6
Maximum size (diameter) of indurated lesion (mm)	6	9	18	6	6	±§	5	8	7	7
Time after inoculation when lesion most strongly indurated (days)	14	41	84	14	20	41**	136	10	55	10
Time after inoculation when lesions fully regressed (days)	55	>365†	>178‡	>365†	218	70	>365†	70	218	70
RPR test at 84 days after inoculation	+	±	+ +	+	+ +	+	±	+	+	±
TPHA at 84 days after inoculation (titre)	1/640	1/1280	>1/5120	1/1280	>1/5120	1/640	1/640	1/1280	1/1280	1/640

* Time between inoculation and first appearance of lesion
** Time of maximum zone of erythema (not induration) as lesion was only slightly indicated
† Experiment terminated at 365 days: lesions still present
‡ Rabbit destroyed at 178 days because of intercurrent ear infection
§ Very slight induration with a maximum zone of erythema of 18 mm diameter
+ + Very strongly positive; + strongly positive; ± weakly positive

The female rabbits developed genital lesions containing large numbers of treponemes about five months later (table III). The lesions developed as generalised inflammation and oedema of the labia, which later became crusted. It was not possible to quantify them. In all four rabbits lesions were transient even though treponemes could be readily isolated from the labial epidermis and mucosa by gentle scraping (table III), indicating that a rabbit may be infected and infectious without showing obvious lesions. Six months after cohabitation with the infected male all four female rabbits had developed specific antitreponemal antibodies, as shown by a positive TPHA titre, and three had also developed non-specific antibodies, as detected by the RPR test. One of the female rabbits (No 91249) may have been spontaneously cured by 10 months, when no lesions were seen and treponemes were not found in scrapings. This is uncertain however as the observation was terminated at that time.

TABLE III *Treponema paraluis-cuniculi infection in four female laboratory rabbits after cohabitation with an infected male rabbit*

	Rabbit No			
Response to infection	*658*	*91326*	*91249*	*91271*
Latent period of infection (months)*	3	6	5	5
Treponemes present in genital lesions or labial scrapings taken at:				
6 months†	+	+	+	+
7 months	+	+‡	+	+
8 months	+	+	+	+‡
9 months	+‡	+	+	+‡
10 months	+	+	−‡	+
RPR test result at:				
6 months	±	−	±	±
8 months	+	−	+	+
10 months	++	−	++	±
TPHA titre at:				
6 months	1/5120	1/640	>1/5120	1/320
8 months	1/5120	1/1280	1/1280	1/320
10 months	1/5120	1/1280	1/640	1/640

* Period between cohabitation with male rabbit and the development of genital lesions containing treponemes
† Infected male rabbit 82673 removed at 6 months and normal male rabbit 83324 placed in the pen to detect female-to-male venereal transmission
‡ No macroscopically visible genital lesions present
++ Very strongly positive; + strongly positive; ± weakly positive RPR test: − negative

Female-to-male transmission

The four venereally infected female rabbits (Nos 658, 91326, 91249, and 91271), with treponeme-containing genital lesions after six months' cohabitation with the originally infected male rabbit (table III), were placed in a pen with one normal male rabbit (No 83324). This male rabbit was also examined monthly. He developed treponeme-containing genital lesions after five months' cohabitation with the four infected female rabbits, when the TPHA gave a positive result at a titre of 1/320 but the result of the RPR test was negative. Observations were terminated at that time as proof of venereal transfer had been obtained.

Reproductive abilities

Male rabbits, infected intratesticularly with *T paraluis-cuniculi,* were able to sire normal healthy litters. Female rabbits, infected venereally, were also able to produce apparently healthy offspring. Two such kittens of infected mothers were examined serologically and found to have negative results to the RPR test even though the mother had treponemal antibodies.

Discussion

For many years rabbits have been used for research into human syphilis, primarily because they were susceptible to the human pathogen *T pallidum* and the disease closely resembled primary syphilis in man. It was not until 1912 however that Ross[7] suggested that the anogenital lesions seen in some rabbits may represent a spirochaetal infection. Bayon[8] examined these lesions and found spirochaetes 8-12 μ in length, with 5-8 spirals, which were indistinguishable from *T pallidum.* He concluded that this previously unrecognised spontaneous spirochaetal disease of rabbits may be a source of error in experiments on human syphilis in the rabbit. Several papers have reviewed the history of work on this disease[4 9 10] and several give extensive clinical and histological details of the disease after natural and artificial infection.[4 11-14]

The disease is apparently widespread in European wild and laboratory rabbits, North American laboratory rabbits, and European hares,[1] although there are no reports of it being present or absent in other parts of the world. On the basis of our serological survey of Victorian rabbits we tentatively conclude that the disease is absent from Australia. The reason for this must presumably be that the rabbits which became successfully established as feral populations were free of the disease, either because the introduced rabbits were carefully selected or fortuitously. It is unlikely that a disease which does not cause rabbits to become systemically ill would have resulted in their keepers killing them off on the long sea voyage to Australia. There are other instances of parasites of European wild or domestic rabbits which either did not arrive or failed to become established in Australia, for example, *Spilopsyllus cuniculi,* the European rabbit flea,[15] and *Encephalitozoon cuniculi.*[16] On the basis of a study of sera from 20 Victorian hares which gave negative serological results treponemal disease is probably

absent from this population also, although 27% of European hares had positive serological results for syphilis.[1]

The reported incidence of rabbit venereal spirochaetosis in naturally infected populations varies considerably. McLeod and Turner[14] found only six rabbits out of 1800 with treponeme-containing genital lesions in a survey of North American laboratory rabbits. Fried and Orlov[17] reported on two epidemics of rabbit venereal spirochaetosis in Moscow in 1927 and 1928. Only 0·7% of rabbits less than 8 months old were infected whereas 46·4% of rabbits older than 1 year were infected, suggesting its venereal mode of transmission. Artz and Kerl[18] found an incidence of 26·9% in adult European rabbits and Noguchi[11] reported incidences of 10% and 30% in North American laboratory rabbits. Adams *et al*[13] reported the disease to be endemic in British wild and hutch rabbits and found 14 of 228 laboratory rabbits with treponeme-containing genital lesions. He further added that "reputable observers" estimated that 10-40% of wild rabbits were affected.

Other surveys have been based on serological methods using tests designed for the diagnosis of human syphilis. A number of these reports are recorded by Smith and Pesetsky[9] using non-specific tests such as the Wassermann reaction and the Venereal Disease Research Laboratory (VDRL) test, both of which detect antibody to cardiolipin, as does the RPR test used in our study.

There is considerable difference of opinion on whether or not the disease gives rise to positive results to cardiolipin tests; some argue that it does[4 19 20] while others have found their results to be uniformly negative.[11 12] Our controlled experiments with venereally and experimentally infected rabbits may have settled this controversy. While most rabbits (17/19) did develop positive results to the RPR test, two did not (rabbits Nos 91326 and 83324), although treponeme-containing genital lesions were present. The result is obviously variable. With the more specific (and probably more sensitive) TPHA however all rabbits with lesions gave positive results (19/19). This is a fairly recent test and has not previously been used in rabbit venereal spirochaetosis. Other specific antitreponemal serological tests however have been used. Pannu *et al*[20] studied 30 normal rabbits, 10 of which had a positive VDRL test result, and found two had weakly reactive results to the fluorescent treponemal antibody absorbed (FTA-ABS) test, although in one case the result subsequently became non-reactive. Twenty-nine of 30 rabbits gave negative results to the *Treponema pallidum* immobilisation (TPI) test and the result on the other rabbit was not stated in the paper. There was no clinical or bacteriological evidence however that these rabbits were infected. Small and Newman[4] detected positive FTA-ABS test results in known infected rabbits with peak titres at the time of appearance of cutaneous lesions. They also detected positive RPR test results in all infected rabbits in contrast to our own observations. Significant titres (in the RPR and FTA-ABS tests) were present six months after inoculation at the end of their experiment.

In retrospect it appears that it may have been wiser to screen the wild Victorian rabbit sera with the TPHA rather than with the RPR test as the former gave uniformly positive results in infection. The RPR test was used however because it is generally considered to be the appropriate screening test in serological surveys of syphilis. Nevertheless, it is unlikely that our conclusions on the absence of this disease in wild Victorian rabbits would be altered by retesting all sera with the TPHA. The eight RPR-positive (or weakly positive) sera were all TPHA-negative and these are the sera most likely to have been from *T paraluis-cuniculi* infected rabbits.

Our demonstration of venereal transmission of *T paraluis-cuniculi* between rabbits was not new; others have previously done so.[11-13] However, our experiments did show that Australian laboratory rabbits were readily susceptible to this bacterium and that the absence of infection in the wild population, including rabbits known to be partly domestic in origin, is probably not due to innate rabbit resistance to the disease but to a failure of the pathogen to be introduced.

Our observation that *T paraluis-cuniculi* could be readily isolated from labial scrapings in infected female rabbits, even in the absence of microscopic lesions, has also been reported.[13] While our rabbits developed genital lesions about five months after cohabitation with infected mates, Noguchi[11] reported that a male rabbit developed lesions after three months in a similar situation. These time periods are not significantly different.

Two of the offspring of an infected, serologically positive female rabbit were themselves RPR-negative and apparently normal in every respect. This suggests that the infection did not become systemic and that a bacteraemia was not established, supporting earlier observations.[12 13] In general, maternal rabbit antibodies of any immunoglobulin class will pass into the fetal circulation,[21] so our failure to detect antibodies reacting in the RPR test in these newborn rabbits is difficult to understand.

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