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Experimental Syphilis and Serological Examination for Treponematosis in Hares

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Of 202 captive hares studied, many of which had lesions on their external genital organs or testicular atrophy or both, 27% had positive serological tests for syphilis although dark-field examination of extracts of atrophic testes was negative. A total of 12 hares that were nonreactive for the serological test for syphilis was inoculated with *Treponema pallidum*, 9 intratesticularly and 3 intradermally. Six of the animals inoculated intratesticularly exhibited orchitis after 7 days with an associated accumulation of treponemes. No chancres developed in the intracutaneously inoculated animals during a 27-day period of observation. These results provide additional evidence to support the contention that endemic treponematosis occurs in wild hares and suggest that hares are moderately resistant to experimental infection with *T. pallidum*.

The hare (*Lepus europaeus*) belongs to a different genus than the rabbit (*Oryctolagus cuniculus*). The resistance of the two species to various infections is different: only the rabbit can be infected with myxomatosis virus and only the hare can be infected with *Pasteurella tularensis*. In vivo (1) and experimental (15, 16) treponematosis of rabbits is well known and has been widely studied. To our knowledge, the pathogenicity of *Treponema pallidum* in hares has not been investigated.

A natural treponematosis in hares was suggested over 100 years ago (3) on the basis of granulomatous lesions on the skin of genital organs and enlargement of the testes, but without the demonstration of the etiological agent. Similar lesions have been reported to present caseous tuberosis (11) and brucellosis (4, 13). The only publication that has provided substantial evidence of a naturally occurring treponematosis of hares is that of Jaksits (7). He demonstrated the existence of *Spirochaeta pallida* subsp. *treponema* by India ink background relief stain in skin lesions on the genital organs of about 20% of the entrapped male hares with purulent skin lesions. The sores cleared up after treatment with neoarsphenamine or penicillin.

The principal purpose of the present study was to investigate the possibility of a naturally occurring treponematosis of hares. We also examined the susceptibility of hares to syphilis by infecting them with a pathogenic strain of *T. pallidum*.

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MATERIALS AND METHODS

From about 15,000 entrapped wild hares, 202 animals were selected. Although some lacked any signs of disease, others presented lesions on the skin of the genital organs or atrophy of the testes or both. Dark-field examination of extracts of atrophic testes failed to demonstrate the presence of treponemes. Blood was taken from the marginal vein of the ear for serological tests for syphilis. A total of 12 seronegative hares were reserved for infection studies with *T. pallidum* conducted after an adaptation period of 5 to 10 days. All animals were maintained on corn, carrots, and oats.

The serological test for syphilis performed included the Rapid Plasma Reagin test (RPR), Kolmer's complement fixation reaction with Reiter protein (RPCFR), cardiolipin complement fixation reaction (CCFR), and the *T. pallidum* immobilization (TPI) test.

The inoculum used for infecting hares was prepared with the Budapest strain (9) of *T. pallidum* harvested from the testes of rabbits 7 days after inoculation. The depilated dorsal skin of female hares was inoculated on each side of the midline with 0.2 ml of a suspension containing 4×10^7 treponemes per ml. In the case of males, the testes were injected with an inoculum of 0.5 ml of the above suspension. The infected testes were removed after 8 days, and a piece of tissue was fixed in formaldehyde and embedded according to the method of Szeky (14). The remaining tissue was extracted in a modified survival medium (6) and examined for the presence of treponemes by dark-field microscopy.

RESULTS

Serology. As shown in Table 1, of the 202 sera initially subjected to the RPR test, 56 (27.7%) were reactive. Subsequently 71 sera were chosen at random for further testing by comple-

ment fixation methods, 35 of which were RPR positive and 36 of which were RPR negative. All of the 35 RPR-positive sera gave positive complement fixation reactions, whereas 6 of the 36 RPR-negative sera gave positive CCFR and RPCFR tests. Another 6 sera were positive only by the RPCFR test. Of the various sera, 19 were selected for TPI testing. The 6 sera that gave positive reactions in all prior tests also gave positive TPI tests. From the remaining 13 sera, 3 were TPI negative (they were negative in all prior tests), and 10 sera were unsatisfactory for TPI testing.

Inoculation of hares with pathogenic *T. pallidum*. (i) Intracutaneous inoculation. Intracutaneous inoculation of three seronegative female hares evoked weakly palpable papules that waned after 4 to 5 days (Table 2). One animal died of unknown cause on day 12. On day 27 no chancres were observed on the two remaining animals.

(ii) Intratesticular inoculation. Intratesticular inoculation of nine seronegative hares resulted in the development of orchitis in six of the animals by day 7 (Table 2). The testes were slightly enlarged, endematous, and hyperemic. Adhesions were observed between membranes of the testes and scrotum in two of the animals. Treponemes were demonstrated in extracts of excised testes by dark-field microscopy (20 to 50 treponemes per microscopic field) and in tissue sections by silver staining (Fig. 1). One animal died on day 7 and another died on day 10 of unknown cause. Motile treponemes were found in the testes of these animals by dark-field examination.

Orchitis did not develop in two animals; their testes, which were atrophic, were free of treponemes 27 days after inoculation. Except for two animals that died and were not examined, all animals challenged intratesticularly became seroreactive as demonstrated by RPR, RPCFR, and CCFR tests. The females infected intracutaneously were serologically nonreactive on day 27 of the experiment.

TABLE 1. *Serological tests for treponematosis in hares*

Sera	No. tested	No. reactive	Percent
RPR	202	56	27.7
CCFR	71	41	57.7
RPCFR	71	47	66.1
RPR-CCFR	71	35	49.2
RPR-RPCFR	71	35	49.2
CCFR-RPCFR	71	41	57.7
TPI	19	6	31.5

DISCUSSION

To date there are no reports in the literature on serological tests for treponematosis in hares. The high percentage (27%) of seroreactivity noted in 202 selected wild animals in the present study would appear to be significant. The reactivity of RPCFR and TPI tests suggests a naturally occurring endemic treponematosis in hares. It appears to confirm the results of Jaksits (7), who demonstrated treponemes in natural skin lesions of hares.

TABLE 2. *Clinical manifestation and serological reactions of hares inoculated with pathogenic T. pallidum*[a]

Animal	Sex[b]	Site	Observation day	Reactivity				
				Result	Dark field	RPR	CCFR	RPCFR
1	F	Skin[c]	12[d]	Papule	−	N[e]	N	N
2	F	Skin[c]	27	Papule	−	N	N	N
3	F	Skin[c]	27	Papule	−	N	N	N
4	M	Testes[f]	7[d]	?	+			
5	M	Testes[f]	10[d]	Orchitis	+			
6	M	Testes[f]	15	Orchitis	+	R[a]	R	R
7	M	Testes[f]	15	Orchitis	+	R	R	R
8	M	Testes[f]	15	Orchitis	+	R	R	R
9	M	Testes[f]	15	Orchitis	+	R	R	R
10	M	Testes[f]	15	Orchitis	+	R	R	R
11	M	Testes[f]	27	Atrophy	−	R	R	R
12	M	Testes[f]	27	Atrophy	−	R	R	R

[a] Inoculum contained 1.7×10^7 to 2.4×10^7 treponemes per ml.
[b] F, Female; M, male.
[c] Challenged with 0.2 ml.
[d] Died.
[e] N, Negative; R, reactive.
[f] Challenged with 0.5 ml.

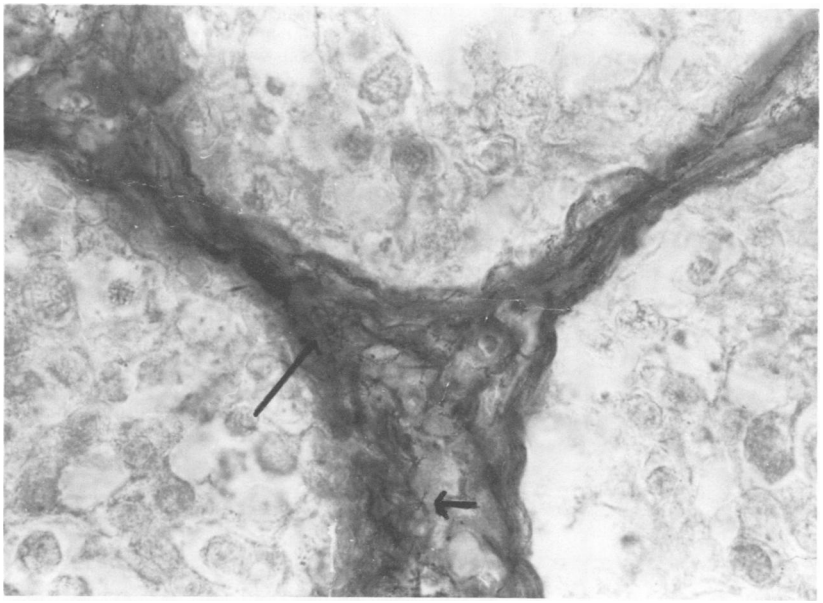

FIG. 1. *Experimental syphilis in hare orchitic testes with silver stain. Numerous treponemes (arrows) are identified by the silver impregnation technique. ×900.*

The testes of some of the male hares that were reactive in serological tests for syphilis were atrophic; however, treponemes could not be detected by dark-field examination.

A high percentage of hares were infected after intratesticular inoculation of *T. pallidum*. An orchitis developed after a short incubation period and large numbers of treponemes were harvested. However, after intracutaneous inoculation of *T. pallidum*, lesions failed to develop after 4 weeks. In contrast, a similar inoculum injected intracutaneously into rabbits usually produces lesions within 5 to 7 days. Additional research will be required to determine whether hares are more resistant to *T. pallidum* than are rabbits. Although the present studies suggested that natural tremponematosis occurs in hares, the serological findings did not give a clear indication of its frequency.

The animals were chosen from a large group of hares based on the presence or absence of lesions and signs suggestive of "hare syphilis." The treponeme responsible for this disease remains to be isolated and characterized. It may be *T. paraluis-cuniculi*, the treponeme that causes "rabbit syphilis," or closely related to it (10, 10a).

Irrespective of their pathogenicity in humans, the study of new strains of treponemes naturally pathogenic for animals is important, particularly because of their possible value as immunizing agents against human syphilis.

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