

Experimental genital trichomoniasis in the squirrel monkey (*Saimiri sciureus*)

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SUMMARY The squirrel monkey (*Saimiri sciureus*) has been proposed as a model for urogenital trichomoniasis in man, but has not been accepted as such because of the purported presence of naturally occurring vaginal trichomonads in this animal. The study published here shows that these are easily eradicated organisms of intestinal origin, which eliminates the potential confusion created by them. In addition, our experiments have shown that the hormonal status of primates seems to be a determinant in successfully establishing experimental trichomoniasis. This experimental infection recapitulates the clinical observations sufficiently to warrant the use of this model for studies of vaginal trichomoniasis.

Introduction

Trichomoniasis due to *Trichomonas vaginalis* is the most common protozoal infestation of man in the United States of America and the most common sexually transmitted disease (STD) throughout the world.¹ Despite its prevalence, there is scant information regarding the pathogenesis of urogenital trichomoniasis in men^{2,3} or women.⁴ Most information about its pathogenesis has been inferred from cytological studies and culture of vaginal secretions. Contributing to the fact that "the scientific community has almost ignored this interesting organism"⁵ has been the absence of a suitable animal model of trichomoniasis in man.

Several attempts have been made to establish a valid animal model. Street *et al* found that experimental infection of female squirrel monkeys with *T vaginalis* provided a number of similarities to infection in the human female genital tract.⁶ Hollander and Gonder, however, questioned the usefulness of the squirrel monkey as a model for human trichomoniasis because of their finding that squirrel monkeys had naturally occurring intravaginal trichomonads.⁷ The current

study, as well as several earlier reports, address these conflicting points of view. We isolated pentatrachomonads and a previously undescribed *Tritrichomonas* sp from vaginal swabs from our squirrel monkeys. These organisms were not, however, present in vaginal samples when meticulous attention had been paid to collection procedures. They were both identified as being of intestinal origin,⁸ and have subsequently been confirmed as being almost universally present in our squirrel monkey colony and in squirrel monkeys in two other primate colonies.⁹ *Tritrichomonas mobilensis* has been characterised and reported elsewhere.¹⁰ Because of the incidence of intestinal trichomonads and the potential for accidentally contaminating vaginal swabs with faeces, we developed a protocol to eradicate pre-existing trichomonads¹¹ before attempting to infect the squirrel monkey experimentally with *T vaginalis*. The occurrence of enteric intestinal trichomonads therefore need not vitiate the suitability of the squirrel monkey as a model of urogenital trichomoniasis in man.

The squirrel monkey (*Saimiri sciureus*) is a non-human primate of South American origin. Adult females weigh 550 to 875 g. These animals have a discrete breeding season of about three months (mid-December to mid-March). During the breeding season females experience 6-12 day ovulatory cycles with high levels of circulating oestrogen and progesterone. The remainder of the year is characterised by very low levels of circulating steroid hormones. Ovulatory cycles occur only during the breeding season.¹²

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Materials and methods

SEQUENCE OF EXPERIMENTS

Investigations followed a sequence that (1) screened animals for the presence of indigenous trichomonads, (2) established a group of animals free of parasites, (3) evaluated the effects of a single inoculum of *T vaginalis* in animals before the onset of oestrus, (4) evaluated the effects of inoculation during oestrus, and (5) assessed the possibility of horizontal transmission of the organism.

ANIMALS

Five adult female squirrel monkeys (*Saimiri sciureus*) of feral origin were selected for study. They were divided into two groups without conscious bias. The control group consisted of two animals that received sham inoculations. The test group consisted of three animals that received inoculation of active *T vaginalis* cultures. All animals were maintained at the Primate Research Laboratory of the University of South Alabama. Each animal was housed in an individual cage, maintained in isolation from the main colony, and fed commercial high protein monkey chow and water in unlimited quantities. A solution of sucrose and electrolytes, vitamins, and fresh fruit were provided as supplements to the basic diet. All animals were maintained in compliance with the *Guide for the care and use of laboratory animals*.¹³

EVALUATION AND TREATMENT PROCEDURES

Vaginal and rectal swabs were obtained using sterile (No 4) Calgiswabs after the perineal area had been carefully cleaned with 70% isopropyl alcohol. Direct smears were stained by the Papanicolaou (Pap) technique, and the remaining material was incubated in flat sided culture tubes containing Diamond's TYM (tripticase, yeast extract, and maltose) medium¹⁴ with 10% horse serum at 37°C. The culture tubes were examined for trichomonads by phase contrast microscopy. A specimen was considered to be negative for organisms only after repeated negative examination for 10 days. Before being experimentally inoculated with *T vaginalis*, all animals had been treated with metronidazole (Flagyl, Searle) 25 mg/kg orally for five days.

INOCULATION PROCEDURES

Three squirrel monkeys received a single inoculum of 5×10^5 Balt 42 *T vaginalis* cells⁴ (supplied by Dr B M Honigberg, University of Massachusetts at Amherst, USA) in 0.5 ml TYM medium without agar. Two animals received control inocula consisting of 0.5 ml sterile TYM medium. Inocula were delivered through sterile feeding tubes inserted fully into the vagina. The

labia were held closed for one minute after inoculation.

HORIZONTAL TRANSMISSION

One month after inoculation with *T vaginalis*, a cotton tipped swab was inserted into the vaginal vault of an infected monkey. The swab was swirled for 15 seconds, withdrawn, and inserted into the vagina of a non-infected control animal, swirled for one minute, and withdrawn. This procedure was repeated two months after inoculation.

Results

PROCEDURES BEFORE INOCULATION

Rectal swabs from all five animals showed *Pentatrichomonas hominis* as well as a previously uncharacterised *Trichomonas* sp. Vaginal swabs obtained after careful cleaning of the perineal area were uniformly negative by direct examination and culture before experimental inoculation.

Intestinal flagellates were cleared from all five animals by the fifth day of treatment, and all animals remained negative for indigenous trichomonads throughout the subsequent studies.

INOCULATION BEFORE BREEDING SEASON

On day three after inoculation several flagellate organisms were identified, but could not be cultured from the vaginal swab of a single experimental animal. Thereafter all Pap smears and vaginal and rectal cultures remained negative for 10 weeks in experimental and control animals. One of the control animals died for reasons unrelated to the experiment.

INOCULATION IN BREEDING SEASON

The three experimental animals described above and one of the controls were reinoculated after 10 weeks of negative culture and smear examination. The control animal remained negative throughout the experiment.

All three inoculated animals yielded positive cultures for *T vaginalis* on the day after inoculation. Two animals remained positive for four weeks (on testing twice a week). At the end of the oestrus cycle both of these animals became negative by vaginal cultures for one week and thereafter one was intermittently positive during the next four months (regular twice weekly cultures). The other animal yielded positive vaginal cultures for an additional three weeks, at which time it underwent hysterectomy with bilateral salpingo-oophrectomy.

Microscopy

Examination of the surgically resected tissues showed acute and chronic cervicitis, predominantly at the level of the squamocolumnar junction (fig. a and b). Dense inflammatory infiltrates of subepithelial monocytes

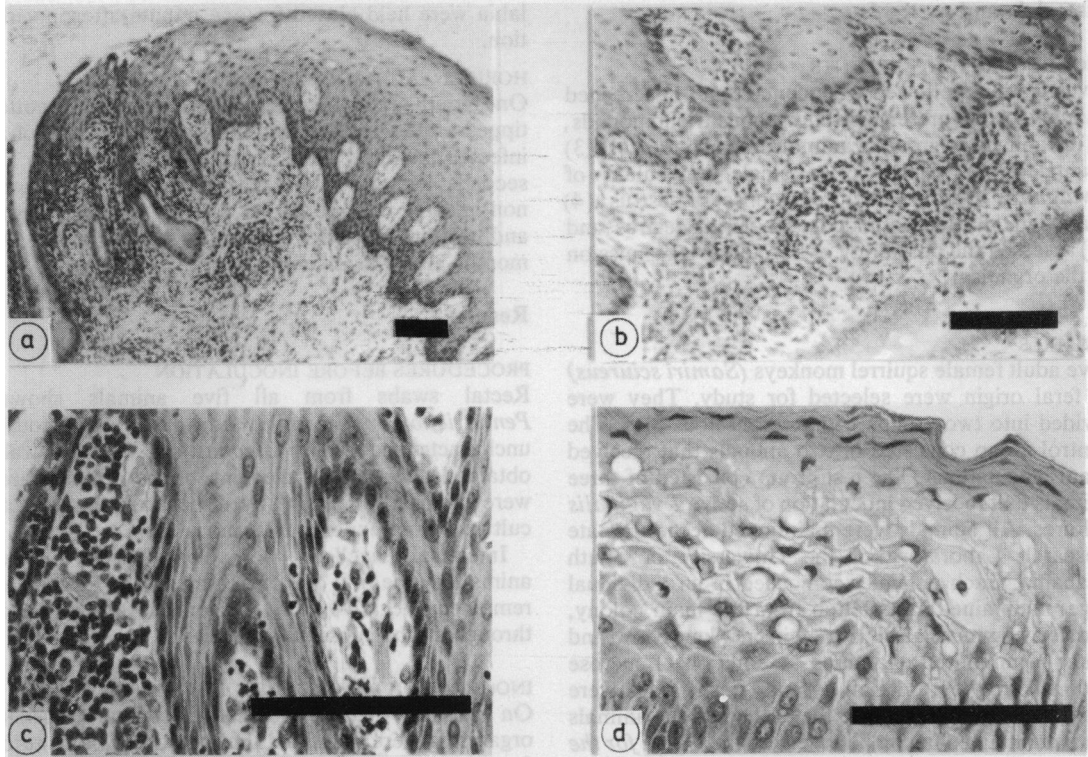


FIGURE Uterine cervix from squirrel monkey with experimental trichomoniasis: (a) stratified squamous epithelium with acute and chronic inflammation and vacuolar change in squamous epithelium; (b) focal acute chronic cervicitis at squamocolumnar junction; (c) high magnification of subepithelial chronic inflammatory cells with prominent capillaries and intraepithelial polymorphonuclear leucocytes; and (d) vacuolar change in squamous epithelium at squamocolumnar junction. All scale bars = 100 μ m.

and lymphocytes with intraepithelial polymorphonuclear leucocytes were accompanied by prominent subepithelial capillaries (fig. c) and focal ballooning degeneration of the epithelium (fig. a and d). The intraepithelial vacuolisation was similar to the vacuolisation described in the vaginal epithelium of trichomoniasis in women.¹⁵ This appearance was in contrast to the uterus of the control monkey, which exhibited only scattered subepithelial lymphocytes. The salpinges contained rare intraluminal polymorphonuclear leucocytes with aggregates of foamy histiocytes. No epithelial abnormalities were noted on sequential Pap smears, and though there was variable polymorphonuclear exudate the appearance of this exudate did not always correlate with positive cultures.

HORIZONTAL TRANSMISSION

No positive cultures were obtained from the recipient animal at the first attempt. The second attempt, however, was followed by intermittent positive cultures for three months.

Discussion

Our initial background studies confirm Hollander and Gonder's finding of indigenous intravaginal trichomonads in the squirrel monkey.⁷ Though our first attempts at culturing indigenous trichomonads from vaginas were successful, we subsequently concluded that these were faecal contaminants (*Pentatrichomonas* and *Tritrichomonas mobilensis*). *T mobilensis* has been characterised and found to be uniformly present in our colony of squirrel monkeys⁸ as well as in two other primate centres.⁹

Oestrogen and progesterone concentrations have been shown to vary dramatically in seasonally polyoestrus animals.¹² Failure to recognise the differential susceptibility related to seasonal endocrine changes could lead to misinterpretation of findings about the ability of these primates to harbour the parasite. Similar misinterpretation might occur regarding the infectivity of different strains of trichomonads. For example, failure to establish infection in several species of primates⁶ has given the impression that

trichomonads may lose their infectivity in primates after prolonged laboratory culture with repeated passages (D Taylor-Robinson, personal communication).

Though there are few published reports on the histopathology of trichomoniasis, the histopathological appearance of the infected uterine cervix of the squirrel monkey does correspond with that described in women in that subepithelial capillaries are prominent (fig. c), which are thought to contribute to the "strawberry" clinical appearance of an infected cervix. The difficulty of identifying organisms in histological sections has been documented by a number of authors.^{2,3,16} We did not undertake a specific immunoperoxidase search for organisms,^{2,17} but organisms were cultured from the vagina at the time of surgery.

Positive cultures for *T vaginalis* in the absence of identifiable organisms on Pap smear as well as recurrent positive cultures in isolated animals that had not been reinoculated both recapitulate the clinical observations of the disease in women. Though horizontal transmission of organisms was shown (further validating the use of squirrel monkeys as a model of genital trichomoniasis in man), sexual transmission was not attempted.

Our findings confirm that the squirrel monkey provides a useful model for the study of human trichomoniasis and point to additional similarities between experimental infection in squirrel monkeys and the disease in man. A full exposition of the variables of the usefulness of this model would address many traditionally unanswered queries about the pathogenesis of this common sexually transmitted organism. These include ways of transmission, pathogenesis in men, immunological reactivity, therapeutic influences, symptomless carriers, the relation of pathogenicity to other organisms in the genital tract, and the role of trichomonads at extragenital sites.

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