Urol Res (1982) 10:45–49

Urological Research

Chlamydial Prostatitis in Dogs: An Experimental Study*

O. S. Nielsen[1], R. Golubjatnikov[2], R. Dodge[2], and P. O. Madsen[1] **

[1] The Urology Section, Williams S. Middleton Memorial Veterans Hospital, 2500 Overlook Terrace, Madison, Wisconsin, USA, and the Department of Surgery, University of Wisconsin School of Medicine, Madison, Wisconsin, USA

[2] The State Laboratory of Hygiene, University of Wisconsin, 465 Henry Mall, Madison, Wisconsin, USA

Accepted: August 14, 1981

Summary. Ten adult male mongrel dogs were inoculated with *Chlamydia trachomatis* by injections of 10^6 or 10^7 organisms directly into the prostate. We were able to recover *Chlamydia* 3 to 7 days later in three of four dogs receiving injections of 10^7 organisms. Eight of ten dogs developed detectable serum antibody to *Chlamydia* 14 to 68 days following inoculation. All dogs receiving 10^7 *Chlamydia* and two of six dogs receiving 10^6 organisms developed histological signs of prostatic hyperplasia. Thus, the dog may be a model for establishment of chlamydial prostatitis and may be used for further investigations of this disease.

Key words: Experimental, Chlamydia, Prostatitis, Dog, Prostatic hyperplasia.

Introduction

The role of *Chlamydia trachomatis* in nongonococcal urethritis (NGU) has been well established. In studies of males with NGU, *Chlamydia* was recovered from approximately 40% as compared to only 0% to 5% of normal controls [9, 11]. Experimental chlamydial urethritis has been established in the male baboon urethra [2], providing strong evidence for the pathogenicity of *Chlamydia* in the human urogenital tract.

Chlamydia has been suggested as the infective agent in a high percentage of cases of chronic nonbacterial prostatitis]3–5], and has been isolated from prostatic secretion in patients with Reiter's disease [12]. Another study, however, has suggested that *C. trachomatis* appears to play a minor aetiological role in non-acute prostatitis [6].

We have used dogs in this study to establish a model for chlamydial prostatitis and to provide support for the pathogenicity of *Chlamydia.* Diseases similar to human chlamydial diseases have only been reproduced in subhuman primates [11].

Materials and Methods

The studies were carried out in 10 male, mongrel dogs (18 to 28 kg) who were under sodium thiopental anaesthesia. The abdominal wall of each dog was opened through a low, transrectal incision. Without opening the peritoneal cavity, the prostate was isolated by blunt dissection. Two biopsies were taken from each lobe with a Tru-Cut® biopsy needle (Travenol Laboratories, Inc., Deerfield, IL). Prostatic secretion was obtained by rectal prostatic massage and placement of a small polyethylene tube in the urethra with the tip just below the prostate. None of the dogs were treated with antimicrobial agents during the study. In the first six dogs, 1 ml of saline containing 10^6 *C. trachomatis* (Type D [75 H 345 passage 35] originally from Department of Microbiology, Harvard School of Public Health, Boston MA) was injected into a small prostatic artery and into the prostate itself through a 26-gauge needle. The other four dogs were injected directly with 1 ml of saline containing 10^7 *C. Trachomatis* into the prostate in six separate areas with a 26-gauge needle. The *C. trachomatis* were harvested after passage in 7 days eggs on day 9. The concentration was determined by the method below and expressed as inclusion forming units.

Following the injection, blood, prostatic tissue, and prostatic secretion were obtained on the days listed in Tables 1 and 2.

Chlamydial cultures were processed according to the methods described by McComb et al. [7] and Ripa and Mårdh [10]. Prostatic tissue biopsies and prostatic secretion were placed in 0.2 M sucrose phosphate solution and cultured immediately or stored at –20 °C. Cultures were made in McCoy cells, which had been layered the previous day on 5-mm cover slips in Cluster^{96} (Costar, Cambridge, MA) microtiter plates. The supportive medium was removed and inoculum was added to the cell layer. After 1h of centrifugation at 36 °C (1,000 x *g*), plates were incubated for 2h at 36 °C, inoculum was removed, and growth medium containing 1.5 μg/ml cycloheximide (Actidione, The Upjohn Co., Kalamazoo, MI) was added. Plates were reincubated for 65 h, growth medium was removed, and cover slips were stained and mounted in iodine solution and observed at a magnification of 400X for the presence of intracytoplasmic brown or purple inclusions typical of *C. trachomatis.*

* This project was supported in part by the Veterans Administration
** Send reprint requests to: Paul O. Madsen, M.D., Chief, Urology Service, Williams S. Middleton Memorial, Veterans Hospital, 2500 Overlook Terrace, Madison, WI 53705, USA

0300-5623/82/0010/0045/$01.00

Table 1. Microimmunofluorescence test (MIF), cultivation for *Chlamydia* in prostatic biopsy and prostatic secretion, and histological Evaluation after injection of 10^6 *Chlamydia trachomatis* into dog prostate

Dog No.	Days after infection	Serology MIF titer 1	Culture		Histology
			Tissue biopsy	Prostatic secretion	
1	0	–	–	–	ND[a]
	14	–	–	–	inflam.
	24[b]	ND	–	ND	inflam.
2	0	–	–	–	ND
	14	–	–	–	normal
	23	–	ND	–	ND
	53	–	ND	ND	inflam.
3	0	–	–	–	ND
	14	–	–	–	normal
	20	16	ND	–	ND
	51	–	ND	ND	inflam.
4	0	–	–	–	ND
	14	16	–	–	normal
	20	32	ND	–	ND
	56	–	ND	ND	normal
5	0	–	–	–	ND
	14	16	–	–	normal
	21	32	ND	–	ND
	68	16	–	ND	inflam. papillation
6	0	–	–	–	ND
	14	16	–	–	normal
	21	16	ND	–	ND
	60	–	ND	ND	papillation

[a] ND = Not done
[b] Sacrificed due to surgical complications

Table 2. Microimmunofluorescence test (MIF), cultivation for *Chlamydia* in prostatic biopsy and prostatic secretion, and histological evaluation after injection of 10^7 *Chlamydia trachomatis* into dog prostate

Dog No.	Days after infection	Serology MIF titer 1	Culture		Histology
			Tissue biopsy	Prostatic secretion	
7	0	–	–	–	normal
	3	ND[a]	ND	positive	ND
	7	ND	ND	–	ND
	14	64	–	–	inflam.
	48	16	–	ND	inflam. + papillation
8	0	–	–	–	normal
	3	ND	ND	positive	ND
	7	ND	ND	–	ND
	14	–	–	–	normal
	48	16	–	ND	papillation
9	0	–	–	–	normal
	3	ND	ND	–	ND
	7	ND	ND	positive	ND
	16	16	–	yeast	inflam.
	49	16	–	ND	papillation
10	0	–	–	–	normal
	3	ND	ND	–	ND
	7	ND	ND	yeast	ND
	16	–	ND	yeast	inflam.
	49	16	–	ND	papillation

[a] ND = Not done

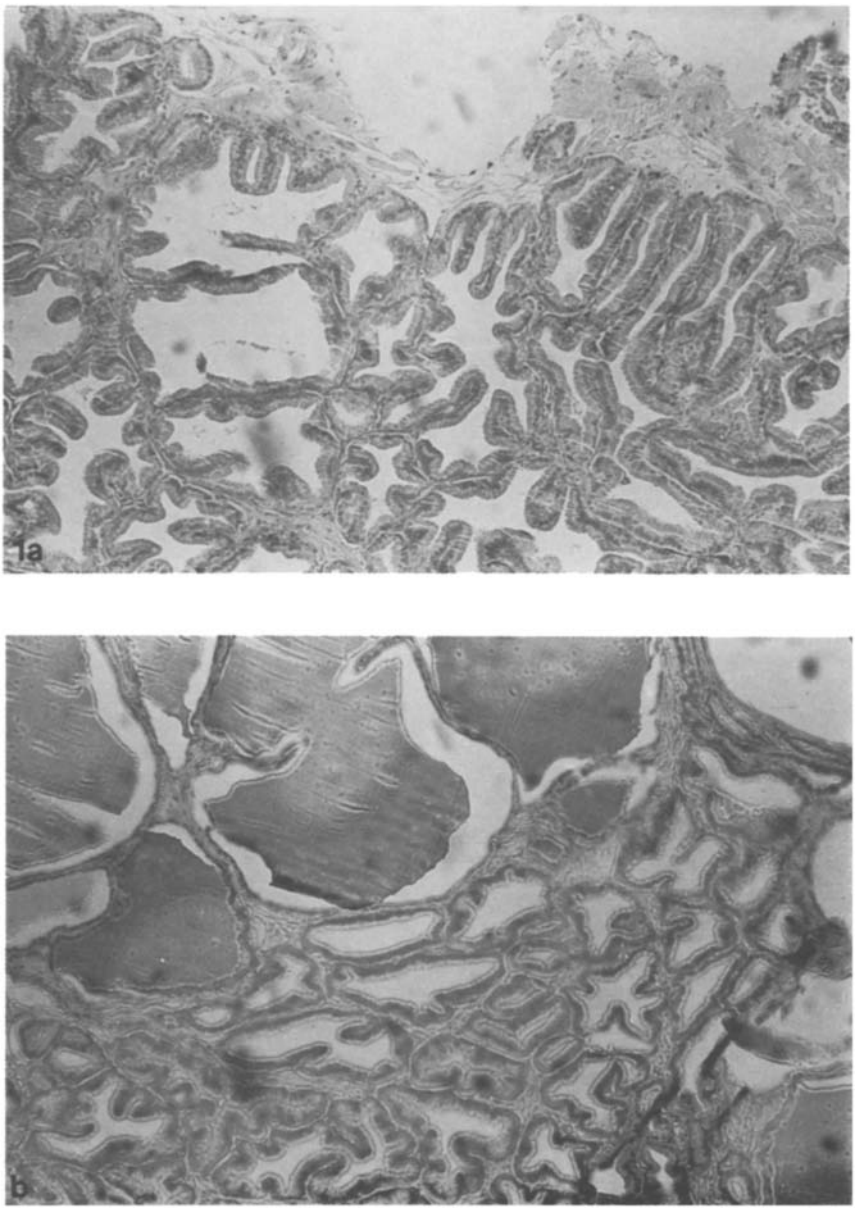

Fig. 1a and b. Dog #6. 14 and 60 days after noculation with 10^6 *Chlamydia* (Giemsa x 100)

The microimmunofluorescent tests were conducted using the following technique. Antigen consisted of egg-passaged *C. trachomatis* and a mixture of serological types C, D, and L. Dots of infected yolk sac were applied with micropipettes, dried, fixed in acetone, and stored at −70 °C until used. The serum dilution used for initial screening was 1:16. Sera was absorbed for 30 min with egg yolk, then incubated with antigen in a moist chamber at 37 °C. Following two 7 min rinses in phosphate buffered saline (pH 7.2), rabbit anti-dog fluorescent conjugate (lot C683416, GIBCO, Grand Island, NY) was added at the optimum dilution, and the slides were reincubated for an additional 30 min. A counterstain of Evans blue dye was added to the first of two PBS washes., and the slides were rinsed with distilled water and mounted with a coverslip. The slides were observed for yellow-green fluorescent dots under a Zeiss microscope using an FITC exciter filter and "50" barrier filter.

Histological examination of the prostate was done after staining the tissue slides with hematoxylin-eosin and Giemsa stain. The tissue samples were examined for cystic formation and papillation, two conditions which signify prostatic hypertrophy in dogs [8, 13].

Results

The results after injection of 10^6 infective units of *C. trachomatis* in 6 dogs are listed in Table 1. Four out of six dogs developed a titre in response to the inoculation. We were unable to culture *Chlamydia* from prostatic tissue or prostatic secretion at any time. At the end of the study three of six dogs has an acute inflammation in the prostate tissue, two dogs showed cystic formation and papillation of the prostate epithelium (Figs. 1a andb), and one dog showed no histological changes. Dog #1 developed a pelvic abscess 24 days after inoculation and had to be sacrificed because of profuse bleeding into the abscess from the iliac artery.

The group of dogs who received 10^7 *Chlamydia* all developed chlamydial infection, as demonstrated by the elevations in titre (Table 2). Three of four dogs excreted

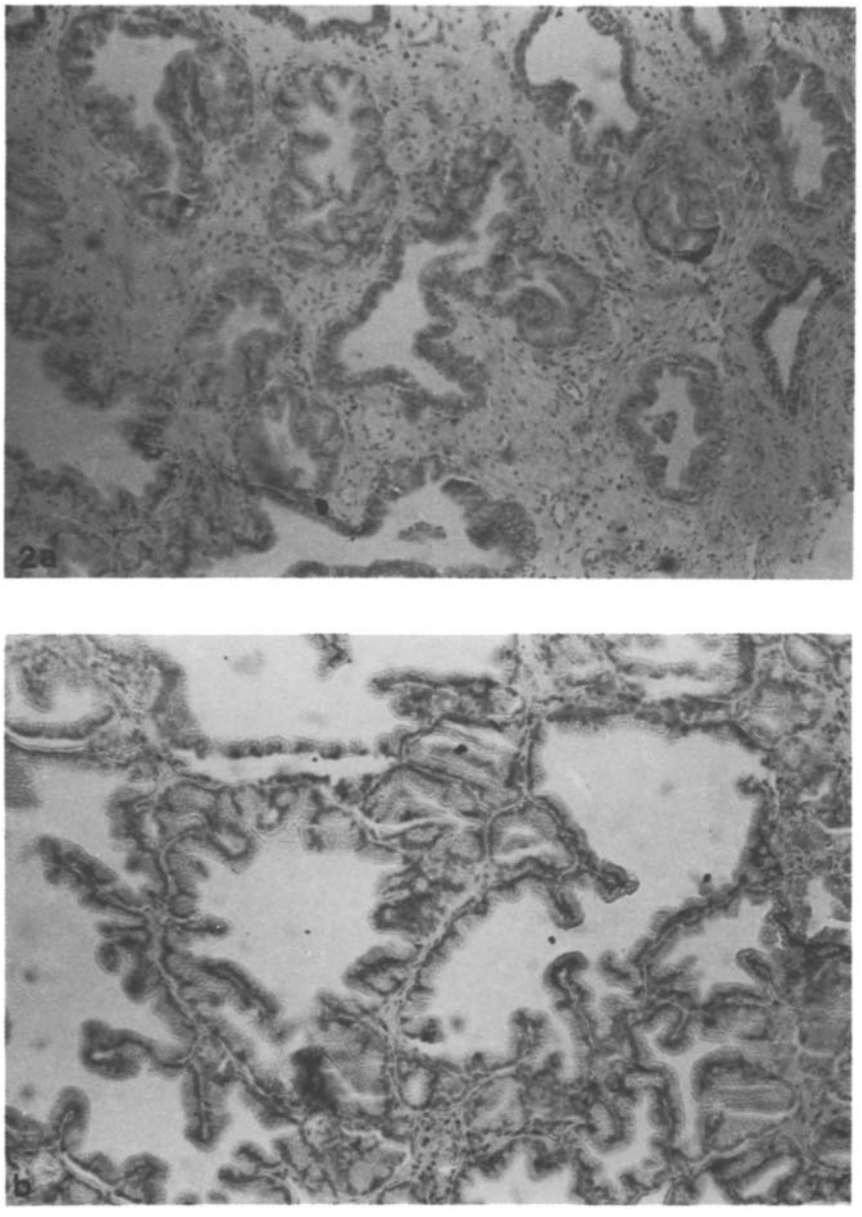

Fig. 2a and b. Dog #8. 0 and 48 days after inoculation with 10^7 *Chlamydia* (Giemsa x 100)

Chlamydia in the prostatic secretion, either on day 3 or 7. Dog #10 could not be evaluated because the prostatic secretion specimen was contaminated with yeast. We were unable to culture *Chlamydia* from the prostatic tissue in all dogs. Three dogs in this group developed infection in their prostatic tissue, and all four developed cystic formation and papillation at the end of the study (Fig. 2a and b).

Discussion

In this study, we were able to infect eight of ten dogs by injecting *C. trachomatis* into the prostate. However, since the dogs ceased to excrete *Chlamydia* after some time (3 to 16 days) and *Chlamydia* organisms were not found in the tissue cultures, the infection in dogs may have been self-limiting.

To our knowledge, this study represented the first time that an animal lower than subhuman primates has been infected with *C. trachomatis. Chlamydia* could be an aetiological factor in human prostatitis, as supported by a study of humans with Reiter's disease in which one of six patients with prostatitis had a positive culture of *Chlamydia* in the expressed prostatic secretion [12]. In another study, 33% of patients with chronic prostatitis had *Chlamydia* antibodies compared to only 5% in a control goup [5]. This finding was later questioned by the same authors [6]. Since *Chlamydia* epididymitis accounts for two-thirds of so-called "idiopathic" epididymitis in young men [1], and since 40% of males with nongonococcal urethritis are infected with *Chlamydia* [11], this microorganism appears to be a more common pathogen in the urinary tract than previously thought.

In our study, we found cystic formation and papillation in the prostates of some of the dogs. Prostatic enlargement can occur in dogs naturally and experimentally [8, 13]. Quantitative predominance of columnar secretory epithelial elements with papillation into dilated alveoli distinguishes canine lesions from human prostatic enlargement. However, on rare occasion, hyperplasia in dogs may be irregular, simulating human lesions [8, 13]. Because our starting point for evalution of the tissue samples was a needle biopsy, we cannot claim that the biopsy represents the whole prostate. However, we feel the demonstrated histological changes are not due to bias in the examining procedure. An accurate histological comparison can probably be made only from examining whole prostates.

In this study, it is not clearly demonstrated that the histological changes are due to infect with *C. trachomatis,* as control experiments with killed *Chlamydia* or with saline were not carried out. However, from other studies in our laboratory, it is known that an injection of saline into the prostate does not produce any alterations in this histology.

Because we have been able to establish chlamysial prostatitis in dogs by infecting them with an organism normally not pathogenic to dogs [11], we find that *C. trachomatis* could have an aetiological role in human prostatitis. On the other hand, an animal model cannot establish the pathogenicity for humans but may serve as a valuable tool in investigating chlamydial prostatitis and treatment of this condition.

References

1. Berger RE, Alexander ER, Harnisch JP, Paulsen CA, Monda GD, Ansell J, Holmes KK (1979) Etiology, manifestations and therapy of acute epididymitis: Prospective study 50 case. J Urol 121:750
2. Digiacomo RF, Gale JL, Wange S-P, Kiviat MD (1975) Chlamydial infection of the male baboon urethra. Br J Vener Dis 51: 310
3. Hofstetter A (1979) The role of *Chlamydiae* in the male urethro-adnexitis. Infection 7 (Suppl 2) p 228
4. Mårdh P-A, Colleen S (1975) Search for uro-genetial tract infections in patients with symptoms of prostatitis. Scand J Urol Nephrol 9:361 (1975)
5. Mårdh P-A, Colleen S, Holmquist B (1972) *Chlamydia* in chronic prostatitis. Br Med J 4:361
6. Mårdh P-A, Ripa KT, Colleen S, Treharne JD, Darougar S (1978) Role of *Chlamydia trachomatis* in non-acute prostatitis. Br J Vener Dis 54:330
7. McComb DE, Puzniak CI (1974) Micro cell culture method for isolation of *Chlamydia trachomatis.* Appl Environ Microbiol 28:727
8. Mostofi FK (1970) Benign hyperplasia of the prostate gland. In: Campbell MF, Harrison JH (eds) Urology. Saunders, Philiadelphia, p 1073
9. Richmond SJ, Sparling PF (1976) Genital chlamydial infections. Am J Epidemiol 103:428
10. Ripa KT, Mårdh P-A (1977) Cultivation of *Chlamydia trachomatis* in cycloheximide treated McCoy cells. J Clin Microbiol 6:328
11. Schachter J (1978) Chlamydial infections. N Engl J Med 298: 428
12. Zachariae H, From E, Zachariae E (1979) Reiter's disease and prostatitis. Treatment with tetracycline. Ugeskr Laeger 141: 2058
13. Zuckerman S, Groome JR (1937) The aetiology of benign enlargement of the prostate in the dog. J Path 44:113

Dr. P. O. Madsen
Urology Service
Williams S. Middleton Memorial Veterans Hospital
2500 Overlook Terrace
Madison, WI 53705
USA