

Urinary-tract infection by *Mycoplasma pulmonis* in mice and its wider implications

By D. TAYLOR-ROBINSON AND PATRICIA M. FURR

*Division of Sexually Transmitted Diseases, MRC Clinical Research Centre,
Watford Road, Harrow, Middlesex HA1 3UJ*

(Received 9 December 1985; accepted 10 January 1986)

SUMMARY

Young adult mice were inoculated intravenously with strains JB or Peter C of *Mycoplasma pulmonis*. A few were inoculated intranasally with strain JB. This strain but not Peter C was isolated for 50 days or more from the urines of more than half of the mice. Those of strains TO, C₃H and CBA, but not CFLP, were susceptible. Recovery of mycoplasmas was intermittent and sometimes the numbers isolated varied within individual mice and between mice of a particular strain, ranging from 5×10^1 to $\geq 5 \times 10^7$ colour-changing units/ml. Fifty serial passes of *M. pulmonis*, strain JB, in mycoplasmal medium resulted in attenuation, the organisms after inoculation of TO mice not being recovered from the urine and excretion not being stimulated by treating the mice with progesterone. At autopsy, the organisms of early passage were usually but not invariably isolated from the kidneys of mice that had been urinary excretors. About half of the latter had no renal histopathological changes. The others had usually minimal renal perivascular lymphocytic infiltrates but occasionally more widespread inflammatory changes. The findings may have relevance to the spread of mycoplasmal infection within mouse colonies and suggest that an association between such infection and nephritis in other species, including man, should be sought more closely.

INTRODUCTION

The murine mycoplasma, *Mycoplasma pulmonis*, is known to produce respiratory and genital tract disease, as well as arthritis, in mice (Cassell & Hill, 1979), but urinary-tract or genitourinary-tract infections have not been documented. However, our finding of *M. pulmonis* organisms in the kidney and urine of a mouse following their intravenous inoculation stimulated an investigation to determine how frequently this mycoplasma might be excreted from the kidney and the extent to which this might be influenced by the strain of mycoplasma and the strain of mouse. The finding of urinary excretion has implications for mycoplasmal containment within animal facilities and for mycoplasmas as a cause of urinary tract infections in species other than the mouse.

MATERIALS AND METHODS

Mice

Young adult male or female mice of strains TO, C₃H and CBA, bred in the Specific Pathogen-free Unit at the Clinical Research Centre, and those of strain CFLP (Carworth), were used. Each animal was checked by a culture technique for indigenous *M. pulmonis* infection of the respiratory and genital tracts before the experiments began.

Mycoplasma medium

Glucose-containing medium used for the growth and isolation of *M. pulmonis* has been described previously (Manchec & Taylor-Robinson, 1968).

M. pulmonis inoculum

Two strains of *M. pulmonis* were used. Strain 'Peter C' had nine passes in medium before mouse inoculation; it had been shown previously to produce pneumonia in mice at this passage level (Denny, Taylor-Robinson & Allison, 1972). Strain JB, at a designated pass 0, was known to produce pneumonia, arthritis and genital-tract disease in mice (Furr & Taylor-Robinson, 1984). This strain was used also after a further 50 serial passes in liquid mycoplasmal medium. The different haemadsorptive properties of the unpassed and serially passed organisms have been described before (Taylor-Robinson & Furr, 1985). The organisms for the inocula were grown in liquid medium incubated at 37 °C for 3 days. The number of organisms in each culture was determined by making serial tenfold dilutions in medium (0.2 ml in 1.8 ml); the highest dilution at which the colour of the medium changed from red to yellow on incubation at 37 °C was considered to contain one colour-changing unit (c.c.u.).

Experimental procedure

All mice, except one group given 0.1 ml of the inoculum intranasally, were inoculated intravenously with 0.2 ml. A few mice were treated with progesterone before and after they had been given *M. pulmonis*, as described previously (Furr & Taylor-Robinson, 1984). After mycoplasmal inoculation urine specimens and sometimes vaginal swabs were collected at about weekly intervals. Some of the mice were autopsied, at which time urine was collected and the kidneys were removed. They were cut in half longitudinally, one portion being used for culture and the other for histological examination. The urine collected before and at autopsy was taken into sufficient liquid mycoplasmal medium to produce a 10⁻¹ dilution and swabs were expressed in 1.8 ml of medium which was then designated a 10⁻¹ dilution. Kidney tissue was homogenized in a Ten-Broeck tissue grinder to give a 10% (w/v) suspension in the medium. Further dilutions of this and the other specimens were made in the medium in tenfold steps (0.2 ml in 1.8 ml), usually up to 10⁻⁷, to assess, as described above, the number of *M. pulmonis* organisms present in the specimen.

Histological procedure

Kidney tissue was fixed in 10% buffered formol saline and sections were stained with haematoxylin and eosin.

Table 1. Isolation of *M. pulmonis* from the urine of different strains of mice

<i>M. pulmonis</i> strain	Mouse strain	Route of inoculation*	Sex	No. of mice in group initially†	Urines positive on indicated day after inoculation (%)							
					1	7	14	21	28	35	42	50
Peter C	TO	i.v.	M	10	·	0	0	0	0	·	0	·
			F	10	·	0	0	0	0	·	0	·
JB	TO	i.v.	F	6	·	0	50	60	·	·	25	25
JB	C ₃ H	i.v.	M	33	13	12	45	47	60	·	·	15
JB	C ₃ H	i.n.	M	16	0	15	25	33	·	0	0	·
JB	CBA	i.v.	M	6	·	66	·	·	·	·	·	·
JB	CFLP	i.v.	M	10	·	0	0	0	·	·	·	0
			F	10	·	0	0	0	·	·	·	0
Peter C	CFLP	i.v.	M	10	·	0	0	0	·	0	·	·
			F	10	·	0	0	0	·	0	·	·

* i.v., intravenous; i.n., intranasal.

† Some mice killed at intervals after inoculation so that urines from a diminishing number were examined in the later stages.

Table 2. Effect of attenuating *M. pulmonis* strain JB and of treating female TO mice with progesterone on mycoplasmal recovery from urine

No. of passes of <i>M. pulmonis</i>	Progesterone treatment	No. of mice in group	Number of urines positive						
			On indicated day after inoculation						At autopsy
			3	7	14	21	35	42	
0	—	10	1	2	1	3	2	1	5
	+	5	0	0	0	0	0	0	0
50	—	10	0	0	0	0	0	0	0
	+	5	0	0	0	0	0	0	0

RESULTS

Influence of the strain of M. pulmonis on its recovery from urine

Inocula of strains Peter C and JB (pass O) of *M. pulmonis*, containing 10⁷ c.c.u., were introduced intravenously into mice of strain TO. Strain Peter C was not isolated from the urine of any of the mice during a period of 42 days whereas strain JB was recovered from the urine of up to half of the mice, recovery from some occurring as long as 50 days after inoculation (Table 1). Strain JB was used almost exclusively in further experiments.

Influence of the mouse strain on the recovery of M. pulmonis from urine

As shown in Table 1, in addition to the recovery of strain JB (pass O) from the urine of TO mice, it was isolated from the urine of C₃H and CBA mice after intravenous inoculation. However, strain JB was not recovered from the urine of

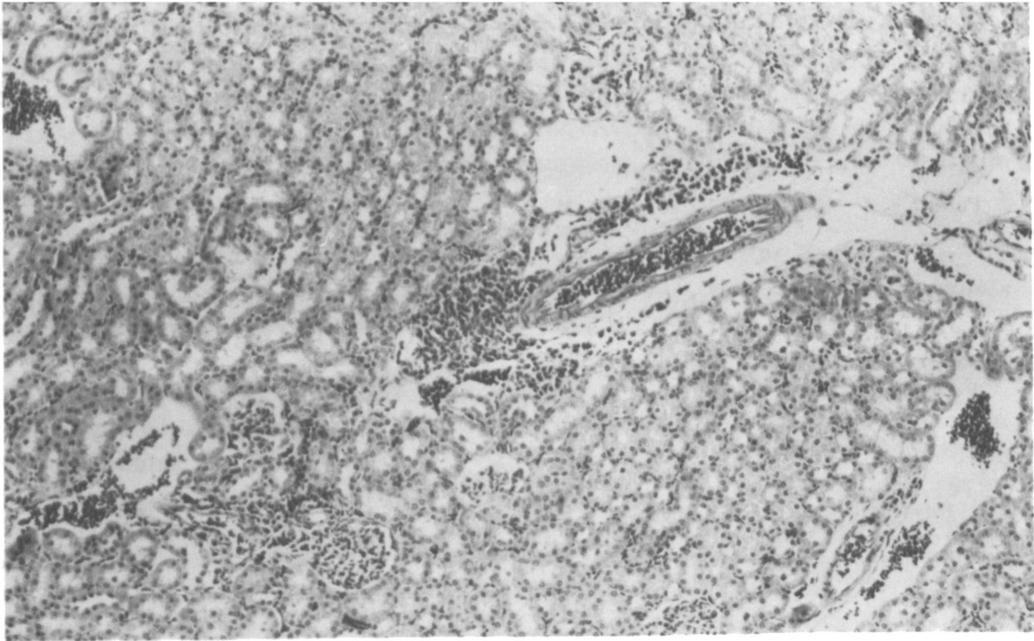


Fig. 1. Perivascular cuffing by lymphocytes in the kidney of a TO mouse 63 days after intravenous inoculation with *M. pulmonis* strain JB (pass O): organisms were recovered from urine before and at autopsy.

CFLP mice examined for 50 days nor, perhaps not surprisingly, was strain Peter C recovered from the urine of mice of this strain.

Route of inoculation

Most of the mice were inoculated intravenously, but the JB strain of *M. pulmonis* (pass O) was isolated also from C₃H mice after intranasal inoculation (Table 1).

Influence of attenuating M. pulmonis and of treating mice with progesterone on recovery of M. pulmonis from urine

As shown in Table 2, strain JB (pass O) was isolated from the urine of 3 of 10 TO mice during a period of 42 days and from the urine of a further 2 at autopsy, undertaken 63 days after inoculation; it was not isolated from the urine of 5 TO mice that had been treated with progesterone and inoculated at the same time. In contrast, strain JB (pass 50), the pathogenicity of which was known to be attenuated for other anatomical sites, was not recovered from the urine of any of 10 TO mice, either before or at autopsy. Furthermore, progesterone treatment of five mice did not promote excretion of the mycoplasma.

Quantitative estimation of M. pulmonis in urine

The numbers of organisms of *M. pulmonis* strain JB (pass O) that were isolated varied within individual mice and between mice of a particular strain, ranging from 10^1 to $\geq 10^7$ c.e.u./0.2 ml. Thus, there was no particular pattern and the numbers

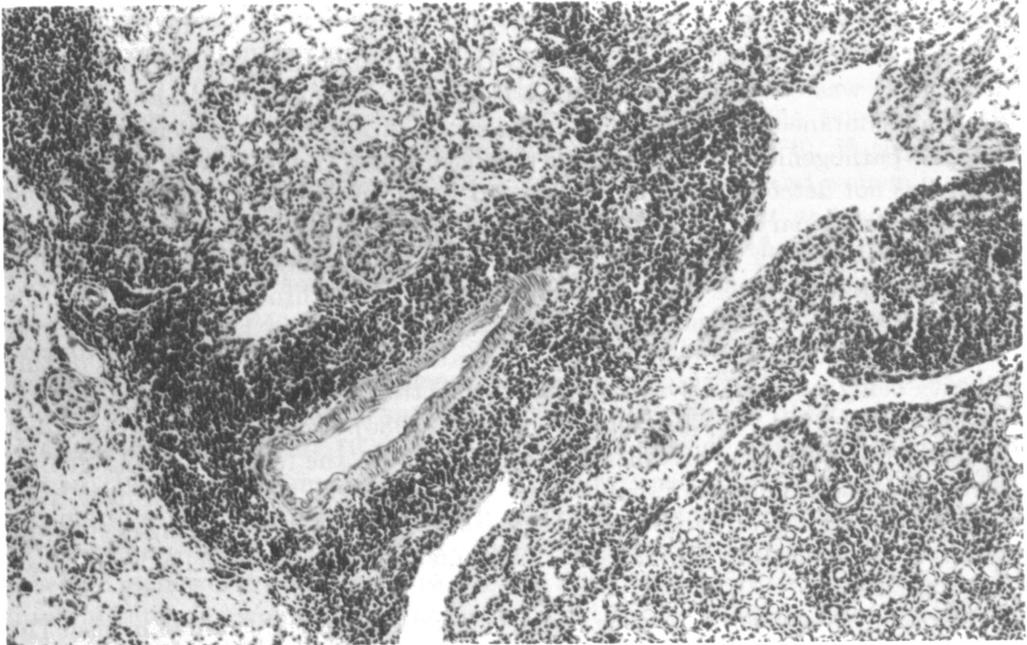


Fig. 2. Perivascular cuffing by lymphocytes and interstitial nephritis with more widespread infiltration of acute and chronic inflammatory cells, including plasma cells, and destruction of kidney architecture. Autopsy 63 days after intravenous inoculation of a TO mouse with *M. pulmonis* strain JB (pass 0): organisms were recovered from urine before and from kidney tissue at autopsy.

isolated were not consistently large or small. For example, the numbers isolated from one mouse were 10^2 , 10^2 , nil, 10^1 , 10^6 and 10^1 c.c.u. on days 3, 7, 14, 21, 35 and 42, respectively, after inoculation. Occasionally, recovery was even less consistent. Thus, the organisms were detected in only 1 of 6 urine samples from one mouse, $\geq 10^3$ c.c.u. being isolated 21 days after inoculation of the animal.

Autopsy and histopathological findings

M. pulmonis organisms (up to 10^7 c.c.u.) were isolated usually from one or both kidneys of mice when the organisms had been recovered from urine either before or at the time of autopsy. However, in some instances in which only a few organisms (10^2 or less c.c.u.) were isolated from the urine, they were not isolated from the kidneys, and vice versa.

M. pulmonis organisms were isolated from the urine of about half of the mice without there being renal histopathological changes. In the others, there were changes which usually were minimal, comprising small perivascular infiltrates of lymphocytes (Fig. 1). Occasionally, however, there was severe interstitial nephritis in which acute and chronic inflammatory cells infiltrated the parenchyma of the kidney, as illustrated in Fig. 2.

DISCUSSION

Excretion of *M. pulmonis* in urine was detected in both male and female mice although it was easier to collect urine from male animals since many of them urinated spontaneously on handling. One strain of *M. pulmonis*, Peter C, known to be less pathogenic in the mouse lung than strain JB (Taylor & Taylor-Robinson, 1975) was not detected in urine. This could be ascertained only over a period of time because urinary excretion appeared to be intermittent even with the more pathogenic JB strain. The latter was known to be capable of causing disease in various anatomical sites and to adhere strongly to erythrocytes and other cells (cytadsorptive). The cytadsorptive capacity of this strain has been shown to correlate with its ability to infect the respiratory and genital tracts, loss of this capacity being associated with loss of both infectivity and pathogenicity (Taylor-Robinson *et al.* 1981; Taylor-Robinson & Furr, 1985). It is interesting, therefore, that the same pathogenic strain when attenuated for the respiratory and genital tracts by passage in medium should also lose its ability to infect the urinary tract after inoculation directly into the blood stream. The mechanism of entry into the kidney is unknown but presumably attachment to renal epithelial cells is an important factor.

The other major factor affecting urinary excretion was the strain of mouse. The influence that this imparted had been seen previously in the development of arthritis (Hannan, 1971; Taylor, Taylor-Robinson & Slavin, 1974; Keystone *et al.* 1978) and in infection of the genital tract by *M. pulmonis* (Furr & Taylor-Robinson, 1984). Clearly, infection of various anatomical sites in the mouse by this mycoplasma is not simply a matter of the organisms attaching to receptive cells but is also under genetic influence.

The results of our previous studies had shown unequivocally that infection of the genital tract was enhanced by treating the mice with progesterone (Furr & Taylor-Robinson, 1984). This hormone caused a cessation of the oestrous cycle and we postulated that, in so doing, it allowed organisms to become better established in the genital tract. Our current observations support this idea because progesterone treatment had no effect on urinary excretion of the organisms after they had been introduced intravenously, so avoiding the genital tract. Any effect that progesterone might have had on the immune system, increasing susceptibility, was not apparent.

Spread of *M. pulmonis* in mouse colonies has been thought to occur directly from nasal contact or through aerosols generated from the respiratory tract. Excretion of the organisms in urine, sometimes in large numbers, in our experiments suggests that the same phenomenon might occur sometimes under natural conditions. This would provide an even more effective means of spread and needs to be considered when attempts are being made to contain or eradicate mycoplasmal infection within colonies.

It is, of course, pertinent to consider whether urinary excretion is a reflection of the organisms causing urinary-tract disease. Certainly, no close correlation between the two was observed. Sometimes organisms were detected in the urine in the absence of inflammatory changes in the kidneys. On the other hand, acute inflammatory changes were seen in some mice. This raises the question of the extent

to which urinary excretion occurs with other mycoplasmas in other species and whether mycoplasmal nephritis is a recognized entity. Regarding the first point, urinary excretion in the mouse is not unique since *M. mycoides* organisms have been isolated from the urine of infected cattle (Masiga, Windsor & Read, 1972) and the numbers recovered correlated to some extent with the severity of the respiratory disease caused by this mycoplasma (Scudamore, 1976). *M. hominis* and ureaplasmas are recovered frequently from the urine of men and women but this is most often a result of their presence in the genital tract and not due to a urinary-tract infection. However, in relation to this and the final point about kidney damage, *M. hominis* is thought to be an infrequent cause of acute pyelonephritis and exacerbations thereof (Thomsen, 1975; 1978a), the organisms probably ascending to the kidneys by reflux. How often they might infect and damage the kidney as a consequence of haematogenous spread is a moot point. Kidney infarcts were seen in cattle with pleuro-pneumonia due to *M. mycoides* (Masiga, Windsor & Read, 1972; Scudamore, 1976), presumably as a result of blood spread. In our experiments, urinary excretion was observed after intranasal inoculation and the human respiratory pathogen, *M. pneumoniae*, has been listed as one of the causes of post-infectious glomerulonephritis (Williams & Peters, 1983). It would seem worthwhile investigating this association more thoroughly since the interval between respiratory infection and renal disease might be considerable and the two events not be linked easily. Antibody to *M. hominis* can be detected in the urine of some patients with acute pyelonephritis (Thomsen, 1978b) and it is possible that the approach of seeking antibodies to *M. pneumoniae* in both serum and urine might help to define whether this mycoplasma has any significant association with glomerulonephritis of otherwise unknown aetiology.

REFERENCES

- CASELL, G. H. & HILL, A. (1979). Murine and other small-animal mycoplasmas. In *The Mycoplasmas* (ed. J. G. Tully and R. F. Whitcomb), vol. 2, pp. 235–273. New York: Academic Press.
- DENNY, F. W., TAYLOR-ROBINSON, D. & ALLISON, A. C. (1972). The role of thymus-dependent immunity in *Mycoplasma pulmonis* infections of mice. *Journal of Medical Microbiology* **5**, 327–336.
- FURR, P. M. & TAYLOR-ROBINSON, D. (1984) Enhancement of experimental *Mycoplasma pulmonis* infection of the mouse genital tract by progesterone treatment. *Journal of Hygiene* **92**, 139–144.
- HANNAN, P. C. T. (1971). Observations on the arthritogenic properties of Sabin's type C murine mycoplasma (*Mycoplasma histotropicum*). *Journal of General Microbiology* **67**, 363–365.
- KEYSTONE, E., TAYLOR-ROBINSON, D., POPE, C., TAYLOR, G. & FURR, P. (1978). Effect of inherited deficiency of the fifth component of complement on arthritis induced in mice by *Mycoplasma pulmonis*. *Arthritis and Rheumatism* **21**, 792–797.
- MANCHEE, R. J. & TAYLOR-ROBINSON, D. (1968). Haemadsorption and haemagglutination by mycoplasmas. *Journal of General Microbiology* **50**, 465–478.
- MASIGA, W. N., WINDSOR, R. S. & READ, W. C. S. (1972). A new mode of spread of contagious bovine pleuropneumonia? *Veterinary Record* **90**, 247–248.
- SCUDAMORE, J. M. (1976). Observations on the epidemiology of contagious bovine pleuropneumonia: *Mycoplasma mycoides* in urine. *Research in Veterinary Science* **20**, 330–333.
- TAYLOR, G. & TAYLOR-ROBINSON, D. (1975). The part played by cell-mediated immunity in mycoplasma respiratory infections. *Developments in Biological Standardization* **28**, 195–210.
- TAYLOR, G., TAYLOR-ROBINSON, D. & SLAVIN, G. (1974). Effect of immunosuppression on arthritis in mice induced by *Mycoplasma pulmonis*. *Annals of the Rheumatic Diseases* **33**, 376–384.

- TAYLOR-ROBINSON, D. & FURR, P. M. (1985). The interplay of host and organism factors in infection of the mouse genital tract by *Mycoplasma pulmonis*. *Journal of Hygiene* **95**, 7–14.
- TAYLOR-ROBINSON, D., FURR, P. M. & DAVIES, H. A., MANCHEE, R. J., MOUCHES, C. & BOVE, J. M. (1981). Mycoplasmal adherence with particular reference to the pathogenicity of *Mycoplasma pulmonis*. *Israel Journal of Medical Sciences* **17**, 599–603.
- THOMSEN, A. C. (1975). The occurrence of mycoplasmas in the urinary tract of patients with chronic pyelonephritis. *Acta Pathologica et Microbiologica Scandinavica* **83 B**, 10–16.
- THOMSEN, A. C. (1978*a*). Occurrence of mycoplasmas in urinary tracts of patients with acute pyelonephritis. *Journal of Clinical Microbiology* **8**, 84–88.
- THOMSEN, A. C. (1978*b*). Mycoplasmas in human pyelonephritis: demonstration of antibodies in serum and urine. *Journal of Clinical Microbiology* **8**, 197–202.
- WILLIAMS, D. G. & PETERS, D. K. (1983). Glomerulonephritis and renal manifestations of systemic disease. In *Oxford Textbook of Medicine* (ed. D. J. Weatherall, J. G. G. Ledingham and D. A. Warrell), pp. 18, 33–18, 52. Oxford: Oxford University Press.