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Serotyping of *Chlamydia psittaci* by the Micro-Immunofluorescence Test: Isolates of Ovine Origin

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The micro-immunofluorescence test was successfully applied to seven ovine isolates which could be separated into two distinct immunotypes. The pattern of reactivity was similar to that elucidated by the plaque reduction test and confirmed by growth characteristics and biotyping.

The micro-immunofluorescence (IF) test has been used for immunoclassification of *Chlamydia trachomatis* strains (12, 14–17, 19). Recently, we used this method for serotyping six strains of *Chlamydia psittaci* recovered from birds, sheep, mice, and humans (2). This note reports the results obtained with this technique applied to seven isolates recovered from sheep and associated with abortion, polyarthritis, and inapparent infection.

Five strains isolated from placentas or aborted lambs and associated with abortion were tested: Q18 and EAE Nice (Edlinger; Institut Pasteur, Paris); EAE Tours (Rodolakis; INRA, Nouzilly, France); A22 (J. D. Treharne; Institute of Ophthalmology, London); and B577 (J. Storz; Colorado State University, Fort Collins, Colo.). The MO907 strain, isolated from the feces of an apparently normal sheep (J. Storz), and the LW679 strain, isolated from a joint and associated with polyarthritis (J. Storz), were also tested, as were the 6BC strain of *C. psittaci* (J. Schachter, University of California, San Francisco) and a human genital strain of *C. trachomatis*, LB1 (TRIC/D/GB/MRC-1/G) (Hanna; Proctor Institute, San Francisco).

Isolates were propagated in chicken embryos; suspensions for slide antigens were titrated by the method of Reeve and Taverne (5) and stored at −70°C. The micro-IF technique and the immunization schedule have been described by Wang and Grayston (16). Some modifications were made: (i) all antigens for immunization were prepared in HeLa 229 or McCoy cell cultures; (ii) antisera were prepared in inbred mice (2) chosen for their resistance to the chlamydial strains (C3H mice for the isolates associated with abortion and the MO907 strain; C57BL mice for the LW679, 6BC and LB1 strains). Solutions of a commercial anti-mouse gamma globulin conjugated with fluorescein isothiocyanate (anti-mouse immunoglobulin G (IgG) (H + L), obtained from Institut Pasteur Production, France, and anti-mouse Fab fragment of normal IgG, obtained from Nordic Immunological Laboratories) were utilized.

The results obtained with anti-mouse IgG (H + L) were converted to percentages of the homologous titers (17) (Table 1). With the anti-mouse Fab fragment of IgG, titers were similar but lower and less specific.

The seven ovine strains could be separated into two antigenic groups. The first group included the five abortion isolates and the single isolate from the feces of an apparently normal sheep; these strains are identical with one another (EAE Tours, A22, MO907) or closely related. There were extensive cross-reactions between this group and the other chlamydial strains tested. The second group included the single isolate associated with ovine polyarthritis. When LW679 antiserum was tested with the group 1 antigens, the level of cross-reaction was only from 3 to 6%; it was about 13% with the 6BC and LB1 strains.

When 6BC antiserum was tested with all of the ovine isolates, the level of cross-reaction ranged from 25 to 50%. LB1 antiserum was also tested against the ovine strains, and no cross-reaction was observed. However, when the antisera against the ovine isolates were tested against the LB1 strain, the amount of cross-reaction ranged from 0 to 25%.

The destructive effect on the group antigen of high dilutions of KIO_4 was reported by Barwell (1). Periodate treatment (6) rendered Q18 antigen completely nonreactive. When other group 1 antisera were tested with group 1 treated antigens, they reacted at lower titers than they did with no treated antigens. Degrees of cross-reac-

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TABLE 1. Results of micro-IF cross-tests with ovine isolates, an avian strain (6BC), and a TRIC strain (LB1)

Mouse antiserum	Antigen reaction (% of homologous titer)									Homologous titer
	Q18	B577	EAE Nice	EAE Tours	A22	MO907	LW679	6BC	LB1	
Q18	100	50	100	200	200	200	25	0	0	32
B577	100	100	50	100	100	100	50	50	25	256
EAE Nice	100	100	100	100	100	100	25	25	13	512
EAE Tours	100	50	100	100	100	100	13	13	6	1,024
A22	50	50	50	100	100	100	25	13	13	1,024
MO907	50	50	50	100	100	100	25	13	6	1,024
LW679	3	3	3	6	6	6	100	13	13	128
6BC	25	25	25	50	50	25	50	100	25	1,024
LB1	0	0	0	0	0	0	0	0	100	128

tivity were found with the avian and LB1 strains, but no cross-reactions were observed with group 2 antigen. However, LW679 antiserum cross-reacted only with homologous antigen.

Previously, IF methods have been used only for serotyping isolates associated with ovine abortion (3; P. Russo, Ph.D. thesis, Université de Nice, France, 1975), and some differences were demonstrated among six French isolates (P. Russo, Ph.D. thesis, 1975). With a neutralization test, the differences among the American and British strains were no greater than were those among the British strains alone (20). With the micro-IF method, none of the strains associated with abortion gave a reaction of identity, but their percentages of homology were not significantly different (50 to 200%). Thus, it appears that strains isolated from different geographical areas are closely related. The type 1-type 2 cross-reactions observed were predominantly one way, suggesting that there are senior and junior strains as demonstrated for type A and type C trachoma strains (17). Nevertheless, after periodate treatment there was no cross-reaction between these two types. The ovine strains of serotypes 1 and 2 also differed in their pathogenicity in inbred mice. When the abortion strains or the MO907 fecal strain were injected intraperitonally, the C57BL mice generally died and the C3H mice lived. When the LW679 isolate was inoculated, the C3H mice died and the C57BL mice lived (unpublished data).

Fraser and Berman (4) clearly demonstrated that the strains associated with EAE were antigenically different from the polyarthritis and ovine pneumonitis isolates by using purification of elementary bodies with fluorocarbon and different procedures involving marked modification of antigens. A yolk sac infectivity neutralization test (11, 20) and more recently a plaque reduction test (7, 8) have also been used. Isolates associated with abortion and the MO907 strain could be separated from isolates associated with polyarthritis and conjunctivitis; there was no cross-reaction between these two serotypes or with several avian strains (7). These two serotypes were not host specific (8).

Similar results were obtained with biological methods by studying inclusion morphology and response to DEAE-dextran and cycloheximide. Ovine strains that caused abortions and inapparent infections (MO907 isolate) were placed into biotype 1; isolates of strains that caused polyarthritis and conjunctivitis, into biotype 2 (9). These two biotypes also differed in their use of host cell resources (10).

Thus, the results obtained with the micro-IF test are in agreement with those obtained with the plaque reduction test and biological methods. It appears that ovine strains could be classified into two major antigen groups and two corresponding biotypes. The advantage of the micro-IF technique is that it allows direct comparison of antigens and measurement of specific antibody titers.

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