

Serotyping of *Chlamydia*

I. Isolates of Ovine Origin

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Eight chlamydial isolates of ovine origin were tested in a plaque reduction system using homologous and heterologous rooster antisera. The eight isolates could be separated into two separate immunotypes. Type 1 included isolates associated with ovine abortion and one agent recovered from the feces of an apparently normal sheep. Type 2 isolates were associated with polyarthritides and conjunctivitis. These two serotypes were not cross-reactive with several chlamydiae of avian origin. Further application of the plaque reduction test may provide a useful means of typing chlamydiae.

The major antigenic determinants of the *Chlamydia (Bedsonia)* appear to be genus specific. Although many studies have demonstrated species-specific antigens (4, 5), only recently has a workable system for serotyping chlamydiae of human origin been developed. The mouse toxicity prevention test (1) and the microimmunofluorescence test (9) have allowed useful, reproducible serological differentiation of chlamydiae of human origin (trachoma-inclusion conjunctivitis agents and lymphogranuloma venereum strains). Although it has been long recognized that chlamydiae of avian origin, as well as those recovered from domestic and feral mammals, contain specific antigens (for a recent review, see Storz [8]), useful tests for serotyping these isolates have not yet been developed. Most tests detecting strain differences employ difficult procedures involving marked modification of antigens and purification of elementary bodies and have not found general acceptance among researchers. The plaque assay of infectivity of chlamydiae (2) has been utilized to demonstrate neutralizing antibody in hyperimmune rooster antiserum, and the plaque reduction test has shown promise in differentiating chlamydiae of avian origin (3). This communication reports the results obtained in applying this technique to isolates recovered from sheep.

The antigenic relationship among some of these isolates has been studied previously by using a yolk sac infectivity neutralization test (7). The results of that study led to the conclu-

sion that specific antigens were disease specific. Chlamydial isolates associated with ovine abortion were separable from those associated with arthritis in sheep.

MATERIALS AND METHODS

Infectivity assay. Chlamydial infectivity was determined by plaque assay in L929 cells as described previously (2).

Antiserum production. Neutralizing antisera were obtained by hyperimmunization of roosters. The technique involved inoculation of each rooster with noninfective (Formalinized) suspensions of yolk sac-propagated chlamydiae followed by a series of inoculations with viable suspensions of the same agent, as described previously (3). Each rooster was pre-bled, and serial bleedings were taken during the immunization course. The sera from exsanguinated positive animals were pooled with neutralizing antisera obtained from other roosters receiving the same agent. Most titrations were performed with such pooled sera, although occasionally only one of the four roosters immunized with each strain had a neutralizing antibody response. Titrations of the sera were expressed as the dilution reducing the plaque count by 50% when compared with controls run with normal sera obtained by pooling prebleedings of these roosters (3).

Chlamydial strains used. The following uncloned chlamydial strains, isolated from sheep, were tested: MO-907 (feces of apparently normal sheep); B577, FITZ-65, and TO-3 (aborted lambs); LW-646 (ileum of polyarthritic lamb); LW-646A (joints of same lamb above); FCS-13 (conjunctival scraping); FCS-12 (blood of sheep with follicular conjunctivitis and polyarthritides).

Yolk sac-grown agent pools were prepared and stored at -70 C. The same pools were used for

immunization and all titrations. These pools titrated 10^7 to 10^8 plaque-forming units/g of yolk sac. The size of the plaques ranged from 0.5 to 2.0 mm in diameter. Although some range in plaque size was shown, some isolates tended to produce larger or smaller plaques than most of this group.

Roosters were immunized with yolk sac suspensions of each of these isolates, and at each bleeding the sera were titrated in the homologous system. Pooled positive sera were then retitrated against homologous and heterologous isolates by checkerboard pattern.

RESULTS

Results of the checkerboard titration are presented in Table 1. Two antigenic groups of chlamydial strains could be clearly differentiated. The first group, designated type 1, included the three abortion isolates and the single isolate from feces of an apparently normal sheep. The second group, designated type 2, included the two strains recovered from different anatomic sites of a lamb with polyarthritis and the two isolates recovered from different sheep in a flock which was experiencing an outbreak of chlamydial conjunctivitis and polyarthritis. There was no cross-reaction between these two types. It is evident that all the isolates belonging within each of these types did not give reactions of identity. For example, MO-907 reacted with two of three antisera of type 1 abortion isolates but not with the third. Antisera to TO-3 did not reduce infectivity in a homologous system but did so with two other abortion isolates. This failure may reflect both the low titer of this antiserum pool and quantitative antigenic differences between isolates. For example, serum against B-577 had a titer of 1:64 against FITZ-65, higher than the 1:35 titer observed in the homologous system. Similar relationships might exist with isolate TO-3 and

B-577 or FITZ-65, but could not be detected because the homologous titer would have been less than 1:10 with this serum of low titer. Infectivity of TO-3 was reduced by sera against the other three type 1 isolates. Since the pattern of reactions was clear and the limitations of this assay system and the method of producing antisera are not yet sufficiently defined, it is preferable to group those strains giving significant cross-reaction.

The antisera against these ovine isolates were tested against three chlamydiae recovered from avian species (Texas turkey, Meyer parrot, and pigeon AU-46) and antisera against the avian species (homologous titers of 1:520, 1:20, and 1:18, respectively) tested against the sheep isolates. No cross-reactions were observed.

DISCUSSION

The genus *Chlamydia* is presently divided into two species on the basis of relatively easily measured laboratory parameters (folate production and glycogen accumulation within the inclusion). Unfortunately, this taxonomic approach lumps a wide variety of isolates of avian and mammalian origin into one species, *Chlamydia psittaci* (6). Such a designation is of no epidemiological value because all chlamydial isolates responsible for zoonotic infections in man and economically important diseases in exotic birds, poultry, and domestic mammals fall within that species. But it is obvious from many studies on biological properties, such as animal pathogenicity, that many of the *C. psittaci* isolates are, in fact, different. The relationship between chlamydiae recovered from a diversity of mammalian species or from different disease conditions in the same host is not clear. In addition, it has been postulated

TABLE 1. *Plaque reduction by antisera to ovine chlamydiae*

Agents tested	Antisera							
	Type 1				Type 2			
	MO-907	FITZ-65	B577	TO-3	LW-646A	LW-646	FCS-12	FCS-13
MO-907	48 ^a	56	11	— ^b	—	—	—	—
FITZ-65	49	63	64	17	—	—	—	—
B577	55	49	35	18	—	—	—	—
TO-3	35	46	16	—	—	—	—	—
LW-646A	—	—	—	—	42	—	—	45
LW-646	—	—	—	—	11	75	31	33
FCS-12	—	—	—	—	20	80	26	52
FCS-13	—	—	—	—	23	58	25	26

^a Reciprocal of serum dilution reducing plaque count by 50%.

^b —, No reduction at 1:10 serum dilution.

that avian species may be involved in transmission of chlamydiae to mammals.

For epidemiological purposes, in order to obtain information on distribution of chlamydiae in the animal reservoir and intraspecies or interspecies transfer, a more specific designation and identification of chlamydial isolates is required. A reproducible and sensitive method of serotyping isolates may provide the necessary tool. The present investigation has allowed differentiation of eight chlamydial isolates of ovine origin into two major serotypes. These serotypes were not cross-reactive with serotypes among isolates of avian origin, nor in other experiments (unpublished) was there any cross-reaction with lymphogranuloma venereum isolates. The two broad antigenic groups of chlamydial isolates associated with either ovine abortion or polyarthritis were previously observed in neutralization tests performed with developing chicken embryos (7). Possibly the antigenic differences reflect pathogenic properties.

The general groupings obtained by the plaque reduction technique and chicken embryo neutralization method are similar, and the few discrepancies probably reflect differing sensitivities. With the chicken embryo technique, there were some low-grade cross-reactions between L-646-1, an arthritis isolate, and some abortion isolates. No such reactions were seen by the plaque reduction method. With both methods occasional higher heterologous titers were found, and not all isolates within a group gave identical reactions.

The tissue culture method offers several advantages. It is more economical of serum and gives more reproducible, precise end points than the chicken embryo technique. Although it is certain that subtypes will eventually be defined, these results clearly differentiate two antigenic types. The selection of appropriately broad reactive antisera (such as anti-FITZ-65 and anti-FCS-13) would have allowed typing all eight isolates simply on a positive or negative result.

The usefulness of this method of typing will be further tested by determining the relationship between the two serotypes of ovine chlamydiae and the chlamydiae recovered from cattle and other domestic mammals. In addition, a screening program on fresh field isolates is planned. Application of this typing method will hopefully allow elucidation of the epizootiology of mammalian chlamydial infections and identification of sources of human infection, and may permit development of a useful and comprehensive system for classification of chlamydial isolates.

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