Factor Analysis of Saprophytic Serogroup Doberdo of *Leptospira biflexa* and Characterization of Serovars *zoo* and *drahovce*

M. CINCO, R. DOUGAN, AND I. STEFANELLI

Institute of Microbiology, University of Trieste, Trieste, Italy

Major and minor antigens of serovar members of the saprophytic leptospiral serogroup Doberdo were defined by factor analysis. This serogroup could be characterized by two main antigens which served to distinguish members of this group from strains of other serogroups which possess other, common antigens. In addition, factor analysis of two new strains of leptospires, V-2 and V-7/2, ascribed to serogroup Doberdo revealed that strain V-2, serovar *drahovce*, is a typical representative of the group, whereas strain V-7/2, serovar *zoo*, is not representative.

Factor analysis, as proposed by Kmety (7), was previously used to identify main and minor agglutinating antigens of Semaranga and Andamana serogroups (4). As part of a continuing program to describe the agglutinating factors of other serogroups of saprophytic leptospires we present here the results of studies on the three serovars, *doberdo*, *rupino*, and *isolasacra*, in the serogroup Doberdo, as well as on strains Farneti, AM13, and AM20. The last three strains share high-titer agglutinins with Doberdo serovars; however, they were previously assigned to other serogroups.

Antigenic factor analysis was carried out on two strains, V-2 and V-7/2, reported by Hlavatá to be new serovars which were designated *drahovce* and *zoo*, respectively (6). Both were assigned to the Doberdo serogroup.

MATERIALS AND METHODS

Strains: Doberdo serogroup. Strain Doberdò 1, belonging to *doberdo* serovar, was isolated from fresh water and classified by Cinco and Petelin (5). Strain Isola Sacra 1, belonging to serovar *isolasacra*, was isolated by Babudieri (1) in 1947 and classified by Nardelli (8). Strain RPE, representing serovar *rupino*, was isolated and classified by Cinco and Petelin (5).

Aurisina serogroup. Strain Farneti, belonging to *farneti* serovar, was isolated by Babudieri (1) and classified by Castellani and Polster (2).

Holland serogroup. Strain AM20, belonging to serovar *acquamarzia*, was isolated by Babudieri (1) and classified by Castellani and Polster (2). Strain AM13, belonging to serovar *tredici*, was isolated by Babudieri (1) and classified by Castellani and Polster (2).

Immune sera and serological tests. The preparation of immune sera and serological procedures were previously described (4).

Serovars *drahovce* and *zoo*. Tests related to sero-

vars *drahovce* and *zoo* were carried out with members of the serogroup Doberdo and Holland by using previously described methods (4).

RESULTS

The results of cross-agglutination reactions of different sera before and after absorption with the various serovar strains studied are reported in Fig. 1. A common main antigen, called Dob 6, was found in all strains except Farneti and AM13. Antibodies against this factor were also present in serum anti-AM20.

Serovars of the serogroup Doberdo were also found to contain another main factor, designated Dob 4. Anti-RPE and anti-Isola Sacra sera after absorption with strain Doberdò 1 retained high homologous titers, thus indicating the presence of another main antigen on RPE and Isola Sacra, called Dob 5, which was not detectable on strain Doberdò 1. This factor would represent another main antigen typical of the group and could not be distinguished from factor Dob 2 and Dob 3 in absorption tests.

Factors Dob 1, Dob 2, and Dob 3 represented the antigens specific for serovars *doberdo*, *rupino*, and *isolasacra*, respectively. They could be obtained by selective absorption with heterologous serovars (Fig. 1). A thermolabile agglutinin which previously was found in strain Isola Sacra was associated with factor Dob 3 (3).

Strains Isola Sacra 1 and RPE were more closely related to each other than to strain Doberdò 1: RPE had more inclusive antigens than Isola Sacra 1 since it was possible to obtain the monospecific anti-Isola Sacra factor by absorbing anti-Isola Sacra serum with RPE in one step. Doberdò 1 differed from these strains in lacking factor Dob 5 and in its serological affinity with AM20 which is a member of serogroup

Holland. Minor factors Dob 01, 02, 04, and 05 were responsible for the cross-agglutination with strain Farneti, and Dob 6, 03, 06, 07, 08, 09, and 010 were responsible for the cross-agglutination with strains AM13 and AM20.

Absorption performed with anti-AM20 and anti-AM13 sera confirmed the existence of the above-listed antigens, but the postabsorption titers indicated that the same minor factors present in strain AM20 were of lower immunogenicity. An antigenic map of the factors of serogroup Doberdo is shown in Table 1. Cross-agglutinations with members of other serogroups revealed that factors Dob 4 and Dob 5 were the specific antigens of the group; consequently the presence of these could be used in assigning a strain to this serogroup.

Serovars *zoo* and *drahovec*. By factor analysis, strain V-2 was found to be a typical representative of the serogroup Doberdo because it has the main factors of its own serovar, which is designated Dob 8, and further other main and minor factors as reported in Table 2. It showed a marked affinity with strain Doberdò 1.

Strain V-7/2 could not be considered a member of Doberdo serogroup, because main factors of the group were not detectable on this serovar, except factor Dob 7. It showed more antigenic affinity with serovars of the Holland serogroup (these antigens are provisionally indicated with letters in Table 2).

Many new "haptene-like" factors are described in the present study (indicated by foot-

FIG. 1. Results of cross-absorption tests among serovars of Doberdo serogroup and related serovars.

TABLE 1. Antigenic map of serovars representing serogroup Doberdo

Serovars	Reference strains	Main antigens	Minor antigens
<i>doberdò</i>	Doberdò	Dob 1,-,4,-,6,...	Dob 01,02, -, -, -,06, -08, -,010,....
<i>rupino</i>	RPE	Dob -,2,-,4,5,6...	Dob 01, -,03,04,05, -,07,08,09, -,....
<i>isolasacra</i>	Isola Sacra 1	Dob -,3,4,5,6,...	Dob 01, -, -, -,05, -,07, -,09, -,....
<i>acquamarzia</i> ^a	AM20	Dob 6	Dob 01,02,03, -, -,06, -, -,09, -,....
<i>tredici</i> ^a	AM13		Dob -, -, -, -, -,07,08,09,010,....
<i>farneti</i> ^a	Farneti		Dob 01,02, -,04,05, -, -, -, -,....

^a Serovars not belonging to Doberdò serogroup.

TABLE 2. *Antigenic map of zoo and drahouce and of the correlated serovars*

Serovars	Reference strains	Main antigens	Minor antigens
<i>drahouce</i> ^a	V-2	Dob -, -, -, 4, ^b 5, 6, ^b 7, 8, 9, ^b ., ., ., -, -, -	Dob 01, 02, -, -, -, 06, -, 08, 09, 010, 011, --, 013, 014, ., ., ., -
<i>zoo</i>	V-7/2	Dob -, -, -, -, -, 7, -, -, . . . A, B, Z	Dob 01, -, 03, -, -, -, 07, 08, -, 010, ^b --, --, --, . . . ,
<i>doberdo</i>	Doberdò 1	Dob 1, -, -, 4, -, 6, -, -, 9, . . . -, -, -	Dob 01, 02, -, -, -, 06, -, 08, -, 010, 011, 012, ^b --, --, ., ., ., -
<i>rupino</i>	RPE	Dob -, 2, -, 4, 5, 6, -, -, -, . . . -, -, -	Dob 01, -, 03, 04, 05, -, 07, 08, 09, --, --, 012, 013, --., ., ., ., -
<i>isolasacra</i>	Isola Sacra 1	Dob -, -, 3, 4, 5, 6, -, -, -, . . . -, -, -	Dob 01, -, -, -, 05, -, 07, -, 09, --, --, 012, --., 014, ., ., ., -
<i>acquamarzia</i> ^a	AM20	Dob -, -, -, -, -, 6, 7, -, -, . . . A, -, Z	Dob 01, 02, 03, -, -, 06, -, -, 09, --, 011, --, 013, --, ., ., ., -
<i>tredici</i> ^a	AM13	Dob -, -, -, -, -, -, -, . . . A, -, -	Dob -, -, -, -, -, -, 07, 08, 09, 010, 011, --, --, --, . . . ,
<i>farneti</i> ^a	Farneti	Dob -, -, -, -, -, -, -, . . . -, -, Z	Dob 01, 02, -, 04, 05, -, -, -, -, --, --, --, 013, --, ., ., ., -

^a Serovars not belonging to Doberdo serogroup.^b Haptene-like factors.

note *b* of Table 2). This suggests that the majority of factors detectable in leptospire are polysaccharide units.

DISCUSSION

Antigenic analysis of serogroup Doberdo does not affect the original taxonomic disposition of the serovars examined. However, it gives a more detailed picture of their relationships with the members of other serogroups. Strain RPE, serovar *rupino*, would appear to be most representative of the serogroup, because it possesses all of the antigens of Isola Sacra and the main factors of the serogroup. Factors Dob 5 and Dob 4 are the specific antigens which characterize the Doberdo group.

REPRINT REQUESTS

Address reprint requests to: Dr. M. Cinco, Institute of Microbiology, University of Trieste, 34 Via Alfonso Valerio, 34127 Trieste, Italy.

LITERATURE CITED

1. Babudieri, B., and I. Archetti. 1947. Le Leptospire acquicole e la loro costituzione antigenica. Rend. Ist. Super. Sanita Ital. Ed. 10:962.
2. Castellani, M., and E. Polster. 1970. Studio sistematico di alcuni ceppi de leptospire acquicole. Ann. Ist. Super. Sanita Ital. Ed. 6:270-279.
3. Cinco, M., and P. Crismani. 1974. Thermolabile agglutinogens in saprophytic letpospirae. Trop. Geogr. Med. 26:417-422.
4. Cinco, M., and R. Dougan. 1975. Factor analysis of saprophytic serogroups Semaranga and Andamana of *Leptospira biflexa*. Int. J. Syst. Bacteriol. 25:138-142.
5. Cinco, M., and N. Petelin. 1970. Serogroups and serotypes in water leptospira strains. Trop. Geogr. Med. 22:237-244.
6. Hlavatà, Z. 1974. Water strains of leptospire isolated in Slovakia and their classification. Folia Fac. Med. Univ. Comenianae Bratisl. 12(Suppl.):157-160.
7. Kmety, E. 1960. Contributions to the antigenic structure of leptospira serotypes forming the Australis Serogroup. J. Hyg. Microbiol. Epidemiol. 2:171-175.
8. Nardelli, M. G., and B. Babudieri. 1972. Studio sistematicodi 20 ceppi de Leptospire acquicoli. Ann. Ist. Super. Sanita Ital. Ed. 8:114.