INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, Jan. 1990, p. 98–99 Vol. 40, No. 1
0020-7713/90/010098-02$02.00/0

NOTES

Leptospira interrogans Serovar Unipertama Isolated in Malaysia

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A leptospiral isolate from a bovine kidney was found to be antigenically different from all previously recognized serovars of *Leptospira interrogans* based on the cross-agglutinin absorption test. The new serovar belongs to the Sejroe serogroup, and the name *Leptospira interrogans* serovar unipertama is proposed for it, with strain K2-1 as the reference strain.

A total of 37 leptospiral serovars have been isolated in Malaysia (1), and rats have been ascribed as the principal maintenance hosts for these leptospires (6). To date, only six leptospiral serovars have been reported in domestic animals in Malaysia. Serovar hardjo is seen as the most common one affecting cattle and has been isolated from a number of herds throughout Malaysia (2). In our attempt to establish the role of domestic animals in the epidemiology of leptospiral infection in Malaysia, we isolated from a bovine kidney a leptospiral strain which was found to be entirely different from the previously established serovars maintained in reference laboratories. In this paper we describe the isolation and identification of this new serovar.

In this study, six bovine kidneys were obtained from an abattoir in Selangor, Malaysia, on 7 October 1982 and were examined for evidence of leptospiral infection. One of the kidneys (kidney K2-1) was obtained from a 3-year-old Kedah-Kelantan (a local breed) bull which was apparently healthy on inspection prior to slaughter. Each kidney sampled was swabbed with 70% alcohol to reduce bacterial contamination, and about 10 g of the organ was then macerated in phosphate-buffered saline with the aid of a kitchen blender. Then 2 drops of the homogenate was inoculated into two bottles of semisolid EMJH medium (7), one containing 200 μg of 5-fluorouracil per ml of medium and the other containing 400 μg of 5-fluorouracil per ml of medium. The remaining homogenate was diluted 10-fold with phosphate-buffered saline and then inoculated into another two bottles of semisolid EMJH medium. The cultures were then incubated at 30°C and examined fortnightly for 12 weeks. Positive cultures were subcultured consecutively into fresh semisolid and fluid EMJH medium until a pure and heavy growth was obtained.

The leptospiral cultures obtained were subjected to the egg yolk test (4), the oxidase test (5), the pathogenicity test in weanling hamsters, and tests for growth at 13°C (7) and in the presence of 8-azaguanine (8). We found that strain K2-1 was susceptible to 8-azaguanine and grew at 30°C but not at 13°C. It was agreed by the Subcommittee on the Taxonomy of *Leptospira* that members of *Leptospira interrogans* need to be confirmed only by susceptibility to 8-azaguanine and lack of growth at 13°C (3, 9).

The cultures were then subjected to the microscopic agglutination test against 16 different group sera (Difco Laboratories, Detroit, Mich.), which represented the 15 important leptospiral serogroups. The hyperimmune sera used were australis (Australis serogroup), autumnalis (Autumnalis serogroup), ballum (Ballum serogroup), bataviae (Bataviae serogroup), canicola (Canicola serogroup), celledoni (Celledoni serogroup), cynopteri (Cynopteri serogroup), grippotyphosa (Grippotyphosa serogroup), icterohaemorrhagiae (Icterohaemorrhagiae serogroup), javanica (Javanica serogroup), mini (Hebdomadis serogroup), pomona (Pomona serogroup), pyrogenes (Pyrogenes serogroup), sejroe, hardjo (Sejroe serogroup), and tarassovi (Tarassovi serogroup).

The results obtained from the microscopic agglutination test showed that strain K2-1 was not homologous with any of the 16 rabbit hyperimmune sera. Cross-agglutinin absorption tests indicated that strain K2-1 is antigenically different from all currently recognized serovars of *L. interrogans*. The rabbit hyperimmune sera to strain K2-1 did not agglutinate any of the 23 serovars representing currently recognized serogroups, but it agglutinated strain K2-1 to a titer of 1/3,200. Conversely, antiserum to each of the 23 serovars did not agglutinate strain K2-1, with the following two exceptions: antisera to wolffi (a member of the Sejroe serogroup) and to borincana (a member of the Hebdomadis serogroup) agglutinated strain K2-1 to titers of 1/400 and 1/100, respectively. Further cross-agglutinin absorption tests with the 36 serovars currently recognized as members of the Hebdomadis serogroup indicated that strain K2-1 is definitely not a member of the Hebdomadis serogroup. The rabbit antiserum against strain K2-1 did not agglutinate any of the 36 serovars, and reciprocal testing with antisera raised to the 36 serovars revealed no agglutination titers in excess of 1/400. Strain K2-1 has a tendency toward roughness, making interpretation of agglutination more difficult than usual.

The results described above indicated that strain K2-1 shares only minor antigens with members of the Hebdomadis serogroup and that its major antigens are not shared with any member of the currently recognized serogroups. Tests initially performed at the Leptospirosis Reference Laboratory, Brisbane, Australia, and later confirmed at the Centers for Disease Control, Atlanta, Ga., found that strain K2-1 is antigenically unrelated to known pathogenic serovars, and therefore we proposed that it be classified as a new serovar in the Sejroe serogroup. The strain was assigned to the Sejroe serogroup because antiserum to wolffi agglutinated strain K2-1 to a titer of 1/400. We propose the name

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unipertama for this new serovar of *L. interrogans* and strain K2-1 as the reference strain.

We thank Unipertama (Universiti Pertanian Malaysia) for sponsoring this study (Project 1803-1-122).

We thank K. R. Sulzer, Centers for Disease Control, Atlanta, Ga., for kindly serotyping our isolates and confirming our findings.

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