An Unusual *Leptospira*, Serotype *illini* (a New Serotype)

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During the course of a periodic epidemiological survey for leptospirosis of a herd of bulls at the University of Illinois Agricultural Research Center, Dixon Springs, a new leptospiral strain was isolated from the urine of a clinically healthy Hereford bull, no. 3055. The strain was found to be antigenically unrelated to known pathogenic (*Leptospira interrogans*) and "saprophytic" (*Leptospira biflexa*) serotypes. Phenotypically, it most closely resembled *L. biflexa* strains; however, in view of the reported distinct genetic characteristics of strain 3055, its species classification is not recommended at this time.

A periodic evaluation of leptospiral antibodies in a herd of 60 2-year-old Hereford bulls at the Dixon Springs Agricultural Center indicated recent *Leptospira interrogans* serotype *pomona* infection in the spring of 1965. (Information available since submission of the manuscript indicates "serovar" is the new official term for "serotype.") As no clinical signs of disease were evident, an attempt was made to isolate leptospires from the urine of selected seropositive and seronegative bulls to determine whether serotype *pomona* was present in the herd. The isolation and characterization of a new leptospiral serotype from a clinically healthy bull (no. 3055) is described.

MATERIALS AND METHODS

Urine was collected from each animal in a sterile container, and approximately 0.1 ml was transferred into 5 ml of liquid and semisolid bovine albumin polysorbate 80 (4) medium. The inoculated medium was incubated at 30 C and examined weekly over a 6-week period with dark-field microscopy for the presence of leptospires.

The leptospiral culture and the serum from the bull from which it was isolated were sent to the WHO/FAO Leptospirosis Reference Laboratory at Walter Reed Army Institute of Research (WRAIR) for typing and additional serological tests, respectively. The submitted culture was cloned by the use of plating medium. The cloned culture used as antigen was tested for cross-agglutination reactions with 85 different serotype-specific antisera selected from all known pathogenic serogroups, and with all except one (serotype *tororo*) of the antisera to the 55 classified *Leptospira biflexa* serotypes (2). Additional tests were made with antisera prepared against strain 3055 and against eight other unidentified "*biflexa*-like" isolates, four of which were from the same geographic region as

strain 3055. One of the latter strains, A-177, isolated from a turtle (R. D. Andrews, Ph.D. thesis, Univ. of Illinois, 1966), had the same growth, physiological, and phage characteristics as strain 3055 (H. E. Ellinghausen and A. Ritchie, personal communication), which it also resembled in genetic characteristics (unpublished data, WRAIR). The microscopic agglutination technique was used for serological tests (1). Homologous titers of test antisera ranged from 1:10,000 to 1:500,000.

Several differential tests, e.g., growth in 2,6-diaminopurine (8), growth at 13 C (7), hemolysis of mouse erythrocytes (9), and egg yolk reaction test (5), were conducted to determine the properties of strain 3055.

RESULTS

A leptospiral strain was isolated in the bovine albumin polysorbate 80 medium from the urine of an asymptomatic bull (no. 3055) in March 1965. A subsequent attempt to isolate leptospires from the urine of bull 3055 was unsuccessful. In preliminary studies, strain 3055 was found to be antigenically unrelated to *L. interrogans* serotypes *autumnalis*, *ballum*, *canicola*, *grippotyphosa*, *hyos*, *icterohaemorrhagiae*, *pomona*, *sejroe*, *georgia*, and *hardjo* and to a few selected *L. biflexa* serotypes when tested against reference antisera. Serum from the bull had a homologous titer of 1:100 but did not react with serotypes *autumnalis*, *grippotyphosa*, *canicola*, *icterohaemorrhagiae*, *pomona*, or *hardjo*.

The initial serological findings were affirmed at WRAIR. Strain 3055 could not be serologically related to any of the known pathogenic and *biflexa* serotypes in extensive cross-agglutination testing. It reacted to titer (1:25,600) with the homologous antiserum and with

antisera against A-177 and A-177 with anti-3055 serum. Otherwise, only minor cross-reactions were elicited against antisera as follows: *copenhageni*, 1:200; *mankarso*, 1:400; *nda-hambukuje*, 1:400; *naam*, 1:800; *weaveri*, 1:400; *bindjei*, 1:100; *sofia*, 1:100; *worsfoldi*, 1:100; *mini*, 1:100; *djatzi*, 1:100; *bratislava*, 1:100; *grippotyphosa*, 1:100; *canalzonae*, 1:100; *garcia*, 1:500; and *fons*, 1:500.

In differential tests the isolate grew well in the presence of 10 µg of 2,6-diaminopurine per ml and at 13 C. Old cultures of this isolate hemolyzed mouse as well as rabbit, horse, hamster, and gerbil erythrocytes to varying titers. Lower titers were obtained with horse and rabbit erythrocytes (1:4 to 1:16) than with mouse, hamster, or gerbil erythrocytes (1:32 to 1:64). It decomposed egg yolk medium in 3 to 4 days.

DISCUSSION

Strain 3055 not only is serologically distinct but also has remarkable genetic and cultural properties. Brendle et al. (3) found that strain 3055 had no deoxyribonucleic acid (DNA) affinities with known pathogenic and "saprophytic" genetic types of leptospires on the basis of DNA annealing tests and appeared to represent a new genetic "complex" of leptospires. Moreover, its guanine and cytosine content, 53 mol%, was found to be considerably higher than that of other leptospires (35.2 to 41.2 mol% range) (3). Strain 3055 also represents the only leptospiral serotype in which a phagelike entity has been demonstrated (10). In addition, Ellinghausen (personal communication) reported that it differed from other leptospires except strain A-177 in its ability to grow in ordinary Trypticase media. Although strain 3055 was isolated from a bull, it behaved like a non-pathogenic leptospira in pathogenicity tests (11) in its ability to grow at 13 C, in its relative resistance to 2,6-diaminopurine, 8-azaguanine, and CuSO₄ (unpublished data, WRAIR), and in its hemolytic properties.

The close antigenic affinities of strain 3055 to strain A-177 was not surprising. Strain A-177 has the same cultural and physiological characteristics as those of strain 3055. Moreover, a phagelike entity has also been demonstrated in strain A-177 (Ritchie and Ellinghausen, personal communication). Like strain 3055, it has a high guanine and cytosine base ratio (51.2 mol%) (J. J. Brendle, M. Rogul, A. D. Alexander, unpublished data).

On the basis of serological findings, strain

3055 can be classified as a new serotype, for which we selected the name *illini*. The species classification of 3055 is problematical because it does not fit into either of the two species, *L. interrogans* ("pathogenic") and *L. biflexa* ("non-pathogenic"), recently proposed by the Leptospira Subcommittee, International Committee on Systematic Bacteriology (6). The two species were established on the basis of pathogenicity and other phenotypic differences as well as genetic differences as determined by DNA-DNA annealing tests. Although strain 3055 is phenotypically similar to *L. biflexa* strains, it cannot be classified in these species in view of its remarkably distinct genetic characteristics. Accordingly, at this time we do not propose species classification of strain 3055.

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