NOTES

A New Leptospiral Serovar in the Icterohaemorrhagiae Serogroup Isolated from an Ox in Zimbabwe

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A strain of *Leptospira interrogans* that was isolated from an ox slaughtered in Zimbabwe and belonged to serogroup Icterohaemorrhagiae could not be identified when we compared it with 18 reference strains belonging to this serogroup by using cross-agglutinin absorption, monoclonal antibody, and restriction endonuclease DNA analyses. The name *zimbabwe* is proposed for the new serovar containing this strain; the type strain of this serovar is strain SBF 23.

Leptospira interrogans strains belonging to serogroup Icterohaemorrhagiae were the first members of the genus *Leptospira* to be isolated (9). Since the initial isolation, the number of serovars belonging to this serogroup has risen to 14 (11), with four other serovars currently being considered (19, 27). Although the majority of these serovars were first isolated from humans, antibodies to serogroup Icterohaemorrhagiae strains have been detected in cattle (18, 24), and strains belonging to serogroup Icterohaemorrhagiae have occasionally been associated with clinical disease in naturally infected cattle (5). In Africa, strains belonging to serogroup Icterohaemorrhagiae have been isolated from humans (8, 10, 15), dogs (15), and rodents (1–3, 14, 17). Reference strains for serovars *mwogolo*, *ndahambukuje*, and *ndambari* in sero-

group Icterohaemorrhagiae were first isolated from humans in Zaire (25).

The current classification of the bacteria that belong to the genus *Leptospira* is based on serological criteria. The microscopic agglutination test and the cross-agglutinin absorption test are the standard reference procedures used for serological classification of leptospires (4). When these methods are used, two strains are considered to belong to different serovars if after cross-absorption with adequate amounts of heterologous antigen, 10% or more of the homologous titer regularly remains in at least one of the two antisera in repeated tests. The methodology cannot be standardized and, therefore, sometimes is not reproducible. It may also fail to distinguish between genetically different but antigenically similar strains.

TABLE 1. Serovar identification of strain SBF 23 by cross-agglutinin absorption

Serovar	Antiserum	Absorbed with strain:	Reciprocal of homologous titer as a % of unabsorbed serum titer
<i>birkini</i>	SBF 23	Birkin	25
	Birkin	SBF 23	12.5
<i>copenhageni</i>	SBF 23	M20	50
	M20	SBF 23	50
<i>dakota</i>	SBF 23	Grand River	25
	Grand River	SBF 23	50
<i>gem</i>	SBF 23	Simon	50
	Simon	SBF 23	12.5
<i>lai</i>	SBF 23	Lai	6.2
	Lai	SBF 23	25
<i>mankarso</i>	SBF 23	Mankarso	50
	Mankarso	SBF 23	50
<i>mwogolo</i>	SBF 23	Mwogolo	25
	Mwogolo	SBF 23	12.5
<i>ndahambukuje</i>	SBF 23	Ndahambukuje	25
	Ndahambukuje	SBF 23	25

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TABLE 2. Reciprocals of agglutination titers of MAbs with serogroup Icterohaemorrhagiae reference serovars and Zimbabwe strain SBF 23

Subgroup	Strain or serovar	Reciprocal of agglutination titer with:		
		MAb F70C7	MAb F20C4	MAb F82C2
I	<i>bogvere</i>	40,960	2,560	— ^a
	<i>copenhageni</i>	81,920	81,920	—
	<i>icterohaemorrhagiae</i>	40,960	20,480	—
	<i>ndahambukuje</i>	81,920	10,240	—
II	<i>dakota</i>	81,920	40,960	20,480
	<i>hongchon</i>	40,960	10,240	10,240
	<i>mankarso</i>	81,920	20,480	81,920
	<i>naam</i>	81,920	10,240	81,920
	<i>yeonchon</i>	81,920	2,560	10,240
III	<i>honghe</i>	81,920	—	—
	<i>nanxi</i>	40,960	—	—
	<i>ndambari</i>	20,480	—	—
	<i>smithi</i>	81,920	—	—
	<i>tonkini</i>	81,920	—	—
IV	<i>birkini</i>	81,920	—	2,560
	<i>gem</i>	81,920	—	1,280
	<i>lai</i>	40,960	—	40,960
	<i>mwogolo</i>	20,480	—	10,240
	<i>SBF 23</i>	20,480	—	20,480

^a —, <20.

FIG. 1. Agglutination profiles (microscopic agglutinin test titers) of MAbs with serogroup Icterohaemorrhagiae serovars *birkini*, *gem*, *lai*, and *mwogolo* and strain SBF 23. The vertical axis indicates serial twofold dilutions, starting from 1:20 (1), 1:40 (2), etc. (0 = <1:20). (A) Cross-hatched bars, strain SBF 23; solid bars, serovar *birkini*. (B) Cross-hatched bars, strain SBF 23; solid bars, serovar *gem*. (C) Cross-hatched bars, strain SBF 23; solid bars, serovar *lai*. (D) Cross-hatched bars, strain SBF 23; solid bars, serovar *mwogolo*.

Mouse monoclonal antibodies (MAbs) have been used as an additional serological method to identify organisms belonging to the genus *Leptospira* (12, 13, 21). The MAb technique is accurate but still depends on the detection of surface antigens and on careful selection of antibodies that discriminate between serovars.

Restriction endonuclease analysis (REA) of leptospiral DNA was first proposed as an alternative method for classifying leptospires by Marshall et al. (16). After improvements in the DNA extraction procedure and resolution of the restricted DNA fragments in agarose gels (22, 23), REA has proven to be a more sensitive and discriminating method than serotyping. However, the banding pattern obtained with REA may be complicated and/or difficult to read. REA may also fail to find differences between antigenically different strains. For example, serovars *wolffi* and *roumanica*, serovars *istricea* and *nyanza*, and serovars *polonica* and *sejroe* in serogroup Sejroe (23) and serovars *tarassovi*,

guidae, *tunis*, and *moldaviae* in serogroup Tarassovi have similar digestion patterns and are not easily differentiated by REA alone (28).

On the basis of DNA relatedness results, Yasuda et al. (26) have proposed that *L. interrogans* should be divided into the following five species: *Leptospira borgpetersenii*, *L. interrogans*, *Leptospira noguchii*, *Leptospira santarosai*, and *Leptospira weilii*. However, although many leptospirosis laboratories have adopted this classification and are trying to develop and test it, the new classification has not been adopted yet by the Subcommittee on the Taxonomy of *Leptospira*.

Pending the adoption of a completely new classification system, Terpstra (20) has proposed that serological methods complemented by REA patterns produced by using one or more restriction enzymes should be used for the allocation of new strains to serovars.

The isolation and serogrouping of a strain belonging to

FIG. 2. Electrophoretic patterns of chromosomal DNAs from reference strains of serogroup Icterohaemorrhagiae and strain SBF 23 digested with *EcoRI*. The reference strains were strains belonging to serovars *mankarso* (lane 1), *ndahambukuje* (lane 2), *dakota* (lane 3), *lai* (lane 4), *mwogolo* (lane 5), and *birkini* (lane 6). Lane 7 contained strain SBF 23. Lane M contains fragment size markers (sizes are given in kilobase pairs).

serogroup Icterohaemorrhagiae in Zimbabwe have been described previously (6). In this paper we describe the identification of this strain to serovar level, which was accomplished by using the cross-agglutinin absorption test, MAb analysis, and REA.

Antisera from 18 reference strains belonging to serogroup Icterohaemorrhagiae and strain SBF 23 for use in the cross-agglutinin absorption tests were produced in paired rabbits and were pooled before use as previously described (7).

Strain SBF 23 was tested against the serogroup Icterohaemorrhagiae reference antisera, and the serogroup Icterohaemorrhagiae reference strains were tested against antiserum to strain SBF 23 by using the microscopic agglutination test. Only those reference strains which had a reciprocal titer of at least 1:1,600 with strain SBF 23 were used for the cross-agglutinin absorption test. Strain SBF 23 had a reciprocal titer of less than 1:1,600 with antisera raised against reference strains belonging to serovars *bogvere*, *icterohaemorrhagiae*, *naam*, *ndambari*, *smithi*, and *tonkini*. Apparently, strain SBF 23 shares little similarity with members of these serovars, and therefore, they were excluded from the cross-agglutinin absorption tests with strain SBF 23.

High-level reactions (>50% of the reciprocal titer of the homologous strain) were observed with strains belonging to serovars *birkini*, *copenhageni*, *dakota*, *gem*, *lai*, *mankarso*,

FIG. 3. Electrophoretic patterns of chromosomal DNAs from reference strains of serogroup Icterohaemorrhagiae and strain SBF 23 digested with *HhaI*. The reference strains were strains belonging to serovars *birkini* (lane 1), *copenhageni* (lane 2), *dakota* (lane 3), *lai* (lane 4), *gem* (lane 5), *mwogolo* (lane 6), and *ndahambukuje* (lane 7). Lane 8 contained strain SBF 23. Lane M contains fragment size markers (sizes are given in kilobase pairs).

mwogolo, *ndahambukuje*, *hongchon*, *honghe*, *nanxi*, and *yeonchon*. These reference strains were included in the cross-agglutinin absorption tests. The cross-agglutinin absorption tests were performed as described previously (7), except that the results were checked by using both the Bratislava and Amsterdam techniques (4).

Only one-side absorption tests were performed with antisera from strains belonging to the recently discovered but not yet officially recognized serovars *hongchon*, *honghe*, *nanxi*, and *yeonchon*. More than 25% of the antibodies remained in their antisera after absorption with strain SBF 23, indicating that the Zimbabwe isolate did not belong to these serovars.

The results of the cross-agglutinin absorption tests with strain SBF 23 and the reference strains are shown in Table 1. These results indicate that although strain SBF 23 appears to be closely related to serovar *birkini*, *gem*, *lai*, and *mwogolo* strains, it is antigenically different from members of all of the previously described serovars of serogroup Icterohaemorrhagiae because more than 10% of the antibodies remained in at least one of the two antisera. Therefore, on the basis of the current definition of a serovar, strain SBF 23 represents a new serovar in serogroup Icterohaemorrhagiae.

MAbs were produced as previously described (12, 21). Three MAbs (MAbs F70C7, F20C4, and F82C2) were used to divide the serogroup Icterohaemorrhagiae reference strains and Zimbabwe isolate SBF 23 into subgroups (Table

2). MAb F70C7 is serogroup specific and reacted at a high level with strain SBF 23, indicating that this strain belongs to serogroup Icterohaemorrhagiae. Two other broadly reactive MAbs (MAbs F20C4 and F82C2) enabled us to divide the serogroup into four subgroups. Strain SBF 23 showed the same pattern as the members of subgroup IV.

The agglutination profiles obtained with five other MAbs (MAbs F52C2, F70C13, F82C1, F82C7, and F82C8) (Fig. 1) separated strain SBF 23 from serovars *birkini*, *gem*, *lai*, and *mwogolo*. Therefore, the results of the MAb analysis are consistent with those of the cross-agglutinin absorption tests, showing that although strain SBF 23 is closely related to serovars *birkini*, *gem*, *lai*, and *mwogolo*, it is antigenically distinct from these serovars.

The restriction endonuclease DNA patterns of strain SBF 23 and serogroup Icterohaemorrhagiae serovar *birkini*, *copenhageni*, *dakota*, *gem*, *lai*, *mankarso*, *mwogolo*, and *ndahambukuje* reference strains were compared. The selection of these serovars was based on the results of the cross-agglutinin absorption tests. Bacterial DNA extraction, restriction endonuclease digestion, gel electrophoresis, and photography were carried out as described by Thiermann et al. (22).

Strain SBF 23 produced a unique pattern which could be differentiated easily from the patterns of all of the reference strains when the DNAs were digested with enzymes *EcoRI* (Fig. 2) and *HhaI* (Fig. 3). This finding supported the serological data and indicated that in addition to representing a new serovar, strain SBF 23 also represents a new genotype in serogroup Icterohaemorrhagiae.

The name *zimbabwe* is proposed for the new serovar; the type strain of this serovar is strain SBF 23, and this strain is available from the collections of Leptospirosis Reference Laboratories.

The significance of this new serovar as a pathogen of cattle, other domestic animals, and humans is unknown since no clinical syndrome was associated with the isolation of strain SBF 23.

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