MAJOR ARTICLE

# Usefulness of Serologic Analysis as a Predictor of the Infecting Serovar in Patients with Severe Leptospirosis

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**The diagnosis of leptospirosis is often made using the microscopic agglutination test (MAT), in which live antigens representing >20 serogroups undergo reaction with patient serum samples to detect agglutinating antibodies. Data derived from this assay are often used to infer the identity of the infecting leptospiral serovar or serogroup; however, paradoxical reactions and cross-reactions between serogroups are common. To evaluate the usefulness of this approach, data on culture-proven cases of leptospirosis that occurred in Barbados from January 1980 through December 1998 were reviewed. A total of 151 isolates of 4 serovars were identified. The sensitivity of MAT for the prediction of the infecting serovar was determined. Overall, the predominant serogroup at a titer of ⩾100 correctly predicted 46.4% of all serovars isolated. If a titer of ⩾800 was used as the cutoff, sensitivity decreased slightly to 44.4%. The overall specificity for all serogroups was 64.8%. Serologic analysis appeared to be of little value for the identification of the infecting serovar in individual cases of leptospirosis in humans. Presumptive serogroup reactivity data should be used only to gain a broad idea of the serogroups present at the population level.**

Leptospirosis is an acute febrile disease caused by infection with pathogenic spirochetes of the genus *Leptospira.* The disease occurs throughout the world, but its incidence is highest in tropical regions [1]. The spectrum of disease caused by leptospiral infection is extremely wide [2] and varies from clinically inapparent to severe multisystemic disease characterized by jaundice and acute renal failure [3]. Aseptic meningitis was once a common presentation [4]. More recently, pulmonary hemorrhage has been recognized as a significant complication in several outbreaks of leptospirosis [5–7]. The broad range of clinical manifestations mandates that leptospirosis is part of the differential diagnosis of many febrile illness syndromes [8]. In patients with exposure in tropical climates, leptospirosis must be differentiated from malaria and dengue fever, but the differential diagnosis inevitably varies depending on the infectious diseases that are prevalent locally.

The mortality rate associated with severe leptospirosis may be as high as 15% [9]. Antibiotic treatment of leptospirosis with either doxycycline or penicillin has been shown to be effective if initiated early [10–12]. The difficulty in diagnosing leptospirosis clinically highlights the need for laboratory diagnosis if unnecessary antibiotic use is to be avoided. Several diagnostic assays based on IgM detection are now available [2], and these assays may be relied upon to provide a diagnosis after 5–7 days of illness in most cases. The definitive diagnostic test is culture, and isolation of leptospires allows for identification of the infecting serovar

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[13]. However, culture is relatively insensitive and requires several weeks' incubation, which limits its use in most laboratories [2]. The microscopic agglutination test (MAT) uses a panel of leptospiral strains as antigens for detection of agglutinating antibodies [14]. This assay requires significant expertise to perform, and interlaboratory variation in results is high. Despite these limitations, the MAT has epidemiological value, and it is often used to give an indication of the presumptive serovar or serogroup of leptospires involved in an infection. However, overinterpretation of MAT serologic findings in the absence of a sound knowledge of the locally prevalent serovars may limit its value.

The genus *Leptospira* is classified into several species defined by DNA-DNA hybridization [15, 16], and it is classified into >200 serovars [17]. For convenience, antigenically related serovars are clustered within serogroups [17]. The purpose of the present study was to evaluate the ability of the MAT to predict the infecting serovar in a population in which leptospirosis has been extensively characterized for >30 years [18–24].

## METHODS

Laboratory records were reviewed retrospectively to identify all cases of leptospirosis for which the onset of symptoms occurred during the period from 1 January 1980 through 31 December 1998, as confirmed by isolation of *Leptospira* species from blood or urine samples or from specimens obtained from other body sites [24]. Data from these cases were used to determine the sensitivity and specificity of MAT for the prediction of the infecting serovar.

The MAT was performed as described elsewhere [24], by use of a panel of antigens representing both ubiquitous serovars and locally prevalent serovars. The initial panel of antigens was expanded to include the 4 serovars isolated in Barbados. Titers of ⩾100 were considered to be positive. The predominant serogroup was defined as a titer of ⩾100, with the maximum titer directed against a single serogroup. Analyses were also performed using a cutoff titer of ⩾800 [25]. Cases of leptospirosis were excluded from the analysis if patients were seronegative, if maximum MAT titers of <100 were detected, if highest titers were detected against *Leptospira biflexa* serovar patoc, or if titers of ⩾100 were detected, with equal titers directed against >1 serogroup.

The sensitivity of the MAT was defined as the proportion of isolates of a single serovar correctly predicted by the corresponding serogroup that was the predominant reactive serogroup in the convalescent-phase serum sample or in the acute-phase sample obtained most recently. The specificity of MAT serologic analysis was defined as the proportion of patients with a predominant serogroup whose isolate was of the corresponding serovar. The serovars isolated in Barbados and their respective species and serogroups are *Leptospira kirschneri* serovar bim (serogroup Autumnalis), *Leptospira interrogans* serovar copenhageni (serogroup Icterohaemorrhagiae), *Leptospira borgpetersenii* serovar arborea (serogroup Ballum), and *Leptospira noguchii* serovar bajan (serogroup Australis) [24].

## RESULTS

During the 19-year period of this study, a total of 155 cases of leptospirosis were confirmed by isolation of *Leptospira* species. For these cases, 151 isolates were identified to the serovar level. The distribution of serovars among the isolates was as follows: *L. kirschneri* serovar bim, 98 isolates (64.9%); *L. interrogans* serovar copenhageni, 34 isolates (22.5%); *L. borgpetersenii* serovar arborea, 17 isolates (11.3%); and *L. noguchii* serovar bajan, 2 isolates (1.3%). Of the 151 patients with isolates identified to the serovar level, 29 died (mortality rate, 19.2%), and, for these patients, only acute-phase and/or postmortem serum samples were available. An additional 18 patients (11.9%) had only an acute-phase serum sample available. Convalescent-phase samples, which were obtained a mean of 15 days after the onset of symptoms, were available for 104 patients (68.9%). A total of 105 patients had a highest titer of ⩾100 against a single serogroup (table 1), including serogroups Autumnalis (43 patients [41%]), Icterohaemorrhagiae (26 patients [24.8%]), Ballum (14 patients [13.3%]), Australis (13 patients [12.4%]), Canicola (6 patients [5.7%]), Grippotyphosa (2 patients [1.9%]), and Panama (1 patient [1%]).

The sensitivity of MAT serologic analysis for the prediction of the infecting serovar was determined. Serogroup Australis was predominant in both patients from whom *L. noguchii* serovar bajan isolates were recovered (sensitivity, 100%), Autumnalis was the predominant serogroup in 39 (39.8%) of 98 patients with *L. kirschneri* serovar bim isolates, serogroup Ballum predominated in 11 (64.7%) of 17 patients with *L. borgpetersenii* serovar arborea infection, and serogroup Icterohaemorrhagiae was the predominant serogroup in 18 (52.9%) of 34 patients from whom *L. interrogans* serovar copenhageni isolates were recovered. Overall, the predominant serogroup at a titer of ⩾100 correctly predicted 70 (46.4%) of all 151 serovar isolates. When a titer of ⩾800 was used as a cutoff, sensitivity slightly decreased to 44.4% (67 of 151 serovar isolates).

For patients with serogroup Autumnalis predominating at titers of ⩾100, the specificity was 90.7% (39 of 43 serovar isolates). The specificity for serogroup Icterohaemorrhagiae was 69.2% (18 of 26 serovar isolates), that for serogroup Ballum was 78.6% (11 of 14 serovar isolates), and that for serogroup Australis was 15.4% (2 of 13 serovar isolates), whereas, for serogroups Canicola, Grippotyphosa, and Panama, the specificity was zero, because no serovars from these serogroups were isolated (table 1). Among patients with serogroup Australis

**Table 1. Predominant serologic responses among 105 patients with culture-proven leptospirosis.**

| Serogroup[a] | No. of patients with serologic response against serogroup | Sensitivity, % | Specificity, % | No. of patients with *Leptospira* serovar isolates | | | |
|---|---|---|---|---|---|---|---|
| | | | | *L. borgpetersenii* serovar arborea ($n$ = 17) | *L. noguchii* serovar bajan ($n$ = 2) | *L. kirschneri* serovar bim ($n$ = 98) | *L. interrogans* serovar copenhageni ($n$ = 34) |
| Australis | 13 | 100 | 15.4 | 0 | 2[b] | 11 | 0 |
| Autumnalis | 43 | 39.8 | 90.7 | 0 | 0 | 39[b] | 4 |
| Ballum | 14 | 64.7 | 78.6 | 11[b] | 0 | 3 | 0 |
| Canicola | 6 | 0 | 0 | 1 | 0 | 1 | 4 |
| Grippotyphosa | 2 | 0 | 0 | 0 | 0 | 2 | 0 |
| Icterohaemorrhagiae | 26 | 52.9 | 69.2 | 2 | 0 | 6 | 18[b] |
| Panama | 1 | 0 | 0 | 0 | 0 | 1 | 0 |

[a] Predominant serogroup reacting at a highest titer of ⩾100.
[b] Determines the corresponding serogroup for each serovar isolated.

predominating, there were 2 isolates of *L. noguchii* serovar bajan and 11 isolates of *L. kirschneri* serovar bim. Thus, the specificity of serogroup Australis reactivity was higher for *L. kirschneri* serovar bim (84.6%) than for *L. noguchii* serovar bajan (15.4%). The overall specificity for all serogroups was 64.8% (68 of 105 patients).

To increase the specificity of serovar prediction by serogroup, the analysis was repeated using only convalescent-phase samples with titers of ⩾800. Only 79 patients had titers of ⩾800. The specificities determined were as follows: for serogroup Ballum, 90.9% (10 of 11 isolates); for serogroup Autumnalis, 88.6% (31 of 35 isolates); for serogroup Icterohaemorrhagiae, 80% (16 of 20 isolates); for serogroup Australis, 22.2% (2 of 9 isolates); and for serogroup Canicola, 0% (0 isolates). The overall specificity was 74.7% (59 of 79 isolates).

## DISCUSSION

In this study, the ability to infer the serovar identity of infecting leptospires from the results of serologic testing by use of the MAT was evaluated. For more than one-half of the patients, this was not possible. In other studies, serological data derived from the MAT were often used to infer the infecting leptospiral serovar [5, 26–36]. This inference may be based on a lack of understanding of the serologic relationships between leptospires.

Agglutination tests for leptospiral antibody were developed soon after the first isolation of leptospires, which occurred >80 years ago. In the early years of diagnosis of leptospiral infection, when few serovars were known, it was customary to include all those serovars known to occur within a region in the antigen panel and to interpret the results of serologic testing as being serovar specific [14, 37]. However, as more serovars were detected, it became apparent that serovar specificity was an erroneous concept [38]. Cross-reactions between serogroups are common [39, 40], as are paradoxical reactions, in which the initial immune response is directed to a heterologous serovar or serogroup [2, 4, 14, 41, 42]. Paradoxical reactions may occur in up to 50% of cases [43]. The potential for overinterpretation of serologic data thus is much greater if only acute-phase or early convalescent-phase serum samples are available for testing [14].

In reference laboratories, a broad range of serogroups has been used in the MAT to maximize the probability of detecting an immune response to a serovar not expected, either because it has not yet been isolated or because a previously known serovar has been introduced into the population [14, 25, 44]. An additional confounding factor in areas of high endemicity is the possibility of coinfection with multiple serovars [14, 45].

In many reports, a narrow range of serogroups is represented in the panel of antigens used in the MAT, which may further reduce the ability of serologic analysis to accurately predict the infecting serogroup [46, 47]. Moreover, most serogroups contain several serovars. Thus, reaction with an individual serovar selected for use as an antigen representing a serogroup cannot be taken to imply infection with the serovar tested but, rather, infection with only an antigenically similar serovar of the same serogroup. When multiple serovars from a single serogroup are included in the MAT, cross-reactivity is the rule rather than the exception [48–50]. Moreover, within a serogroup, antigens of different serovars may not detect identical titers [50, 51].

Several authors have made the observation that understanding the epidemiology in a geographical region requires isolation and serological characterization of leptospires [52, 53], as does the identification of the leptospires that cause infection in an individual patient [13, 39, 40, 54]. Rarely, a point-source outbreak of leptospirosis occurs, allowing for serologic identification of the presumptive infecting serogroup [49, 55]. In one outbreak, serogroup Australis was the predominant serogroup reacting in 30 of 33 patients, and, in the remaining 3 patients, equal reactivity to serogroups Australis, Javanica, and Sejroe was recorded [49]. Three serovars were used to represent sero-

group Australis in the panel of antigens used in the MAT in this investigation. Of the 33 patients, 12 had reactions against only *L. interrogans* serovar bratislava, and 2 had reactions against only *L. interrogans* serovar lora, whereas the remaining 19 patients had reactions against either 2 or all 3 of the Australis serovars. Cross-agglutinin absorption testing [56], used in the long-term follow-up of patients involved in this outbreak, failed to discriminate between serovars australis and lora [51]. Thus, in this example, extensive testing allowed for the presumptive serogroup to be identified with a high degree of confidence, but it could not identify a probable infecting serovar.

In another small outbreak, 2 isolates of different serovars and serogroups were recovered. The serogroup of one of the isolates was not represented in the MAT panel initially used to test the patients' serum samples, and 6 of the 8 patients were found to be nonreactive by use of the MAT [57]. When another serovar that was from the same serogroup to which the isolate belonged was included in the MAT antigen panel and the samples were retested, all 6 patients were found to be seroreactive.

Only one previous study related serologic findings to the identity of the infecting serovar, by use of data from 93 patients with culture-confirmed cases of leptospirosis in Malaysia [45]. Results of serologic testing were consistent with the serovar identification for 69% of patients and were consistent with the serogroup for an additional 20% of patients, whereas, for the remaining 11% of patients, there was no correlation between serologic findings and the identity of the isolate at any level. In one patient with culture-proven leptospirosis, no antibody response was detected. These data were derived from extensive serologic testing with a panel of MAT antigens that were selected after the identification of isolates obtained from the patients, and they thus represent optimized performance, which is unlikely to be achieved in a laboratory using a restricted range of antigens.

During the past 30 years, leptospirosis has been studied extensively in Barbados, with ~30 severe cases of leptospirosis diagnosed annually. Repeated surveillance has identified only 4 serovars that infect humans and 6 animal species [24]. Each of these leptospiral serovars (*L. borgpetersenii* serovar arborea, *L. noguchii* serovar bajan, *L. kirschneri* serovar bim, and *L. interrogans* serovar copenhageni) belongs both to a different serogroup and to a different species, and the animal reservoir of each has been identified [58–64]. The continued existence of a laboratory that performed cultures and identified isolates allowed for a sufficiently large number of culture-proven cases to be evaluated. During this study, no new serovars were introduced into Barbados. Thus, this island presents a relatively simple epidemiological pattern and is an ideal setting in which to evaluate the usefulness of serovar prediction by serologic testing.

However, use of the predominant serogroup (as determined by MAT serologic analysis) to predict the infecting serovar is relatively insensitive in this population; the prediction matches the serovar for only 46.4% of patients from whom isolates are recovered, and it has low specificity as a predictor of the infecting serovar. Specificity can be increased by applying a more stringent definition of serogroup predominance, but it was still <80%.

Because the range of serovars and serogroups in Barbados is so narrow and well defined, it is probable that the sensitivity and specificity in this population are higher than are those that might be encountered in other populations, in which multiple serovars from a single serogroup or previously undetected serovars might be present. Increasing the number of antigens in the panel used in the MAT probably would not improve sensitivity or specificity, because the panel used in this study already contains strains of all 4 serovars isolated in Barbados. Conversely, in some well-studied settings [9], the range of serovars that cause infection in humans is remarkably restricted, and the specificity of serovar prediction is not high enough to justify use of the MAT for this purpose. The data presented in this report suggest that presumptive serogroup data should be used only to give a broad idea of the common serogroups present in a population and cannot be interpreted reliably in individual patients.

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