Leptospirosis is an acute febrile illness caused by pathogenic members of the genus *Leptospira*. This disease has a worldwide distribution but is most common in tropical regions, including Thailand.\(^1\) In a prospective observational study of undifferentiated fever in 845 patients in rural Thailand, leptospirosis was reported to be responsible for 36.9% of cases.\(^4\) Leptospirosis isolation is the gold standard for confirmation of leptospirosis in humans. This provides definitive identification of the infecting serovar and is an important technique for the study of outbreaks and global epidemiology. It has a number of significant drawbacks, however, including low diagnostic sensitivity, prolonged culture period, and the associated expertise necessary for identification of the infecting serovar together with related costs.

The microscopic agglutination test (MAT) is commonly used to reach a serologic diagnosis of leptospirosis and is performed by detecting agglutinating antibodies by mixing patient serum with a panel of *Leptospira* serovars that are considered to be representative of the endemic strains for a given region.\(^8\) A positive diagnostic result for the MAT is a 4-fold change in titer or a single pre-defined titer. MAT has also been used to provide an indication of the presumptive serovars causing leptospirosis in a given region. The ability of MAT to accurately determine the prevalent serovars was called into question by a retrospective study conducted in Barbados where disease in 151 individuals was caused by four serovars (*L. kirschneri* serovar Bim, *L. interrogans* serovar Copenhageni, *L. borgpertseriensii* serovar Arborea, and *L. noguchii* serovar Bajan); serologic analysis was found to have a low degree of accuracy for determining the infecting serovar in this setting.\(^9\) The epidemiology of infecting isolates in Thailand differs markedly with a recent study showing that the majority of human disease was caused by *L. interrogans* serovar Autumnalis.\(^7\) The aim of this study was to determine whether MAT provides an accurate guide to the infecting serovars of *Leptospira* in Thailand.

A prospective study was conducted in hospitals situated in six provinces in northeast Thailand (Udon Thani, Buriram, Loei, Nakhon Ratchasima, Maha Sarakham, and Yasothon) between October 2000 and December 2006 to identify patients with culture-proven leptospirosis. The study protocol was approved by the Ethical Committee of the Ministry of Public Health, Royal Government of Thailand. Admitting physicians were asked to recruit patients of all ages who they suspected on clinical grounds to have leptospirosis. Clinical features considered were those specifically referred to in the national guidelines (fever with chills and headache together with at least one of the following symptoms or signs: severe muscle pain or muscle tenderness especially calf muscle, meningism or alteration of consciousness, conjunctival suffusion, dry cough, hemoptysis).\(^3\) A 10-mL blood sample was collected into a sterile tube containing 250 units of heparin sodium (Heparin Leo; Leo Pharma, Buckinghamshire, UK) for *Leptospira* culture on the day of admission. A further 5-mL sample was taken on admission and again during the convalescent period for serologic testing using the MAT. Serum was stored at –80°C until analysis. *Leptospira* culture was performed using Ellinghausen McCullough Johnson Harris (EMJH) medium (Difco Laboratories, BD, NJ) supplemented with 3% rabbit serum and 0.1% agarose, as previously described.\(^9\) Positive cultures were sent to the WHO/FAO/OIE Collaborating Center for Reference & Research on Leptospirosis, Australia, for serovar identification using the cross-agglutinin absorption test (CAAT).\(^10\) MAT was performed by the WHO/FAO/OIE Collaborating Center for Reference and Research on Leptospirosis, Australia, as previously described,\(^10\) using a live panel of antigens representing both ubiquitous and locally prevalent serovars (Table 1). A positive MAT was taken as a single titer of ≥1:400 or more or a 4-fold rise in titer between acute and convalescence samples taken up to 60 days after the onset of symptoms.

A total of 149 patients with culture-proven leptospirosis known to be infected with a defined serovar were recruited during the study period. Median age was 35 years (range, 13–72 years; interquartile range [IQR], 25–47 years); 81% were men. The median duration of symptoms before hospital presentation was 3 days (range, 1–8 days; IQR, 2–4 days). Five patients (3%) died during hospital admission. A second serum sample was not collected in 43 patients; this included the 5 fatal cases and 38 patients (26%) who were lost to follow-up. None of these 43 patients had a raised titer on the admission sample. These 43 patients were excluded from further analysis. Convalescent samples were obtained from the remaining 106 cases a median of 15 days (range, 3–53 days; IQR, 9–20 days) after the onset of symptoms. Convalescent samples were used for assigning serovar specificity of MAT.

The seven serovars identified by CAAT for the 106 infecting *Leptospira* were as follows: *L. interrogans* serovar (sv.)
Autumnalis, 91 isolates (86%); L. interrogans sv. Pyrogenes, 7 isolates (7%); L. borgpetersenii sv. Javanica, 3 isolates (3%); L. interrogans sv. Medanensis, 2 isolates (2%); L. interrogans sv. Bataviae, 1 isolate (1%); L. interrogans sv Pomona, 1 isolate (1%); and L. kirschneri sv Grippotyphosa 1 isolate (1%).

A total of 78/106 patients (74%) had a positive MAT titer, and the remaining 28 patients had a negative MAT result. The 78 positive MAT results included 72 patients with a 4-fold rise in titer, 3 patients with raised titers of 1:400 or more in both acute and convalescent samples, and 3 patients with one diagnostic titer. Convalescent samples were obtained a median of 17 days (range, 5–53 days; IQR, 12–21 days) after the onset of symptoms in patients with a positive MAT and a median of 7 days (range, 3–21 days; IQR, 6–14 days) after the onset of symptoms for those with a negative MAT result (P = 0.0001). All 28 patients with a negative MAT were infected with L. interrogans serovar (sv) Autumnalis.

Positive MAT results were divided into three groups: 1) MAT correctly assigned the serovar of the infecting isolate (highest MAT titer against the infecting serovar), which was observed in 26/78 cases (33%); 2) MAT failed to assign the serovar of the infecting isolate (highest MAT titer against a different serovar; 41/78 cases; 53%); and 3) MAT could not be interpreted in relation to the infecting serovar, because shared highest MAT titer were observed for both the correct serovar plus one or more other serovars (11/78 cases, 14%).

Of the 63 patients infected with L. interrogans sv. Autumnalis who had a positive MAT titer, the most common reactions occurring at a titer of ≥ 1:400 for serovars other than Autumnalis were seen against serovar Copenhageni (N = 29), Australis (N = 22), Cynopteri (N = 12), and Djasiman (N = 9). Fifty L. interrogans sv. Autumnalis isolates from this study have been genotyped previously using multilocus sequence typing, and all were found to belong to a single clone (sequence type [ST] 34). One possible explanation for this variable cross-reactivity is that minor variability between isolates in structure and/or expression of the proteins associated with a given serovar can be detected by the configuration of MAT but not by CAAT. It is also possible that determining the cut-off point in the agglutination reaction of MAT, a subtle and subjective measure, is prone to interobserver and intraobserver error.

Failure of MAT to correctly predict the infecting serovar has several possible explanations. We propose that seropositivity is high in the general population in rural Thailand, because leptospirosis is a leading cause of fever in adults presenting to hospital, and such severe cases probably represent a fraction of infections associated with milder clinical symptoms. The sera tested may have been collected too soon to detect antibodies to the strain causing the current infection and may have detected antibodies from a previous infection. It is also possible that sera were collected too soon after infection when more cross-reactive IgM antibody predominated. Median time to the second sample in this study was 15 days after presentation, and further studies are needed in our setting using an extended follow-up to determine whether this would improve the predictive accuracy of MAT for the infecting serovar.

One criticism of the study is that the MAT panel strains contained the correct serovars for the region but did not contain the actual infecting strains in Thailand during the study period. The strain of sv. Autumnalis used in the MAT panel was Akiyami A, which is genetically distant from the dominant sv. Autumnalis clone ST34 (different alleles at all seven MLST loci). We would argue, however, that strains used in the MAT panel are rarely an exact match for those causing disease in the developing world. This is because prior knowledge of the prevalent pathogenic strains is often lacking in resource-poor settings where leptospirosis occurs most commonly but where facilities to culture the causative organisms are usually lacking. Furthermore, the serovars causing disease in a given area can change over time, and the serovars causing leptospirosis even in neighboring countries may be distinctive and non-overlapping. We suggest that it is impractical to repeatedly alter the MAT strain panel over a short time frame and that this could be associated with improved accuracy for some countries while reducing the accuracy for others.

In conclusion, this study showed that serovar data derived from the MAT test offers a poor reflection of infecting serovars in Thailand. Culture of infecting isolates and CAAT identification of serovar remains the technique of choice during epidemiologic studies in Thailand.
REFERENCES


