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. 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. Leptospira 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. 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. borgpetersenii 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. 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. 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). A 10-mL blood sample was collected into a sterile tube containing 250 units of heparin sodium (Heparin Leo; Leo Pharma, Buckinghamshire, UK) for Leptospirosis 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 –40°C until analysis. Leptospirosis 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. 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). MAT was performed by the WHO/FAO/OIE Collaborating Center for Reference and Research on Leptospirosis, Australia, as previously described, 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.)
is also possible that determining the cut-off point in the agglu-
tation 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 sero-
positivity is high in the general population in rural Thailand,
because leptospirosis is a leading cause of fever in adults pre-
senting to hospital,1 and such severe cases probably represent
a fraction of infections associated with milder clinical symp-
toms. 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 infec-
tion when more cross-reactive IgM antibody predominated.1
Median time to the second sample in this study was 15 days
after presentation, and further studies are needed in our set-
ting using an extended follow-up to determine whether this
would improve the predictive accuracy of MAT for the infect-
ing serovar.

One criticism of the study is that the MAT panel strains
contained the correct serovars for the region but did not con-
tain 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 domi-
nant sv. Autumnalis clone ST34 (different alleles at all seven
MLST loci).7 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 know-
ledge of the prevalent pathogenic strains is often lacking in
resource-poor settings where leptospirosis occurs most com-
monly 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 caus-
ing leptospirosis even in neighboring countries may be distinc-
tive 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 sero-
vars in Thailand. Culture of infecting isolates and CAAT iden-
tification of serovar remains the technique of choice during
epidemiologic studies in Thailand.

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