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Six New Leptospiral Serovars Isolated from Wild Animals in Peru

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Six new serovars of *Leptospira interrogans* were isolated from opossums (*Didelphis marsupialis* and *Philander opossum*) trapped in the Peruvian jungle. The proposed names, type strain designation, and serogroup of the serovars, respectively, were: *huallaga*, strain M-7, Djasiman serogroup; *luis*, strain M-6, Tarassovi serogroup; *machiguenga*, strain MMD-3, Icterohaemorrhagiae serogroup; *rioja*, strain MR-12, Bataviae serogroup; *rupa rupa*, strain M-3, Sejroe serogroup; and *tingomaria*, strain M-13, Cynopteri serogroup.

During the 1970s, studies were conducted on leptospirosis in wild animals in the Peruvian jungle (1–3; J. Liceras de Hidalgo, Rev. Assoc. Microbiol., in press). Six strains of *Leptospira interrogans* isolated from the kidneys of opossums *Didelphis marsupialis* and *Philander opossum* eventually proved to be new serovars.

Primary isolation was made by inoculation of opossum kidney tissue into 7 to 10 tubes containing 1 ml of either Ellinghausen-McCullough-Johnson-Harris medium (Difco Laboratories), modified polysorbate 80 medium, or modified Vervoort medium (5). The isolates were classified into serogroups by the cross-agglutination pattern in microscopy-agglutination tests at the Institute de Salud Publica, Lima, Peru (4). Serovar status of the isolates was determined by cross-agglutination and reciprocal agglutinin absorption tests by the microscopy-agglutination technique at the Centers for Disease Control, Atlanta, Ga. (4). Because the results indicated that the isolates were new serovars, subcultures of the type strains were sent for confirmatory tests to the Leptospirosis Reference Laboratory, Department of Health and Human Affairs, Brisbane, Australia, and the Institute of Epidemiology, Medical Faculty of Komensky University, Bratislava, Czechoslovakia.

To be considered a new serovar within an existing serogroup, an isolate must react to at least 6% of the homologous titer with antiserum to all members of the serogroup. Conversely, antiserum against the isolate must retain 10% or more of its homologous titer after cross-absorption with other members of the serogroup. Each of the isolates met these criteria.

Serovar *huallaga* was found to be a member of the Djasiman serogroup but was different from the other recognized members (*djasiman*, *gurungi*, and *sentot*). Serovar *luis* was a member of the Tarassovi serogroup but was different from *atchafalaya*, *atlantae*, *bakeri*, *bravo*, *chagres*, *darien*, *gatuni*, *guidae*, *kaup*, *kisuba*, *langati*, *navet*, *rama*, *tarassovi*, and *tunis*.

Serovar *machiguenga* was a member of the Icterohaemorrhagiae serogroup but was different from *birkini*, *bogvere*, *budapest*, *copenhageni*, *dakota*, *gem*, *mankarso*, *mwogolo*, *naam*, *ndahambukuje*, *ndambari*, *sarmini*, *smithi*, *tonkini*, and *weaveri*. Serovar *rioja* was a member of the Bataviae serogroup but was different from *argentiniensis*, *balboa*, *bataviae*, *brasiliensis*, *claytoni*, *djatzi*, *kobbe*, and *paidjan*.

Serovar *rupa rupa* was a member of the Sejroe serogroup but was different from *balcanica*, *caribe*, *gorgas*, *haemolytica*, *hardjo*, *medanensis*, *nyanza*, *polonica*, *recreo*, *ricardi*, *saxkoebing*, *sejroe*, *trinidad*, and *wolffi*. Serovar *tingomaria* was a member of the Cynopteri serogroup but was different from *cynopteri*, the only other member of this serogroup.

Tests at the two reference laboratories confirmed our results on the new serovars. Identifying information for the six serovars, including source of the original isolate, is listed in Table 1. Cultures of these serovars are maintained in the permanent *L. interrogans* serovar collection at the Centers for Disease Control.

Each serovar was tested for virulence in guinea pigs weighing 150 to 200 g each. None of the guinea pigs gained weight in the 28-day period after inoculation. All guinea pigs developed antibody to their respective challenge strain, but only serovar *machiguenga* was recovered from kidney cultures at the end of the virulence study.

Three of the new serovars have been isolated from kidney cultures of additional wild opossums. Serovar *luis* was isolated from two *P. opossum*; serovar *rupa rupa* was isolated from one *D. marsupialis* and three *P. opossum*; and serovar *tingomariensis* was isolated from one *D. marsupialis* and one *P. opossum*.

The significance of these new serovars as health hazards for humans or domestic animals is unknown, but the abundant population of opossums near human habitation in the study areas indicates at least a potential for transmission.

In the course of the Peruvian field studies, 10 leptospiral serovars, including the 6 new ones, were isolated. As investigations of leptospirosis continue to expand into new geographic areas, this study suggests that a rapid increase in the number of recognized serovars is to be expected. The limited availability of laboratories with the necessary serotyping capability and the amount of work required to establish the identity of new serovars points to the need for improved technology in the identification of leptospires.

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TABLE 1. New serovars of *L. interrogans* isolated from opossums in Peru

Serogroup	Serovar	Type strain	Animal source	Location
Djasiman	*huallaga*	M-7	*D. marsupialis*	Tingo Maria
Tarassovi	*luis*	M-6	*P. opossum*	Tingo Maria
Icterohaemorrhagiae	*machiguenga*	MMD-3	*P. opossum*	Puerto Maldonado
Bataviae	*rioja*	MR-12	*P. opossum*	Rioja
Sejroe	*rupa rupa*	M-3	*D. marsupialis*	Tingo Maria
Cynopteri	*tingomaria*	M-13	*D. marsupialis*	Tingo Maria

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