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# Development of a novel ELISA for serodiagnosis of Leptospirosis and additional detection of pathogenic *Leptospira* by polymerase chain reaction for veterinary routine diagnostics

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**SUMMARY**

A multi-serovar ELISA based on the outer membrane Lipoprotein L41 (LipL41) of pathogenic *Leptospira* was developed to increase sensitivity by using a single test antigen. A sensitivity of 99 % and a specifity of 92 % could be achieved. The established diagnostic polymerase chain reaction is also able to detect fast and reliably pathogenic *Leptospira* in different clinical samples.

**Key words**: Leptospirosis, leptospira, ELISA, PCR, diagnosis.

Since a reliable clinical diagnosis is frequently not possible because of unspecific symptoms and the identification of the etiologic agent by culture is very difficult, serological diagnosis is of great importance. Different serological methods are able to detect antibodies directed against the test antigen of the corresponding serovar only. In addition they do not achieve the sensitivity of the MAT,[1-5] the reference method, that allows a serovar diagnosis. A fast and sensitive identification of *Leptospira spp.* is possible with different PCR methods, that are based on the amplification of segments of the 16S- and 23S-RNA sequences; however, these PCR methods can not always assure specifity for pathogenic *Leptospira spp.* only.[2,3]

A multi-serovar ELISA based on the outer membrane Lipoprotein L41 (LipL41) of pathogenic Leptospira was developed to increase sensitivity by using a single test antigen. The ELISA was evaluated with 343 Sera from pigs in comparison to MAT results.

A sensitivity of 99 % (Cut-off 5) and a specifity of 92 % (Cut-off 30) could be achieved. Additional Evaluation with 28 Sera from experimentally infected and 28 Sera from not infected mongolian Gerbils by using the state of infection as reference showed 100% sensitivity and specifity dependent on stage of infection. For the specific detection of the *lipl41*-gene of pathogenic *Leptospira* PCR primers were selected and PCR was successfully performed with clinical samples. LipL41[4] shows to be an interesting candidate for diagnostics of Leptospirosis by ELISA-techniques. The established diagnostic PCR based on the *lipl41* gene sequence occurring only in pathogenic *Leptospira spp.*[4] is able to detect fast and reliably

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pathogenic *Leptospira* in different clinical samples and represents an alternative to the time-consuming and difficult identification of the causative agent by culture.

## Desarrollo de un ELISA novedoso para el serodiagnóstico de Leptospirosis y la detección adicional de *Leptospira* patogénica mediante la reacción en cadena de la polimerasa para diagnóstico veterinario de rutina

**RESUMEN**

Se desarrolló un ELISA multiserovar basado en la lipoproteína de membrana externa L41 (LipL 41) de *Leptospira* patogénica para incrementar la sensibilidad mediante el uso de un antígeno de prueba. Se logró 99 % de sensibilidad y 92 % de especificidad. La reacción en cadena de la polimerasa diagnóstica establecida es capaz de detectar de una manera rápida y confiable la *Leptospira* patogénica en diferentes muestras clínicas.

**Palabras clave**: Leptospirosis, leptospira, ELISA, RCP, diagnóstico.

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