technique for determining the cellular localization of proteins to *Leptospira* spp. **Methods:** Four recombinant proteins, previously predicted by reverse and structural vaccinology as surface proteins, as well as three flagellar proteins, were produced. From these, it was produced policlonal antibodies, which were used in the localization of leptospiral proteins. Leptospires were subjected to immunofluorescence analysis with methanol, immunofluorescence in agarose beads and surface immunofluorescence. **Results:** The immunofluorescence in agarose beads confirmed the localization of LigB, LipL32 and LcpA as surface exposed proteins; and the surface immunofluorescence erroneously identified the location of FcpA, a bacterial flagellar component, as a surface protein. **Conclusion:** The approach based on the encapsulation of leptospires in agarose microdroplets, although needing further improvement, provided promising results for determining the cellular localization of proteins in *L. interrogans*. **CEEA/UFPEL**: nº 4336-2015. **Funding:** Capes, CNPq.

## 33. MARSUPIALS AS MAINTENANCE HOSTS OF PATHOGENIC LEPTOSPIRES IN PARANAÍBA RIVER'S VALLEY, GOIÁS AND MINAS GERAIS STATES, BRAZIL

Marsupiais como hospedeiros de manutenção de leptospiras patogênicas no Vale do Rio Paranaíba, estados de Goiás e Minas Gerais, Brasil

MOREIA, R. Q.;[1,2] RAMOS, V. N.;[3] VIEIRA, R. F;[1] MANGUEIRA, R. A. F.;[4] PIRES, B. C.;[5] LIBONATI, H.;[5] LILENBAUM, W.;[5] LIMA, A. M. C.[2]
[1]Veterinary Medicine Course, Instituto Master de Ensino Presidente Antônio Carlos (Imepac) Araguari/MG, Brazil.
[2]Laboratory of Infectious and Contagious Diseases, School of Veterinary Medicine (Famev), Universidade Federal de Uberlândia (UFU), Brazil.
[3]Laboratory of ixodology, Universidade Federal de Uberlândia (UFU), Brazil.
[4]Department of Physical Sciences, School of Agriculture Luiz de Queiros (Esalq), Universidade de São Paulo (USP), São Paulo/SP, Brazil.
[5]Laboratory of Veterinary Bacteriology, Universidade Federal Fluminense (UFF), Niterói/RJ, Brazil.
E-mail: annalimaufu@yahoo.com.br

**Introduction:** Small non-flying mammals hold the largest number of species within the Mammalia class in Brazil. Mainly rodents and marsupials, important reservoirs hosts of pathogenic serovars of leptospires, represent this group of animals. **Objective:** The aim of this study was to identify the frequency of wild marsupial carriers of pathogenic leptospiras in three different areas of the Paranaíba river's valley, Brazil. **Methods:** Two campaigns were carried out to capture marsupials, one at the end of the rainy season and another at the end of the drought. Eight traps of the Tomahawk, and 116 traps of the Sherman types were used, baited with banana slices covered with peanut flour. PCR assays were performed to detect the lipL32 gene in renal tissue of marsupials captured in three distinct areas along the Paranaíba river's valley: Low Paranaíba (Ipiaçu/MG, 18.7770833S; 49.8978889W); Middle Paranaíba (Goiandira/GO, 18,1630556S; 48,1354722W); and High Paranaíba (Guimarânia/MG, 18.8101944S, 46.6755278W). It was applied the non-parametric chi-square association test to verify the significance of the association of the factors studied. The procedures performed were authorized by CEUA-UFU under protocol number 151/16. **Results:** Thirty-nine specimens belonging to the Marsupialia order were captured, and of these, 14 (35.89%) presented the *lipL32* gene in their renal tissues at the PCR. The Middle Paranaíba area had a higher frequency of renal carriers (9/14) than Alto Paranaíba (3/17) and Lower Paranaíba (2/8), with p = 0.0086. **Conclusion:** Marsupials presented as pathogenic leptospire maintainers in the Paranaíba river's valley. The Middle Paranaiba's area was characterized as the one of greater challenge to the marsupials by pathogenic leptospires. **CEUA:** 151/16 **Funding:** Fapemig, mostly in own resources.

## 34. MOLECULAR COMPARISON OF FOUR VIRULENCE-RELATED GENES IN LEPTOSPIRAL STRAINS OF ICTEROHAEMORRHAGIAE SEROGROUP

Comparação molecular de quatro genes relacionados à virulência nas estirpes leptospirais do sorogrupo Icterohaemorrhagiae

CORREIA, L.;[1] MALLIAGROS, L. V.;[1] PIRES, B. C.;[1] BRASIL, T.;[1] JAEGER, L. H.;[1] CARVALHO-COSTA, F. A.;[2] LILENBAUM, W.[1]
[1]Laboratory of Veterinary Bacteriology, Microbiology and Parasitology Department, Universidade Federal Fluminense (UFF), Niterói/RJ, Brazil.

[2]Laboratory of Epidemiology and Molecular Systematics, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz (IOC-Fiocruz), Rio de Janeiro/RJ, Brazil.
E-mail: lucascorreia@id.uff.br

**Introduction:** Pathogenesis of leptospirosis is related to agent characteristics as well the ability to escape immune system of the host. However, the leptospires biology and virulence factors are not enough characterized and the factors for the pathogenesis and that trigger the development of the disease are still unclear. **Objective:** Compare the occurrence and expression of four virulence-related genes in leptospiral strains of serogroup Icterohaemorrhagiae that were virulent or not in hamster model (*Mesocricetus auratus*). **Methods:** Eight strains of serogroup Icterohaemorrhagiae belonging to two species were studied: four of *L. interrogans* (virulent) and four of *L. kirschneri* (not virulent). DNA was obtained using Wizard SV Genomic DNA Purification System® (Promega) and RNA using Trizol Reagent (Invitrogen). PCR was performed with GoTaq® DNA Polymerase (Promega) and RT-PCR using OneStep RT-PCR Kit (QIAGEN): for two genes for surface protein (*lig*A and *lip*L32), one for motile-associated flagella (*fli*Y) and one for adhesin (*len*A). The PCR products of partial region of the genes were purified using Wizard SV Gel and PCR Clean-up System (Promega) and sequenced using Big Dye terminator v3.1 kit (Applied Biosystems) in the ABI 3730XL Genetic Analyzer (Life Technologies, Carlsbad, CA, USA) on the RPT01A DNA sequencing platform (Laboratório de Genômica Funcional e Bioinformática, IOC/FIOCRUZ). **Results:** All virulent and non-virulent strains studied showed the target genes in DNA. Regarding expression of the virulence-related genes in RNA, all presented positive result for these with exception of a non-virulent strain for the *fli*Y gene. In nucleotide sequence analyzes, no polymorphisms were observed which may be related by differences in strain virulence. **Conclusion:** The targets studied may not be related to differences in virulence in strains of serogroup Icterohaemorrhagiae, making it necessary evaluate a larger set of different targets for a better understanding of the leptospiral virulence mechanisms. **CEUA:** 611/2015.**Funding:** Capes (Finance code 001), Faperj.

## 35. *NECTOMYS SQUAMIPES* AS MAINTENANCE HOSTS OF PATHOGENIC LEPTOSPIRES IN PARANAÍBA'S RIVER VALLEY, GOIÁS AND MINAS GERAIS STATES, BRAZIL

*Nectomys squamipes* como hospedeiros de manutenção de leptospiras patogênicas no Vale do Rio Paranaíba, estados Goiás e Minas Gerais, Brasil

MOREIA, R. Q.;[1,2] RAMOS, V. N.;[3] VIEIRA, R. F.;[1] PIRES, B. C.;[4] LIBONATI, H.;[4] LILENBAUM, W.;[4] LIMA, A. M. C.[2]
[1]Veterinary Medicine Course, Instituto Master de Ensino Presidente Antônio Carlos (Imepac) Araguari/MG, Brazil.
[2]Laboratory of Infectious and Contagious Diseases (Famev), Universidade Federal de Uberlândia (UFU), Uberlândia/MG, Brazil.
[3]Laboratory of Ixodology, Universidade Federal de Uberlândia, Uberlândia/MG, Brazil.
[4]Laboratory of Veterinary Bacteriology, Universidade Federal Fluminense (UFF), Niterói/RJ, Brazil.
E-mail: annalimaufu@yahoo.com.br

**Introduction:** Leptospirosis is a disease that occurs more frequently in regions of tropical climate, which favor the survival of the bacteria in the environment. *Nectomys squamipes* is a wild rodent of semiaquatic behavior, which favors its contact with leptospires. **Objective:** The aim of this study was to identify the renal carrier status of three specimens of Nectomys squamipes captured in the Paranaíba's river valley, Brazil. **Methods:** MAT test was performed for 21 serovars of standard strains, and four strains of isolates of small and wild synanthropic mammals in Brazil. PCR assays were also performed for the detection of the *lipL32* gene in *Nectomys squamipes* renal tissue, captured in two areas along the Paranaíba's river valley (Goiandira/GO, 18,1630556S; 48,135,472W and Guimarânia/MG, 18,8101944S; 46,6755278W). **Results:** Three specimens of *Nectomys squamipes* were captured in riverside forest environment, and all presented the *lipL32* gene in their renal tissues against a PCR, but none reacted to MAT. **Conclusion:** *Nectomys squamipes* is an important maintainer of circulating pathogenic leptospires in the studied environment, being configured as prominent agents in the epidemiology of leptospirosis in the Paranaíba's river valley, Goiás and Minas Gerais states, since they promote an interface between the aquatic and terrestrial environments. **CEUA:** 151/16. **Funding:** Fapemig, mostly in own resources.