similarly. In challenge assays, the majority of hamsters immunized with the flagellins and LigAC survived the lethal challenge. However, they were not protected against kidney colonization. Control animals vaccinated with PBS died with symptoms of leptospirosis and hamsters immunized with commercial vaccine survived after challenge. ELISA demonstrated that with exception of FlaB5, all flagellins were recognized by sera from infected hamsters, sera from hamsters immunized with the commercial vaccine and with recombinant flagellin pool. **Conclusions:** These results indicate that in spite of leptospiral flagellins to be immunogenic and able to activate the TLR-5, none succeeded in preventing renal colonization. **CEUA:** 2151/2011. **Funding:** Fapesp.

15. EVALUATION OF PRESENCE OF A PUTATIVE MULTIDRUG EFFLUX PUMP GENE (*NORM*) IN *LEPTOSPIRA* SPP. STRAINS FROM BOVINE ORIGIN

Avaliação da presença de um gene de multidrogas putativo de bomba de efluxo (norM) em estirpes de *Leptospira* spp. de origem bovina

MALLIAGROS, L. V.;[1] CORREIA, L.;[1] PIRES, B. C.;[1] BRASIL, T.;[1] CARVALHO-COSTA, F. A.;[2] JAEGER, L. H.;[1] 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: wlilenbaum@id.uff.br

Introduction: Leptospirosis in livestock is associated with large economic losses. In order to minimize these losses, different control strategies have been applied, including antibiotic therapy. Besides, failure of antibiotic therapy may be related to reduced susceptibility and the presence of genes associated with antimicrobial resistance. **Objective:** To evaluate the presence of a putative multidrug efflux pump gene in *Leptospira* spp. strains from bovine origin with susceptibility profile previously described. **Methods:** Twenty-five strains of *Leptospira* spp. were studied. DNA was obtained using Wizard SV Genomic DNA Purification System® (Promega) and PCR was performed with GoTaq® DNA Polymerase (Promega) for putative *nor*M gene encoding a multidrug efflux. The PCR products of partial region of the gene 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) to confirm the studied target. **Results:** Twenty-four strains showed a positive result for the presence of the target gene. One strain that had a negative result belongs to a saprophytic specie (*L. meyeri*). Analysis of the nucleotide sequences demonstrated that the amplified region belongs to the gene studied. **Conclusion:** The presence of a putative multidrug efflux pump gene present in other microorganisms may also influence antimicrobial susceptibility in *Leptospira* spp. More refined studies focusing the molecular structure and function are necessary to elucidate this putative multidrug efflux pump. **CEUA:** Not applicable. **Funding:** Capes (Finance code 001), Faperj.

16. EXPRESSÃO DIFERENCIAL DA PROTEÍNA DE MEMBRANA EXTERNA OMPL36 EM BIOFILME DE *LEPTOSPIRA*

Differential expression of external membrane protein ompl36 in *Leptospira* biofilm

GUIMARÃES, J. M. J.;[1] RIBEIRO, P. S.;[1] FRANÇA, G. V. F.;[1] SILVA, T. M. R.;[1] RISTOW, P.[1]
[1]Laboratório de Bacteriologia e Saúde, Instituto de Biologia, Universidade Federal da Bahia (UFBA), Salvador/BA, Brasil.
E-mail: paularistow3@gmail.com.

Introdução: A leptospirose é uma zoonose negligenciada causada por bactérias patogênicas do gênero *Leptospira*. A doença afeta mais de um milhão de pessoas por ano em todo o mundo. As leptospiras formam biofilmes caracterizados por comunidades microbianas envoltas por uma matriz de exopolimérica. As proteínas de membrana externa (OMPs) bacterianas podem participar da adesão celular em biofilmes. **Objetivo:** Avaliar a expressão diferencial da proteína de membrana externa OmpL36 em biofilmes de *Leptospira biflexa*. **Métodos:** *Leptospira biflexa* serovar Patoc (saprófita) foi cultivada em meio EMJH a 29ºC por 48 h (tempo correspondente à formação do biofilme maduro). Os biofilmes foram cultivados sem agitação e células planctônicas sob agitação. Após a lise celular, foi realizado o *western blot* com os extratos proteicos totais usando anticorpos específicos anti-OmpL36,

seguido de análise das membranas pelo programa ImageJ. A expressão do gene OmpL36 foi avaliada durante a formação do biofilme de *L. biflexa*, acessando os dados do transcriptoma (BioProject PRJNA288909). **Resultados:** Por *western blot*, OmpL36 teve maior expressão em biofilme quando comparado ao fenótipo planctônico. A partir da análise do transcriptoma e corroborando este resultado, foi constatado que o gene OmpL36 foi regulado positivamente no fenótipo biofilme em comparação com o planctônico no biofilme tardio de 120h (FDR 7, 00E-3; p < 0,05). No biofilme maduro de 48h, houve regulação positiva, porém essa não foi estatisticamente significativa (FDR 1,60E-2; p < 0,05). **Conclusões:** Os resultados obtidos demonstram que OmpL36 é mais expressa em biofilme que no estado planctônico, o que sugere que essa proteína desempenha um papel em biofilmes de *Leptospira*. **CEUA:** Não aplicável. **Financiamento:** Projeto universal CNPq 425526/2016-0, Fapesb, Capes.

17. EXPRESSION OF THREE VIRULENCE-RELATED GENES IN LEPTOSPIRAL STRAINS OF SEROGROUP SEJROE AFTER WEEKLY SUBCULTURES

Expressão de três genes relacionados à virulência em estirpes leptospirais do sorogrupo Sejroe após subculturas semanais

CORREIA, L.;[1] MALLIAGROS, L. V.;[1] BRASIL, T.;[1] PIRES, B. C.;[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: Bovine leptospirosis is characterized mainly by reproductive problems associated with infections by strains of serogroup Sejroe. Pathogenic strains belonging to same serogroup could present differences in pathogenicity, suggesting existence of unknown molecular mechanisms involved in virulence. Furthermore, it is known that subculture of strains can lead to attenuation of virulence by changes in protein coding genes. **Objective:** Compare the occurrence and expression of three virulence-related genes in leptospiral strains of serogroup Sejroe that were virulent or not in hamster model (*Mesocricetus auratus*) after recovery of strain post infection (first moment) and after twenty weekly subcultures in EMJH media (second moment). **Methods:** Four strains of serogroup Sejroe belonging to *Leptospira santarosai* specie was studied: three of them were 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) and one for motile-associated flagella (*fli*Y). **Results:** All virulent and non-virulent strains studied showed the target genes in DNA. Regarding expression of the virulence-related genes in RNA, the *lip*L32 and *lig*A targets obtained positive results in all strains tested in the two moments of this study. For *fli*Y, all strains tested did not express at the first moment. While in the second moment, two virulent strains were positive for the expression of this gene. **Conclusion:** The *lip*L32 and *lig*A targets studied may not be related to differences in virulence in strains of serogroup Sejroe. The result of the *fli*Y gene in strains of the serogroup Sejroe was unexpected and could be related to differences in infection by strains of this serogroup. It is necessary to compare strains of serogroup Sejroe with to other serogroups. **CEUA:** 611/2015. **Funding:** Capes (Finance code 001), Faperj.

18. GENOMIC FEATURES OF *LEPTOSPIRA INTERROGANS* SEROVAR HARDJO STR. NORMA: POTENTIAL RECOMBINATION SITE GENOME DEPICTED BY COMPARATIVE GENOMICS

Características genômicas da *Leptospira interrogans* sorovar Hardjo str. Norma: genoma do local de recombinação potencial representado pela genômica comparativa

COSATE, M. R. V.;[1] MOREIRA, E. C.;[2] ORTEGA, J. M.;[3] LEITE, R. C.;[2] HADDAD, J. P.;[2] MENDES, T. A. O.[1]
[1]Laboratory of Molecular Biology, Biochemistry Department, Universidade Federal de Viçosa (UFV), Viçosa/MG, Brazil.
[2]Department of Preventive Veterinary Medicine, Veterinary School, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil.
[3]Institute for Biological Sciences, Biochemistry and Immunology Department, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil.
E-mail: raquelcosate@ymail.com

Introduction: Leptospirosis is caused by pathogenic spirochetes of the genus *Leptospira* spp. with