

13. EFFECTS OF A STRATEGIC APPROACH *IN SITU* ON THE CONTROL OF LEPTOSPIROSIS IN A NATURALLY INFECTED DAIRY HERD

Efeitos de uma abordagem estratégica *in situ* sobre o controle da leptospirose em um rebanho leiteiro naturalmente infectado

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Introduction: Bovine leptospirosis has a great economic impact, mainly in chronic form, when it is asymptomatic but causes reproductive failures.

Objective: Verify the reflexes of adopting a preventive and strategic treatment *in situ*, in the serologic profile, and in the reproductive indexes of a dairy herd, naturally infected with *Leptospira* spp., and with reproductive disorders background. **Methodology:** The preventive and strategic treatment approach was based on the division of the herd in specific groups, according to the serologic status for leptospirosis, and the background of reproductive disorders (abortions, repeat breeding, and increase in the parturition intervals). Since the lactating animals were housed in climate controlled barns (compost bedding pack barn), it was used a sanitary barrier for maintaining the physical separation between groups. The non-lactating groups were housed in separated pens. The animals reactive to the MAT were treated with streptomycin, and vaccinated afterwards.

The non-reactive animals were only vaccinated; and the non-reactive animals with a background of reproductive disorders, also received antibiotic treatment and vaccination. **Results:** The antibody titers ranged from 100 to 800. Nine months after the adoption of the preventive and strategic treatment, the highest titers were 100. It was noticed that the repeat breeding decreased. Before the control measures application, it was observed 20 cases of repeat breeding, but after 12 cases were observed, but this reduction didn't present a statistical significance ($p > 0,05$). It was also noticed a decrease in the abortions. Before the strategic conduct, 19 abortion cases were detected, and after the experiment, only seven cases were observed. This reduction is statistically significant ($p < 0,05$).

Conclusion: The control measures applied were able to promote a decrease of antibody titers against *Leptospira* sp. and in the reproductive failures investigated. **CEUA:** n. 035/17. **Funding:** Fapemig, UFU.

14. EVALUATION OF LEPTOSPICAL FLAGELLINS AS ADJUVANTS FOR THE DEVELOPMENT OF A SUBUNIT VACCINE AGAINST LEPTOSPIROSIS

Avaliação das Flagelinas Leptospirais como adjuvantes para o desenvolvimento de uma vacina subunitária contra a leptospirose

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Introduction: Leptospirosis is a zoonosis of global importance caused by pathogenic leptospires that colonize the renal tubules of wild and domestic animals. Commercial bacterines are being used, but they can promote protection only against the serovar included in the preparation and they I can induce a shortterm immunity. The LigAC is able to induce immunoprotection against leptospirosis. However, immunization with LigAC did not confer sterilizing immunity. Flagellins have been considered a promising adjuvant for vaccine development. Leptospires have two periplasmic flagella that are formed by two classes of proteins (FlaA and FlaB); only FlaB proteins show homology with important regions that elicit TLR5-dependent responses.

Objectives: Evaluate the ability to induce the TLR5 activity and the adjuvant activity of five *L. interrogans* sorovar Copenhageni flagellins (FlaB1, FlaB2, FlaB3, FlaB4 and FlaB5) in the protective immunity of LigAC against lethal challenge in hamsters. **Methods:** The recombinant 6xHis-tagged flagellins expressed in *E.coli* were purified by affinity chromatography. Hamsters were immunized subcutaneously with purified flagellins with LigAC as well as in combination with alum or a cocktail of five flagellins. **Results:** Experimental data showed that all flagellins activated both the TLR-5 receptor and the secretion of cytokines in stimulated macrophages,

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

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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 *norM* 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 OMP136 EM BIOFILME DE *LEPTOSPIRA*

Differential expression of external membrane protein omp136 in *Leptospira* biofilm

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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 leptospirosas formam biofilmes caracterizados por comunidades microbianas envolvidas 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 Omp136 em biofilmes de *Leptospira biflexa*. **Métodos:** *Leptospira biflexa* serovar Patoc (saprófita) foi cultivada em meio EMJH a 29°C 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-Omp136,