

imunoistoquímica detectou marcação positiva para antígeno de *Leptospira* spp. em 60 animais. A técnica de PCR revelou sete animais positivos. O diagnóstico de leptospirose é complexo, pois, dos três métodos utilizados, apenas a IMH detectou antígeno onde a sorologia também detectou infecção, mas a recíproca não é verdadeira, e a sorologia e IMH detectaram infecção onde a PCR não detectou. A comparação dos três testes utilizados para o diagnóstico de infecção por leptospirosas revelou que a imunoistoquímica apresentou sensibilidade de 53,6% e 53,1% comparada à sorologia e PCR, respectivamente, e especificidade de 100%. **Conclusão:** Os resultados mostraram que a imunoistoquímica constitui um diagnóstico específico e sensível e pode ser usada para complementar o diagnóstico de leptospirose quando for possível a colheita de amostras de tecido.

06. BASIC MICROBIOLOGY OF LEPTOSPIRA SPP.: A TOOL FOR IMPROVING STUDIES ON LEPTOSPIROSIS

Microbiologia básica de *Leptospira* spp.: uma ferramenta para melhorar estudos sobre leptospirose

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Introduction: Leptospirosis is a zoonosis with a high incidence worldwide, caused by spirochetes belonging to the genus *Leptospira*. The maintenance of the strains and the *in vitro* growth of the microorganism remain time-consuming and difficult to be performed. Demonstrating the need to optimize laboratory culture techniques, towards improving studies in leptospirosis.

Objective: The objective of this work was to evaluate different conditions for the *in vitro* growth of *Leptospira* spp. and the corresponding virulence in an animal model for acute leptospirosis. **Methods:** *L. interrogans* strains L1-130 and RCA and *L. kirschneri* strain UFPel-61H were cultured under different conditions *in vitro* using commercial EMJH culture medium (Difco) and homemade EMJH supplemented with rabbit serum (EMJH++) using different temperatures (28 °C and 37 °C), flasks and inocula (10⁰ to 10⁴) to evaluate the growth

dynamics of the bacteria. Leptospire at different stages of bacterial growth were used to evaluate their impact on virulence in the hamster model for acute infection.

Results: All of the *in vitro* conditions were viable for leptospiral growth, with the exception of the Difco culture medium at 37 °C. The highest bacterial densities (10⁹ leptospire/ml) and the best doubling times were obtained with the Difco medium at 28 °C, especially when associated with orbital agitation. The EMJH++ medium was the most efficient with a low initial inoculum (1 leptospire), especially for *L. interrogans* strains. The *in vivo* experiments demonstrated that growth in Difco medium at 28 °C was more suitable for maintaining the stability and virulence of leptospire across the different bacterial growth stages. **Conclusion:** In conclusion, *in vitro* growth conditions influenced leptospiral virulence, demonstrating the importance of understanding the dynamics of *in vitro* growth of the microorganism. The standardization of leptospire culture techniques will improve the reproducibility of experiments involving pathogenic leptospire. **CEEA UFPel:** 4337-2015. **Funding:** Capes, CNPq.

07. BOVINE LEPTOSPIROSIS: MOST PREVALENT SEROGROUPS IN THE MUNICIPALITY OF NOVO REPARTIMENTO - PARÁ

Leptospirose bovina: sorogrupos mais predominantes no município de Novo Repartimento, Pará

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Introduction: In cattle, leptospirosis is particularly manifested by reproductive disorders, leading to losses in the production of these animals. Once the disease is present in a herd, control becomes difficult, especially by the adaptation of the bacterium to the animal species and can become a reservoir and/or maintenance host, such as serovars of the Sejroe serogroup for cattle. **Objective:**

Identify the serogroup(s) most prevalent(s) in bovine females in the municipality of Novo Repartimento/PA, using the microscopic agglutination test (MAT).

Methods: Blood samples were collected from 208 bovine females, raised in the municipality of Novo Repartimento/Pará, during slaughter in a slaughterhouse in the Baixo Tocantins/PA region. After the blood serum was obtained, the samples were submitted to microscopic agglutination test (MAT) using 24 serovars of *Leptospira* spp. that represents 18 serogroups. **Results:** From the total of 208 samples, 97 (46.63%) were reagents, titers ranged from 100 to 3.200 for 11 of the 18 serogroup tested. Sejroe was the most prevalent, with frequency of 65.00%; followed by Shermani (10.30%); Canicola (6.18%); Tarassovi (4.12%); Grippotyphosa, Australis and Pomona (3.09%); Autumnalis (2.04%); Celledoni, Icterohaemorrhagiae and Javanica, were the least prevalent, with a frequency of 1.03% each one of them.

Conclusion: Bovine leptospirosis is present in the municipality of Novo Repartimento/PA, with Serogroup Sejroe as the most prevalent. **CEUA:** CEUA/FMVZ/USP Nº 5893100816. **Funding:** CNPq (MBH fellowship), Capes (Finance code 001).

08. CERVICO-VAGINAL MUCUS PCR AS AN ALTERNATIVE TOOL TO DETECT UTERINE LEPTOSPIRAL INFECTION

RCP do muco cervico-vaginal como ferramenta alternativa para a detecção de infecção leptospiral uterina

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Introduction: Bovine leptospirosis is characterized by reproductive failure, mainly embryonic death and abortion, leading to economic losses. Nevertheless, the majority of the studies focus on the kidneys, and little is known about genital infection. **Objective:** Investigate the uterine leptospiral infection among slaughtered non-pregnant cows and if its correlation with the infection of cervico-vaginal mucus and urine from the same cows. **Methods:** Samples of cervico-vaginal mucus (CVM), uterine fragments (UF) and urine (n = 34) were

collected from non-pregnant cows at a slaughterhouse. A *LipL32* PCR was performed in all samples in order to detect positivity for pathogenic *Leptospira* in these samples. A correlation between positivity for CVM-UF and the positivity for CVM-urine was assessed by chi-squared analysis and logistic regression. **Results:** It was verified that, among the eight PCRpos CVMs, six were also positive on uterine fragment and one was urine positive. Thus, CVM-UF positivity occurred in 14.3% of the samples, while CVM-urine positivity occurred in 2.3%, a significant difference ($p < 0.05$). Logistic regression indicated that positivity in the uterus is a risk factor for positivity in CVM (OR = 3.4), while positivity in urine is not. **Conclusion:** Although not as sensitive as performing PCR on uterine fragments, CVM may be recommended as a relatively easy to perform, reliable sample that can facilitate the diagnosis of genital leptospirosis by PCR. **CEUA:** Number 863 (CEUA/UFF). **Funding:** CNPq, Faperj.

09. CHRONIC EXPERIMENTAL GENITAL LEPTOSPIROSIS WITH AUTOCHTHONOUS LEPTOSPIRA SANTAROSAI STRAINS OF SEROGROUP SEJROE

Leptospirose genital experimental crônica com estirpes autóctones de *Leptospira santarosai* do sorogrupo Sejroe

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Introduction: Leptospirosis in livestock is usually a chronic infection that leads to reproductive problems. Few studies succeeded on reproducing the chronic genital infection on experimental conditions. **Objective:** To assess the chronic experimental genital leptospirosis with *Leptospira santarosai* strains of serogroup Sejroe.

Methods: Six Santa Inês lamb ewes were inoculated with 1×10^8 leptospire, with three different strains of the serogroup Sejroe: FV237 (Group A), FV52 (Group B) and U81(Group C). Blood samples were collected daily for 28 days, and urine and vaginal fluid were collected at D0, D7, D16 and D22. Laparotomy was performed at D30, D60 and D90. On those days, all samples were collected, in addition to uterus fragment, uterine fluid and follicular aspirate samples. Study was conducted