Molecular demonstration of intermittent shedding of *Leptospira* in cattle and sheep and its implications on control

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**ABSTRACT:** For a long time, it has been stated that urine leptospiral shedding is intermittent, which was observed primarily by culturing. However, culturing presents serious limitations, mainly low sensitivity, and failure on detection of leptospires cannot be neglected. PCR presents several advantages, mainly higher sensitivity. The present study aimed to analyze the occurrence of intermittency on leptospiral shedding by PCR in naturally and experimentally infected animals. In this study two experiments were conducted, the first with 60 cows naturally infected from an endemic herd. The second one was conducted in three sheep experimentally infected, each one with a different strain of Leptospira (strains Copenhageni L1-130, Canicola LO-4 and Pomona Fromm). Considering cattle, 43.3% presented negative in all tests, the remaining (56.7%) were positive at least once. From these, only one (1.6%) was positive in all samples, and seven (11.8%) were positive only in the last sampling, making it impossible to evaluate the intermittency. Noteworthy, 26 cows (43.3%) presented the typical intermittent pattern of leptospiral shedding in urine. In sheep, all experimentally infected animals presented the typical intermittent shedding patterns, independently of the inoculated leptospiral strain. We considered that a careful serial analysis of urine samples for a more definitive and reliable individual diagnosis would be required for a successful antimicrobial therapy and control of leptospirosis on a herd.

**Key words:** cows, intermittent, PCR, sheep, strain.

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**INTRODUCTION**

Leptospirosis is a zoonotic disease of worldwide distribution, affecting both wildlife and domestic animals. In livestock it often presents as a silent and asymptomatic infection (DIRECTOR et al., 2014). Leptospirosis in livestock leads to reproductive failure, such as oestrus repetition, abortion, stillbirths and weak offspring (ELLIS, 2015). The real impact of these affections has not yet been estimated, but it is well known that these reproductive symptoms are related to economic losses (AYRAL, 2013).

Leptospires penetrate the host for lesions on the skin and mucous membranes. After penetration, the bacteria invade the circulation (leptospiremia), spreading throughout the animal (ADLER, 2014). After

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**Demonstração molecular da intermitência eliminando *Leptospira* em vacas e ovelhas e suas implicações no controle**

**RESUMO:** Durante muito tempo, foi afirmado que a eliminação de leptospiras na urina era intermitente, o que havia sido demonstrado principalmente por meio do cultivo microbiano. No entanto, a cultura apresenta graves limitações, principalmente com relação à baixa sensibilidade. Em contraste, a PCR apresenta várias vantagens em relação ao cultivo bacteriológico para leptospiras, sendo esta ferramenta cada vez mais utilizada para o diagnóstico de animais eliminadores da bactéria em diversos sítios. Assim, o presente estudo teve como objetivo analisar a ocorrência de intermitência na eliminação de leptospiras por meio de PCR em animais naturais e experimentalmente infectados. Para este estudo foram realizados dois experimentos, sendo o primeiro com 60 vacas naturalmente infectadas de um rebanho sabidamente endêmico e o segundo em três ovelhas experimentalmente infectadas, cada uma com uma estirpe diferente de Leptospira (estirpes Copenhageni L1-130, Canicola LO-4 e Pomona Fromm). Considerando-se os bovinos, 43,3% apresentaram negatividade em todos os testes, sendo os demais 56,7% positivos ao menos uma vez. Deste, apenas um (1,6%) foi positivo em todas as amostras, e sete (11,8%) foram positivos somente na última coleta, o que impossibilitou a avaliação da intermitência. No entanto, 26 vacas (43,3%) apresentaram o padrão de eliminação tipicamente intermitente de leptospiras na urina. Das três ovelhas experimentalmente infectadas, todas apresentaram eliminação intermitente da bactéria na urina, independentemente da estirpe inoculada. Consideramos que seria necessária uma cuidadosa análise seriada de amostras de urina para um diagnóstico individual mais definitivo e confiável para uma terapêutica antimicrobiana bem-sucedida e o controle de leptospirose em um rebanho.

**Palavras-chave:** vacas, intermitência, PCR, ovelhas, estirpes.
this initial phase, it is known that leptospires lodge in
the renal tubules of infected animals, being shed for long
periods (leptospiruria), contaminating the environment
and other animals (ELLIS, 2015). In this context, it has
been reported that naturally infected cattle may shed
the bacteria for about 40 weeks, while experimentally
infected calves for up to 32 weeks (LEONARD et al., 1992).
Independently of the use of host-adapted (e.g. Hardjo in ruminants), or incidental (e.g. Pomona in ruminants) serovars for experimental infections, renal colonization has been reported, in general 10-25 days after the infectious challenge (SLEIGHT et al., 1964; LITTLE & SALT, 1976; RINEHART et al., 2012).

For a long time, it has been stated that urine
leptospiral shedding to be intermittent (FAINE et al.,
2000). It was first determined by the inconstant recovery
of this microorganism by culturing (INADA et al.,
1916). Nevertheless, it must be considered that culturing
of leptospires presents serious limitations, mostly low
sensitivity (CHIDEROLI et al., 2016), so failure on
detection of leptospires cannot be neglected. Thus, it
may be unclear if intermittence really occurs or if the
failure on recovering leptospires from infected animals
is a reflex of the low sensitivity of culturing. PCR has
been widely used for the diagnosis of leptospirosis, with
high sensibility and specificity (PICARDEAU, 2013;
TAYLOR et al., 2015). It is a rapid and reliable method
that may be used in large-scale. Additionally, frozen
samples may be used for PCR without compromising
the reaction (HAMOND et al., 2014). Considering
the advances of PCR on the detection of carriers in
leptospirosis, this study aimed to analyze the occurrence
of intermittency on leptospiral shedding in naturally and
experimentally infected animals.

MATERIALS AND METHODS

Study Design

In this study two experiments were conducted, both
with the approval of the Ethics Committee of Universidade Federal Fluminense (UFF), Brazil (number 814/2016). The first one was conducted in naturally infected cows belonging to the Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Gado de Leite - Valença, Rio de Janeiro, while the other was conducted on experimentally infected sheep in the Unidade de Pesquisa Experimental em Caprinos e Ovinos (Unipeco) on the school farm of the Universidade Federal Fluminense (UFF) - Cachoeiras de Macacu, Rio de Janeiro.

Experiment I

In the first experiment (naturally infected cows), 60 adult cows from a herd known to be endemically
infected were studied. The animals remained altogether in
the same pasture. Six urine samplings were made from
each cow, at weekly intervals, totaling 360 samples.
Urine was collected after intravenous furosemide
administration, in sterile conical tubes (50ml) and then
1ml aliquots were transferred into microtubes, identified,
conditioned at 4°C and sent to the laboratory.

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RESULTS

Considering cattle, 26/60 (43.3%) animals
tested negative in all samples and were considered
as non-infected. For the remaining 34 (56.7%) cows that were PCR-positive at least once, only one
(1.6%) presented positive in all samples, and seven
(11.8%) were positive only in the last sampling,
making impossible to evaluate the intermittency.
Noteworthy, 26 of the naturally infected cows
(43.3%) presented the typical intermittent pattern of
urine leptospiral shedding (Table 1). In relation to
the three experimentally infected sheep, all of them

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presented the typical intermittent pattern of urine leptospiral shedding, independently of the inoculated leptospiral strain (Table 1).

**DISCUSSION**

The results confirmed that when employing molecular tools there was intermittent shedding of leptospires, independently of the leptospiral strain, host species, natural or experimental infection. This shedding intermittency may have strong implications for the control of leptospirosis on livestock, mainly on the strategic decision about employing or not antimicrobial agents. Although, the whole-herd treatment approach has been recommended (MUGHINI-GRAS et al., 2014) it may be expensive and economically non-feasible. Besides the cost, other important aspects should be reminded, as the grace period of milk/meat after the usage of antibiotic therapy, as well as the environmental impact of the antibiotics usage. Therefore, employing antibiotic therapy cannot be performed indiscriminately and must be proceeded by a thorough identification of infected animals (MARTINS & LILENBAUM, 2017). Isolation of leptospires in tissues, urine and blood is considered the gold standard for the definitive diagnosis of leptospirosis (OIE, 2014). However, this isolation is usually achieved after weeks or months (VERMA et al., 2012), what makes this method not ideal for a rapid diagnosis (ADLER & DE LA PEÑA MOCTEZUMA, 2010). In contrast, PCR seems to be an alternative for a rapid and direct diagnosis of the infection. Nevertheless, this method is a poor indicator for the infecting serovar in a herd, and its implication for epidemiological studies is limited, expensive and difficult (ELLIS, 2015).

Results of our experiments clearly demonstrated that after negative results on PCR analysis of urine samples for a more definitive and reliable individual diagnosis would be required for a successful control program of leptospirosis on a herd.

The low number of experimentally infected animals used in the presented study represents a limitation. Despite that, intermittence was clearly observed in all infected ewes. Other possible bias of this study was the possible interference of environmental conditions in naturally infected animals. Transmission of leptospirosis is influenced by environmental conditions, such as rainfalls (CORREIA et al., 2017), which may interfere in the exposure of those animals to leptospires, also influencing the reinfection and intermittency.

In conclusion this is the first study to describe the intermittent shedding of leptospires in urine by PCR. We suggest that control strategies may incorporate a serial analysis of urine samples for a more reliable individual diagnosis and treatment.

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**Molecular demonstration of intermittent shedding of Leptospira in cattle and sheep and its implications on control.**

<table>
<thead>
<tr>
<th>Status Species</th>
<th>Positive on all samplings</th>
<th>Negative on all samplings</th>
<th>Intermittent shedding</th>
<th>Inconclusive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows</td>
<td>1 (1.7%)</td>
<td>26 (43.3%)</td>
<td>26 (43.3%)</td>
<td>7 (11.7%)</td>
</tr>
<tr>
<td>Sheep</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>3 (100%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

**REFERENCES**


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