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Comparison of different DNA extraction and polymerase chain reaction methods to detect *Leptospira* spp. on field samples

Dr. Maria Vitale,[1] Dr. Fabrizio Vitale,[2] Dr. Stefano Reale,[3] Dr. Gesualdo Vesco,[3] Dr. Vittoria Currò[2] y Dr. Santo Caracappa[2]

SUMMARY

It was carried out a comparison on two reference *Leptospira* strains DNA between different extraction methods and two polymerase chain reaction protocol. The DNA was quantified and serial dilutions were tested by polymerase chain reaction. The results showed difference in terms of recovery and sensitivity between this methods.

Key words: Leptospira, DNA extraction, PCR.

In Italy leptospirosis is diffused in the northern regions but only very limited serological survey had been conducted until now in Sicily. To obtain more data on animal leptospirosis in Sicily we decided to begin a general survey on herds and wild animals, not only by serological methods (MAT) but also by molecular biology technique. To choose for a high sensitive and specific method we performed comparison on two reference Leptospira strains DNA between different extraction methods and two PCR protocol.

L. pomona and *L. sejoroe* reference strains were kindly provided by the Italian animal leptospirosis reference centre in IZS of Brescia.

Leptospira cells were counted on a Burker chamber and serial dilution were performed up to 50 cells/100 microliters. The suspension was introduced with a syringe in 25 mg of negative bovine Kidney samples and DNA extraction had been performed by the following methods:

A) Proteinase K treatment followed by phenol-chloroform extraction.
B) Quiagen column affinity following manufacture extraction.
C) Rybolyser homogenization followed by affinity column.

The DNA was quantified and serial dilutions were tested by PCR.

Two PCR method were used for sensitivity assay targeting *fla* B gene (primers flaB-Forward-TCTCACCGTTCTCTAAAGTTCAAC and flaB--Reverse CTGAATTCGGTTTCATATTTGCC with an amplicon of 793bp[1] and 16S rRNA gene (primers: LEPTO E1 GGGAAAAATAAGCAGCGATGTG and LEPTO E2-ATTCCACTCCATGTCAAGCC amplicon 571 bp).[2]

The analysis, performed in double, showed that not much difference in terms of recovery and sensitivity are present between the methods A and

[1] Doctor in Veterinary Medicine. Molecular Biology.
[2] Doctor in Veterinary Medicine.
[3] Doctor in Biological Sciences.

B but method C gives a higher sensitivity probably due to a better lysis and a more efficient proteinase K digestion.

DNA extracted by homogenization with ribolyser showed a sensitivity of up to 5 cell with flaB PCR but a further 5 fold dilution could be detected by 16 ribosomal RNA gene PCR.

Comparación de diferentes métodos de extracción de ADN y reacción de polimerasa en cadena para detectar *Leptospira* spp. en muestras de campo

RESUMEN

Se realizó una comparación entre diferentes métodos de extracción y 2 protocolos de reacción en cadena de la polimerasa en 2 ADN de cepas de *Leptospira* de referencia. El ADN se cuantificó y se probaron diluciones seriadas mediante la reacción en cadena de la polimerasa. Los resultados mostraron diferencias en términos de recuperación y sensibilidad entre estos métodos.

Palabras clave: Leptospira, ADN, extracción, RCP.

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Dr. *Maria Vitale*. Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri" Palermo, Italy. Fax: 39-0916565313 e-mail: mvitale@pa.izs.it