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DETECTION OF THE *LEPTOSPIRA* GENOME IN A ROMANIAN PIG FARM

DETECȚIA GENOMICĂ A *LEPTOSPIRA* ÎNTR-O FERMĂ DE PORCI DIN ROMÂNIA

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ABSTRACT

Leptospirosis is a worldwide zoonosis, produced by a spirochete of the genus *Leptospira*, which affects a large number of animal species (fish, reptiles, birds, mammals). Leptospirosis is an economic burden on society because of the costs related to public health and farm animals, with abortions, falling production and deaths being encountered. The purpose of this study was to determine the presence of leptospirosis in a breeding pig farm in Romania.

Twentyfour pigs were tested by collecting urine samples for the detection of *Leptospira*. DNA extraction was performed by using the PureLink® Genomic DNA Kits kit that allows for rapid and efficient purification of genomic DNA. The generated electrophoretic images revealed the presence of the specific amplicon for *Leptospira sp*. to 25% of the samples. PCR detection technique of *Leptospira sp*. from urine is a rapid diagnosis method of high accuracy and sensitivity that can be used successfully in completing common diagnostic tests. Infection with *Leptospira sp*. of some pigs from a congregate intensive system, in the absence of any clinical sign of disease, is a biological reality in breeding farms due to immunoprophylaxis for the prevention of leptospirosis that is practiced.

Keywords: *Leptospira*, molecular biology, PCR, diagnostic

REZUMAT

Leptospiroza este o zoonoză întâlnită pe tot globul, produsă de o spirochetă din genul *Leptospira*, care afectează un număr mare de specii de animale (pești, reptile, păsări, mamifere). Leptospiroza este o povară economică pentru societate din cauza costurilor legate de sănătatea publică și de cele legate de animalele de fermă, fiind întâlnite avorturi, scăderea producției și decese. În studiul de față s-a avut ca scop determinarea prezenței leptospirozei într-o fermă de porci de reproducție din România.

S-au testat 24 de animale din specia suină, de la care s-au recoltat probe de urină în vederea detecției leptospirelor. Extracția ADN s-a făcut utilizând kitul PureLink® genomic DNA Kits ce permite purificarea rapidă și eficientă de ADN genomic. Imaginea electroforetică generată a evidențiat prezența ampliconului specific pentru *Leptospira sp*. la 25 % din probe. Tehnica PCR de detecție a *Leptospira sp.* din urină este o metodă de diagnostic rapidă, de mare acuratețe și sensibilitate care poate fi folosită cu succes în completarea testelor uzuale de diagnostic. Infecția cu *Leptospira sp*. a unor suine dintr-un efectiv din sistemul intensiv, în absența oricărui semn clinic de boală este o realitate biologică în fermele de reproducție, dată fiind imunoprofilaxia pentru prevenirea leptospirozei care este practicată.

Cuvinte cheie: *Leptospira*, biologie moleculară, PCR, diagnostic

Leptospirosis is a worldwide zoonosis, produced by a spirochete of the genus *Leptospira*, which affects several animals (fish, reptiles, birds, mammals) (2).

Despite the fact that the epidemiology of *Leptospira* infections has been extensively studied, changes in animal husbandry, climate and human behavior continuously alter the epidemiological characteristics of leptospiroses (1).

Over the last decades, the interest in *Leptospira* infections has increased, which has led to the reclassification of *Leptospira* strains in the new genomic classification system (8), and the development of new diagnostic tests (4).

Leptospira transmission occurs when infected animals spread bacteria through urine, contaminating the environment (9). *Leptospira* penetrates the host's organism through skin or affected mucous membranes (12). Carrier rats and mice are considered important reservoirs of the disease (7).

Leptospirosis is an economic burden to society, due to costs related to public health and farm animals (3). Leptospirosis in humans can be manifested either as a

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flu-like syndrome or as a severe, acute, renal, hepatic and cardiac failure that can lead to death (6). The main effects recorded in the infected pig farms are abortions, stillbirths, and reduction in farrowing rates (3). Considering the negative effects of the infection mainly in the reproduction sector, prevention of leptospirosis should be taken in consideration mainly in gilts and boars. Because anti-*Leptospira* vaccines have low efficiency (do not provide a solid immunity), in prevention it is very important to apply also strict biosecurity measures. Considered the natural reservoir of *Leptospira*, a major objective of biosecurity is the rodent control (13).

The purpose of this paper is to detect the presence of *Leptospira* strains in a farrow-to-wean pig farm by using the molecular biology technique of diagnostic.

MATERIALS AND METHODS

Farm and animals

The farrow-to-wean pig farm is located in Ialomița county, and it has one farrowing unit with four buildings: two pregnant sow halls, a maternity hall, and a post-weaning hall. The farm has 8541 animals (Large White breed) of which: 1113 sows, 182 pregnant gilts, 2637 suckling piglets, 4440 weaned piglets, 167 young gilts, and two boars.

Biological samples

A number of 24 urine samples were collected from pregnant sows of different ages, in 50 ml sterile tubes. The urine samples have been preserved at 4°C until testing.

DNA extraction

DNA extraction was carried out by using the Pure Link Genomic DNA Kit (Invitrogen, Thermo Fisher Scientific Inc., Carlsbad, California, USA). The kit was designed for efficient genomic DNA isolation from mammalian tissues, blood samples, oral swabs, bacteria, yeast, and formalin-fixed paraffin-embedded tissues. The principle of the kit is based on the DNA selective binding to a silica membrane in the presence of guanidine-HCL. RNA digestion was performed in order to prevent contaminating the DNA sample. For an efficient lysis of the tissues/cells, the proteinase K was used by incubating samples at 55° C. DNA was selectively bound to the silicon membrane and then was washed in two steps to remove all contaminating cellular components. In the last elution step, DNA from the silica membrane was released.

PCR technique

The samples were diluted to have about 50ng DNA per reaction. The primer sequences used in the present study are shown in table 1 and the locus of the genome fragment for the sense primer is from codon 38 to codon 57 and for the antisense primer from codon 348 to codon 369.

Table 1

Primer sequences for *Leptospira sp*. detection

Primer A	5 µM 5'GGCGGCGCGTCTTAAACATG-3'
Primer B	5µM 5'TTCCCCCCATTGAGCAAGATT-3'

In a 0.5 ml PCR tube the following reagents were mixed: 17.75 µL water (RNase free), 2.5 µL PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 8.0), 0.75 µL MgCl2 (1.5 mM), 0.5 µL dNTP solution (200 µM) (Invitrogen, Thermo Fisher Scientific Inc., Carlsbad, California, USA), 0.5 µL Primer A (10 pmol), 0.5 µL Primer B (10 pmol), 0.5 µL Taq platinum polymerase (5 U/µL)(Invitrogen, Itapevi, São Paulo, Brazil).

The tubes with the reagents were vortexed and centrifuged. Then, 23 µl of the above mixture was distributed into 0.2 ml tubes for each tested sample. To this mixture 2 µl of DNA (50 ng/reaction) sample was added and, for the negative control, ultra-pure water was added. The tubes with the PCR mix content were introduced in a Thermo Fisher PCR thermocycler.

The PCR tempera-tures conditions were: 94° C-3 min, 1 cycle; 94° C-1 min, 63° C -1.5 min, 72° C-2 min, 45 cycles; 72° C-10 min, 1 cycle. The DNA fragment was visualized on a UV device after a 1.5% agarose gel electrophoresis was performed.

RESULTS

The electrophoretic migrations show the targeted DNA fragment (289 bp) in 8 samples (Fig. 1).

In our study, 3 electrophoreses were performed in order to visualize all the samples.

A 100 bp molecular ladder was used.

For the positive control we used an inactivated *Leptospira* culture (*Leptospira icterohaemorrhagiae* and *Leptospira canicola*), and for the negative control we used ultra-purified water. All samples were run in duplicate.

From the 24 urine samples tested, 18 samples were negative, three were positive and three were weakly positive. The percentage of the positive samples was 25% and of the negative samples was 75%.

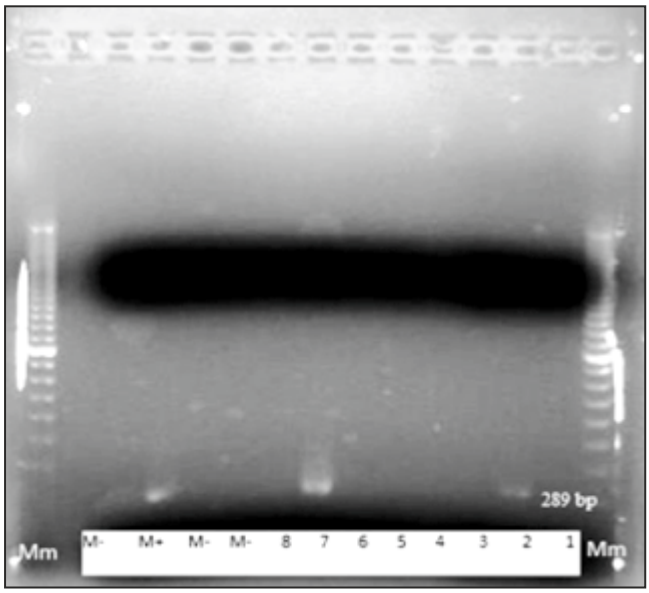

Fig. 1. Electrophoretic image with the results in classical PCR testing; Mm = molecular ladder, M- = negative control, M+ = positive control, 8 = negative sample 8, 7 = sample no.7 positive, 6 = sample no. 6 negative, 5 = 5 negative sample, 4 = negative sample 4, 3 = negative sample 3, 2 = low positive sample 2, 1 = sample no. 1 negative. Electrophoretic migration of the DNA fragment to 289 bp (base pairs)

DISCUSSIONS

The sows from which the urine samples were collected showed no clinical sign of the disease, but the results revealed the presence of a *Lepstospira* sp. infection in the studied farm.

In a 2014 study of 20 sows from 5 farms in eastern, western and southern Poland, the prevalence of leptospirosis by PCR testing was 100% (11).

It should be noted that in our study the intensity of the positive sample is low, comparable to the positive control. In sows breeding flocks, it is expected / desirable for the *Leptospira* elimination rate in the urine to be low or zero. This must happen because the vaccination against leptospirosis recommended in this animals category, has this objective /effect alongside the protection against abortion and illness.

Another study in Sicily, Italy, on 70 pigs, by PCR technique, showed a 40% prevalence of leptospirosis (10).

In 2013, a study in Rio de Janeiro, Brazil, on 15 pigs, revealed 33.33% leptospirosis prevalence (5).

Compared to these studies, the 25.00% percentage of positive samples obtained by the PCR method is a relatively low percentage.

CONCLUSIONS

PCR detection technique of *Leptospira sp*. from urine is a rapid diagnosis method of high accuracy and sensitivity that can be used successfully in completing common diagnostic tests. The prevalence of positive samples for the presence of the DNA fragment specific for *Leptospira* sp in the studied group is 25% and the prevalence of negative samples is 75%, which shows the presence of leptospirosis in the studied farm. The amount of *Leptospira* sp. excreted appears to be low, which is correlated with the amount of DNA: the phenomenon, very likely, is the consequence of the immunoprophylaxis practiced.

Infection with *Leptospira* sp. of pigs in an intensive system in the absence of any clinical sign of the disease is a biological reality in breeding farms due to immunoprophylaxis for the prevention of leptospirosis that is practiced.

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