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Prevalence of Shiga toxin-producing *Escherichia coli* and pathogenic *Leptospira* spp. in rodents from outdoor farms in western Spain

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Abstract: Sixty-five rats (*Rattus norvegicus*) and 5 mice (*Mus musculus*) were trapped from 8 outdoor farms and the presence of Shiga toxin-producing *Escherichia coli* was not detected in any individual. Nevertheless, using the microscopic agglutination test, it was found that 20% (13/65) of rat serum samples were positive for anti-*Leptospira* antibodies, and DNA of pathogenic leptospires was detected in 3.07% (2/65) of the rat kidneys by real-time polymerase chain reaction. The most common infecting serovar was Castellonis (7.69%), followed by Canicola (6.15%) and Mangus (6.15%). According to these results, rats from outdoor farms may be considered a reservoir host for *Leptospira* in the study area.

Key words: Rodents, Shiga-toxin producing *Escherichia coli*, *Leptospira* spp., outdoor farms

Rodents are abundant on outdoor farms, where they may cause structural damage to farm building and equipment. Furthermore, rodents may play a major role in the transmission of infectious diseases.

Rodents, especially rats, are considered to be the most important reservoir or maintenance host of *Leptospira* spp. and different species of rats have been reported to carry different pathogenic leptospiral serovars (1). Pathogenic leptospires that infect these maintenance hosts can persist in their kidneys, causing either little or no harm, and therefore they may play an important risk in the environmental contamination.

In the same way, *Escherichia coli* O157 has already been identified in farm-trapped rats (2). The authors even considered from an epidemiological point of view that infected rodents can spread these microorganisms from one part of the farm to another.

Despite these facts, only very limited information is available on the role of rats inhabiting outdoor farms as carriers of these zoonotic agents. The aim of this study was to analyze the prevalence of Shiga toxin-producing *E. coli* (STEC) and pathogenic *Leptospira* spp. in peridomestic rodents caught on different outdoor farms in western Spain.

Livestock production in western Spain is mainly based on autochthonous breeds, usually reared on outdoor farms under extensive conditions where they live side by side with wild and peridomestic animal species like rodents. The climate is continental with extreme heat in summers and cold winters; the average rainfall is 475 mm, with very little rainfall during July and August.

From summer 2010 to spring 2011, 65 rats and 5 mice were randomly trapped alive on 8 outdoor farms including 2 pig farms, 2 mixed pig and cattle farms, 2 mixed pig and sheep farms, and 2 mixed pig, cattle, and sheep farms, using Tomahawk traps (15 × 15 × 45 cm).

An average of 10 traps were used during the study period to conduct 1 capture session every fortnight. Traps were usually placed in the storage area for livestock feed. Captured rodents were anesthetized with a mixture of ketamine hydrochloride (Leti & Merieux, Spain) and xylazine hydrochloride (Bayer, Spain) (9:2) using 0.1 mL/50 g. Rats were bled by heart puncture with disposable syringes (BD, Spain) and blood samples were allowed to clot at room temperature. After centrifuging for 20 min at 2000 rpm, serum was separated and stored at –20 °C until used. It was not possible to collect serum samples from the mice.

Animals were killed with an overdose of sodium pentobarbital (Vetoquinol, Spain). Necropsy was performed under biosafety conditions and both kidneys

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were extracted using sterilized scissors and tweezers, and the internal organs were examined for any signs of clinical illness.

From each rodent, 2 rectal swabs were taken. One of each of the samples was enriched in 5 mL of buffered peptone water (Oxoid, UK) supplemented with vancomycin (8 mg/L), cefixime (0.005 mg/L), and cefsulodin (10 mg/L) and was incubated for 18 h at 37 °C. About 1 mL of this enrichment culture was added to 20 µL of magnetic beads coated with O157-antibodies (Dynabeads anti-*E. coli* O157, Dynal, Norway) and immunomagnetic separation was performed following the manufacturer's instructions. The concentrates obtained were then inoculated onto sorbitol MacConkey agar (Oxoid, UK) containing cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L) and incubated at 37 °C overnight.

For isolation of non-O157 STEC, rectal swab samples were plated directly onto MacConkey agar (Oxoid). Following overnight incubation, a loopful of bacterial growth from the first streaking area of the culture plates were tested for the genes encoding O157 and H7 antigens (O157*rfbE* and *fliCh7* genes) and Shiga-toxins (stx_1 and stx_2 genes) by polymerase chain reaction (PCR) as previously described (3).

Microscopic agglutination test (MAT), as a gold standard method for serological diagnosis of leptospirosis, was performed on sera from the 65 trapped rats using 24 live leptospiral strains as antigens.

The antigen panel consisted of the following serogroups (serovars are given in parentheses): Australis (Australis, Bratislava, and Munchen), Autumnalis (Akiyami A and Bim), Ballum (Castellonis), Bataviae (Bataviae), Canicola (Canicola), Icterohaemorrhagiae (Icterohaemorrhagiae and Copenhageni), Grippotyphosa (Grippotyphosa and Vanderhoedoni), Hebdomadis (Kremastos), Panama (Panama and Mangus), Pomona (Pomona and Mozdok), Pyrogenes (Pyrogenes), Sejroe (Sejroe, Saxkoebing, Wolffi, and Hardjo), Tarassovi (Tarassovi), and Cynopteri (Cynopteri). The leptospiral cultures were adjusted to a cell density of 1.5×10^8 cells/mL (0.5 McFarland standard) with phosphate-buffered saline (pH 7.4) (1). Positive sera at a titer of 1:20 were further titrated until 1:5120. All the strains were maintained in Ellinghausen–McCullough–Johnson–Harris medium (Indicia Biotechnology, France) with periodic subculturing. Seven-day-old cultures free from contamination were utilized for the performance of MAT. A serum sample was considered positive at a titer of 1:40.

DNA was extracted from kidney samples of rodents using the QIAamp DNA Mini Kit (QIAGEN, Germany). Extracted DNAs were subjected to real-time PCR for detection of pathogenic *Leptospira* species using the PathoLept TaqVet™ Kit (LSI, France) according to the manufacturer's instructions. All the reactions were performed using a 7500 Fast Real-Time PCR thermocycler (Applied Biosystems, USA).

The dependent variable used throughout this study was the presence of antibodies against *Leptospira* in captured rats. The independent variables were sex, animal species on the farm, rainfall, temperature, and pregnancy.

The relationship between the dependent and the independent variables was studied by calculating chi-square values and odds ratios. The data were analyzed using the SPSS 15.0 (SPSS Inc., USA) and Win Episcope 2.0 (Epidecon, USA).

In total, 43 (66.2%) female and 22 (33.8%) male adult rats (*Rattus norvegicus*) and 4 females and 1 male mice (*Mus musculus*) were trapped. After being dissected, 26 rat livers (40%) showed parasitic cysts and 8 of the 43 female rats were pregnant (18.6%).

Neither non-O157 Shiga toxin-producing *E. coli* nor *E. coli* O157:H7 was detected in any of the individuals analyzed (65 rats and 5 mice).

MAT was used to identify the most probable infecting serovar. Thus, in our study, 20% (13/65) of the rats were positive for leptospirosis. Castellonis was the most frequently reactive serovar with rat sera (7.69%), followed by Canicola (6.15%) and Mangus (6.15%).

Leptospira-seropositive rats were detected from 5 out of the 8 farms (62.5%) and MAT titers ranged from 1:40 to 1:280. The highest titer (1:1280) was found in 2 samples, 1 of which was due to serovar Mangus and other to serovar Castellonis.

There was no statistically significant association ($P > 0.05$) between the independent rat variables and the dependent variable, with the exception of a significant positive association between the presence of anti-*Leptospira* antibodies and rat pregnancy (Table 1).

Since leptospires are difficult to culture, several PCR methods have been used to facilitate early diagnosis. In this study, a commercially available real-time PCR kit able to detect pathogenic *Leptospira* was used. Of the 70 samples tested by real-time PCR, pathogenic *Leptospira* spp. was only demonstrated in kidneys of 2 rats from 1 of the mixed pig and sheep farms. Both rats were also positive by MAT due to serovar Castellonis with titers of 1:640 and 1:1280 (Table 2).

In agreement with previously reported studies of trapped rodents from farms (4,5), we did not find any positive samples for STEC in our study. Rodents presumably show a low prevalence of STEC; however, some authors consider that they may become infected from farm animals or vice versa (6).

The detection rate of *Leptospira* antibodies in rats was similar to the prevalence found by PCR (20.3%) in an earlier study carried out in the Canary Islands of Spain (7).

Table 1. Statistical data.

Variable		Category	Odds ratio	χ^2	P
Sex		Male vs. female	1.287	0.155	0.694
Animal species on farms	Cattle	Presence vs. absence	1.08	0.015	0.901
	Sheep	Presence vs. absence	0.567	0.824	0.518
Rainfall		High vs. low	4.00	1.843	0.267
Temperature		High vs. low	0.36	2.677	0.102
Pregnancy		Pregnant vs. not pregnant	7.750	6.397	0.028*

*: Statistically significant ($P < 0.05$).

Table 2. Data, serovars, and PCR results from *Leptospira* MAT-positive rats.

Rat no.	Sex	Farm type*	Season**	Presence of liver cyst	Pregnant	Serovars	MAT titers	PCR result
7	♀	3	3	-	+	Castellonis	1:160	-
21	♂	4	4	+		Canicola	1:40	-
32	♀	2	4	-	-	Mangus	1:160	-
35	♀	2	4	-	+	Mangus	1:1280	-
38	♀	2	4	+	-	Canicola	1:160	-
39	♂	2	4	+		Canicola	1:80	-
51	♂	2	2	+		Mangus	1:40	-
57	♀	2	2	+	+	Canicola	1:160	-
62	♂	1	2	-		Mangus	1:640	-
64	♀	1	2	-	-	Castellonis	1:1280	+
66	♀	1	2	+	+	Castellonis	1:80	-
69	♂	1	2	-		Castellonis	1:160	-
70	♀	1	2	-	-	Castellonis	1:640	+

*Farm type: 1, pig and sheep; 2, pig and cattle; 3, pig, cattle, and sheep; 4, pig.
**Season: 1, winter; 2, spring; 3, summer; 4, autumn.

However, in our study, only the 3.07% of rat samples were positive by real-time PCR.

There was great variation in the results obtained by the 2 different diagnostic tests, which could be explained by probably only the 2 positive rats by real-time PCR being temporary shedders; meanwhile, antibodies were the only remaining evidence of a previous leptospiral contact in the rest of the rats positive by MAT.

From an epidemiological point of view it is essential to establish which animal species are reservoirs and which *Leptospira* serovars are involved in a particular area of study. In our study, serovar Castellonis was the major serovar detected in the serum samples of rats caught. The Castellonis (serogroup Ballum) serovar is found to be associated with rodents worldwide (8). However, human and animal leptospirosis caused by *Leptospira* spp. belonging to serogroup Ballum has increased worldwide in the past decade (9). Furthermore, the Castellonis serovar was previously detected in the same area of study with 1.15% seroprevalence in pigs (10).

The isolation of serovar Canicola was an unexpected finding, because dogs are recognized as the natural maintenance hosts for this serovar (11). However, some authors have already warned about the possible adaptation of an old and well-established serovar to new and abundant hosts. In such circumstances the authors suggest that these new hosts are a serious risk, for both veterinary and public health (12). In fact, Canicola, together with other serovars, has been implicated as the cause of reproductive disorders in cattle and swine.

Finally, there are few reports on *Leptospira* serovar Mangus; it has been found in wild animals, including opossum, monkeys, and mongooses, but to the authors' knowledge this is the first time that high antibody titers

for serovar Mangus (1:1280) have been detected in rats of Spain. Leptospirosis causes major economic losses in the livestock industries through its adverse effects on reproductive performance. Livestock can be infected by several leptospiral serovars, the particular ones depending on the occurrence of reservoir hosts, environment, and climate in the particular area (13).

Until now, the status of outdoor farm rats in western Spain as leptospirosis reservoirs remained unknown; however, the results from this study suggest that common peridomestic wildlife rodents should be considered as potential sources of leptospirosis for animals and humans on outdoor farms. This risk may be even greater in outdoor production, where habitat and food are available and rodenticides are used less often.

In addition, there are not noticeable consequences for wild rats, and, unlike in other animal species, pathogenic leptospires such as *Leptospira canicola* may persist in these carriers without causing any kind of reproductive problems, as has been shown by the positive association between the presence of anti-*Leptospira* antibodies and pregnancy in trapped rats.

Thus, it is important to impress upon outdoor farmers the need for rodent control and protective barriers to prevent contamination of feedstuffs and water supplies with the excretions and secretions of these animals as an important method of leptospirosis control.

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