

SHORT REPORT

First isolation of *Leptospira noguchii* serogroups Panama and Autumnalis from cattle

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SUMMARY

Prevention and control of leptospirosis are based on the knowledge of locally circulating strains. Thus, efforts to obtain local isolates are paramount to the epidemiological understanding of leptospirosis. We report and discuss here the first isolation of members of serogroups Autumnalis and Panama from cattle, both belonging to *Leptospira noguchii* species. Urine samples ($n = 167$) were collected directly by puncture of the bladder from randomly selected cows from a slaughterhouse in Rio de Janeiro, Brazil, for bacteriological culture. Isolates were characterized by serogrouping and sequencing (*rrs* and *secY* genes). Overall, 10/167 positive urine samples (6%) were obtained. Sequencing of amplicons targeting for both *rrs* and *secY* genes identified two of them (2013_U73 and 2013_U232) as *L. noguchii*. Serogrouping of those strains indicated that 2013_U73 belonged to the Panama serogroup (titre 1600), and 2013_U232 to the Autumnalis serogroup (titre 12800). Both Panama and Autumnalis are known agents of incidental leptospirosis in cattle. This group of leptospires could be particularly important in tropical countries. This is the first report of members of serogroups Autumnalis and Panama belonging to *L. noguchii* species from cattle. Although related to previously reported strains, these isolates have been shown to be genetically diverse from them.

Key words: Autumnalis, cattle, *Leptospira noguchii*, Panama.

Leptospirosis is a major infectious disease in cattle with direct impacts on animal production, leading to important economic hazards, mainly associated with reproductive failure. Besides the economic impact on

livestock, leptospirosis is also an emerging zoonosis worldwide [1]. Rodents are commonly described as the main reservoirs of leptospires, but other species can also harbour this bacterium. However, the real role of cattle in the epidemiology of human leptospirosis in rural areas remains to be elucidated [2].

More recently, advances in molecular characterization of leptospires has demonstrated a wide variability of circulating species and genotypes in different regions [3]. In this scenario, *Leptospira*

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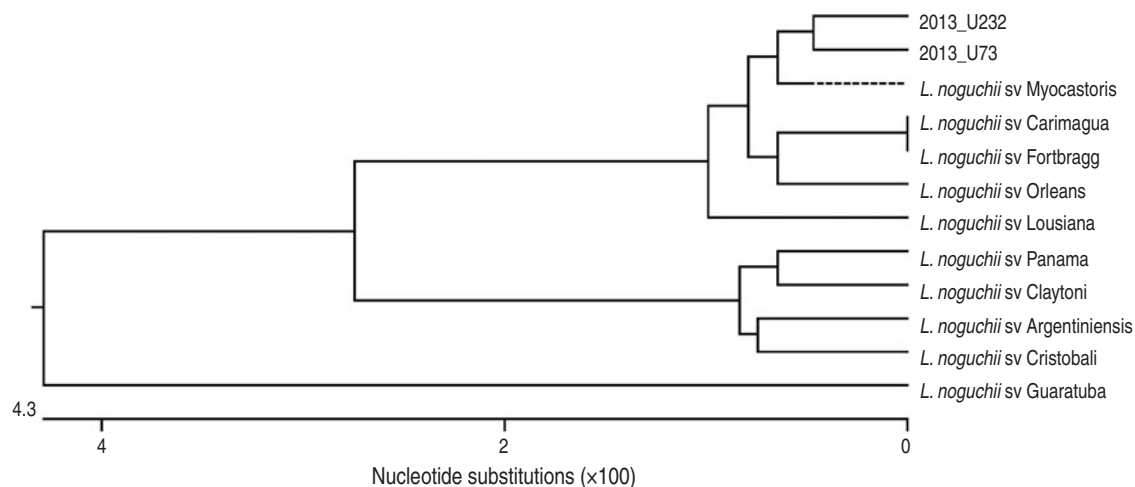


Fig. 1. Phylogenetic tree of leptospiral *secY* gene sequences of reference strains (*L. noguchii* serovars Argentinensis strain Peludo, Claytoni strain 1348 U, Panama strain CZ 214 K, Cristobali strain 1996 K, Myocastoris strain LSU 1551, Fortbragg strain Fort Bragg, Louisiana strain LSU 1945, Orleans strain LSU 2580, Carimagua strain 9160, Guaratuba strain An 7705) and a set of clinical isolates from cattle (2013_U73 and 2013_U232). The tree was drawn using the unweighted pair-group method with arithmetic average (UPGMA) algorithm.

noguchii strains have been isolated worldwide from different hosts, including humans [4], domestic [5] and wild [6] animals. Yet the only reference regarding *L. noguchii* in cattle concerns a strain from serogroup Australis (serovar Peruviana) identified in Peru [6].

Since strategies for prevention and control of leptospirosis are based on the knowledge of the circulating strains in a specific host and region, efforts to obtain local isolates are crucial for understanding the local epidemiology of leptospirosis [1]. This paper aimed at reporting and discussing the first isolation of members of serogroups Autumnalis and Panama, both belonging to *L. noguchii* species, from cattle.

During 2013, in six monthly visits, 167 bovines were randomly selected in a slaughterhouse located close (130 km) to Rio de Janeiro, Brazil. Animals were adults from ten herds located nearby the slaughterhouse. Although the individual history of each animal was not provided, there was no particular reference to reproductive or sanitary problems in these herds. From the selected animals, urine samples ($n = 167$) were collected directly by puncture of the bladder for bacteriological culture. A few drops of urine were immediately inoculated into 5 ml Ellinghausen-McCullough-Johnson-Harris (EMJH) liquid medium (Difco, BD, USA), and 5 ml semi-solid Fletcher medium (Difco). Culture tubes were maintained at room temperature and transported to the laboratory.

Cultures were incubated at 28 °C and evaluated weekly by dark-field microscopy for up to 4 months [6]. If contamination (other bacteria and fungi) was observed, 5-fluorouracil (Sigma-Aldrich, USA) and filtration (using a 0.22-µm sterile syringe filter; Millipore Corporation, USA) were used to decontaminate cultures. The obtained isolates were serogrouped using a panel of 32 specific antisera provided by the Royal Tropical Institute (KIT, The Netherlands) [6].

DNA of isolates was extracted using the Promega Wizard SV Genomic DNA Purification System® (Promega, USA) for genetic characterization. Primers F (5'-ATGCCGATCATTTTTGCTTC-3') and R (5'-CGTCCCTTAATTTTAGACTTCTTC-3') were used for amplification of the partial *secY* gene. For amplification of the partial *rrs* gene, the primers LA (5'-GGCGGCGCGTCTTAAACATG-3') and LB (5'-TTCCCCCATGAGCAAGATT-3') were used. The obtained amplicons of the partial *secY* gene and partial *rrs* gene were sequenced [3].

Overall, ten (6%) isolates were obtained from the 167 urine samples. Sequencing of amplicons targeting for both *rrs* and *secY* genes identified two of them (named 2013_U73 and 2013_U232) as *L. noguchii* species. Serogrouping of those strains indicated that 2013_U73 belonged to the Panama serogroup (titre 1600) and 2013_U232 to the Autumnalis serogroup (titre 12800).

Genetic characterization based on the variability of the *secY* gene (Fig. 1) showed these isolates to be very closely related, but genetically diverse to previously reported strains. Although 2013_U73 belongs to the

Panama serogroup, it is diverse from the other members of the same species (*L. noguchii*) and serogroup (Panama), as serovars Cristobali strain 1996 K and Panama strain CZ 214 K (both obtained from opossums in Panama), and do not share the same origin. Curiously, they are related to the *L. noguchii* serovar Myocastoris, from serogroup Pyrogenes (obtained from nutria in the USA).

This is the first isolation of strains of *L. noguchii* serogroups Panama or Autumnalis from cattle. The only other report of this species from cattle was a strain from serogroup Australis (serovar Peruviana) [6]. These two isolates are related to another *L. noguchii* strain, *L. noguchii* serovar Myocastoris, probably representing genotypes from that serovar. With the advent of molecular characterization, a large genotypic diversity of leptospires has been revealed, varying by different hosts and geographical regions [3]. Despite the low number of *L. noguchii* isolates reported, almost all of them were obtained in American countries, such as Brazil, Argentina, Peru, Panama, Barbados, Nicaragua, and the USA [6], where this species is considered to be an important zoonotic agent [5].

Members of serogroup Panama can be found in two species, *L. noguchii* and *L. inadai* [6]. With the exception of serovar Peruviana, all reports regarding that serogroup in cattle are based on serology, and usually at low frequencies [7]. Nevertheless, the role of this serogroup as a zoonotic agent cannot be neglected. A clinical case of lymphocytic meningitis has been described [8], and serological findings have also been reported. In Mexico, it was reported as the most common in anicteric cases of human leptospirosis [9] and, in a retrospective study conducted in Barbados, serogroup Panama accounted for over half of the seropositive reactions [10].

Although *L. noguchii*, belonging to serogroup Autumnalis, was obtained only once in Brazil [5], that serogroup, which can be found in five different species (*L. interrogans*, *L. borgpetersenii*, *L. santarosai*, *L. noguchii*, *L. kirshneri*), had never been isolated from cattle before. In fact, the presence of antibodies against Autumnalis in bovines is known to be sporadic [7] and has been associated with the presence of wild mammals close to the herds [11]. By contrast, isolates belonging to serogroup Autumnalis have been reported in humans from tropical countries, such as India [12] and Brazil [5].

Although members of serogroups Panama and Autumnalis have been recovered from various hosts, mainly wildlife (particularly opossums and field rats),

their main reservoir(s) remains unknown. Incidental leptospirosis is particularly important in tropical countries, due to the role of wildlife as reservoirs and environmental circumstances [6, 7]. Opossums are widespread in Brazil, and although leptospires from *L. noguchii* species have been isolated from that species, their real role in the transmission of leptospires remains to be elucidated [13]. It is of note that while these leptospires have been reported in wildlife and humans, they have now been recovered from livestock. This reinforces the 'one health' concept, and demonstrates the wide circulation of these bacteria among various hosts and the environment.

In conclusion, this is the first isolation of strains of *L. noguchii* serogroups Panama or Autumnalis from cattle. Genetic analysis showed those isolates to be diverse from previously reported strains.

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DECLARATION OF INTEREST

None.

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