Isolation and characterization of pathogenic leptospires associated with cattle

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ABSTRACT

Pathogenic leptospires colonize the renal tubules of reservoir hosts of infection, including cattle, and are excreted via urine. In order to identify circulating serovars of pathogenic leptospires in beef cattle, and their associated rates of urinary excretion, a cross sectional study was performed. Fifty urine samples were collected one day each month over 12 consecutive months (N = 600), directly from the bladder of beef cattle at a single slaughter facility and assessed for the presence of leptospires by culture and the fluorescent antibody test (FAT). Where possible, a matched serum sample was also collected for the microscopic agglutination test (MAT). Forty-three urine samples were either culture positive or FAT positive, indicating that 7.2% of sampled beef cattle were actively excreting leptospires in urine. Twenty-three urine samples were culture positive. Sequence analysis of 16S ribosomal DNA and secY indicated that all isolates were Leptospira borgpetersenii. Typing by serology indicated that all isolates were serogroup Sejroe. An overall seroprevalence of 20% (MAT ≥ 1:25) was determined; positive bovine sera was most reactive to serogroup Sejroe (serovar Hardjo) (8.1%), and serogroup Australis (serovar Bratislava) (6.7%). There was poor correlation between seroprevalence and excretion of leptospires since 18/43 (41.9%) cattle, which were positive by culture or FAT, were seronegative. The virulence of two selected isolates of L. borgpetersenii was confirmed by experimental infection in small animal models of infection. Results confirm that L. borgpetersenii continues to circulate in beef cattle and that multiple diagnostic assays are required to detect active shedding. These findings also highlight beef cattle as a reservoir host for the potential zoonotic transmission of leptospires.

1. Introduction

Leptospirosis is a global zoonotic disease of domestic and wild animal species caused by pathogenic species of Leptospira (Adler, 2014; Bharti et al., 2003). Pathogenic leptospires colonize the renal tubules of reservoir hosts of infection, including cattle, and are excreted via urine into the environment where they survive in suitable moist conditions. Transmission of disease is via contact with contaminated environments or direct contact with infected urine.

Bovine leptospirosis results in abortion, stillbirth, premature birth and reproductive failure (Ellis, 2015; Guitian et al., 1999). Although cattle are recognized as a reservoir host for L. borgpetersenii serovar Hardjo (Ellis et al., 1981), multiple species and serovars of leptospires are associated with bovine leptospirosis including L. interrogans serovar Pomona and L. kirschneri serovar Grippotyphosa (Miller et al., 1991; Rajeev et al., 2014). More recently, infection of cattle with L. noguchii was demonstrated for the first time (Martins et al., 2015). Seroprevalence studies, in the U.S. and other countries, indicate that a large proportion (up to 49%) of cattle are exposed to multiple serovars of pathogenic leptospires (Benkirane et al., 2016; Martins et al., 2010; Miller et al., 1991; Pinna et al., 2018; Talpada et al., 2003).

The gold standard diagnostic test to detect reservoir hosts of leptospirosis is culture (Ellis, 2015). However, given the fastidious nature of pathogenic leptospires, time required, the use of highly specialized media and a relatively laborious process, culture is rarely performed (Girault et al., 2017). Nonetheless, actual isolates of pathogenic leptospires derived from naturally infected animal species are essential for research purposes: to understand pathogenic mechanisms of infection, for epidemiological typing, for diagnostic purposes (MAT), and for updating bacterin-based vaccine strategies to prevent infection and transmission of leptospirosis in domestic animals (Arent et al., 2013; Girault et al., 2017; Schuller et al., 2015). The kidney is often a
preferred tissue for culture of leptospires from cattle, but considerable effort in obtaining, handling, and sampling, limits processing to a relatively few samples per day (Rajeev et al., 2014). In addition, leptospires are not evenly distributed throughout an infected kidney and the relatively small part of the kidney (grams) that is actually cultured, further reduces chances of successful isolation.

In contrast, urine is a relatively easy sample to collect and culture. In theory, urine represents an entire flush of the kidney and thus the potential to culture any leptospires excreted from colonized renal tubules. In this report, a cross sectional study was performed on bovine urine samples collected from an abattoir in order to directly culture and characterize isolates of *Leptospira* associated with renal colonization in cattle.

2. Methods

2.1. Sample collection

A beef cattle (< 30 months of age) slaughter facility was visited once per month for twelve consecutive months. At each visit, approximately fifty urine samples (up to 35 ml each) were collected for a total of six hundred. The bladder was identified after removal of the abdominal contents and urine collected by direct aspiration using a 50 ml syringe with an 18 gauge needle. Where possible (580/600), 2–3 ml of cardiac blood was also collected directly from the heart of the same animal, by syringe aspiration, immediately after Food Safety and Inspection Service (FSIS) inspection.

2.2. Microscopic agglutination test (MAT)

Cardiac blood samples were transported back to the laboratory at room temperature where they were allowed to clot overnight at 4 °C. Serum was harvested by centrifugation and stored at −20 °C prior to analysis. The MAT was performed according to OIE guidelines (Cole et al., 1973), at two-fold dilutions from an initial dilution of 1:25, to 1:800, using six serovars of pathogenic *Leptospira* representative of the following serogroups: *L. interrogans* serogroup Australis serovar Bratislava strain Jez Bratislava, *L. interrogans* serogroup Canicola serovar Portlandvere strain 12-001, *L. kirschneri* serogroup Grippotyphosa serovar Grippotyphosa strain GR-01-082, *L. interrogans* serogroup Sejroe serovar Hardjo strain Hardjoprajitno, *L. interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni strain IC-02-001, and *L. interrogans* serogroup Pomona serovar Pomona strain Pomona (Talpada et al., 2003).

2.3. Culture

Two media types were used for the culture of leptospires from six hundred bovine urine samples; urine sample numbers 1–600 were cultured in semi-solid T80/40/LH media. PDMS media comprised Probumin 5X Vaccine Grade Solution (EMD Millipore, Billerica, MA) which was diluted 5-fold in autoclaved distilled water, supplemented with 0.15% (w/v) purified agar, Dulbecco’s Modified Eagle’s Medium/Ham’s Nutrient Mixture F12 (10% v/v) (Sigma, St. Louis, MO) and the antibiotic cocktail STAFF consisting of sulfamethoxazole, 40 μg/ml; trimethoprim, 20 μg/ml; amphotericin B, 5 μg/ml; fosfomycin, 400 μg/ml; and 5-fluorouracil, 100 μg/ml (Chakraborty et al., 2011). T80/40/LH media was prepared as previously described (Ellis et al., 1985) with 0.15% (w/v) purified agar, 5-fluorouracil at 100 μg/ml, and without nalidixic acid. Media was dispensed in 6 ml aliquots in 14 ml-poly-styrene tubes. As soon as each urine sample was collected, the needle was removed from the syringe and 3–4 drops of urine were inoculated directly onto the surface of each media type. Media tubes were sealed immediately after inoculation and transported back to the laboratory at room temperature where they were incubated at 30 °C in the dark and periodically examined by dark-field microscopy for the presence of leptospires, up to twenty-six weeks. Samples were considered positive as soon as a Dinger zone began to be observed, and as confirmed by the detection of spirochetes by dark-field microscopy. Samples demonstrating growth were expanded in liquid Probumin 5X Vaccine Grade Solution (200 ml/L) and 5-fluorouracil (100 μg/ml) for serotyping and genomic sequencing.

2.4. Fluorescent antibody test (FAT)

After inoculation of media for culture, the remaining urine was transferred to a 50 ml-conical tube and transported back to the laboratory at room temperature. Urine volumes for each sample were recorded and urinary pellets collected by centrifugation at 6100 × g for 60 min at 4 °C, or 10,000 × g for 30 min at 4 °C. Supernatant was removed, the urinary pellet resuspended in 2 ml H2O2, and 1 ml transferred to a 1.5 ml-microfuge tube. The urinary pellet was washed by centrifugation at 12,000 × g for 10 min at 4 °C. Supernatant was removed until approximately 100 μl remained and this was resuspended in 500 μl H2O2. The pellet was again harvested by centrifugation at 12,000 × g for 10 min at 4 °C. The supernatant was removed until approximately 50μl remained which was resuspended. An aliquot of 15 μl of the suspension was placed on a glass slide with a 7 mm well for FAT, performed in duplicate, as previously described (Zuerner, 2005). In brief, slides were air dried and fixed in acetone for 10 min. Slides were then placed in a humid chamber and 20 μl of high-titer rabbit anti-*Leptospira* sera (National Veterinary Services Laboratories, APHIS, USDA, Ames, IA) conjugated with fluorescein isothiocyanate (FITC) applied to each 7 mm spot. Slides were incubated at 37 °C for 1 h. Slides were washed for 10 min in PBS with gentle rocking, dried and counterstained for 30 s with FluoroOrange (National Veterinary Services Laboratory). Slides were rinsed with PBS, blotted dry and mounted using Vectashield™ mounting medium (Vector Laboratories, Burlingame, CA). Microscopic examination was done using a Nikon Eclipse E800 microscope and B2-A filter (excitation, 450–490 nm; emission, 520 nm) at 200 × magnification.

2.5. Typing of leptospires by serology

Cultured isolates were propagated in liquid Probumin medium to a density of 2.5 × 10⁶/ml, as determined by dark-field microscopy (Miller, 1971). The isolates were serotyped by the MAT with a panel of
Polyclonal rabbit reference antisera representing the six serogroups Australis, Canicola, Grippotyphosa, Sejroe, Icterohaemorrhagiae, and Pomona (National Veterinary Services Laboratories, APHIS, USDA, Ames, IA).

2.6. Typing of leptospires by genomic sequence

Genomic DNA from each cultured isolate was extracted and purified with QIAamp\textsuperscript{®} DNA Mini Kit (Qiagen, Hilden, DE). Libraries were prepared using the Tru-Seq PCR-free DNA library preparation kit (Illumina Inc., San Diego, CA). Libraries were pooled, loaded into a single flow-cell lane, and 150-cycle sequencing was performed using a HiSeq 3000 (Illumina Inc., San Diego, CA). Draft assemblies of each genome were mined to retrieve the full length 16S rRNA and secY coding regions. Previously (Nally et al., 2016), we had obtained a curated set of leptospire 16S rRNA and secY coding sequences. In each case, sequences were aligned with MUSCLE (Edgar, 2004), and divergent and ambiguously aligned alignment blocks were removed with Gblocks (Talavera and Castresana, 2007). The modelTest feature of Phangorn (Schliep, 2011) was used to calculate the Bayesian Information Criterion (BIC) for a variety of models, and guided the selection of the HKY + I model. The model parameters for computing the maximum likelihood of phylogeny were optimized using optim.pml, and bootstrap.pml was used to perform a bootstrap analysis (Schliep, 2011). The phylogenetic reconstruction with bootstrapped values assigned to the edges was graphically rendered with TreeDyn (Chevenet et al., 2006). The nucleotide sequence for 16S and secY has been deposited in Genbank, accession numbers MH059524 and MH059525 respectively.

2.7. Evaluation of virulence

All animal experimentation was conducted in accordance with protocols as reviewed and approved by the Animal Care & Use Committee at the National Animal Disease Center, and as approved by USDA Institutional guidelines. Two bovine isolates (designated strain TC129 and strain TC273) were propagated in T80/40/LH semi-solid medium and evaluated for virulence by intraperitoneal injection into two groups of 3 golden Syrian hamsters (Mesocricetus auratus), as previously described (Zuerner et al., 2012). Liver and kidney tissue were harvested for culture of leptospires in T80/40/LH semi-solid medium at 8 days (for hamsters infected with TC129) or 3 weeks (for hamsters infected with TC273) post-infection. Tissue homogenates were also directly observed by dark-field microscopy for the presence of leptospires. Kidney tissues were fixed in neutral buffered 4% formaldehyde, processed routinely, embedded in paraaffin, cut into 4 μm sections and stained for the presence of leptospires by immunohistochemistry. After dewaxing, tissue sections were blocked with 10% normal goat serum in PBS for 30 min at room temperature. Samples were incubated with antibody specific for the outer membrane lipoprotein LipL32 at 1:200 in blocking solution and incubated overnight at 4 °C. After 3 x 5 min washes in PBS, samples were incubated in goat anti-rabbit IgG conjugated to AlexaFluor 546 (Invitrogen) and DAPI (Invitrogen) 1:3000 in blocking solution, for 60 min at room temperature in the dark. Samples

Table 1

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Bratislava</th>
<th>Canicola</th>
<th>Grippotyphosa</th>
<th>Hardjo</th>
<th>Icterohaemorrhagiae</th>
<th>Pomona</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:25</td>
<td>20 (6.7%)</td>
<td>0 (0%)</td>
<td>3 (2.6%)</td>
<td>3 (8.1%)</td>
<td>42 (8.1%)</td>
<td>9 (1.6%)</td>
</tr>
<tr>
<td>1:50</td>
<td>12 (2.6%)</td>
<td>0 (0%)</td>
<td>6 (1.6%)</td>
<td>5 (1.6%)</td>
<td>3 (0.9%)</td>
<td>2 (0.5%)</td>
</tr>
<tr>
<td>1:100</td>
<td>4 (0.7%)</td>
<td>0 (0%)</td>
<td>6 (1.2%)</td>
<td>11 (2.5)</td>
<td>1 (0.2%)</td>
<td>3 (0.7%)</td>
</tr>
<tr>
<td>1:200</td>
<td>3 (0.5%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>20 (5.2)</td>
<td>0 (0.1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>1:400</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (0.5)</td>
<td>0 (0.1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>1:800</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (0.2)</td>
<td>0 (0.1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>39 (6.7%)</td>
<td>0 (0%)</td>
<td>15 (2.6%)</td>
<td>42 (8.1%)</td>
<td>22 (3.8%)</td>
<td>9 (1.6%)</td>
</tr>
</tbody>
</table>
were again washed in PBS before the addition of ProLong Gold antifade (Molecular Probes) mounting media per slide and covered with a 24 × 50 coverslip. Samples were viewed using a Nikon Eclipse E800 and images captured using Nikon Elements Software.

2.8. Statistical analyses

Cohen’s Kappa was performed on FAT and culture samples to determine the observed proportion of agreement of both positive and negative results from the same set of six hundred urine samples (Agresti, 2003).

3. Results

3.1. Detection of leptospires in bovine urine

Six hundred urine samples were cultured for the presence of leptospires, of which twenty-three (3.8%) urine samples were culture positive. Of these, thirteen were culture positive in both media types; one sample was culture positive only in PDMS while nine were culture positive only in T80/40/LH media. Cultures became positive from 2 to 16 weeks post-inoculation (median = 4 weeks).

Out of six hundred urine samples, thirty-three (5.5%) were considered to be positive by the FAT. Samples were only considered FAT positive if reactive antibody conformed to the morphology expected of a leptospire, Fig. 1 (arrow). Reactive debris only, without corresponding morphology as illustrated in Fig. 1 (triangle), was considered negative.

Cumulatively, forty-three of six hundred urine samples were either culture positive or FAT positive, indicating that 7.2% of sampled beef cattle were actively excreting leptospires in urine. Of the forty-three positive samples, thirteen were both culture and FAT positive. Ten of forty-three positive urine samples were culture positive but FAT negative, and conversely, 20 were FAT positive but culture negative, Fig. 2A. The observed proportion of agreement between FAT and culture for both positive and negative results was calculated to be 95% using Cohen’s Kappa test. The probability that positive results from both FAT and culture would occur randomly was very low (0.2%), indicating strong association between them.

Of 1200 inoculated culture tubes, twenty-five were considered contaminated. In four samples, both PDMS and T80/40/LH media were contaminated. In two samples, only the PDMS media was contaminated and in 15 samples, only the T80/40/LH media was contaminated.

3.2. Serology

Given that the MAT was being used to determine levels of exposure to representative pathogenic leptospires, and not to diagnose acute disease, a starting dilution of 1:25 for sera was used (Arent et al., 2013; Benkirane et al., 2016). Of 580 sera tested, 116 (20%) had an MAT of 1:25 or greater against 1 or more serovars of Leptospirosis, Table 1. In our panel, the most reactive serogroup was Sejroe (8.1%), followed by Australis (6.7%), Icterohaemorrhagiae (3.8%), Grippotyphosa (2.6%), Pomona (1.6%) and Canicola (0.0%). Only nine of 580 sera samples were reactive to more than one serovar, Table 2. Out of 116 sera samples positive by MAT at 1:25 or higher, twenty-five were positive by culture or FAT, Fig. 2B. Four culture-positive urine samples came from animals that had no detectable MAT titer. Fourteen FAT-positive urine samples came from animals that had no detectable MAT titer.

Sera samples from cattle that were culture or FAT positive, but MAT negative, were retested by MAT using one of the newly cultured bovine isolates, i.e. cultured from one of the urine samples, but all such sera samples remained MAT negative.

3.3. Typing of leptospires

All newly cultured bovine isolates of leptospires presented high agglutination titers to reference sera specific for serogroup Sejroe. None of the isolates demonstrated any agglutination to any of other reference antisera indicating that all were typed as serogroup Sejroe. Phylogenetic analysis of 16S rDNA and secY from each isolate indicated that all were typed as L. borgpetersenii which aligned most closely with serovar Hardjo type bovis, Supplementary Figs. 1 and 2.

3.4. Evaluation of virulence

No experimentally infected hamsters showed clinical signs of infection or weight loss after inoculation with L. borgpetersenii. All kidneys from hamsters experimentally infected with isolate TC129 were positive by culture and immunohistochemistry at 8 days post-infection, Fig. 3A. All kidneys from hamsters experimentally infected with isolate TC273 were positive by culture and immunohistochemistry at 3 weeks post-infection, Fig. 3B. At 3 weeks post-infection, sera samples were positive by MAT at 1:800 against serogroup Sejroe. All livers from experimentally infected hamsters were culture negative.

4. Discussion

In order to obtain isolates of Leptospira associated with renal colonization in cattle, a cross sectional study was performed on urine samples collected from a single abattoir facility over the course of one year. Bovine urine samples were collected directly from the bladder by syringe and needle, and within minutes, three to four drops of urine were used to inoculate media. Out of 600 urine samples tested, 23 were culture positive. All 23 isolates were identified as L. borgpetersenii serovar Hardjo type bovis. Two of these isolates (designated TC129 and TC273) were tested in the golden Syrian hamster model of infection and, as with previous isolates of L. borgpetersenii serovar Hardjo type bovis, and similar to persistent infection in cattle, animals remained asymptomatic despite the fact that leptospires colonized renal tubules (Zuerner et al., 2012).

Analysis of the genome of L. borgpetersenii indicates that it is smaller.

Table 2

Bovine sera samples that were reactive with more than one serovar (N = 9). The complete MAT panel for those 9 bovine sera that were reactive to more than one serovar is provided.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Bratislava</th>
<th>Canicola</th>
<th>Grippotyphosa</th>
<th>Hardjo</th>
<th>Icterohaemorrhagiae</th>
<th>Pomona</th>
<th>Culture/FAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>63</td>
<td>1:100</td>
<td>–</td>
<td>1:25</td>
<td>–</td>
<td>–</td>
<td>1:100</td>
<td>–/–</td>
</tr>
<tr>
<td>115</td>
<td>1:25</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1:400</td>
<td>–</td>
<td>+/+</td>
</tr>
<tr>
<td>190</td>
<td>1:25</td>
<td>–</td>
<td>1:100</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–/+</td>
</tr>
<tr>
<td>258</td>
<td>–</td>
<td>–</td>
<td>1:50</td>
<td>–</td>
<td>1:100</td>
<td>–</td>
<td>–/+</td>
</tr>
<tr>
<td>308</td>
<td>1:25</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1:25</td>
<td>–</td>
<td>–/+</td>
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<tr>
<td>312</td>
<td>1:50</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1:25</td>
<td>–/+</td>
</tr>
<tr>
<td>322</td>
<td>1:50</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1:25</td>
<td>–</td>
<td>–/+</td>
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<td>–</td>
<td>1:25</td>
<td>–</td>
<td>–/+</td>
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<tr>
<td>542</td>
<td>1:200</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1:25</td>
<td>1:50</td>
<td>–/+</td>
</tr>
</tbody>
</table>
Hardjo (Hotka et al., 2007). Increased sensitivity of the MAT panel can be commended for the most sensitive detection of exposure to serovar Hardjo strain Hardjoprajitno as retainting antibodies by the MAT, Fig. 2B. Our MAT panel used persistently colonize renal tubules in the absence of detectable agglutinating antibodies by the MAT. Fig. 2B. Our MAT panel used L. interrogans serogroup Sejroe serovar Hardjo strain Hardjoprajitno as recommended for the most sensitive detection of exposure to serovar Hardjo (Hotka et al., 2007). Increased sensitivity of the MAT panel can sometimes be accomplished when local isolates are used (Pinto et al., 2015). Similar to previous analyses of bovine sera (Libonati et al., 2017) and when the MAT was repeated using one of our newly acquired isolates, no reactivity was detected in samples from animals that were culture positive but serologically negative.

Multiple assays are required to detect active excretion of leptospires in urine samples (Wagenaar et al., 2000). In our study, several samples were culture positive but FAT negative. This was interpreted as culture requiring fewer numbers of leptospires to have a positive result. Conversely, when samples were FAT positive but culture negative, this was interpreted as a limitation of the media for satisfying the growth requirements of these fastidious bacteria. The exacting nature of culturing leptospires from primary host tissue of naturally infected animals is well recognized and several media types are often used, which contain multiple variables e.g. differing concentrations of 5-FU, nalidixic acid, and fatty acids (Ellis et al., 1985; Rajeev et al., 2014). However, our culture attempts were limited to two media types inoculated with only 3–4 drops of urine: in contrast, FAT was performed on concentrated urine volumes that were originally as high as 35 ml. Cultured isolates are similar to the most comprehensive prevalence study of bovine leptospirosis in the U.S. which attempted culture from over 5000 kidneys and recovered 88 isolates of Leptospira: of these, 73 were serovar Hardjo, 11 were serovar Pomona and 4 were serovar Grippotyphosa (Miller et al., 1991). However, it is important to note that our results are confined to one single abattoir facility and should not be used to extrapolate which serovars are being excreted in bovine urine samples in other parts of the country. Understanding the changing epidemiology of bovine leptospirosis is dependent on studies that identify those serovars associated with infection, not by serology, but by culture (Pinto et al., 2017). The major risk factors for Hardjo infection in cattle are open herds, co-grazing with sheep, access to contaminated water sources, use of natural service and herd size. Previous seroprevalence studies confirm that cattle are exposed to pathogenic leptospires. In addition, they also confirm that a majority of MAT positive bovine sera samples (65% at MAT of ≥1:100) react with more than one serovar in the MAT (Miller et al., 1991; Talpada et al., 2003). This is in direct contrast to our results in which only 9/580 (1.6%) of sera samples (at an MAT of ≥1:25) reacted to more than one serovar, Table 2. This is likely a function of our sample population comprising cattle < 30 months of age. It also implies that these animals were not vaccinated against leptospirosis. Since the use of the MAT was directed at identifying exposure and not active infection, sera samples were tested at an initial dilution of 1:25 (Arent et al., 2013; Benkirane et al., 2016). Our results confirm exposure to serogroup Sejroe in 8.1% of cattle tested. Surprisingly, 6.7% of samples also indicated exposure of a large number of cattle to serogroup Australis serovar Bratislava. The source of serovar Bratislava is not clear; it is rarely isolated in the U.S. whereas in Europe, it has been identified in horses, pigs and various wildlife sources (Arent et al., 2016; Milas et al., 2013). Exposure is not indicative of infection but given that current bovine bacterin vaccines in the U.S. do not contain serovar Bratislava, this needs to be explored further.

Cattle are recognized as a reservoir host for serovar Hardjo which may be classified as L. interrogans serovar Hardjo type prajitno or L. borgpetersenii serovar Hardjo type bovis. While multiple seroprevalence studies confirm exposure of cattle to leptospires, few studies actually culture the strains involved, a necessary requirement to confirm active excretion and to make isolates available for phenotypic and genotypic comparison. Analysis of 16S rDNA and secY coding sequences indicates that our newly obtained isolates align most closely with that of L. borgpetersenii serovar Hardjo type bovis strain JB197 and L. borgpetersenii serovar Hardjo type bovis strain L550, Supplementary Figs. 1 and 2. Given that strain JB197 was originally isolated in cattle more than 25 years ago in the U.S., and that strain L550 was a human isolate in Australia, our results highlight the ubiquitous, and conserved, nature of serovar Hardjo as a global disease (Bulach et al., 2006; Miller et al., 1991). Strains JB197 and L550 are considered identical when typed by serology, and they have a nearly identical gene content; yet they cause different clinical diseases in experimentally infected hamsters (Bulach et al., 2006). In order to further understand pathogenic mechanisms of
L. borgpetersenii, it will be necessary to assess not just gene content, but gene expression pathways that modulate protein and lipopolysaccharide expression during disease (Nally et al., 2017).

5. Conclusion

Collectively, our results indicate that 7.2% of beef cattle were positive for active excretion of pathogenic leptospires. Multiple assays are required to detect urinary excretion of whole leptospires. The availability of 23 new isolates of L. borgpetersenii serovar Hardjo provides for the opportunity to determine how these serovars have evolved over the last 30 years as a cause of renal colonization in cattle by comparative genomic, transcriptomic and proteomic analyses. Results also highlight beef cattle as a reservoir host for the potential zoonotic transmission of leptospires.

Conflicts of interest

None.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: https://doi.org/10.1016/j.vetmic.2018.03.023.

References