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Seroprevalence and renal carriage of pathogenic *Leptospira* in livestock in Cotonou, Benin

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Abstract

Background: Leptospirosis is a zoonotic disease. It is particularly prevalent in tropical countries and has major consequences for human and animal health. In Benin, the disease's epidemiology remains poorly understood, especially in livestock, for which data are lacking.

Objectives: To characterise *Leptospira* seroprevalence and locally circulating serogroups in livestock from Cotonou and to estimate the prevalence of *Leptospira* renal carriage in cattle.

Methods: We conducted a cross-sectional study in February 2020 during which livestock were sampled at an abattoir and in an impoverished city district. We analysed blood samples from 279 livestock animals (i.e. cattle, sheep, goats and pigs) using the microscopic agglutination test. Additionally, samples of renal tissue from 100 cattle underwent 16s rRNA (*rrs*) real-time PCR analysis.

Results: For the 131 cattle, 85 sheep, and 50 goats tested, seroprevalence was 18% (95% confidence interval [CI] [12%, 26%]), 9% (95% CI [4%, 17%] and 2% (95% CI [0%, 9%]), respectively, and most of the seropositive animals were associated with 1:100 titres. All 13 pigs were seronegative. *Leptospira* DNA was found in the renal tissue of 10% (95% CI [5%, 18%]) of the cattle tested ($n = 100$). *Leptospira borgpetersenii* was the main species present ($n = 7$), but *Leptospira interrogans* ($n = 2$) and *Leptospira kirschneri* ($n = 1$) were also detected. Various serogroups (Canicola, Grippotyphosa, Sejroe, Icterohaemorrhagiae, Pomona, Pyrogenes, Australis and Autumnalis) were detected using microscopic agglutination test without a clear predominance of any of them.

Conclusions: These results suggest that abattoir workers and people living in close contact with livestock in poor urban areas are exposed to the risk of *Leptospira* infection.

KEYWORDS
abattoir, Akaike's information criterion, leptospirosis, microscopic agglutination test, PCR, slum, West Africa, zoonosis

1 | INTRODUCTION

Leptospirosis is one of the world's most widespread zoonotic bacterial diseases, and it has a significant impact on both human and animal health (Ellis, 2015; Levett, 2015). The disease's etiological agents are spirochetes in the genus *Leptospira*, which comprises over 250 pathogenic serovars organised into at least 32 serogroups (Caimi & Ruybal, 2020). At present, the genus contains 69 phylogenetically defined species, including 41 that are pathogenic (Fernandes et al., 2022; Vincent et al., 2019). *Leptospira* bacteria can infect multiple hosts. Some serovars are regionally endemic and predominant in certain wild mammals (e.g. rodents) and domestic mammals, and there may be selective carriage of some *Leptospira* strains (Thiermann, 1981). *Leptospira* infections are frequently chronic in ruminants and pigs, with transient bacteraemia giving way to the prolonged colonisation of the kidney or uterus (Loureiro & Lilenbaum, 2020). Consequently, carriers are likely to transmit leptospires to other animal species, including humans, either directly, via contact with contaminated urine, or indirectly, via contact with water or soil contaminated by urine. The clinical signs of leptospirosis vary among species, from severe disease (e.g. in dogs) to reproductive failure (e.g. in pigs and ruminants) to subclinical disease or even the absence of clinical signs (e.g. in Norway rats) (Ellis, 2015; Loureiro & Lilenbaum, 2020; Schuller et al., 2015; Thiermann, 1981). This spectrum is also seen within humans, in whom the consequences of leptospirosis may range from asymptomatic infection to renal failure and death (Ashford et al., 2000; Levett, 2001).

Globally, it has been estimated that leptospirosisannually results in 1 million cases of disease and 59,000 deaths in humans, with most (73%) occurring in tropical regions (Costa et al., 2015). A model developed by Costa et al. (2015) has suggested that Benin is among the top 10 countries most impacted by leptospirosis, given its high degree of estimated disease incidence (as many as 2800 cases and 200 deaths per year) compared to the other 29 stratum D countries identified by the World Health Organisation (WHO). Recent studies have shown that pathogenic leptospires are broadly circulating in Cotonou, Benin's largest city and economic capital, with prevalence ranging from 12.9% to 18.9% in rodents (Houéménou et al., 2013, 2019, 2021) and seroprevalence reaching 54.7% in abattoir workers ($n = 503$) (Koundé & Zohoun, 1994).

Past work in various African countries has highlighted that abattoir workers are at higher risk of *Leptospira* infection (Alinaitwe et al., 2019; Dreyfus et al., 2014; Ezeh et al., 1991; Onyemelukwe, 1993). Another at-risk population is the inhabitants of inner-city neighbourhoods, particularly because the living conditions associated with poverty promote contact between people and rats (chiefly *Rattus norvegicus*) as well as disease transmission by the latter (Himsworth et al., 2013; Lau et al., 2010; Reis et al., 2008). Yet, commensal rodents are not the only animals to occur in such areas. Recent studies, including some in Benin, have hypothesised that the free-ranging livestock frequently found in these neighbourhoods could also play a prominent role in transmitting pathogenic *Leptospira* (Houéménou et al., 2019; Reis et al., 2008). It is necessary to characterise *Leptospira* circulation among domestic animals, including livestock, to have a comprehensive understanding of the public health risks; data of this type are lacking for Benin.

To characterise past or present *Leptospira* infections in livestock, the World Organisation for Animal Health (WOAH) recommends using the microscopic agglutination test (MAT) on blood samples from a subset of animals (WOAH (founded as OIE), 2018). However, at the individual level, the MAT has a limited ability to detect infected animals (low sensitivity) and to identify the infecting serogroups (low serogroup specificity) (Chappel et al., 2004; WOAH (founded as OIE), 2018). In contrast, direct methods, such as PCR, can characterise the *Leptospira* species circulating in individuals or populations (Ferreira et al., 2014). Here, we used both methods on samples obtained at an abattoir and in Ladji, an impoverished inner-city district of Cotonou. The objectives of the study were (1) to estimate the *Leptospira* seroprevalence and describe the serogroup distribution in cattle, goats, sheep and pigs from two locations at Cotonou, Benin, (2) to investigate the relationship between some factors and the seropositivity and (3) to estimate the prevalence of *Leptospira* renal carriage and describe the diversity of *Leptospira* species in slaughtered cattle.

2 | RESEARCH METHODS AND DESIGN

2.1 | Ethical considerations

The fieldwork was conducted in Benin in accordance with the procedure approved by an Ethics Committee (agreement n°2349). Abattoir veterinary authorities, local government authorities and livestock owners consented to the sampling procedure beforehand and at the time of sample collection. The informed owner consent was obtained using door-to-door to explain the study objectives and request access to cattle, goats, sheep and pigs, which were found within households.

2.2 | Study design

In February 2020, we conducted a cross-sectional study at two sampling sites in Cotonou, Benin (Figure 1). Cotonou lies along the coast, with the Atlantic Ocean to the south and Lake Nokoué to the north. This part of Benin has a subequatorial climate and a dense hydrographic network; more than 60% of the city and its suburbs may remain partly flooded for up to two months each year.

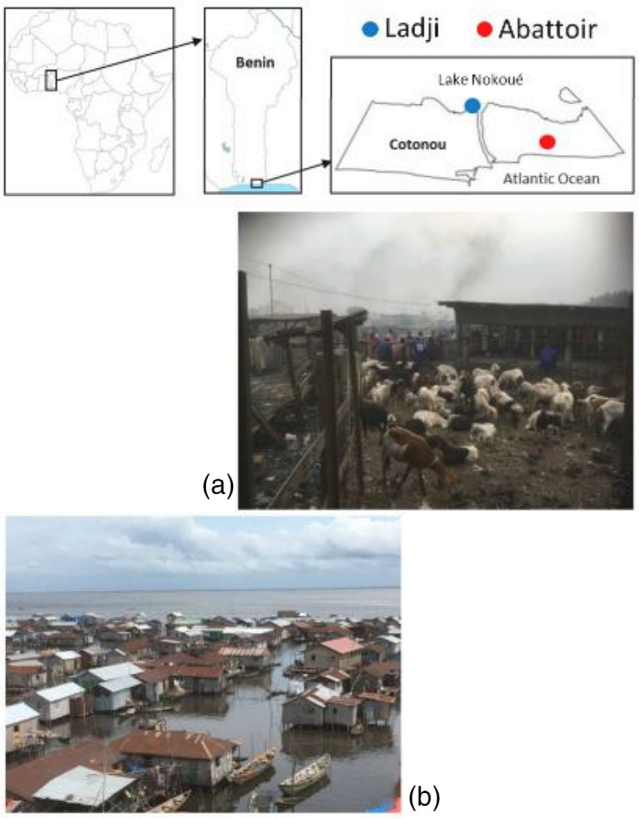

FIGURE 1 The two sampling sites. Above: map showing the sites' locations. Below: Cotonou abattoir – (A) livestock pens. Ladji district – (B) houses on solid ground vs. stilts.

The first sampling site was the municipal abattoir of Cotonou. It was expected that useful data could be obtained from such facilities, which process large numbers of animals of various species and geographical origins on a daily basis (Figure 1A). Each day, the Cotonou abattoir slaughters about 30 cattle and 15 pigs as well as 100 goats and 100 sheep. The animals are brought to the site by independent traders, who purchase the livestock individually or in herds from farmers in certain regions of Benin.

The second sampling site was Ladji, an impoverished district of Cotonou. People live both along the lake, in houses built on solid ground, as well as above the lake, in pile dwellings (Figure 1B). Ladji is densely populated. The area has largely been developed without any formal urban planning, and basic public services are lacking. We chose to sample in this area because it contains free-ranging livestock, and preliminary research has detected *Leptospira* in rodents (Houéménou et al., 2019) and water sources (Houéménou et al., 2021).

2.3 | Animal selection

At the Cotonou abattoir, serum and kidney samples were taken from all ($n = 100$) cattle that were slaughtered over 3 days in February 2020. The sampling size was compatible with the *Leptospira* prevalence estimate including a precision of 10% and a confidence of 95% (Humphry et al., 2004). Additionally, serum samples were taken from the goats, sheep and pigs that were also being processed. For the latter animals, the slaughter process was faster, and animals were selected based on the ability of the researchers to collect and trace the samples. If traceability conditions were not met, the animal was not sampled. Therefore, they were selected conveniently for the serum sampling. The kidney tissue was not collected because of logistical constraints related to the slaughter line and the inability to ensure the traceability. Information on the animals' owners and geographical origins was unavailable because there were no formalised or reliable records for the animals.

In Ladji, cattle belonging to several owners were grouped together. Cattle were kept in a single enclosure at night and were left to freely forage within the district during the day. At the time of sampling, 40 cattle were present, and it was possible to sample 31 cattle across 2 separate days in February 2020. Overall, serum samples were collected from 19 goats, 10 sheep and 2 pigs.

The sex and age of all the animals sampled were determined based on their degree of sexual dimorphism and morphological traits (i.e. size and appearance).

2.4 | Sample collection

At the abattoir, soon after slaughter for each animal, approximately 10 mL of blood was collected using a plain vacutainer tube. The tubes were labelled with a code that was specific to each individual, and they were left in a tilted position for 2–4 h at room temperature to allow for clotting. Then, the samples were centrifuged (3000 *g* for 20 min). The resulting sera were stored at −20°C until the samples were transported to the laboratory and analysed with MATs.

In the case of the kidney samples, three 1-cm^3 cubes of tissue were aseptically collected from the corticomedullar area of each animal using a scalpel blade (no. 22). They were stored at room temperature in a 5-mL screw-cap tube containing 1.5 mL of 100% ethanol. The latter was removed the day before shipping.

In Ladji, blood collected for the prophylaxis program was used in this study for further investigation of *Leptospira* infection and exposure.

2.5 | Serological testing

MATs were carried out in accordance with WOAH standards (WOAH (founded as OIE), 2018). A panel of 22 antigens was used that represented ubiquitous serovars; the log2 dilution series ranged from 1:100 to 1:6400. Specifically, the MATs tested for the presence of antibodies directed against the following *Leptospira* serogroups (specific serovars in brackets): Australis (Munchen, Australis, Bratislava), Autumnalis (Autumnalis, Bim), Ballum (Castellonis), Bataviae (Bataviae), Canicola (Canicola), Grippotyphosa (Grippotyphosa, Vanderhoedoni), Icterohaemorrhagiae (Icterohaemorrhagiae, Copenhageni), Panama (Panama, Mangus), Pomona (Pomona, Mozdok), Pyrogenes (Pyrogenes), Sejroe (Sejroe, Saxkoebing, Hardjo, Wolffi) and Tarassovi (Tarassovi) (Supplementary file 1). This panel includes 14 of the 16

serogroups that have been described in ruminants or pigs in Africa (de Vries et al., 2014). The two other serogroups, Celledoni and Mini, are more locally distributed and correspond to less than 1% and 8% of the serogroups seen in seropositive small ruminants, cattle and pigs, respectively. The Shermani serogroup was also observed in 2.6% of seropositive cattle in another study (Alinaitwe et al., 2020). Given their low levels of occurrence, the latter three serogroups were not included in the panel.

Sera with titres ≥1:100 against any *Leptospira* serovar were considered positive, as recommended by the WOAH (WOAH (founded as OIE), 2018). The predominant serogroup was then defined based on the maximum titre directed against a given serovar, which needed to be twofold higher than that of any other titre (Chappel et al., 2004). Otherwise, MAT results were categorised as co-agglutinations. Therefore, we determined the putative serogroup infecting an individual when the maximum titre was sufficiently high. In other cases, we considered co-agglutinations to the panel of serogroups and interpreted it at the population level.

2.6 | DNA extraction and real-time PCR

Each subsample of renal tissue was aseptically homogenised using a syringe. A small amount of the result (~25 mg) was incubated with 180 µL of ATL buffer and 25 µL of proteinase K (QIAamp, Qiagen, Courtaboeuf) for 3 h. Following protein digestion, the DNA was extracted from 200 µL of lysed tissue using a Nucleospin Tissue Kit (QIAamp, Qiagen, Courtaboeuf) in accordance with the manufacturer's instructions. All DNA samples were stored at −20°C until further processing.

The presence of *Leptospira* DNA in renal tissue was assessed using a *Leptospira* TaqMan Real-Time PCR Kit (AgPath-ID One-Step RT-PCR Reagents, Life Technologies), which targets a specific region of the *Leptospira* 16S rRNA (*rrs*) gene (Waggoner et al., 2014).

The 16S-positive samples were subsequently tested using species-specific assays (total of four probe/primer sets) for *Leptospira interrogans*, *Leptospira noguchii*, *Leptospira borgpetersenii* and *Leptospira Kirschneri* (29). The amplification reactions were optimised by exploring the best probe/primer ratios using the Real-Time PCR Kit (AgPath-ID One-Step RT-PCR Reagents, Life Technologies). The Mx3000P Real-Time PCR System (Agilent Technology) was used for all the assays. Each reaction was conducted in a total volume of 25 µL, which consisted of 2× RT-PCR Buffer; 400 or 300 nM of each primer; 400, 120 or 150 nM of TaqMan probe; and 4 µL of DNA template solution. Amplification conditions were as follows: 95°C for 10 min, 40 cycles of 15 s at 95°C, and 1 m at the probe's optimal annealing temperature. Each PCR run included positive and negative controls. The positive controls were DNA from the reference strains *L. interrogans* serogroup Icterohaemorrhagiae serovar 19, *L. borgpetersenii* serogroup Sejroe serovar Sejroe, and *L. kirschneri* serogroup Grippotyphosa serovar Grippotyphosa; the negative controls were water. Samples were considered to be positive if Ct <40, as per the manufacturer's instructions (RT-PCR system).

2.7 | Data analysis

We determined seroprevalence for each livestock species by dividing the number of seropositive animals by the total number of animals tested using MATs. We determined the prevalence of *Leptospira* renal infection by dividing the number of cattle for which the renal tissue was tested positive by the total number of cattle tested via PCR. Exact distribution with the mid *p*-value was used to calculate the 95% confidence intervals (CIs) (Agresti & Coull, 1998) using SAS (SAS Institute Inc. 2012. SAS/STAT Software, Version 9.4. Cary, NC). These prevalence and seroprevalence estimates were expressed in percentages (%), which contrasts with the probabilities used in the seropositivity analyses below, expressed in proportions.

We then explored three factors – sex, sampling site and livestock species – that could impact an individual's probability of being seropositive. We only included livestock species for which there were at least two seropositive individuals, which were the cattle and sheep. We excluded age because few young animals (9 cattle and 3 sheep) were sampled (Table 1). We used Firth logistic regression models (PROC LOGISTIC in SAS; SAS Institute Inc. 2012. SAS/STAT Software, Version 9.4. Cary, NC.) (Firth, 1993). We ran all the models combining the three factors and their two-way interactions (i.e. total of 18 models). Following the information-theoretic approach described in Burnham and Anderson (2002), we carried out model selection based on Akaike's information criterion (AIC): the smaller the criterion, the better the model. To help evaluate the fit of each model, we also calculated the Akaike weights as well as the difference in AIC (ΔAIC) between a given model and the model with the lowest AIC score. Additionally, we calculated the relative importance weight of each factor, defined as the sum of the Akaike weights from all the models containing that factor. Interestingly, the information-theoretic approach can be used to make inferences about several models when model selection cannot identify a single best model. To do so, it averages parameter estimates over the models deemed to be important. As a rule of thumb, 'All models with a ΔAIC value <2 are all likely to be the best model, and hence, they should all be used when making further inferences. Models with a ΔAIC value in the range 4–7 are less likely to be best but probably should not be discounted. Models with a ΔAIC value >10 are extremely unlikely to be the best model and can be ignored'.(Richards, 2005).That said, a recent review has highlighted that model averaging has some unresolved statistical concerns (Dormann et al., 2018). Consequently, we present the estimate of the probability of being seropositive (and its approximate unconditional CI, see Equation [4] in Burnham & Anderson, 2004) based on model averaging over the entire set of 18 models as well as the 'individual' estimates from the four top-ranked models.

To provide context for our results, we also investigated the differences between estimated prevalence in this study vs. in other studies. To this end, we calculated the 95% CI of the difference proposed by Agresti-Caffo (hereafter, 95% AC CI) (Agresti & Caffo, 2000). This simple CI has been found to perform satisfactorily in simulations (Fagerland et al., 2015).If the 95% AC CI contained 0, the two estimates of prevalence were not considered to be significantly different with an alpha level of 0.05.

TABLE 1 Number of animals sampled from two locations (abattoir and Ladji district of Cotonou) in 2020, tested using microagglutination test and Leptospira seropositive by livestock species, sampling site, age and sex.

	Abattoir					Ladji					
	N tot	Adult	Young	Female	Male	*N* tot	Adult	Young	Female	Male	Total
Cattle	100 (18)	100 (18)	0	9 (1)	91 (17)	31 (6)	22 (4)	9 (2)	27 (5)	4 (1)	131 (24)
Sheep	75 (5)	73 (4)	2 (1)	72 (5)	3 (0)	10 (3)	5 (3)	5 (0)	6 (3)	4 (0)	85 (8)
Goats	31 (0)	30 (0)	1 (0)	24 (0)	7 (0)	19 (1)	11 (1)	8 (0)	13 (0)	6 (1)	50 (1)
Pigs	11 (0)	10 (0)	1 (0)	0	11 (0)	2 (0)	2 (0)	0	2 (0)	0	13 (0)

Note: *N* tot is the total number of animals tested. The number of seropositive animals is in brackets. There were seropositive animals within all the ruminant species, and seroprevalence was greatest in cattle: 18% (95% CI [12%, 26%]) (Table 2). All 13 pigs were seronegative.

TABLE 2 Leptospira seroprevalence estimates (%) and the related 95% Confidence interval (CI) according to livestock species from two locations in Cotonou, in 2020.

	Seroprevalence (%)	$_{95\%}$CI
Cattle	18	[12–26]
Sheep	9	[4–17]
Goats	2	[0–9]
Pigs	0	[0–21]

Note: The seroprevalence estimates and 95% confidence intervals in square brackets are expressed in percentage (%).

3 | RESULTS

3.1 | Serum sampling and seroprevalence

Across the two sampling sites, we tested the serostatus of 279 animals that differed in species, age and sex (Table 1). Overall, the sample was markedly unbalanced. The sites differed in their relative numbers of animals: Ladji had more cattle, fewer pigs and smaller sample sizes in general. Across the board, adults were more frequently sampled. The same was true for females, except in the case of cattle and pigs at the abattoir.

3.2 | Information-theoretic approach

3.2.1 | Model selection

Model selection could not single out a best model among all 18 models fitted (see Supplementary file 2 for the characteristics of every models). The model with the lowest AIC score contained sampling site and sex in an additive way (Model 1: AIC = 155.36; $k = 3$; Akaike weight = 0.20; Table 3). Model 1 indicated that males were more likely than females to be seropositive, as were animals in Ladji compared to animals at the abattoir. However, three other models (Models 2–4) were within two AIC units (max ΔAIC = 0.61) of Model 1 and had similar Akaike weights (0.20, 0.17 and 0.15, respectively) which means that these models were thus similar in their ability to describe the data. Furthermore, Models 1–3 were all simplified versions of Model 4, and their AIC-based ranks echoed their ranks based on parameter number. This pattern is characteristic of sparse data sets and suggests that Model 4 may well be the model that best describes the data, with its relatively high number of parameters (5 vs. 3) having hindered then its ranking as the best model. Given the uncertainty around model selection, we worked out both the probability of being seropositive for each four top-ranked models (Table 3) and the model-averaged estimates across all the models (Table 4).

3.2.2 | Factors and estimates of probability of being seropositive

Sampling site was the factor most strongly supported by the data, as shown by its relatively large Akaike weight (0.97) and its presence in all top 10 models ranked by AIC. Among the seven model-averaged estimated probabilities of being seropositive, two allowed a comparison between Ladji and the slaughterhouse with reasonable precision. This comparison indicated that female cattle were more likely to be seropositive in Ladji than at the slaughterhouse (0.21 [0.09; 0.44] and 0.09 [0.02; 0.38], respectively). The two other comparisons led to the same conclusion, though their level of precision was lower (Table 4). There was less support for factors sex and species (relative importance weight of 0.76 and 0.74, respectively). Both factors were present in 8 of the top 10 models ranked by AIC. There was a pronounced difference in the probability of being seropositive between males and females at the abattoir. For the cattle, whose model-averaged estimates were relatively precise, the estimates for males and females were 0.18 (95% CI [0.12, 0.28]) and 0.09 (95% CI [0.02, 0.38]), respectively. For the sheep, whose model-averaged estimates were less precise, these values were 0.17 (95% CI [0.02, 0.64]) and0.06 (95% CI [0.03, 0.15]), respectively. The same qualitative conclusion was reached based on estimates from the three top-ranked models in which sex was present. Results for species were less clear-cut, and we defer this point to the discussion.

3.3 | Serogroup distributions

The seropositive animals were 24 cattle, 8 sheep and 1 goat. These results were based on titres between 1:100 and 1:400 (Table 5). Most

TABLE 3 Factors influencing the estimated probability of being Leptospira seropositive in cattle and sheep from abattoir and Ladji district (Cotonou, 2020) and its conditional 95% confidence interval [CI], from the four top-ranked models with the lowest AIC scores.

			Factors and/or interactions included in the models			
Animal species	Location	Sex	Sex Location	Location Animal species Location × animal species	Sex Location Animal species	Sex Location Animal species Location × animal species
Cattle	Abattoir	F	0.06 [0.03–0.14]	0.18 [0.12–0.27]	0.03 [0.01–0.15]	0.09 [0.02–0.37]
Cattle	Abattoir	M	0.19 [0.12–0.28]	0.18 [0.12–0.27]	0.19 [0.12–0.28]	0.20 [0.13–0.29]
Cattle	Ladji	F	0.26 [0.13–0.45]	0.20 [0.08–0.41]	0.22 [0.10–0.43]	0.19 [0.07–0.40]
Cattle	Ladji	M	0.55 [0.25–0.82]	0.20 [0.08–0.41]	0.66 [0.29–0.91]	0.37 [0.07–0.82]
Sheep	Abattoir	F	0.06 [0.03–0.14]	0.06 [0.02–0.14]	0.07 [0.03–0.15]	0.06 [0.02–0.14]
Sheep	Abattoir	M	0.19 [0.12–0.28]	0.06 [0.02–0.14]	0.34 [0.10–0.70]	0.14 [0.02–0.54]
Sheep	Ladji	F	0.26 [0.13–0.45]	0.58 [0.19–0.89]	0.39 [0.14–0.72]	0.58 [0.19–0.89]

TABLE 4 Model-averaged estimated probabilities of being Leptospira seropositive (unconditional 95% confidence intervals) in cattle and sheep from two locations (abattoir and Ladji district in Cotonou) in 2020.

Category	Estimate [95% CI]
Females – Cattle – Ladji	0.21 [0.09, 0.44]
Females – Cattle – Abattoir	0.09 [0.02, 0.38]
Males – Cattle – Ladji	0.49 [0.08, 0.91]
Males – Cattle – Abattoir	**0.18 [0.12, 0.28]**
Females – Sheep – Ladji	0.43 [0.10, 0.83]
Females – Sheep – Abattoir	**0.06 [0.03, 0.15]**
Males – Sheep – Abattoir	0.17 [0.02, 0.64]

Note: In bold are the estimates with the greatest precision.

sera were associated with a maximum titre of 1:100 ($n = 18$) and three with a maximum titre of 1:400. The latter were detected only in cattle.

In the cattle, 14 MAT profiles suggested exposure to the following *Leptospira* serogroups: Canicola ($n = 5$), Grippotyphosa ($n = 3$), Sejroe ($n = 2$), Icterohaemorrhagiae ($n = 1$), Australis ($n = 1$), Pomona ($n = 1$) and Pyrogenes ($n = 1$). In 10 MAT profiles, there were crossreactions directed against two to five of these serogroups. In cattle, most of the seropositive samples reacted against the serogroup Canicola ($n = 10$). Such reactions were observed at both sampling sites. In contrast, reactions against the serogroup Sejroe were only retrieved in cattle at the abattoir.

In the sheep, three MAT profiles suggested exposure to Grippotyphosa ($n = 2$) and Icterohaemorrhagiae ($n = 1$). In five other MAT profiles, there were crossreactions directed against two to four serogroups. Overall, the most commonly observed serogroups were Icterohaemorrhagiae ($n = 5$), Grippotyphosa ($n = 4$), Canicola ($n = 3$) and Pomona ($n = 3$); Grippotyphosa was only observed in sheep at the abattoir.

In the goats, the only MAT profile we obtained displayed crossreactions directed against Canicola, Pomona and Pyrogenes.

3.4 | *Leptospira* in cattle renal tissue

Among the 100 cattle studied, 10 individuals (10%, 95% CI [5%, 18%]) tested positive for *Leptospira* DNA via PCR. The analyses detected *Leptospira* DNA related to *L. borgpetersenii* ($n = 7$), *L. interrogans* ($n = 2$) and *L. kirschneri* ($n = 1$) (Table 6). Three of the PCR-positive samples were also seropositive (Supplementary file 3).

4 | DISCUSSION

To our knowledge, our study is the first to explore *Leptospira* prevalence, seroprevalence and diversity in livestock in Benin. Based on the kidney samples, 10% of cattle were infected with pathogenic *Leptospira* species, mainly *L. borgpetersenii*. This finding suggests that this livestock group could promote *Leptospira* maintenance and transmission. The levels of *Leptospira* seroprevalence in cattle and sheep indicate that *Leptospira* may circulate to a certain extent in livestock in this country. Our results shed light on *Leptospira* ecology and risk in Benin. The discussion mainly addresses the importance and distribution of *Leptospira* in cattle and sheep, given the small sample sizes for goats and pigs.

The tests used to estimate prevalence often have limitations due to their imperfect sensitivity (Se) or specificity (Sp) that may induce a bias compared to the true prevalence (Alinaitwe et al., 2020; Allan, 2016; Ellis, 2015). To date, in absence of Gold standard for leptospiral infection diagnosis in livestock, no study has provided sensitivity and specificity estimates for the MAT or PCR. To take this limit into account, we try to implement a latent class model which would have enabled us to estimate simultaneously the Se and Sp of the MAT and PCR and the true prevalence of leptospiral infection using a method described elsewhere (Lurier et al., 2021). However, the small number of PCR-positive animals impeded model convergence (data not shown). Additional data could help address this issue and studies on the sensitivity and specificity of diagnostic tests for leptospirosis are needed to better assess its prevalence and epidemiology.

TABLE 5 Serological titres, animal species and their location among the tested positive animals using microscopic agglutination test (MAT).

ID	Animal species	Location	Serological titres							
			AUS	AUT	CAN	GRI	IH	POM	PYR	SJ
A003	Cattle	Abattoir	0	0	1:400	0	0	0	0	1:200
A004	Cattle	Abattoir	0	0	1:200	0	0	0	0	0
A015	Cattle	Abattoir	0	0	0	1:100	0	0	0	0
A016	Cattle	Abattoir	0	0	1:200	0	0	0	0	0
A017	Cattle	Abattoir	0	0	1:100	0	0	0	0	0
A020	Cattle	Abattoir	0	0	1:100	0	0	0	0	0
A029	Cattle	Abattoir	1:100	0	1:100	0	1:100	1:100	1:100	0
A131	Cattle	Abattoir	0	0	0	0	0	0	0	1:400
A211	Cattle	Abattoir	0	0	1:100	0	0	0	0	1:100
A213	Cattle	Abattoir	0	0	0	0	1:100	0	0	0
A218	Cattle	Abattoir	0	0	0	1:200	0	0	0	0
A221	Cattle	Abattoir	0	0	0	0	0	1:100	0	1:100
A227	Cattle	Abattoir	0	0	0	1:100	0	0	0	1:100
A233	Cattle	Abattoir	0	0	0	0	0	0	0	1:200
A237	Cattle	Abattoir	0	0	0	0	0	0	1:200	0
A242	Cattle	Abattoir	0	0	0	1:100	0	0	0	0
A246	Cattle	Abattoir	1:100	1:100	0	0	0	1:100	0	0
A249	Cattle	Abattoir	0	0	0	0	0	200	0	0
L14	Cattle	Ladji	0	0	1:100	0	1:100	1:100	1:100	0
L20	Cattle	Ladji	1:100	0	0	0	0	0	0	0
L34	Cattle	Ladji	0	0	0	0	0	1:100	1:100	0
L38	Cattle	Ladji	0	0	1:100	0	0	0	1:200	0
L40	Cattle	Ladji	0	0	0	0	1:100	0	1:100	0
L42	Cattle	Ladji	0	0	1:100	0	0	0	0	0
L3	Goat	Ladji	0	0	1:100	0	0	1:200	1:200	0
A070	Sheep	Abattoir	0	0	0	0	1:100	1:200	0	0
A083	Sheep	Abattoir	0	0	0	1:400	0	0	0	0
A084	Sheep	Abattoir	0	0	0	1:100	0	0	0	0
A089	Sheep	Abattoir	0	0	0	1:100	1:100	1:200	0	0
A207	Sheep	Abattoir	0	0	1:100	1:200	0	1:100	0	0
L13	Sheep	Ladji	1:100	0	1:100	0	1:100	0	1:200	0
L49	Sheep	Ladji	0	0	1:100	0	1:100	0	0	0
L50	Sheep	Ladji	0	0	0	0	1:100	0	0	0

Note: Abbreviation of the *Leptospira* serogroups tested positive using a threshold of 1:100, Australis (AUS), Autumnalis (AUT), Canicola (CAN), Grippotyphosa (GRI), Icterohaemorrhagiae (IH), Pomona (POM), Pyrogenes (PYR) and Sejroe (SJ).

We found that 10% (95% CI [5%, 18%]) of cattle carried *Leptospira* in their renal tissue, which is likely an underestimate of prevalence because of the matrix and tissue sampling method used. Indeed, urine samples were not tested, but they may be positive when kidney samples are negative (Ayralet al., 2015; Allan, 2016; Alinaitwe et al., 2019). For instance, two abattoir surveys elsewhere in Africa found different PCR-based prevalence values depending on whether the metric was based on renal tissue only vs. renal tissue plus urine samples (7% vs. 8.6% [n = 453] in Tanzania; 7.2% vs. 8.8% [n = 500] in Uganda) (Alinaitwe et al., 2019; Allan, 2016). Regardless, it is important to note that the prevalence estimated in our study was not significantly different from the prevalence estimated from the renal tissue only, in the Tanzanian and Ugandan studies (for both comparisons – 95% AC CI: [−0.03, 0.10]). Furthermore, during sampling, just 25 mg were collected out of an estimated 500 g of total kidney mass (<0.005%). Although sampling was targeted to include the tubules, which have been reported to contain the bacteria, *Leptospira* can be missed because it may display an aggregated distribution pattern (Yamaguchi

TABLE 6 Leptospira species determination using species-specific assays among the 10 abattoir cattle that were tested positive using real-time PCR.

ID	*Leptospira* species
A024	*L. borgpetersenii*
A025	*L. borgpetersenii*
A214	*L. borgpetersenii*
A215	*L interrogans*
A216	*L. kirschneri*
A221	*L. borgpetersenii*
A237	*L. borgpetersenii*
A239	*L. borgpetersenii*
A244	*L. borgpetersenii*
A246	*L. interrogans*

et al., 2018). Additional studies suggested that *Leptospira* prevalence can be higher and up 32% [n = 130] in cattle slaughterhouses from Nigeria (Udechukwu et al., 2023).

Another factor that may have influenced our results is the time of year. We sampled in February, during the long dry season. Consequently, environmental conditions were not conducive to *Leptospira* survival, transmission or infection. In contrast, during the rainy season, infection risks climb for humans and animals because *Leptospira* survival in wet soils increases (Desvars et al., 2011). Mean annual rainfall in Cotonou is 1300 mm (Yabi & Afouda, 2012), but most precipitation falls during the long rainy season, from mid-April to mid-July, and the short rainy season, from mid-September to mid-November. Therefore, dry-season prevalence may not express the risks faced at other times of year, especially in southern Benin, which experiences major rainfall events and flooding. For example, 43% of Cotonou may experience floods that last weeks (Anonymous, 2012). Seroprevalence can convey the rate of previous or current infection in animals. To a certain extent, this metric can be compared among studies. At the Cotonou abattoir, 18% (95% CI [11%, 27%]) of cattle were seropositive. MAT-derived estimates for abattoirs vary across other African countries, from 27.8% in Uganda (n = 500) to 51% (n = 51) in Tanzania (Dreyfus et al., 2016; Swai & Schoonman, 2012). These figures are significantly higher than our estimate of seroprevalence (Uganda – 95% AC CI [−0.17, −0.01] and Tanzania – 95% AC CI [−0.47, −0.17]). However, it is unlikely that our results differed due to test methodology because we used a similar MAT threshold (i.e.1:100) as others (i.e.1:100 or 1:160) and serogroup panel. Although our panel lacked the serogroups Celledoni, Mini and Shermani, previously reported to be present in Africa, all occur at low levels and therefore should not have greatly influenced our findings. However, as mentioned above, sampling season may have been important (Desvars et al., 2011; Schmidt et al., 2021). The Ugandan study also sampled during the dry season, whereas the Tanzanian study sampled across multiple years and seasons (Dreyfus et al., 2016; Swai & Schoonman, 2012).

The use of an information-theoretic approach, never before used with *Leptospira* data to our knowledge, allowed us to identify the factors explaining variability in seroprevalence. Differences between sampling sites and, to a lesser extent, differences between males and females were reasonably supported by the data. Results for species were less clear-cut. In the four top-ranked models, species was not included in the lowest AIC model, appeared once as a main effect and twice alongside the sampling site × species interaction. This interaction was well supported by the data (relative importance weight of 0.44 vs. 0.15 and 0.09 for sex × sampling site and sex × species, respectively). Estimates of the probability of being seropositive from models with interaction site × species suggested that cattle were less likely than sheep to be seropositive in Ladji, whereas the reverse was true for the abattoir (second and fourth top-ranked models in Table 3). The third best model that did not include the site × species interaction estimated instead that the probability of being seropositive was higher for sheep than cattle (Table 3). Finally, reasonably precise model-averaged estimates highlighted that, at the abattoir, female cattle were only slightly more likely than sheep to be seropositive, whereas less precise model-averaged estimates conveyed that female sheep were more likely than cattle to be seropositive in Ladji but that there was essentially no difference for males at the abattoir (Table 4). It is thus necessary to sample a larger number of sheep in Ladji if we wish to better estimate local seroprevalence and improve our understanding of *Leptospira* epidemiology for these two species and sites.

L. borgpetersenii serovar Hardjo (Hardjobovis) is the most common strain maintained in cattle, although the *L. interrogans* serovar Hardjo (Hardjoprajitno) also occurs in certain parts of the world (Ellis, 2015). In this study, the 10 PCR-positive cattle were predominantly infected with *L. borgpetersenii*, which is consistent with global observations as well as with genotyping studies conducted specifically in Tanzania (n = 13 *L. borgpetersenii* out of 15 *Leptospira* spp) and Uganda (n = 3 *L. borgpetersenii* out of 5 *Leptospira* spp) (Alinaitwe et al., 2019; Allan, 2016). We also detected *L. kirschneri*, whose occurrence has been reported in Tanzania and Uganda. More surprisingly, in Benin, we detected *L. interrogans* DNA in two cattle, which were not found in the studies above but were predominant among the *Leptospira* species described in Nigeria (Udechukwu et al., 2023). These observations underscore the locally distribution of *Leptospira* species in cattle.

Recent research on rodents in Ladji found that *L. interrogans* and *L. borgpetersenii* were the most common species in renal tissue (Dossou et al., 2022). The fact that we detected the same bacterial species in cattle raises questions about how both hosts contribute to *Leptospira* maintenance. Future work should characterise the genetic profiles of the bacteria circulating in rodents, livestock and humans with a view to tracing the source(s) of *Leptospira* infections.

According to the MAT results, the predominant serogroup in cattle was Canicola, which contrasts with the results of other studies conducted in Africa (Dreyfus et al., 2016; Kelly et al., 2021; de Vries et al., 2014). The latter found the most common serogroups to be Sejroe, Pomona and Tarassovi. Variation in serogroup distribution is expected between countries, as the epidemiology is different.

In Uganda, the MAT results for Tarassovi were associated with the highest titres (1:800 and 1:1600). In cattle, higher titres can be observed when animals are poorly exposed or adapted to a particular

serogroup (Ellis, 2015). In our results, MAT titres were low (mainly 1:100 and 1:200), as previously reported including in other African studies (Alinaitwe et al., 2020; Allan, 2016). Thus, compared to the Ugandan results, our results suggest that the serogroups circulating in the sampled cattle may be endemic.

Past research exploring the genotypes of known serogroups has shown some Canicola strains to be related to *L. interrogans* and others to be related to *L. kirschneri* (5). We observed both species in Benin. As described elsewhere, neither the seropositivity nor the titre levels predicted *Leptospira* infection (Harran et al., 2023). Thus, further work must be carried out – using bacterial cultivation and additional molecular tools –to determine whether Canicola's predominance is a specificity of Benin or a spurious result of MAT crossreactions.

A diversity of serogroups has been observed in small ruminants: Icterohaemorrhagiae and Grippotyphosa, found in our study and in a Tanzanian study (Assenga et al., 2015), as well as Autumnalis and Sejroe, which are common in many African countries but did not show up in our samples (de Vries et al., 2014). According to our results, only nine of the small ruminants were seropositive (eight sheep and one goat), which limits interpretation. Additional sampling is required. We did not collect cattle urine at the abattoir because of logistical constraints. However, the presence of *Leptospira* DNA in the kidney samples indicates that the bacteria could be shed via urine. Thus, this source of contamination may present health risks to other animals and people. Indeed, there are occupational health risks for abattoir workers, as well as for individuals tasked with animal obstetrics, milking, and transportation, as underscored elsewhere (Alinaitwe et al., 2019; Dreyfus et al., 2014, 2016; Swai & Schoonman, 2012). Based on our results, workers may face greater exposure when handling male cattle vs. female sheep. However, health and safety measures can help reduce the likelihood of *Leptospira* transmission.

We currently have a limited picture of the health threat posed by *Leptospira* in abattoirs in Benin vs. other countries (Dreyfus et al., 2016; Ezeh et al., 1991). In Benin, unpublished seroprevalence data were collected for abattoir workers in 1994 (Koundé & Zohoun, 1994), and their level of seroprevalence (54.7%, $n = 503$ workers) was not significantly different from that of abattoir workers in Nigeria (29%, $n = 112$ workers) (95% AC CI [0.15, 0.34]) and in Uganda (35%, $n = 359$ workers) (95% AC CI [0.13, 0.26]) (Agunloye et al., 2001; Dreyfus et al., 2016). Thus, the risks appear to be of the same magnitude in all three countries, although the seroprevalence in livestock was significantly higher in Uganda than in Benin, as discussed before. That said, epidemiological conditions and the public health situation have likely changed over recent decades. This important issue should be explored in future research.

Finally, we found evidence that pathogenic *Leptospira* are circulating in livestock found in the district of Ladji. These animals roam freely and are kept in close proximity to residences, a common situation in Benin and many tropical countries in Africa. For example, 47% of households in Dakar contain small ruminants (Wilson, 2018). Thus, it is likely that the urban environment is contaminated by *Leptospira* shed by cattle or sheep and that infection risks in people are amplified by the infrastructure disrepair and seasonal flooding (Lau et al., 2010). Ladji harbours also many goats and pigs and both groups might play a role in *Leptospira* transmission. To our knowledge, information is lacking on the risks associated with animals being kept within households in urban zones. We hypothesise that the health risks may be elevated, and future work should focus on the inhabitants of such areas.

5 | CONCLUSIONS

To our knowledge, our study is the first to find *Leptospira* DNA or antibodies in livestock (cattle, goats and sheep) in Benin. Cattle had the greatest seroprevalence, and molecular analysis revealed 10% (95% CI [4.9%, 17.6%]) of cattle carried *Leptospira* DNA in their renal tissue. These findings underscore the public health risks associated with this livestock group. Consequently, further research should focus on better understanding human exposure at the Cotonou abattoir as well as in the densely populated and impoverished Ladji district, where leptospirosis may be a major but massively overlooked health concern. In conclusion, exploring leptospirosis epidemiology and *Leptospira* diversity in such settings can provide insights into transmission that can inform effective control and prevention measures.

AUTHOR CONTRIBUTIONS

Conceptualisation: Florence Ayral, Gauthier Dobigny, Rebecca Her, Laurent Crespin. *Methodology*: Florence Ayral, Laurent Crespin. *Formal analysis*: Laurent Crespin, Karine Groud, Audrey Chapron, Thibaut Lurier. *Investigation*: Rebecca Her, Jonas Etougbétché, Mathias Gnolonfoun, Camille Evenamia, Gualbert Houéménou, Gauthier Dobigny; Writing—original draft: Laurent Crespin, Gauthier Dobigny, Florence Ayral. *Visualisation*: Gauthier Dobigny. *Project administration*: Florence Ayral. *Software*: Laurent Crespin, Thibaut Lurier. *Validation*: Audrey Chapron, Karine Groud, Laurent Crespin, Thibaut Lurier. *Data curation*: Florence Ayral. *Resources*: Florence Ayral. *Writing – review and editing*: Rebecca Her, Laurent Crespin, Jonas Etougbétché, Karine Groud, Mathias Gnolonfoun, Audrey Chapron, Camille Evenamia, Gualbert Houéménou, Thibaut Lurier, Julien Cappelle, Gauthier Dobigny, Florence Ayral. *Supervision*: Laurent Crespin, Julien Cappelle, Gauthier Dobigny, Florence Ayral. *Funding acquisition*: Florence Ayral, Laurent Crespin.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no financial or personal relationship that may have inappropriately influenced them in writing this article.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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PEER REVIEW

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ETHICS STATEMENT

We assert that the sampling protocol adhered to French and European legislation on the treatment and usage of animals (Directive 2010/63/EC and French Administrative Decision 118-2013/02/01). The fieldwork was conducted in accordance with the procedure approved by VetAgro Sup Ethic Committee (agreement n° 2349).

DISCLAIMER

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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